PHYSIOLOGICAL AND HISTOPATHOLOGICAL EFFECTS OF CHRONIC INTRACOCHLEAR ELECTRICAL STIMULATION

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Introduction

Direct and r.f. currents are known to result in destruction of neural tissue. However, it is now apparent that non-destructive electrical stimulation can be achieved by the use of biphasic pulsatile stimuli (Lilly, 1960; Mortimer et al., 1970; Hughes et al., 1980). Although maximum biologically safe stimulation regimes have yet to be clearly defined, the evidence of a number of investigators suggests that charge density per phase and charge injection per phase are important parameters when establishing biologically safe levels of electrical stimulation (Pudenz et al., 1975; Pudenz et al., 1977; Brown et al., 1977; Babb et al., 1977). Furthermore, considerable attention has been given to ensure that the stimulus is not producing adverse electrochemical reactions that could result in physical or toxic injury to the biological environment. Brummer et al. (1977) have defined the upper limit of electrochemically safe electrical stimulation for platinum electrodes as charge balanced biphasic pulses at a maximum charge density of 300 μC/cm² geom./phase.

The present study was designed to examine in detail the effect of chronic intracochlear electrical stimulation on the spiral ganglion cell population using a stimulation regime within the upper operating range of the Melbourne Cochlear Prosthesis. The study used electrically evoked auditory brainstem responses (EABR's) to monitor the status of the auditory nerve and histological techniques to examine the cochleas following stimulation. The use of EABR's to non-invasively monitor the status of the auditory nerve has been reported by a number of investigators. Merzenich and White (1977) observed that the growth of the amplitude of the EABR with stimulus current (the growth response) is directly proportional to the number of fibres being stimulated. Smith and Simmons (1983) have supported this finding by demonstrating a correlation between the slope of the EABR growth response, and the percentage of surviving spiral ganglion cells.

Methods

Ten normal hearing adult cats were used in the present study. Using sterile conditions, a bipolar electrode array was inserted 5 mm into the scala tympani, via the round window. The surgical procedures have been described elsewhere (Shepherd et al., 1983). Both cochleas of each animal were implanted; one side was stimulated while the other was a control. The electrode array consisted of two 0.3 mm wide platinum band electrodes on a 0.6 mm O.D. silicone rubber carrier. Teflon insulated platinum: iridium (90 : 10) lead wires were welded to the underside of each electrode and were encapsulated within the silicone carrier. The inter-electrode spacing was approximately 0.45 mm. Each animal commenced a continuous electrical stimulation program ten days following surgery. The stimulus regime consisted of biphasic constant current pulses presented at 500 pulses per second (pps) at 0.2 ms/phase. The asymmetry of the biphasic pulse was within 0.01-0.1%. A stimulus current level midway between threshold and a current level that gave an aversive response was used, and was confirmed to be supra-threshold by EABR. In this study, stimulus current levels varied from 0.5 to 0.9 mA with corresponding charge densities of 18 to 32 μC/cm² geom./phase. EABR's were recorded at regular periods throughout each animal's stimulation program, thus EABR thresholds and growth responses were monitored. EABR recording techniques used in the present study have been described elsewhere (Black et al., 1983). On completion of the stimulation program the animals were sacrificed and their cochleas sectioned. Sections every 120 μm were collected and stained with haematoxylin and eosin. The platinum electrodes were accurately located along the scala tympani using a graphic reconstruction technique (Schuknecht, 1953). Spiral ganglion cell densities for both control and stimulated cochleas were determined for cell populations within a 1 mm region of the electrode pair - a distance within the electrically excited field for mid-dynamic range currents.
(Black et al., 1982). The degree of inflammation in each cochlea was graded from I-V on the basis of the number of polymorphonuclear and mononuclear leukocytes and the fibrous tissue reaction. When assessing the inflammation the investigator had no knowledge of whether the slide was from a stimulated or control cochlea. The mean spiral ganglion cell densities for stimulated cochleas were compared statistically with those from control cochleas using a multiple linear regression analysis. The same statistical analysis was used to compare the mean spiral ganglion cell densities with the degree of acute inflammation.

Results
Electrode implantation times varied from 32 to 113 days, and total electrical stimulation times varied from 424 to 2029 hours. Table 1 summarizes the histopathological results.

Cochlear Histopathology
Moderate to severe inflammatory reactions were observed in ten of the cochleas in this study, the remaining ten cochleas exhibited reactions varying from mild to absent. The degree of inflammation was not related to the degree of electrical stimulation. Mild inflammation did not, in general, result in extensive hair cell loss or atrophy of the organ of corti (Fig. 1a). Occasionally, eosinophilic exudate was present although it was generally restricted to the scala tympani close to the round window. In a number of cochleas there was no apparent inflammation reaction (Fig. 2a).

