PHARMACOLOGICAL ASPECTS OF β-ADRENOCEPTOR STIMULANTS

A Thesis submitted for the Degree of
DOCTOR OF PHILOSOPHY

by

ERROL MALTA B.Sc. (Hons.)

Department of Pharmacology,
University of Melbourne

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Page 29, paragraph 2, 3rd line should read:
"dose are 5-10 times less than those required to sensitise the dog"

Page 91, Table 9:
"(±)-Soterenol" should replace "(±)-Soterenol"

Example of the method used to compare the drugs with (-)-isoprenaline as described in Section 2.2.1, p 57 of the thesis.

For illustrative purposes the trace shown in Fig 1, p 57 will be used with hypothetical data.

a) Before the first concentration-effect curve, the resting atrial rate was 235 and the maximum after (-)-isoprenaline was 297 beats min⁻¹

<table>
<thead>
<tr>
<th>CONC.</th>
<th>0.1</th>
<th>0.2</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>5</th>
<th>10</th>
<th>20μgml⁻¹</th>
</tr>
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<tbody>
<tr>
<td>*Incr</td>
<td>5</td>
<td>10</td>
<td>20</td>
<td>35</td>
<td>45</td>
<td>55</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td>† %</td>
<td>8.1</td>
<td>16.2</td>
<td>32.4</td>
<td>56.5</td>
<td>72.6</td>
<td>88.7</td>
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<td>100</td>
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</table>

*These figures are the increases (in beats min⁻¹) above the resting rate produced by the various concentrations of (-)-isoprenaline.

†This line gives the increases produced at each concentration expressed as a percentage of the maximal increase elicited (i.e. 62 beats min⁻¹).
b) For the second concentration-effect curve the resting rate was for example 220 beats min\(^{-1}\) and the maximum after (−)-isoprenaline was 297 beats min\(^{-1}\). Calculations are carried out in the same way as in (a) except that 77 beats min\(^{-1}\) (297-220) represents 100%.

c) The percentage increases produced at the same concentrations in both curves are then averaged to give the mean curve for (−)-isoprenaline.

d) The resting rate before the MJ9184-1 curve was 233 beats min\(^{-1}\). It would be expected that the absolute maximum atrial rate produced by MJ9184-1 would be 297 beats min\(^{-1}\) on the basis of the absolute maximum rates produced by (−)-isoprenaline.

<table>
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<th>CONC</th>
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<th>0.1</th>
<th>0.2</th>
<th>0.5</th>
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<th>2</th>
<th>5</th>
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<tr>
<td>Incr</td>
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<td>6</td>
<td>8</td>
<td>10</td>
<td>20</td>
<td>28</td>
<td>30</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>%</td>
<td>4.8</td>
<td>9.4</td>
<td>12.5</td>
<td>15.6</td>
<td>31.3</td>
<td>43.8</td>
<td>46.9</td>
<td>51.6</td>
<td>51.6</td>
</tr>
</tbody>
</table>

*This line is now calculated on the basis that a 100% response would represent a 64 beats min\(^{-1}\) (297-233) increase above the resting rate.

e) The mean values for the (−)-isoprenaline curve (from c)) and the above values for MJ9184-1 are plotted in terms of percentages versus log (agonist concentration).
From these curves a 100% response for (-)-isoprenaline represents an intrinsic activity of 1.00 and therefore the intrinsic activity of MJ9184-1 in this experiment is 0.516.

To calculate the activity ratio: the concentration required to produce the 50% response in the case of (-)-isoprenaline is divided by the concentration required to produce 25.8% response for MJ9184-1 i.e. the concentration for half-maximal effect of MJ9184-1. Note: the calculation of the activity ratio is made using the equivalent molar concentrations.
SUMMARY

Structure-activity relationships of non-catecholamine sympathomimetics have been studied in isolated tissue preparations from the guinea-pig and in anaesthetized cats. The results indicate that the cat most closely resembles man with respect to $\beta_1/\beta_2$-receptor selectivity. Investigations show that the subtype of $\beta$-adrenoceptor involved in inhibiting antigen-induced histamine release in guinea-pig lung differs from that found in guinea-pig atria and trachea. Hydrocortisone appears to potentiate catecholamine-induced effects in the cat by blocking the extraneuronal uptake mechanisms.
PREFACE

The work presented for examination in this Thesis is that of the candidate alone. No portion of the work has been submitted by the candidate for the award of any other degree.
ACKNOWLEDGEMENTS

I would like to thank Professor M.J. Rand for inspiration and encouragement and for allowing me to complete this degree within his Department. I would also like to thank the Victorian College of Pharmacy for making available the facilities required to perform some of the experiments.

Special thanks are due to my supervisors, Dr. C. Raper, Dean of Pharmacology, Victorian College of Pharmacy, and Dr. Marian W. McCulloch, Senior Lecturer, Department of Pharmacology, University of Melbourne, without whose help, guidance and encouragement, many inspirations would not have evolved.

Others to whom I wish to express my thanks are Dr. E.J. Cornish and Mr. R.G. Goldie of the Department of Pharmacology, Victorian College of Pharmacy for ideas, help and discussion during the experiments concerned with the actions of hydrocortisone reported in Chapter 9; Mr. Trevor Davey for performing the experiments with (-)-isoprenaline and MJ9184-1 on guinea-pig pulmonary resistance which were included in Chapter 4 to facilitate discussion; Mr. Edward Stewart of the Department of Pharmaceutical Chemistry at the Victorian College of Pharmacy for the synthesis of the 3-methoxy compounds used in the experiments cited in Chapter 2; Boehringer-Ingelheim Pty. Ltd., Mead Johnson Pty. Ltd., Allen & Hanburys,
Astra Chemicals Pty.Ltd. and Wyeth Pharmaceuticals Pty. Ltd. for generous gifts of drugs; Miss Jeannie Baird for excellent technical assistance, expertise in the drawing of the diagrams and in the photographic work; Ms. Ilse Rand for competent typing of this Thesis and to those around me who have made work enjoyable during excruciating times.

Thanks are also due to Sigma (Pharmaceutical) Co. Ltd. for a scholarship held during the time of my candidature for this degree and to the National Heart Foundation of Australia for support in the form of a Grant-in-Aid to Dr. C. Raper.
ABSTRACT

The sympathomimetic amines studied in this Thesis are used or have potential use for the treatment of bronchial asthma. Since these bronchodilators appear to be the major form of treatment and were implicated in the increased in asthma deaths amongst young people in the 1960's, the first half of the introductory Chapter deals with asthma as a disease, the theories which have been proposed to account for the manifestations of asthma and the treatment of the disease with special emphasis placed on the sympathomimetic bronchodilators. The dual \( \beta \)-adrenoceptor hypothesis proposed by Lands and co-workers (1967) was supported by the discovery of salbutamol and other selective \( \beta_2 \)-adrenergic agonists. Since this Thesis is concerned in the large part with the structure-activity relationships (SAR) of such compounds, the second half of the first Chapter surveys the literature in an attempt to rationalize the SAR of such drugs, and also the concepts of the receptors on which these agents act.

In a number of isolated atrial preparations from different species, non-catechol phenylethanolamines produce concentration-effect curves reminiscent of those produced by partial agonists or dualists, while in tracheal preparations the compounds are full agonists. From the structural similarity of \( \beta \)-adrenergic antagonists and the newly synthesized selective \( \beta \)-adrenergic agonists it was decided to assess both the agonistic and antagonistic actions of a number of compounds in isolated guinea-pig atrial and tracheal
preparations. Compounds which are substituted catechols possess antagonistic actions which are of a competitive nature. The structure-activity relationships for both the agonistic and antagonistic actions of these compounds are discussed in Chapter 2.

Salbutamol was shown to possess antagonistic actions \textit{in vitro} and due to the possibility of the production of tolerance to the drug by such an action, it was decided to investigate its possible antagonistic effects \textit{in vivo} (Chapter 3). Although \((-\)), isoprenaline responsiveness was reduced during an infusion of salbutamol it appeared unlikely that this was due solely to a \(\beta\)-adrenoceptor blocking action.

It was apparent from the literature that species differences existed with regard to the potencies and \(\beta_1/\beta_2\)-receptor selectivity of non-catechol sympathomimetic amines. Chapter 4 illustrates the marked species differences that exist with regard to the actions of MJ9184-1 \textit{in vivo} and \textit{in vitro} using cat and guinea-pig preparations. In view of these differences it was decided to test a number of compounds studied in Chapter 2 in the anaesthetized cat (Chapter 5). Once again there were marked differences in the relative potencies of the compounds, their selectivity for \(\beta_2\)-receptors, and in the structure-activity relationships involved. A number of the compounds are used in man, and therefore comparisons as to the best animal model to predict the actions of a drug in man could be made. The results suggest that the cat resembled man more closely than the guinea-pig with respect to relative potency and \(\beta\)-receptor
selectivity.

Of the compounds tested in isolated tissue preparations from the guinea-pig, clenbuterol displayed the most potent antagonistic actions. Chapter 6 deals with the possible antagonistic actions of this compound in the anaesthetized cat. Although agonistic actions were displayed in cardiac, bronchial and skeletal muscle, no evidence of β-receptor antagonistic effects were seen in the cat. Thus this shows further evidence for a species difference between cats and guinea-pigs with respect to drug action on β-adrenoceptors.

Since there is a close relationship between the various results obtained in Chapters 2–6, a general discussion with regard to the overall results obtained has been included as Chapter 7 of this Thesis.

It has been claimed that sympathomimetic bronchodilators, in addition to producing relaxation of bronchial smooth muscle, may also inhibit the release of mediators from mast cells in anaphylaxis, thereby having a dual effect in those forms of asthma which have an immunological basis. Previous investigations into the subtype of β-adrenoceptor were not controlled as well as they might have been, and therefore it was decided to reinvestigate the subtype of receptor involved in inhibition of mediator release (Chapter 8). The conclusion reached was that in the guinea-pig the β-receptor involved in the inhibition of histamine release from sensitized lung differed from those found in guinea-pig atrial or tracheal preparations.

In the final Chapter (Chapter 9) investigations were carried out to test the hypothesis that hydrocortisone may be
producing its beneficial effect in status asthmaticus by blockade of the extraneuronal uptake of circulating adrenaline. Although the results discounted this possibility the potentiated response seen with bronchodilator drugs after hydrocortisone in severe asthma may be due in part to such an action.
PUBLICATIONS AND COMMUNICATIONS

The following publications and communications have arisen out of the work contained in this Thesis.

PUBLICATIONS:


[*Denotes publications arising out of communications presented to learned Societies in Australia.]*

COMMUNICATIONS:


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LITERATURE SURVEY
1. **Definition of asthma**

The diverse and seemingly unrelated factors which can precipitate an asthmatic attack were described nearly 300 years ago (Willis, 1684). Salmon (1686) recognised that these precipitating factors produced one end result - a variable degree of obstruction in the airways of the lungs.

In recent times attempts to define asthma have emphasized the narrowing of the airways, the spontaneous changes in severity of this constriction, and the fact that these effects are produced by a bronchial hyperreactivity to various stimuli (Fletcher, Gilson, Hugh-Jones & Scadding, 1958; American Thoracic Society, 1962). These factors remain the essential features associated with asthma although in certain conditions they may be difficult to distinguish from forms of chronic bronchitis (Fletcher, Howell, Pepys & Scadding, 1971).

2. **Clinical manifestations of asthma**

Even during symptom-free periods there is evidence of airway obstruction and/or abnormal imbalances in ventilation-perfusion ratios in asthmatics (Mcfadden & Lyons, 1968; Cade & Pain, 1973; Rebuck, 1974; McFadden, 1975; Palmer & Kelman, 1975). As recently pointed out by McFadden (1975), subtle degrees of bronchospasm can exaggerate responses produced by exposure to various stimuli, and therefore treatment should be continuously maintained until there is no evidence of bronchospasm.
During attacks of asthma there are dramatic falls in the FEV\textsubscript{1} (force expiratory volume in one second) and vital capacity (VC), and increases in functional residual capacity (FRC), residual volume (RV) and total lung capacity (TLC) (Permutt, 1971; Rebuck, 1974; McFadden, 1975). Thus marked hyperinflation of the lung occurs with respiration occurring at higher lung volumes presumably to maintain patent airways in the face of the bronchial smooth muscle constriction which tends to collapse the bronchi (Permutt, 1971). Arterial hypoxia occurs and this is associated with normo- or hypocapnia due to the maintenance of alveolar ventilation (Rebuck, 1974). Generally speaking hypercapnia is only observed in the late stages of status asthmaticus and usually heralds imminent death (Rees, Millar & Donald, 1968).

The cardiovascular effects associated with an asthmatic attack are sinus tachycardia, pulsus paradoxus, temporary enlargement of the right ventricle, pulmonary hypertension and S-T segment and T wave ECG abnormalities (Rees et al., 1968; Permutt, 1971; Rebuck & Read, 1971).

The lungs of patients who have died in status asthmaticus [defined as intense and persistent asthma lasting for hours or days (Davidson & Macleod, 1972)] are grossly overdistended and fail to collapse when the chest wall is opened. Macroscopic examination of the airways indicate obstruction by numerous mucus plugs. Microscopic studies on the bronchial lumen, mucous membrane, submucosa, wall and lung parenchyma have been described by Dunnill (1971).
3. Theories of asthma

3.1. β-Adrenergic Theory

Szentivanyi (1968) proposed the "Beta-Adrenergic Theory of Bronchial Asthma" on the basis of experiments in which reactivity to a number of general stimuli were tested in rats and mice sensitized with Bordetella pertussis. Apart from displaying many asthma-like symptoms these animals were hyposensitive to catecholamines. These facts led to further investigations and resulted in the idea that there was some imbalance between the α- and β-adrenoceptor effector systems in asthma.

Szentivanyi proposed that the defect was in the β-adrenergic bronchodilator system, and more specifically in the enzyme adenyl cyclase. He suggested that irrespective of the precipitating factors, asthma was produced by a relatively unopposed α-receptor mediated bronchoconstriction.

Due to the difficulty in obtaining lung samples from living asthmatic patients, most investigations into the Szentivanyi hypothesis have used isolated human leucocytes obtained from asthmatic and normal individuals. Measurements of leucocyte adenyl cyclase levels in asthmatics indicate that basal activity is lower and in the presence of isoprenaline increases in activity are lower or even non-existent when compared with normal controls (Smith & Parkes, 1970; Logsdon, Middleton & Coffey, 1972; Logsdon, Carnright, Middleton et al., 1973; Parker & Smith, 1973; Alston, Patel & Kerr, 1974;
Coffey, Hadden & Middleton, 1974). When studied, the lack of isoprenaline responsiveness was most evident in leucocytes that had been obtained from asthmatics experiencing "active" asthma for some time (Parker & Smith, 1973; Alston et al., 1974). If extrapolations can be made from the leucocyte studies then it would appear that there is some malfunction in the adenyl cyclase system in asthma.

Although not always observed (Leeks, Wood & Donsky, 1969) in asthmatics catecholamine-induced changes in blood glucose, lactic acid and eosinopenia are depressed when compared with non-asthmatic individuals (see Middleton, 1972; Reed, 1974 for references). Myocardial responses to adrenaline or isoprenaline do not appear to differ in asthmatic and normal persons (Cookson & Reed, 1963; Grieco, Pierson & Pi-Sunyer, 1968; Leeks et al., 1969; Makino, Oullette, Reed et al., 1970; Middleton, 1972). Thus it has been suggested that only $\beta_2$-adrenoceptor mediated responses are impaired in asthma (Makino et al., 1970; Middleton, 1972; Reed, 1974). Apart from the in vitro evidence involving adenyl cyclase levels, the in vivo evidence although scanty with respect to the lung, suggests there may be a lack of $\beta$-adrenergic responsiveness in asthma.

While the above evidence suggests that there may be a defect in the $\beta$-receptor system in asthmatics the second part of Szentivanyi's theory states that the asthmatical symptoms are due to unopposed $\alpha$-receptor mediated bronchoconstriction. $\alpha$-Adrenoceptor mediated broncho-
constriction has only been demonstrated after \( \beta \)-receptor blockade, thus it would appear that only a small population of \( \alpha \)-receptors are present and that \( \beta \)-receptor mediated activity predominates (Adolphson, Abern & Townley, 1971; Townley, Honrath & Guirgis, 1972; Bewtra, Longo, Adolphson et al., 1975). Evidence for overreactivity of \( \alpha \)-receptors in asthma is seen in experiments where isoprenaline-induced increases in adenyl cyclase activity in leucocytes from asthmatic patients was restored towards normal values after the addition of phentolamine (Logsdon, Carnright et al., 1973; Alston et al., 1974). Based on the studies by Belleau (1967) and Coffey, Hadden, Hadden et al. (1971) who showed that membrane bound ATPase was associated with the \( \alpha \)-receptor, Coffey, Hadden & Middleton (1974) investigated the \( Mg^{++} \) and \( Ca^{++} \)-dependent ATPase enzymes and found increased levels of these enzymes in leucocytes from asthmatic as opposed to normal children. Thus there may be more than one enzyme abnormality involved in asthma.

Non-asthmatic subjects show no changes in airway conductance with noradrenaline infusions after prior \( \beta \)-receptor blockade (Stone, Sarkar & Keltz, 1973). However in asthmatics the administration of non-selective \( \beta \)-receptor antagonists alone can precipitate severe bronchoconstriction (see Beumer, 1974 for references) which can in some cases be blocked by \( \alpha \)-adrenoceptor antagonists (Patel & Kerr, 1973; Skinner, Gaddie & Palmer, 1975). Indeed \( \alpha \)-adrenoceptor blockade has been claimed to be beneficial in the treatment of asthma (Kerr, Govindaraj

Since the α-adrenoceptor antagonists so far tested have many actions (e.g., antihistaminic, antiserotoninergeric and anticholinergic activities) (Benfey & Grillo, 1963; Boyd, Burnstock, Campbell et al., 1963; Birmingham & Szoksányi, 1965; Nickerson & Hollenberg, 1967; Kohli, 1968; Alps, Hill, Johnson et al., 1972) and in addition block neuronal uptake and prejunctional α-receptors (Rand, Story, Allen et al., 1973) it is difficult to determine the mechanism underlying any beneficial effect.

In man, α-receptor stimulation in the presence of β-receptor blockade, produces increases in plasma cyclic GMP levels (Ball, Kaminsky, Hardman et al., 1972). This nucleotide is now being recognised as the second messenger of cholinergic receptor activation (Lewis, Douglas & Bouhuys, 1973; Eichhorn, Salzman & Silen, 1974). Since atropine has been shown to prevent propranolol-induced bronchoconstriction, presumably via blockade of unmasked vagal activity (Macdonald, Ingram & McNeil, 1967; Greco & Pierson, 1971; Beumer, 1974) and if α-blockers can prevent increases in cyclic GMP produced by α-stimulants, then there may be a common pathway between the actions of α-receptor antagonists and anticholinergic compounds in preventing propranolol-induced bronchoconstriction.

From the available evidence it is apparent that Szentivanyi's theory is a little simplistic. Biochemically
the evidence points to the increased importance of the nucleotide cyclic GMP and possibly the enzyme ATPase (Polson, Krzanowski & Szentivanyi, 1974; Coffey, Hadden & Middleton, 1974) rather than adenyl cyclase (Patel, Alston & Kerr, 1974). The findings of depressed adenyl cyclase activity in asthmatics may well be due to the alterations in the levels of these other enzyme systems (Haddock, Patel, Alston & Kerr, 1975).

3.2. Immunological theory

Asthmatics have been classed into 2 groups - "intrinsic" and "extrinsic". In extrinsic as opposed to intrinsic asthma, there is a clearly identifiable allergen which provokes the asthmatic attack.

Exposure of the extrinsic asthmatic to the causal allergens can produce either or both Type I and Type III hypersensitivity reactions (see Pepys, 1973; Brogden, Speight & Avery, 1974 for references). In Type I (immediate onset) hypersensitivity reactions it is mast cell bound immunoglobulin E (IgE) which, on combining with its specific allergen, sets into motion a number of reactions which leads to the release of mediators from the mast cell (see Austen, 1974 for references). The released mediators have both direct and indirect actions (Orange, 1973; Drazen & Austen, 1975) which result in severe bronchoconstriction, mucous plugging and possibly some of the pathological features seen in asthma (Orange, 1973). Asthmatics displaying Type I reactions are referred to as atopic since they show an immediate reaction to the allergen
In Type III (late onset) reactions, immunoglobulins G, M and A have been implicated (Pepys, 1973; Brogden, Speight & Avery, 1974). These antibodies are of the circulating precipitin-type and union with the antigen occurs independently of tissues and cells triggering off a chain reaction as described by Pepys (1973). The mediators finally released in these reactions constitute most of those released in Type I reactions (Brocklehurst, 1971). Asthmatics showing Type III reactions have been referred to as non-atopic individuals (Pepys, 1971).

Both Type I and III reactions can be elicited in some individuals in response to an allergen and it is considered that these people have mixtures of IgE and IgG antibodies (Pepys, 1971, 1973).

Asthmatics suffering from intrinsic asthma show no demonstrable allergic reactions in skin tests with common allergens, and unlike extrinsic asthmatics, their blood levels of IgE are not generally elevated (Pepys, 1972; see Brogden, Avery & Speight, 1974 for references).

In some intrinsic asthmatics there is circumstantial evidence of Type I reactions since sputum and/or blood eosinophil levels are raised (Pepys, 1972, 1973; Austen, 1974; Brogden, Avery & Speight, 1974). Donovan, Johnasson, Bennich & Soothill (1970) came to the conclusion that although IgE levels in plasma may indicate the extent of the allergic response, the amount of IgE bound to tissue membranes was probably more important in terms of the
production of local hypersensitivity reactions. Support for this view came from Assem, Turner Warwick, Cole & Shaw (1971) who showed that the membranes of human leucocytes from intrinsic asthmatics were coated in the same proportion with IgE and IgG, as those on cells from patients with extrinsic asthma. Assem & co-workers (1971) further suggested that the IgE on these cells in intrinsic subjects may be non-specific IgE, and hence autoantibodies could be raised to the immunoglobulin.

Corticosteroids are beneficial in both intrinsic and extrinsic asthma (Rebuck, 1974) and since they have been shown to produce their effects by inhibition of the late onset of Type III reactions (Pepys, Davies, Breslin et al., 1974) it is possible that there is some allergic reaction in the underlying cause of intrinsic asthma.

Thus the terms "intrinsic" and "extrinsic" asthma represent an oversimplification of the view of asthma. Pepys (1972) suggested that there was no justification in terming extrinsic asthma as allergic and intrinsic asthma as non-allergic since the intrinsic group is probably heterogeneous in terms of an underlying mechanism. Overall it would seem that the terms extrinsic and intrinsic represent only the broad extremes of types of asthma.

3.3. Prostaglandin theory

Prostaglandins of the E₂ and F₂α types (PGE₂, PGF₂α) are found in the lung (Karim, Sandler & Williams, 1967) and are released in anaphylaxis (Piper & Vane, 1969) and by non-specific mechanical stimuli (Piper & Vane, 1971). Both
types of prostaglandin are metabolized to large extents in one passage through the lungs (Ferriera & Vane, 1967; Piper, Vane & Wyllie, 1970).

On the basis of results published by Sweatman & Collier (1968) showing that PGF$_{2\alpha}$ contracted whilst PGE$_2$ relaxed isolated human bronchial muscle, Horton (1969) proposed that bronchoconstriction might be produced by (i) overproduction of PGF$_{2\alpha}$ at the expense of E$_2$, or (ii) an increase in the sensitivity of muscle to prostaglandin F$_{2\alpha}$, or (iii) decreased F$_{2\alpha}$ metabolism or (iv) the conversion of PGE to PGF$_{2\alpha}$.

Prostaglandins F$_{2\alpha}$ and E$_2$ produce bronchoconstriction and bronchodilatation in both normals and asthmatics. Although asthmatics are ten times more sensitive to the bronchoconstrictor action of histamine they are eight-thousand times more sensitive to PGF$_{2\alpha}$ than normals. These facts have led some of the authors cited below to suggest that prostaglandins are implicated in the pathogenesis of asthma (Cuthbert, 1969; Herxheimer & Roetscher, 1971; Smith & Cuthbert, 1972, 1973; Kawakami, Uchiyama & Murao, 1973; Mathé, Hedqvist, Holmgren et al., 1973). Further evidence arose from the findings that within a few minutes of provoking an asthmatic attack there was an 8-fold increase in the major metabolite of PGF$_{2\alpha}$ in peripheral venous blood, the level of which seemed to be correlated with the severity of the attack (Green, Hedqvist & Sanborg, 1974). It is of interest that one of the PGF$_{2\alpha}$ metabolites (15-keto PGF$_{2\alpha}$) is a potent bronchoconstrictor agent (Dawson, Lewis, McMahon et al., 1974). Non-steroidal
anti-inflammatory drugs which prevent prostaglandin synthesis have been claimed to be beneficial in treating asthmatics who are not allergic to aspirin (see Smith, 1971 for references). However in a recent study Smith (1975) has cast doubt on the efficacy of indomethacin in asthma.

Arguments against prostaglandins playing a major role in the pathogenesis of asthma were presented by Smith (1973) who claimed that bronchoconstriction in asthma differed to that produced by PGF$_2$$_a$ in terms of its reversal by PGE$_2$ and isoprenaline. Dawson & Sweatman (1975) showed that sodium cromoglycate alleviated mediator release in anaphylaxis without affecting prostaglandin release, and that blockade of prostaglandin synthesis alone had no effect on anaphylactic responses. In addition Patel (1975) showed that PGF$_2$$_a$-induced bronchoconstriction was not inhibited by sodium cromoglycate. Since this drug is effective in both allergen- and exercise-induced asthma, he concluded that PGF$_2$$_a$ was not implicated in the pathogenesis of extrinsic asthma.

It would thus seem that PGF$_2$$_a$ does not play a major role in extrinsic asthma. However its possible role in intrinsic asthma is still unknown (Smith, 1972). PGF$_2$$_a$ may participate in both types of asthma via sensitization of bronchial smooth muscle to the other mediators which are released (Parker & Snider, 1973; Piper, 1973; Dawson & Sweatman, 1975; Patel, 1975).
3.4. Vagal stimulation

Woolcock, Macklem, Hogg, Wilson, Nadel, Frank & Brain (1969) using a modified retrograde catheter technique partitioned the pulmonary resistance of open-chest anaesthetized dogs into central (>3 mm internal diameter) and peripheral (<3 mm i.d. bronchioles) components. Stimulation of vagal nerves produced an increase in resistance in both the central and peripheral airways. In some dogs there was a greater increase in central than peripheral resistance and in others the reverse situation occurred.

The major resistance to airflow occurs in the 3-8 mm internal diameter bronchi (Macklem et al., 1969) so that if the effects of vagal stimulation occur predominantly in the central airways there will be a greater increase in total pulmonary resistance than if the predominant vagal effect was in the peripheral airways.

Since most of the bronchoconstriction produced by the inhalation of a specific antigen or a non-specific irritant is mediated via vagal reflex bronchoconstriction (Nadel, 1973; Gold, 1973) the results of Woolcock et al. (1969) might suggest that the site of major bronchoconstriction could vary.

Woolcock et al. (1969) suggested that if the pattern of vagally-induced responses seen in dogs also occurs in humans, and if the major response to reflex bronchoconstriction occurs in the central airways, then there would be a large increase in pulmonary resistance
and the person might be termed a hyperreactor to exogenous stimuli. Thus the site rather than the degree of bronchoconstriction may be important.

Gardiner et al. (1974) measured maximum expiratory flow volume curves (MEFV) in dogs and mentioned that in those dogs which showed predominant bronchoconstriction in the central airways the MEFV curves were similar to those obtained from asthmatic patients. Further support for the theory of Woolcock et al. (1969) came from the results of McFadden & Lyons (1969) who studied various airway parameters in asthmatics recovering from acute attacks. These authors noted that in three out of four cases the principal site of bronchoconstriction was in the central airways.

An alternative explanation for the bronchial hyperreactivity in asthma came from Woolcock, Macklem, Hogg & Wilson (1969) who studied the effect of vagal stimulation on lung mechanics in anaesthetized dogs treated with propranolol. After propranolol the increase in airway resistance to vagal stimulation was greater in the peripheral than central airways and thus they concluded that the major site for sympathetically-mediated bronchodilatation was in the peripheral airways. Since the vagus innervates both the central and peripheral airways, removal of the sympathetic influence could produce a state of hyperreactivity of the lungs. Thus the manifestations of a malfunction in the sympathetic nervous system in the lung, as a cause of asthma, would be in accord with the original theory proposed by Szentivanyi (see section 3.1 of this
Chapter). However when humans who are not asthmatic are tested for bronchial reactivity to acetylcholine aerosols before and after propranolol, the small degree and variability of the potentiation of acetylcholine makes it evident that complete loss of sympathetic tone to the lung cannot fully explain the bronchial hyperreactivity seen in asthmatics (Orehek, Gayrard, Grimaud & Charpin, 1975).

3.5. Summary

This section has attempted to outline the major theories which have been proposed to explain the production of the asthmatic state. The theory outlined by Mathé (1971) (decreased circulating adrenaline in asthma) has not been included since it is only a supplementary hypothesis and little direct evidence has been published to either support or dismiss the hypothesis.

It is evident that the biochemical abnormality originally proposed by Szentivanyi (1968) is not as simple as was first thought, however, the stimulus that this theory has invoked may well lead to a better understanding of the biochemical abnormalities involved in asthma.

4. Treatment of asthma

4.1. Miscellaneous Forms

Hyposensitization (immunotherapy) has been used for many years with variable success in an attempt to alleviate allergen-induced asthma (Norman, 1973). Recent trials using antigen extracts of greater specificity have yielded more promising results (Assem & McAllen, 1973a; D'Souza
et al., 1973). The various hypotheses proposed as to the mechanisms involved in immunotherapy have recently been outlined by Assem & McAllen (1973a).

Bacterial infection can precipitate asthmatic attacks by either specific (antigenic) or non-specific irritant processes (Howell, 1971). If asthma attacks are associated with respiratory infection then the use of antibiotics or if necessary autogenous bacterial vaccines to control the infective state is warranted (Rebuck & Read, 1971; Millman & Goldstein, 1972).

Barbiturates are included in many anti-asthmatic formulations to antagonize the CNS stimulant actions of the xanthines and/or centrally acting sympathomimetic amines which are present as the major therapeutic agents. Due to the ability of barbiturates to suppress central respiratory centres they should not be given to those who have severe bronchoconstriction (Anon., 1973a). In a recent review the anti-anxiety drug hydroxyzine was claimed to alleviate the anxiety associated with asthma, the CNS stimulation produced by the xanthines and ephedrine etc. which are present in the formulation, and to possess direct smooth muscle relaxant actions. Despite the fact that the drug possesses some CNS depressant actions it has been considered to be superior to the barbiturates and the drug of choice for inclusion in these formulations (Fiedelman & Martens, 1974).

Ingestion of non-steroidal anti-inflammatory drugs, particularly aspirin, may be beneficial in some asthmatics (see Smith, 1971; Anon., 1973b for references), yet
deleterious to the point of death in other subjects (Samter & Beers, 1967, 1968; Smith, 1971; Anon., 1973b; Szczklik, Gryglewski & Czerniawaska-mysik, 1975). The characteristics of aspirin-sensitive asthmatic individuals have already been outlined (Samter & Beers, 1967, 1968; Chaffee & Settipane, 1974). Initially it was proposed that aspirin acted as an agonist rather than an antagonist on kinin receptors (Samter & Beers, 1967), however recently Walker (1973) and Liebig, Bernauer et al. (1974) showed that indomethacin enhanced SRS-A release from lungs in anaphylaxis and suggested that by blocking prostaglandin synthesis, there was removal of a negative feed-back inhibition on mast cell mediator release. Whatever the mechanism involved, aspirin should be administered with caution to all asthmatics.

Anticholinergic compounds have been used in the treatment of asthma for many years (see Herxheimer, 1959). Atropine has been shown to produce bronchodilatation in man (Chamberlain, Muir & Kennedy, 1962; Yu, Galant & Gold, 1972; Patel, 1975), and to inhibit the bronchospasm produced by antigen inhalation (Yu et al., 1972), histamine, non-specific irritants (Simonsson, Jacobs & Nadel, 1967), suggestion (McFadden, Luparello, Lyons et al., 1969), and in some cases exercise-induced asthma (Kiviloog, 1973; Godfrey, 1975). The effects of atropine in asthma have been recently described (Gold, 1973). In two studies (Altounyan, 1964; Itkin & Anaud, 1970) atropine was shown to be ineffective, suggesting that the influence of the parasympathetic nervous system on bronchial smooth muscle
The therapeutic use of atropine is limited by its unpleasant side-effects, but a recently introduced atropine derivative, ipratropium (Sch1000), has been claimed to be a selective tracheo-bronchial muscarinic antagonist (see Emirgil, Dwyer, Baskette et al., 1975 for references) with a quick onset, a long duration of action and a freedom from side-effects (Emirgil et al., 1975; Storms, DoPico & Reed, 1975; Simonsson, Jonson & Ström, 1975).

4.2. Sodium cromoglycate

Since sodium cromoglycate prevents the release of mediators from mast cells induced by antigen, phospholipase A, compound 48/80, dextran and mechanical agitation, it is said to act by a non-specific stabilization of the cell membrane (Cox, 1971; Piper & Walker, 1973; see Brogden, Speight & Avery, 1974 for references). It is effective as a prophylactic agent in "extrinsic" as well as some cases of "intrinsic" asthma (Irani, Jones, Gent et al., 1972; Blumenthal, Schoenwetter, Macdonald et al., 1973) and therefore it is impossible to predict without trial which patients will benefit by its use. The clinical pharmacology of sodium cromoglycate has recently been extensively reviewed (Brogden et al., 1974).

Three new orally active sodium cromoglycate-like compounds AH 7079, AH 7725 and doxantrazole may be advantageous over sodium cromoglycate in terms of dosage control and lack of transient bronchoconstriction (Assem & McAllen, 1973b; Assem, Evans & McAllen, 1974; Haydu,
Bradley & Hughes, 1975; Batecholar, Garland, Green et al., 1975). Doxantrazole has both direct smooth muscle relaxant properties and inhibits phosphodiesterase (Batecholar et al., 1975). Since sodium cromoglycate has also been claimed to produce relatively strong phosphodiesterase inhibition (Roy & Warren, 1974) it has been suggested that it produces mast cell membrane stabilization via an increase in intracellular cyclic AMP (Taylor, Francis, Sheldon & Riott, 1974).

4.3. Corticosteroids

Corticosteroids are utilized in the therapy of asthma in two ways: (i) when the patient is severely ill and large doses are used, and (ii) in the form of low dose maintenance therapy. Although the mode of action of steroids is unknown their anti-inflammatory properties appear to be important in terms of asthma therapy (Rebuck, 1974). Other possible modes of action of the steroids when used in large doses are discussed in Chapter 9. Pepys, Davies, Breslin et al. (1974) showed that low doses of steroids inhibited the late asthmatic reaction (Type III) in patients showing either dual or only late reactions to various provocation tests. The mode of action at the cellular level is thought to be due to stabilization of neutrophil polymorphonuclear leucocyte lysosomal membranes against circulating immune complexes (Weissman, 1973; see Pepys, 1973 for further discussion).

The introduction of aerosol topical steroids for maintenance therapy has enabled many asthmatics to control
their symptoms without suffering from the side-effects frequently seen after administration of the compounds (McAllen, Kochanowski & Shaw, 1974; Williams, Kane & Shim, 1974; Rebuck, 1974; Wilcox & Avery, 1974).

Asthmatic patients who are not steroid-dependent are usually well controlled by 400 µg/day of beclomethasone dipropionate, whilst steroid-dependent patients can usually have the oral dose reduced, if not withdrawn altogether when given beclomethasone (Clark, 1972; Morrow Brown, Storey & George, 1972; Chatterjee, Ross, Carroll et al., 1972; Cameron, Cooper, Crompton et al., 1973; Dickson, Hall, Ellis 1973., Gaddie, Petrie, Reed et al., 1973; Brompton Hospital Report, 1974).

The side-effects of aerosol steroids are an unmasking of atopic symptoms in some individuals (Clark, 1972; Morrow Brown et al., 1972; Smith, 1973), and candida albicans infection in the mouth or throat. This is easily treated with anti-fungal agents (Brompton Hospital Report, 1974; McAllen et al., 1974; Wilcox & Avery, 1974).

Evidence for hypothalamic-pituitary-adrenal axis suppression occurs only at much higher daily doses than those required for adequate maintenance therapy (see Harris, Martin, Harrison et al., 1973 for references).

Thus aerosol steroids present a major advance in asthma treatment.

4.4. Xanthine derivatives

An account of the history of the introduction of xanthine compounds in asthma therapy has been recently
published (May, 1974).

As with steroids, theophylline can be given to relieve acute severe bronchospasm or as maintenance therapy (Pain, 1973; Rebuck, 1974; Piafsky & Ogilvie, 1975). In severe bronchospasm aminophylline is given in doses of 250 to 500 mg i.v. over 5-10 min in order to reach clinically effective plasma levels (5-15 µg ml⁻¹) (Taylor, Siegel, Busser et al., 1968; Howard, 1971; Hartnett & Marlin, 1974; Piafsky & Ogilvie, 1975). Toxic effects occur with plasma levels >20 µg ml⁻¹. Oxygen therapy is frequently instituted along with aminophylline in order to prevent further arterial hypoxia (Rees, Borthwick, Millar et al., 1967). In maintenance therapy for chronic asthma similar plasma levels are required. Due to side-effects such as nausea and vomiting, theophylline is formulated in many different ways in an attempt to reduce these side-effects which occur on oral administration (Martindale, 1972).

The mechanism of action of the xanthine derivatives has been thought to be due to inhibition of phosphodiesterase enzymes (see Piafsky & Ogilvie, 1975 for references). However some workers do not agree that this is the only mechanism involved (Robison, Butcher & Sutherland, 1971; Sheppard, 1973). Blockade of the extraneuronal uptake of catecholamines by methylxanthines may provide an alternative explanation since the concentrations required for this action are similar to the plasma concentrations required for effective therapy (Kalsner, 1971).
4.5. **Expectorants and mucolytics**

Expectorants and mucolytics are frequently included in preparations used in the treatment of asthma. However, their clinical value has not been substantiated (Fiedelman & Martens, 1974).

Potassium iodide has been used extensively, however toxic side-effects usually preclude continuous use for long periods (Thomson & Cohen, 1972). The onset of action is rapid (12-24 h) when compared with corticosteroids (2-3 weeks) (Herdheimer, 1969). One suggested mode of action is that iodides are excreted in part by the mucous glands of the respiratory tract and that increasing secretion of watery mucous allows more effective expectoration (Crossland, 1970). Ammonium chloride has also been used its mode of action being assigned to irritation of the gastric mucosa which produces a reflex increase in vagal tone and hence increased bronchial secretion (Crossland, 1970).

Proteolytic enzymes (deoxyribonucleases) are of questionable value as mucolytics due to their many side-effects. Depolymerization of the mucous fibre network is held responsible for their effectiveness. Sodium acetylcysteine has been used as an adjunct to asthma therapy to reduce viscid secretions. Its mode of action appears to be due to breaking of disulphide bonds in the mucoproteins, but its clinical effectiveness is reduced owing to its frequent ability to precipitate bronchospasm (Martindale, 1972).
One drug which has received wide attention as a mucolytic agent is bromhexine. Its action within the mucous glands of the respiratory tract is to prevent formation of acid mucopolysaccharide fibres which are held responsible for the tough, thick sputum which is difficult to expectorate. Of all the mucolytics available it appears to have the greatest beneficial effect in asthma (Today's Drugs, 1971).

