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Neural substrate essential for suppression of vasopressin secretion and excretion of a water load

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Abstract

Suppression of vasopressin secretion to very low levels is essential for the excretion of excess water. To investigate a role for the preoptic brain region in the suppression of vasopressin secretion and excretion of a water-load, lesions were made in the vicinity of the lamina terminalis in ewes (LTX-sheep) and responses to water-loading or reduction of cerebrospinal fluid NaCl by intracerebroventricular (ICV) isotonic mannitol solution were investigated. In normal conscious sheep, intraruminal water-loading resulted in urine flow rate increasing and urine osmolality decreasing within 1 hour so that renal free water clearance (C_{H2O}) increased from -1.02 ± 0.16 ml/min (mean ±SEM) to a maximum of +4.99 ± 0.62 at 2.5 hours post-water-loading ($p < 0.05$, n = 6). Plasma vasopressin levels fell from 0.88 ± 0.17 pg/ml to undetectable levels (< 0.4 pg/ml, n = 4). In LTX-sheep (n = 6), C_{H2O} did not change significantly after water loading (-1.78 ± 0.13 to -2.03 ± 0.49 ml/min at 2.5 h post water load). Plasma vasopressin levels were inappropriately elevated in water-loaded LTX-sheep (n = 3). ICV mannitol (1 ml/h for 2h) resulted in a water diuresis and increase in C_{H2O} (-1.16 ± 0.12 to +2.81 ± 0.58 ml/min, p <

0.05) after 2h in normal sheep, and plasma vasopressin levels fell significantly from to 0.88 ± 0.23 pg/ml to < 0.4 pg/ml ($p < 0.05$, $n = 6$). However, in LTX-sheep there was no change in C_{H2O} (-1.31 ± 0.14 to -1.35 ± 0.12 ml/min) or plasma vasopressin concentration (1.47 ± 0.18 to 1.60 ± 0.44 pg/ml, n.s.) with ICV mannitol. The results suggest that an inhibitory pathway from the vicinity of the median preoptic nucleus to the supraoptic and hypothalamic paraventricular nuclei plays an important role in the suppression of vasopressin secretion and excretion of excess water.

Introduction

Maintenance of normal body hydration needs adequate water intake and regulation of water excretion by vasopressin (1-3). Suppression of vasopressin secretion is essential when excess water needs to be unloaded (4). If this fails, dangerous hyponatremic/hyposmolar states may result (3, 5-7). The overhydration caused by excessive intake or administration of water results in suppression of AVP secretion that is presumed to be due to withdrawal of osmotic or volemic stimuli (4). However, a possibility that has received insufficient attention is that it may also involve an active neural inhibition of the magnocellular vasopressin-secreting neurons.

How might this be mediated? Hypothalamic magnocellular vasopressin-secreting neurons receive direct afferent inhibitory GABAergic neural input from several brain regions. These regions include the median preoptic nucleus (MnPO), the perinuclear zone around the supraoptic nucleus (SON), nucleus accumbens, arcuate and suprachiasmatic nucei, and a disynaptic inhibitory input from the diagonal band of Broca (8-12). The MnPO is located in the lamina terminalis (LT) (forming the anterior wall of the third ventricle), and this structure has been shown in the rat and sheep to have extensive efferent neural projections to magnocellular neurons of the SON and hypothalamic paraventricular nucleus (PVN) (13-17). While a significant proportion of these efferent projections to the SON are glutamatergic, and have been shown to play an important role in the osmotic stimulation of vasopressin secretion (18-22), a greater proportion are inhibitory GABAergic neurons (9). Such an inhibitory pathway from MnPO to SON could have a role in the suppression of vasopressin secretion after fluid loading.

Consistent with this suggestion are experimental results in goats (23,24) and rats (25) in which the anterior wall of the third ventricle was extensively ablated. These animals incurred damage to the MnPO along with other preoptic and anterior hypothalamic tissue, and approximately half of these animals did not adequately excrete excess body fluid following

water-loading by either intravenous or intraruminal route (23-25). In explanation of these results, it was suggested that an inhibitory neural input to the vasopressin secreting cells, associated with either hypervolemia (23) or hypotonicity (25), was disrupted by the lesions.

Here we have sought to clarify the brain regions underlying these deficits by investigating the effect of ablating the lamina terminalis region on (i) the suppression of vasopressin release and renal excretion of free water in response to gastric water loading and (ii) the inhibition of vasopressin release and production of a water diuresis in response to an experimental reduction of CSF NaCl concentration, an experimental manoeuver shown previously to inhibit vasopressin release thereby causing a water diuresis (26-28).

Materials and Methods

Experiments were performed on 24 adult merino crossbreed ewes (body weight 32-52 kg). All experiments were approved by the Animal Ethics Committee of the Florey Institute which adheres to the Australian National Health and Medical Research Council's Code of Practice for the Care and Use of Experimental Animals. During experiments, sheep were housed in individual metabolism cages in the laboratory; room temperature was maintained at ~20^{o}C. Water was available at all times and a daily ration of 0.8 kg oaten/lucerne chaff was provided at 1700h each day.

Surgical preparation

Prior to experiments, these sheep were surgically prepared on two different occasions while under the influence of general anaesthesia (induction with sodium thiopentone, maintenance with isoflurane/oxygen inhalation). During the first operation, the ovaries were removed (to abrogate any effects of the estrous cycle on fluid balance) and the carotid arteries enclosed in skin loops in the neck (to enable arterial blood sampling and blood pressure measurement). At the second operation, the head was held in a stereotaxic frame and guide tubes (17g stainless steel needles) were implanted over each lateral cerebral ventricle, and either 1 or 2 stainless steel 20g electrodes (for lesions) were implanted into the region of the MnPO in 18 of the sheep. Ventriculography using contrast medium injected into a lateral ventricle was used to accurately determine the stereotaxic position of the lesion electrodes, and normal communication of CSF between lateral and third ventricles. The ventricular guide tubes and electrodes were held in

place with dental acrylic moulded around them and 4-5 stainless steel screws inserted into the skull.