Table 1
Histopathological results

<table>
<thead>
<tr>
<th>Cochlea</th>
<th>Stimulus hours (mA)</th>
<th>Inflammation Grade</th>
<th>New bone</th>
<th>Hair cells</th>
<th>Dendrites</th>
<th>Mean spiral ganglion cell density (cell/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>98R</td>
<td>2029</td>
<td>0.5</td>
<td>I</td>
<td><strong>/</strong>*</td>
<td><strong>/</strong>*</td>
<td>1193</td>
</tr>
<tr>
<td>98L</td>
<td>control</td>
<td>IV</td>
<td>++</td>
<td>-/ -</td>
<td>-/ -</td>
<td>736</td>
</tr>
<tr>
<td>99R</td>
<td>1011</td>
<td>0.8</td>
<td>II</td>
<td><strong>/</strong>*</td>
<td><strong>/</strong>*</td>
<td>1348</td>
</tr>
<tr>
<td>99L</td>
<td>control</td>
<td>I</td>
<td></td>
<td>-/ -</td>
<td><strong>/</strong>*</td>
<td>1183</td>
</tr>
<tr>
<td>101R</td>
<td>1115</td>
<td>0.6</td>
<td>IV</td>
<td>+ -</td>
<td>-/ -</td>
<td>630</td>
</tr>
<tr>
<td>101L</td>
<td>control</td>
<td>I</td>
<td></td>
<td>***</td>
<td>***</td>
<td>1183</td>
</tr>
<tr>
<td>107R</td>
<td>1728</td>
<td>0.5</td>
<td>II</td>
<td>-/ -/ ***</td>
<td>-/ -/ ***</td>
<td>1334</td>
</tr>
<tr>
<td>107L</td>
<td>control</td>
<td>I</td>
<td>+ -/ ***</td>
<td>-/ -/ ***</td>
<td>-/ -/ ***</td>
<td>1100</td>
</tr>
<tr>
<td>108R</td>
<td>1189</td>
<td>0.9</td>
<td>III</td>
<td>+ -</td>
<td></td>
<td>839</td>
</tr>
<tr>
<td>108L</td>
<td>control</td>
<td>I</td>
<td></td>
<td>-/ -/ ***</td>
<td>-/ -/ ***</td>
<td>927</td>
</tr>
<tr>
<td>109R</td>
<td>control</td>
<td>I</td>
<td></td>
<td>*/ ***</td>
<td>***</td>
<td>1483</td>
</tr>
<tr>
<td>109L</td>
<td>1529</td>
<td>0.6</td>
<td>II</td>
<td>-/ -/ *</td>
<td>*/ **</td>
<td>1134</td>
</tr>
<tr>
<td>112R</td>
<td>424</td>
<td>0.8</td>
<td>I</td>
<td>+ */ **</td>
<td>*/ **</td>
<td>905</td>
</tr>
<tr>
<td>112L</td>
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<td>IV</td>
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<td>+ -</td>
<td></td>
<td>538</td>
</tr>
<tr>
<td>117R</td>
<td>1514</td>
<td>0.6</td>
<td>II</td>
<td>+ -</td>
<td>-/ -/ **</td>
<td>1085</td>
</tr>
<tr>
<td>117L</td>
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<td>I</td>
<td></td>
<td>-/ -/ **</td>
<td>**/ ***</td>
<td>1250</td>
</tr>
<tr>
<td>132R</td>
<td>539</td>
<td>0.6</td>
<td>V</td>
<td>- -</td>
<td></td>
<td>129</td>
</tr>
<tr>
<td>132L</td>
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<td>*/ ***</td>
<td>1141</td>
</tr>
<tr>
<td>134R</td>
<td>568</td>
<td>0.5</td>
<td>I</td>
<td>- -</td>
<td>*/ ***</td>
<td>1062</td>
</tr>
<tr>
<td>134L</td>
<td>control</td>
<td>I</td>
<td></td>
<td>- -</td>
<td>-/ ***</td>
<td>848</td>
</tr>
</tbody>
</table>

KEY: New bone: - absent; + mild; ++ moderate.
Hair cell and dendrite populations: - absent; * moderate loss; ** mild loss; *** normal.
More severe inflammation was marked by a significant increase in the number of polymorphonuclear and mononuclear leukocytes and more pronounced and widespread exudate. Associated with these reactions were more extensive hair cell loss and atrophy of the organ of corti (Fig. 3a). The loss of dendrites was closely associated with atrophy of the organ of corti. New bone was observed in nine of the 20 cochleas, occupying small regions of the scala tympani in the basal turn. The new bone was typically associated with a fibrous tissue reaction, and appeared to originate from the endosteal lining (e.g. Fig. 3a). Electrical stimulation did not appear to affect new bone growth as four of the nine cochleas containing new bone were controls. Fracture of the osseous spiral lamina as a result of electrode insertion trauma was observed in two cochleas (108R and 112L), resulting in moderate to severe spiral ganglion cell loss localised to the damaged region, and new bone growth associated with the fractured spiral lamina.

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**Fig. 1.** a) Basal turn of cochlea 99R. This cochlea was continuously stimulated for 1011 hours, and had an acute inflammation grade of II. Hair cells appeared normal throughout all turns. e, electrode tract (x 30). b) EABR growth responses for cat 99. The recording days are days post-surgery.