4.6. Sympathomimetic bronchodilators

Aerosol sympathomimetic bronchodilators constitute the major drug treatment used in asthma. Sympathomimetic amines are effective in bronchial asthma since they produce smooth muscle relaxation via a direct action on the \( \beta_2 \)-adrenoceptors of the lung and possibly by inhibiting the release of the mediators of anaphylaxis and/or decreasing spontaneous and allergen-induced increases in histamine forming capacity (see Assem, 1974).

When administered by aerosol, sympathomimetic amines are effective in mild to moderate asthma and where the airway obstruction is largely due to bronchiolar muscle constriction (Clark, 1971). In severe asthma and in status asthmaticus the aerosols are relatively ineffective and this is thought to be due to the intense muscular constriction, oedema and mucous plugs in the airways which prevent the agent from reaching its site of action (Hume & Gandevia, 1957; Hume & Rhys Jones, 1960, 1961; Clark, 1971). However in severe asthmatic states, bronchodilatation via \( \beta \)-adrenoceptor stimulation is very effective when the drugs
are given either intravenously or with intermittent positive pressure ventilation (Wood, Downes, Scheinkopf et al., 1972; Choo-Kang, Tribe & Grant, 1974; Choo-Kang & Grant, 1975; Pitchett, McNicol & Riordan, 1975; Klaustermeyer, Di Bernado & Hale, 1975).

The major side-effects associated with the use of sympathomimetic bronchodilators are cardiac stimulation and skeletal muscle tremor (Rebuck, 1974). Further discussion on the prediction of side-effects based on animal experiments is presented in Chapters 5 and 7.

During the middle to late 1960's numerous reports appeared in the literature which showed that there had been a marked and disproportionate increase in asthma mortality amongst young asthmatics (see Aviado, 1975 for references). The increases in mortality were claimed to be due to abuse of the aerosols, since there appeared to be a positive correlation between the increased death rate and sales of aerosol bronchodilators (see Stolley, 1972 for references). That there was this increase is not disputed, however the relationship between the increased mortality and sales of aerosol preparations is a point of controversy. Various hypotheses have been proposed and some of these are outlined below.

Gandevia (1973, 1974) has argued against the relationship between increase in sales of aerosols and asthma deaths; however one factor overlooked in his argument is that although sales may continue to rise, physician awareness has increased as to the hazards associated with abuse of aerosols and therefore the
"epidemic" has subsided.

Stolley (1972) has implicated the availability of a high-dose formulation of isoprenaline (which would probably be prescribed to the more severely affected asthmatics and therefore be more prone to overuse) as one of the contributory reasons for the asthma epidemic only occurring in countries where the high-dose formulation was available.

Paterson, Conolly, Davies & Dollery (1968) found that the dose of isoprenaline required to produce a given increase in heart rate was greater in an asthmatic group which abused an isoprenaline aerosol than in the non-asthmatic group. These authors implicated tolerance to the bronchodilator effects of the sympathomimetics as a cause of asthma deaths. They found that a metabolite of isoprenaline (3-methoxyisoprenaline) possessed β-receptor blocking actions and therefore suggested that accumulation of this metabolite might be the cause of isoprenaline resistance. However quantitative determinations showed that the β-receptor blocking activities of the 3-methoxy derivatives of isoprenaline, isoetharine and rimiterol were extremely weak, and this together with the fact that only small quantities of the metabolite were formed negated this idea as the cause of isoprenaline tolerance (Blackwell, Conolly, Davies et al., 1970; Bassett, 1971; Hornsey, Gailer, Turner et al., 1971).

Conolly, Davies, Dollery & George (1971) produced tolerance to isoprenaline in non-asthmatic subjects by
infusing low doses of the amine and showing that superimposed bolus doses of isoprenaline had to be increased in order to produce a given increase in heart rate during the infusion. Kingsley, Littlejohns & Pritchard (1972) suggested that Conolly's results might be explained by the differing dose-response relationships which would occur when a given increase in heart rate was assessed on differing parts of a dose-response curve. However this cannot explain all of Conolly's results since in four of the volunteers resistance was shown despite the fact that the isoprenaline infusions produced no increases in basal heart rate. Conolly & co-workers (1971) also showed that in animals, particularly guinea-pigs, resistance to sympathomimetic amines could be produced. The conclusion from the study was that prolonged β-adrenoceptor stimulation may cause a degree of resistance to sympathetic drive in the lung, a drive which asthmatics rely upon heavily.

The underlying mechanisms involved in the production of this resistance have yet to be clarified. However further investigations along the lines described by Tothill (1972) regarding the influence of prostaglandins on sympathetic transmission, Hopkins (1975) concerning the levels of cAMP during continued infusions of sympathomimetics and particularly Mukherjee, Caron & Lefkowitz (1975) showing decreases in the number of β-receptor binding sites during prolonged exposure to isoprenaline, should be carried out.
Although Paterson et al. (1968) and Conolly et al. (1971) demonstrated isoprenaline tolerance in the myocardium, other investigators have failed to substantiate these results in both dog and man (Kingsley et al., 1972; Lichterfield & Löllgen, 1974; see Minatoya & Spilker, 1975 for references). Experimental evidence for diminished isoprenaline responsiveness in the lungs of man and dog are also lacking (Lichterfield & Löllgen, 1974; Minatoya & Spilker, 1975). Thus production of tolerance to isoprenaline in acute experiments is not easily demonstrable. In experiments in man chronic administration of the recommended doses of isoprenaline, salbutamol, orciprenaline and terbutaline do not appear to diminish peak responses to the amines (Chervinsky & Belinkoffs, 1969; Gibson, Tattersfield & Pride, 1972; Sims, 1974; Svedmyr, Larsson & Thiringer, 1974; Bhatia & Davies, 1975; Jenne, Strickland, Chick et al., 1975), however a shortened duration of action with isoprenaline and terbutaline was observed (Chervinsky et al., 1969; Jenne et al., 1975). The significance of this latter effect is not known.

Investigations of possible tolerance to the bronchodilator effects associated with chronic abuse of the aerosols, i.e., use of above recommended doses, have yet to be carried out, and on the basis of the original hypothesis (see Stolley, 1972 for references) these are the crucial experiments.

It has frequently been reported that adverse reactions such as severe bronchospasm can be induced by inhalation of aerosol sympathomimetic amines particularly isoprenaline (Keighley, 1966; Keighley, 1969; Lorenzen & Andersson,
1968; Caplan & Haynes, 1969; Van Metre, 1969; Reisman, 1970). Aviado (1975) has discussed three possible mechanisms and suggested that altered responsiveness to isoprenaline by the production of mucosal congestion as the likely reason. It cannot be overlooked that such reactions may have been involved in some of the asthma deaths.

Sympathomimetic amines, particularly isoprenaline and adrenaline, produce falls in the arterial oxygen tension ($PaO_2$). These decreases are smaller with selective $\beta_2$-receptor stimulants and in some cases increases in $PaO_2$ have been observed (see Harris, 1970; Flenley, 1971; Palmer, 1971; Radwan & Koziorowski, 1973; Holten, 1974 for references). Possible mechanisms for the production of this effect have been investigated by Harris (1970, 1972), Palmer (1971), Chick, Nicholson & Jönsson (1973) and Holten (1974). Although it has been argued that falls in $PaO_2$ are small and are therefore of little clinical importance (Anon., 1968, 1972a), a small drop in $PaO_2$ in persons who are already slightly hypoxic may produce large decreases in oxygen saturation. This, when coupled with the increased sensitivity of the hypoxic myocardium to isoprenaline, may precipitate cardiac arrhythmias (Palmer, 1971) and could therefore play a role in the rise in asthma deaths (see Harris, 1970 for references).

In a series of publications Collins, McDevitt, Shanks et al. (1968), Shanks & Swanton (1971) and McDevitt, Shanks & Swanton (1974) have shown an increased ability of sympathomimetic amines (isoprenaline, orciprenaline, salbutamol) to produce cardiac asystole in the presence of
hypoxia (PaO₂ < 40 mmHg) in anaesthetized dogs. The authors argued that this evidence supported the idea that the increase in asthma deaths may have been due to a combination of worsening hypoxia induced by the sympathomimetic amines together with the toxic effects of the amines themselves. Greenberg & Pines (1967) suggested that death in status asthmaticus was preceded by ventricular fibrillation but only one or two dogs in the former studies died for this reason. However McDevitt et al. (1974) and Rees, Millar & Donald (1968) have reported on 2 asthmatics dying with cardiac asystole. There is little documented evidence of cardiac disturbances associated with status asthmaticus. However, it is apparent that asthmatics undergoing an acute attack exhibit cardiac abnormalities associated with hypoxia and thus sympathomimetics may be able to exaggerate these conditions (Thomas & Valabhj, 1969; Riding, Dinda & Chatterjee, 1970; Rebuck & Read, 1971; Goldman, 1973).

Although no studies have been reported as yet for bronchial responsiveness to sympathomimetics under hypoxic conditions, chronic hypoxia in dogs has been shown to decrease the sensitivity of the myocardium to isoprenaline and to increase sensitivity to blockade by propranolol (Maher, Manchanda, Cymerman et al., 1975). Further work in this area with regard to a possible isoprenaline tolerance in asthmatics would be of interest.

Sensitization of the myocardium to isoprenaline by the halogenated propellants (trichlorofluoromethane, F-11 and dichlorodifluoromethane, F-12) used in the aerosols
and a consequent precipitation of cardiac arrhythmias has been suggested as another cause of the rise in asthma mortality associated with abuse of the aerosols.

The levels of the propellants in arterial blood indicate that the peak levels attained after one recommended dose are 5-10 times those required to sensitize the dog myocardium to adrenaline, and this safety factor is probably larger if isoprenaline is used (see Dollery, Williams, Draffan et al., 1974 for references). In addition, the plasma half-lives of the propellants are very short, indicating that when used in the recommended manner the propellants are unlikely to be exerting any detrimental effect on the myocardium (Dollery et al., 1974; Anon., 1975). In order to reach the required plasma concentrations of propellants to sensitize the myocardium one volunteer had to take one inhalation per breath of a propellant alone for 2 min. If a few breaths were taken in between aerosol inhalations, plasma levels failed to reach high concentrations (Draffan, Dollery, Williams et al., 1974). In an earlier study another volunteer took one inhalation of the propellant every 10 min for 6 h and no accumulation of the propellant was observed (Dollery, Draffan, Davies et al., 1970).

Thus unless the aerosols are very heavily abused there appears to be little likelihood of precipitating cardiac arrhythmias through myocardial sensitization to sympathomimetic amines.

Although many theories as to why there was an increase in asthma mortality have been proposed, the answer
is still unknown. Due to the lack of documented evidence surrounding those deaths the answer may never be known. It is unlikely that one single factor was involved, however no matter what the cause deleterious effects due to high doses of sympathomimetic amines appear to be implicated in the rise in asthma mortality in the 1960's. After the implication of aerosol abuse in the rise in asthma mortality physicians realized that the ineffectiveness of normal doses of sympathomimetics indicated an increasing severity of asthma which demanded other forms of therapy. This, together with increased patient counselling against overuse of the aerosols probably represents the major factor in the decline in the asthma mortality rates.

5. **Species**

Brittain, Jack & Ritchie (1970) in their review on 8-receptor stimulants commented on the difficulty of making reliable comparisons between various agonists since different methods and species have been utilized in the reports appearing in the literature. However this particular aspect has to be overlooked in order to discuss the structure-activity relationships presented in the following section.

For non-catechol agonists which are selective for 82-adrenoceptors, qualitative and quantitative differences do exist between species with regard to relative potencies and the degree of selectivity observed. While structure-activity relationships have been used to indicate broad structural requirements for agonistic or
antagonistic activity and selectivity of action at β-adrenoceptors, in practical terms the aim is to produce a compound with clinical utility in man.

Thus there is a need to find an animal model which can be utilized at the drug screening stage to predict the efficacy, relative potency and β₁/β₂-receptor selectivity of drugs in man. A detailed discussion on this point is presented in Chapter 7 of this Thesis.

6. Structure-activity relationships at β-adrenoceptor sites

Since the topic of this Thesis is concerned with structure-activity relationships of compounds acting on β-adrenoceptors, particularly with reference to their selectivity of action, the following discussions will be directed towards molecular modifications which affect the selectivity of action of agents affecting β-adrenoceptors.

6.1. β-Carbon Substitution

Removal of the β-hydroxy group or oxidation of this group leads to a dramatic loss of potency for positive chronotropic, bronchodilator and vasopressor actions compared to those produced by isoprenaline (Lands, Nash, Dertinger et al., 1948; Lands & Brown, 1967; Chapter 2 of this Thesis). Recent studies with dobutamine suggest that there is a selectivity towards positive inotropic as opposed to chronotropic actions in catecholamine compounds lacking a β-hydroxyl group (Tuttle, 1974; Vatner, McRitchie & Braunwald, 1974; Jewitt, Birkhead,
Mitchell et al., 1974; Beregovich, Bianchi, D'Angelo et al., 1975). Thus this modification may lead to a new series of clinically useful inotropic agents.

The desoxy derivative of propranolol is also much weaker as an antagonist than the parent compound (Howe & Shanks, 1966). Thus in general, a β-hydroxy group is required for potency activity of both agonists and antagonists at β-receptor sites.

The β-hydroxy group produces an assymetric carbon which confers optical isomerism on the compounds. The laevorotatory isomers of both β-adrenoceptor agonists and antagonists account for nearly all of the actions of racemic mixtures. An extensive review on the subject of adrenergic geometry has recently been published (Patil, Miller & Trendelenburg, 1974).

Patil (1969) suggested that under appropriate experimental conditions, the difference in activity between the optical isomers of one compound (isomeric ratio) would be different if different receptors were present in a number of tissues. Two publications (Buckner & Patil, 1971; Buckner & Abel, 1974) using this criteria concluded that the β-adrenoceptors in guinea-pig atria and trachea were of the same type and not of two different types (β₁ and β₂) as proposed by Lands, Arnold, McAuliff et al. (1967). The converse of Patil's (1969) hypothesis, i.e., for one agonist in a number of tissues, different isomeric ratios reflect different receptor types is valid. However the basic premise that similar isomeric ratios imply
identical receptors is not true since an investigation of the results of Patil, Patil & Krell (1971) and Buckner & Patil (1971) shows that when using noradrenaline isomeric ratios, \( \alpha \)- and \( \beta \)-receptors would in fact be identical! Thus the use of isomeric ratios to differentiate subtypes of \( \beta \)-receptors in various tissues does not appear to be a satisfactory method.

6.2. \( \alpha \)-Carbon Substitution

The introduction of \( \alpha \)-methyl or \( \alpha \)-ethyl groups in the ethanolamine side-chain of adrenaline or noradrenaline or in the corresponding amines in the 3-methanesulphonamido, 4-hydroxy ring substituted series, changes the spectrum of activity from predominant actions at \( \alpha \)- to \( \beta \)-adrenoceptor sites (Lands & Brown, 1967; Larsen, Gould, Roth et al., 1967).

In compounds having N-isopropyl substituents and either ethanolamine or oxypropanolamine side-chains, substitution of an \( \alpha \)-methyl or \( \alpha \)-ethyl group generally reduces the potency of the compound, but to a greater extent for \( \beta_1 \)- than \( \beta_2 \)-receptor mediated effects. Thus the compounds become \( \beta_2 \)-selective agents (Van Deripe, Åblad & Moran, 1964; Van Deripe & Moran, 1965; Lands & Brown, 1967; Collier & Dornhorst, 1969; Somani, 1969; Levy, 1973a; Rodger, 1973; Banitt, Coyne, McGurran et al., 1974; Daly, Flock & Levy, 1975; Fitzgerald & Missirlis, 1975).

In \( \beta \)-adrenoceptor agonists which because of their chemical structure already display selectivity for \( \beta_2 \)-adrenoceptors, the addition of \( \alpha \) -alkyl groups can have
varying effects, i.e., selectivity can be increased (Kaiser, Colella, Schwartz et al., 1974), remain unchanged (Collin, Hartley, Jack et al., 1970) or decrease (Kaiser, Schwartz, Colella et al., 1975). However potency is invariably reduced. Methylation of the α-carbon in the side-chain of the cardioselective antagonist practolol, did not alter its β₁-adrenoceptor selectivity (Levy, 1973a).

In conclusion, alkylation of the α-carbon atom can convert a compound from a non-selective β-adrenoceptor agent to one which shows β₂-adrenoceptor selectivity. The degree of selectivity attained appears to depend on the nature of the ring substituents. The variable results produced by α-alkyl substitution on compounds which already possess β₂-selective actions suggests the ring and amine substituents are more important than α-carbon substitution in determining selectivity.

6.3. Oxymethylene link

The inclusion of an oxymethylene group (−OCH₂−) between the ethanolamine side-chain and the ring structure is a feature of most recently synthetized β-adrenoceptor antagonists. However little quantitative work has been reported on the effects produced by oxymethylene substitution in compounds having the same amine and ring substitutions of such an addition.

The addition of such a group to the isoprenaline molecule produces little change in β-receptor potency (Raper & Keh, personal communication), however marked changes in the spectrum of activity were seen when such a group was
added to the 3-hydroxymethyl,5-hydroxy derivative of salbutamol. Activity was changed from a selective \( \beta_2 \)-receptor agonist to a potent non-selective \( \beta \)-adrenoceptor antagonist with no intrinsic sympathomimetic activity (Schwender, Sunday, Shavel et al., 1974; Schwender, Pike & Shavel, 1975). Since salbutamol possesses weak \( \beta \)-receptor blocking activity (see Chapter 2 of this Thesis), it would be of interest to investigate the addition of an oxymethylene group to this molecule.

Increased \( \beta \)-receptor blocking potency is found in compounds with 3-CH; 4-CN; 4-CH\(_3\)CONH (practolol); 1-naphthyl (propranolol) and 2-naphthyl (pronethalol) ring substitutions when the side-chains are oxypropanolamines rather than ethanolamine (compare Pratesi, Grana & Villa, 1968 with Mylecharane & Raper, 1974 and Buckner & Patil, 1971; Biel & Lun, 1966). No change in \( \beta \)-receptor blocking potency was found in 3-NO\(_2\) ring substituted compounds whilst a decrease in potency was seen in the 4-NO\(_2\) series. Abolition of \( \beta \)-receptor blockade was reported for the series of heterocyclic compounds synthetized by DaRe, Valenti et al. (1972) and DaRe, Primofiore et al. (1972).

Replacement of the oxymethylene group by groups such as CH\(_2\)CH\(_2\); OCH\(_2\)CH\(_2\); OCH(CH\(_3\)) - produces a reduction in potency (Biel & Lum, 1966; Crowther & Smith, 1968; Howe, 1969, 1970).

It is not known how the introduction of such a group produces its effects. Two possible mechanisms have been outlined by Barrett (1972) and by Ammon, Balsamo, Macchia
For β-adrenoceptor antagonists it would appear that an oxymethylene group generally enhances potency, the degree of which is governed by the ring substituents.

6.4. Amine substitution

Since both tertiary and quaternary derivatives of both sympathomimetic amines and β-receptor antagonists are virtually devoid of activity at β-adrenoceptor sites (Bovet & Bovet-Nitti, 1948; Cuthbert, 1964; Paton, 1967; Lahiri & Hardman, 1974), the following discussions will only deal with primary and secondary amines.

In agonists which contain a catechol or a methanesulphonanilide ring substitution there is an increase in β-receptor potency when the primary amine is converted to the N-methyl secondary amine. No such effect on potency is observed if the ring structures are 3,5-dichloro, 4-amino or saligenin. β-Receptor potency is either unchanged or diminished by further increases in chain lengths. However branching of the chain to form an isopropyl group greatly increases β-receptor potency whilst diminishing α-receptor activity, for the catechol, methanesulphonanilide, saligenin and 3,5-dichloro,4-amino ring substituted compounds (Ariëns, 1960; Lands & Brown, 1967; Larsen, Gould, Roth et al., 1967; Collin, Hartley, Jack et al., 1970; Engelhardt, 1972).

Increasing antagonistic activity at β-adrenoceptors is also seen when the -H,- CH,-CH(CH₃)₂ amine substituents are compared (Pratesi & Grana, 1965; Uloth, Kirk, Gould
et al., 1966; Crowther & Smith, 1968; Howe, Crowther, Stephenson et al., 1968; Chodnekar, Crowther, Hepworth et al., 1972; Mylecharane & Raper, 1973).

Phenylethanolamines with an N-t-butyl amine group possessing either a catechol, 2-chloro catechol, methanesulphonanilide, saligenin, 3,5-dichloro,4-amino or 3-NH₂, 4-OH ring substituents have generally a similar order of potency or are more potent than their corresponding N-isopropyl cogeners as β-receptor agonists. Except for the 2-chloro catechol compound, the other ring substituted compounds are more β₂-adrenoceptor selective than the N-isopropyl derivatives. This is due to an increased potency on β₂-receptors which is generally coupled with a decrease in β₁-receptor potency (Farmer & Levy, 1968; Engelhardt, 1972; Kaiser, Colella, Schwartz et al., 1974; Kaiser, Colella, Pavloff et al., 1974; O'Donnell & Wanstall, 1974; Chapter 2 of this Thesis).

The N-t-butyl derivative of 2-Cl,3,4-dihydroxy phenylethanolamine although more potent than the N-isopropyl derivative, exhibits a decreased selectivity due to a disproportionate increase in atrial compared to tracheal potency (Kaiser, Colella, Pavloff et al., 1974).

The potencies of β-adrenoceptor antagonists are usually increased, albeit the increase is small in some cases, when the amine substituent is changed from N-isopropyl to N-t-butyl (Uloth et al., 1966; Crowther & Smith, 1968; Howe, Crowther, Stephenson et al., 1968; Boissier et al., 1970; Schwender et al., 1970; Nakanishi et al., 1972; Ban, 1973; Levy, 1973b; Mylecharane & Raper,
1973, 1974; Nakagawa et al., 1974). No change in potency has been reported for the 2-ethoxy ring substituted series and practolol, while for ring 2-cyclopropyl and α-methyl pronethalol a decrease in potency was noted when the N-t-butyl was compared with the N-isopropyl substituted compounds (Crowther, Gilman, McLoughlin et al., 1969; Howe, 1969; Boissier et al., 1970; Crowther, Howe & Smith, 1971).

In terms of selectivity for β1- and β2-adrenoceptors little has been published regarding this N-alkyl modification for antagonists. The N-isopropyl derivatives of 2,4-dichloro and o-cyclopropyl ring substituted compounds were not selective and neither were the N-t-butyl cogeners (Boissier et al., 1970; Levy, 1973b). The change from N-isopropyl to N-t-butyl substitution in compounds possessing a ring nitrilo substitution depends on the position of the CN group. This alteration in amine loading resulted in a change from non-selective to selective with the 4-CN compound, no change in selectivity with the 3-CN compound, and an enhancement of β2-receptor selectivity with the 2-CN compound (Mylecharane & Raper, 1973, 1974). For the 2-nitrilo compound, increased β2-receptor selectivity was attained by a larger increase in tracheal than atrial potency, whilst β2-receptor selectivity for 4-CN (Mylecharane & Raper, 1974) and the 4-cyclopropyl derivatives (Boissier et al., 1970) is induced via increased tracheal and decreased atrial potency. In the 2,5-dimethoxy compounds selective β2-adrenoceptor blocking activity was produced by the N-t-butyl (butoxamine)
and there was a lesser selectivity with the N-isopropyl derivative (Levy, 1964; Wilkenfeld & Levy, 1969; Wasserman & Levy, 1974a; Daly, Flook & Levy, 1975; Fitzgerald & Missirilis, 1975).

In β-receptor agonists further increases in amine substitution usually consist of the addition of a phenyl or substituted phenyl moiety (4-hydroxy or 3,4-dimethoxy phenyl) onto the N-isopropyl and N-t-butyl substituents. Although large N-aralkyl groups produce variable effects on potency and selectivity, the changes produced are small and therefore of no advantage in the saligenin and 2-chloro catechol ring substituted compounds (Collin et al., 1970; Kaiser, Colella, Pavloff et al., 1974). Similar additional substitution produces increases in tracheal potency in sulphonalkyl and methanesulphonanilide compounds, however the selectivity for β₂-receptors decreased in the sulphonalkyl series and increased in the methanesulphonanilides due to a disproportionate increase in atrial and a large decrease in atrial potency, respectively (Kaiser, Schwartz, Colella et al., 1975; Chapter 2 of this Thesis).

N-tertiary butyl noradrenaline is more β₂-selective than isoprenaline and the addition of 4-hydroxyphenyl group, in addition to increasing the potency of both compounds, increases the β₂-selectivity to a greater extent in the substituted isopropyl than in the corresponding t-butyl compound so that both large N-alkyl compounds (Cc25 and Me454) are approximately equal in their β₂-receptor selectivity. An analogous situation
occurs in the resorcinol series except that the phenol isopropyl compound (fenoterol) remains less selective than the phenol-\textit{t}-butyl compound (Me506) (O'Donnell & Wanstall, 1974). Further discussion on the structure-activity relationships of these compounds is contained in Chapter 2.

For a number of \(\beta\)-adrenoceptor antagonists addition of a phenyl or substituted phenyl group to the N-isopropyl or N-\textit{t}-butyl amine moiety generally produces a small decrease in potency (Crowther & Smith, 1968; Howe, Crowther \textit{et al.}, 1968; Crowther \textit{et al.}, 1969, 1971; Hoefle, Hastings, Meyer \textit{et al.}, 1975; Schwender, Pike & Shavel, 1975).

Uloth \textit{et al.} (1966) reported that \(\text{CH(CH}_3\text{)}\text{CH}_2\text{OC}_6\text{H}_5\) amine substitution did not alter the potency of the compound from that seen with sotalol and therefore in that series the increase in potency with the change from N-isopropyl to N-\textit{t}-butyl was more influential than the addition of the large substituent.

Comparison of the salicylamide compounds AH3474 and AH5158 indicated that a large amine substituent increased the potency of the compounds as \(\beta\)-adrenoceptor antagonists (Blackburn, Byrne, Cullum \textit{et al.}, 1969; Farmer, Kennedy, Levy \textit{et al.}, 1972).

Practolol analogs with large N-aralkyl groups show a decreased cardioselectivity (Crowther \textit{et al.}, 1971). However with the amine substituent \(-\text{CH}_2\text{CH}_2\text{C}_6\text{H}_5\text{3,4-(CH}_3\text{O)}_2\) practolol's \(\beta_1\)-receptor selectivity is enhanced (Hoefle
Indeed with this substituent cardioselective blockade was produced with compounds related to propranolol and alprenolol, and altered the spectrum of activity of a $\beta_2$-receptor antagonist (Kö 1560) (Mylecharane & Raper, 1973) to that of a cardioselective ($\beta_1$) antagonist (Hoefle et al., 1975). A similar substituent [-CH$_2$CH$_2$C$_6$H$_4$4-CNH] was reported by Augstein et al. (1973) to produce cardioselective antagonists, however it appears that this substituent is not as universal in its production of selective $\beta_1$-receptor antagonism as that discovered by Hoefle et al. (1975). These amine substituents are relatively unique in terms of their effects on antagonistic activity compared with other large amine substituents.

In conclusion most $\beta$-receptor agonists and antagonists possess similar amine but differing ring substitutions. When considering the diversity of action of the compounds with respect to agonistic and antagonistic potency and selectivity at $\beta$-adrenoceptor sites, it would appear that the nature of the ring substituent is more important than the amine substituent in determining the spectrum of action of a compound.

6.5. Ring substitution

Due to the vast amount published concerning different ring substituents, this section will deal only with those compounds in which the combination of substitutions on the ring and ethanolamine side-chain produce selective $\beta$-adrenoceptor actions.
Removal of both the 3- and 4-hydroxyls from the ring of isoprenaline produces a marked drop in potency (Pratesi, Grana & Villa, 1968). The removal of a single hydroxy group also produces compounds which are much weaker agonists than isoprenaline. 3-Hydroxyisoprenaline and 4-hydroxyisoprenaline are equiactive as vasodepressor and cardiac stimulant agents, however relative to isoprenaline more activity is retained by the 3-hydroxy than the 4-hydroxy compound for bronchodilator and lactic acidemia effects (Lands & Tainter, 1953; Pratesi & Grana, 1965; Pratesi et al., 1968). Thus 3-hydroxyisoprenaline may be able to differentiate β-receptor subtypes in different tissues.

The introduction of a 2-hydroxy into noradrenaline converts the spectrum of activity from mainly α- to β-receptor stimulation (Bovet & Bovet-Nitti, 1948). Recently, Kaiser, Colella, Pavloff et al. (1974) showed that a 2-chloro catechol ring structured analog of isoprenaline marginally increased potency in atrial and tracheal preparations and increased β₂-receptor selectivity over that seen with isoprenaline. Comparison of this analog with clorprenaline indicated increased potency on the addition of a catechol group, but decreased β₂-selectivity, but unlike the addition of 2-methoxy group as in Sm220Cl (Bohmer, 1975) produced no increase in the duration of action of the compound.

Rearrangement of the hydroxyl groups in a catechol ring to produce a resorcinol ring (3,5-dihydroxy) produces compounds which although weaker as β-receptor agonists, are
selective for $\beta_2$- as opposed to $\beta_1$-adrenoceptors (O'Donnell & Wanstall, 1974; Chapters 2 and 5 of this Thesis). To gauge the importance of a second meta-hydroxyl group the actions of orciprenaline and 3-hydroxyisoprenaline should be compared.

Uloth et al. (1966) theorized that substitution of a moiety which had comparable acidity and therefore proton mobility and a similar spatial arrangement to a phenolic hydroxyl group should lead to the production of a compound with catecholamine-like actions. In this respect the authors chose the methanesulphonamido group. When this group was substituted for the 3-hydroxyl in isoprenaline a compound (soterenol) with potent $\beta$-receptor agonistic actions as well as selectivity for $\beta_2$- as opposed to $\beta_1$-receptor sites was produced (Dungan et al., 1968; Chapters 2 and 5 of this Thesis). Increasing the size of the substituent to ethylsulphonamido was shown to reduce $\beta$-receptor activity (Larsen et al., 1967).

Substitution of the methanesulphonamido group in the 4-position of isoprenaline produces what may be tentatively called a cardioselective agonist (MJ6987-1, Chapter 2). Removal of the 3-hydroxyl group from MJ6987-1 produces a non-selective antagonist with no intrinsic sympathomimetic activity (i.e., sotalol) (Barrett & Carter, 1970). Thus from these results, together with those of Kaiser, Colella, Schwartz et al. (1974) with a 3,5-bismethanesulphonamido compound, it is evident that such a group does not closely resemble the phenolic hydroxyls. Recent studies (Banitt et al., 1974) have
shown that the group can be made even more acidic without
drastic loss of potency.

Kaiser, Schwartz, Colella et al. (1975) when
considering methanesulphonamidos, proposed that due to the
adjacent sulphonyl group, a mobile proton would still be
produced even if the nitrogen atom of the methanesulphon-
amido group was replaced by a carbon atom. Replacement
of a CH$_3$CO$_2$NH$^-$ with a CH$_3$SO$_2$CH$_2$- group did not produce a
loss of activity, and appeared to enhance $\beta_2$-receptor
selectivity.

Preliminary studies by the above authors with the
N-$t$-butyl compound possessing a 3-CH$_2$SO$_2$CH$_3$,4-OH ring substitution
(sulforterol) showed that it was more selective for actions
in bronchial than in cardiac and skeletal muscle. In
contrast the methanesulphonanilide analogue was equipotent
in bronchial and skeletal muscle but had a lower cardiac
potency (see Chapter 5 of this Thesis). In contrast to
the methanesulphonamides maximal $\beta_2$-receptor selectivity
without loss of potency was seen when R=ethyl in a series
of RSO$_2$CH$_2^-$ substituted compounds. Larger groups (R>ethyl)
reduced potency and selectivity. In a series of
CH$_3$SO$_2$(CH$_2$)$_n$- analogs maximum potency and selectivity
occurred when n=1 (Kaiser, Schwartz, Colelli et al., 1975).

In addition to conferring $\beta$-receptor selectivity
in stimulant actions, the presence of other than a hydroxy
group in the meta-position also induces stability to
metabolic degradation by catechol-0-methyl transferase,
thereby prolonging the duration of $\beta$-adrenoceptor mediated
effects. It was for this reason that other workers
(Hartley, Jack, Lunts & Ritchie, 1968) were attempting to find meta ring substituents which would mimic the action of phenolic hydroxyl groups.

Although a m-COOH group theoretically fulfilled the required criteria, it was inactive as a β-receptor agonist. However testing of the compounds with the less acidic 3-hydroxymethyl group led to the discovery of salbutamol (Collin, Hartley, Jack et al., 1970). A labile proton capable of H-bonding was still essential since the ether (-CH₂OCH₃) and methyl (-CH₃) analogs were inactive (Hartley et al., 1968).

Apart from using a hydroxyethyl instead of a hydroxymethyl group, the replacement of the hydroxymethyl oxygen atom by a nitrogen atom or having 3-hydroxymethyl, 5-hydroxy ring substituents little can be changed in the meta-function without producing near complete loss of β-receptor stimulant activity (Brittain et al., 1970; Collin et al., 1970; Schwender, Sunday, Shavel et al., 1974).

The study by Kaiser, Colella, Schwartz et al. (1974) illustrates that compounds which have basic as well as weakly and strongly acidic meta substituents can be potent and selective β₂-adrenoceptor agonists. Therefore it appears that the acidic properties of meta substituents are not as important as was first suggested by Larsen et al. (1967).

Additionally the study of Kaiser, Colella, Schwartz et al. (1974) illustrates that a mobile proton is not an absolute requirement, since the compounds with an
N,N-dimethyl meta substituent, although 1000 times weaker than the extremely potent N-methyl analog, still possess reasonable β-adrenoceptor stimulant activity and in fact exhibits a larger selectivity for β₂ as opposed to β₁ receptors than the m-NHCH₃ substituted compound. The maintenance of β-receptor agonistic actions is also evident in quinterenol (Scriabine, Moore, Iorio et al., 1968) and NAB-365 (Engelhardt, 1972; Chapter 6 of this Thesis), compounds which do not have a mobile proton in the meta substituted moieties.

With a few exceptions, β-adrenoceptor antagonists exhibit sympathomimetic actions (Barrett & Carter, 1970) and as will be illustrated in Chapter 2, selective β-adrenoceptor agonists possess antagonistic effects.

From the preceding discussion it would appear that in some compounds substituents possessing a mobile proton near the meta-position are necessary to achieve agonistic actions, while in others this is not the case. One conclusion that covers all the above aspects for both β-adrenoceptor agonists and antagonists is that it is the nature of the chemical groups which constitute the ring substitution, rather than their ability to fulfill certain criteria, e.g., possession of a mobile proton near the meta-position, which will determine whether the compound behaves as an agonist or an antagonist at β-receptor sites.
Dale (1906) injected ergot alkaloids into cats and found that the adrenaline pressor response was converted into a depressor response. Other experiments showed that ergot alkaloids did not affect most of the inhibitory responses to sympathomimetic amines. This result plus structure-activity relationship studies of compounds related to adrenaline (Barger & Dale, 1910) led to the first indication that sympathomimetic amines might produce their actions through activation of differing types of adrenoceptors.

This idea was formalized by Ahlquist (1948) who compared the rank order of potencies of 6 sympathomimetic amines on a variety of parameters using both in vivo and in vitro preparations from many different species. On the basis of the two different rank orders of potency obtained, Ahlquist suggested that two types of adrenoceptors were involved. These he designated as α- and β-receptors. At the time of Ahlquist's publication only antagonists to the α-receptor mediated effects were available and thus little attention was paid to his dual adrenoceptor hypothesis until Moran & Perkins (1958) and Powell & Slater (1958) reported on the actions of 3,4-dichloroisoprenaline (OCI). This agent was found to block the effects of adrenaline-induced relaxation of bronchial smooth muscle, the cardiac chronotropic and inotropic effects of sympathetic stimulation and of catecholamines, catecholamine-induced vasodepressor responses and increases in femoral artery blood flow. Thus this agent antagonized
actions which Ahlquist classified as being due to \( \beta \)-receptor stimulation without affecting so-called \( \alpha \)-receptor mediated responses.

The discovery that the \( \alpha \)-methyl analogs of DCI and other compounds (Van Deripe et al., 1964, 1965) selectively antagonized vasodepressor as opposed to the cardiac stimulant actions of isoprenaline led Moran (1966) to suggest that the \( \beta \)-receptors in these two tissues might be different.

Later work by Furchgott (1967) also suggested the possible existence of multiple \( \beta \)-adrenoceptors. The criteria advanced by Furchgott (1967) for delineating receptors was that at equilibrium, similar antagonist dissociation constants (\( K_B \) values) would be obtained in different tissues if a single type of \( \beta \)-adrenoceptor was involved. In carefully controlled experiments using the non-selective antagonist pronethalol Furchgott was able to demonstrate at least 3 types of \( \beta \)-receptor, even within tissues from one species. In addition the relative potencies of isoprenaline, adrenaline, noradrenaline and phenylephrine differed in the tissues.

In the same year, Lands, Arnold, McAuliff, Luduena & Brown (1967) published the correlation coefficients of the relative potencies of a number of sympathomimetic amines for the production of \( \beta \)-receptor mediated responses in both \textit{in vivo} and \textit{in vitro} preparations from many species. These workers concluded that there were two types of \( \beta \)-adrenoceptor which they designated \( \beta_1 \)- and \( \beta_2 \)-receptors.

A summary of most of the tissues where responses have
been classified as being due to either $\beta_1$- or $\beta_2$-receptor activation has recently been published by Furchgott (1972).

Support for Land's hypothesis was soon forthcoming with the discovery of selective $\beta$-adrenoceptor agents such as salbutamol, terbutaline, practolol and butoxamine.

However in the review by Brittain, Jack & Ritchie (1970) the authors noted that there were differences in the responsiveness of "unnatural" agonists in cardiac and smooth muscle preparations. They rationalized this difference by introducing exo-receptor sites which differ in the various tissues containing $\beta$-receptors. By analogy with isoenzyme forms of a specific enzyme they suggested the term "isoreceptors" to distinguish between $\beta$-receptors that show differing specificities for the same $\beta$-agonist." Thus they concluded that Land's $\beta_1$- and $\beta_2$-receptor classification may represent only the extreme of a variable spectrum of different isoreceptors.

Spilker, McKeon & Arnold (1974) argued against the use of non-catecholamine agonists, antagonists, a combination of these two, or comparisons of potency which are made against any other compound than isoprenaline, to define $\beta_1$- and $\beta_2$-receptors, since they would not conform to the experimental approach originally used in sub-classifying the adrenoceptor. It is unquestionable that Land's original hypothesis has provided a classification of $\beta$-adrenoceptors which is of practical value as well as academic importance. However if the conditions laid down by Spilker et al. (1974) were followed it would restrict the development of hypotheses and conclusions such that no
other subtypes of receptors other than the broad based $\beta_1$- and $\beta_2$-receptors could be delineated. The corollary of Spilker and co-workers' hypothesis lends support to the view that there may be more than two subtypes of $\beta$-adrenoceptors.

