Effect of individual or combined ablation of the nuclear groups of the lamina terminalis on water drinking in sheep

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McKinley, M. J., M. L. Mathai, G. Pennington, M. Rundgren, and L. Vivas. Effect of individual or combined ablation of the nuclear groups of the lamina terminalis on water drinking in sheep. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R673–R683, 1999.—The subfornical organ (SFO), organum vasculosum of the lamina terminalis (OVLT), and median preoptic nucleus (MnPO) were ablated either individually or in various combinations, and the effects on drinking induced by either intravenous infusion of hypertonic 4 M NaCl (1.3 ml/min for 30 min) or water deprivation for 48 h were studied. Ablation of either the OVLT or SFO alone did not affect drinking in response to intravenous 4 M NaCl, although combined ablation of these two circumventricular organs substantially reduced but did not abolish such drinking. Ablation of the MnPO or MnPO and SFO together also substantially reduced, but did not abolish, drinking in response to intravenous hypertonic NaCl. Only near-total destruction of the lamina terminalis (OVLT, MnPO, and part or all of the SFO) abolished acute osmotically induced drinking. The large lesions also reduced drinking after water deprivation, whereas none of the other lesions significantly affected such drinking. None of these lesions altered feeding. The results show that all parts of the lamina terminalis play a role in the drinking induced by acute increases in plasma tonicity. The lamina terminalis appears to play a less crucial role in the drinking response after water deprivation than for the drinking response to acute intravenous infusion of hypertonic saline.

hypertonicity; subfornical organ; organum vasculosum of the lamina terminalis; median preoptic nucleus; dehydration

THE SUBFORNICAL ORGAN (SFO), median preoptic nucleus (MnPO), and organum vasculosum of the lamina terminalis (OVLT), which are situated serially in the anterior wall of the third ventricle, together form the lamina terminalis (LT). Extensive evidence has implicated these nuclei in the neural control of water intake. For instance, it is widely recognized that circulating angiotensin (ANG II) acts on neurons in the SFO to induce water drinking in some species (18, 40, 41, 44). Thus ablation of the SFO results in reduced drinking responses to systemically administered ANG II in rats (40) and dogs (44). However, this does not occur in sheep (23). It is possible that the SFO may also participate in osmotically stimulated water intake (14, 18, 23), but the evidence in favor of this is less clear cut (see Refs. 18, 40, and 46).

In regard to the MnPO, if this nucleus is ablated, drinking responses to either systemically or centrally administered ANG II or hypertonic saline may be severely impaired (4, 7, 9, 19). Despite the proposals that the osmoreceptors subserving drinking are situated in a region lacking the blood-brain barrier, such as the OVLT (24, 43), few studies have investigated the role of the OVLT per se in drinking. This is principally because lesions of this circumventricular organ usually also encroach on the ventral part of the MnPO and periventricular preoptic nucleus, as is the case with the widely studied anteroventral third ventricle (AV3V) lesion in rats (3, 17) or with ablation of the optic recess in sheep (22). These AV3V lesions caused widespread disruption of body fluid homeostasis, and it is likely that damage to the OVLT was a contributing factor to the observed deficits in fluid intake (3, 22). In dogs, ablation of the OVLT with little damage elsewhere was achieved and this disrupted the drinking response to systemic infusion of concentrated saline solution (43). It has long been known that such infusions induce drinking because the toxicity of the body fluids is increased and an osmoreceptor mechanism is stimulated (5, 12, 48). A diencephalic location of osmoreceptors subserving drinking was also proposed (1, 31).

Further indications of the importance of the tissue in and around the LT for water drinking are demonstrations that more global ablation of this region in goats often results in permanent adipsia (2, 36), as is sometimes the case in rats with AV3V lesions (3). Recent studies of c-fos expression, used as an indicator of neuronal activity, show that water deprivation or systemic administration of hypertonic saline, ANG II, or isoproterenol, which are all dipsogenic, cause neurons throughout the LT to be activated (10, 11, 15, 25, 30, 34).

The aim of the present study was to determine the relative importance of each of the structures in the LT either individually or together for osmotically stimulated water drinking. Thus we have ablated each part of the LT, either individually or in combination to determine its involvement in the water drinking responses to intravenous infusion of hypertonic saline or water deprivation.

METHODS

Animals

Fifty-seven merino crossbred female sheep were studied. They were housed in individual metabolism cages, which allowed separation of urine and feces. Access to water was provided at all times, except when the effects of water deprivation were studied. Sheep were fed 0.8 kg chaff each day at 1630. Room temperature was maintained at 20°C.