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**Fig. 2.** a) Basal turn of cochlea 134R. This cochlea was continuously stimulated for 568 hours and had an acute inflammation grade of I. The organ of corti was present in all turns, however inner and outer hair cells were absent. There was no inflammation reaction associated with this cochlea (x 30). b) EABR growth responses for cat 134.
Loss of hair cells and atrophy of the organ of corti were not associated with electrical stimulation per se. Furthermore, spiral ganglion cell densities were not adversely affected by electrical stimulation. Analysis of the spiral ganglion cell densities of both control and stimulated cochleas showed no statistically significant difference, however the correlation between cell density and the degree of acute inflammation was highly significant (Table 2).

**Table 2**

Multiple linear regression analysis of mean spiral ganglion cell densities

<table>
<thead>
<tr>
<th>Factors</th>
<th>Levels</th>
<th>% of total variance</th>
<th>t value (DF = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrical stimulation</td>
<td>2</td>
<td>1.4</td>
<td>0.99 N.S.</td>
</tr>
<tr>
<td>Degree of inflammation</td>
<td>5</td>
<td>61.1</td>
<td>-5.32 p &lt;0.01</td>
</tr>
</tbody>
</table>

**Auditory brainstem responses**

Immediate post-operative EABR growth response curves were similar for each animal in this study. Growth responses were characterised by a low gradient (large dynamic range) limb with a threshold typically between 50 μA - 0.1 mA, followed by a high gradient limb at stimulus currents of greater than 0.75 mA (e.g. day 0, Fig. 2b). The low gradient limb was identified as electrophonic in origin, whereas the high gradient limb resulted from direct electrical excitation of the auditory nerve (Black et al., 1983). Three distinct trends appeared in the EABR growth responses during the course of the chronic stimulation program, representative growth response curves are shown in Figs. 1b, 2b and 3b. The first group (cats 98, 99, 107 and 112) maintained growth responses characteristic of the immediate post-operative response (Fig. 1b). The second group (cats 109, 117 and 134) showed a progressive increase in EABR thresholds to 0.4-0.5 mA associated with a gradual loss of the low gradient limb, and a shift in the high gradient limb towards lower current levels (Fig. 2b). The third group (cats 101, 108 and 132) developed higher thresholds than experienced with the second group of animals; typically 0.7-0.8 mA, a complete loss of the low gradient limb, and in contrast with the second group of animals, a significant reduction in the slope of the second, high gradient limb, of the EABR growth response (Fig. 3b).
Discussion

The results of the present study indicate that long-term intracochlear electrical stimulation, using a carefully controlled biphasic stimulation regime, does not adversely affect the spiral ganglion cell population. The work does, however, highlight the adverse effect of infection on the neural population of the cochlea.

These results are in agreement with the results of two other investigations examining the effects of electrical stimulation on neural tissue. Agnew et al. (1982) stimulated the cortex of cats for four hours using a balanced biphasic stimulation regime. No histological damage was reported at a charge density of 20 μC/cm² geom./phase at 50 pps, slight local neural degeneration was observed at 100 μC/cm² geom./phase at 20 pps with more extensive neural damage occurring at 100 μC/cm² geom./phase at 50 pps. Walsh and Leake-Jones (1982) formed similar conclusions following chronic intracochlear electrical stimulation for periods of up to 800 hours. They used a charge balanced biphasic current source stimulus and monitored EABR threshold and growth responses, concluding that stimulus induced damage of the auditory nerve occurred at charge densities of 100-200 μC/cm² real/phase with no stimulus damage at 20-40 μC/cm² real/phase. These results, together with the results of the present study where charge densities of up to 32 μC/cm² geom./phase were used, suggest that the maximum biologically safe charge density for platinum electrodes is significantly less than the electrochemically safe limit of 300 μC/cm² geom./phase (Brummer et al., 1977).

Furthermore, in contrast to the conclusion of Agnew et al. (1982), the results of the present study suggest that the total charge injection per se is not related to neural damage, as the total charge injection in this study was as high as 365 coulomb without apparent neural damage.

The EABR results from the present study demonstrated the viability of the spiral ganglion cell population throughout the electrical stimulation program. Furthermore, the results also showed good correlation with cochlear pathology following infection. For example, cochleas with complete or near complete hair cell loss with normal spiral ganglion populations always produced EABR's with an elevation in threshold together with a gradual loss of the low growth (electrophonic) limb of the growth response. In addition, these growth responses typically showed a shift of the high gradient limb towards lower stimulus currents, presumably indicating a change in the electrical resistance patterns within the cochlea as a result of the deafening process (Black et al. 1983). In contrast however, cochleas exhibiting spiral ganglion cell loss in addition to hair cell loss, showed a significant increase in threshold and a reduction in the slope of the high gradient limb of the growth response. It is probable that the higher thresholds experienced in this group of animals was due to a marked loss of dendrites thus resulting in higher stimulus currents in order to directly excite the residual spiral ganglion cells.

Although there is a qualitative correlation between the slope of high gradient limb of the growth response and severe spiral ganglion cell loss, a quantitative correlation as suggested by Smith and Simmons (1983) could not be demonstrated in the present study. This may be due to the differences in electrode geometries and therefore the current distribution in the two studies.

Acknowledgments

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