Lesions

Sheep were allowed at least 1 month recovery following the surgery, then a lesion was made in the region of the lamina terminalis by passage of radiofrequency current (LM4 Lesion Maker; Grass Instruments, Quincy MA, USA.) between the electrode tip and a subcutaneously located uninsulated 18 g needle, so that temperature at the electrode tip increased to 55-60^{o}C for 2-3 min. A panting response (increased shallow breaths) to the increased preoptic temperature occurred during this time, but no adverse behavioural responses were observed. Food and water intakes were measured each day. Blood samples were obtained from the carotid artery in the ensuing weeks for measurement of plasma [Na], [K] and osmolality so that the hydrational status of animals could be monitored. Several of the sheep exhibited periods of temporary hypodipsia or adipsia in the days immediately after lesions were made, but they maintained their food intake. These sheep were administered water by rumen tube at 1-2 day intervals to prevent dangerous levels of dehydration. By 2 months all but one sheep had resumed drinking and experiments were commenced.

Experimental protocols

Experiment 1. Water loading. Six normal and 11 lesioned sheep were administered an intraruminal water load. At 0900-1000h, the sheep was taken from its cage, weighed, and a Foley retention catheter inserted into the bladder for collection of urine. A soft plastic naso-gastric tube was also inserted via a nostril into the esophagus and then into the rumen where it was held in place. The sheep was returned to its cage and after approximately 30 min, timed urine collections of 20-30 min commenced. A blood sample (12 ml) was obtained during the first urine collection, then warm water (~30^{o}C) was infused by gravity flow into the rumen over the subsequent 20-40 min so that a total of 75 ml/kg was administered. One of the lesioned sheep that had the most pronounced hypernatremic plasma (plasma [Na] of 158 mmol/l, plasma osmolality 320 mosmol/kg) pre-water-loading was given additional water (1 litre) by nasogastric tube to lower plasma osmolality and [Na] to levels equivalent to the other animals. Urine collections were continued for a further 4-5 hours and blood samples obtained usually at hourly

intervals during this time. Arterial pressure was not recorded. The bladder catheter, carotid needle cannula and naso-gastric tube were removed at the end of the experiment.

Experiment 2. Reduction of CSF [NaCl] by intracerebroventricular infusion of 0.3 mol/l mannitol artificial CSF. A bladder catheter was inserted on the morning of an experiment as described above. In addition a cannula (18g needle) was positioned in a carotid artery in most sheep and connected via a polyethylene tube (filled with heparinized saline) to a pressure transducer. This cannula was used for continuous recording of arterial blood pressure (Gould 2400 chart recorder; Gould Inc, Cleveland OH, USA) and obtaining blood samples. CSF [NaCl] was reduced by an intracerebroventricular (ICV) infusion of an artificial CSF solution containing 300 mmol/l mannitol but no NaCl. This solution also contained other major ions in concentrations based on levels present in natural sheep CSF (29). The ions and their concentrations in mmol/l were as follows; Mg^{++} 1.0, K^{+} 2.8, Ca^{++} 1.2, and HPO_4^{--} 0.5. Control experiments were carried out on animals prepared surgically in the same way as lesion animals except that no electrodes were implanted. These sheep were infused with either the 300 mmol/l mannitol (zero NaCl) artificial CSF solution or artificial CSF solution containing 150 mmol/l NaCl and no mannitol at 1 ml/h for 2h into the lateral cerebral ventricle (via a ventricular guide tube) using a syringe pump (Perfusor IV, B. Braun, Melsungen Germany). Contact between the 20g stainless steel ventricular probe needle tip and CSF was considered adequate if CSF backflow was obtained prior to and at the end of infusions. A pre-infusion period of 1 h was followed by ICV infusion for 2 h, and a post-infusion period of 2h 40min. Urine was collected at 20 min intervals. Blood samples (12 ml) were obtained during the urine collection periods immediately prior to and at the end of the ICV infusion.

Histology

On completion of experiments (usually 3-4 months after making lesions), sheep were killed by intravenous injection of sodium pentobarbitone (100 mg/kg). Cannulae were placed in each carotid artery, the head severed, and 3 litres of isotonic saline solution perfused through the head, followed by 3 litres of 4% formalin in 0.9% NaCl solution. The brain was removed, a block containing the lesion prepared and embedded in paraffin. Sections (10µm) of the block were cut usually in the coronal plane (except for 5 blocks cut in parasagittal plane) with a rotary microtome, mounted on glass slides, and stained with cresyl violet. Glass coverslips were applied prior to examination under a microscope. Lesions were then plotted onto a set of

standard sheep brain sections prepared in the laboratory. Those with more than 75% of the lamina terminalis ablated were designated as LTX-sheep.

Ionic and hormonal assays

Blood samples (12 ml into ice-cold heparinized tubes) obtained from a polyethylene cannula inserted into a carotid artery that had been enclosed surgically in a skin loop, were spun in a refrigerated centrifuge, the plasma decanted and divided into two aliquots. One aliquot was frozen for subsequent arginine vasopressin (AVP) radioimmunassay, while the Na concentration and osmolality were measured on the other non-frozen aliquot. Plasma Na and K concentrations were measured using ion selective electrodes (Beckman System E2A) and urine Na and K concentrations by flame photometry (Model 410C, Corning, Medfield, USA). Osmolality was measured by the freezing-point depression method (Digimatic 3DII Advanced Instruments, Norwood MA, USA).

The renal free water clearances (C_{H2O}) were calculated using the formula $C_{H20} = V(1-U_{osm}/Pl_{osm})$ where V denotes urine volume/time, U_{osm} denotes urine osmolality, and Pl_{osm} denotes plasma osmolality. Renal Na ($U_{Na}.V$) and K ($U_K.V$) excretion rates were also calculated where U_{Na} and U_K denote urine Na and K concentrations respectively.