Surgery

Before being studied, these sheep underwent surgical preparation while they were anesthetized. Anesthesia was induced with an intravenous injection of thiopentone sodium (15 mg/kg) and maintained by inhalation of fluothane-oxygen.
gas mixture. The first operation involved oophorectomy (to reduce influences of the estrous cycle on fluid balance) and enclosure of the common carotid arteries in skin loops in the neck to enable the sampling of arterial blood and measurement of arterial pressure. In a second operation, a guide tube consisting of a 17-gauge needle was implanted over the lateral ventricle while the head of the sheep was held rigid in a stereotaxic frame. Contrast medium (0.5 ml of sodium ioephendylate, Iopamiro, Schering) was then injected into the lateral ventricle to enable radiographic visualization of the third ventricle in all planes. After drilling small holes in the skull with a dental drill, we then implanted either one or two electrodes (19-gauge stainless steel tubing blocked at one end) into or near various parts of the anterior wall of the third ventricle. Electrodes were aimed at either the SFO, OVLT, MnPO, or a combination of any two of these sites. The electrodes were insulated with epoxylite, except for the tips, where the length of the uninsulated electrode varied from 1 to 7 mm, depending on the size of the lesion intended. The electrode(s) and guide tube were then fixed in position with dental acrylic molded around them and four stainless steel screws, which had been inserted into the skull. Animals recovered well from this surgery as indicated by their general demeanor and their resumption of normal food and water intakes within 1–2 days. All surgical and experimental procedures were approved by the Institute's Animal Experimentation Ethics Committee, which adheres to the Australian National Health and Medical Research Council's Code of Practice for the care and use of animals in experiments.

Experiments

At least 1 wk after the surgery for implantation of electrodes, day food and water intakes were measured and a series of presion experiments were conducted. These were to measure the drinking responses to 1) water deprivation, 2) intravenous infusion of hypertonic saline, and 3) (in 8 sheep) the water drinking associated with feeding. Daily food and water intake of the animals was also recorded.

Water deprivation. A blood sample (10 ml) was obtained from the carotid artery (for measurement of plasma osmolality and Na concentration ([Na]+)), the sheep was weighed, then the water bin was removed from the cage. After 48 h, these procedures were repeated, then a bin of fresh water at room temperature was presented to the sheep. Water intake was measured at 30 min after presentation. The duration of 48 h for water deprivation was chosen because merino sheep are well adapted to a dry environment, and in our experience 1 day of water deprivation does not consistently result in immediate water drinking in all sheep. In addition, sheep grazing in the field in hot dry conditions and eating dry food are known to voluntarily refrain from drinking water for up to 24 h, depending on the distance required to travel to a source of water (42).

Intravenous infusion of hypertonic saline. Several hours before an experiment, the sheep was removed from the cage and a polyethylene cannula was inserted into a jugular vein and sutured in place. This cannula was filled with heparinized saline until it was used to infuse 4.0 mol/l NaCl at 1.3 ml/min for 30 min. Water was removed from the cage during the infusion, and blood samples (10 ml) were obtained from the carotid artery before the infusion and at 10, 20, and 30 min after commencement of the infusion to monitor the gradual increase in plasma [Na+] and osmolality. When the infusion was terminated, a bin of water was presented to the sheep and the cumulative volume of water drunk during the subsequent 30 min was measured.

Water intake associated with feeding. This was measured in eight sheep before and several weeks after the production of lesions. Sheep were presented with 800 g of food, and the amount of food eaten and water drunk was measured each hour for the ensuing 4 h.

Production of lesions. After daily water intake had been measured for 4–5 days and blood samples (10 ml) were obtained during this period by puncture of the carotid artery with a 20-gauge needle, a lesion was made in tissue in or near the anterior wall of the third ventricle. The lesion was produced by application of radio frequency current (100 kHz, 12–15 V, 50–60 mA) between the electrode and an indifferent electrode placed under the skin. The electrode tip was heated to 58–60°C, as measured by a thermistor inserted into the tip of the tube that formed the electrode. Apart from hyperventilation (probably due to heating of the preoptic region) no other disturbance or any adverse effect to animals was noticed.

In the following days, water and food intakes were measured and blood samples were obtained at regular intervals. At least 2–3 wk were allowed to elapse so that the water intake of animals had reached a steady state before the dipsogenic challenges were repeated that were tested before ablation of the brain tissue. In some cases where there was more prolonged adipsia or hypodipsia, 1–3 mo elapsed before the daily water intake stabilized and dipsogenic challenges were tested. These dipsogenic challenges were separated by at least 5 days. In several of the animals, other experiments testing the effect of the lesions on plasma renin levels and renal Na excretion were also carried out in pre- and postlesion periods. These have been reported elsewhere (26, 28).

Brain Histology

When all experimental protocols were completed (normally 3–4 mo postlesion), the animals were killed with an intravenous injection of pentobarbitone sodium (Lethabarb, Arnold of Reading). The head was perfused via the carotid arteries with 3 liters of isotonic 0.9% NaCl solution followed by 3 liters of 4% Formalin in 0.9% NaCl solution. Brains were then removed from the skull, and a block of tissue containing the lesion site was prepared and embedded in paraffin. Sections of this block were cut (usually in the coronal plane, but sometimes in the parasagittal plane) on a rotary microtome, and after being mounted on glass slides, they were stained with cresyl violet and covered with glass coverslips. The site of the lesion was then examined microscopically and mapped by drawing the projected image using a microfilm reader. To show some representative lesions, the lightly stained slides were scanned (SprintScan 35, Polaroid) and computer enhanced to increase contrast, which enabled the lesion site and anatomic features to be observed more readily.