Over recent years Åblad and co-workers (see Åblad, Borg, Carlsson et al., 1975 for references) have presented evidence which indicates that most tissues do not contain homogenous populations of either $\beta_1$- or $\beta_2$-receptor subtypes. Their studies with selective $\beta$-adrenoceptor antagonists in cardiac preparations showed that there was a differential blockade of responses to noradrenaline, adrenaline and isoprenaline. $\alpha$-Adrenoceptor antagonists blocked responses to noradrenaline to a greater extent than those of isoprenaline, while responses to adrenaline were only weakly inhibited. Conversely with the $\beta_2$-receptor antagonists the rank order for the greatest blockade was adrenaline > isoprenaline > noradrenaline. The results could be explained in two ways: (i) both $\beta_1$- and $\beta_2$-receptors are present in cardiac tissues, or (ii) the relative frequencies or concentrations of $\beta_1$- and $\beta_2$-receptors vary in different effector organs (Åblad et al., 1975). Thus for the latter proposal the population of $\beta_1$-receptors would be greatest in heart and adipose tissues, whilst from bronchial and vascular smooth muscle $\beta_2$-receptors would predominate. These workers have now established differential blockade in many tissues (see Åblad et al., 1975).
Recent work by Furchgott and colleagues (Furchgott, Wakade, Sorace & Stollak, 1975) supports the general concept outlined by Ablad et al. (1975) and at the same time suggests that within the one tissue in the one species there may be a variation in the ratios of $\beta$-receptor subtypes that are present.

Thus the isoreceptor concept of Brittain et al. (1970) can be envisaged in terms of varying heterogeneous populations of $\beta_1$- and $\beta_2$-receptors as proposed by Ablad and co-workers (1975).

Ideally in vitro preparations should be used to delineate $\beta$-receptors (see Furchgott, 1972 for references), however possible clinical implications of findings in which new subclasses of $\beta$-receptors are proposed can only be determined in the in vivo situation. One possible example is the recent finding that the ability of the cardioselective antagonists practolol and acebutolol to induce bronchospasm in asthmatic patients is variable and appears to be patient-selective, i.e., asthmatics sensitive to practolol will also be sensitive to acebutolol (see Beumer, 1974 for references; Skinner, Palmer & Kerridge, 1975). It is tempting to speculate that these effects are due to variations in the proportions of $\beta_1$- and $\beta_2$-receptors present or a slightly different $\beta$-isoreceptor in the lungs of these asthmatics.

Isolation of $\beta$-adrenoceptors has recently been achieved (see Lefkowitz, 1975) and possibly from the binding properties and chemical characterisation of the receptor will come the answer to the question: "Do
β-adrenoceptors constitute a wide ranging variable spectrum of isoreceptors in which β₁- and β₂-subtypes are the extremes, or can the available knowledge on β-receptors in different tissues and in the one tissue, be rationalized as being due to variable mixtures of only two receptor subtypes?"
CHAPTER 2:

NON-CATECHOL PHENYLETHANOLAMINES: AGONISTIC AND ANTAGONISTIC ACTIONS ON β-ADRENOCEPTORS IN ISOLATED TISSUES FROM THE GUINEA-PIG, AND ON α-ADRENOCEPTORS IN THE ISOLATED RAT VAS DEFERENS PREPARATION
SUMMARY

1. The β-adrenoceptor agonistic and antagonistic actions of a number of non-catechol phenylethanolamines with varying N-alkyl substitutions have been assessed in isolated atrial (β₁) and tracheal (β₂) preparations from the guinea-pig.

2. The α-adrenoceptor actions of some of these compounds have been evaluated using the isolated vas deferens preparation of the rat.

3. When series of compounds containing either a 3-methanesulphonamido ,4-hydroxy (methanesulphonanilide) or a 3,5-dihydroxy (resorcinol) ring substitutions with varying N-alkyl substituents are compared, there are marked differences in the structure-activity relationships between the two series with respect to both agonistic and antagonistic actions in isolated guinea-pig preparations.

4. The marked influence of the nature and position of the ring substitution upon the activity and selectivity of both β-adrenoceptor agonistic and antagonistic actions was seen when compounds with the same N-alkyl but different ring substitutions were compared.

5. Increasing the size of the amine group in the methanesulphonanilide series altered the pattern of α-receptor activity from agonist to antagonist. In
contrast the resorcinol compounds were without effect on α-adrenoceptors.

6. The results of this study show that the non-catechol phenylethanolamines exhibited β-adrenoceptor agonistic and antagonistic activity in both preparations and that the potency and selectivity seen depended on the nature and position of the ring substituents.

7. The results from the study on isolated vas deferens preparations of the rat showed that as far as the requirements for activity at α-receptors is concerned, the methanesulphonanilide moiety resembled the catechol moiety to a much greater extent than did the resorcinol moiety.
1. INTRODUCTION

It is well documented that compounds possessing some degree of selectivity for $\beta_2$- as opposed to $\beta_1$-adrenoceptors (e.g., salbutamol, soterenol, terbutaline) have lower intrinsic activity values than isoprenaline in isolated atrial preparations from the guinea-pig (see Brittain, Jack & Ritchie, 1970, for references; O'Donnell, 1972). This type of activity does not appear to be confined to guinea-pig preparations since similar findings have been reported in isolated rat atrial preparations and in experiments where positive chronotropic activity has been assessed in pithed rat preparations in vivo (Brittain et al., 1970).

From a structural viewpoint $\beta$-adrenoceptor antagonists are similar to $\beta$-receptor agonists, such as isoprenaline, the main difference being the nature of the ring substituents (see Barrett, 1972; Patil, Miller & Trendelenburg, 1974, for references). The selective $\beta$-adrenoceptor agonists mentioned previously, have other than catechol ring substitutions. Since the concentration-effect curves produced by many of these agents in isolated guinea-pig atrial preparations are reminiscent of those produced by partial agonists or dualists (Ariëns, Simonis & van Rossum, 1964; Barlow, 1964) it was decided to quantify the agonistic and antagonistic actions of such compounds. Guinea-pig atrial and tracheal preparations were used in these studies and the influence of the amine and ring substitution on $\beta$-adrenoceptor activity assessed.
2. METHODS

2.1. General

Isolated tissue preparations from guinea-pigs were mounted in Krebs solution (NaCl, 6.9; KCl, 0.4; MgSO_4.7H_2O, 0.14; Na_H_2PO_4, 0.14; NaHCO_3, 2.1; CaCl_2, 0.28; glucose, 2.0 g l\(^{-1}\)) maintained at 37°C and aerated with 5% carbon dioxide in oxygen. The bathing solution contained ascorbic acid (20 µg ml\(^{-1}\)) to reduce oxidation of catecholamines. In all experiments a 45 min equilibration period was allowed before addition of drugs to the tissue bath.

2.2. Guinea-pig atrial preparations: agonistic actions

Guinea-pigs (250 - 500 g) of either sex were killed by a blow on the head and the hearts removed and placed in oxygenated Krebs-Henseleit solution maintained at 37°C.

Whole atrial preparations were dissected free and attached to a platinum punctate tissue hook which acted as an anchor for the preparation. A resting tension of 2 g was applied to the tissues and spontaneous contractions were recorded using a force-displacement transducer (Grass FT03c) coupled through either a strain gauge coupler (Beckman type 9803) or Grass (7PLD) preamplifier to an ink-writing recorder. (Beckman type RP Dynograph or Grass Model 79). The output from the strain gauge coupler or Grass preamplifier was used to trigger a cardiotachometer (Beckman type 9857B or Grass 7P4DF) the integrated output of which was fed to the pen.

After the 45 min equilibration period full cumulative concentration-effect curves for (-)-isoprenaline were obtained at 20 min intervals until constant. The effects of
the drug under test were then examined, cumulative doses being administered until the maximum response was obtained. Figure 1 shows traces from an experiment in which the positive chronotropic actions of (-)-isoprenaline and MJ9184-1 were compared. In each experiment only one curve for the drug under test was established since washout of the drug was difficult.

Fig. 1. Traces from an experiment in which (-)-isoprenaline (ISO) and MJ9184-1 were compared for their ability to produce positive chronotropic actions in isolated spontaneously beating guinea-pig atrial preparations.

2.2.1. Evaluation of Results in Atrial Preparations

In control experiments it was found that the maximal atrial rate obtained in concentration-effect curves to (-)-isoprenaline was remarkably constant over a period of 6 hours. However the resting atrial rates were more variable. Because of the variability in the beats min\(^{-1}\) increase which occurred as a result of the changes in resting rate, the effects produced by submaximal concentrations of (-)-isoprenaline were calculated as a percentage of the maximal increase in beats min\(^{-1}\) obtained in each curve. Concentration-effect curves to (-)-isoprenaline were considered to be constant when there
was less than 5% variation in the percentage of the maximal response produced by the individual cumulative concentrations from three curves. In each experiment a mean concentration-effect curve to (-)-isoprenaline was calculated and this was used for comparative purposes when the activities of the other drugs were assessed.

In evaluating the agonistic actions of the drug under test, the difference in beats min\(^{-1}\) between the resting atrial rate and the absolute maximum obtained for (-)-isoprenaline was taken as 100%. Thus the drug-induced increases in atrial rates were assessed as percentages of the expected maximum response to (-)-isoprenaline.

Since most of the drugs tested did not produce the same maximal response as (-)-isoprenaline, and for the most part concentration-effect curves were not parallel, activity ratios rather than potency ratios with respect to (-)-isoprenaline were calculated. The activity ratio as used in this Thesis is defined as the ratio of the concentrations required to produce 50% of each drug's individual maximal response ((-)-isoprenaline = 1). The pD\(_2\) values are the negative logarithms of these concentrations. From these definitions it can be seen that the activity ratio approaches the potency ratio (Ariëns, Simonis & van Rossum, 1964) as the intrinsic activity approaches unity.

2.3. Guinea-pig tracheal preparations: agonistic actions

Guinea-pigs were killed by a blow on the head and the tracheas removed and placed in cold Krebs-Henseleit solution. The tracheas were cleared of connective tissue
and cut longitudinally, the incision being made in the cartilaginous band opposite the smooth muscle. Transverse cuts were then made alternately from each side of the preparation. Each cut was made through approximately three-quarters of the width of the preparation at two to three ring intervals. This method of cutting, which is very similar to that used in the rat fundus strip preparation (Vane, 1957) allows a near vertical alignment of the smooth muscle fibres in the preparation. Each tracheal preparation was set up in an organ bath, one end being attached to a tissue hook, the other to a lightly weighted frontal writing lever by means of a nylon thread. Contractions and relaxations were measured isotonically and recorded on smoked paper.

Carbachol \((10^{-6} \text{ M})\) was used to induce tone in the preparations. Once the response to carbachol had plateaued \(\beta\)-receptor mediated relaxations were produced by the cumulative administration of the drug under test. The concentration of carbachol used produced a submaximal increase in tension within the preparation. As in atrial preparations (section 2.2) constant responses were first obtained to \((-)\)-isoprenaline and thereafter a cumulative concentration-effect curve was established to the drug under test. Figure 2 shows traces from an experiment in which the effects of \((-)\)-isoprenaline and MJ9184-1 were compared.

In each experiment only one curve to the drug under test was established since the drugs were difficult to wash out.
2.3.1. Evaluation of Results in Tracheal Preparations

In preliminary experiments it was found that constant responses could be obtained to carbachol at 45 min intervals for periods up to 7 hours. The increase in tension produced by carbachol was maintained at a constant level for periods in excess of those required to establish cumulative concentration-effect curves for the relaxant actions of the sympathomimetic amines used in the experiments. (-)-Isoprenaline produced complete relaxation of the response to carbachol. After constant responses to (-)-isoprenaline had been obtained the mean maximal relaxation was calculated and given a value of 100%. A mean concentration-effect curve for (-)-isoprenaline was then calculated. The responses then produced by various concentrations of the test drug were expressed as percentages of the mean maximal relaxation produced by (-)-isoprenaline.
in each experiment.

Activity ratios and pD\textsubscript{2} values were determined in the same was as described for atria (section 2.2.1).

For each compound tested in either atrial or tracheal preparations, a pD\textsubscript{2} value was obtained, and an activity ratio and value for intrinsic activity \((-\)-isoprenaline = 1) calculated. At least four experiments were performed with each drug in each preparation and then means (±s.e.m.) calculated.

2.4. Selectivity ratio

In this Thesis in isolated guinea-pig atrial and tracheal preparations, the selectivity of a compound has been defined as the ratio of the activity ratios found in atria:trachea. Thus a value greater than unity indicates selective β\textsubscript{2}-adrenoceptor actions.

2.5. Guinea-pig atrial preparations: antagonistic actions

After constant cumulative concentration-effect curves had been established to \((-\)-isoprenaline one concentration of the drug under test was added to the bath and left in contact with the tissue for 45 min. All the drugs tested produced initial increases in atrial rate which were either maintained or declined towards the resting atrial rate during the contact period. With the drug still in contact with the tissue an \((-\)-isoprenaline curve was superimposed. This process was then repeated using increasing concentrations of the drug under test. Figure 3 illustrates traces from
an experiment in which soterenol was used.

The degree of intrinsic activity produced by the drug was taken into account in the plotting of the results. The increase in atrial rate which was present at the end of 45 min contact period was calculated as a percentage of the maximal response expected to occur with (-)-isoprenaline. The effects produced in superimposed (-)-isoprenaline concentration-effect curves were calculated with respect to the resting atrial rate before the addition of the partial agonist.

The shifts in the (-)-isoprenaline curves were assessed using the method of Arunlakshana & Schild (1959). In each curve a point was taken from a position midway between the residual intrinsic chronotropic activity produced by the drug and the maximum response obtained in the superimposed (-)-isoprenaline curve. The concentration corresponding to this point was then divided by the EC$_{50}$ value for (-)-isoprenaline in the control curves for the calculation of dose-ratios. This dose-ratio was used in the calculation of slope [$\log$(dose-ratio-1) vs. $\log$(molar antagonist concentration)] and pA$_2$ values for the compound.

Rather than using the EC$_{50}$ value for (-)-isoprenaline in the control curve as the divisor for the dose-ratio, an alternative method is to use the same percentage Emax level corresponding to the midway point calculated for the antagonist plus isoprenaline curve. However in terms of pA$_2$ and slope values there was little difference between the two methods and it was decided to use the former method for all calculations. At least four experiments were performed
with each compound and the means of the pA₂ and slope values calculated.

![Graph showing atrial rate in response to cumulative administration of (-)-isoprenaline (Iso), before (left) and in the presence (right) of soterenol [Sot, 2 µg ml⁻¹ (6.2 x 10⁻⁶ M), centre panel]. Lower records: relaxant effects in a tracheal preparation produced by the cumulative administration of (-)-isoprenaline (Iso), before (left) and in the presence (right) of soterenol [Sot, 1 µg ml⁻¹ (3 x 10⁻⁶ M) final concentration, centre panel.]

Fig. 3. **Upper records:** traces showing rise in atrial rate in response to the cumulative administration of (-)-isoprenaline (Iso), before (left) and in the presence (right) of soterenol [Sot, 2 µg ml⁻¹ (6.2 x 10⁻⁶ M), centre panel]. **Lower records:** relaxant effects in a tracheal preparation produced by the cumulative administration of (-)-isoprenaline (Iso), before (left) and in the presence (right) of soterenol [Sot, 1 µg ml⁻¹ (3 x 10⁻⁶ M) final concentration, centre panel.]

2.6. **Guinea-pig tracheal preparations:** antagonistic actions

Constant cumulative concentration-effect curves to (-)-isoprenaline were first established. Concentrations of the drug under test were then added in a cumulative fashion. With each dose added the relaxant effects either stabilized or waned. When 10-15 min was allowed between each cumulative concentration the relaxant effects of the drugs were
minimized. High concentrations of the drugs were then left in contact with the tissue for 45 min after which an (-)-isoprenaline curve was superimposed. Figure 3 shows traces from an experiment in which the antagonistic effects of soterenol were assessed in a tracheal preparation. After completion of the superimposed (-)-isoprenaline curve the tissue was washed, 30 min later carbachol was added to induce tone and the scheme then repeated for higher concentrations of the drug under test.

The method of evaluation of the shifts was the same as that described for atria in section 2.5 of this Chapter. Experiments in which the relaxant effects of the "antagonist" were greater than 50% of the (-)-isoprenaline maximum were discarded.

In those experiments in which the antagonistic actions of the compounds were weak, antagonistic activity was assessed in the presence of a higher concentration of carbachol (3 x 10^{-6} M). This enabled higher concentrations of the "antagonist" to be used when assessing shifts in concentration-effect curves to (-)-isoprenaline. The contraction produced by carbachol at 3 x 10^{-6} M was submaximal. In these experiments cumulative concentration-effect curves to (-)-isoprenaline did not always produce complete abolition of the carbachol-induced tone, however the extent of the relaxation was constant in any one experiment and responses were evaluated as a percentage of the maximum response to the catecholamine. For reasons such as availability of the compound, limited solubility and the possibility that other non-specific actions might interfere
with the evaluation of results, compounds which showed no evidence of blockade of (-)-isoprenaline responses at $10^{-5}$ M were not quantified for their antagonistic actions.

2.7. *Isolated rat vas deferens preparations*

Rats were killed by a blow on the head and the vasa deferentia removed and set up in an organ bath as described in section 2.1. Control cumulative concentration-effect curves to the effects of (-)-noradrenaline were established and then the effects of the drug were tested in the same manner. Results were plotted in terms of the maximal contraction produced by (-)-noradrenaline.

Antagonistic actions of the compounds were assessed by incubating the drug with the tissue for 45 min and then repeating the (-)-noradrenaline curves. Shifts in the curves were assessed using the method of Arunlakshana & Schild (1959).

2.8. *Drugs used*

The drugs used were (-)-isoprenaline bitartrate (Wyeth), (+)-isoprenaline bitartrate (Winthrop), carbachol chloride (British Drug Houses), (-)-noradrenaline bitartrate (Sigma), (-)-soterenol, (+)-soterenol, (±)-soterenol, (±)-MJ9184-1, MJ7858-1, (±)-MJ7999-1, (±)-MJ9184-1, (±)-sotalol, all as hydrochloride salts (Mead Johnson), (±)-orciptrenaline sulphate, (±)-fenoterol, (±)-Me506 hydrobromide and (±)-clenbuterol hydrochloride (Boehringer-Ingelheim), (-)-terbutaline and (+)-terbutaline hydrobromide, (±)-terbutaline sulphate (Astra), (-)-salbutamol and
(±)-salbutamol base (Allen & Hanburys), phentolamine hydrochloride (Ciba-Geigy), azapetine hydrochloride (Roche Products), propranolol hydrochloride (Imperial Chemical Industries). The N-isopropyl and N-t-butyl analogs of normetanephrine were kindly synthesized as the hydrochloride salts by Mr. Edward Stewart in the Department of Pharmaceutical Chemistry at the Victorian College of Pharmacy, Melbourne, Australia.

Stock solutions of the catecholamines and resorcinol compounds were freshly prepared and made up in N/100 HCl, while the other drugs were dissolved in distilled water. Appropriate dilutions were made with the Krebs-Henseleit solution. Concentrations in the text are stated in molar terms.
3. RESULTS

3.1. General

The agonistic actions of all the drugs tested in both guinea-pig atrial and tracheal preparations were antagonized by propranolol (10^{-6} M).

The mean values (±s.e.m.) for the β-adrenoceptor agonistic and antagonistic actions of the compounds tested are contained in Tables 1 to 6. The drugs have been arranged in these Tables according to the nature of the chemical group substituted in the benzene ring. In order to illustrate some points more clearly, the various mean values from these Tables have been reproduced in the Discussion.

3.1.1. Time Course for Onset of β-Adrenoceptor Antagonistic Actions

In 6 preliminary experiments in isolated guinea-pig atrial preparations, calculations of pA₂ values and values for the slope of the relationship log(dose-ratio-1) vs. log(molar antagonist concentration) were obtained with (±)-soterenol, (±)-salbutamol and (±)-MJ9184-1 after 5 or 20 min incubation periods. The pA₂ values obtained fell into the same range as those obtained when a 45 min incubation period was used. Thus in terms of β-adrenoceptor blockade, there was a fast equilibration between the concentration of the drugs in the bathing solution and that in the vicinity of the β-receptors.

The necessity for a slow build-up of concentrations of the drugs in tracheal preparations (see section 2.6)
precluded any attempts to establish the time of onset of β-receptor blockade in this tissue. However on the basis of the results found in atrial preparations, a 45 min equilibration period was used in tracheal preparations.

3.2. β-Adrenoceptor agonistic and antagonistic actions of the optical isomers of some sympathomimetic amines

The optical isomers of terbutaline, soterenol, salbutamol and isoprenaline were tested for their agonistic actions in atrial and tracheal preparations from the guinea-pig. The effects of desoxysoterenol (MJ7858-1) were also assessed in this study. The values obtained for pD₂, intrinsic activity and activity ratios are shown in Table 1. For all compounds tested the agonistic activity was seen to be greatest with the laevo-rotatory isomers.

In addition to evaluating the agonistic actions of the optical isomers the isomers of soterenol, salbutamol and isoprenaline were tested for their β-receptor blocking actions in atrial preparations. The values obtained for soterenol and salbutamol are shown in Table 2. As with the agonistic actions, the (-)- were much more potent than the (+)-isomers. Desoxysoterenol (MJ7858-1) was also very weak as an antagonist, having a low pA₂ value of the same order as that found with the (+)-isomer of soterenol. No antagonistic effects were found with the (-)- or (+)-isomers of isoprenaline. In atrial preparations the chronotropic actions produced by concentrations of (-)-isoprenaline
TABLE 1. β-Adrenoceptor agonistic actions of the optical isomers of terbutaline, soterenol, salbutamol, isoprenaline and of MJ7858-1 (desoxysoterenol) in isolated spontaneously beating guinea-pig atrial preparations and in isolated carbachol-stimulated guinea-pig tracheal preparations. Shown are the mean values with s.e.m. in parentheses, calculated from at least four comparisons with each drug. Activity ratios are calculated with respect to (-)-isoprenaline.

<table>
<thead>
<tr>
<th></th>
<th>ATRIA pD2</th>
<th>α</th>
<th>ACTIVITY RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-Terbutaline</td>
<td>5.72 (0.14)</td>
<td>0.86 (0.02)</td>
<td>531 (75)</td>
</tr>
<tr>
<td>(+)-Terbutaline</td>
<td>4.03 (0.09)</td>
<td>0.63 (0.07)</td>
<td>30,617 (6,900)</td>
</tr>
<tr>
<td>(-)-Soterenol</td>
<td>7.40 (0.06)</td>
<td>0.71 (0.05)</td>
<td>11.6 (1.2)</td>
</tr>
<tr>
<td>(+)-Soterenol</td>
<td>4.44 (0.06)</td>
<td>0.29 (0.04)</td>
<td>10,224 (1,280)</td>
</tr>
<tr>
<td>MJ7858-1</td>
<td>5.46 (0.04)</td>
<td>0.14 (0.03)</td>
<td>1,057 (235)</td>
</tr>
<tr>
<td>(-)-Salbutamol</td>
<td>6.17 (0.02)</td>
<td>0.51 (0.07)</td>
<td>198 (29)</td>
</tr>
<tr>
<td>(+)-Salbutamol</td>
<td>4.47 (0.07)</td>
<td>0.56 (0.11)</td>
<td>11,542 (2,833)</td>
</tr>
<tr>
<td>(-)-Isoprenaline</td>
<td>8.45 (0.06)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>(+)-Isoprenaline</td>
<td>6.79 (0.08)</td>
<td>0.96 (0.02)</td>
<td>42.9 (3.0)</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>TRACHEA pD2</th>
<th>α</th>
<th>ACTIVITY RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-Terbutaline</td>
<td>6.14 (0.08)</td>
<td>1.09 (0.03)</td>
<td>6.8 (1.0)</td>
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<tr>
<td>(+)-Terbutaline</td>
<td>3.50 (0.06)</td>
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<td>2,599 (347)</td>
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<tr>
<td>(-)-Soterenol</td>
<td>5.95 (0.15)</td>
<td>0.24 (0.04)</td>
<td>15.2 (9.7)</td>
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<td>(+)-Soterenol</td>
<td>4.57 (0.17)</td>
<td>0.12 (0.05)</td>
<td>396 (192)</td>
</tr>
<tr>
<td>MJ7858-1</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>(-)-Salbutamol</td>
<td>6.20 (0.10)</td>
<td>1.00 (0.01)</td>
<td>9.6 (1.7)</td>
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<tr>
<td>(+)-Salbutamol</td>
<td>3.75 (0.07)</td>
<td>0.90 (0.07)</td>
<td>2,593 (151)</td>
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<td>(-)-Isoprenaline</td>
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<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>(+)-Isoprenaline</td>
<td>5.87 (0.09)</td>
<td>1.04 (0.03)</td>
<td>20.4 (1.0)</td>
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*pD₂ < 4
TABLE 2. β-Adrenoceptor antagonistic actions of the isomers of soterenol, salbutamol and of MJ7858-1 (desoxysoterenol) in isolated spontaneously beating guinea-pig atrial preparations and soterenol in isolated carbachol-stimulated tracheal preparations from guinea-pigs. Shown are the mean pA2 values and values of slope (log(DR-1) vs. log(molar antagonist concentration) obtained from at least 4 experiments with each drug. Numbers in parentheses are standard errors of the mean.

<table>
<thead>
<tr>
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<th>ATRIA pA2</th>
<th>Slope</th>
</tr>
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<tr>
<td>(-)-Soterenol</td>
<td>6.47 (0.02)</td>
<td>0.95 (0.03)</td>
</tr>
<tr>
<td>(+)-Soterenol</td>
<td>4.49 (0.09)</td>
<td>1.12 (0.29)</td>
</tr>
<tr>
<td>MJ7858-1</td>
<td>4.18 (0.07)</td>
<td>1.34 (0.22)</td>
</tr>
<tr>
<td>(-)-Salbutamol</td>
<td>5.44 (1.0)</td>
<td>0.96 (0.09)</td>
</tr>
<tr>
<td>(+)-Salbutamol</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>TRACHEA pA2</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-Soterenol</td>
<td>5.88 (0.10)</td>
<td>0.74 (0.15)</td>
</tr>
<tr>
<td>(+)-Soterenol</td>
<td>4.14 (0.07)</td>
<td>1.27 (0.16)</td>
</tr>
<tr>
<td>MJ7858-1</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

*No β-adrenoceptor antagonistic actions at 10^-4 M.

producing 25-50% of the maximal chronotropic activity (2-5 x 10^-9 M) were well maintained throughout the whole of the 45 min contact period. The EC_{50} values for superimposed (-)-isoprenaline curves were virtually unchanged being 96% (s.e.m. = 6.0, n = 4) of control values. Maximal responses to (-)-isoprenaline were slightly reduced, a mean reduction of 6.4 ± 2.7% being obtained. The (+)-isomer of isoprenaline also produced no evidence of a reduction in maximal response to (-)-isoprenaline and EC_{50} values were unchanged.
As in atrial preparations the pA$_2$ value for the (+)-isomers of soterenol in tracheal preparations was lower than that found with the (-)-isomer (Table 2). MJ7858-1 was without antagonistic effects in tracheal preparations in concentrations up to $10^{-4}$ M. For both the (-)- and (+)-isomers of isoprenaline there was no change in the EC$_{50}$ values or maximum response in superimposed (-)-isoprenaline curves after 45 min incubations with concentrations producing 25-50% of the maximum response.

3.3. Methanesulphonanilide compounds

In all, five compounds possessing a methanesulphonanilide group in the benzene ring were evaluated for their agonistic and antagonistic actions at $\beta$-adrenoceptor sites.

Table 3a shows the mean values (±s.e.m.) obtained with (+)-soterenol, MJ7999-1 and MJ9184-1. These compounds have a 3-methanesulphonamido, 4-hydroxy ring substitution but differ in the groups attached to the amine nitrogen. In terms of their agonistic actions soterenol and MJ7999-1 are less active than (-)-isoprenaline and are partial agonists in both atrial and tracheal preparations. Figure 4 shows the mean concentration-effect curves for soterenol and MJ7999-1 in both preparations. The aralkyl substituted compound MJ9184-1 is a relatively weak partial agonist in atrial preparations but shows strong agonistic actions in tracheal preparations. When the three compounds are compared, increasing the size of the amine substituent leads to a progressive decrease in atrial ($\beta_1$) and an
TABLE 3. Quantitative data for the β-adrenoceptor agonistic and antagonistic actions of five compounds possessing a methanesulphonamido substituent in the benzene ring in isolated guinea-pig atrial (AT) and tracheal (TR) preparations. Mean values for \( pD_2 \), intrinsic activity (\( \alpha \)), activity ratio, \( pA_2 \) and slope of the relationship \( \log(\text{dose-ratio}-1) \) vs. \( \log (\text{molar antagonist concentration}) \) are shown. Numbers in parentheses are the s.e.m. from at least four experiments with each drug. (a) Compounds with a 3-methanesulphonamido, 4-hydroxy ring substitution but with differing amine substituents are shown. (b) Compounds with N-isopropyl amine substitution: sotalol possesses a 4-methanesulphonamido and MJ6987-1 4-methanesulphonamido, 3-hydroxy ring substitution. * Denotes no β-receptor blocking action at \( 10^{-4} \) M.

(a)

<table>
<thead>
<tr>
<th>Compound</th>
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<th>TR</th>
</tr>
</thead>
<tbody>
<tr>
<td>((\pm)-\text{soterenol})</td>
<td>((\pm)-\text{MJ7999}-1)</td>
<td>((\pm)-\text{MJ9184}-1)</td>
</tr>
<tr>
<td>R=CH(CH(_3))(_2)</td>
<td>R=C(CH(_3))(_3)</td>
<td>R=C(CH(_3))(_2)CH(_2)C(_6)H(_5)</td>
</tr>
<tr>
<td>(pD_2)</td>
<td>7.26 (0.19)</td>
<td>7.26 (0.09)</td>
</tr>
<tr>
<td>(\alpha)</td>
<td>0.33 (0.04)</td>
<td>0.68 (0.10)</td>
</tr>
<tr>
<td>Activity Ratio</td>
<td>13.8 (2.5)</td>
<td>22.2 (4.2)</td>
</tr>
<tr>
<td>(pA_2)</td>
<td>6.48 (0.05)</td>
<td>6.52 (0.06)</td>
</tr>
<tr>
<td>Slope</td>
<td>0.97 (0.11)</td>
<td>1.06 (0.07)</td>
</tr>
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</table>

(b)

<table>
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<th>Compound</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Sotalol</td>
<td>MJ6987-1</td>
<td>MJ6987-1</td>
</tr>
<tr>
<td>R=C(CH(_3))(_3)</td>
<td>R=C(CH(_3))(_3)</td>
<td>R=C(CH(_3))(_3)</td>
</tr>
<tr>
<td>(pD_2)</td>
<td>6.71 (0.09)</td>
<td>6.71 (0.09)</td>
</tr>
<tr>
<td>(\alpha)</td>
<td>0.91 (0.03)</td>
<td>0.91 (0.03)</td>
</tr>
<tr>
<td>Activity Ratio</td>
<td>63.3 (19.4)</td>
<td>63.3 (19.4)</td>
</tr>
<tr>
<td>(pA_2)</td>
<td>4.76 (0.03)</td>
<td>4.76 (0.03)</td>
</tr>
<tr>
<td>Slope</td>
<td>1.08 (0.03)</td>
<td>1.08 (0.03)</td>
</tr>
</tbody>
</table>
increase in tracheal ($\beta_2$) activity. Thus MJ9184-1 is the most selective $\beta_2$-adrenoceptor agonist in this series.

Fig. 4. Mean concentration-effect curves for the positive chronotropic and smooth muscle relaxant effects of (-)-isoprenaline (Iso, □), MJ7999-1 (○), soterenol (SOT, ■), terbutaline (TERB, ●) and orciprenaline (ORCI, ♦) in (a) guinea-pig atrial and (b) tracheal preparations. Results are expressed in terms of the maximal effects ($E_{\text{max}}$) produced by (-)-isoprenaline in each experiment. Individual points show mean concentrations from four to six experiments required to produce a given percentage of the maximal response to (-)-isoprenaline.

All three compounds produced parallel shifts to the right of (-)-isoprenaline concentration-effect curves without affecting the maximum response to the catecholamine. Figure 5 shows concentration-effect curves to (-)-isoprenaline in the absence and presence of various concentrations of soterenol in both atrial and tracheal preparations. Evaluations of the shifts using the method of Arunlakshana & Schild (1959) showed that the antagonism was
of a competitive type in both atrial and tracheal preparations since the slope of the relationship between log(dose-ratio-1) and log (antagonist concentration) is close to the theoretical value of unity. The pA₂ values for all compounds were obviously not significantly different in atria. Although there was no significant difference between the pA₂ values for MJ9184-1 and MJ7999-1 in trachea there was a significant difference between MJ9184-1 and soterenol (0.1<P<0.20, 9 d.f., t-test and 0.025<P<0.05, 8 d.f., t-test, respectively). The pA₂ values for MJ9184-1 were not whilst those for MJ7999-1 and soterenol were significantly different when atrial and tracheal pA₂ values were analysed for each compound (0.20<P<0.30, 9 d.f., t-test; 0.025<P<0.05, 8 d.f., t-test; P<0.001, 7 d.f., t-test, respectively).

The other two compounds studied had an N-isopropylamino substitution and a 4-methanesulphonamido group with (MJ6987-1) and without (sotalol) hydroxyl substitution in the meta position. In contrast to sotalol which possesses no agonistic activity in either atrial or tracheal preparations, MJ6987-1 is 63 times less active than (-)-isoprenaline and has an intrinsic activity (α) of 0.91 in atrial preparations. In tracheal preparations MJ6987-1 is some 14,000 times less active than (-)-isoprenaline but is a full agonist (see Table 3b). Thus on the basis of these results it would appear that MJ6987-1 is a cardioselective β-receptor agonist.

Both sotalol and MJ6987-1 possessed competitive β-receptor blocking actions in atrial preparations, the
latter compound being 35 times less potent than sotalol. In tracheal preparations sotalol was a competitive antagonist with a pA2 value of 5.96, however at 10^{-4} M MJ6987-1 showed no evidence of ß-adrenoceptor blockade.

3.4. **3,5-Dihydroxy ring substituted compounds (resorcinols)**

Four compounds possessing a 3,5-dihydroxy ring substitution (resorcinol) but having different amine substituents were studied in both isolated preparations. The mean values for pD2, intrinsic activity, activity ratio, pA2 and slope of the relationship log(dose-ratio-1) vs. log(molar antagonist concentration) are shown in Table 4a. Figure 4 shows mean concentration-effect curves for orciprenaline and terbutaline in isolated guinea-pig atrial and tracheal preparations.

All compounds except terbutaline possessed high levels of intrinsic activity in isolated atrial preparations. The rank order for the compounds as cardiac stimulants, as judged by activity ratios is fenoterol>orciprenaline>Me506>>terbutaline. In tracheal preparations all four sympathomimetics possessed high levels of intrinsic activity and the rank order of activity found was fenoterol>>Me506=terbutaline=orciprenaline.

The activity ratios for orciprenaline and terbutaline in atrial preparations indicate that increasing the size of the amine substituent from N-isopropyl to N-t-butyl leads to an 11-fold decrease in cardiac stimulant activity. Further increase in the size of the amine substitution to that present in Me506, increases the cardiac stimulating actions
such that Me506 is half as active as orciprenaline. The influence on atrial agonist activity produced by the addition of a p-hydroxyphenyl group can be seen when fenoterol is compared to orciprenaline, and Me506 is compared with terbutaline. In both cases there is a similar and marked increase in activity at β₁-adrenoceptor sites.

In contrast to the pattern seen in atrial preparations, the change in amine loading through the series orciprenaline, terbutaline, Me506 produces only a very small increase in activity in tracheal preparations. In terms of activity ratios in tracheal preparations, the addition of a p-hydroxyphenyl ring leads to a very marked increase in activity when the addition is to an N-isopropyl substituent (cf. fenoterol & orciprenaline), and virtually no effect when added to an N-t-butyl group (cf. Me506 & terbutaline).

Of the three compounds orciprenaline, terbutaline and Me506, terbutaline (the N-t-butyl substituent resorcinol) was the most selective for β₂-adrenoceptors as judged by the ratio of the activity ratios atria:trachea. Since these three compounds all had similar activities in the trachea, selectivity for β₂-receptors depended almost entirely on their agonistic activity in atrial preparations. Thus because of the weak β₁-receptor action of terbutaline, it was the most selective.

When fenoterol and Me506 are compared to orciprenaline and terbutaline respectively, the addition of the p-hydroxyphenyl ring produces an increase in selectivity for β₂-receptors when the amine substituent is N-isopropyl (orciprenaline) and a decrease in selectivity when the
TABLE 4. β-Adrenoceptor agonistic and antagonistic actions of a series of compounds possessing a 3,5-dihydroxy ring substitution (resorcinol) and varying amine substitutions in isolated atrial (AT) and tracheal (TR) preparations from the guinea-pig. (a) The mean values for pD₂, intrinsic activity (α), activity ratio, pA₂ and slope of the relationship log(dose-ratio-1) vs. log(molar antagonist concentration) are shown. (b) The mean percentage reductions in the maximum response to (-)-isoproterenol produced by the four resorcinols in isolated atrial preparations are shown. Numbers in parentheses are s.e.m. from at least 4 experiments with each drug.

---

(a)

<table>
<thead>
<tr>
<th>Compound</th>
<th>AT pD₂</th>
<th>TR pD₂</th>
<th>Activity Ratio</th>
<th>AT pA₂</th>
<th>TR pA₂</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orciprenaline</td>
<td>6.36 (0.05)</td>
<td>7.20 (0.10)</td>
<td>167 (47)</td>
<td>6.39 (0.08)</td>
<td>1.06 (0.23)</td>
<td></td>
</tr>
<tr>
<td>R=CH(CH₃)₂</td>
<td>5.11 (0.15)</td>
<td>6.77 (0.10)</td>
<td>1882 (374)</td>
<td>6.22 (0.14)</td>
<td>1.10 (0.15)</td>
<td></td>
</tr>
<tr>
<td>Terbutaline</td>
<td>6.10 (0.07)</td>
<td>5.52 (0.14)</td>
<td>333 (56)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>R=C(CH₃)₃</td>
<td>6.75 (0.07)</td>
<td>7.10 (0.12)</td>
<td>37.0 (9.0)</td>
<td>6.86 (0.55)</td>
<td>1.53 (0.66)</td>
<td></td>
</tr>
<tr>
<td>Me506</td>
<td>6.10 (0.07)</td>
<td>5.52 (0.14)</td>
<td>333 (56)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>R=C(CH₃)₂CH₂-</td>
<td>6.2 x 10⁻⁷</td>
<td>1.6 x 10⁻⁶</td>
<td>16.3 (3.8)</td>
<td>41.5 (6.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-OHC₆H₄</td>
<td>1.3 x 10⁻⁷</td>
<td>2.6 x 10⁻⁷</td>
<td>16.8 (6.1)</td>
<td>39.9 (7.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fenoterol</td>
<td>7.1 x 10⁻⁸</td>
<td>3 x 10⁻⁶</td>
<td>6.8 x 10⁻¹</td>
<td>17.3 (3.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-OHC₆H₅</td>
<td>6.9 x 10⁻⁸</td>
<td>3 x 10⁻⁶</td>
<td>6.8 x 10⁻¹</td>
<td>17.3 (3.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Concentrations required to show β-receptor blockade could not be attained due to marked relaxant effects of amines.