Plasma AVP concentration was measured by radio-immunoassay. Duplicated 1.5ml samples of plasma were extracted with acetone and ether. They were incubated with AVP antibodies (final dilution 1:500,000) and ^{125}I-AVP. The AVP antibodies were raised in rabbits and had no cross-reactivity with oxytocin (<0.1%) or arginine vasotocin (<0.2%). The sensitivity was 0.4 pg/ml. The intra- and interasssay coefficients of variation were 4.4% and 8.0%, respectively.

Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). For evaluation of excretion rates and plasma ionic and osmolar concentrations, two factor repeated measures on one factor (time) analysis of variance was performed followed by Bonferroni multiple comparisons test for between and within group differences. Differences within groups were performed by comparing average pre-infusion values with individual post-infusion values. Mann Whitney U-test was used for non-parametric analysis of plasma AVP data.

Results

Acute and general effects of lesions

Following the production of lesions, several sheep with extensive lesions in the lamina terminalis (Fig 1, Figs S1-S4) exhibited either hypodipsia or adipsia temporarily for periods of a few days up to 2 months post-lesion. During this time, these sheep were maintained by administration of water into the rumen by tube. When daily water intake and food intake had returned to pre-lesion levels (usually within a few weeks), experiments were performed. Plasma [Na] and osmolality tended to be more variable in these groups post-lesion compared with the control normal sheep (see in Fig 2 and Fig. S5). The results reported below are based on 18 lesioned sheep (11 were used in Experiment 1 and 10 in Experiment 2, i.e. 3 of the sheep were common to both experiments) and 6 normal sheep that were used in both Experiment 1 and 2.

Experiment 1. Effect of ablation of the median preoptic nucleus on the excretion of an intraruminal water load and on plasma vasopressin levels.

Normal sheep. Prior to water loading, urine flow rate was less than 0.7 ml/min and urine osmolality ranged from 441-2390 mosmol/kg in the 6 sheep studied. Plasma [Na] and osmolality were 143.0 ± 0.3 mmol/l and 287.2 ± 0.7 mosmol/kg respectively. Following the administration of warm water (75 ml/kg) into the rumen of sheep, plasma [Na] and osmolality had fallen to 141.7 ± 1.2 mmol/l and 282.8 ± 2.1 mosmol/kg by 2 h post water-loading. A water diuresis commenced within 1 hour in all 6 sheep (Fig 2). The urine flow rate increased to > 5 ml/min (Fig 2) and urine osmolality fell below 102 mosmol/kg in all 6 normal sheep. As a result, the initial negative values for renal free water clearance changed to positive values, increasing to levels of up to 6 ml/min that persisted for the ensuing 4 hours of observations (Fig 2). Plasma [AVP] was measured in 4 of these sheep and fell from 0.88 ± 0.17 pg/ml prior to water loading to undetectable levels (<0.4 pg/ml) by 2 hour after water loading in all 4 normal animals, remaining at undetectable levels during the remaining 2 h. Renal Na excretion rate increased following water loading, while renal K excretion rate fell during the course of the experiment in the control normal animals (see Fig S5).

Lesion sheep. Prior to water loading, plasma [Na] and osmolality ranged from 140-158 mmol/l and 280-318 mosmol/kg respectively in these animals. Plasma [Na] and osmolality (140-146 mmol/l and 280-297 mosmol/kg) in 6 of the LTX-sheep prior to water loading were within the

range observed in normal animals, and were grouped together for statistical analysis. One of the other two sheep was given additional supplemental water so that plasma [Na] and osmolality would fall to levels seen in the other 6 sheep. The lesions, individual results, including plasma AVP levels, for these 2 sheep are shown in Figs 3 and 4, together with one other LTX-sheep in which plasma [AVP] was measured.

Neither urine flow rate increased or urine osmolality decreased to diuretic levels during 4 hours following water loading in the LTX-sheep (Fig 2). This was the case also in the sheep that commenced with a high plasma [Na] and osmolality. Renal free water clearance remained at negative values during the subsequent 4 hours following water-loading (Figs 2 and 4), despite greater reductions in the plasma [Na] and osmolality (to 135.7 ± 0.9 mmol/l and 274.8 ± 2.2 mosmol/kg respectively) than in normal sheep.

The plasma [AVP] measured in 3 of the LTX-sheep are shown in Fig 4. Whereas plasma [AVP] fell to undetectable levels (< 0.4 pg/ml) in the normal water-loaded sheep, plasma [AVP] in the 3 sheep with LT lesions remained at detectable levels that were greater than those of any of the water-loaded normal sheep, despite the plasma [Na] and osmolality of LTX-sheep falling to levels equal to or less than those of the normal water loaded group.

Water-loading in the other 3 sheep that had tissue ablated in the dorsal LT and medial septum, but left MnPO ventral to the anterior commissure and diagonal band ventral to the anterior commissure largely intact, resulted in a water diuresis within 80-100 min, with renal free water clearance increasing to > 3-5 ml/min during the ensuing 2 hours (see Fig S6).

Experiment 2. Effect of ablation of the lamina terminalis on water diuresis and reduced plasma AVP in response to ICV infusion of 0.3M mannitol (low [Na] artificial CSF).

In normal control sheep (n = 6), ICV infusion of 0.3M mannitol caused a large increase in urine flow and reduction in urine osmolality approximately 1 hour after commencing the ICV infusion. This resulted in a significant increase in renal free water clearance in all 6 sheep so that by 60-90 min after the start of the infusion of 0.3M mannitol, renal free water clearance had become positive and remained so for the remainder of the ICV infusion and for up to 60 min following its cessation (Fig 5). Plasma AVP concentration was 0.88 ± 0.23 pg/ml prior to the ICV 0.3 mol/l mannitol infusion and fell significantly to below detectability levels (< 0.4 pg/ml) in control normal sheep by the end of infusions (Fig. 6). Plasma [Na] and osmolality increased significantly by the end of the ICV infusion of mannitol (Table 1). Urine Na excretion rate did not change

significantly during ICV infusion of mannitol, or during the subsequent hour; urine potassium excretion rate tended to fall during the course of observations (Fig. S7). No significant change in arterial pressure was observed during ICV mannitol (Fig. S8). ICV infusion of artificial CSF ([Na] = 150 mM, [mannitol] = 0 mM) in normal sheep did not significantly change urine flow rate, renal free water clearance (Fig. 5), renal Na excretion rate or mean arterial pressure; renal K excretion rate fell significantly during the experiment (Fig S7, S8). No changes in plasma [Na] or osmolality were observed with ICV artificial CSF (Table 1).