Experimental Groups and Statistical Treatment of Results

After histological examination of the lesions, the results from each animal were allocated to a particular group, depending on the amount and the position of the ablated tissues. They were designated as 1) SFO group, in which the SFO was destroyed (90–100% damage), 2) OVLT group, in which the OVLT was ablated (100% damage), 3) MnPO group, which had a lesion throughout the median preoptic nucleus (90–100% damage), 4) SFO/OVLT group, in which the SFO and OVLT were ablated (>90% ablation) but the MnPO was mostly intact (<20% damage), 5) SFO/MnPO group, which sustained combined destruction of the SFO (100% ablated) and the MnPO dorsal to the anterior commissure (100% ablated), 6) SFO/OVLT group, in which the lesion...
encompassed the SFO (100% ablated) and the entire MnPO (100% ablated), 7) OVLT/MnPO, in which the OVLT and MnPO were entirely ablated but parts (60–100%) of the SFO were left intact, 8) LT group, in which the entire LT (OVLT, MnPO, and SFO) was completely destroyed, 9) DMS group, in which midline septal tissue either dorsal or rostral to the SFO was ablated, but the LT was undamaged, and 10) VL group, in which preoptic tissue lateral to the midline lamina terminalis and ventral to the anterior comissure was ablated.

For each group, the postlesion response to either infusion of hypertonic saline or water deprivation was compared with the prelesion values within individual sheep by means of a paired t-test with Bonferroni correction. Across-group comparison was made by nonparametric statistical analysis of those groups in which lesions reduced drinking to intravenous hypertonic saline. Because of the small number of observations in some groups, the OVLT/MnPO and LT groups were combined, giving a group combining all sheep in which 85% of the LT was ablated and another group in which the SFO/dMnPO and SFO/MnPO were combined. Postlesion results were expressed as the percentage of the prelesion response and subjected to Kruskal Wallis analysis and subsequent multiple-comparison test for nonparametric data (49).

Chemical Analysis

Plasma was obtained from blood samples by centrifugation, then stored at 4°C. The sodium and potassium concentration was measured by ion-selective electrode using a Beckman Clinical Analyser. Freezing-point depression was the method used to measure osmolality of plasma by osmometer (Advanced Instruments, model 3 CII).

RESULTS

Histological Examination of Brains

Examination of brain tissue in the vicinity of the LT of sheep with lesions showed that variable amounts of the LT had been ablated, as judged by gross loss of brain tissue or extensive gliosis. Histological examples of several lesions are shown in Figs. 1–4, and a diagrammatical representation of the common area of overlap of lesion for each group is shown in Fig. 5. In five of the animals (SFO group), the SFO incurred total or near total (>90%) ablation, with the lesion extending dorsally in the midline 2–3 mm dorsal to the SFO. No other parts of the LT were damaged, but in three of these animals there was damage unilateral to the midline in the medial preoptic region. In four sheep (OVLT group), the OVLT was completely ablated, leaving the MnPO and SFO intact, but some dorsal septal tissue was usually damaged; whereas in another seven sheep (MnPO group), the MnPO dorsal and ventral to the anterior commissure was severely damaged with the OVLT and SFO left intact (Fig. 1). Six sheep incurred total or near-total damage to both the OVLT and SFO (Figs. 2 and 5), but most of the MnPO was left intact (>80% undamaged), and these were designated as the SFO/OVLT group. Another group of four sheep (SFO/dMnPO) had the SFO and MnPO dorsal to the anterior commissure totally ablated, but the remaining tissue in the LT (i.e., ventral MnPO and OVLT) was intact (Fig. 5); whereas in four sheep (SFO/MnPO group), only the OVLT remained intact, with the SFO and MnPO incurring extensive damage (Fig. 5). The most extensive lesions were incurred by seven sheep with total destruction of the LT (LT group; Figs. 3 and 5). Only slightly less damaged were another four animals in which the OVLT and MnPO were completely ablated, but only parts of the SFO were destroyed (OVLT/MnPO group; Figs. 4 and 5). Two other groups in which tissue lateral and/or dorsal to the LT was ablated but the midline SFO, MnPO, and OVLT were left undamaged served as controls. The first group of six sheep (DMS group) had midline tissue ablated that was rostral and/or dorsal to the SFO, mainly in the medial septal region (Fig. 3). Another four animals (VL group) had more ventral lesions lateral to the midline,
which damaged variable amounts of tissue in the medial and periventricular preoptic regions or nucleus of the diagonal band (Fig. 3). Six other sheep did not fall into any of these groups, three of them had parts of the dorsal MnPO and medial septal nucleus ablated, but the remainder of the LT remained intact, another two had total ablation of the SFO, MnPO, and approximately half the OVLT in its dorsal aspect ablated, and one animal had a lesion in the ventral MnPO.