(b)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (M)</th>
<th>% Depression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orciprenaline</td>
<td>6.5 x 10⁻⁷</td>
<td>21.1 (3.3)</td>
</tr>
<tr>
<td></td>
<td>1.6 x 10⁻⁶</td>
<td>39.8 (4.0)</td>
</tr>
<tr>
<td>Terbutaline</td>
<td>6.2 x 10⁻⁷</td>
<td>16.3 (3.8)</td>
</tr>
<tr>
<td></td>
<td>1.6 x 10⁻⁶</td>
<td>41.5 (6.4)</td>
</tr>
<tr>
<td>Fenoterol</td>
<td>1.3 x 10⁻⁷</td>
<td>16.8 (6.1)</td>
</tr>
<tr>
<td></td>
<td>2.6 x 10⁻⁷</td>
<td>39.9 (7.0)</td>
</tr>
<tr>
<td>Me506</td>
<td>3 x 10⁻⁵</td>
<td>9.1 (3.6)</td>
</tr>
<tr>
<td></td>
<td>10⁻⁵</td>
<td>17.3 (3.6)</td>
</tr>
</tbody>
</table>
addition is to an N-t-butyl substituent (terbutaline). For fenoterol and orciprenaline, the increased \( \beta_2 \)-selectivity is due to a comparatively greater increase in activity in tracheal than in atrial preparations, whereas for Me506 and terbutaline the decreased selectivity is almost wholly due to an increased atrial activity with Me506 compared to terbutaline.

In isolated guinea-pig atrial preparations orciprenaline, terbutaline and fenoterol produced competitive \( \beta \)-adrenoceptor antagonistic actions according to the experiments stated by Arunlakshana & Schild (1959). Although Me506 did not produce competitive blockade, it together with the other three resorcinols, produced a depression in the maximum response of the superimposed (-)-isoprenaline concentration-effect curves. Figure 5 shows concentration-effect curves for (-)-isoprenaline in the absence and presence of various concentrations of terbutaline in both atrial and tracheal preparations. The mean values together with the standard errors of the mean for \( pA_2 \) and slope of the relationship \( \log(\text{dose-ratio}-1) \) vs. \( \log(\text{molar antagonist concentration}) \) are shown in Table 4a. The mean percentage depressions in maximum response produced at the concentrations indicated are shown in Table 4b. In terms of \( pA_2 \) values fenoterol is not significantly different from either orciprenaline and terbutaline (0.4<\( P <0.5, 6 \text{ d.f.}, t \)-test and 0.3<\( P <0.4, 6 \text{ d.f.}, t \)-test, respectively). However when the percentage depressions in the maximum response are compared fenoterol is more active in this regard than either orciprenaline or terbutaline. Me506 is
considerably weaker than the other three resorcinol compounds (Table 4b) in both types of antagonistic action.

In isolated tracheal preparations β-adrenoceptor antagonistic actions could be displayed with orciprenaline and terbutaline. The values of slope for these two compounds is slightly greater than unity, but this may be due to some non-competitive actions which also occur in tracheal as well as in atrial preparations. The mechanical factors involved in measuring a response as a relaxation
(rather than an increase as in atrial preparations), may preclude the exhibition of a non-competitive action of these resorcinol compounds.

Due to the marked relaxant effects of Me506 and fenoterol, it was impossible to achieve blocking concentrations of the compounds even when a very slow build-up procedure was used. However since $10^{-5}$ M concentrations of both amines showed no evidence of blockade it can be concluded that antagonistic actions, if they do exist, are very weak.

3.5. 3-0-Methyl,4-hydroxy ring substituted compounds

Two compounds possessing a 3-methoxy,4-hydroxy ring substitution with N-isopropyl (3-methoxyisoprenaline) and N-t-butyl amine groups were tested in the two isolated guinea-pig preparations.

In isolated guinea-pig atrial preparations both compounds were weak partial agonists when compared to (-)-isoprenaline. As judged by the standard errors of the mean of their agonistic activity ratios, 3-methoxyisoprenaline was much more variable in its activity from preparation to preparation than was its N-t-butyl analog. In isolated tracheal preparations 3-methoxyisoprenaline was a very weak agonist with a low intrinsic activity ($\alpha = 0.12$, s.e.m. = 0.05, n = 4) whilst the N-t-butyl compound produced no relaxation in concentrations up to $10^{-3}$ M in four preparations. The mean values for $pD_2$, intrinsic activity ($\alpha$), and activity ratio together with the standard errors of the mean are shown in Table 5.
TABLE 5. β-Adrenoceptor agonistic and antagonistic actions of 3-O-methyl,4-hydroxy ring substituted compounds with N-isopropyl and N-t-butyl amine substituents in isolated guinea-pig atrial (AT) and tracheal (TR) preparations. Shown are the mean pD2 values, values for intrinsic activity, activity ratios, pA2 values and values of slope for the relationship log(dose-ratio-1) vs. log(molar antagonist concentration) from at least 4 experiments with each drug. Numbers in parentheses are s.e.m. of these mean values.

\[
\begin{array}{|c|c|c|c|c|}
\hline
& pD_2 & \alpha & \text{ACTIVITY RATIO} & pA_2 & \text{SLOPE} \\
\hline
\text{CH(CH}_3)_2 & \text{AT} & 5.78 & 0.44 & 588 & 5.23 & 0.97 \\
& & (0.30) & (0.12) & (354) & (0.02) & (0.04) \\
& \text{TR} & 3.82 & 0.12 & 1502 & \dagger & \dagger \\
& & (0.12) & (0.05) & (165) & & \\
\text{C(CH}_3)_3 & \text{AT} & 5.89 & 0.27 & 308 & 4.94 & 1.10 \\
& & (0.08) & (0.20) & (60) & (0.08) & (0.04) \\
& \text{TR} & * & * & * & \dagger & \dagger \\
\hline
\end{array}
\]

*No relaxation in concentrations up to 10^{-3} M.
†No antagonism of (−)-isoprenaline responses in concentrations up to 10^{-4} M.

Neither of the compounds possessed antagonistic actions in tracheal preparations when used at concentrations up to 10^{-4} M. However, in atrial preparations the compounds exhibited competitive β-receptor blockade with pA2 values of 5.23 and 4.94 for 3-methoxyisoprenaline and the N-t-butyl analog, respectively.

Thus the 3-methoxy compounds are weak agonists and antagonists in these preparations.
3.6. Clenbuterol and salbutamol

The β-adrenoceptor agonistic and antagonistic actions of clenbuterol and salbutamol are discussed more fully in Chapters 6 and 3 respectively, of this Thesis.

The compounds have been included in this Chapter in order to facilitate discussion on the effects of ring substitution in compounds with the same N-alkyl group. The quantitative data for the actions of the two compounds are shown in Table 6.

**TABLE 6. β-Adrenoceptor agonistic and antagonistic actions of 2 compounds possessing an N-t-butyl amine group and ring substitutions of 3,5-dichloro,4-amino (clenbuterol) and 3-hydroxymethyl,4-hydroxy (salbutamol) in atrial (AT) and tracheal (TR) preparations from the guinea-pig. Shown are the mean values for pD2, intrinsic activity (α), activity ratio, pA2 and slope of the relationship log(dose-ratio-1) vs. log(molar antagonist concentration) from four experiments with each drug. Numbers in parentheses are s.e.m. of the means.**

<table>
<thead>
<tr>
<th></th>
<th>ACTIVITY RATIO</th>
<th>pA2</th>
<th>SLOPE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pD2</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>Clenbuterol</td>
<td>8.07 (0.34)</td>
<td>0.25 (0.06)</td>
<td>3.36 (2.36)</td>
</tr>
<tr>
<td>AR</td>
<td>7.58 (0.21)</td>
<td>0.39 (0.10)</td>
<td>4.17 (1.85)</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>5.90 (0.11)</td>
<td>0.51 (0.06)</td>
<td>494 (162)</td>
</tr>
<tr>
<td>TR</td>
<td>6.53 (0.08)</td>
<td>0.98 (0.01)</td>
<td>8.7 (1.0)</td>
</tr>
</tbody>
</table>

3.7. α-Adrenoceptor agonistic and antagonistic actions in isolated rat vas deferens preparations

In isolated vas deferens preparations noradrenaline was used as the agonist against which the compounds were evaluated for their agonistic and antagonistic actions.
Three methanesulphonanilide (soterenol, MJ7999-1, MJ9184-1) and three resorcinol compounds (orciprenaline, terbutaline, fenoterol) were tested, along with (-)-isoprenaline, phentolamine and azapetine.

Of the above compounds only noradrenaline, (-)-isoprenaline and soterenol produced contractions in the rat vas deferens and these were antagonized by the prior addition of phentolamine \(3 \times 10^{-6} \text{ M}\) to the bath. The other compounds studied produced no contractions of the vas deferens in concentrations up to \(4 \times 10^{-3} \text{ M}\). The mean \(pD_2\) values, values for intrinsic activity \(\alpha\), and activity ratios for the active compounds are shown in Table 7. On a molar basis soterenol was 2.1 times and (-)-isoprenaline 28.4 times less active than noradrenaline.

Of the six non-catechol \(\beta\)-adrenoceptor agonists only MJ9184-1 exhibited competitive \(\alpha\)-adrenoceptor blocking activity. MJ9184-1 is approximately 10 times less potent than phentolamine and azapetine as an \(\alpha\)-receptor antagonist. The other 5 non-catechol \(\beta\)-agonists (soterenol, MJ7999-1, orciprenaline, terbutaline, fenoterol) had variable effects on the concentration-effect curves to noradrenaline. Shifts in the noradrenaline curves were only obtained with high concentrations \(10^{-4} - 10^{-3} \text{ M}\) of the agents and the effects produced were not concentration-dependent. These high concentrations also variably affected the maximal response to noradrenaline.
TABLE 7. α-Adrenoceptor agonistic and antagonistic actions of compounds which were active in the isolated rat vas deferens preparation. Shown are the mean pD$_2$ values, intrinsic activity (α), activity ratios, pA$_2$ values and slope of the relationship log(dose-ratio-1) vs. log(molar antagonist concentration) from at least 4 experiments with each drug. Numbers in parentheses are s.e.m. of these values.

<table>
<thead>
<tr>
<th>Compound</th>
<th>pD$_2$</th>
<th>α</th>
<th>Activity Ratio</th>
<th>pA$_2$</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noradrenaline</td>
<td>5.17</td>
<td>1.00</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(0.04)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soterenol</td>
<td>4.88</td>
<td>0.84</td>
<td>2.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(0.04)</td>
<td>(0.09)</td>
<td>(0.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MJ9184-1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.72</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.05)</td>
<td>(0.10)</td>
</tr>
<tr>
<td>(-)-Isoprenaline</td>
<td>3.74</td>
<td>1.22</td>
<td>28.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(0.07)</td>
<td>(0.10)</td>
<td>(5.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phentolamine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.83</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.10)</td>
<td>(0.15)</td>
</tr>
<tr>
<td>Azapetine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.68</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.22)</td>
<td>(0.43)</td>
</tr>
</tbody>
</table>
4. **DISCUSSION**

In general, the results of the present experiments show that compounds which do not possess a catechol (3,4-dihydroxyphenyl) ring can act as both agonists and antagonists at β-adrenoceptor sites in isolated guinea-pig atrial and tracheal preparations. In addition the type of antagonism shown, competitive or non-competitive, depends on the nature and position of the substituted chemical groups.

In terms of agonistic actions the (-)-isomers of terbutaline, soterenol, salbutamol and isoprenaline are more active than their corresponding (+)-isomers. These results are in agreement with previously published reports (Buckner & Patil, 1971; Wetterlin, 1972; Buckner & Abel, 1974). Buckner & Patil (1971) found differences of 500- and 1000-fold between the two isomers of isoprenaline in guinea-pig tracheal and atrial preparations respectively, whilst in the present experiments these differences were only 20- and 42-fold, respectively. A comparison of the pD₂ values for (-)-isoprenaline show that there is little difference between Buckner & Patil's (1971) values and those obtained in the present experiments. This indicates that the discrepancies are due to the activity of the (+)-isomers. Although the (+)-isomers were obtained from different sources, "impurity" of the (-)-isomer in the (+) sample can not account for the high activity of the (+)-isomer [Nachod (Sterling-Winthrop), personal communication]. Thus there is no apparent reason for the differences observed in the two studies.
The pA2 values for the isomers of soterenol and salbutamol in the two guinea-pig preparations (Table 2) indicate that the β-adrenoceptor blocking activity is associated with the (-)-isomers. These observations are in accord with the many previously published reports dealing with the isomers of β-receptor antagonists (see Patil, Miller & Trendelenburg, 1974 for references).

These results also indicate that in the racemates the antagonistic actions shown are not due to the (+)-isomers inhibiting the agonistic actions of the (-)-isomers, a situation which has been shown for the (+)- and (-)-isomers of isoprenaline on α-receptors in the rat vas deferens preparation (Ariëns, Simonis & van Rossum, 1964).

When initially performing the experiments the possibility was raised that selectivity for agonistic actions at β2-receptor sites might be due to a selective antagonism at β1-adrenoceptors. With the exception of the resorcinols, the pA2 values in tracheal preparations were close to those found in atria. Thus the possibility of selective β1-receptor blockade being responsible for β2-receptor selectivity of the agonists must be dismissed as a general principle.

When comparing the results obtained with the methanesulphonanilides and resorcinols which have differing amine substituents, the importance of ring substitution or agonistic activity and the β1/β2-receptor selectivity of the compounds is clearly delineated (Table 3 and 4). Thus in the methanesulphonanilides MJ9184-1 is the most
$\beta_2$-selective of the agents investigated and this selectivity is based on decreasing atrial activity coupled with increasing tracheal activity, as amine loading is increased. In contrast the resorcinols orciprenaline, terbutaline and Me506 have similar tracheal activities and $\beta_2$-selectivity is largely determined by atrial activity.

With the methanesulphonanilides (Table 8) it was noted that within the series there is a 15-fold difference in agonistic actions in tracheal and a 40-fold difference in atrial preparations. In terms of antagonistic actions within each tissue there is only a 1- and 2-fold difference in the concentrations required to produce a dose-ratio of two in atrial and tracheal preparations, respectively. The affinity of a drug for the receptor is related to its pA$_2$ values and is independent of the efficacy of the agonist employed. Although pD$_2$ values have also been claimed to provide a measure of affinity, when measuring an agonistic response the values obtained depend not only on the affinity of the drug for the receptor, but also the efficacy of the drug-receptor complex in initiating the response.

The similar pA$_2$ values and the divergent pD$_2$ values obtained with the methanesulphonanilides suggests that the differing agonistic activities and selectivities are related to changes in efficacy rather than affinity with increasing amine loading.

A similar argument could be advanced for orciprenaline, terbutaline and fenoterol in the resorcinol series in
TABLE 8. Mean pD₂ and pA₂ values for soterenol, MJ7999-1 and MJ9184-1 in isolated guinea-pig atria and trachea preparations. Values taken from Table 3.

<table>
<thead>
<tr>
<th></th>
<th>SOTERENOL</th>
<th>MJ7999-1</th>
<th>MJ9184-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>pD₂ Values:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atria</td>
<td>7.26</td>
<td>7.22</td>
<td>6.05</td>
</tr>
<tr>
<td>Trachea</td>
<td>6.82</td>
<td>7.11</td>
<td>7.91</td>
</tr>
<tr>
<td>pA₂ Values:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atria</td>
<td>6.48</td>
<td>6.52</td>
<td>6.48</td>
</tr>
<tr>
<td>Trachea</td>
<td>6.02</td>
<td>6.02</td>
<td>6.32</td>
</tr>
</tbody>
</table>
guinea-pig atria, however within this series the non-competitive actions of the compounds complicate the picture.

Thus the basis for the dual $\beta$-receptor hypothesis of Lands et al. (1967) may involve not only differences in the $\beta$-receptors themselves, but also the ability of a particular agonist-receptor complex to initiate the measured response in different tissues.

Of the other compounds possessing a methanesulphonanilide substitution sotalol possesses only antagonistic actions and MJ6987-1 cardioselective agonistic actions with considerably weaker $\beta$-receptor blocking actions. The latter compound, which can be considered as soterenol with the positions of the ring substituents reversed, shows marked differences in its spectrum of activity to soterenol. These results also indicate the importance of ring substitution on agonistic and antagonistic activity.

Within the resorcinol series fenoterol is more active as an agonist than Me506 in both atrial and tracheal preparations. Since a $p$-hydroxyphenyl substituent is common to both agonists this difference must be due to the nature of the alkyl substituent connecting it to the amine, $N$-isopropyl in fenoterol and $N$-t-butyl in Me506. This raises the possibility that the reason for the greater activity of fenoterol may be due to the ability of the $N$-isopropyl group to rotate and allow the drug to achieve a conformation which will decrease the steric hindrance and allow the phenol ring to interact and increase the binding of the drug to the receptor. Rotation of chemical bonds
will have no effect on the degree of steric hindrance associated with Me506 due to the symmetrical nature of the t-butyl group.

The methanesulphonanilide and resorcinol structure-activity relationships demonstrate the marked influence that the ring substitution has on the activity and pattern of selectivity of compounds as the size of the amine substituent is increased.

When a number of compounds with identical amine substitutents but differing ring structures are compared the influence of the ring substitution on agonistic and antagonistic activity and on the selectivity of action at $\beta_1$- and $\beta_2$-receptor sites is even more striking (Table 9).

Ariëns (1960) showed that increasing the size of the amine substituent from N-isopropyl to t-butyl to phenyl-l-t-butyl in a series of catecholamine compounds altered the spectrum of activity from $\alpha$-agonistic to neither agonistic or antagonistic to competitive antagonistic actions in the isolated rat vas deferens preparation. In the present Chapter the methanesulphonanilides showed a similar trend for the same changes in the amine substitution whilst for the resorcinol compounds orciprenaline, terbutaline and fenoterol neither agonistic nor competitive antagonistic actions were present.

The large aralkyl compound MJ9184-1 exhibited competitive antagonism which was similar in potency to that observed with the identical catechol analog Mc5 (Ariëns, 1960). In addition MJ9184-1 was only approximately ten times less potent
TABLE 9. (a) Comparisons of the β-adrenoceptor agonistic and antagonistic activity of a number of sympathomimetic amines possessing an N-isopropyl group but differing ring substitutions. Values have been taken from Tables in the Results section and only the means are shown.

<table>
<thead>
<tr>
<th></th>
<th>ATRIA ACTIVITY RATIOS</th>
<th>ATRIA pA₂</th>
<th>TRACHEA ACTIVITY RATIOS</th>
<th>TRACHEA pA₂</th>
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</thead>
<tbody>
<tr>
<td>(-)-Isoprenaline</td>
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<td>-</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>(+)-Soterenol</td>
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<td>6.48</td>
<td>7.6</td>
<td>6.02</td>
</tr>
<tr>
<td>MJ6987-1</td>
<td>63.3</td>
<td>4.76</td>
<td>14046</td>
<td>*</td>
</tr>
<tr>
<td>Orciprenaline</td>
<td>167</td>
<td>6.39</td>
<td>30.8</td>
<td>4.97</td>
</tr>
<tr>
<td>3-Methoxy-isoprenaline</td>
<td>538</td>
<td>5.23</td>
<td>1502</td>
<td>*</td>
</tr>
</tbody>
</table>

(b) Comparisons of the β-adrenoceptor agonistic and antagonistic activity of a number of sympathomimetic amines possessing an N-t-butyl amine substituent but differing ring substitutions. Values have been taken from Tables in the Results section and again only the mean values are shown.

<table>
<thead>
<tr>
<th></th>
<th>ATRIA ACTIVITY RATIOS</th>
<th>ATRIA pA₂</th>
<th>TRACHEA ACTIVITY RATIOS</th>
<th>TRACHEA pA₂</th>
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</thead>
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<td>Terbutaline</td>
<td>1882</td>
<td>6.22</td>
<td>28.4</td>
<td>4.81</td>
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<tr>
<td>MJ7999-1</td>
<td>22.2</td>
<td>6.52</td>
<td>2.4</td>
<td>6.02</td>
</tr>
<tr>
<td>N-t-butyl-normetanephrine</td>
<td>308</td>
<td>4.94</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Clenbuterol</td>
<td>3.4</td>
<td>7.03</td>
<td>4.2</td>
<td>6.90</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>494</td>
<td>5.40</td>
<td>8.7</td>
<td>5.64</td>
</tr>
</tbody>
</table>

*Denotes no activity at $10^{-4}$ M concentration of the drug.
than phentolamine in its α-receptor blocking actions.

These structure-activity relationships indicate that the methanesulphonanilides resemble the catechol moiety with regard to α-receptor actions more than the resorcinol moiety and highlight the difference in structural requirements required for α- as opposed to β-receptor mediated actions.
CHAPTER 3:

THE $\beta$-ADRENOCEPTOR AGONISTIC AND ANTAGONISTIC ACTIONS OF SALBUTAMOL IN ISOLATED PREPARATIONS AND IN ANAESTHETIZED GUINEA-PIGS
SUMMARY

1. The β-adrenoceptor agonistic actions of (-)-isoprenaline and salbutamol have been compared in isolated guinea-pig atrial and tracheal preparations and on heart rate and intratracheal pressure in urethane anaesthetized guinea-pigs.

2. Salbutamol produced the same maximal response as (-)-isoprenaline in tracheal preparations and when its bronchodilator effects were assessed in vivo. On a molar basis it was 8.7 and 28 times less potent than (-)-isoprenaline in the in vitro and in vivo preparations, respectively.

3. Salbutamol produced lower maximal chronotropic effects than (-)-isoprenaline and on a molar basis was 493 and 608 times less active than the catecholamine in the in vitro and in vivo preparations, respectively.

4. Salbutamol possessed competitive β-adrenoceptor antagonistic actions towards (-)-isoprenaline in isolated atrial and tracheal preparations. The mean pA2 values were 5.40 and 5.64, respectively.

5. In anaesthetized guinea-pigs infusions of salbutamol at 100 ng kg⁻¹ min⁻¹ produced small shifts to the right in (-)-isoprenaline dose-response curves, however the predominant effect produced was a reduction in the
maximum response to (-)-isoprenaline.

6. Measurements of intratracheal pressure showed that salbutamol (100 ng kg\(^{-1}\) min\(^{-1}\)) produced initial bronchodilator actions which were no longer evident 180 min after the commencement of the infusion. At the same time the bronchodilator actions of (-)-isoprenaline were antagonized by salbutamol.

7. The results show that resistance or tolerance to the effects of salbutamol occur in the whole animal. However the mechanism by which this is produced is unclear and may be due, only in part, to a \(\beta\)-receptor blocking action.
1. **INTRODUCTION**

Salbutamol is a sympathomimetic amine which preferentially stimulates $\beta_2$- as opposed to $\beta_1$-adrenoceptors (see Brittain, Jack & Ritchie, 1970 for references). The drug is now widely used for the treatment of asthma since it is orally active, has a long duration of action and displays selectivity at the receptor level in man (Warrell, Robertson, Newton Howes, Conolly, Paterson, Beilen & Dollery, 1970; Watson & Richens, 1974).

Controversy still surrounds the conclusion of Speizer, Doll, Heaf & Strang (1968) that sympathomimetic aerosols were the reason for the rise in asthma mortality in young people in the 1960's (for opposing views see Chapter 1).

In this Chapter the agonistic and antagonistic actions of salbutamol have been quantified using isolated guinea-pig atrial and tracheal preparations and the possibility that salbutamol may produce resistance or tolerance due to $\beta$-receptor blocking actions *in vivo* has also been investigated.
2. METHODS

2.1. Isolated guinea-pig preparations

The methods used for assessing the β-adrenoceptor agonistic and antagonistic actions of salbutamol in isolated atrial and tracheal preparations are described fully in Chapter 2.

2.2. Anaesthetized guinea-pigs

Guinea-pigs of either sex weighing 250-400 g were anaesthetized by the intraperitoneal injection of urethane (1.5 g kg\(^{-1}\)). In all experiments an external jugular vein was cannulated for administration of drugs. The animals were artificially respired using a Palmer small animal respirator at a rate of 35 cycles min\(^{-1}\) and a stroke volume of 3.5-4.5 ml.

2.2.1. Heart Rate: Agonistic Actions

Heart rate was recorded using a Beckman (Type 9857) or Grass (7P4DF) cardiotachometer which was triggered by the QRS complex of lead I electrocardiograms. In these experiments arterial blood pressure was monitored from a cannulated carotid artery using a Statham P23Ac pressure transducer coupled to a Beckman (Type 9853) or Grass (7P1D) preamplifier. Traces of both parameters were displayed on a two-channel Beckman (RP) or Grass (79C) pen recorder.

Constant cumulative dose-response curves to the effects of (-)-isoprenaline on heart rate were first obtained and then the agonistic actions of salbutamol were assessed in the same
2.2.2. Heart Rate: Antagonistic Actions

In these experiments the animals were prepared as described in sections 2.2 and 2.2.1, and in addition a second jugular vein was cannulated for the infusion of salbutamol. Salbutamol was infused at a rate of 100 ng kg\(^{-1}\) min\(^{-1}\) in a volume of 0.01 ml min\(^{-1}\) using a Harvard 944 slow infusion apparatus. The pH of the salbutamol solution was adjusted to 7.4.

Prior to the infusion of salbutamol constant cumulative dose-response curves to \((-\)-isoprenaline were obtained. These were then repeated at varying times after the commencement of the salbutamol infusion. The increase in heart rate produced by the cumulative administration of \((-\)-isoprenaline during the salbutamol infusion were expressed as percentages of the maximal increase in heart rate produced by \((-\)-isoprenaline in the control situation.

2.2.3. Intratracheal Pressure: Agonistic Actions

The experimental set-up was similar to that described by Konsett & Rössler (1940) except that pressure rather than volume overflow was monitored. An 18-gauge needle was inserted into the sidearm of the tracheal cannula which was connected via pressure tubing to a Statham P23Ac pressure transducer. Heart rate, recorded as described in section 2.2.1, and intratracheal pressure were recorded on a Grass polygraph (model 79C).
In all experiments guinea-pigs received gallamine triethiodide (1-3 mg kg\(^{-1}\), i.v., every 60 min) to suppress spontaneous respiration. Intravenous injections of histamine (0.5-3.0 \(\mu g\) kg\(^{-1}\) in different experiments) produced 3- to 5-fold increases in intratracheal pressure. In any one experiment constant and reproducible increases in intratracheal pressure to histamine could be obtained at 15 min intervals. \(\beta\)-Agonistic activity was assessed in terms of the reduction in responses to histamine.

In preliminary experiments it was found that maximal reductions in histamine response with any one dose of the bronchodilators occurred when \((-\))-isoprenaline and salbutamol were injected 30 s and 60 s respectively, before the histamine challenge.

The \(\beta\)-adrenoceptor agonistic actions of \((-\))-isoprenaline and salbutamol were assessed by using four randomized doses of each compound which spanned the dose-response curve for that agonist. Single doses of the agonists were administered and the next dose in the series was not injected until histamine responses had returned to control levels.

Percentage reductions in histamine effect were plotted against the doses of the compounds and relative potency compared at the level of 50% reduction in histamine response.
2.2.4. Intratracheal Pressure: Antagonistic Actions

In these experiments the bronchodilator effects of (-)-isoprenaline and salbutamol were assessed in terms of shifts to the right in histamine dose-response lines rather than reductions in responses to single doses of the bronchoconstrictor. Bronchoconstrictor responses were obtained using two cumulative doses of histamine, the second being injected at the peak of the response obtained with the first. In the initial part of each experiment responses were obtained to histamine alone and to histamine in the presence of (-)-isoprenaline (50 ng kg\(^{-1}\)). The latter compound was injected 30 s before histamine challenge. When constant responses had been obtained, salbutamol (100 ng kg\(^{-1}\) min\(^{-1}\)) was infused and alternate responses to histamine alone and to (-)-isoprenaline plus histamine were monitored. Results were plotted as histamine dose-ratios vs. time after commencement of the salbutamol infusion. The dose-response lines were close to parallel and dose-ratios were taken approximately midway between the responses obtained.

2.3. Drugs used

The drugs used were (-)-isoprenaline bitartrate (Wyeth), urethane (Unilab), gallamine triethiodide (May & Baker), salbutamol base substance (Allen & Hanburys), histamine acid phosphate and carbachol chloride (British Drug Houses). Stock solutions of (-)-isoprenaline were made up in N/100 HCl and suitably diluted with either Krebs-Henseleit solution (in vitro experiments) or 0.9% w/v NaCl (in vivo experiments) containing 20 μg ml\(^{-1}\) ascorbic acid. Salbutamol, histamine
and carbachol were dissolved in distilled water and diluted with Krebs-Henseleit solution or 0.9% w/v NaCl to the desired concentrations. With the exception of gallamine triethiodide doses of drugs are expressed in terms of the base.
3. RESULTS

3.1. Isolated guinea-pig atrial and tracheal preparations

3.1.1. Atria: Agonistic and Antagonistic Actions

Salbutamol produced positive chronotropic and inotropic effects in spontaneously beating guinea-pig atrial preparations. The mean maximal chronotropic activity produced by salbutamol was 0.51 (s.e.m. = 0.06, n = 4) of that elicited by (-)-isoprenaline (= 1.00). The ratios of the molar concentrations required to produce 50% of each drug's individual maximal response (EC$_{50}$) showed that salbutamol was 493 (s.e.m. = 162, n = 4) times less active than (-)-isoprenaline.

In the presence of concentrations of salbutamol greater than 10$^{-5}$ M, cumulative concentration-effect curves to (-)-isoprenaline were displaced to the right of the control curves. Analysis of the shifts produced by a number of concentrations of salbutamol, using the method of Arunlakshana & Schild (1959) indicated that the antagonism was of a competitive type. The mean value of the slope of the relationship between log (dose-ratio-1) and log (salbutamol concentration) was 0.99 (s.e.m. = 0.13, n = 4). Table 1 shows values for intrinsic activity ($\alpha$), pD$_2$, pA$_2$ and the slope of the above relationship.

3.1.2. Trachea: Agonistic and Antagonistic Actions

Salbutamol produced concentration-dependent relaxations of carbachol (10$^{-6}$ M) stimulated isolated guinea-pig tracheal preparations. Both (-)-isoprenaline and salbutamol produced
TABLE 1. Agonistic effects of (-)-isoprenaline and salbutamol in isolated atrial and tracheal preparations from guinea-pigs expressed as mean pD₂ values, intrinsic activity (α) and activity ratios (ratio of concentrations required to produce 50% of each drug's individual maximal response). The pA₂ values for salbutamol and the slope of the relationship between log (dose-ratio-1) and log (molar salbutamol concentration) are also shown. Numbers in parentheses are standard errors of the mean from four to six experiments.

<table>
<thead>
<tr>
<th></th>
<th>pD₂</th>
<th>α</th>
<th>Activity ratios</th>
<th>pA₂</th>
<th>Slope</th>
</tr>
</thead>
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<td>ATRIA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-)-Isoprenaline</td>
<td>8.53</td>
<td>1.00</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(0.11)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Salbutamol</td>
<td>5.90</td>
<td>0.51</td>
<td>493 (162)</td>
<td>5.40 (0.09)</td>
<td>0.99 (0.13)</td>
</tr>
<tr>
<td></td>
<td>(0.15)(0.06)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TRACHEA</td>
<td></td>
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</tr>
<tr>
<td>(-)-Isoprenaline</td>
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<td>1.00</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(0.09)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salbutamol</td>
<td>6.53</td>
<td>0.98</td>
<td>8.7 (1.0)</td>
<td>5.64 (0.10)</td>
<td>1.22 (0.34)</td>
</tr>
<tr>
<td></td>
<td>(0.08)(0.01)</td>
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<td></td>
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</tr>
</tbody>
</table>

Similar maximal responses in these tissues. Comparisons of the molar concentrations required to produce 50% of the maximum response showed that salbutamol was 8.7 (s.e.m. = 1.0, n = 5) times less potent than (-)-isoprenaline.

β-Adrenoceptor antagonistic actions of salbutamol were evident when concentrations greater than 3 x 10⁻⁶ M were used. As with atrial preparations, analysis of shifts in (-)-isoprenaline concentration-effect curves using the method of Arunlakshana & Schild (1959) showed that the antagonism produced by salbutamol was of a competitive type (slope 1.22, s.e.m. = 0.34, n = 7).

The values for intrinsic activity, pD₂, pA₂ and slope are shown in Table 1.
3.2. Anaesthetized guinea-pigs: agonistic actions

3.2.1. Heart Rate

The mean resting heart rate of anaesthetized guinea-pigs was 348 beats min\(^{-1}\) (s.e.m. = 5.3, n = 4). Cumulative intravenous injections of (-)-isoprenaline produced maximal increases in heart rate of 84 (s.e.m. = 15, n = 4) beats min\(^{-1}\) above the resting heart rate. The maximum increase in heart rate produced by salbutamol was 0.47 (s.e.m. = 0.13, n = 4) of that elicited by (-)-isoprenaline (= 1). On a mole kg\(^{-1}\) basis the ratio of the doses producing 50% of each drug's individual maximal response showed that salbutamol was 608 (s.e.m. = 240, n = 4) times less active than (-)-isoprenaline.

**Response**

\[
\text{% E}_{\text{max}}
\]

---

**Fig. 1.** Mean dose-response curves for the ability of (-)-isoprenaline (○) and salbutamol (○) to produce positive chronotropic effects in anaesthetized guinea-pigs. Responses are plotted as the mean doses required to produce a given response (n = 4). In each experiment responses were expressed as a percentage of the maximum response ($\text{E}_{\text{max}}$) obtained with (-)-isoprenaline.
In these experiments the ED₅₀ values for (-)-isoprenaline were relatively constant (mean 16.5 ng kg⁻¹, s.e.m. = 1.7, n = 4) whilst responses to salbutamol in terms of both ED₅₀ values (mean 10.5 µg kg⁻¹, s.e.m. = 3.8, n = 4) and maximal responses were much more variable.

Table 2 shows mean ED₅₀ values and activity ratios.

## 3.2.2. Intratracheal Pressure

Both (-)-isoprenaline and salbutamol produced complete abolition of the histamine-induced increase in intratracheal pressure. On a molar basis salbutamol was 28.0 (s.e.m. = 6.7, n = 4) times less potent than (-)-isoprenaline. Figure 2

![Graph showing response percentage against mole/kg](image)
shows the dose-response curves from one experiment in which the two β-adrenoceptor agonists were compared. Table 2 shows the mean ED$_{50}$ values and the potency ratio for the two agonists.

TABLE 2. The mean ED$_{50}$ values (µg kg$^{-1}$, base) and activity ratios (ratios of doses required to produce 50% of each drug's individual maximal response) on a molar basis for (-)-isoprenaline and salbutamol-induced increases in heart rate (HR) and reductions in histamine-induced increases in intratracheal pressure (ITP) in anaesthetized guinea-pigs. Numbers in parentheses are standard errors of the mean from four comparisons.

<table>
<thead>
<tr>
<th></th>
<th>(-)-Isoprenaline</th>
<th>Salbutamol</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED$_{50}$ HR</td>
<td>0.016 (0.002)</td>
<td>10.5 (3.8)</td>
</tr>
<tr>
<td>Activity ratio</td>
<td>1</td>
<td>608 (240)</td>
</tr>
<tr>
<td>ED$_{50}$ ITP</td>
<td>0.006 (0.002)</td>
<td>0.155 (0.036)</td>
</tr>
<tr>
<td>Activity ratio</td>
<td>1</td>
<td>28 (6.7)</td>
</tr>
</tbody>
</table>

3.3. Anaesthetized guinea-pigs: antagonistic actions

3.3.1. Heart Rate

Infusions of 0.9% w/v saline (pH 7.4) given at a rate equivalent to that of the salbutamol infusion (0.01 ml min$^{-1}$) for periods up to 270 min produced little or no change in the (-)-isoprenaline cumulative dose-response curves as judged by ED$_{50}$ values and maximal increases in heart rate. With increasing time of infusion, salbutamol produced small shifts to the right of the (-)-isoprenaline dose-response curves, however the predominant effect was a fall off in the maximum response to the catecholamine. Figure 3 shows the results from one experiment in which (-)-isoprenaline curves were
Fig. 3. Results from an experiment showing cumulative dose-response curves for the positive chronotropic effects of (-)-isoprenaline before (dotted line) and at various times following the start of an infusion of salbutamol (100 ng kg$^{-1}$ min$^{-1}$). Responses are expressed in terms of the maximal increase in heart rate produced under control conditions.

obtained in the absence and presence of an infusion of salbutamol. The infusion of salbutamol itself produced only small changes in basal heart rate (less than 5 beats min$^{-1}$ variation). Although in individual experiments the salbutamol-induced depression of the maximal response to (-)-isoprenaline was, at any given time variable, in all experiments the onset of this action was evident approximately 100 min after the commencement of the salbutamol infusion.
3.3.2. Intratracheal Pressure

Figure 4 shows traces from an experiment in which rises in intratracheal pressure induced by two doses of histamine were monitored before and during an infusion of salbutamol. Twenty minutes after the commencement of the salbutamol infusion greater doses of histamine were required to induce a similar degree of bronchoconstriction to that obtained under control conditions whereas 300 min later histamine responses had returned towards control levels despite the continued infusion of the sympathomimetic amine. After plotting the responses to individual doses of histamine dose-ratios were calculated. Mean results from these are shown in Fig. 5.

Infusions of normal saline (pH 7.4) over a period of 240 min had little or no effect on responses to histamine as
shown by dose-ratios which remained close to unity throughout the experiments. After commencing the infusion of salbutamol the histamine dose-ratios increased progressively over the first hour and thereafter stabilized for a further 30 min. Continuation of the infusion then led to a progressive waning of the bronchodilator actions of salbutamol as shown by the decrease in dose-ratios. At 180 min the bronchodilator action of salbutamol was no longer evident.

**Histamine Dose-Ratio**

Fig. 5. Changes in the mean histamine dose-ratios during infusions of normal saline adjusted to pH 7.4 (○, n = 3) and salbutamol (□, 100 ng kg⁻¹ min⁻¹, n = 5). Dose-ratios to single injections of (-)-isoprenaline (▲, 50 ng kg⁻¹) before and during the continuing infusion of salbutamol are also shown (n = 4). The normal saline and salbutamol were infused at a rate of 0.01 ml min⁻¹.
(-)-Isoprenaline (50 ng kg\(^{-1}\)) alone produced a shift to the right in the dose-response lines to histamine (mean dose-ratio = 2.64, s.e.m. = 0.15, n = 4). Following the start of the salbutamol infusion there was a progressive and marked increase in the histamine dose-ratios obtained over the first 90 min due to the combined bronchodilator effects of the two sympathomimetic amines. Thereafter the dose-ratios decreased progressively and fell towards the control levels. At 280 min the histamine dose-ratio to (-)-isoprenaline in the presence of the salbutamol infusion had returned to control levels.

The standard errors have not been included in Fig. 5 since the actual dose-ratios obtained at various times after commencing the salbutamol infusion varied from experiment to experiment. However the general shape of the curves were similar and the lack of bronchodilator actions of salbutamol after 3-4 hours was present in all but one of the guinea-pigs used. In this one guinea-pig there was a slow progressive increase in bronchodilator action of salbutamol from time zero to 240 min.