In LTX-sheep, ICV infusion of 0.3M mannitol did not cause any significant change in urine flow rate or renal free water clearance during or after the infusion (Fig 5). Plasma AVP levels were measured in 6 of the LTX-sheep and there was no significant change during this period (Fig 6), nor were there any significant changes in plasma [Na] or osmolality in LTX-sheep (Table 1). There were no significant changes in renal Na excretion rate (Fig. S7) during ICV infusion of 0.3M mannitol although some changes occurred in Na excretion after cessation of infusion. Renal K excretion rate fell significantly during the experiment in each group (see Fig S7). Mean arterial pressure was recorded in 7 LTX-sheep and was 88 ± 4 mmHg prior to ICV mannitol in these sheep, not significantly different to 90 ± 4 mm Hg in 6 control normal sheep. There was no significant change in arterial pressure with ICV mannitol (Fig. S8).

Summary of lesion histology.

Of the 24 sheep used in these studies, 18 had lesions that encompassed part or all of the median preoptic nucleus. The effect of water-loading was tested in 11 lesion-sheep. Of these 11 sheep, 8 (LTX-sheep) were classified as having >75-100% of the median preoptic nucleus ablated, with variable amounts of damage to the remainder of the lamina terminalis (i.e. the OVLT and subfornical organ), medial septum (MS), diagonal band of Broca (DB), preoptic periventricular nucleus, medial preoptic region, anterior commissure and midline parts of optic chiasma. There was little or no damage to the SON or PVN. Photomicrographs of typical lesions are shown in Fig 1, and detailed diagrams of the lesions in 5 of the sheep used for grouped data shown in Fig 2 are provided in Supporting Information (Fig. S1). Diagrams of the lesions in the other 3 LTX-sheep are shown in Fig.3.

Another 3 animals, not included in the LTX-sheep group all incurred extensive damage to the MnPO dorsal to the anterior commissure, MS and subfornical organ, but the MnPO

ventral to the anterior commissure remained largely intact (see Fig S2). In 1 of these 3 sheep, all of the OVLT and the vertical limb of the DB unilaterally were also damaged. It was estimated that approximately 50% of the MnPO remained intact in these 3 sheep.

For Experiment 2 (ICV mannitol), 3 of the LTX-sheep studied in the water loading protocol of Experiment 1 (shown by the dotted lines in Fig. 3 and Fig. S1) were used as well as another 7 sheep with lamina terminalis lesions; i.e. a total of 10 LTX-sheep. Of these 10 sheep, 5 incurred damage to part or all of the MnPO, subfornical organ, OVLT, medial septum, diagonal band, anterior commissure, optic chiasma and periventricular and medial preoptic regions. Lesions in the other 5 sheep were more confined to the MnPO with at least 75% of this nucleus both dorsal and ventral to the anterior commissure being ablated (see Fig. S3 and S4 for diagrams of lesions in 7 individual sheep used in Experiment 2). Some damage to medial septum and periventricular preoptic tissue was also observed.

Discussion

To excrete a water load, plasma vaopressin concentration must be reduced to a very low level (< 0.4 pg/ml in sheep). Our experiments show that the integrity of the ventral part of the MnPO and adjacent medial septal and diagonal band region (MnPO/MS/DB) is essential for sheep to adequately excrete a gastric water load or to develop a water diuresis in response to an experimental reduction of CSF sodium concentration. Normal sheep were able to reduce plasma AVP levels to very low concentrations in response to water-loading or ICV mannitol, whereas plasma AVP levels did not fall in response to these experimental procedures in LTX-sheep. This shows that their failure to elicit water diuresis was almost certainly due to an inability to suppress vasopressin secretion. Renal Na and K excretion levels were similar to or higher than normal in LTX-sheep. We have previously observed that glomerular filtration rate and renal blood flow were not reduced in sheep with lesions of the lamina terminalis (30), so the impairment of water diuresis in LTX-sheep cannot be attributed to low levels of solute excretion. Our data suggest that neurons within the MnPO/MS/DB, or nerve tracts passing through it, provide either directly or indirectly, a significant inhibitory influence on vasopressin-secreting magnocellular neurons of the hypothalamic SON and PVN. Although there is evidence of an intrinsic osmosensitivity of the magnocellular vasopressin-secreting neurons (31,32), these cells were evidently not silenced in LTX-sheep, despite the plasma osmolality falling by more than 20-30 mosmol/kg and to levels as low as 262 mosmol/kg after water-loading. By contrast, plasma AVP levels were suppressed to undetectable levels in non-lesioned water-loaded sheep

when plasma osmolality had fallen by only 3-5 mosmol/kg to ~ 280 mosmol/kg. Therefore, we deduce that the vasopressin-secreting neurons are unable to acutely suppress basal vasopressin release without neural input from the MnPO/MS/DB region.