**Water Intakes of Sheep**

Effect of individual ablation of the SFO, OVLT, or MnPO on water drinking. Apart from two sheep (1 from each group), who ceased drinking for 1 day, ablation of the OVLT or SFO had little effect on daily water drinking in the days immediately after production of the lesion. There was no reduction in daily drinking in any of these sheep in the longer term (Table 1). Five of the seven sheep with lesions in the MnPO were severely hypodipsic for 2–3 days after production of the lesion, but after this time normal intakes recovered. Ablation of the SFO or OVLT had no significant effect on water intake in response to intravenous infusion of hypertonic NaCl (Fig. 6) or after water deprivation for 2 days (see Table 3). Ablation of the MnPO (dorsal and ventral to the anterior commissure) consistently reduced water drinking in response to intravenous infusion of hypertonic saline without usually abolishing it (Fig. 6); however, the drinking response to water deprivation was not reduced (see Table 3). In three sheep, the MnPO dorsal to the anterior commissure was ablated, leaving the SFO and also the ventral part of the MnPO intact. In these sheep, daily water intake was not affected by the lesion (Table 1). Water drinking in response to intravenous hypertonic saline was reduced markedly in two sheep and marginally in the other. Drinking after water deprivation was similar to prelesion levels in two of these sheep and abolished in the other. In the sheep with damage to the ventral MnPO, water intake in response to intravenous hypertonic saline was 800 ml prelesion and fell to 425 ml postlesion, whereas water intake after water deprivation was not reduced postlesion.

Effect of combined ablation of the SFO and OVLT on water drinking. Combined ablation of the SFO and the OVLT with only minor damage to the MnPO resulted in
marked hypodipsia (daily water intake 0–250 ml) in
one animal for a period of 7 days, and this sheep had to
be maintained with intraruminal administration of
water during this time to prevent severe dehydration.
Two other sheep were adipsic for 1 day immediately
after combined ablation of these circumventricular
organs, and the other three were unaffected. In the
longer term, when a steady state was reached, there
was no significant change in daily water intake with
this lesion (Table 1). Five of the six sheep with SFO/
OVLT lesion showed a drinking response to intrave-
rous hypertonic saline, but this response was greatly
reduced in comparison to the prelesion period (Fig. 6).
However, this lesion did not reduce drinking after
water deprivation (Table 3). Daily water intake was not
reduced by these lesions.
Effect of combined ablation of the SFO and the MnPO
on water drinking. When the SFO was ablated together
with either MnPO dorsal to the anterior commissure or
MnPO dorsal and ventral to the anterior commissure,
there was no acute reduction of water drinking immedi-
ately after the production of these lesions nor was there
a significant change in daily water drinking in the
longer term (Table 1). Both lesions caused a significant
reduction in the drinking response to intravenous hypotonic saline, with the reduction in drinking tend-
ing to be greater with the larger lesion (Fig. 6). One of
four sheep in the SFO/dMnPO group and two of four
sheep of the SFO/MnPO group exhibited no water
intake in response to intravenous hypertonic saline.
Drinking after water deprivation for 2 days was not
inhibited significantly by these lesions (Table 3). In the
two sheep with ablation of the SFO, MnPO, and dorsal
part of the OVLT, water intake in response to intrave-
rous hypertonic NaCl was 1,680 and 1,740 ml, respec-
tively, prelesion and was abolished postlesion. Water
intake in response to 2-day water deprivation fell from
4.18 prelesion to 3.2 liters postlesion in one of these
sheep and from 3.59 to 1.31 liters in the other.
Effect of combined ablation of the MnPO and OVLT
on water drinking. When the OVLT and MnPO were
ablated in combination, leaving parts of the SFO intact
(OVLT/MnPO group), all four sheep became severely
hypodipsic for periods that ranged from 3 to 18 days
after production of lesions. During this time they were
maintained by intraruminal administration of water.
Water drinking eventually recovered to a level similar
to that observed before the lesion (Table 1). Combined
OVLT/MnPO ablation resulted in a total abolition of
water drinking in response to intravenous hypertonic
saline in all four sheep (Fig. 6). Water intake after
water deprivation was not significantly reduced; how-
ever, the responses were quite variable between ani-
mals (Table 3).
Effect of total ablation of the LT on water drinking.
In the LT group of sheep, the SFO, OVLT, and MnPO were
all ablated in combination. All sheep exhibited adipsia

Fig. 3. Computer scans of coronal sections from the brain of a sheep
of the OVLT/MnPO group. Lesion (dashed lines) is shown to have
damaged the OVLT (A), MnPO (B and C), and parts of the SFO (D).
Part of the SFO remains intact (C). Boundary of the anterior
commissure marked with a solid line. Bar = 1 mm.