In one further experiment the initial increase in histamine dose-ratio produced by an infusion of (-)-isoprenaline at 100 ng kg\(^{-1}\) min\(^{-1}\) was maintained for a period up to 240 min there being no evidence of decreasing activity with time.
4. DISCUSSION

The measurement of changes in intratracheal pressure reflect the alterations in dynamic compliance rather than airway resistance (James, 1969; see Widdicombe, 1963 for more discussion). As pointed out by Widdicombe (1963) this method can be used when the mechanism of action of a drug is known but should not be used if investigations are being performed to determine a mechanism of action. Unfortunately the equipment was not available to measure airway resistance or compliance so intratracheal pressure had to be used in this study.

Dose-ratios with respect to (-)-isoprenaline for the agonistic actions of salbutamol in isolated tracheal preparations and for bronchodilatation in vivo are of the same order as those reported by other workers (Brittain, Jack & Ritchie, 1970; Apperley & Levy, 1975).

The ratio found for the agonistic activity of salbutamol and (-)-isoprenaline in isolated guinea-pig atrial preparations is similar to those reported by other workers (O'Donnell, 1972; Wardell, Colella, Shetzline & Fowler, 1974). However in anaesthetized animals the cardiac activity ratio of salbutamol indicates weaker agonistic actions than those found by Apperley & Levy (1975). This difference in activity may be due to the anaesthetic used: urethane in this study and allobarbitone by Apperley & Levy. Although the maximum cardiac response produced by salbutamol is not quoted in the latter study it would be of interest to know whether salbutamol is a partial agonist in allobarbitone as well as in urethane.
anaesthetized guinea-pigs.

Salbutamol produced competitive β-adrenoceptor antagonism in isolated guinea-pig atrial and tracheal preparations. That this action may possibly occur in vivo was studied using salbutamol infusions and monitoring the chronotropic and bronchodilator responses of (-)-isoprenaline.

The chronotropic responses to the injections of (-)-isoprenaline in vivo were antagonized during an infusion of salbutamol. However instead of the expected parallel shift to the right of (-)-isoprenaline curves characteristic of a competitive antagonist, the major effect observed was a reduction in the maximal response, an effect which might be expected of a non-competitive antagonist. The reduction observed is not due to an increase in background heart rate produced by the salbutamol infusion since the resting heart rates were little affected by the infusion. Until more rigidly controlled experiments are performed a possible mechanism of action cannot as yet be proposed.

Autoinhibition to the bronchodilator action of salbutamol was produced during the salbutamol infusion. In addition the bronchodilator effects of (-)-isoprenaline were also antagonized. With the methodology used it is again, impossible to discern whether the antagonism was of a competitive or non-competitive type.

Conolly, Davies, Dollery & George (1971) showed that repeated injections of salbutamol and isoprenaline produced an increase in mortality to histamine-induced bronchospasm in guinea-pigs. The mechanism by which these sympathomimetics
produced this effect was not elucidated. However the authors suggested that long periods of postjunctional $\beta$-receptor stimulation would lead to a decrease in endogenous sympathetic drive and that this would still be lowered 2 h later when the histamine challenge occurred, thus increasing the mortality. The results of the present study do not support the hypothesis proposed by Conolly et al. (1971) in that there was no tolerance to the effect of isoprenaline (infusion) over that period in which tachyphylaxis developed to the effects of salbutamol. Since no antagonistic actions for (-)-isoprenaline could be displayed in vitro and salbutamol does possess $\beta$-receptor blocking actions, it is possible that the in vivo effects may be due, in part, to $\beta$-receptor antagonism.

Clinical investigations have produced no concrete evidence that tolerance or resistance occurs with salbutamol in man. Choo-Kang, Simpson & Grant (1969) noticed that after 1-3 weeks of treatment with oral salbutamol the increases in peak expiratory flow rates were less than those obtained in the initial part of the trial. However as stated by the authors this could be due to variations in the severity of the asthma. Gibson, Tattersfield & Pride (1972) examined the effects of therapeutic doses of oral salbutamol (2 mg and 4 mg q.d.s.) on the bronchodilator action of inhaled isoprenaline. The conclusion of this study was that there was no evidence of resistance to salbutamol. Recent studies (Sims, 1974; Bhatia & Davies, 1975) have further confirmed these findings.
Thus the clinical results show that no tachyphylaxis to salbutamol is seen when therapeutic doses are administered. However the question as to whether salbutamol produces tachyphylaxis in situations of gross misuse and therefore overdosage of sympathomimetic amines is still unresolved. Further discussion relating to tolerance and asthma deaths has already been presented in Chapter 1.
CHAPTER 4:

A COMPARISON OF THE ACTIVITIES OF THE β-ADRENOCEPTOR AGONISTS MJ9184-1 AND (-)-ISOPRENALINE IN GUINEA-PIG AND CAT PREPARATIONS
SUMMARY

1. The β-adrenoceptor stimulants MJ9184-1 and (-)-isoprenaline have been compared for their ability to produce agonistic actions in isolated atrial and tracheal preparations from both guinea-pigs and cats.

2. The ability of the compounds to produce positive chronotropic actions and to reduce serotonin-induced increases in pulmonary resistance have been assessed in anaesthetized guinea-pigs. These results have been compared with those obtained previously in anaesthetized cats.

3. In isolated tissues from both species there was a marked difference in the relative β₁- and β₂-agonistic activity of MJ9184-1 compared to (-)-isoprenaline. In whole animal preparations the relative potency of MJ9184-1 as a bronchodilator was similar in the two species whereas the compound was much less active in producing positive chronotropic actions in the guinea-pig than in the cat.

4. When in vivo and in vitro results were compared from the same species relative differences in β₁- and β₂-agonistic activity for MJ9184-1 were still apparent.

5. The results suggested that the cardiac β₂-R's in the cat and the guinea pig differ.
5. The results suggest that the cardiac $\beta$-adrenoceptors in the cat and guinea-pig differ.
1. **INTRODUCTION**

In the search for selective $\beta_2$-adrenoceptor agonists, observations are frequently made on cardiac and bronchial effects produced *in vivo* and *in vitro* using various animal preparations from different species.

MJ9184-1 (2'-hydroxy-5'-(1-hydroxy-2-(2-methyl-1-phenyl-2-propylamino)ethyl) methanesulphonanilide) (Fig. 1) is a potent $\beta$-adrenoceptor agonist which in anaesthetized cats produces vasodepressor and positive chronotropic effects, antagonizes serotonin-induced increases in pulmonary resistance and depresses soleus muscle contractility (Gwee, Nott, Raper & Rodger, 1972). In terms of the dual $\beta$-adrenoceptor classification developed by Lands and his coworkers (Lands, Arnold, McAuliff, Luduena & Brown, 1967), it would appear that MJ9184-1 possesses some degree of selectivity in its ability to stimulate $\beta_2$- (bronchodilator) as opposed to $\beta_1$- (cardiac) adrenoceptors (Gwee *et al.*, 1972). In view of the possibility that species differences might
exist in the selectivity of action of compounds at $\beta_1$- and $\beta_2$-adrenoceptors the effects of MJ9184-1 and (-)-isoprenaline in antagonizing serotonin-induced increases in pulmonary resistance and in producing positive chronotropic effects have been assessed in anaesthetized guinea-pigs. The results obtained in guinea-pigs differed from those previously obtained in cats (Gwee et al., 1972). Since marked species differences in the $\beta_1/\beta_2$ selectivity of MJ9184-1 were obtained in vivo, further experiments were performed in which the effects of the drugs were tested on isolated atrial and tracheal preparations from the two species. The in vitro results obtained in the guinea-pig preparations have been cited in Chapter 2.
2. METHODS

2.1. In vivo experiments

Guinea-pigs of either sex (250 to 500 g) were anaesthetized by the intraperitoneal injection of a mixture of \( \alpha \)-chloralose (30 mg kg\(^{-1}\)) and urethane (0.725 g kg\(^{-1}\)). Tracheal cannulae were inserted and drugs were injected through a cannulated jugular vein. Traces of the various parameters studied were recorded using Beckman (Type RP and R) Dynographs.

2.1.1. Heart Rate

In all experiments where cardiovascular responses were studied the animals were artificially respired at a rate of 35 cycles min\(^{-1}\) and a stroke volume of approximately 4 ml using a Palmer small animal positive pressure respirator. Arterial blood pressure was recorded from a cannulated carotid artery using a Statham (P23AA) pressure transducer. Heart rate was recorded using a cardiotachometer (Beckman type 9857B) triggered by the QRS complex from Lead II electrocardiograms.

2.1.2. Pulmonary Resistance

Pulmonary resistance was assessed by a modification of the method described by Amdur & Mead (1958) in which the guinea-pig was placed in a whole body pressure plethysmograph. Artificial respiration was applied as described in Section 2.1.1. Measurements were made of tidal volume, air flow and oesophageal and tracheal
pressure for calculation of pulmonary resistance (Amdur & Mead, 1958). When necessary gallamine triethiodide (2 mg kg\(^{-1}\)) was injected to suppress spontaneous respiration. Doses of serotonin were injected at 15 min intervals to produce increases in pulmonary resistance. After establishing constant responses to serotonin the bronchodilator effects of MJ9184-1 and (-)-isoprenaline were assessed in terms of their ability to reduce the response to serotonin.

2.2. In vitro experiments

Guinea-pigs (250 to 500 g) and cats (400 to 800 g) of either sex were killed by stunning and exsanguination. Isolated atrial and tracheal preparations were set up in Krebs solution (NaCl, 6.9; KCl, 0.4; MgSO\(_4\)\(\cdot\)7H\(_2\)O, 0.14; Na\(_2\)HPO\(_4\), 0.14; NaHCO\(_3\), 2.1; dextrose, 2.0 and CaCl\(_2\), 0.28 g l\(^{-1}\)) maintained at 37°C and aerated with 5% CO\(_2\) in oxygen. The bathing solution contained ascorbic acid (20 µg ml\(^{-1}\)) to reduce the breakdown of the catecholamines. With all preparations a 45 min equilibration period was allowed before addition of drugs to the organ bath.

2.2.1. Atrial Preparations

For the assessment of the positive inotropic activity of the drugs, driven left atrial preparations were used. A resting tension of 2 g was applied to the tissues and the force of contraction was measured using an isometric transducer. Stimuli were applied between a platinum punctate electrode which anchored the tissue to the electrode block and an indifferent electrode which was fixed alongside
the atrial preparation. The preparations were driven at a frequency of 5 Hz using square wave pulses of 1 ms duration and twice the threshold voltage required for driving (0.5 to 4.0 V).

Positive chronotropic activity of the drugs was assessed using spontaneously beating whole atria as described in Chapter 2.

In both types of atrial preparation control cumulative concentration-effect curves were first established for (-)-isoprenaline and a cumulative curve for MJ9184-1 was then obtained.

2.2.2. Tracheal Preparations

Guinea-pig tracheal preparations were set up as described in Chapter 2. Cat tracheal chain preparations were prepared as described by Ackasu (1959). Unless otherwise stated, tone was induced in the preparations by the use of carbachol ($10^{-6}$ M). Both guinea-pig and cat tracheal preparations maintained the carbachol-induced tone over equivalent periods to those used when the relaxant effects of the agonists were studied. Constant cumulative concentration-effect curves were initially obtained using (-)-isoprenaline and then a cumulative curve for MJ9184-1 was established.
2.3. **Drugs used**

The drugs used were (-)-isoprenaline bitartrate (Wyeth); MJ9184-1 hydrochloride (Mead Johnson); carbachol chloride, α-chloralose and urethane (British Drug Houses); propranolol hydrochloride (Imperial Chemical Industries); serotonin (5-hydroxytryptamine) creatinine sulphate (Sigma) and gallamine triethiodide (May & Baker). In the text, doses of the drugs refer to the base and all concentrations are expressed in molar terms. Stock solutions of the compounds were freshly prepared and diluted appropriately with either 0.9% w/v sodium chloride solution or Krebs solution for *in vivo* and *in vitro* experiments, respectively.
3. RESULTS

3.1. Guinea-pig: Chronotropic Activity In Vivo

Preliminary experiments showed that MJ9184-1 produced vasodepressor and positive chronotropic responses which were abolished by the prior administration of propranolol (0.5 mg kg⁻¹). As in cats (Gwee et al., 1972) the tachycardia produced by MJ9184-1 was long lasting. To compare the effects of MJ9184-1 and (-)-isoprenaline, control cumulative dose-response curves to (-)-isoprenaline were obtained at 20 min intervals until constant, after which the effects produced by cumulative doses of MJ9184-1 were determined. Figure 2 shows the mean results obtained from four such comparisons.

![Graph showing dose-response curves](image)

**Fig. 2.** Results showing mean dose-effect curves from four experiments for the positive chronotropic actions of cumulatively administered (-)-isoprenaline (○) and MJ9184-1 (▲) in anaesthetized guinea-pigs. Results were expressed in terms of the maximal response (Emax) to (-)-isoprenaline in each experiment. Individual points show the mean doses required to produce a given percentage of the maximal response.
In these experiments the mean resting heart rate was 308 beats min\(^{-1}\) (s.e.m. = 11, n = 9). The mean maximal increase in heart rate produced by (-)-isoprenaline was 61 beats min\(^{-1}\) (s.e.m. = 6, n = 9) and the time to half-return of the heart rate to control levels was 3.1 min (s.e.m. = 0.3, n = 9). The maximum increase in heart rate produced by MJ9184-1 was less than that obtained with (-)-isoprenaline. The mean maximal positive chronotropic response to MJ9184-1 was 63.2% (s.e.m. = 3.4, n = 4) of that obtained with (-)-isoprenaline. After maximal positive chronotropic responses had been obtained with MJ9184-1, the time to half-return of the heart rate to control levels was greater than 30 min in all experiments.

The results obtained in the present experiments with anaesthetized guinea-pigs differ from those obtained when the chronotropic actions of (-)-isoprenaline and MJ9184-1 were compared in anaesthetized cats (Gwee et al., 1972). In the cat experiments both drugs produced similar maximal increases in heart rate. Furthermore in cats MJ9184-1 was approximately 4 times less potent than (-)-isoprenaline on a molar basis, whereas in guinea-pigs the ratio of the doses of the compounds producing 50% of their individual maximal responses indicates that MJ9184-1 is 25 times less active than (-)-isoprenaline.

Table 1 shows the mean doses of (-)-isoprenaline and MJ9184-1 required to produce 50% of the individual maximal increases in heart rate with each drug and the mean ratio of these doses (MJ9184-1/(-)-isoprenaline).
TABLE 1. Comparison of the positive chronotropic and bronchodilator activities of (-)-isoprenaline and MJ9184-I in anaesthetized cats and guinea-pigs.

| Dose* (ng kg⁻¹) | CAT† | | | GUINEA-PIG | | |
| | Bronchial | Cardiac | | Bronchial | Cardiac |
| (-)-Iso | 16 ± 5 | 55 ± 5 | | 40 ± 10 | 82 ± 21 |
| MJ9184-I | 41 ± 13 | 370 ± 60 | | 103 ± 26 | 4480 ± 2040 |
| Ratio | | | | | |
| MJ9184-I Weight | 2.4 ± 0.4 | 6.8 ± 2.2 | | 3.33 ± 1.03 | 44.4 ± 14.2 |
| (-)-Iso Molar | 1.41 | 3.70 | | 1.86 ± 0.58 | 24.8 ± 7.9 |

†Data from Gwee et al., 1972.

*For bronchodilator activity doses refer to those required to produce a 50% decrease in serotonin-induced increases in pulmonary resistance, and for cardiac activity doses required to produce 50% of the maximal chronotropic activity (ED50) with the individual compounds. Ratios of these doses and those calculated on a molar basis (MJ9184-I/(-)-isoprenaline) were calculated in each experiment. Results in the Table are expressed means (±s.e.m.) from four to six experiments.

3.2. Guinea-Pig: Antagonism of Serotonin-Induced Increases in Pulmonary Resistance

Doses of 6.5 to 9.0 μg kg⁻¹ of serotonin were used in different experiments and these produced two- to twelve-fold increases in pulmonary resistance. In all experiments, the responses to serotonin were submaximal.

In preliminary experiments doses of MJ9184-I or (-)-isoprenaline were given at various times before serotonin. The maximal effect produced by (-)-isoprenaline was observed when the compound was injected 35 to 45 s before serotonin, whereas with MJ9184-I maximal activity was apparent when it
was injected 3-4 min before serotonin. In all further experiments in which bronchodilator effects were compared, MJ9184-1 and (-)-isoprenaline were injected 3.5 min and 40 s, respectively before the serotonin challenge.

In each experiment a number of doses of MJ9184-1 and (-)-isoprenaline were used. Dose-response curves were plotted in which the response at any dose-level was expressed as the % reduction of control serotonin-induced increases in pulmonary resistance. Figure 3 shows the results from a single experiment. In any one experiment the dose-response curves for the two drugs were close to parallel. The doses of

![Graph showing dose-response curves for MJ9184-1 and (-)-isoprenaline compared to serotonin challenge.](image)

**Fig. 3.** Dose-response curves from an experiment in which the effects of (-)-isoprenaline (●) and MJ9184-1 (▲) were compared for their ability to reduce the increase in pulmonary resistance produced by serotonin 8.7 μg kg⁻¹. Each point shows the effect produced by a single dose of a β-adrenoceptor stimulant expressed as a percentage reduction of the control response to serotonin.
MJ9184-1 and (-)-isoprenaline used in different experiments varied in accordance with the dose of serotonin used and the degree of bronchoconstriction produced. However, as noted previously by Gwee et al. (1972) and Bowman & Rodger (1972) who used cats, the relative potencies of the compounds were similar in all experiments. MJ9184-1 appeared to possess a longer lasting bronchodilator action than did (-)-isoprenaline; after MJ9184-1, return of serotonin responses to control levels occasionally took 30 min or more, whereas after (-)-isoprenaline responses to serotonin invariably returned to control levels within 15 min.

When comparing the doses of the compounds required to produce a 50% reduction in the response to serotonin, MJ9184-1 was shown to be 1.86 times less potent than (-)-isoprenaline on a molar basis (Table 1). This mean value is similar to that found in anaesthetized cats (Gwee et al., 1972).

3.3. Isolated Atrial Preparations

Preliminary studies showed that reproducible cumulative concentration-effect curves to (-)-isoprenaline could be obtained at 45 min intervals in both cat and guinea-pig atrial preparations. In each curve dosage was continued until a maximal response had been obtained. After constant control responses to (-)-isoprenaline had been established the effects of cumulatively administered MJ9184-1 were assessed. In cat atrial preparations MJ9184-1 and (-)-isoprenaline produced similar maximal positive inotropic and positive chronotropic responses, whereas in guinea-pig preparations the maximal
responses to MJ9184-1 were less than those obtained with
(-)-isoprenaline (Fig. 4a,b).

Table 2 shows the pD₂ values (i.e., negative log of
molar concentration producing 50% of an agonist's maximal
response; Ariëns, Simonis & van Rossum, 1964) for
(-)-isoprenaline and MJ9184-1 in the atrial preparations and
values for intrinsic activity (Ariëns et al., 1964) of MJ9184-1
(intrinsic activity, α, of (-)-isoprenaline = 1). Table 2 also
shows the relative activities of MJ9184-1 and (-)-isoprenaline
expressed as the ratio of the molar concentrations (MJ9184-1:
(-)-isoprenaline) required to produce 50% of their individual
maximal responses (EC₅₀).

**TABLE 2. Comparison of the effects of MJ9184-1 and
(-)-isoprenaline in in vitro preparations from guinea-pigs
and cats.**

<table>
<thead>
<tr>
<th></th>
<th>(-)-Iso</th>
<th>MJ9184-1</th>
<th>Ratio MJ9184-1/(-)-Iso</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Atria (force)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>8.43±0.10</td>
<td>6.41±0.11</td>
<td>1.06±0.06</td>
</tr>
<tr>
<td>Guinea-pig</td>
<td>8.35±0.21</td>
<td>5.37±0.10</td>
<td>0.45±0.03</td>
</tr>
<tr>
<td><strong>Atria (rate)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>8.40±0.14</td>
<td>7.92±0.09</td>
<td>1.00±0.03</td>
</tr>
<tr>
<td>Guinea-pig</td>
<td>8.60±0.07</td>
<td>6.05±0.10</td>
<td>0.65±0.02</td>
</tr>
<tr>
<td><strong>Tracheal relaxation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>7.01±0.05</td>
<td>5.16±0.19</td>
<td>1.00±0.01</td>
</tr>
<tr>
<td>Guinea-pig</td>
<td>7.58±0.14</td>
<td>7.91±0.13</td>
<td>0.94±0.02</td>
</tr>
<tr>
<td></td>
<td>9.21±0.05</td>
<td>9.51±0.08</td>
<td>1.02±0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.56±0.12</td>
</tr>
</tbody>
</table>

Mean pD₂ values for (-)-isoprenaline and MJ9184-1, and
values for the intrinsic activity (α) of MJ9184-1
((-)-isoprenaline = 1). Molar ratios of EC₅₀ values
(MJ9184-1/(-)-isoprenaline) are also shown. Values in
parentheses are those obtained in guinea-pig tracheal
preparations where intrinsic rather than carbachol-induced
tone was used. Each value shows the mean (±s.e.m.) from
4 to 6 experiments.
Fig. 4. Concentration-effect curves to (-)-isoprenaline (○) and MJ9184-1 (▲) in isolated tissue preparations from cats (solid lines) and guinea-pigs (dashed lines). Results show (a) positive chronotropic activity in whole atrial preparations, (b) positive inotropic activity in driven left atrial preparations, and (c) relaxant effects in tracheal preparations. Results expressed in terms of the maximal effects (E_max) produced by (-)-isoprenaline. Individual points show mean concentrations from four to six experiments required to produce a given percentage of the maximal effect produced by (-)-isoprenaline.
The pD2 values for (-)-isoprenaline are similar for both positive inotropic and positive chronotropic activity in both guinea-pig and cat preparations. However, the pD2 values for both the inotropic and chronotropic actions of MJ9184-1 differ markedly within and between species (Table 2).

3.4. Isolated Tracheal Preparations

Guinea-pig tracheal preparations developed spontaneous tone, so that the relaxant effects of β-adrenoceptor agonists could be assessed directly. Intrinsic tone was not present in tracheal preparations from cats, and therefore, in experiments where the effects of (-)-isoprenaline and MJ9184-1 were compared using preparations from the two species, tone was induced using carbachol. Control cumulative concentration-effect curves to (-)-isoprenaline were obtained first, and thereafter the effects of cumulatively administered MJ9184-1 were tested. In tracheal preparations from both species (-)-isoprenaline and MJ9184-1 produced similar maximal relaxant effects (Fig. 4c and Table 2). In guinea-pig preparations the compounds had a similar potency, whereas in cat preparations MJ9184-1 was less potent than (-)-isoprenaline. Table 2 shows the pD2 values of the two compounds and values for the intrinsic activity of MJ9184-1 ((-)-isoprenaline = 1). The ratios of potency of the compounds (MJ9184-1:(-)-isoprenaline) calculated from their respective molar EC50 values are also shown. The potency ratio obtained from experiments using guinea-pig preparations where intrinsic rather than carbachol-induced tone was used is also shown in Table 2.
Although the pD$_2$ values were greater in the former preparations, the ratio of potency of the compounds was similar in experiments where intrinsic and carbachol-induced tone was utilized.
4. DISCUSSION

The results of the present experiments show that in guinea-pigs, as in cats (Gwee et al., 1972), MJ9184-1 possesses some selectivity in its stimulant activity on β-adrenoceptors in bronchial as opposed to those in cardiac tissue. Although the relative activities of (-)-isoprenaline and MJ9184-1 are of the same order in terms of their effects in bronchial muscle of cats and guinea-pigs in vivo, there are marked differences in the cardiostimulant effects of MJ9184-1 in the two species. Thus in cats, cumulative dose-effect curves for the positive chronotropic actions of (-)-isoprenaline and MJ9184-1 are close to parallel and have similar maxima, whereas in guinea-pigs the maximal response to MJ9184-1 is less than that obtained with (-)-isoprenaline. In addition, comparisons of doses producing 50% of the maximal rise in heart rate (ED50) show that while values for (-)-isoprenaline are similar in both species, MJ9184-1 is much less active in producing positive chronotropic actions in guinea-pigs than in cats. These differences do not appear to be entirely dependent on the in vivo situation in which the drugs were tested, since similar trends in relative activity and maximal responses were also apparent in isolated atrial preparations from the two species.

In addition to the species differences outlined above, there are also differences between results from in vivo and in vitro experiments within species. Thus the relative activity of MJ9184-1 to (-)-isoprenaline is similar for chronotropic activity in vivo and in vitro in the cat, but not in the guinea-pig. When comparing antagonism of
serotonin-induced bronchoconstriction (in vivo) and tracheal relaxation (in vitro), the relative activities of the drugs are similar in the guinea-pig, but not in the cat.

In isolated tracheal preparations from cats and guinea-pigs, both compounds produced similar maximal relaxant effects, but the relative activity of MJ9184-1 to (-)-isoprenaline was much less in cat than in guinea-pig tracheal preparations.

Olsson, Persson, Persson & Sörenby (1974) compared terbutaline with (-)-adrenaline and found similar dose-ratios in both isolated cat bronchial muscle strips and guinea-pig tracheal chains. The dose-ratios found for a number of selective $\beta_2$-receptor agonists on guinea-pig tracheal preparations (see Chapter 2) are similar to the dose-ratios reported for isolated human bronchial smooth muscle (Svedmyr, Malmberg & Thiringer, 1972). Thus from the results of the present experiments plus the already published data cited above, it may be tentatively suggested that cat tracheal muscle responds quantitatively in a different way to cat bronchial muscle, human bronchial muscle and guinea-pig tracheal chains with respect to dose-ratios for selective $\beta_2$-adrenoceptor agonists.

Although it is generally thought that cardiac responses are mediated through $\beta_1$-adrenoceptor stimulation, the results of the present experiments show that both in vivo and in vitro pacemaker responses to MJ9184-1 differ to a marked extent in guinea-pigs and cats.

Brittain, Jack & Ritchie (1970) have suggested, by analogy with the existence of isoenzymic forms of specific
enzymes, that differing β-isoreceptors may show differing specificities for a given β-adrenoceptor agonist. Thus it is possible that differing isoreceptors in the pacemaker tissues from the two species are responsible for the divergent results obtained. On the other hand the β-isoreceptors in the bronchial smooth muscle of the two species appear to be similar.

Another possibility for the difference in cardiac β-adrenoceptor responsiveness comes from the work of Åblad, Carlsson, Carlsson et al. (1974) and Åblad, Borg, Carlsson et al. (1975). They suggested that both β₁- and β₂-receptors mediate the chronotropic effects in the cat; however, no evidence was found for such a situation in rat atrial or guinea-pig atrial preparations (J.L. Wale, personal communication). Thus the inhomogeneity of β-receptors in the cat heart may explain the species difference observed.

Further discussion as to the best animal model to predict selective bronchodilators in man is contained in the General Discussion (Chapter 7). Suffice it to say, from the data obtained in this Chapter it would appear that selective β-adrenoceptor agonists can show widely varying degrees of selectivity depending on the species used and whether the results have been obtained from in vivo or in vitro experiments.
CHAPTER 5:

INFLUENCES OF CHANGES IN AMINE SUBSTITUTION ON THE 
β-ADRENOCEPTOR STIMULANT ACTIVITY OF COMPOUNDS 
RELATED TO ORCIPRENALINE AND SOTERENOL IN THE 
ANAESTHETIZED CAT
SUMMARY

1. Three 3,5-dihydroxy ring substituted phenylethanolamine derivatives (resorcinols) with N-isopropyl (orcioprenaline), N-t-butyl (terbutaline) and N-p-hydroxyphenyl-1-t-butyl (Me506) amine substituents and three 3-methanesulphonamido,4-hydroxy ring substituted phenylethanolamines (methanesulphonanilides) with N-isopropyl (soterenol) N-t-butyl (MJ7999-1) and N-(1-phenyl-t-butyl) (MJ9184-l) amine substituents have been compared with (-)-isoprenaline for their ability to produce $\beta_2$-receptor mediated reduction in serotonin-induced increases in pulmonary resistance ($\beta_2$), decreases in soleus muscle contractility ($\beta_2$), and increases in heart rate ($\beta_1$) in anaesthetized cats.

2. For all parameters studied each compound produced a similar maximal response to (-)-isoprenaline and dose-response curves for the sympathomimetic amines were close to parallel. From the graphs doses of the compounds producing 50% of the maximal response (ED$_{50}$) were interpolated, and from these dose-ratios with respect to (-)-isoprenaline (drug ED$_{50}$:(-)-isoprenaline ED$_{50}$) were calculated on a molar basis.

3. In the resorcinol series increasing the size of the amine substituent from N-isopropyl to N-t-butyl led
to an increase in β-receptor stimulant activity in bronchial and skeletal muscle, but not in the heart. For the methanesulphonanilides the same change in the amine substituent produced an increase in the potency of the compounds as cardiac stimulants, but only small increases in potency were seen in the bronchial and skeletal muscle systems.

4. In both series of compounds the addition of a phenyl ring (methanesulphonanilides) or phenol ring (resorcinols) to the N-t-butyl substituent did not further affect stimulant activity in any of the tissues studied.

5. Calculation of selectivity ratios [molar dose-ratio (heart):molar dose-ratio (pulmonary resistance)] showed that orciprenaline was non-selective, and that terbutaline and Me506 showed a similar degree of selectivity for β₂- as opposed to β₁-receptor mediated actions.

6. Selectivity ratios showed that soterenol was the most β₂-selective compound in the methanesulphonanilides series. MJ7999-1 and MJ9184-1 showed a similar but lower selectivity for β₂-receptor mediated effects.
7. Of the six non-catechol compounds studied, soterenol was the most selective $\beta_2$-adrenoceptor agonist. Terbutaline, Me506, MJ7999-1 and MJ9184-1 all exhibited similar degrees of $\beta_2$-receptor selectivity whilst orciprenaline was non-selective in its actions.

8. The results are discussed in the light of the structure-activity relationships shown in Chapter 2 where the six compounds were tested in isolated guinea-pig atrial and tracheal preparations. In addition the clinical relevance of the results with orciprenaline and terbutaline are discussed.
1. **INTRODUCTION**

The major side effects associated with the use of sympathomimetic bronchodilators are skeletal muscle tremor and cardiac stimulation (Rebuck, 1974).

Previous workers have shown that the cardiac stimulant actions of sympathomimetic amines are mediated through $\beta_1$-adrenoceptor stimulation whereas $\beta_2$-adrenoceptors are involved in the pulmonary and skeletal muscle effects produced by the amines (Lands, Arnold, McAuliff, Luduena & Brown, 1967; Bowman & Nott, 1970).

In the development of new $\beta$-adrenoceptor agonists it would be advantageous to have an animal model which could predict the selectivity of action of compounds for $\beta_1$- and $\beta_2$-adrenoceptor mediated effects in man. The chloralose-anaesthetized cat has been suggested as a suitable model in this respect (Bowman & Rodger, 1972; Rodger, 1973) since the bronchodilator, cardiac and skeletal muscle actions of sympathomimetic amines can all be tested, and in addition dose-ratios with respect to isoprenaline for a variety of $\beta$-receptor mediated effects are similar for salbutamol (Rodger, 1973; Warrell, Robertson, Newton Howes, Conolly, Paterson, Bielin & Dollery, 1970; Marlin & Turner, 1975), isothaerarine (Collier & Dornhorst, 1969; Rodger, 1973), orciprenaline (Shanks, Brick, Hutchinson & Roddie, 1967; Apperley & Daly, 1972; McEvoy, Vall-Spinosa & Paterson, 1973) and rimiterol (Bowman & Rodger, 1972; Marlin & Turner, 1975) in both cat and man.

In the present experiments three resorcinol
(3,5-dihydroxyphenyl) derivatives with N-isopropyl (orci­presinaline), N-t-butyl (terbutaline) and N-p-hydroxyphenyl-t-butyl (Me506) amine substitutes and three 3-methanesulphonamido, 4-hydroxy ring substituted phenylethanolamines (methanesulphonanilides) with N-isopropyl (soterenol), N-t-butyl (MJ7999-1) and N-(1-phenyl-t-butyl) (MJ9184-1) amine substitutes (Table 1) have been compared with (-)-isoprenaline for their bronchial, skeletal muscle and cardiac effects in anaesthetized cats.

TABLE 1. Structures of the β-adrenoceptor agonists used in this study.

<table>
<thead>
<tr>
<th>DRUG</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-Isoprenaline</td>
<td>HO</td>
<td>HO</td>
<td>H</td>
<td>CH(CH₃)₂</td>
</tr>
<tr>
<td>Orciprenaline</td>
<td>HO</td>
<td>H</td>
<td>HO</td>
<td>CH(CH₃)₂</td>
</tr>
<tr>
<td>Terbutaline</td>
<td>HO</td>
<td>H</td>
<td>HO</td>
<td>C(CH₃)₃</td>
</tr>
<tr>
<td>Me506</td>
<td>HO</td>
<td>H</td>
<td>HO</td>
<td>C(CH₃)₂CH₂-p-OHC₆H₄</td>
</tr>
<tr>
<td>Soterenol</td>
<td>H</td>
<td>HO</td>
<td>H₃CSO₂HN</td>
<td>CH(CH₃)₂</td>
</tr>
<tr>
<td>MJ7999-1</td>
<td>H</td>
<td>HO</td>
<td>H₃CSO₂HN</td>
<td>C(CH₃)₃</td>
</tr>
<tr>
<td>MJ9184-1</td>
<td>H</td>
<td>HO</td>
<td>H₃CSO₂HN</td>
<td>C(CH₃)₂CH₂C₆H₅</td>
</tr>
</tbody>
</table>

Since the agonistic actions of MJ9184-1 have already been reported on the three parameters (Gwee, Nott, Raper & Rodger, 1972) and that for soterenol on the cat soleus muscle (Nott & Raper, 1972), these experiments were not duplicated and the ratios obtained in the published studies were used to
facilitate discussion in this Chapter.

As yet there are no published data on the clinical effectiveness of the methanesulphonanilides studied, however two of the resorcinols (orciprenaline and terbutaline) are used clinically as bronchodilators.
2. METHODS

2.1. General

Cats of either sex were anaesthetized by the intra-peritoneal injection of a mixture of α-chloralose (80 mg kg\(^{-1}\)) and pentobarbitone sodium (6 mg kg\(^{-1}\)). After tracheal cannulation the animals were artificially respired at 27 cycles min\(^{-1}\) using a stroke volume of 13 ml kg\(^{-1}\). Blood pressure was recorded from either a cannulated carotid or femoral artery using a Statham P23Dc pressure transducer, and heart rate recorded using a Grass 7P4DF tachograph triggered by the arterial pulse. All drugs were injected intravenously through a cannulated brachial vein.

2.2. Heart rate

Cumulative dose-response curves for the positive chronotropic actions of the β-adrenoceptor agonists were obtained using the method of Rodger (1973). In some experiments with the resorcinols and in all experiments with the methanesulphonanilides, it was possible to establish concurrent cumulative dose-response curves for the cardiac and skeletal muscle effects of the amines, since with each dose in the series, peak effects in the two tissues occurred simultaneously. This was not possible with (−)-isoprenaline since the time to reach peak effects in the two tissues differed.
2.2.1. Soleus Muscle

After clearing the soleus from neighbouring muscles, its tendon of insertion was cut and attached to a Grass FT10C or FT03C transducer for the recording of muscle contractions. The muscle was set up as described by Bowman & Nott (1970) and stimulated indirectly at a frequency of 8 Hz for 1 s once every 10 s using supramaximal voltages. Cumulative dose-response curves were established for assessing β-receptor mediated depression in soleus muscle contractility using the method of Nott & Raper (1972).

2.2.2. Pulmonary Resistance

The animals were bilaterally vagotomized and when necessary gallamine triethiodide (2–10 mg kg⁻¹ intravenously) was given to suppress spontaneous respiration.

Air flow was measured using a Fleisch Type 1 pneumotachograph connected to a Grass PT5 volumetric pressure transducer. A further Grass PT5 transducer was used to measure transpulmonary pressure (using the difference between oesophageal and intratracheal pressure). Tidal volume was obtained by integration of the air flow signal using a Grass 7P10B integrator. Records of air flow, transpulmonary pressure and tidal volume, were used for the calculation of pulmonary resistance as described by Amdur & Mead (1958). Increases in pulmonary resistance were obtained by infusing serotonin (2–23 μg kg⁻¹ min⁻¹ in different experiments) and the bronchodilator assessed by calculating the decrease in pulmonary resistance produced by the cumulative administration of the amines (Rodger, 1974). The serotonin infusions
were terminated as soon as maximal sympathomimetic-induced bronchodilatation had been achieved. With a given infusion rate of serotonin the increases in resistance were generally repeatable. However when necessary the infusion rate was altered so that constant responses could be obtained. Infusions of serotonin were performed at 45 min intervals.

2.3. Calculations

In all experiments constant cumulative dose-response curves to (-)-isoprenaline were first obtained and thereafter responses to the other drugs tested. In each experiment dose-response curves were plotted in terms of the maximal response produced by (-)-isoprenaline and doses of the compounds required to produce 50% of their individual maximal responses (ED$_{50}$) calculated. From these results dose-ratios (drug ED$_{50}$: (-)-isoprenaline ED$_{50}$) were calculated on a molar basis. Differences between mean molar dose-ratios were assessed using Student's $t$-test; significance was taken at the 5% level in all cases.

2.4. Drugs Used

Drugs used were (-)-isoprenaline bitartrate (Wyeth), propranolol hydrochloride (Imperial Chemical Industries), orciprenaline sulphate and Me506 hydrobromide (Boehringer-Ingelheim), terbutaline sulphate (Astra), serotonin creatinine sulphate (Koch-Light), soterenol hydrochloride, MJ7999-1 hydrochloride and MJ9184-1 hydrochloride (Mead Johnson), gallamine triethiodide (May & Baker), $\alpha$-chloralose (British Drug Houses) and pentobarbitone sodium (Abbot).
Drug solutions were freshly prepared and were diluted to appropriate concentrations with 0.9% w/v sodium chloride solution containing 20 µg ml$^{-1}$ ascorbic acid. All doses in the text refer to the weight of the drugs in terms of their bases.
3. RESULTS

3.1. Heart rate

All the β-adrenoceptor agonists produced increases in heart rate and reductions in systemic arterial blood pressure which were abolished by the intravenous administration of propranolol (0.5 mg kg⁻¹). The mean resting heart rate of anaesthetized cats was 135 beats min⁻¹ (s.e.m. = 8, n = 29). The mean maximal increase in heart rate produced by (-)-isoprenaline was 55 beats min⁻¹ (s.e.m. = 3, n = 29). The three resorcinols and the three methanesulphonanilides produced similar maximal increases in heart rate as those produced by (-)-isoprenaline. Figure 1 shows the effects on heart rate and blood pressure produced by the cumulative administration of (-)-isoprenaline and terbutaline. The doses of (-)-isoprenaline produced the most rapid rise in heart rate; the resorcinols were intermediate and the methanesulphonanilides the slowest in this respect. The time to half-return from maximal cardiac responses to control levels was 4 to 5 min for (-)-isoprenaline, 10-12 min for the resorcinols and greater than 120 min for the methanesulphonanilides. Both series of agonists produced dose-response curves parallel to those elicited by (-)-isoprenaline. Figure 2a shows mean dose-response curves for the ability of the three resorcinols and (-)-isoprenaline to produce increases in heart rate in anaesthetized cats.