Various amounts of other preoptic and anterior hypothalamic tissue (e.g. OVLT, medial septum, anterior commissure, subfornical organ, periventricular preoptic area) were usually damaged together with the MnPO and diagonal band in lesioned sheep, so it is possible that fibres projecting to magnocellular neurons, and passing through these regions, contributed to the deficit in these animals. Earlier investigations in goats and rats showed that more extensive ablation of the midline periventricular tissue in the anterior wall of the third ventricle that included MnPO resulted in impaired renal excretion of a water load in about half of these animals (23-25), consistent with a failure of these animals to inhibit vasopressin secretion (24,25). By contrast, we consistently observed impaired suppression of vasopressin release in LTX-sheep and show that it is the extent of damage to the MnPO/MS/DB region ventral to the anterior commissure, not the other preoptic or hypothalamic sites, that correlates with the degree of disruption of water diuresis after water-loading or reduced CSF sodium levels. Three sheep, in which the medial septal nucleus and MnPO dorsal to the anterior commissure were extensively damaged, still showed a water diuresis (although somewhat delayed) on water loading. Thus, when > 75% of MnPO tissue was ablated in sheep, the acute diuretic response to these experimental procedures was severely disrupted or abolished, but if ~ 50% of the MnPO remained intact, despite complete destruction of either subfornical organ or OVLT, the diuretic responses were only delayed. The results from these 3 sheep with more dorsal lesions, when contrasted with those from LT-X sheep, show that the MnPO/MS/DB region ventral to the anterior commissure is crucial for the suppression of vasopressin release in overhydrated animals.

Putative inhibitory GABAergic pathways to magnocellular neurons have been identified in the MnPO and surrounding regions. These include the DB and MS, perinuclear zones of the SON and PVN, suprachiasmatic nucleus, arcuate nucleus and lateral hypothalamic area (9-12). Of these regions, only afferent pathways from the MnPO/MS/DB region are likely to be disrupted by the present lesions. Neither magnocellular vasopressin-secreting neurons of the SON and PVN, nor their perinuclear zones were included in lesions. We cannot exclude a role for the diagonal band. However, inhibitory influences of the diagonal band are driven by baroreceptors (10), and because this should not be a factor with ICV mannitol suppression of

vasopressin release, we consider it less likely that damage to the diagonal band is an explanation of our results.

Electrophysiological as well as tract tracing studies show that there are direct efferent neural pathways from the MnPO to vasopressin-secreting neurons of supraoptic nucleus in sheep (15) as well as in rats (9,16). Indeed, adjacent sites in the OVLT and subfornical organ, as well as the MnPO provide extensive excitatory neural inputs from osmoreceptors therein to the SON (2,20-22). Thus, it might have been expected that removal of these glutamatergic links to the vasopressin-secreting neurons would make suppression of vasopressin secretion more amenable. However, our findings suggest that a net inhibitory influence was removed by ablating the MnPO. There is evidence that the MnPO contains many GABA-containing neurons (33), and a major proportion of these have been shown to provide a direct link to vasopressin-containing neurons within the supraoptic nucleus (8,9). *In vitro* electrophysiological studies in hypothalamic slices, cultured magnocellular neurons, or neurohypophysial explants have shown vasopressin magnocellular neurons are inhibited by application of GABA agonists (8,9,34-36). It seems likely that the inhibitory link from the MnPO to vasopressin-containing magnocellular neurons needs to be operational in water-loaded animals for vasopressin release to be suppressed and the water excreted.

Our results suggest also that inhibition of vasopressin secretion that occurs in response to a reduction of CSF sodium concentration is also mediated by the MnPO. Rather than just the removal of a stimulatory influence of the sodium ion (37) on vasopressin release (that would have been achieved by the MnPO lesion alone), our data suggest that reduced CSF sodium levels (resulting from either ICV mannitol or water-loading) may activate an inhibitory influence of the MnPO on vasopressin secretion. It is possible that sodium sensors, which have been shown to be present in neurons within the MnPO (37,38), may control an inhibitory neural pathway from the MnPO to the supraoptic and paraventricular nuclei.

Before concluding, the likely mechanisms at play in these experiments should be considered. It is probable that osmotically stimulated vasopressin secretion by hypothalamic magnocellular neurons depends on the balance of excitatory and inhibitory afferent neural signals influencing these cells (39), as well as their intrinsic osmosensitivity that depends on mechanosensitive non-selective cation channels (2,31,32). In addition, taurine release from local glial cells within the SON and PVN in response to hypotonicity may also have an inhibitory influence on vasopressin secretion in water-loaded animals (2,32,40), although it seems unlikely

that taurine can adequately suppress vasopressin release unless an inhibitory neural influence from the MnPO is extant. There is also evidence in the rat that in addition to excitatory input, the MnPO also receives inhibitory GABAergic neural projections from both the subfornical organ and OVLT (41,42). If these inhibitory GABAergic inputs are activated by hypertonicity and connect synaptically to tonically active GABAergic neurons in MnPO (8) projecting to the SON, we speculate that stimulation of vasopressin secretion may involve inhibition of the tonically active GABAergic pathway from MnPO to SON, i.e. disinhibition of magnocellular neurons, as well as stimulation of excitatory glutamatergic inputs (see Fig 7 for diagram). There is neuropharmacological evidence in conscious rats to support such a proposal. Yamaguchi and Yamada (43) have shown that while microinjection of the $GABA_B$ receptor antagonist baclofen into the MnPO stimulates AVP secretion under isosmotic conditions (due to disinhibition of a tonically active inhibitory input to the glutamatergic pathway from MnPO to SON), the same procedure inhibits vasopressin secretion in response to intravenous infusion of hypertonic saline (due to disinhibition of the inhibitory MnPO to SON pathway). This proposal is shown diagrammatically in Fig 7. We speculate that destruction of such a tonically active inhibitory pathway from MnPO to SON (and PVN), that would have occurred in the current experiments in lesioned sheep, may be the explanation for the impaired suppression of vasopressin secretion during overhydration in these animals.

Active suppression of vasopressin release can occur in water deprived animals and humans just by the act of drinking, even before ingested water has been absorbed into the systemic circulation (44-47). These data also suggest that the suppression of AVP release following rehydration may be caused in part by inhibitory neural influences on the magnocellular vasopressin-containing neurons of the SON and PVN. The neural pathways that mediate this rapid inhibition of vasopressin release associated with drinking have yet to be defined, although at the outset, part of the neural signal appears to involve the superior laryngeal nerve in rats (48). Whether the inhibitory pathway from the MnPO to the SON has a role in drinking-induced suppression of vasopressin secretion is yet to be investigated.