Fig. 4. Computer scans of coronal sections from
brain of a sheep of the LT group showing destruction
of the OVLT and MnPO (A) and MnPO and SFO (B
and C). Lesion is indicated by dashed line. Bar = 1 mm.
or hypodipsia immediately after the production of the lesion. Four of these sheep were severely hypodipsic for periods of 3–12 mo and had to be maintained with regular intraruminal administration of water, whereas others eventually regained or exceeded levels of daily water intake observed in the prelesion period. Only two of these seven sheep showed any drinking responses to intravenous hypertonic NaCl, and these responses were

**Fig. 6.** Histogram showing water intake in response to intravenous infusion of 4 mol/l NaCl during the prelesion period (open bar) and postlesion (filled bar) in each of the different groups of sheep. Mean and SE shown. Difference from preinfusion value for a particular group is indicated by *P < 0.05; **P < 0.01.

**Table 1.** Average daily water intake in sheep prelesion and postlesion

<table>
<thead>
<tr>
<th>Lesion Site</th>
<th>n</th>
<th>Daily Water Intake, liters</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Prelesion</td>
</tr>
<tr>
<td>SFO</td>
<td>5</td>
<td>1.70 ± 0.20</td>
</tr>
<tr>
<td>OVL T</td>
<td>4</td>
<td>1.85 ± 0.17</td>
</tr>
<tr>
<td>MnPO</td>
<td>7</td>
<td>1.87 ± 0.23</td>
</tr>
<tr>
<td>SFO/OVL T</td>
<td>5</td>
<td>1.76 ± 0.33</td>
</tr>
<tr>
<td>dMnPO/SFO</td>
<td>4</td>
<td>1.41 ± 0.54</td>
</tr>
<tr>
<td>MnPO/SFO</td>
<td>4</td>
<td>2.22 ± 0.46</td>
</tr>
<tr>
<td>OVL T/MnPO</td>
<td>4</td>
<td>1.36 ± 0.19</td>
</tr>
<tr>
<td>LT</td>
<td>7</td>
<td>1.99 ± 0.30</td>
</tr>
<tr>
<td>DMS</td>
<td>6</td>
<td>1.97 ± 0.35</td>
</tr>
<tr>
<td>VL</td>
<td>4</td>
<td>2.50 ± 0.61</td>
</tr>
</tbody>
</table>

Values are means ± SE; water intakes on 4–6 successive days averaged to obtain values for each sheep. Prelesion, before ablation of brain tissue; postlesion, when sheep with lesions in various parts of the lamina terminalis (LT) or elsewhere had recovered from periods of adipsia and reached a steady state of water intake (usually 3 wk to 2 mo); SFO, subfornical organ; OVL T, organum vasculosum of the lamina terminalis; MnPO, median preoptic nucleus; DMS, ablation of midline septal tissue either dorsal or rostral to the SFO; VL, ablation of preoptic tissue lateral to midline LT and ventral to the anterior commissure; dMnPO, ablation of the MnPO dorsal to the anterior commissure.
very low (50 and 100 ml). Four of the sheep were not spontaneously consuming water at the time of testing the response to intravenous hypertonic saline. Their responses (no drinking) are included in the group data. When the LT and OVLT/MnPO groups were combined (making a group with either total or near-total destruction of the LT), postlesion response was 1.4 ± 1.1% of the prelesion response, which was statistically less than the postlesion response of the OVLT/SFO group (37.1 ± 12.4%, P < 0.05) or MnPO group (33.2 ± 7.7%, P < 0.05) but not significantly different from the combined SFO/MnPO, dMnPO group (14.3 ± 4.8%). Water drinking after 2 days of water deprivation was significantly reduced in the LT group of sheep despite the much larger increase in plasma [Na] and osmolality that resulted from the period of water deprivation (Table 3).

Effect of lesions lateral or dorsal to the LT. Sheep with lesions lateral to the ventral part of the LT (VL group) or in the dorsal part of the medial septal region (DMS group) did not show reduced water intakes after lesions (Table 1), nor were their water intakes in response to intravenous hypertonic saline (Fig. 6) or water deprivation (Table 3) changed by these lesions.

Effect of lesions on daily food intake. Water drinking associated with feeding was measured in eight of the sheep that had shown large deficits in the drinking response to intravenous hypertonic saline. Before ablation of either the total LT (4 sheep of the LT group) or median preoptic nucleus (3 sheep of the MnPO group and 1 of the OVLT/MnPO group), all sheep exhibited a copious water intake during the 4 h after commencement of feeding, when they consumed most of their daily food ration (Fig. 7). In animals with either ablation of the LT in total or the MnPO, water intake was virtually absent during the first 2 h after feeding, then began to recover in the group with MnPO lesions. The water intake of the LT group remained low during the 4 h associated with feeding, although by 24 h after feeding more drinking had occurred. There was no reduction in food intake in either group of sheep (Fig. 7).

Effect of lesions on plasma [Na] and osmolality. Similar increases in plasma sodium concentration (~6 mmol/l) and osmolality (~12 mosmol/kg) occurred with intravenous infusion of 4 M NaCl in the postlesion experimental trials compared with the prelesion result (Table 2). However, in comparison with the response obtained in the prelesion period, there was a significantly larger increase in plasma sodium concentration and osmolality in response to water deprivation in the LT group of sheep with lesions in the LT, but the reduction in body weight was not significantly different from the other groups (Table 3).