Table 2 shows the mean ED₅₀ values and the mean molar dose-ratios with respect to (-)-isoprenaline for the cardiac actions of the six compounds. The values indicate
Fig. 1. Traces from an experiment showing the effects on blood pressure (BP) and heart rate (HR) produced by the cumulative administration of (-)-isoprenaline [(-)-ISO] and terbutaline [TERB].

that increasing the size of the amine substituent in the resorcinol series leads to a moderate increase in the potency of the compounds as stimulants at cardiac β-adrenoceptors. Thus although the dose-ratio for Me506 (N-p-hydroxyphenyl-<i>t</i>-butyl) is significantly less than that for orciprenaline (N-isopropyl) (0.01<P<0.02, 8 d.f.) neither of these compounds has a molar dose-ratio which
Fig. 2. Mean cumulative dose-effect curves (n = 4) to (-)-isoprenaline (●), orciprenaline (■), terbutaline (○) and Me506 (□) for their ability to (a) increase heart rate, (b) depress soleus muscle contractility, and (c) inhibit serotonin-induced increases in pulmonary resistance in anaesthetized cats. Responses are expressed in terms of the maximal response produced by (-)-isoprenaline for each parameter.
differs significantly from that of terbutaline (N-t-butyl) (0.2<P<0.3, 8 d.f. and 0.2<P<0.3, 6 d.f., respectively).

In contrast, within the methansulphonanilide series soterenol (N-isopropyl) is significantly less potent than MJ7999-l (N-t-butyl) (P<0.001, 6 d.f.). Due to lack of data from individual experiments with MJ9184-l no tests of significance between molar dose-ratios for MJ7999-l and MJ9184-l could be performed. However, the similarities in mean ED$_{50}$ values and the molar dose-ratios suggests that the molecular change from N-t-butyl (MJ7999-l) to N-(1-phenyl-t-butyl) (MJ9184-l) produces little or no change in agonistic activity.

3.2. Soleus muscle contractility

All the sympathomimetic amines tested produced decreases in the tension and fusion of incomplete tetanic contractions of the cat soleus muscle. These actions were antagonized by the intravenous administration of propranolol 0.5 mg kg$^{-1}$. The mean maximal depression in soleus muscle contractility produced by (-)-isoprenaline was 46.3% (s.e.m. = 2.4, n = 15) of the original evoked tension. The six non-catechol β-adrenoceptor agonists produced similar maximal responses to those obtained with (-)-isoprenaline. Figure 3 shows cumulative dose-response curves to (-)-isoprenaline and terbutaline on the cat soleus muscle. As for the effects on heart rate, the rank order for the speed at which the effects were elicited was (-)-isoprenaline>resorcinols>methanesulphonanilides.
TABLE 2. Mean dose-ratios on a molar basis [drug \( ED_{50} \) : \((-\)-isoprenaline \( ED_{50} \)] and mean \( ED_{50} \) values (\( \mu g \ kg^{-1} \)) in terms of the base for the six non-catechol compounds and \((-\)-isoprenaline for their actions in increasing heart rate, decreasing soleus muscle contractility and reducing serotonin-induced increases in pulmonary resistance in anaesthetized cats. Numbers in parentheses are s.e.m. from at least 4 comparisons with each drug. The selectivity ratios (SR) for the compounds [molar dose-ratio (heart rate) : molar dose-ratio (pulmonary resistance)] are also shown.

<table>
<thead>
<tr>
<th></th>
<th>HEART RATE</th>
<th>SOLEUS CONTRACTILITY</th>
<th>PULMONARY RESISTANCE</th>
<th>SR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ratio</td>
<td>( ED_{50} )</td>
<td>Ratio</td>
<td>( ED_{50} )</td>
</tr>
<tr>
<td>Orciprenaline</td>
<td>47.2 (3.2)</td>
<td>2.06 (0.58)</td>
<td>74.6 (8.9)</td>
<td>2.13 (0.33)</td>
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<tr>
<td>Terbutaline</td>
<td>38.8 (5.5)</td>
<td>3.06 (0.75)</td>
<td>18.2 (4.7)</td>
<td>0.48 (0.15)</td>
</tr>
<tr>
<td>Me506</td>
<td>29.6 (2.4)</td>
<td>2.24 (0.35)</td>
<td>17.8 (0.2)</td>
<td>0.79 (0.11)</td>
</tr>
<tr>
<td>Soterenol</td>
<td>24.8 (2.8)</td>
<td>1.38 (0.20)</td>
<td>4.1* (0.5)</td>
<td>0.54 (1.2)</td>
</tr>
<tr>
<td>MJ7999-1</td>
<td>3.2 (0.5)</td>
<td>0.16 (0.05)</td>
<td>1.7 (0.2)</td>
<td>0.072 (0.016)</td>
</tr>
<tr>
<td>MJ9184-1†</td>
<td>3.8 (0.06)</td>
<td>0.37 (0.06)</td>
<td>1.5 (0.089)</td>
<td>0.089 (0.019)</td>
</tr>
<tr>
<td>((-)-Isoprenaline</td>
<td>0.052 (0.008)</td>
<td>0.026 (0.003)</td>
<td>0.019 (0.004)</td>
<td></td>
</tr>
</tbody>
</table>

† Adapted from Gwee et al. (1972)
* Adapted from Nott & Raper (1972)

After doses of the compounds which produced maximal effects the times to half-return of responses to control values were \((-\)-isoprenaline, 7-9 min; resorcinols, 12-15 min and for MJ7999-1 greater than 2 h. Since MJ9184-1 and soterenol have previously been assessed for their effects on soleus muscle contractility (Gwee et al., 1972; Nott & Raper, 1972)
Fig. 3. Traces from an experiment showing the effects on soleus muscle contractility produced by the cumulative administration of (-)-isoprenaline [(-)-ISO] and terbutaline [TERB]. Responses taken at fast trace speed are shown before and at the height of the response produced by the amines.

the only compound examined in this study was MJ7999-1. As with MJ7999-1 the above authors found that MJ9184-1 and soterenol had a very long duration of action. The dose-response curves for (-)-isoprenaline, the resorcinols and the methanesulphonanilides were close to parallel. Mean dose-response curves for (-)-isoprenaline and the three resorcinols are shown in Fig. 2b. Table 2 shows the mean molar dose-ratios and the mean ED$_{50}$ values for the six non-catechol $\beta$-receptor agonists on the cat soleus muscle. Comparisons of the molar dose-ratios obtained show that both terbutaline and Me506 are significantly more potent than
orcioprenaline (0.001<P<0.01, 6 d.f. and P<0.001, 6 d.f., respectively) whilst there is no significant difference in the ratios for terbutaline and Me506 (P>0.9, 6 d.f.). Thus alteration of the N-alkyl group from that in orciprenaline to that in terbutaline produces a marked increase in β-adrenoceptor activity on the soleus muscle, whilst the addition of a p-hydroxyphenyl ring to the t-butyl group of terbutaline (as in Me506) produces no further increase in activity.

In the methanesulphonanilide series, soterenol was significantly less potent than MJ7999-1 (0.001<P<0.005, 7 d.f.). Since the ED50 values and molar dose-ratios of MJ7999-1 and MJ9184-1 are similar, it appears that increasing the size of the amine substituent from N-t-butyl to N-(1-phenyl-t-butyl) produces little or no change in the activity of the compounds in the soleus muscle.

3.3. Pulmonary resistance

The mean resting pulmonary resistance of anaesthetized cats was 9.3 cmH2O l⁻¹ s⁻¹ (s.e.m. = 1.7, n = 17). In each experiment the infusion rate of serotonin was initially adjusted to produce an increase of 300-400% in pulmonary resistance. The mean increase in all experiments was 344% (s.e.m. = 23, n = 17).

In three preliminary experiments cumulative dose-response curves to (-)-isoprenaline were established under conditions where the infusion rate of serotonin was altered so that the range of increase in pulmonary resistance varied
from 450-750, 280-600 and 200-400%. In these experiments the ED$_{50}$ values ranged from 20-29, 10-15 and 5-6 ng kg$^{-1}$, respectively. Thus marked changes in the degree of serotonin-induced bronchoconstriction have relatively little effect on the ED$_{50}$ values obtained. Nevertheless, in experiments where the effects of the non-catechol compounds and (-)-isoprenaline were compared, the degree of bronchoconstriction produced by serotonin was maintained at a constant level by altering its infusion rate whenever necessary in order to minimize quantitative variations in ED$_{50}$ values.

All the drugs produced a complete abolition of the serotonin-induced response and the effects of the sympathomimetic amines were antagonized by the intravenous injection of propranolol 0.5 mg kg$^{-1}$. Figure 4 shows the effect of a serotonin infusion and its reversal by cumulative intravenous injections of (-)-isoprenaline. The trace in this Figure was run at a slow paper speed. However in experiments where antagonism of serotonin-induced increase in resistance was assessed the paper was run at fast speed so that calculations of resistance for individual breaths could be made as described by Amdur & Mead (1958).

In terms of onset and rate of production of bronchodilator activity, the same rank order as that found for the cardiac and skeletal muscle effects of the drugs was observed. Times to half-return could not be assessed accurately since the serotonin infusions were stopped as soon as maximal bronchodilator activity had been achieved. However the effects of (-)-isoprenaline and the resorcinols
Fig. 4. Traces from an experiment showing transpulmonary pressure (P), airflow (V), tidal volume (Vt), blood pressure (BP) and heart rate (HR) in the anaesthetized cat. Changes in these parameters induced by a continuous intravenous infusion of serotonin are shown together with the effects of the cumulative administration of (-)-isoprenaline.

were not evident at the start of the next serotonin infusion given 45 min later, whereas maximal bronchodilator activity for the methanesulphonanilides was still present at this time.
Dose-response curves for (-)-isoprenaline, the resorcinols and the methanesulphonanilides were close to parallel. Figure 2c shows the mean dose-response curves for the ability of the resorcinols and (-)-isoprenaline to reduce serotonin-induced increases in pulmonary resistance. Mean molar dose-ratios and \( ED_{50} \) values for the six compounds are shown in Table 2.

The dose-ratios for the resorcinols and methanesulphonanilides show the same pattern as that seen in experiments on the cat soleus muscle. Thus, in the resorcinols, the change from N-isopropyl (orciprenaline) to N-t-butyl (terbutaline) substitution produces a significant increase in bronchodilator activity \((0.001<P<0.01, 6 \text{ d.f.})\) whilst further increase in the size of the amine substitution as in Me506 does not enhance the activity above that seen with terbutaline \((0.8<P<0.9, 6 \text{ d.f.})\).

A similar pattern is found with the methanesulphonanilides where soterenol (N-isopropyl) is significantly less potent than MJ7999-1 \((N-t\text{-butyl}) \) \((0.02<P<0.05, 6 \text{ d.f.})\) and as judged by \( ED_{50} \) values and molar dose-ratios, MJ9184-1 \([N-(1\text{-phenyl}-t\text{-butyl})]\) has a similar activity to MJ7999-1.

3.4. **Selectivity for \( \beta \)-adrenoceptors**

For each individual compound the molar dose-ratios on the three parameters measured were compared using the Student's \( t \)-test.

A selectivity ratio, [i.e., the molar dose-ratio (heart rate):molar dose ratio (pulmonary resistance)], was calculated for each compound.
3.4.1. Resorcinols

With orciprenaline the molar dose-ratios for cardiac and pulmonary effects are not significantly different (0.4 < \( P < 0.5 \), 6 d.f.). However the molar dose-ratios for the effects on the soleus muscle are significantly different from either the cardiac or bronchial muscle dose-ratios (0.02 < \( P < 0.05 \), 6 d.f. and 0.01 < \( P < 0.02 \), 6 d.f., respectively).

In the case of terbutaline the dose-ratios for heart rate and pulmonary resistance are significantly different (0.02 < \( P < 0.05 \), 6 d.f.) and for Me506 these ratios border on significance (0.05 < \( P < 0.10 \), 8 d.f.). Dose-ratios for effects on the soleus muscle and on heart rate are significantly different for terbutaline and Me506 (0.02 < \( P < 0.05 \), 6 d.f. and 0.02 < \( P < 0.05 \), 8 d.f., respectively). However there are no significant differences between soleus and pulmonary dose-ratios for terbutaline and Me506 (0.8 < \( P < 0.9 \), 6 d.f. and 0.6 < \( P < 0.7 \), 6 d.f., respectively). Orciprenaline has a selectivity ratio of 1.0, indicating that the compound shows no selectivity in its ability to produce \( \beta_1 \)- and \( \beta_2 \)-receptor mediated effects in cardiac and bronchial smooth muscle, respectively. Terbutaline and Me506 show selectivity for \( \beta_2 \)- as opposed to \( \beta_1 \)-receptor actions since ratios of 2.3 and 1.9, respectively are obtained with these compounds.

3.4.2. Methanesulphonanilides

For all three compounds the molar dose-ratios [(\( \pm \))-isoprenaline = 1] for the effects of the drugs on soleus muscle contractility and pulmonary resistance are similar,
and are smaller than those obtained for the cardiac actions of the compounds.

The molar dose-ratios for the cardiac and pulmonary effects of both MJ7999-1 and soterenol are significantly different (0.02 < P < 0.05, 6 d.f.; P < 0.01, 6 d.f., respectively). There is no significant difference between the molar dose-ratios for the effects of MJ7999-1 or soterenol on soleus muscle contractility and pulmonary resistance (0.4 < P < 0.5, 6 d.f.; 0.6 < P < 0.7, 7 d.f., respectively).

As mentioned earlier, due to lack of data from individual experiments, t-tests could not be performed for MJ9184-1. However from the molar dose-ratios obtained no significant differences would be expected between soleus and pulmonary resistance dose-ratios. The dose-ratio for the cardiac action of MJ9184-1 is greater than that found in bronchial or skeletal muscle.

All the methanesulphonanilides show selectivity for β₂- as opposed to β₁-receptor mediated effects. Soterenol is the most selective of the compounds studied (selectivity ratio = 5.3). MJ7999-1 and MJ9184-1 have similar but lower selectivity ratios (2.5 and 2.9, respectively).
4. DISCUSSION

With the resorcinols and methanesulphonanilides studied in this Chapter, changes in the amine substitution not only affect the β-receptor stimulant activity of the compounds but also their selectivity of action for β₁- and β₂-adrenoceptor mediated effects.

In the resorcinols, the change from N-isopropyl (orciptrenaline) to N-t-butyl (terbutaline) substitution produces an increase in activity in the soleus muscle (β₂) and in the bronchi (β₂) but no significant effect in the heart (β₁). Thus this molecular modification leads to a change in the selectivity of action of the compounds, orciprenaline being non-selective and terbutaline selective for β₂- as opposed to β₁-adrenoceptors. In contrast, with the methanesulphonanilides it is apparent that increasing the size of the amine substituent from N-isopropyl (soterenol) to N-t-butyl (MJ7999-1) increases agonistic activity for β-adrenoceptor mediated effects not only in the soleus muscle and bronchi but also in the heart. Since the increase in activity in the heart is greater than that in the bronchi or the soleus, the change from N-isopropyl to N-t-butyl substitution in the methanesulphonanilide series leads to a decrease in β₂-receptor selectivity.

In addition to the differences in activity produced by the change from N-isopropyl to N-t-butyl substitution in the resorcinols and the methanesulphonanilides, the selectivity of action of the N-isopropyl derivatives differ. Thus on the basis of selectivity ratios (heart:bronchi) orciprenaline is non-selective, whereas soterenol shows a
greater selectivity than any of the compounds studied.

For both series of compounds further increases in the amine substituent from N-t-butyl, terbutaline and MJ7999-1, to N-P-hydroxyphenyl-t-butyl (Me506) or N-1-phenyl-t-butyl (MJ9184-1) respectively, produces no further increases in the potency or selectivity of the compounds for $\beta_2$- as opposed to $\beta_1$-adrenoceptor mediated effects. In this respect, two series of compounds show identical trends.

The results using the six non-catechol $\beta$-adrenoceptor agonists indicate that ring substitution plays an important role in determining the degree of selectivity of non-catechol sympathomimetic amines for actions at $\beta_1$- and $\beta_2$-receptor sites. Optimal $\beta_2$-receptor selectivity can be attained with either an N-isopropyl or N-t-butyl substitution depending on the nature of the ring substituent. In both the resorcinols and the methanesulphonanilides increases in the amine loading greater than N-t-butyl, do not further affect either the selectivity or activity of the compounds as $\beta$-receptor stimulants.

Of the many compounds which have been studied for their bronchodilator and soleus muscle effects in anaesthetized cats, similar dose-ratios with respect to (-)-isoprenaline have been found in the two tissues with salbutamol and isoetharine (Rodger, 1973), rimiterol (Bowman & Rodger, 1972), MJ9184-1 (Gwee et al., 1972), trimetaquinol (Houston & Rodger, 1974), soterenol, MJ7999-1, terbutaline, Me506 (this study). Results such as these led Bowman & Nott (1970) and Bowman & Rodger (1972) to suggest that effects in both tissues were due to activation of a
similar type of $\beta$-receptor. However, evidence that drugs might distinguish between these $\beta$-receptors has recently been given by Kaiser, Schwartz, Colella & Wardell (1975) who showed that relative to isoprenaline, sulfonterol was much more potent in the bronchi than in the soleus muscle. In the present experiments dose-ratios for orciprenaline were also found to be higher for the effects of the drug in the soleus muscle than in the bronchi. However this difference is much smaller than that found for sulfonterol. In addition the present result with orciprenaline disagrees with that reported by Apperley & Daly (1972) who found similar dose-ratios for orciprenaline in the two tissues. The reason for this discrepancy is not known, but may be related to the fact that the latter authors used single doses of the compounds whereas a cumulative dose regime was used in the present experiments. It is of interest that in anaesthetized dogs Brittain (1972) found that orciprenaline distinguished between bronchial and skeletal muscle effects. However in that study Brittain found that orciprenaline showed a selectivity for the skeletal rather than the bronchial actions of the compound.

The ability of drugs to differentiate between $\beta$-receptors in these two tissues is of interest since it may give a lead to the production of compounds which produce bronchodilatation without the troublesome side effect of skeletal muscle tremor.

In determining the selectivity of a compound for $\beta_1$- or $\beta_2$-adrenoceptor mediated actions two main methods can be used. In the first the doses or concentrations of the
drug required to produce a given response in tissues where actions are said to be due to $\beta_1$- or $\beta_2$-receptor stimulation are compared; in the second the actions of a drug in various tissues are compared with an internal standard (e.g., isoprenaline). The apparent selectivities of compounds when assessed by the above procedures frequently differ and a number of points must be considered in choosing one or other of the methods.

In the present experiments molar dose-ratios with respect to (-)-isoprenaline were used in assessing selectivity and structure-activity relationships since in the in vivo situation animal variation generally produces a wider scatter of results in ED$_{50}$ values than in dose-ratios. This can be seen when comparing the standard errors of the mean ED$_{50}$ doses and the mean molar dose-ratios (Table 2). This scatter is in part due to technical difficulties in measuring the three parameters in any one animal and the long duration of action of the compounds which precludes multiple drug assessments in one experiment.

A further difficulty with assessing selectivity on the basis of ED$_{50}$ values is that so many $\beta$-receptor mediated responses are inhibitory in nature. Thus when "tone" within the system must be produced before inhibitory responses can be elicited, ED$_{50}$ values increase as the degree of tone is raised. This fact has been commented on by Bowman & Rodger (1972) with reference to assessing the bronchodilator responses to sympathomimetic amines. In the present experiments changes in the degree of bronchoconstriction produced caused only minor changes in ED$_{50}$ values. However,
if large variations in bronchoconstriction had been elicited there is no doubt that a considerable variation in ED$_{50}$ could have been obtained.

While the reasons outlined above might well indicate that dose-ratios rather than ED$_{50}$ values would give a better assessment of selectivity, they suffer from one major disadvantage. The perfect internal standard should possess completely non-selective actions itself. (-)-Isoprenaline is generally used as an internal standard, not only because of its "classical" actions as a $\beta$-receptor stimulant, but also because of its use by Lands et al. (1967) when the authors propounded the $\beta_1/\beta_2$-hypothesis. Although in the present experiments mean ED$_{50}$ values for (-)-isoprenaline reduce in the rank order heart:soleus:bronchi, the differences in the ED$_{50}$ values in these parameters are small compared to the other compounds tested. Therefore, (-)-isoprenaline appears to be relatively non-selective in its actions. However the criteria by which a drug might be classified as non-selective suffer from the deficiencies outlined above with respect to induced "tone" when inhibitory responses are elicited.

When considering the results obtained with the resorcinols and the methanesulphonanilides in the present experiments in the anaesthetized cat, some differences are apparent when relative potencies and selectivities are compared with those in isolated guinea-pig tissues (see Chapter 2).

The results obtained with the resorcinols show that in the cat the order of the $\beta_2$-receptor selectivity is
Me₅₀₆=terbutaline>orciprenaline and in the isolated guinea-pig tissues terbutaline>Me₅₀₆>orciprenaline. In quantitative terms the degree of selectivity of the compounds in guinea-pig tissues was greater than that found in the cat.

In isolated tracheal preparations the three resorcinols had similar activities (Chapter 2) whilst in the cat, orciprenaline is less active than terbutaline or Me₅₀₆ as a bronchodilator. In contrast chronotropic activities of the three compounds in guinea-pig atria are markedly different whilst the differences in the cat are relatively minor. Thus in the guinea-pig changes in selectivity which occur through increasing amine substitution are largely determined by differences in the cardiac actions of the compounds, whereas in the cat, changes in bronchial activity are the most important determinant of selectivity.

Also with the methanesulphonanilides, there is virtually no similarity between the results obtained in the cat and in guinea-pig tissues. In tracheal preparations the order of potency of the compounds as relaxants was soterenol<MJ7999-1<MJ9184-1, while in atria the rank order for positive chronotropic activity was soterenol=MJ7999-1>MJ9184-1. Thus the rank order of β₂-selectivity in guinea-pig tissues is MJ9184-1>MJ7999-1>soterenol. This order is achieved through decreasing atrial and increasing tracheal activity as the size of the amine substituent increases. Cardiac potency for the three methanesulphonanilides in the cat was MJ9184-1=MJ7999-1>soterenol and in bronchial smooth muscle the order was MJ9184-1=MJ7999-1>
soterenol. There is little alteration in the bronchodilator activity of the three methanesulphonanilides compared with the changes found in the heart. Thus soterenol displays the greatest $\beta_2$-receptor selectivity of the compounds tested due to its weak cardiac potency.

Reflex increases in heart rate do not appear to be a cause of the differences found between the isolated guinea-pig preparations and the anaesthetized cat, since potency ratios and $ED_{50}$ values are not different when performed in the presence of adrenergic neurone blockade (Gwee et al., 1972).

In clinical practice selective $\beta$-adrenoceptor agonists may produce bronchodilatation as opposed to cardiac stimulation through their selectivity of action at the receptor level. However separation of bronchial and skeletal muscle effects is more difficult since both actions appear to be mediated by $\beta_2$-adrenoceptors. Selectivity for the bronchial actions of the compounds may be further enhanced when the drugs are given by aerosol rather than oral administration (Schumann & Herxheimer, 1971; Watson & Richens, 1974) since lower doses can be employed and the drugs are given directly to the target organ. However evidence has now accumulated which suggests that most of an aerosol dose is swallowed (see Evans, Shenfield & Paterson, 1974, for references) so that some of the drug will enter the general circulation regardless of its route of administration. In this study, in order to investigate the relative selectivities of the sympathomimetic amines at the receptor level comparisons were made using intravenous
injections of the compounds.

The results from this study show that orciprenaline has very little selectivity in the cat. On this basis increase in heart rate would be predicted as the major side effect for orciprenaline in man, and this is confirmed by the results reported by McEvoy, Vall-Spinosa & Paterson (1973) who showed that in man orciprenaline has little selectivity for bronchial as opposed to cardiac effects when given intravenously.

From the results presented in this Chapter the predicted major side effect for terbutaline and Me506 when used as bronchodilators would be skeletal muscle tremor since the soleus and pulmonary resistance ratios are similar and less than those found in the heart. As yet there have been no clinical reports on Me506, but for terbutaline tremor has been reported as the major side effect in a number of studies (Arner, Berther, Karlefor & Westling, 1970; Legge, Gaddie & Palmer, 1971; Simonsson, Svedenblad, Axelsson & Anderson, 1974).

Further discussion on the species difference with respect to selectivity of β-adrenoceptor stimulants is presented in Chapter 7.
CHAPTER 6:

EFFECTS OF CLENBUTEROL (NAB365) ON THE β-ADRENOCEPTORS FOUND IN ISOLATED GUINEA-PIG ATRIAL AND TRACHEAL PREPARATIONS AND IN THE ANAESTHETIZED CAT
SUMMARY

1. The β-adrenoceptor agonistic and possible antagonistic actions of clenbuterol have been investigated in isolated guinea-pig atrial and tracheal preparations and on heart rate, soleus muscle contractility and pulmonary resistance in anaesthetized cats. The effects of clenbuterol have been compared to those produced by (-)-isoprenaline.

2. In both isolated guinea-pig preparations clenbuterol produced agonistic actions at concentrations similar to those found for (-)-isoprenaline. In both preparations clenbuterol was a partial agonist. Lower values for intrinsic activity were found in atrial than tracheal preparations.

3. Competitive β-adrenoceptor antagonistic actions to the effects of (-)-isoprenaline were present in both atrial and tracheal preparations, the pA₂ values being 7.03 and 6.90, respectively.

4. In anaesthetized cats, clenbuterol produced similar maximal responses to those found with (-)-isoprenaline in all three preparations. Calculations of dose-ratios on a molar basis showed that clenbuterol was 34.9, 18.2 and 17.8 times less active than (-)-isoprenaline on heart rate, soleus muscle
contractility and pulmonary resistance, respectively.

5. There was no evidence of \( \beta \)-adrenoceptor antagonistic actions when clenbuterol was either infused or given as large bolus injections in the anaesthetized cat.

6. The results demonstrate the difference in species responsiveness to clenbuterol's agonistic actions and that antagonistic actions can be shown in acute experiments in isolated guinea-pig preparations but not in anaesthetized cats.
1. **INTRODUCTION**

In 1972, Keck, Krüger, Noll & Machleidt reported on the synthesis of a number of compounds which contained a 4-amino-3,5-dichloro ring substitution. Engelhardt (1972) investigated the pharmacology of a number of compounds in the series and found that the N-t-butyl substituted analog, clenbuterol (NAB365) (Fig. 1), was the most potent in antagonizing acetylcholine-induced bronchoconstriction in anaesthetized guinea-pigs. In this respect it was equipotent with salbutamol. In addition Engelhardt (1972) showed that in anaesthetized cats clenbuterol was relatively potent as a vasodepressor agent but possessed little cardiac stimulant activity. In this study clenbuterol was also reported to exhibit selective β-adrenoceptor antagonism since it reduced isoprenaline-induced effects on heart rate but appeared to show no such blocking activity against the vasodepressor actions of the amine.

A number of clinical reports on clenbuterol have been published (Curti, 1974; Nolte, Ulmer & Krieger, 1974; Uhl, 1974; Salorinne, Stenius, Tukiainen & Poppius, 1975). In
the paper by Curti (1974) a 15-42 day trial on clenbuterol was undertaken and the results showed that there was no tachyphylaxis to its bronchodilator effects over this period. Although Curti (1974) reported on the lack of side-effects of clenbuterol, Salorinne et al. (1975) using half the dose that Curti utilized, found that palpitations and tremor were the main side-effects. These occurred at about the same frequency as those reported for salbutamol. These clinical studies showed that clenbuterol was a potent bronchodilator which had some selectivity for $\beta$-adrenoceptors in man.

From a structural viewpoint clenbuterol is a non-catechol $\beta$-adrenoceptor agonist which on the basis of the results in Chapter 2 would be expected to possess $\beta$-adrenoceptor antagonistic actions in isolated guinea-pig atrial and tracheal preparations. In the present Chapter the agonistic and possible antagonistic actions of clenbuterol have been investigated in isolated guinea-pig tracheal and atrial preparations. These results showed it to be a potent $\beta$-adrenoceptor antagonist and on this basis the study was enlarged. The agonistic actions of clenbuterol were assessed on heart rate, soleus muscle contractility and pulmonary resistance of anaesthetized cats. Engelhardt (1972) showed that clenbuterol antagonized the cardiac stimulant actions of isoprenaline in the cat. It was therefore decided to quantify possible $\beta$-receptor blocking actions in the cat heart ($\beta_1$) and soleus muscle ($\beta_2$).
2. METHODS

2.1. Guinea-pig isolated atrial and tracheal preparations

The methods used in this Chapter to assess the β-adrenoceptor agonistic and antagonistic actions of clenbuterol in isolated guinea-pig atrial and tracheal preparations have already been described in Chapter 2.

2.2. Anaesthetized cat preparations: agonistic actions

The agonistic actions of clenbuterol and (-)-isoprenaline at β-adrenoceptor sites in the anaesthetized cat were assessed using the methods outlined in Chapter 5.

2.3. Anaesthetized cat preparations: antagonistic actions

In these experiments heart rate and soleus muscle contractility were recorded simultaneously as described in Section 2.2.1. and 2.2.2. of Chapter 5. After control cumulative dose-response curves to (-)-isoprenaline had been established, clenbuterol was infused intravenously at a rate of 0.1 μg kg⁻¹ min⁻¹ in a volume of 0.1 ml min⁻¹ using a Harvard 944 slow infusion pump. Cumulative dose-response curves to (-)-isoprenaline on both parameters were superimposed at varying times during the clenbuterol infusion. In further experiments large bolus doses of clenbuterol were administered intravenously and the effects on heart rate monitored (see Results section for further details).

2.4. Drugs used

The drugs used were α-chloralose and carbachol chloride (British Drug Houses), pentobarbitone sodium (Abbot),
gallamine triethiodide (May & Baker), propranolol hydrochloride (Imperial Chemical Industries), (-)-isoprenaline bitartrate (Wyeth), clenbuterol (NAB365; Boehringer-Ingelheim) and serotonin creatinine sulphate (Koch-Light). Doses in the text refer to the compounds in terms of the base substance. Stock solutions of (-)-isoprenaline were made up in N/100 HCl and diluted with normal saline (in vivo experiments) or Krebs-Henseleit solution (in vitro experiments) containing ascorbic acid (20 µg ml⁻¹). Since clenbuterol is unstable to light, fresh stock solutions were prepared daily in distilled water and these, together with solutions diluted with either normal saline or Krebs solution, were protected from light during the course of the experiments.
3. RESULTS

3.1. Guinea-pig atrial preparations

Clenbuterol produced positive chronotropic actions in spontaneously beating guinea-pig atrial preparations which were antagonized by the addition of propranolol (10^{-6} M) to the bathing solution. In 4 out of 5 experiments clenbuterol had an intrinsic activity (\(a; (-)-isoprenaline = 1\)) which ranged from 0.13 to 0.36. In the remaining experiment, clenbuterol exhibited no agonistic activity at concentrations up to 10^{-6} M. Activity ratios (clenbuterol EC_{50}:(-)-isoprenaline EC_{50}) showed that clenbuterol ranged from 2.5 times more active to 10.4 times less active than (-)-isoprenaline. The mean pD_{2} values for clenbuterol and (-)-isoprenaline and mean values for intrinsic activity and activity ratios are shown in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>(-)-ISO</th>
<th>CLEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATRIA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\alpha)</td>
<td>8.3 (0.1)</td>
<td>8.1 (0.3)</td>
</tr>
<tr>
<td>Activity ratio</td>
<td>1.00</td>
<td>0.25 (0.06)</td>
</tr>
<tr>
<td>TRACHEA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\alpha)</td>
<td>7.8 (0.2)</td>
<td>7.6 (0.2)</td>
</tr>
<tr>
<td>Activity ratio</td>
<td>1.00</td>
<td>0.40 (0.10)</td>
</tr>
</tbody>
</table>

*pd2 values are defined as the negative logarithm of the concentration of the drug required to produce 50% of its own maximum response (EC_{50}).

Activity ratios are defined as the ratios clenbuterol EC_{50}:(-)-isoprenaline EC_{50} determined on a molar basis.
When concentrations of clenbuterol greater than $2 \times 10^{-7}$ M were left in contact with the atria for 45 min antagonism to the effects of a superimposed cumulative concentration-effect curve to (-)-isoprenaline were observed. The results from one experiment are shown in Fig. 2a. The (-)-isoprenaline curves were shifted to the right in a parallel fashion, with little effect on $E_{\text{max}}$. The shifts in the (-)-isoprenaline curves were assessed using the method of Arunlakshana & Schild (1959). This analysis showed that the antagonism was of a competitive type since the mean slope of a plot of $\log$ (dose-ratio-1) vs. $\log$ (molar clenbuterol concentration) was 1.01 (s.e.m. = 0.03, n = 10). The mean $\text{pA}_2$ values and slope values are shown in Table 2.

### Table 2. Mean $\text{pA}_2$ values and values of the slope of the relationship $\log$ (dose-ratio-1) vs. $\log$ (molar clenbuterol concentration) obtained for clenbuterol using (-)-isoprenaline as the agonist on isolated guinea-pig atrial and tracheal preparations. Numbers in parentheses are the standard errors of the mean from at least four comparisons on each preparation.

<table>
<thead>
<tr>
<th></th>
<th>ATRIA</th>
<th>TRACHEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{pA}_2$</td>
<td>7.03 (0.06)</td>
<td>6.90 (0.05)</td>
</tr>
<tr>
<td>Slope</td>
<td>1.01 (0.03)</td>
<td>1.00 (0.17)</td>
</tr>
</tbody>
</table>

### 3.2. Guinea-pig tracheal preparations

Clenbuterol produced a concentration-dependent relaxation of carbachol-induced tone in isolated guinea-pig tracheal preparations. These relaxations were antagonized by the prior addition of propranolol ($10^{-6}$ M). The value for
intrinsic activity of clenbuterol ranged from 0.26 - 0.70
\((-\text{-isoprenaline} = 0.1\). Activity ratios determined in the
same way as in the preceding section indicated that
clenbuterol activity ranged from 1.2 times more active to 8
times less active than \((-\text{-isoprenaline}. Mean values for
activity ratios, intrinsic activity and \(pD_2\) values are shown
in Table 1.

As in atrial preparations, concentrations of
clenbuterol greater than \(2 \times 10^{-7}\) M antagonized the relaxant
effects of \(-\text{-isoprenaline. The antagonism after 45 min
incubation with the tissue was of a competitive type, since
analysis of the shifts in the superimposed \(-\text{-isoprenaline
curves using the method of Arunlakshana & Schild gave a mean
slope value of 1.00 (s.e.m. = 0.17, \(n = 4\)). Figure 2b
shows the results from one experiment illustrating the shifts
in the concentration-effect curves to \(-\text{-isoprenaline. The
corresponding mean \(pA_2\) value and value of slope is shown in
Table 2.

3.3. Anaesthetized cats

3.3.1. Heart Rate.

In anaesthetized cats clenbuterol produced increases in
heart rate and decreases in arterial blood pressure which were
antagonized by the administration of 0.5 mg kg\(^{-1}\) propranolol.
With clenbuterol the time to reach maximal response with each
dose was 4-5 times longer than with \(-\text{-isoprenaline. After
maximal responses had been established to clenbuterol,
responses were still near maximum 1-1/2 - 2 h after the
administration of the compound. The clenbuterol and
Fig. 2. Graphical plots from one experiment in guinea-pig atria (2a) and one in trachea (2b) in which the antagonistic actions of clenbuterol were evaluated. Responses are expressed as a percentage of the maximum response to (-)-isoprenaline (●) in the control situation. Shown are the superimposed (-)-isoprenaline concentration-effect curves on both atria and trachea in the presence of $10^{-6}$ M (○) and $5 \times 10^{-6}$ M (□) of clenbuterol.

(-)-isoprenaline dose-response curves were close to parallel and both drugs produced a similar maximal response. Potency ratios calculated on a molar basis at the 50% $E_{\text{max}}$ level
showed that clenbuterol was 34.9 times less potent than (-)-isoprenaline. The \( \text{ED}_{50} \) values (\( \mu g \ \text{kg}^{-1} \)) and molar potency ratios for the drugs are shown in Table 3.

### TABLE 3. Mean molar potency ratios (clenbuterol \( \text{ED}_{50} \): (-)-isoprenaline \( \text{ED}_{50} \)) for the effects produced by clenbuterol on the heart rate, soleus muscle contractility and pulmonary resistance of anaesthetized cats. Also shown are the \( \text{ED}_{50} \) values (\( \mu g \ \text{kg}^{-1} \)) for the two drugs in terms of their bases. Numbers in parentheses are standard errors of the mean taken from at least four comparisons on each parameter.

<table>
<thead>
<tr>
<th></th>
<th>HEART RATE</th>
<th>SOLEUS</th>
<th>PULMONARY RESISTANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clenbuterol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio</td>
<td>34.9 (4.9)</td>
<td>18.2 (2.0)</td>
<td>17.8 (4.6)</td>
</tr>
<tr>
<td>( \text{ED}_{50} )</td>
<td>2.12 (0.36)</td>
<td>0.68 (0.08)</td>
<td>0.51 (0.20)</td>
</tr>
<tr>
<td>(-)-Isoprenaline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{ED}_{50} )</td>
<td>0.047 (0.007)</td>
<td>0.029 (0.003)</td>
<td>0.022 (0.008)</td>
</tr>
</tbody>
</table>

#### 3.3.2. Soleus Muscle Contractility

Clenbuterol produced decreases in the tension and fusion of incomplete tetanic contractions of the cat soleus muscle which were antagonized by the administration of propranolol (0.5 mg kg\(^{-1}\)). As for heart rate effects, clenbuterol was slow in producing \( \beta \)-receptor mediated responses in comparison with (-)-isoprenaline and had an extremely long duration of action. Dose-response curves to both drugs were close to parallel and similar maximal responses were obtained. On a molar basis clenbuterol was 18.2 times less active than (-)-isoprenaline. The \( \text{ED}_{50} \) values and molar potency ratios for clenbuterol and
(-)-isoprenaline are shown in Table 3.

3.3.3. Pulmonary Resistance

Serotonin infusions which produced constant degrees of bronchoconstriction were antagonized in a dose-dependent manner by clenbuterol. The bronchodilating actions of clenbuterol were antagonized by propranolol (0.5 mg kg\(^{-1}\), i.v.). As for the other two parameters measured, clenbuterol had a slower rate of action than (-)-isoprenaline and a long duration of action. Both (-)-isoprenaline and clenbuterol produced complete abolition of the bronchoconstrictor effects of serotonin and the slopes of dose-response curves for the two drugs were close to parallel. On a molar basis clenbuterol was 17.8 times less potent than (-)-isoprenaline. ED\(_{50}\) values (\(\mu g\ kg^{-1}\)) and potency ratios are indicated in Table 3.