Finally, these results may have relevance to understanding causes of hyponatremia and the syndrome of inappropriate antidiuretic hormone secretion (SIADH), the incidence of which may be as high as 7-20% of the nursing home aged population (49). In addition to several known causes of SIADH such as lung carcinomas and other neoplasms in the gastrointestinal tract and kidneys (50), our results suggest that damage to inhibitory neural influences regulating neurohypophysial vasopressin secretion should also be taken in to consideration as well as

excitatory neural influences. If median preoptic inhibitory signals to vasopressin-secreting neurons are impaired with aging, SIADH with resultant hyponatremia could be a consequence.

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Legends to Figures

Fig. 1. Low power photomicrographs showing typical lesions of the midline lamina terminalis region in 2 different sheep with lesions. Left panel shows a midsagittal section through the third ventricle showing a lesion (outlined by dotted line) encompassing the median preoptic nucleus both dorsal and ventral to the level of the anterior commissure which was also ablated. Right panel shows a coronal section through the anterior wall of the third ventricle showing a lesion (dotted line denotes its boundaries) extending 1-2 mm on either side of the midline destroying the entire lamina terminalis. Abbreviations. ac, anterior commissure; E, electrode track; f, fornix; lv, lateral ventricle; MI, massa intermedia;MPA, medial preoptic area; MS, medial septum; oc, optic chiasm; OVLT, organum vasculosum of the lamina terminalis; SO, supraoptic nucleus; 3v, third ventricle.

Fig. 2. The effect of an intraruminal water-load (75 ml/kg over 20-40 min; denoted by the dotted lines) on renal free water clearance, urine flow rate and plasma osmolality in normal sheep (n= 6, blue line) and sheep with lesions of the lamina terminalis (LTX-sheep, n = 6,

red line). Stars signify significantly different from average pre-water loading values $p < 0.05$, asterisk indicates significant difference ($p < 0.05$ from normal sheep values).

Fig. 3. Diagram of lesions in the 3 LTX-sheep in which plasma [AVP] was measured before and after water loading. A different colour is used to denote each animal and repeated in Fig. 4. The interrupted lines indicate 2 sheep that also were used in Experiment 2 (ICV mannitol.

Fig. 4. The effect of an intraruminal water-load (75 ml/kg over 20-40 min; denoted by the dotted lines) on renal free water clearance, plasma AVP concentration and urine flow rate in 3 sheep with lesions of the lamina terminalis. The colour coding for the individual animals is the same as used in Fig. 3. The mean ± SEM data for normal sheep from Fig. 2 is repeated and indicated by the blue line. An arbitrary value of 0.2 pg/ml (the mid-point of the non-detectable levels of 0-0.4 pg/ml) is given for non-detectable levels normal water-loaded sheep.

Fig. 5. Renal free water clearance and urine flow rate in reponse to intracerebroventricular (ICV) infusion of 0.3 mol/l mannitol CSF at 1 ml/h for 2 h in sheep with lamina terminalis lesions (n = 10, LTX-sheep, red line), or normal sheep (n = 6, blue line). Effect of ICV infusion of artificial CSF at 1 ml/h for 2 h is also shown (n = 4, black line). Star indicates significant difference ($p < 0.05$) from pre-infusion values, asterisk indicates significant difference from normal sheep infused with ICV mannitol.

Fig. 6. Plasma AVP concentrations prior to and at the end of intracerebroventricular infusion of 0.3 mol/l mannitol CSF at 1 ml/h for 2 h in normal control sheep and in 6 LTX-sheep (n = 6, both groups). An arbitrary value of 0.2 pg/ml was given for values below the level of detectability (0.4 pg/ml) that were observed only in normal sheep.

Fig. 7. A diagram of proposed inhibitory and excitatory pathways from the lamina terminalis that may influence vasopressin secretion by neurons of the hypothalamic supraoptic and paraventricular nuclei.

Table 1. Effect of intracerebroventricular (ICV) 0.3 mol/l mannitol on plasma [Na] (pNa) and osmolality (pOsm) in normal or LTX-sheep, and in normal sheep administered ICV artificial cerebrospinal fluid (CSF). * = $p < 0.05$ for comparision between normal sheep given ICV 0.3 mol/l mannitol and other groups. # = $p < 0.05$ comparing pre- and post-ICV infusion values within groups.

ICV infusate	Group		Pre-infusion	Post infusion
Artificial CSF	Normal Sheep (n=4)	pNa (mmol/l)	144.5 +/- 0.3	143.3 +/- 1.0*
		pOsm (mosmol/kg)	294.3 +/- 0.3	293.3 +/- 0.9
0.3M Mannitol (zero Na)	Normal Sheep (n=6)	pNa	144.2 +/- 0.3	147.0 +/- 0.6#
		pOsm	291.3 +/- 0.6	295.5 +/- 1.4#
	LTX-Sheep (n=10)	pNa	143.8 +/- 0.9	142.8 +/- 0.9*
		pOsm	288.4 +/- 2.2	286.9 +/- 2.2*

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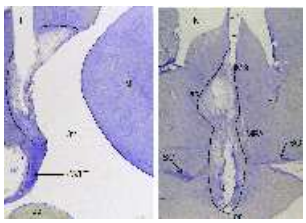