**DISCUSSION**

This study in sheep is the first systematic study of the role of all the parts of the LT in osmotically induced drinking. It confirms and extends the findings of earlier studies in several species (2–4, 7, 9, 14, 17, 18, 22, 23, 40, 43), showing an important role of tissue in the LT for such drinking. The major finding of the present study is that near-total ablation of the LT is necessary to abolish water drinking in response to an acute intravenous infusion of hypertonic saline. Although ablation of considerable parts of the LT, e.g., the MnPO or combinations of the MnPO and parts of the SFO causes significant disruption of hypertonic saline-induced drinking, it is rarely abolished by such lesions. This is also the case in rats, where individual ablations of the SFO, MnPO, or AV3V region (which includes OVLT and ventral MnPO) reduce but do not abolish drinking in response to acute administration of hypertonic saline (3, 7, 14, 17, 22) and in some cases, MnPO lesions (6, 7) and SFO lesions (40, 46).
osmotically stimulated drinking in sheep, it would be premature to conclude that these circumventricular organs are not involved in osmotically stimulated drinking. This is because 1) combined ablation of these two circumventricular organs with a mostly intact MnPO severely disrupted drinking in the six sheep in which this lesion was achieved and 2) if the OVLT remains the only intact part of the LT, a drinking response (albeit greatly reduced) can still be obtained, whereas combined ablation of the SFO, OVLT, and MnPO abolishes the drinking response to acute intravenous infusion of hypertonic saline. The resilience of the drinking response to acute hypertonicity suggests to us that there may be considerable redundancy of this important homeostatic function within the LT, and it is also possible that compensatory mechanisms or neural pathways come into play in the weeks after the production of lesions in parts of the LT. In this regard, many sheep showed a gradual return of day-to-day water drinking, particularly in the first month after ablation of this tissue. Of relevance to this discussion are observations that specific neurons in both the OVLT and SFO are activated (as indicated by expression of c-fos) in response to peripherally administered hypertonic solutions (10, 11, 15, 30).

Although these lesions often caused quite severe effects, they do not enable conclusions to be drawn as to the precise function of the roles of the OVLT, SFO, and MnPO in osmotically induced drinking, and these studies need to be considered in the context of relevant results obtained with other methodologies. Lesions destroy fibers of passage as well as neuronal cell bodies in the LT. The SFO, OVLT, and MnPO are reciprocally interconnected with each other and with a number of other sites outside the LT (28, 29, 32, 37), and these neural connections will also be disrupted in varying measure by the various lesions made in these experiments. However, there is considerable data from investigations of expression of c-fos in rats (10, 11, 15, 30) and electrophysiological studies both in vivo and in vitro to show that hypertonicity results in activation of neurons in the SFO (8, 39), OVLT (33, 38, 45), and MnPO (13), i.e., throughout the LT in rats.

With regard to the proposal that osmoreceptors subserving drinking and vasopressin secretion are located in the OVLT and/or the SFO (24, 43), neurons in both these sites are activated by systemic administration of hypertonic saline in rats (8, 10, 11, 15, 30) and there is evidence from in vitro studies using low [Ca]-high [Mg] medium that neurons in the OVLT are directly sensitive to altered tonicity (45). If the reduction in osmotically stimulated drinking caused by combined ablation of the OVLT and SFO is due to disruption of osmoreceptors in these sites, they are not the only sites of sensors detecting hypertonicity or NaCl concentration, because responses were not abolished. Earlier studies using

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### Table 2. Plasma Na and K concentrations and osmolality immediately before and at the end of intravenous infusions of NaCl