3.3.4. Selectivity of Clenbuterol in Cats

Using the Student's t-test it was found that the molar potency ratios for clenbuterol on soleus muscle contractility and pulmonary resistance were significantly different from that found on heart rate (0.01<P<0.02, 6 d.f. and 0.02<P<0.05, 6 d.f.). However there was no significant difference between potency ratios for soleus muscle and pulmonary effects (P>0.9, 6 d.f.). If selectivity is taken as the value of heart rate molar dose-ratio:pulmonary resistance molar dose-ratio then clenbuterol has a selectivity factor of 1.95.
3.3.5. Antagonistic Actions of Clenbuterol

The infusion rate of 0.1 μg kg\(^{-1}\) min\(^{-1}\) was selected since bolus doses of 0.1 μg kg\(^{-1}\) of clenbuterol produced increases in heart rate and depressions in soleus muscle contractility of less than 10% of the maximal response to (-)-isoprenaline. It was hoped that by using an infusion high enough doses could be reached so that the stimulant effects of clenbuterol would be minimized and that antagonism to superimposed (-)-isoprenaline dose-response curves could be observed in the same way as antagonistic actions were assessed in isolated guinea-pig tracheal preparations (see Chapter 2). In three experiments the infusion of clenbuterol at 0.1 μg kg\(^{-1}\) min\(^{-1}\) produced a slow and progressive increase in heart rate and depression of soleus muscle contractility. There was no evidence of a waning of these stimulant effects and consequently after 1-1/2 - 2 h of infusion maximal responses similar to those produced by (-)-isoprenaline in the control situation were obtained on both tissues. Lower infusion rates were not used since it was thought that high enough plasma concentrations of clenbuterol would not be achieved. Higher infusion rates would presumably have only accelerated the attainment of maximal responses on the two tissues.

Nevertheless in the three experiments mentioned, (-)-isoprenaline curves were obtained at varying times from 30 min to 70 min after the commencement of the clenbuterol infusion. Times at which (-)-isoprenaline curves could be superimposed were variable since the time before another curve could be obtained depended on the rate of recovery of
the tissues from the effects of (-)-isoprenaline. The superimposed (-)-isoprenaline curves showed no evidence of any antagonism by the clenbuterol infusion when the background stimulant activity of the infused drug was taken into account in the plotting of the curves.

In terms of pA₂ values in isolated guinea-pig atria, clenbuterol with a value of 7 is more potent than dichloro-isoprenaline (DCI) (Pratesi, Grana & Villa, 1968). Powell & Slater (1958) and Bowman, Goldberg & Raper (1962) have shown that injection of DCI in mg kg⁻¹ dose ranges produces initial sympathomimetic effects which then wane over a short period of time, responses returning to control levels. Responses to injections of isoprenaline or adrenaline are antagonized at this time. It was thus decided to administer large doses of clenbuterol (5 mg kg⁻¹, i.v.) and to observe if there was any waning of the initial sympathomimetic effect. However, after periods of up to 1 - 1-1/2 h after injection, maximal sympathomimetic effects on heart and soleus muscle contractility were still present there being no evidence of the response returning towards control values.
4. DISCUSSION

O'Donnell (1975) investigated the actions of clenbuterol on the isolated guinea-pig trachea, uterus, ileum, atria and isolated perfused hind-limb. In that study it was found that clenbuterol was similar in activity on the uterus, hind-limb and carbachol-stimulated trachea and at least 100 times less active on guinea-pig atria and ileum.

The results presented in this Chapter are in accord with O'Donnell's finding for the trachea but are at variance when guinea-pig atrial results are compared. There appears to be no clear-cut explanation for this discrepancy; however, the variable intrinsic activity exhibited by clenbuterol in this study may be one reason. O'Donnell (personal communication) found that clenbuterol had a higher intrinsic activity than in the experiments reported in this Chapter. By virtue of the strong dependence of the calculated activity-ratio (as defined in this Chapter) on the magnitude of the intrinsic activity, a low value for the latter would make the compound more active in relation to (-)-isoprenaline. Indeed in some of the present experiments the intrinsic activity is so low that it is dubious whether activity-ratios should be calculated at all.

Whereas clenbuterol shows no selectivity for tracheal as opposed to atrial preparations from the guinea-pig on the basis of activity-ratios, it does show selectivity for $\beta_2$- as opposed to $\beta_1$-adrenoceptor mediated responses in the anaesthetized cat. The selectivity ratio of 1.95 for clenbuterol indicates that it is slightly less selective than
terbutaline, MJ7999-1 and salbutamol (see Chapter 5 and Rodger (1973), respectively). In this regard Salorinne et al. (1975) reported that salbutamol and clenbuterol had almost the same profile of side-effects in man. In terms of the molar dose-ratios on the 3 parameters, clenbuterol is approximately equipotent with terbutaline but is less potent than MJ7999-1 and salbutamol. Thus compounds with the same N-alkyl substitution, i.e., N-t-butyl, appear to show selectivity ratios of approximately 2 to 3 although the potencies with respect to (–)-isoprenaline are variable. Possibly in the cat, the N-t-butyl substituent will predispose a drug to have a selectivity ratio of 2 to 3 whilst the nature of the ring substitution will determine how potent the compound will be relative to (–)-isoprenaline.

Clenbuterol exhibited relatively strong β-adrenoceptor antagonistic actions in isolated guinea-pig preparations. The similar pA2 values found in atrial and tracheal preparations show that it has no selectivity in its β-receptor blocking profile. The pA2 value of around 7 is similar to or greater than that seen with INPEA and sotalol on both tissues and practolol in atrial preparations (Farmer & Levy, 1970; Buckner & Patil, 1971; Horii, Kawada, Takeda & Imai, 1974).

The results of the present experiments performed in the anaesthetized cat are at variance with those previously reported by Engelhardt (1972). He found that clenbuterol was a partial agonist in the cat and exhibited antagonistic actions to the tachycardia produced by single doses of isoprenaline. In the present experiments clenbuterol
exhibited full agonistic actions compared to (-)-isoprenaline and did not show any antagonistic actions. The methodologies used in these two reports differ, but with the lack of information supplied by Engelhardt in his Method section with respect to doses used to assess both of these actions, little can be concluded as to the reasons for the differences. In this Chapter where full dose-response curves to (-)-isoprenaline were obtained in the absence and presence of an infusion of clenbuterol there was no evidence of any antagonistic actions. Furthermore large single bolus injections produced maximal effects on both heart rate and soleus muscle contractility, but unlike DCI these responses remained maximal for 1-1-1/2 hours.

If indeed clenbuterol can act as an antagonist in the cat, this cannot be demonstrated in an acute experiment. Possibly chronic treatment with clenbuterol may be needed to show any β-adrenoceptor antagonism.

In conclusion, clenbuterol produced both agonistic and antagonistic actions in isolated guinea-pig preparations, but only agonistic actions could be demonstrated in the anaesthetized cat. Thus again there appears to be marked differences between the cat and guinea-pig in their responsiveness to selective β2-adrenoceptor agents.
CHAPTER 7:

SURVEY OF SOME SPECIES DIFFERENCES WITH RESPECT TO $\beta$-ADRENOCEPTOR AGONISTS
The comparisons of relative potency and $\beta_1/\beta_2$-receptor selectivity made using the six non-catechol sympathomimetic compounds in isolated tissue preparations from the guinea-pig and in the anaesthetized cat (Chapter 5, Discussion) indicate marked differences in drug response between the two species. In addition the more detailed study using MJ9184-1 (Chapter 4) and the results from published material on salbutamol, trimetaquinol, rimiterol, terbutaline and Sm220-Cl confirm this species difference (see Table 1). Although these comparisons have been made between *in vitro* guinea-pig and *in vivo* cat results, differences although less marked, still exist when results

| TABLE I. Selectivity ratios [molar dose-ratio(heart):molar dose-ratio (lung)] for a number of $\beta$-adrenoceptor agonists in cats *in vivo* and guinea-pigs *in vivo* and *in vitro*. Values greater than unity indicate selective $\beta_2$-receptor stimulation. |
|---|---|---|
| CAT | GUINEA-PIG |
| *in vivo* | *in vivo* | *in vitro* |
| Salbutamol | 2.9$^4$ | 21.7$^3$ | 85$^2$ |
| Terbutaline | 2.3$^8$ | 9.0$^3$ | 66$^9$ |
| MJ9184-1 | 2.9$^{26}$ | 13.3$^1$ | 104$^1$ |
| Sm220-Cl | 1.0$^7$ | 52.6$^7$ | 22$^7$ |
| Rimiterol | 2.4$^5$ | - | 667$^6$ |
| Trimetaquinol | 1.0$^{10}$ | - | 50-100$^{10}$ |

In this and the following Tables in this Chapter references to various authors are indicated as superscripts. A key to these numbers is given at the end of the Chapter. In all cases where molar dose-ratios are quoted values have been given in terms of (t)-isoprenaline (= 1). (−)-Isoprenaline has been assumed to have twice the potency of the racemate. The molar dose-ratios used to evaluate the selectivity ratio were obtained by dividing the drug ED$_{50}$ by the isoprenaline ED$_{50}$.

from anaesthetized guinea-pigs and cats are compared (see Table 1). The reason for the difference between the
in vivo and in vitro guinea-pig results is probably due to the presence of operational cardiovascular reflexes in the former situation.

Reflex increases in heart rate produced by the vasodepressor actions of sympathomimetic amines do not appear to be a complicating factor in the positive chronotropic actions of the drugs in chloralose-anaesthetized cats (Bowman & Rodger, 1972; Gwee, Nott, Raper & Rodger, 1972; Rodger, 1973; Housten & Rodger, 1974). In fact molar dose-ratios for the positive chronotropic effects of adrenaline, MJ9184-1, salbutamol, Sm220-Cl and trimetaquinol are similar both in vivo and in vitro in the cat (Table 2).

TABLE 2. Molar dose-ratios for the positive chronotropic effects in anaesthetized cats and in isolated cat atrial experiments, calculated relative to the (±)-form of isoprenaline (= 1). The dose-ratios in this and the following Tables were calculated by dividing Drug ED$_{50}$ by Isoprenaline ED$_{50}$.

<table>
<thead>
<tr>
<th></th>
<th>in vivo</th>
<th>in vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenaline</td>
<td>8.8$^{11}$</td>
<td>10.3$^{12}$</td>
</tr>
<tr>
<td>MJ9184-1</td>
<td>1.9$^{26}$</td>
<td>1.7$^{1}$</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>8.8$^{4}$</td>
<td>18.4$^{12}$</td>
</tr>
<tr>
<td>Sm220-Cl</td>
<td>1.1$^{7}$</td>
<td>1.0$^{7}$</td>
</tr>
<tr>
<td>Trimetaquinol</td>
<td>0.84$^{10}$</td>
<td>0.71$^{10}$</td>
</tr>
</tbody>
</table>

The observations that there are differences in the responsiveness of cat and guinea-pig preparations to $\beta_2$-receptor selective sympathomimetic amines is not restricted to these two species.

Table 3 shows molar dose-ratios ((±)-isoprenaline = 1)
for a number of agonists for their cardiac actions in different species. An indication of whether the drug produces a lower Emax than isoprenaline is also given.

The results show that in general, the cardiac chronotropic responses to selective sympathomimetic agents tend to possess similar characteristics with regard to responsiveness in the guinea-pig, dog, rat and rabbit. These characteristics are that the bronchodilators in relation to isoprenaline, are either weak agonists and/or partial agonists in cardiac preparations. In isolated cat atrial preparations and in the anaesthetized cat the selective sympathomimetic amines are more active than in other species and produce the same maximal response as isoprenaline. These points are best illustrated with the salbutamol ratios shown in Table 3. In addition differences between cat and guinea-pig isolated atrial preparations in terms of intrinsic activity and potency have also been reported for trimetaquinol and Sm220-C1 (Housten & Rodger, 1974; Bohmer, 1975).

In anaesthetized dogs it would appear that the cardiac chronotropic responses resemble those found for the selective bronchodilator agents in guinea-pig preparations in that salbutamol and terbutaline are generally weak agonists (see Table 3 for references). However weak positive chronotropic activity for salbutamol is not observed by all investigators as shown by the ratios reported by Wardell, Colella, Shetzline et al. (1974) and Kelly & Shanks (1975).
TABLE 3. Molar dose-ratios (with respect to (±)-isoprenaline = 1) for the myocardial actions of a number of β-adrenoceptor agonists in different species.

<table>
<thead>
<tr>
<th></th>
<th>SALBUTAMOL</th>
<th>SOTERENOL</th>
<th>ORCIPRENALINE</th>
<th>RIMITEROL</th>
<th>MJ9184-1</th>
<th>TERTUTALINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea-pig atria (chronotropic)</td>
<td>160(^2), 247(^{16})</td>
<td>26.8(^2), 6.6(^3)</td>
<td>125(^{13}), 83(^9)</td>
<td>250(^6)</td>
<td>270(^1)</td>
<td>991(^9)</td>
</tr>
<tr>
<td>Rat atria (chronotropic)</td>
<td>57(^{13})</td>
<td>&gt;10,000(^{13})</td>
<td>24(^{13})</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rabbit atria (chronotropic)</td>
<td>2100(^{14})</td>
<td>-</td>
<td>-</td>
<td>790(^{14})</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dog papillary muscle (inotropic)</td>
<td>5000(^{15})</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cat atria (chronotropic)</td>
<td>18.4(^{12})</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.7(^{1})</td>
<td>-</td>
</tr>
<tr>
<td>Anaesthetized dog (heart rate)</td>
<td>100(^{15}), 575(^{16})</td>
<td>10(^{16}), 40(^{17})</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100(^{18})</td>
</tr>
<tr>
<td>Anaesthetized cat (heart rate)</td>
<td>10(^{4})</td>
<td>12.5(^8)</td>
<td>23.7(^8)</td>
<td>19(^5)</td>
<td>1.9(^{26})</td>
<td>19.5(^{8})</td>
</tr>
</tbody>
</table>

*Partial agonist when full dose-response curves have been established.
Kelly & Shanks (1975) reasoned that the difference between their results and other published values (e.g., Kofi Ekue, Shanks & Zaidi, 1971) with regard to the relative potency of salbutamol, was due to the fact that they used infusions rather than single doses and obtained maximal responses to the two drugs. However Wardell et al. (1974) used single doses and although they did not obtain maximal responses, the relative potency ratio was calculated from the straight line portions of parallel dose-response curves. In addition Hughson & Ledsome (1975) used infusions of the two drugs and from the straight line portions of parallel dose-response curves found that salbutamol was a weak agonist. Thus the method of intravenous administration either by single dose or infusion does not appear to be responsible for the differences found in the salbutamol potency ratios by various workers.

Daly, Farmer & Levy (1971) showed the marked effects that barbiturate anaesthesia had on the level of activity of cardiovascular reflexes and hence the relative potency of salbutamol. However the choice of anaesthetic cannot explain the wide ranging potency ratios seen with salbutamol since all groups but one used pentobarbitone sodium. Hughson & Ledsome (1975) used chloralose but observed similar potency ratios for salbutamol to those obtained under barbiturate anaesthesia.

Thus the methodologies cannot account for the range in salbutamol potencies for positive chronotropic actions observed by various workers in the anaesthetized dog. The reason for the divergent results is unknown.
In contrast to the marked species differences found for the cardiac activities of the non-catecholamine $\beta$-receptor agonists, their relaxant effects on smooth muscle within the respiratory tract are remarkably similar (Table 4). In bronchial tissues selective $\beta$-receptor amines produce similar maximal responses to those produced by isoprenaline and have relatively high potencies. In addition human isolated bronchial muscle and measurements \textit{in vivo} show similar potency ratios to those seen in the various species.

It would thus appear that the difference between species lies not so much in alterations of bronchodilator activity, but in the wide ranging variability of the cardiac chronotropic response. Thus the $\beta_2$-receptor selectivity found in different species depends mainly on the myocardial response evoked by the non-catechol phenylethanolamines. The species differences in cardiac responses may be due to (i) the presence of different $\beta$-isoreceptors (Brittain, Jack & Ritchie, 1970) in the myocardium of various species, or (ii) that binding characteristics of the drugs to the cardiac $\beta$-receptors are similar, but differences arise through variation in the efficacies of the drug-receptor complexes to produce the measured response (an extrapolation of the hypothesis presented in Chapter 2), or (iii) a combination of the previous two alternatives, or (iv) differences arising through variations in the proportions of $\beta_1$- and $\beta_2$-receptors in the sino-atrial nodes of different species as has been postulated in the cat and
TABLE 4. Molar dose-ratios (with respect to (+)-isoprenaline = 1) for bronchial smooth muscle relaxant effects of a number of β-adrenoceptor agonists in different species.

<table>
<thead>
<tr>
<th>Species</th>
<th>SALBUTAMOL</th>
<th>SOTERENOL</th>
<th>ORCIPRENALINE</th>
<th>RIMITEROL</th>
<th>MJ9184-1</th>
<th>CARBUTEROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea-pig</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in vivo</td>
<td>4.8&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2&lt;sup&gt;13&lt;/sup&gt;</td>
<td>40&lt;sup&gt;13&lt;/sup&gt;</td>
<td>5&lt;sup&gt;6&lt;/sup&gt;</td>
<td>0.92&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;19&lt;/sup&gt;</td>
</tr>
<tr>
<td>in vitro</td>
<td>1.6&lt;sup&gt;2&lt;/sup&gt;</td>
<td>5&lt;sup&gt;13&lt;/sup&gt;</td>
<td>15&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2&lt;sup&gt;6&lt;/sup&gt;</td>
<td>0.26&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;19&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dog</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in vivo</td>
<td>12.5&lt;sup&gt;16&lt;/sup&gt;</td>
<td>0.67&lt;sup&gt;24&lt;/sup&gt;</td>
<td>18&lt;sup&gt;16&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>5.2&lt;sup&gt;19&lt;/sup&gt;</td>
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<td>Cat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in vivo</td>
<td>3.4&lt;sup&gt;4&lt;/sup&gt;</td>
<td>2.4&lt;sup&gt;8&lt;/sup&gt;</td>
<td>21.5&lt;sup&gt;8&lt;/sup&gt;</td>
<td>4&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.65&lt;sup&gt;9&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;19&lt;/sup&gt;</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in vivo</td>
<td>5&lt;sup&gt;22&lt;/sup&gt;</td>
<td>-</td>
<td>23&lt;sup&gt;21&lt;/sup&gt;</td>
<td>7&lt;sup&gt;22&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>in vitro</td>
<td>7&lt;sup&gt;23&lt;/sup&gt;</td>
<td>-</td>
<td>16&lt;sup&gt;23&lt;/sup&gt;</td>
<td>5&lt;sup&gt;23&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
man (Åblad, Borg, Carlsson et al., 1975).

When selective sympathomimetic amines are administered to humans, the selectivity of action at the $\beta$-adrenoceptor level is small (see Table 5). Previously it has been suggested that the cat provides a suitable animal model to test the selectivity of action of sympathomimetic agents (Rodger, 1973; Housten & Rodger, 1974). Table 5 illustrates the similarity in relative potency and selectivity for bronchial effects as opposed to heart rate effects in both cat and man. In addition to the similar

<table>
<thead>
<tr>
<th></th>
<th>BRONCHI</th>
<th>HEART RATE</th>
<th>SELECTIVITY RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salbutamol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat$^4$</td>
<td>3.0</td>
<td>8.8</td>
<td>2.9</td>
</tr>
<tr>
<td>Man$^{22}$</td>
<td>5</td>
<td>10</td>
<td>2.0</td>
</tr>
<tr>
<td>Isoetharine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat$^4$</td>
<td>4.4</td>
<td>10.8</td>
<td>2.4</td>
</tr>
<tr>
<td>Man$^{25}$</td>
<td>3.9*</td>
<td>10.6</td>
<td>2.7</td>
</tr>
<tr>
<td>Orciprenaline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat$^8$</td>
<td>21.5</td>
<td>23.6</td>
<td>1.0</td>
</tr>
<tr>
<td>Man$^{21}$</td>
<td>23</td>
<td>14</td>
<td>0.6</td>
</tr>
<tr>
<td>Rimiterol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat$^5$</td>
<td>4</td>
<td>9.5</td>
<td>2.4</td>
</tr>
<tr>
<td>Man$^{22}$</td>
<td>7</td>
<td>14</td>
<td>2.0</td>
</tr>
</tbody>
</table>

*Peripheral blood flow which is approximately equal to bronchodilatation (Collier & Dornhorst, 1969)

relative potency ratios for bronchodilator and cardiac actions in cat and man, the effects produced by $\beta$-adrenoceptor agonists are similar in human skeletal
muscle and the slow contracting skeletal muscles of the cat (Marsden & Meadows, 1970; Bowman & Nott, 1970; Bowman & Rodger, 1972). Furthermore when selective sympathomimetic amines are given orally to man or intravenously to cat and man, bronchodilatation cannot be achieved without concurrent effects on the skeletal muscle systems in both species (Rodger, 1973; Watson & Richens, 1974; Marlin & Turner, 1975; Chapter 5 of this Thesis). A similar situation also appears to occur with the guinea-pig soleus muscle (Apperley & Levy, 1975; Bohmer, 1975).

In conclusion, on the basis of relative potencies and the degree of \( \beta_2 \)-receptor selectivity the cat appears to be the best animal model to be able to predict the actions of new sympathomimetics in man. However this conclusion does not necessarily apply to the bronchodilators when they are prescribed in practice. The degree of "selectivity" obtained will depend on the dosage of the drug and how it is administered. If the drug is given by aerosol then the selectivity can be further increased since the drug is administered to the target organ. The dose-response curves illustrated in Fig. 1 represent the bronchodilator (left) and heart rate (right) effects and are drawn to give a selectivity of 2 at the ED_{50} levels. However if dose A is given then the selectivity is 5 although the same degree of bronchodilator activity is present as before. Single dose administration of drug concentrations greater than A will result in more bronchodilatation with an
accompanying increase in heart rate. Alternatively the use of a concentration less than A will result in only a decreased bronchodilator activity. Thus the choice of dose and the route of administration can greatly affect the clinical selectivity of the drug.
### KEY TO TABLES IN THIS CHAPTER

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Chapter 4 of this Thesis</td>
<td>2.</td>
</tr>
<tr>
<td>7.</td>
<td>Bohmer &amp; Raper, personal communication</td>
<td>8.</td>
</tr>
</tbody>
</table>
CHAPTER 8:

β-ADRENOCEPTORS INVOLVED IN THE INHIBITION OF HISTAMINE RELEASE FROM SENSITIZED GUINEA-PIG LUNG
SUMMARY

1. Selective β-adrenoceptor agonists and antagonists were used to study the type of β-adrenoceptors involved in inhibiting antigen-induced histamine release from actively sensitized guinea-pig lung.

2. Results obtained with six non-catechol β-adrenergic agonists were compared with those found in guinea-pig atrial (β₁) and tracheal (β₂) preparations. In terms of rank order the relative activities of the compounds differed in the three preparations.

3. Dissociation constants (K_B values) for the cardio-selective antagonist H93/26 were assessed using (-)-isoprenaline as an agonist. The K_B value for inhibition of histamine release was significantly different from, and intermediate between, the K_B values obtained in atria and trachea.

4. In the guinea-pig tissues H35/25 was not a selective β₂-adrenoceptor antagonist; K_B values were not significantly different in the three preparations.

5. The results using the β-adrenoceptor agonists and antagonists suggest that the β-receptors involved in inhibition of antigen-induced histamine release in the guinea-pig lung differ from those found in guinea-pig atria and trachea.
1. **INTRODUCTION**

In 1920 Hanzlik & Karsner demonstrated that adrenaline protected guinea-pigs against anaphylactic shock induced by horse-serum whereas no protection was afforded against anaphylactoid phenomena induced by the injection of various colloids. Using isolated perfused lungs from egg-albumen sensitized guinea-pigs Schild (1936) showed that adrenaline, in addition to producing bronchial smooth muscle relaxation, inhibited the release of histamine induced by antigen challenge.

Since the late 1960's the effects of catecholamines and other β-adrenoceptor stimulants have been investigated in many models of immediate-type anaphylaxis (see Assem, 1974 for references). In addition to preventing histamine release in anaphylaxis, β-adrenoceptor agonists also reduce the antigen-induced increase and spontaneous histamine forming capacity of isolated human leucocytes. However the effects of β-adrenoceptor agonists on histamine formation show different characteristics to those seen when reduction in histamine release is studied (Assem & Feigenbaum, 1974).

In actively sensitized chopped guinea-pig lung stimulation of β-adrenoceptors has been shown to inhibit antigen-induced histamine release (Assem, 1971; Assem, Pickup & Schild, 1970; Assem & Schild, 1971a,b; 1973). Evidence that the inhibition of histamine release in anaphylaxis is mediated via β-adrenoceptors comes from experiments in which the rank order for reduction in histamine release is isoprenaline>adrenaline>noradrenaline, and from
investigations which showed that propranolol antagonized the inhibitory effect of isoprenaline but not that of disodium cromoglycate (Assem & Schild, 1969, 1971a). In attempting to classify the type of $\beta$-adrenoceptor involved in isoprenaline-induced inhibition of histamine release from the guinea-pig lung, Assem & Schild (1971a) used practolol and butoxamine, antagonists which are selective for $\beta_1$- and $\beta_2$-receptors, respectively. The $pA_2$ values found with these two antagonists were similar and the values obtained were extremely high when compared to those found by other workers in guinea-pig atrial and tracheal preparations (Wasserman & Levy, 1970; Buckner & Patil, 1971). In discussing the results obtained in sensitized guinea-pig lung, Assem & Schild (1971a) compared the effects they obtained with butoxamine and practolol with those published by other workers for the \textit{in vivo} effects of the antagonists. They concluded that the $\beta$-adrenoceptors involved in the inhibition of histamine release from isolated sensitized guinea-pig lung differed from those responsible for cardiac chronotropic effects in cats ($\beta_1$) and vasodepression ($\beta_2$) in the dog, but resembled those involved in lipolysis in the intact dog.

The validity of extrapolating results from \textit{in vivo} experiments on other species to \textit{in vitro} results in guinea-pig tissues is questionable (see Chapter 4). It was therefore considered worthwhile to re-examine the type of $\beta$-adrenoceptor involved in the inhibition of histamine release (anti-anaphylactic effect) in the isolated guinea-pig lung, and to compare the results obtained with
those found in guinea-pig atrial and tracheal preparations in vitro.

Six non-catechol β-adrenoceptor agonists (Table 1) which possess varying degrees of selective stimulant activity in guinea-pig atrial and tracheal preparations have been used. In addition the β-adrenoceptor antagonists H93/26 and H35/25 have been used in an attempt to classify the type of receptor involved. These compounds have been reported to be selective β1- and β2-receptor antagonists respectively in other species (Levy, 1967; Levy & Wilkenfeld, 1969; Åblad, Carlsson & Ek, 1973).

**TABLE 1. Structures of the β-adrenoceptor agonists used in this study.**

<table>
<thead>
<tr>
<th>DRUG</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-Isoprenaline</td>
<td>HO</td>
<td>HO</td>
<td>H</td>
<td>CH(CH₃)₂</td>
</tr>
<tr>
<td>Orciprenaline</td>
<td>HO</td>
<td>H</td>
<td>HO</td>
<td>CH(CH₃)₂</td>
</tr>
<tr>
<td>Terbutaline</td>
<td>HO</td>
<td>H</td>
<td>HO</td>
<td>C(CH₃)₃</td>
</tr>
<tr>
<td>Me506</td>
<td>HO</td>
<td>H</td>
<td>HO</td>
<td>C(CH₃)₂CH₂-P-OHCH₆H₄</td>
</tr>
<tr>
<td>Soterenol</td>
<td>H</td>
<td>HO</td>
<td>H₃C₆O₂HN</td>
<td>CH(CH₃)₂</td>
</tr>
<tr>
<td>MJ7999-1</td>
<td>H</td>
<td>HO</td>
<td>H₃C₆O₂HN</td>
<td>C(CH₃)₃</td>
</tr>
<tr>
<td>MJ9184-1</td>
<td>H</td>
<td>HO</td>
<td>H₃C₆O₂HN</td>
<td>C(CH₃)₂CH₂C₆H₅</td>
</tr>
</tbody>
</table>
2. METHODS

2.1. General

Guinea-pigs (150-200 g) were sensitized using spray dried egg albumen suspended in Freund's incomplete adjuvant. The animals received two single injections of 100 mg (i.p.) given two days apart. Two to three weeks after the second injection they were killed by a blow on the head and a tracheal cannula inserted. A small animal respirator was connected and the lungs respired at 35 min⁻¹ with a stroke volume of 4.5 ml. After thoractomy the pulmonary artery was cannulated and blood removed from the lungs by perfusion for 3 to 5 min with Tyrode solution (NaCl, 8.0; KCl, 0.2; MgCl₂, 0.015; CaCl₂, 0.2; NaH₂PO₄, 0.05; NaHCO₃, 1.0; dextrose, 1.0 g l⁻¹) maintained at 37°C. The lungs were then removed and chopped finely with a sharp scalpel. Eight aliquots of chopped lung tissue (≈200 mg wet weight) were obtained from each animal and these were weighed accurately.

2.2. β-Adrenoceptor agonists

Two tissue samples were each suspended in 4 ml of Tyrode solution. One sample was boiled in a water bath for 5 min and was used to estimate total histamine content in the tissue, and the other sample, which was subjected to the same general procedure as that outlined below for the remaining samples, was used to estimate spontaneous histamine release.

The remaining six samples were incubated with Tyrode solution (3 ml) and maintained at 37°C for a period of 5 to 10 min. At the end of this initial incubation period, antigen alone was added to one of the six samples for
estimation of uninhibited histamine release. Each of the other samples received antigen plus varying concentrations of an agonist. In all cases the final incubation volume was 4 ml. Maximum β-receptor-mediated inhibition of histamine release occurred when the sympathomimetic and antigen were added concurrently to the tissue samples. In 4 experiments using (-)-isoprenaline and MJ9184-1, preincubation of the tissue with the amines for periods of up to 60 min before antigen administration did not enhance the inhibition of histamine release above that obtained when drug and antigen were added together. The samples were shaken gently for 15 min at 37°C in a Gallenkamp water bath shaker. Solutions were then filtered through gauze, and the incubation fluid stored on ice for subsequent assay of histamine. Greaves & Mongar (1968) have previously shown that maximal antigen-induced histamine release from sensitized guinea-pig lung occurs within 7-10 min of antigen administration.

2.3. β-Adrenoceptor antagonists

A similar procedure to that described above was used when assessing the effects of β-receptor antagonists. Three samples were used for assessment of total histamine content, spontaneous histamine release, and uninhibited antigen-induced histamine release. Three samples each containing a different concentration of (-)-isoprenaline served as controls. After the initial incubation, a β-receptor antagonist was added in a volume of 0.1 ml to the remaining two samples, and incubation continued for 1 h. Antigen and (-)-isoprenaline were then added to give a final incubation volume of 4 ml. A
different (-)-isoprenaline concentration was present in each sample containing the antagonist.

Mean dissociation constants (K_B values, Furchgott, 1967) were calculated using the dose-ratios obtained from experiments where the effects of (-)-isoprenaline were tested in the presence and absence of various concentrations of the antagonist. Experiments were discarded if the inhibitions of release produced by (-)-isoprenaline in the presence of the antagonist did not fall within the limits of inhibition produced by the control (-)-isoprenaline concentrations.

2.4. Biological assay

The incubation fluids were assayed for histamine in a 2 ml organ bath using terminal guinea-pig ileum suspended in Tyrode solution maintained at 32°C. Contractions were recorded on a smoked drum using an isotonic frontal writing lever. Propranolol (0.1 μg ml⁻¹) was added to the bathing solution to preclude any interference in the histamine assay produced by the β-adrenergic agonists. Further increase in the concentration of propranolol used reduced the sensitivity of the tissue to histamine. Regardless of the concentration of propranolol used, concentrations of >10⁻⁶ M of the agonists interfered with the assay. Two or three estimations of histamine content were made for each sample using a simple bracketing assay. Final results were expressed as μg of histamine released per g wet weight of the lung tissue.
2.5. Guinea-pig atria and trachea

The agonistic and antagonistic actions of the β-adrenoceptor stimulants and blockers were assessed using previously described methods (see Chapter 2). In atrial preparations positive chronotropic activity was assessed. In tracheal preparations tone was induced using carbachol (10^{-6} M) and smooth muscle relaxation used as an indicator of β-receptor stimulant activity. Drugs were administered cumulatively in both preparations. \( K_B \) values for the β-receptor antagonists were calculated from shifts in concentration-effect curves to (-)-isoprenaline.

2.6. Drugs used

(-)-Isoprenaline bitartrate (Wyeth); noradrenaline bitartrate (Koch-Light); phentolamine hydrochloride (Ciba-Geigy); propranolol hydrochloride (Imperial Chemical Industries); H93/26 bitartrate \((±)-1-(\text{isopropylamino})^3-[p-(2\text{-methoxyethyl})\text{phenoxy}]^2-\text{propanol}\), H35/25 hydrochloride \((1-(4'\text{-methylphenyl})-2\text{-isopropylamino}-\text{propanol})\), terbutaline sulphate (Astra); soterenol hydrochloride, MJ7999-1 hydrochloride and MJ9184-1 hydrochloride (Mead Johnson); orciprenaline sulphate, Me506 hydrobromide (Boehringer Ingelheim) and histamine acid phosphate (British Drug Houses). When molar concentrations are not used, concentrations refer to the base.
3. RESULTS

3.1. Characteristics of histamine release

The total histamine content of sensitized guinea-pig lung was found to be \(5.49 \mu g \text{g}^{-1}\) wet weight (s.d. 2.17, \(n = 30\)). Antigen-induced histamine release was 20.4\% (s.d. 3.3, \(n = 30\)) and spontaneous release 3.53\% (s.d. 0.37, \(n = 38\)) of the total histamine content of the tissue. Experiments in which antigen-induced release was less than 10\% of total histamine content were discarded. In preliminary experiments using tissues from 10 animals, varying antigen concentrations (10 \(\mu g \text{ml}^{-1}\) to 25 mg \(\text{ml}^{-1}\)) were tested for their ability to induce histamine release. Maximal release occurred with an antigen concentration \(\geq 100 \mu g \text{ml}^{-1}\). Maximal release was invariably produced when 250 \(\mu g \text{ml}^{-1}\) was used. A similar release was obtained with antigen concentrations up to 10 mg \(\text{ml}^{-1}\), while evidence for autoinhibition was obtained with the highest antigen concentration that was tested (25 mg \(\text{ml}^{-1}\)). In 7 further experiments the greatest percentage inhibition of histamine release produced with \(10^{-7} \text{M} \) (-)-isoprenaline occurred when an antigen concentration of 250 \(\mu g \text{ml}^{-1}\) was used. These results indicated that the use of 250 \(\mu g \text{ml}^{-1}\) of the antigen would provide optimal conditions for the assessment of the anti-anaphylactic actions of the \(\beta\)-agonists. This antigen concentration was therefore used in all experiments. The percentage inhibition of histamine release by the \(\beta\)-adrenoceptor agonists was calculated using the formula of Assem et al. (1970), i.e.,

\[
\text{Uninhibited release - inhibited release} \times 100 \\
\text{uninhibited release - blank release}
\]
3.1.1. α-Adrenoceptors

Propranolol (10^{-6} M) given 1 h before antigen challenge completely antagonized the inhibition of histamine release produced by noradrenaline in concentrations up to 10^{-6} M. This suggests that α-receptor stimulation does not affect histamine release under the conditions used in these experiments.

3.2. β-Adrenoceptor agonists

Of the compounds tested (-)-isoprenaline produced the most consistent concentration-dependent anti-anaphylactic action. The maximum inhibition of histamine release produced by (-)-isoprenaline occurred at a concentration of 10^{-6} M. The inhibition using this concentration of (-)-isoprenaline was 70.7% (s.e.m. 9.5, n = 4).

Results with the six non-catechol compounds having N-isopropyl, N-t-butyl and N-phenyl (or phenol)-t-butyl N-alkyl substitutions are shown in Fig. 1.

All the compounds, except orciprenaline at the two lowest concentrations tested, were weaker in their ability to inhibit histamine release than (-)-isoprenaline. In the present experiments none of the non-catechol compounds produced the same maximum inhibition of histamine release as that obtained with (-)-isoprenaline. Studies with higher concentrations of the compounds were precluded because of interference in the assay system (see Methods). There is no apparent explanation for the reduction in the orciprenaline response at 10^{-8} M compared to 10^{-9} M.
Fig. 1. Graphs showing the % inhibition of antigen-induced histamine release produced by the non-catechol N-isopropyl [soterenol (SOT); orciprenaline (ORCI)], N-t-butyl [MJ7999-1, terbutaline (TERB)], phenyl-t-butyl [MJ9184-1] and phenol-t-butyl [Me506] substituted compounds. The effects produced by (-)-isoprenaline (ISO) are also shown. Points shown are the means with standard error bars obtained from at least 4 experiments with each compound.
The rank order of potency of the compounds as β-receptor agonists in guinea-pig atrial and tracheal preparations may be deduced from their pD₂ values (Table 2). Determination of the relative activities of the compounds as anti-anaphylactic agents is made difficult by the fact that the standard errors of the mean are large compared to the maximal inhibition produced by the compounds, and the relative activities of the compounds are dependent on which level of inhibition is chosen for comparison. Therefore the rank order of activity of the compounds was based on the area under the curves relating concentration and inhibition of histamine release. The area under the curve gives a comparison of the activities based on the overall consistency and magnitude of the inhibitory response. On this basis the rank order of activity obtained for inhibition of histamine release from actively sensitized guinea-pig lung is

**TABLE 2.** Table showing the pD₂ values for the indicated compounds in guinea-pig atria (positive chronotropic effect) and trachea (relaxation of carbachol-induced tone), taken from results reported in Chapter 2. Also shown are the anti-anaphylactic effects of the drugs (inhibition of histamine release from actively sensitized guinea-pig lung) using the mean areas under the curves for the compounds.

<table>
<thead>
<tr>
<th></th>
<th>ATRIA</th>
<th>TRACHEA</th>
<th>ANTI-ANAPHYLACTIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pD₂</td>
<td>pD₂</td>
<td>AREA x 10³</td>
</tr>
<tr>
<td>(-)-Isoprenaline</td>
<td>8.45</td>
<td>7.42</td>
<td>3.49</td>
</tr>
<tr>
<td>Orciprenaline</td>
<td>6.36</td>
<td>5.75</td>
<td>2.36</td>
</tr>
<tr>
<td>Terbutaline</td>
<td>5.11</td>
<td>5.77</td>
<td>1.89</td>
</tr>
<tr>
<td>Me506</td>
<td>6.10</td>
<td>5.52</td>
<td>1.92</td>
</tr>
<tr>
<td>Soterenol</td>
<td>7.27</td>
<td>6.82</td>
<td>1.98</td>
</tr>
<tr>
<td>MJ7999-1</td>
<td>7.26</td>
<td>7.11</td>
<td>2.70</td>
</tr>
<tr>
<td>MJ9184-1</td>
<td>6.05</td>
<td>7.91</td>
<td>2.44</td>
</tr>
</tbody>
</table>

pD₂ values shown are the means from 4 to 6 experiments with each drug.
(-)-isoprenaline > MJ7999-1 = MJ9184-1 = orciprenaline > soterenol > Me506 = terbutaline. When the rank orders of activity are based on pD2 values, in guinea-pig atria (β1) the order is (-)-isoprenaline > MJ7999-1 = soterenol > orciprenaline > Me506 = MJ9184-1 > terbutaline, and in guinea-pig trachea the order is MJ9184-1 = isoprenaline > MJ7999-1 = soterenol > terbutaline = orciprenaline = Me506. From these results it can be seen that the rank orders of activity of the compounds in the three preparations differ.