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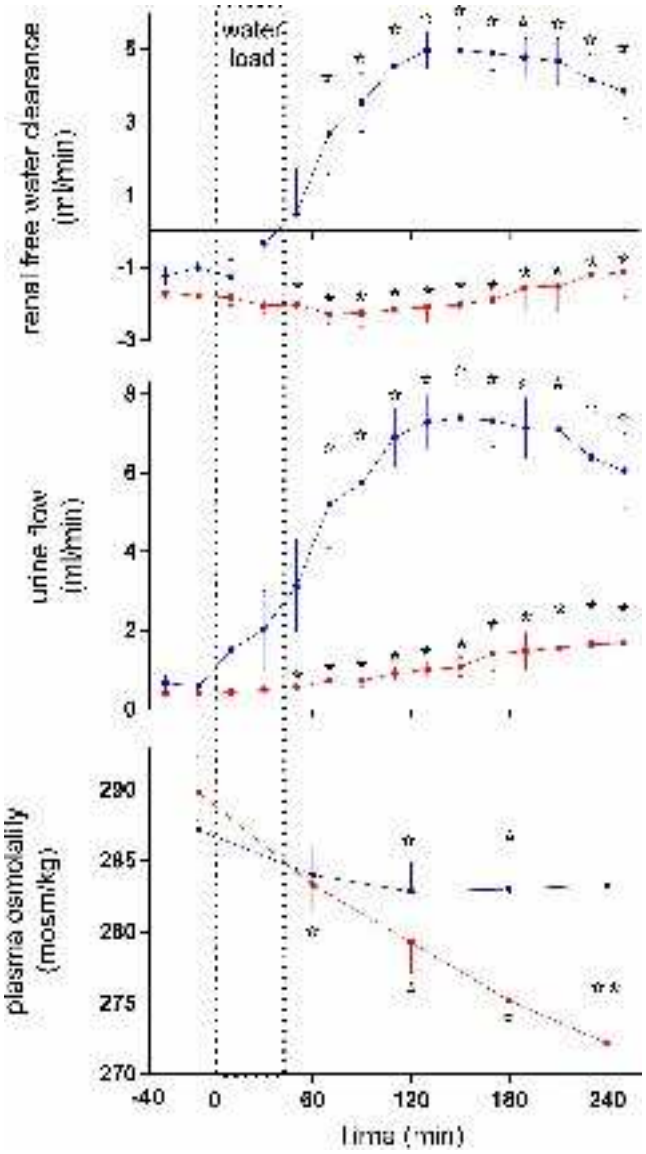

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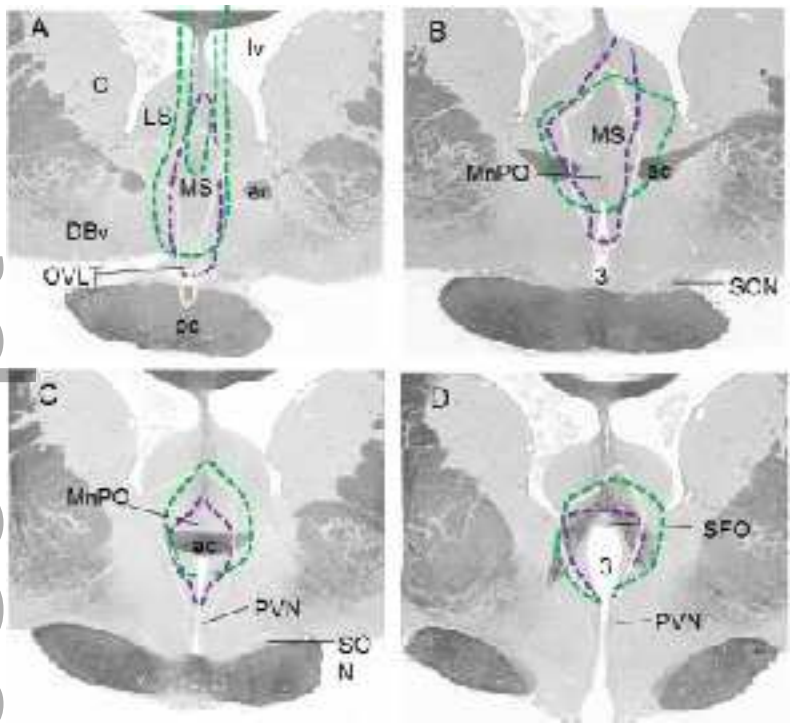

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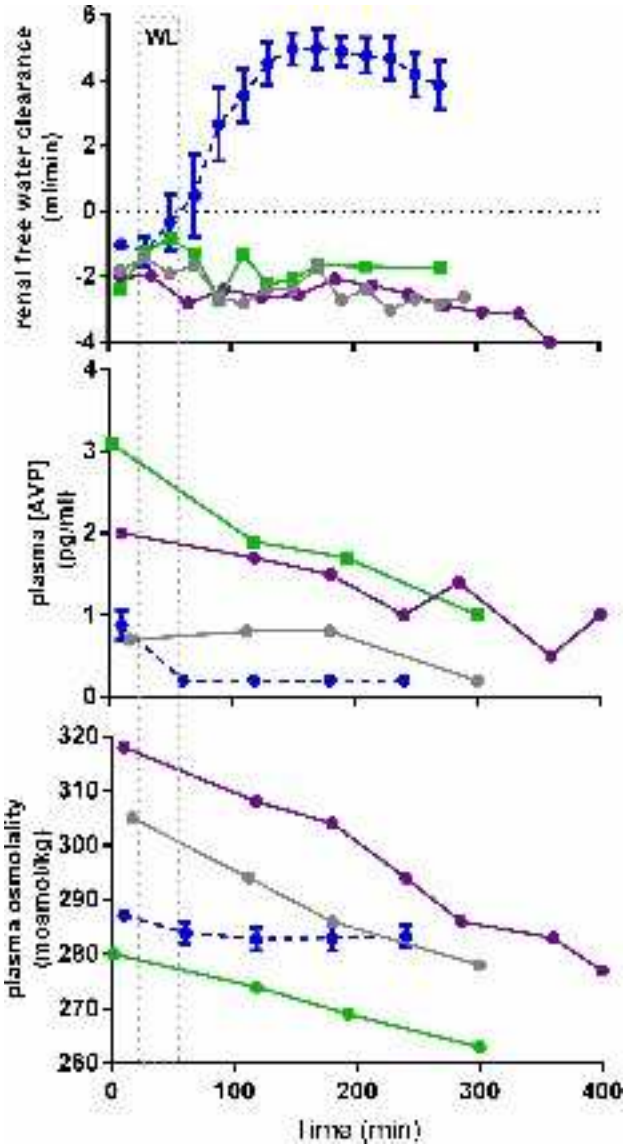