<table>
<thead>
<tr>
<th>Lesion Site</th>
<th>Plasma Na, mmol/l</th>
<th>Plasma K, mmol/l</th>
<th>Plasma Osmolality, mosmol/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preinfusion</td>
<td>Postinfusion</td>
<td>Preinfusion</td>
</tr>
<tr>
<td>SFO (n = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prelesion</td>
<td>145 ± 1</td>
<td>155 ± 1</td>
<td>4.2 ± 0.1</td>
</tr>
<tr>
<td>Postlesion</td>
<td>144 ± 1</td>
<td>155 ± 1</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td>OVLT (n = 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prelesion</td>
<td>145 ± 1</td>
<td>155 ± 1</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td>Postlesion</td>
<td>145 ± 3</td>
<td>158 ± 2</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td>MnPO (n = 7)</td>
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<tr>
<td>Prelesion</td>
<td>146 ± 1</td>
<td>156 ± 1</td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td>Postlesion</td>
<td>146 ± 1</td>
<td>156 ± 0</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>SFO/OVLT (n = 6)</td>
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<td></td>
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<tr>
<td>Prelesion</td>
<td>145 ± 1</td>
<td>155 ± 1</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>Postlesion</td>
<td>146 ± 1</td>
<td>156 ± 0</td>
<td>4.1 ± 0.2</td>
</tr>
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<td>SF/O/dMnPO (n = 4)</td>
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<td>Prelesion</td>
<td>145 ± 1</td>
<td>155 ± 1</td>
<td>4.2 ± 3.6</td>
</tr>
<tr>
<td>Postlesion</td>
<td>143 ± 1</td>
<td>153 ± 1</td>
<td>4.3 ± 0.1</td>
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<tr>
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<td>144 ± 2</td>
<td>153 ± 2</td>
<td>4.4 ± 0.1</td>
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<tr>
<td>Postlesion</td>
<td>142 ± 2</td>
<td>151 ± 3</td>
<td>4.1 ± 0.1</td>
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<tr>
<td>OVLT/MnPO (n = 4)</td>
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<td></td>
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<tr>
<td>Prelesion</td>
<td>146 ± 1</td>
<td>154 ± 1</td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>Postlesion</td>
<td>146 ± 1</td>
<td>158 ± 1</td>
<td>4.0 ± 0.2</td>
</tr>
<tr>
<td>LT (n = 7)</td>
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<td></td>
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<tr>
<td>Prelesion</td>
<td>144 ± 0</td>
<td>154 ± 0</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td>Postlesion</td>
<td>144 ± 1</td>
<td>153 ± 1</td>
<td>4.2 ± 0.0</td>
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<tr>
<td>DMS (n = 6)</td>
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<td></td>
</tr>
<tr>
<td>Prelesion</td>
<td>145 ± 1</td>
<td>155 ± 1</td>
<td>4.0 ± 0.1</td>
</tr>
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<td>Postlesion</td>
<td>144 ± 1</td>
<td>155 ± 1</td>
<td>4.2 ± 3.7</td>
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<td>VL (n = 4)</td>
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<tr>
<td>Prelesion</td>
<td>144 ± 1</td>
<td>154 ± 1</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>Postlesion</td>
<td>142 ± 2</td>
<td>152 ± 2</td>
<td>4.4 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. Preinfusion, postinfusion, immediately before and at end of infusion of 4 mol/l NaCl at 1.3 ml/min for 30 min, respectively.
intracerebral injections of hypertonic solution to stimulate drinking in goats and rats also suggested a more widespread distribution of the diencephalic sites at which injected hypertonic solutions induced drinking (1, 31); however, the hypertonicity of the solutions used in these studies was supraphysiological.

With regard to the MnPO, previous studies, mainly in rats, have shown also that ablation of this nucleus by electrolysis or by injection of neurotoxin causes a reduced drinking response to systemic hypertonicity (4, 7, 9, 19) and the present results confirm this. Neurons in the MnPO of sheep increase electrical activity (21) in response to acute increases in plasma tonicity, which suggests that ablation of the MnPO disrupts responses to systemic hypertonicity by destroying such neurons rather than fibers of passage. Whether or not neurons in the MnPO are directly stimulated by hypertonicity and/or NaCl concentration has not been determined yet, although recent data with injection of 0.2 or 0.6 M NaCl into the MnPO suggest they are (13). However, such concentrations of NaCl are outside normal physiological bounds and other osmotic agents were not tested, leaving it possible that Na or Cl specifically (see Refs. 2, 24, 35) may have stimulated these neurons. Neurons in the MnPO may also be indirectly stimulated via synaptic input from neurons stimulated in the OVLT and SFO that have copious efferent connections projecting to the MnPO (28, 29, 37). The subsequent efferent neural circuitry from the MnPO that may subserve osmotically induced drinking remains to be elucidated.

In several sheep with extensive ablation of the LT, water drinking associated with feeding was delayed considerably. This may reflect the fact that an acute increase in plasma osmolality occurs with feeding (20), which may be part of the stimulus for postprandial drinking in the sheep and therefore should be affected by lesions that disrupt drinking responses to intravenous hypertonic saline. It is also possible that destruction of central angiotensinergic mechanisms subserving drinking may be involved in the disruption of postprandial drinking (16), because central angiotensin receptors in the MnPO and other parts of the LT (27) would be destroyed by these lesions. There is evidence that central angiotensin may play a role in postprandial drinking in sheep as well as rats (20).

An interesting observation in the present studies was the lack of effect of most of the lesions on the drinking response after water deprivation for 48 h, despite the fact that many of these lesions substantially disrupted hypertonic saline-induced drinking. Only total ablation of the LT consistently reduced dehydration-induced drinking. However, it should be noted that significantly greater increases in plasma osmolality occurred in

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**Table 3.** Water intake and body weight loss after deprivation from water for 48 h during either the prelesion or postlesion period for sheep in which tissue in various parts of the lamina terminalis were ablated