3.3. β-Adrenoceptor antagonism

In sensitized guinea-pig lung the β-adrenoceptor antagonists H93/26 and H35/25 antagonized the inhibitory effect of (-)-isoprenaline on histamine release. The results from an experiment using H35/25 are shown in Fig. 2. The shifts in

![Graph](image)

Fig. 2. Graph from an experiment showing the inhibition of histamine release from sensitized guinea-pig lung produced by (-)-isoprenaline alone (○) and in the presence (●) of 1.24 x 10^-4 M H35/25.
the (-)-isoprenaline curves were usually close to parallel, however in some experiments there was some non-parallelism in the slope of the (-)-isoprenaline curves in the presence and absence of the antagonists. In these cases a number of dose-ratios were obtained at different levels, $K_B$ values calculated, and the mean of these $K_B$ values used as the calculated value for that experiment. H93/26 was used in concentrations of $1.24 \times 10^{-6}$ M and $1.24 \times 10^{-5}$ M, and H35/25 in concentrations of $1.24 \times 10^{-5}$ M and $1.24 \times 10^{-4}$ M. Mean $K_B$ values are shown in Table 3. In guinea-pig atrial and tracheal preparations H93/26 and H35/25 produced concentration-dependent parallel shifts to the right of the cumulative concentration-effect curves to (-)-isoprenaline. Table 3 shows the $K_B$ values obtained in these two preparations.

### TABLE 3. $K_B$ values (±s.e.m.) for the β-receptor antagonists H93/26 and H35/25 in guinea-pig atria, trachea and sensitized guinea-pig lung. Figures in brackets show the number of observations made in each preparation.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>H35/25 $\times 10^{-6}$</th>
<th>H93/26 $\times 10^{-8}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atria</td>
<td>1.65 ± 1.10 (6)</td>
<td>3.70 ± 0.77 (6)</td>
</tr>
<tr>
<td>Anaphylaxis</td>
<td>3.37 ± 1.57 (5)</td>
<td>28.0 ± 9.7 (6)</td>
</tr>
<tr>
<td>Trachea</td>
<td>1.85 ± 1.10 (6)</td>
<td>102.4 ± 26.1 (9)</td>
</tr>
</tbody>
</table>

Student's t-test H93/26: 
- trachea vs. atria $P<0.005$
- trachea vs. anaphylaxis $P<0.005$
- atria vs. anaphylaxis $0.01<P<0.025$

H35/25: 
- trachea vs. atria $0.40<P<0.45$
- trachea vs. anaphylaxis $0.20<P<0.25$
- atria vs. anaphylaxis $0.10<P<0.15$

All three $K_B$ values for H93/26 were significantly different from each other, and confirm that H93/26 is a cardio-selective antagonist in the guinea-pig. In contrast the $K_B$ values for
H35/25 in guinea-pig atria and trachea (Table 3) were not significantly different. This indicates that H35/25 is not a selective β-adrenoceptor antagonist in these two tissues from the guinea-pig. In addition, the $K_B$ value found for antagonism of the anti-anaphylactic effect of (-)-isoprenaline in actively sensitized guinea-pig lung is not significantly different from those found in atria or trachea (Table 3).

For both antagonists in all three preparations, the antagonism was of a competitive type since similar $K_B$ values were found whether high or low concentrations of the antagonists were employed.
4. DISCUSSION

Previous workers have shown that the non-catechol sympathomimetics salbutamol, orciprenaline (Assem, 1971) and terbutaline (Sörenby, 1974) inhibit antigen-induced histamine release from sensitized guinea-pig lung. On the basis of the relative potencies of (+)-isoprenaline and terbutaline in sensitized guinea-pig lung, and those quoted by others for the actions of the drugs in guinea-pig atrial and tracheal preparations, Sörenby (1974) suggested that the β-adrenoceptors involved in the inhibition of histamine release "seem to be more related to those in tracheal muscle than those in the heart."

Soon after the results of the present experiments were published (Malta & Raper, 1975), Sörenby (1975) extended his results to include orciprenaline and ITP (dl-1-isopropylamino-3-(2-thiazoloxy)-2-propanol). In these experiments he attempted to categorize the β-adrenoceptor involved in anti-anaphylactic activity by comparing the relative orders of potency of the compounds for (i) inhibition of antigen-induced histamine release from guinea-pig lung, (ii) relaxation of guinea-pig preparations, and (iii) increase in heart rate and contractile force of guinea-pig Langendorff hearts. His conclusion in this paper was similar to that cited in his 1974 publication.

In the present experiments, six non-catechol sympathomimetics were used. These were chosen since they display varying degrees of selectivity in their β-adrenoceptor stimulant actions in guinea-pig atrial (β₁) and tracheal (β₂)
preparations. When these agonists are quantified for their ability to inhibit histamine release from sensitized lung, and placed in a rank order of activity, there are few similarities with the rank orders found in guinea-pig atrial and tracheal preparations.

Thus, on the basis of the results found with agonists, it might be concluded that the \( \beta \)-adrenoceptor involved in the anti-anaphylactic action of the compounds differs from those found in atrial (\( \beta_1 \)) and tracheal (\( \beta_2 \)) preparations. This view is further substantiated by the results obtained with the \( \beta \)-adrenoceptor antagonists H93/26 and H35/25. These antagonists have been reported to be selective for \( \beta_1 \)- and \( \beta_2 \)-adrenoceptors, respectively. In the present experiments the dissociation constants (\( K_B \) values) for the antagonist-receptor complexes were calculated. Furchgott (1967) implied that if for a given antagonist the \( K_B \) values varied in different tissues, this was indicative that different forms or types of a receptor were present.

On the basis of the results obtained with the cardio-selective antagonist H93/26, it would therefore appear that the \( \beta \)-adrenoceptors in atria, trachea and sensitized lung differ in their characteristics. On the other hand, H35/25, which in other tissues has been shown to possess \( \beta_2 \)-selective actions, does not differentiate between the \( \beta \)-adrenoceptors in these three tissues.

In conclusion, our results with the \( \beta \)-adrenoceptor agonists and antagonists indicate that the \( \beta \)-receptors involved in the inhibition of histamine release from actively sensitized guinea-pig lung differ from those found
in guinea-pig atria and trachea. These results are in accord with the suggestion by Brittain Jack & Ritchie (1970) that $\beta_1$- and $\beta_2$-receptors as defined by Lands et al. (1967) represent extremes of a variable spectrum of $\beta$-isoreceptors which have differing affinities for $\beta$-receptor agonists and antagonists.

On the other hand, further work would be required before results compatible with the suggestion that the differences obtained in each tissue are due to a variation of the ratio of $\beta_1$- and $\beta_2$-receptors (Åblad, Borg, Carlsson et al., 1975).
CHAPTER 9:

POTENTIATION OF β-ADRENOCEPTOR MEDIATED RESPONSES TO ADRENALINE AND (-)-ISOPRENALINE BY HYDROCORTISONE IN THE ANAESTHETIZED CAT
SUMMARY

1. Investigations were carried out to test the hypothesis that in status asthmaticus hydrocortisone produces its acute beneficial effects by potentiating the response to administered and circulating catecholamines. Doses of hydrocortisone, comparable to those used clinically, were tested for their effects on cumulative dose-response curves to adrenaline and (-)-isoprenaline for increase in heart rate, decreases in soleus muscle contractility, antagonism of serotonin-induced increases in pulmonary resistance and decreases in the perfusion pressure of the hind-limb in anaesthetized cats. Potentiation was assessed by using the dose-ratio taken at the ED$_{50}$ values obtained with the sympathomimetics before and after 5 and 20 mg kg$^{-1}$ of the steroid.

2. Both doses of hydrocortisone potentiated the positive chronotropic response to adrenaline by a mean ratio of 1.73 and 2.65, whilst for (-)-isoprenaline these ratios were 1.37 and 2.32, respectively. In 2 out of 4 experiments with adrenaline the maximum response was potentiated by both doses of the steroid while in the other experiments with adrenaline and all experiments with (-)-isoprenaline there was no change in the
maximum response. No potentiation of the reductions in hind-limb resistance produced by either catecholamine was present after either the high or the low dose of the steroid.

3. The two doses of hydrocortisone potentiated adrenaline-induced decreases in soleus muscle contractility by a mean ratio of 1.73 and 2.09, and by 1.32 and 2.25 for (-)-isoprenaline. There was no effect on the maximum response for either catecholamine.

4. Dose-response curves for adrenaline-induced decreases in pulmonary resistance were potentiated in only 3 out of 5 experiments. Mean dose-ratios following the low and high dose of the steroid were 1.24 and 1.57. (-)-Isoprenaline dose-response curves were potentiated in all experiments by a mean ratio of 2.47 and 4.06 after the two doses of the steroid.

5. The mechanism of action for the potentiation of catecholamine responses by hydrocortisone appeared to be due to the blockade of the extraneuronal uptake process.

6. Maximal potentiation for the catecholamines was observed 5-10 min after injection of hydrocortisone and by 45 min was reduced by 50%.
7. The small degree of potentiation shown together with the short duration of the potentiation suggests that blockade of the extraneuronal uptake of circulating adrenaline cannot account for the beneficial effect of the steroid when used in status asthmaticus.

8. The potentiation of the bronchodilator responses to isoprenaline by blockade of the extraneuronal uptake process may play a part in the clinically observed potentiation of bronchodilator responses to sympathomimetic amines after hydrocortisone in severe asthma.
1. INTRODUCTION

The use of intravenous steroids in the treatment of status asthmaticus has been regarded as mandatory (Today's Drugs, 1972). Despite many published reports regarding clinical efficacy the mechanism of action by which steroids produce their clinical effects in this situation are still unknown.

To achieve any beneficial effect in status asthmaticus it has been recommended that plasma levels of at least 100-150 μg 100 ml⁻¹ are needed (Dwyer, Lazarus & Hickie, 1967; Collins, Clark, Harris & Townsend, 1970). However the view that a specific minimum plasma level is required is not shared by all investigators (Cayton & Howard, 1973).

In both human and laboratory animals in vivo, acute hydrocortisone treatment has been shown to potentiate the actions of catecholamines in some (Franklin, Michelson, Lowell & Schiller, 1958; Mendlowitz, Gitlow & Naftchi, 1958; Hume & Jones, 1960; Besse & Bass, 1966; Kadowitz & Yard, 1971; Osswald & Branco, 1973; Pun, McCulloch & Rand, 1973), but not all experiments (Margolis, Reid, Mohammed & Gaffney, 1967; Carillo & Aviado, 1968). In in vitro experiments where hydrocortisone has been shown to potentiate catecholamine responses, blockade of the extraneuronal uptake process has been implicated (Kalsner, 1969; Iversen & Salt, 1970; Kaumann, 1972; Almgren & Jonasson, 1973; Pun, McCulloch & Rand, 1973; Mathé & Levine, 1974; Luchelli-Fortis & Langer, 1975; Graefe, 1975).

In the clinical situation plasma concentrations measured
one hour after the intravenous administration of 4 or 8 mg kg\(^{-1}\) hydrocortisone (Collins et al., 1970) are of the same order as those implicated to produce blockade of the extraneuronal uptake process. It was thus thought that in status asthmaticus hydrocortisone, through an extraneuronal uptake blocking action, might potentiate the bronchodilator actions of circulating adrenaline and administered catecholamines. Since hydrocortisone has been shown to block the extraneuronal process in isolated cat myocardial preparations (Kaumann, 1972; Graefe & Trendelenburg, 1974; Goldie, 1975), it was decided to investigate the effects of hydrocortisone on catecholamine-induced responses in the anaesthetized cat. Assessments have been made of the actions of adrenaline and isoprenaline in cardiac, bronchial and skeletal muscle before and after the administration of 5 and 20 mg kg\(^{-1}\) hydrocortisone. On a mg kg\(^{-1}\) basis the lower dose of the steroid used in this study is similar to the recommended clinical dose in status asthmaticus (Today's Drugs, 1972).
2. METHODS

2.1. General

Cats of either sex were anaesthetized by an intraperitoneal injection of a mixture of α-chloralose (80 mg kg\(^{-1}\)) and pentobarbitone sodium (6 mg kg\(^{-1}\)). The trachea was cannulated and the animals respired by a Palmer large animal respirator at a rate of 27 breaths min\(^{-1}\) with a stroke volume of 14 ml kg\(^{-1}\). A brachial vein and a femoral or carotid artery were cannulated for the administration of drugs and recordings of blood pressure, respectively.

2.2. Effects on cardiac, skeletal and bronchial smooth muscle

Heart rate, soleus muscle contractility and pulmonary resistance were measured as described in Chapter 5. In all experiments constant cumulative dose-response curves to adrenaline and/or (-)-isoprenaline were first established. Responses to the catecholamines were then reassessed 5 min after intravenous injections of hydrocortisone (5 and 20 mg kg\(^{-1}\)). In the present experiments where the effects on heart rate were monitored, the cats were bilaterally vagotomized and received bethanidine sulphate (6 mg kg\(^{-1}\)) to eliminate cardiovascular reflexes.

2.3. Hind-limb resistance

In these experiments blood was taken from the left femoral artery and pumped into the right femoral artery using
a Harvard (944) peristaltic pump. The perfusion pressure of the limb was measured using a Statham P23Dc pressure transducer connected as a side-arm to the post-pump section of the circuit. At constant flow the perfusion pressure equals the hind-limb resistance (Nakano & McClay, 1967).

After commencement of the experiment the flow rate of the pump was adjusted such that the pressure in the perfused limb approximated the systemic arterial pressure. The pump flow rate was then unaltered for the rest of the experiment. Drugs were injected intra-arterially in volumes <20 µl into the pre-pump circuit using a 100 µl syringe (S.G.E.). In these experiments the cats received 6 mg kg i.v. bethanidine sulphate and phentolamine mesylate (1 mg kg\(^{-1}\) every 20 min) to prevent \(\alpha\)-receptor mediated effects and sympathetic reflexes. In addition the perfused limb was acutely denervated by sectioning of the femoral and sciatic nerves. Heparin (1000 U kg\(^{-1}\)) was injected intravenously as an anticoagulant.

Decreases in perfusion pressure were obtained with a series of doses of both (-)-isoprenaline and (-)-adrenaline. When these responses were constant, hydrocortisone 5 mg kg\(^{-1}\) was given intravenously and 5 min later the dose schedules of both amines repeated. The same procedure was utilized for the 20 mg kg\(^{-1}\) hydrocortisone dose.

2.4. Evaluation of results

In experiments where cumulative dose-response curves were used to assess the effects of hydrocortisone, the ratio
of the ED$_{50}$ values (Drug:Drug + hydrocortisone) was used. In experiments in which hind-limb resistance was assessed single doses of the amine were used, and the ratio of the response obtained before and after hydrocortisone was calculated.

2.5. Drugs used

The drugs used were (-)-adrenaline bitartrate (Koch-Light), (-)-isoprenaline bitartrate (Wyeth), salbutamol base (Allen & Hanburys), MJ9184-1 hydrochloride (Mead Johnson), hydrocortisone sodium hemisuccinate (Glaxo), serotonin creatinine sulphate (Sigma), gallamine triethiodide (May & Baker), bethanidine sulphate (Burroughs Wellcome), phentolamine mesylate (Ciba), $\alpha$-chloralose (British Drug Houses), pentobarbitone sodium (Abbot), and heparin (Commonwealth Serum Laboratories). Stock solutions of the catecholamines were prepared using N/100 HCl; all other stock solutions were made up in distilled water. Stock solutions were diluted to the appropriate concentrations using 0.9% w/v NaCl. Hydrocortisone solutions were made up daily. All doses in the text refer to the free substances and refer to the salt when the drug and salt are named.
3. RESULTS

3.1. General

Injections of 5 or 20 mg kg$^{-1}$ hydrocortisone when given over 2-5 min did not produce any changes in the basal levels of the parameters studied. Fast injections of hydrocortisone produced sympathomimetic-like effects on heart rate, blood pressure, pulmonary and hind-limb resistance. These effects were variable in magnitude in different cats. The effects produced by fast injections were not seen in two animals which had been bilaterally adrenalectomized, suggesting that release of catecholamines was occurring from the adrenal medulla as has been shown in the dog (Critchley et al. (1975). In the experiments in which the effects of the steroid on catecholamines induced responses were assessed, injections of hydrocortisone were given slowly to preclude the effects of changes in basal levels of the parameters studied.

In preliminary experiments the effects of the catecholamines were assessed at varying times after injection of hydrocortisone. In these studies it was shown that maximal potentiation of the responses to adrenaline and (-)-isoprenaline were present 5 min after injection of the steroid. The potentiations found at 5 and 10 min after hydrocortisone were similar and thereafter responses to the catecholamines returned towards control levels. The potentiation was reduced by approximately 50% after 45 min.
3.2. Heart rate

After hydrocortisone, dose-response curves to adrenaline and (-)-isoprenaline were shifted to the left. The extent of the shift was variable in different experiments. In terms of dose-ratios calculated from the $ED_{SO}$ values, hydrocortisone (5 mg kg$^{-1}$) potentiated the positive chronotropic activity of adrenaline by a factor ranging from 1.00 to 2.42 ($\bar{x} = 1.73$, s.e.m. = 0.36, $n = 4$). After 20 mg kg$^{-1}$ of hydrocortisone the potentiations observed varied from 1.36 to 3.50 ($\bar{x} = 2.65$, s.e.m. = 0.46, $n = 4$). The potentiation produced by the high steroid dose was not significantly greater than that produced by the low dose ($0.1 < P < 0.2$, 6 d.f., $t$-test).

In two of the four experiments the maximum responses to adrenaline were increased by 15 and 20% after 5 mg kg$^{-1}$ of hydrocortisone. In one of these experiments $E_{max}$ increased from 15 to 30% after injection of the high dose of the steroid.

Chronotropic responses to (-)-isoprenaline were also variably potentiated by the two doses of hydrocortisone. The range of values obtained with the low dose varied from 0.96 to 1.83 ($\bar{x} = 1.37$, s.e.m. = 0.14, $n = 5$) and with the high dose from 1.78 to 3.66 ($\bar{x} = 2.32$, s.e.m. = 0.35, $n = 5$). Hydrocortisone at either dose did not potentiate the maximum response to (-)-isoprenaline. After 20 mg kg$^{-1}$ of hydrocortisone there was a significantly greater potentiation ($0.025 < P < 0.05$, 8 d.f., $t$-test) than that occurring at the 5 mg kg$^{-1}$ level.
Figure 1 shows the results from one experiment in which hydrocortisone produced a dose-related shift to the left of the dose-response curves to adrenaline. In this experiment hydrocortisone did not affect the maximal response to adrenaline. Table 1 shows the mean dose-ratios (±s.e.m.) for the steroid-induced potentiation of responses to adrenaline and (−)-isoprenaline.

<table>
<thead>
<tr>
<th>Response</th>
<th>% E max</th>
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<td></td>
<td></td>
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</table>

![Graph showing dose-response curves for adrenaline and (−)-isoprenaline with hydrocortisone doses of 5 and 20 mg kg⁻¹.](image)

**Fig. 1.** Results from one experiment in the anaesthetized cat in which the effects of 5 mg kg⁻¹ (○) and 20 mg kg⁻¹ (□) hydrocortisone was tested for its ability to potentiate the positive chronotropic actions of adrenaline (control, ⬤). Responses are plotted as a percentage of the maximum response obtained in control curves for adrenaline against dose of the catecholamine in terms of the base.

No significant difference existed between the adrenaline and (−)-isoprenaline dose-ratios at either the 5 or 20 mg kg⁻¹ dose levels of hydrocortisone (0.3<P<0.4, 7 d.f.,
t-test and 0.5<P<0.6, 7 d.f., t-test, respectively).

The ability of hydrocortisone to potentiate the actions of the catecholamines does not appear to be due to an effect on postjunctional β-adrenoceptors, since in two experiments in bilaterally adrenalectomized animals, doses of hydrocortisone which potentiated the actions of (-)-isoprenaline had no effect on chronotropic responses to MJ9184-1 or salbutamol. In these experiments injections of two single doses of (-)-isoprenaline were first monitored. The doses were chosen to give approximately 10 and 20 beats min\(^{-1}\) increases in heart rate. Doses of MJ9184-1 or salbutamol were then given to produce an increase in heart rate of approximately 15 beats min\(^{-1}\). After constant responses had been obtained hydrocortisone (5 and 20 mg kg\(^{-1}\)) was injected and responses to the sympathomimetics reassessed. Figure 2 shows the quantitative results obtained for the experiments using MJ9184-1. The responses to (-)-isoprenaline were potentiated whilst those to MJ9184-1 or salbutamol were unchanged.

On a mole kg\(^{-1}\) basis adrenaline was 17.5 times less potent than (-)-isoprenaline in producing positive chronotropic actions indicating that its β\(_1\)-adrenoceptor stimulating effect is approximately the same as that for salbutamol (Rodger, 1973).

### 3.3. Soleus muscle contractility

The actions of hydrocortisone on (-)-adrenaline-induced decreases in tension and fusion of incomplete tetanic stimuli of the cat soleus muscle were less variable
than those seen on heart rate. Hydrocortisone produced a shift to the left in the dose-response curves to adrenaline in the soleus muscles. The dose-ratios ranged from 1.57 to 1.96 (x = 1.73, s.e.m. = 0.09, n = 4) and from 1.71 to 2.41 (x = 2.09, s.e.m. = 0.15, n = 4) with 5 and 20 mg kg\(^{-1}\) hydrocortisone doses, respectively. There was no significant difference between these two means (0.05 < P < 0.10, 6 d.f., t-test). No increase in the maximum response to
(-)-adrenaline occurred after injection of hydrocortisone. Figure 3 shows the results from one experiment in which the steroid produced a dose-related shift to the left in the dose-response curves to adrenaline.

For (-)-isoprenaline the mean dose-ratios were 1.32 (range 0.71 - 1.71, s.e.m. = 0.19, n = 4) and 2.25 (range 1.37 - 3.00, s.e.m. = 0.38, n = 5) after the low and high doses of hydrocortisone, respectively. As for adrenaline there was no significant difference between the potentiation produced by the two doses of the steroid (0.05 < P < 0.1, 8 d.f., t-test). The variability of the potentiation seen in the soleus muscle is similar to that observed for heart rate responses to (-)-isoprenaline. As for heart rate, the steroid did not increase the maximum response to the catecholamine.

Tests of significance showed that there was no difference between the dose-ratios when the hydrocortisone-induced effects on responses to the two catecholamines were compared at the low and high dose of the steroid (0.05 < P < 0.10, 6 d.f., t-test and 0.7 < P < 0.8, 7 d.f., t-test, respectively).

Table 1 shows the mean values (±s.e.m.) for the potentiations of responses to adrenaline and (-)-isoprenaline produced by hydrocortisone in the cat soleus muscle.

3.4. Pulmonary resistance

In 2 of the 5 cats studied, hydrocortisone at both 5 and 20 mg kg⁻¹ failed to potentiate the bronchodilator
Fig. 3. Graphical plot from one experiment showing the effect of two doses of hydrocortisone (5 mg kg\(^{-1}\), ○ and 20 mg kg\(^{-1}\), □), on cumulative dose-response curves obtained for adrenaline (●, control) in the soleus muscle of the cat. Responses are expressed as a percentage of the maximum depression in contraction height obtained with adrenaline in the control period.

action of adrenaline. Potentiation was seen only after 20 mg kg\(^{-1}\) hydrocortisone in one cat, and in the other two cats after both low and high doses of the steroid. Thus the hydrocortisone-induced effect was very much more variable than that seen on either heart rate or the soleus muscle. The mean potency rates taken over the 5 experiments showed that 5 mg kg\(^{-1}\) hydrocortisone produced a 1.24 (s.e.m. = 0.19, n = 5)-fold potentiation whilst the 20 mg kg\(^{-1}\) dose produced a 1.57 (s.e.m. = 0.33, n = 5)-fold potentiation. There is no significant difference between
the mean dose-ratios \((0.4<P<0.5, 8 \text{ d.f.}, t\text{-test})\).

In all experiments in which (-)-isoprenaline was used, hydrocortisone produced potentiation of these responses. Although the values varied from cat to cat the potentiation observed with (-)-isoprenaline was always greater than that for (-)-adrenaline in the same cat. In experiments where there was no potentiation of adrenaline responses, the potentiation observed with (-)-isoprenaline was smaller than in experiments where adrenaline was potentiated. For (-)-isoprenaline the mean ratios were 2.47 (s.e.m. = 0.37, \(n = 4\)) and 4.06 (s.e.m. = 1.02, \(n = 4\)) after 5 and 20 mg kg\(^{-1}\) hydrocortisone, respectively. However there was no significant difference between these two mean values \((0.1<P<0.2, 6 \text{ d.f.}, t\text{-test})\). Table 1 shows the mean values \((±\text{s.e.m.})\) for both catecholamines at the low and high doses of the steroid.

Since in all experiments complete reversal of the serotonin-induced bronchoconstriction was obtained in the control curves, the effects of hydrocortisone on the maximum responses to the catecholamines could not be determined.

Use of the Student's \(t\)-test showed that there was no significant difference between the dose-ratios obtained for either catecholamine at the low or high dose of hydrocortisone \((0.3<P<0.4, t\text{-test}, 7 \text{ d.f.}, \text{ and } 0.1<P<0.2, t\text{-test}, 7 \text{ d.f.})\).

On a molar basis (-)-adrenaline was 6.25 times less potent than (-)-isoprenaline in its bronchodilator actions.
TABLE 1. Mean ratios for the potentiation of responses to adrenaline and (-)-isoprenaline in the anaesthetized cat produced by the i.v. injection of 5 mg kg\(^{-1}\) and 20 mg kg\(^{-1}\) hydrocortisone (HC) 5 min prior to the establishment of cumulative dose-response curves for the catecholamines. The potentiation ratio is defined as the ED\(_{50}\) value for the catecholamine in the control situation: ED\(_{50}\) value after hydrocortisone. Values in parentheses are the standard errors of the mean from at least 4 comparisons.

<table>
<thead>
<tr>
<th>HC dose</th>
<th>ADRENALINE</th>
<th>(-)-ISOPRENALINE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 mg kg(^{-1})</td>
<td>20 mg kg(^{-1})</td>
</tr>
<tr>
<td>Heart rate</td>
<td>1.73 (0.36)</td>
<td>2.65 (0.46)</td>
</tr>
<tr>
<td>Soleus muscle</td>
<td>1.73 (0.09)</td>
<td>2.09 (0.15)</td>
</tr>
<tr>
<td>Pulmonary resistance</td>
<td>1.24 (0.19)</td>
<td>1.57 (0.33)</td>
</tr>
</tbody>
</table>

This dose-ratio is similar to that observed by Nott & Raper (1972) when they compared the relative potencies for the effects of adrenaline and (-)-isoprenaline in the cat soleus muscle.

Table 2 shows the mean molar potency ratios (±s.e.m.) and ED\(_{50}\) values for both catecholamines on the three cat parameters.

3.5. Hind-limb resistance

Adrenaline and (-)-isoprenaline produced dose-dependent reductions in the perfusion pressure in the hind-limbs of anaesthetized cats. In three experiments with (-)-isoprenaline and two with adrenaline the injection of 5 or 20 mg kg\(^{-1}\) hydrocortisone did not alter the responses of the hind-limb to the catecholamines.
TABLE 2. Mean potency ratios on a molar basis and mean ED$_{50}$ values (µg kg$^{-1}$) for adrenaline and (-)-isoprenaline on cat heart rate, soleus muscle contractility and pulmonary resistance in anaesthetized cats. Values in parentheses are s.e.m. from at least four comparisons. Potency ratio is defined as the ED$_{50}$ value adrenaline:ED$_{50}$ value (-)-isoprenaline on a mole kg$^{-1}$ basis.

<table>
<thead>
<tr>
<th></th>
<th>ADRENALINE</th>
<th>(-)-ISOPRENALINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ratio</td>
<td>ED$_{50}$</td>
</tr>
<tr>
<td></td>
<td>17.5 (2.3)</td>
<td>0.92 (0.25)</td>
</tr>
<tr>
<td>Soleus*</td>
<td>Ratio</td>
<td>ED$_{50}$</td>
</tr>
<tr>
<td></td>
<td>5.5 (0.5)</td>
<td>Not cited</td>
</tr>
<tr>
<td></td>
<td>0.068 (0.005)</td>
<td></td>
</tr>
<tr>
<td>Pulmonary resistance</td>
<td>Ratio</td>
<td>ED$_{50}$</td>
</tr>
<tr>
<td></td>
<td>6.25 (1.01)</td>
<td>0.12 (0.03)</td>
</tr>
<tr>
<td></td>
<td>0.020 (0.004)</td>
<td></td>
</tr>
</tbody>
</table>

*Values from Nott & Raper (1972)
4. DISCUSSION

Hydrocortisone produced a potentiation of the responses to (-)-isoprenaline and adrenaline on heart rate, soleus muscle contractility and pulmonary resistance in anaesthetized cats. The lack of such an effect with (-)-isoprenaline on the perfused hind-limb of the cat agrees with the findings of Yard & Kadowitz (1972). However the observation that adrenaline-induced vasodilatation is also not affected by hydrocortisone suggests that the lack of effect probably applies to all sympathomimetic vasodilator compounds. On the other parameters maximal hydrocortisone-induced potentiation of the responses to adrenaline and (-)-isoprenaline occurred 5 min after injection of the steroid and the effect was relatively short lasting, the time to half-return of the potentiated response to control levels being approximately 45 min.

Although quick injection of the steroid frequently produced weak sympathomimetic effects, the potentiation of responses to adrenaline and (-)-isoprenaline does not appear to be due to an additive effect of injected and endogenously released catecholamine, since potentiation of responses occurred in bilaterally adrenalectomized animals.

Besse & Bass (1966) and Yard & Kadowitz (1972) have suggested that steroid-induced changes in responses to catecholamines are due to some alteration in the characteristics of the adrenoceptor resulting in "receptor sensitization". In the present experiments the positive chronotropic responses of the non-catecholamine compounds salbutamol and MJ9184-1 were not potentiated while those
to (-)-isoprenaline were increased. This differential potentiation of agonists suggests that the characteristics of the postjunctional \( \beta \)-receptor are not affected by hydrocortisone. Further evidence for the lack of effect of hydrocortisone on \( \beta \)-receptors had been advanced by Kaumann (1972). This author assessed the blockade of isoprenaline-induced responses in kitten right atrial preparations with the \( \beta \)-adrenoceptor antagonist KL255. Similar \( K_B \) values were found in the absence and in the presence of hydrocortisone.

From a structural standpoint salbutamol and MJ9184-1 would not be expected to be substrates for the extraneuronal uptake system. However experiments by Salt (1972) have shown that salbutamol is an extremely weak inhibitor of the extraneuronal uptake process in the rat heart. The fact that the non-catecholamines are not potentiated by hydrocortisone while those to (-)-isoprenaline are, suggests that the steroid-induced potentiation results from blockade of the Uptake_{2} process. This suggestion is also supported by the finding of Goldie (1975) that in cat atria there was a clear correlation between the hydrocortisone-induced blockade of \( ^3 \)H-isoprenaline uptake and potentiation of the chronotropic actions of the amine in cat atria.

As stated in the Introduction, the potentiation of catecholamine-induced responses by hydrocortisone has not been observed in all acute experiments. Explanations such as variations in the extent of the extraneuronal uptake process in different tissues even from the one species, the failure to block Uptake_{1} when noradrenaline is the
catecholamine under investigation, and the production of maximal responses to the catecholamine before hydrocortisone is tested, can be invoked to account for the failure of some investigators to show potentiation with hydrocortisone. Some examples will be discussed in the following paragraphs.

Carillo & Aviado (1968) who used anaesthetized rabbits, found that the decrease in airway resistance and increase in compliance produced by isoprenaline was not potentiated by hydrocortisone (25 mg kg$^{-1}$ i.v.) whilst potentiation of isoprenaline-induced decreases in aortic blood pressure did occur in 3 of 5 experiments. These results may be in part explained if the extraneuronal uptake process is very weak in rabbit lung as has been reported for rabbit heart (Bönisch & Trendelenburg, 1974). Other workers have shown that hydrocortisone potentiates isoprenaline responses in rabbit aortic strips (Besse & Bass, 1966; Kalsner, 1969) and this may explain the effect seen with isoprenaline on rabbit aortic blood pressure in the presence of hydrocortisone (Carillo & Aviado, 1968). Thus the possibility arises that the extraneuronal uptake process can vary from tissue to tissue even within the one species. Such evidence is also forthcoming from a comparison of the results of Pun, McCulloch & Rand (1973) who found block of the extraneuronal uptake by hydrocortisone in guinea-pig trachea and those of Goldie (1975) who showed no such effect in guinea-pig atrial preparations, respectively.

Osswald & Branco (1973) using the perfused dog hind-limb preparation showed that cortexone (desoxycorticosterone) had no effect on the removal of noradrenaline from the
circulation unless Uptake$_1$ had been blocked, the leg chronically denervated, or the concentration of infused noradrenaline increased from 1 to 4 $\mu g \text{kg}^{-1} \text{min}^{-1}$. The data presented by Margolis et al. (1967) showed that doses of hydrocortisone from 4.7 to 8.7 $\text{mg kg}^{-1}$ had no effect on the increase in heart rate produced by cardioaccelerator nerve stimulation or bolus injections of noradrenaline. However, as shown by Osswald & Branco (1973) and by Hughes (1972) the effect that steroids can exert on the Uptake$_2$ process is best seen when Uptake$_1$ is inactivated. Margolis et al. (1967) did not attempt to block the neuronal uptake process.

Similarly the same argument applies to the results of Kadowitz & Yard (1971) and Yard & Kadowitz (1972). In these two papers conclusions as to a mechanism of action of hydrocortisone have been made when the Uptake$_1$ process was still operational. Thus Yard & Kadowitz (1972) concluded that hydrocortisone altered the confirmation of the adrenergic $\alpha$-receptor since adrenaline was the only vasoconstrictor agent which was potentiated by hydrocortisone. If the experiments had been repeated in the presence of cocaine then the constrictor responses to nerve stimulation and to noradrenaline may well have been potentiated.

Besse & Bass (1966) also arrived at a similar conclusion to that of Yard & Kadowitz (1972); however in their experiments they had blocked the neuronal uptake process. Their results showed that hydrocortisone in the presence of cocaine potentiated the blood pressure responses to adrenaline and isoprenaline but not the pressor response to noradrenaline. These discrepancies can be explained by the data presented in Table 1B of that paper. From
this it can be seen that 2 μg kg⁻¹ noradrenaline is potentiated by cocaine to such a degree that a maximal response is produced, thus the administration of hydrocortisone cannot produce any further potentiation. Maximal responses were not obtained for adrenaline and isoprenaline after cocaine and therefore potentiation could be observed with these amines.

Thus consideration of the factors discussed in the preceding paragraphs have to be taken into account before conclusions can be made as to the mode of action of hydrocortisone.

The results from the present Chapter show that the potentiations produced by hydrocortisone were variable from one animal to another even when the same parameter was studied. This variability could be a reflection of a number of factors. In these present experiments the neuronal uptake process was still operational and this could explain the variability of effects seen with adrenaline, but not (-)-isoprenaline. It would be of interest to perform experiments using a suitable Uptake₁ inhibitor to investigate the influence of neuronal uptake on hydrocortisone-induced potentiation in the cat.

The results of Graefe & Trendelenburg (1974) raise another possible explanation as to the variability in the potentiations. These authors found that in the isolated cat nictitating membrane there were two extraneuronal O-methylating compartments, only one of which was sensitive to blockade by hydrocortisone. If this two compartment system exists in other cat tissues then the inactivation of
the amines can be viewed as a dynamic situation in which there is competition by these two compartments for the amine. To produce potentiations the hydrocortisone-sensitive compartment must (i) be in close proximity to the β-receptors so that increases in the concentration of the catecholamine in the biophase are produced, before the hydrocortisone-insensitive uptake process can inactivate the amine, and (ii) the compartment must be relatively large so that blockade of the uptake process will increase the biophase concentration to an extent that a potentiated response is observed. Thus the cat-to-cat variability in potentiation may reflect differing capacities for these two compartments which compete for the amines.

Additional experiments in which catechol-O-methyl transferase is blocked would be of interest in order to establish the relationship between the extent of hydrocortisone potentiation and the maximal possible potentiations which can be observed with blockade of COMT.

However experiments to determine possible modes of action of hydrocortisone in status asthmaticus should be performed in animals which do not have neuronal uptake process inhibited since this is the situation which occurs clinically.

In preliminary experiments in which pulmonary resistance was measured in cats it was found that approximately half of the potentiation of (-)-isoprenaline responses seen at 5 min after 5 mg kg\(^{-1}\) hydrocortisone had disappeared in 45 min. This coupled with the fact that potentiations to adrenaline were seen in only 2 out of 5 experiments with the
low dose of hydrocortisone suggests that an extraneuronal uptake blocking action of the steroid, as part of its mechanism of action in status asthmaticus, is highly unlikely. In status asthmaticus corticosteroids take many hours to act (Rees, 1967; Anon., 1972b; Ellul-Micallef, Borthwick & McHardy, 1972; Sheehy et al., 1972; Today's Drugs, 1972). However an extraneuronal uptake blocking action may be responsible for part of the increased responsiveness seen with bronchodilators in severe asthma (Franklin et al., 1958; Hume & Jones, 1960; Rebuck & Read, 1971; Dwyer, 1973; Shenfield, Hodson, Clark et al., 1975).

Possible modes of action of high dose steroids have been investigated in isolated human bronchial muscle preparations. Townley, Reeb, Fitzgibbons & Adolphson (1970) found that hydrocortisone potentiated the bronchodilator responses in isolated human bronchial smooth muscle and suggested that the steroid acted by lowering the threshold of β-receptors to catecholamines. However an alternative explanation is that in this case the steroid is preventing the extraneuronal uptake of the catecholamine and for the reasons outlined above, this idea has been dismissed. Andersson & Kövesi (1974) found that a 1 μM concentration of hydrocortisone produced complete relaxation of tone in isolated human bronchial smooth muscle and antagonized the increase in tone produced by histamine. The relaxing effect of hydrocortisone was explained by release of adrenergic transmitter coupled with a direct smooth muscle action. Townley, Hobraath & Guirgis (1972) showed that in isolated human tracheal muscle adrenaline produced relaxation in the
presence of hydrocortisone and propranolol, whilst with propranolol alone, adrenaline contracted the muscle due to $\alpha$-receptor stimulation. However the concentration of the steroid used in these experiments with the human muscle preparations was not stated and therefore no conclusions can be made until this concentration is known. The action of steroids in status asthmaticus is probably due to an intracellular mechanism which produces an anti-inflammatory effect. Claman (1975) has recently reviewed some of the contradictions found with respect to the anti-inflammatory actions of steroids and has highlighted the species differences that occur. However as yet it is not known how steroids act in inflammation or produce their beneficial effects in status asthmaticus.

Finally the ratios of potency for adrenaline against (-)-isoprenaline in the three cat parameters heart rate, soleus muscle contractility and pulmonary resistance show that adrenaline although weaker in potency is relatively more selective than (-)-isoprenaline for $\beta_2$- rather than $\beta_1$-receptor mediated effects. The selectivity ratio, i.e., heart rate dose-ratio:pulmonary resistance dose-ratio is 2.8. Therefore adrenaline is more $\beta_2$-receptor selective in its actions than is terbutaline (Chapter 5). This is somewhat surprising but is evidence for the role of circulating adrenaline in maintaining patent airways in the whole animal.


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