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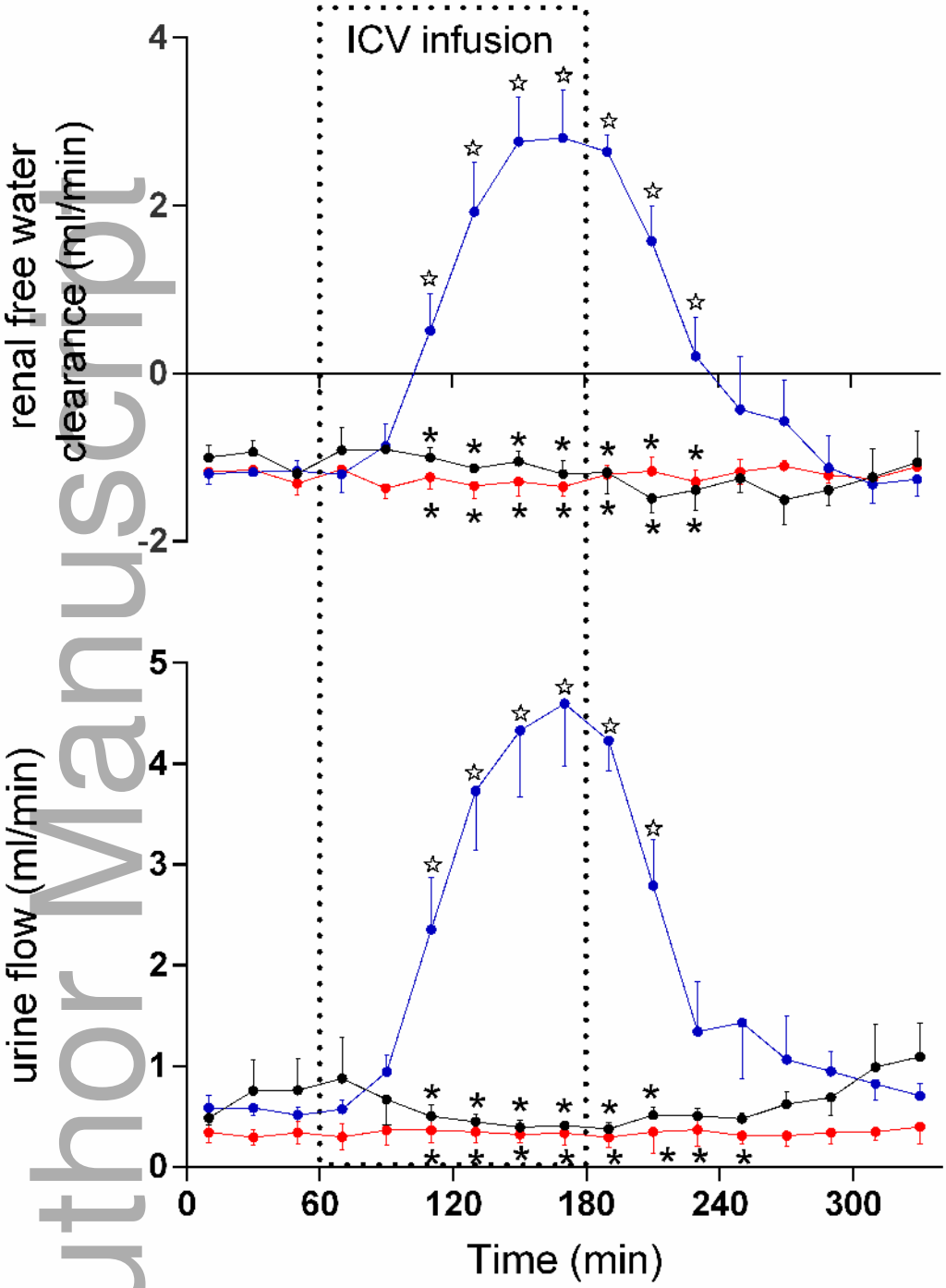

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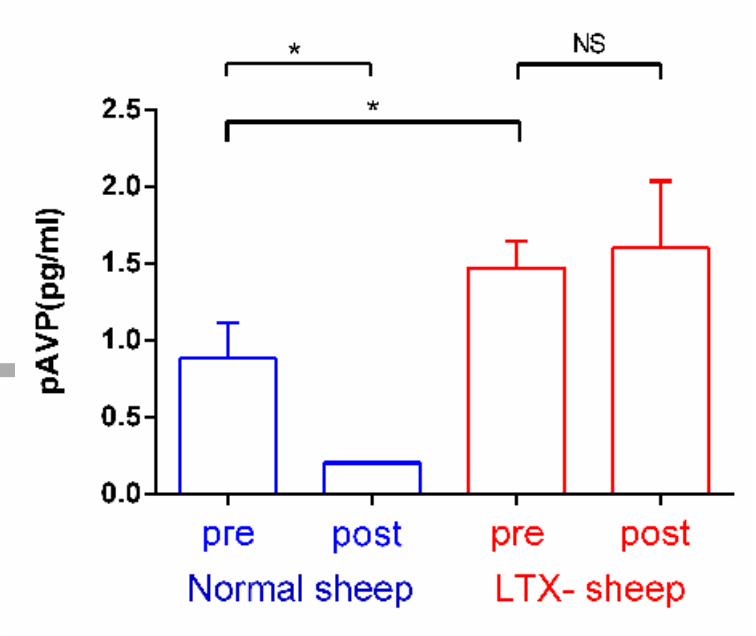

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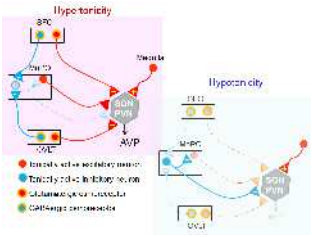

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