<table>
<thead>
<tr>
<th>Lesion Site</th>
<th>Water Intake, liters</th>
<th>Weight Loss, kg</th>
<th>Plasma Na, mmol/l</th>
<th>Plasma Osmolality, mosmol/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Dehydr</td>
<td>Normal</td>
<td>Dehydr</td>
</tr>
<tr>
<td></td>
<td>Prelesion</td>
<td>Postlesion</td>
<td>Prelesion</td>
<td>Postlesion</td>
</tr>
<tr>
<td>SFO (n = 5)</td>
<td>Prelesion 1.95 ± 0.27</td>
<td>Postlesion 2.42 ± 0.43</td>
<td>145 ± 1</td>
<td>152 ± 1</td>
</tr>
<tr>
<td></td>
<td>Prelesion 2.20 ± 0.24</td>
<td>Postlesion 2.28 ± 0.17</td>
<td>145 ± 1</td>
<td>153 ± 1</td>
</tr>
<tr>
<td></td>
<td>OVLT (n = 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prelesion 2.10 ± 0.42</td>
<td>Postlesion 2.56 ± 0.65</td>
<td>143 ± 1</td>
<td>150 ± 2</td>
</tr>
<tr>
<td></td>
<td>Prelesion 2.56 ± 0.26</td>
<td>Postlesion 2.32 ± 0.25</td>
<td>145 ± 1</td>
<td>151 ± 1</td>
</tr>
<tr>
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<td>MnPO (n = 7)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Prelesion 2.54 ± 0.32</td>
<td>Postlesion 2.28 ± 0.17</td>
<td>143 ± 1</td>
<td>156 ± 1</td>
</tr>
<tr>
<td></td>
<td>Prelesion 2.32 ± 0.25</td>
<td>Postlesion 2.27 ± 0.15</td>
<td>143 ± 1</td>
<td>156 ± 1</td>
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<td>OVLT/MnPO (n = 4)</td>
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<tr>
<td></td>
<td>Prelesion 1.55 ± 0.55</td>
<td>Postlesion 1.56 ± 0.47</td>
<td>146 ± 1</td>
<td>153 ± 1</td>
</tr>
<tr>
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<td>Prelesion 1.86 ± 0.19</td>
<td>Postlesion 2.42 ± 0.28</td>
<td>144 ± 1</td>
<td>150 ± 1</td>
</tr>
<tr>
<td></td>
<td>SFO/MnPO (n = 4)</td>
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</tr>
<tr>
<td></td>
<td>Prelesion 1.85 ± 0.32</td>
<td>Postlesion 2.04 ± 0.50</td>
<td>144 ± 1</td>
<td>152 ± 2</td>
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<tr>
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<td>Prelesion 2.20 ± 0.43</td>
<td>Postlesion 2.13 ± 0.29</td>
<td>143 ± 1</td>
<td>152 ± 2</td>
</tr>
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<td>Prelesion 2.53 ± 0.33</td>
<td>Postlesion 1.45 ± 0.48</td>
<td>143 ± 2</td>
<td>148 ± 1</td>
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<td>Prelesion 2.28 ± 0.48</td>
<td>Postlesion 2.58 ± 0.34</td>
<td>140 ± 1</td>
<td>153 ± 1</td>
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<td>LT (n = 5)</td>
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<tr>
<td></td>
<td>Prelesion 2.46 ± 0.34</td>
<td>Postlesion 0.30 ± 0.14*</td>
<td>143 ± 1</td>
<td>149 ± 1</td>
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<td>Prelesion 2.74 ± 0.14</td>
<td>Postlesion 3.00 ± 0.25</td>
<td>148 ± 2</td>
<td>171 ± 2*</td>
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<td>DMS (n = 6)</td>
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<td>Prelesion 2.35 ± 0.29</td>
<td>Postlesion 2.05 ± 0.13</td>
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<td>152 ± 2</td>
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<td>Postlesion 2.05 ± 0.17</td>
<td>144 ± 1</td>
<td>151 ± 1</td>
</tr>
<tr>
<td></td>
<td>VL (n = 4)</td>
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<td></td>
<td>Prelesion 2.91 ± 0.66</td>
<td>Postlesion 3.14 ± 0.55</td>
<td>142 ± 2</td>
<td>152 ± 2</td>
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<tr>
<td></td>
<td>Prelesion 2.78 ± 0.67</td>
<td>Postlesion 3.23 ± 0.44</td>
<td>142 ± 2</td>
<td>152 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE. Plasma Na concentration and osmolality at commencement (Normal) and end (Dehydr) of the periods of water deprivation are shown. No significant difference between corresponding pre- and postlesion values occurred unless indicated by an asterisk (P < 0.05).
postlesion dehydration trials compared with the prelesion trials in four of the groups. These were the SFO/OVLT group (24.3 ± 3.4 cf. 10.9 ± 2.2 mosmol/kg, P < 0.05), the SFO/MnPO group (22.7 ± 2.6 cf. 9.5 ± 1.6 mosmol/kg, P < 0.01), MnPO group (22.7 ± 1.9 cf. 11.6 ± 1.8 mosmol/kg, P < 0.05), and the OVLT/MnPO group (30.5 ± 1.9 cf. 12.8 ± 1.0 mosmol/kg, P < 0.01). Therefore, relative to the degree of cellular dehydration that resulted postlesion with water deprivation, the drinking response to dehydration may be considered inappropriately low with these lesions. Although ablation of the AV3V region and to a lesser extent the SFO resulted in a reduced drinking response after water deprivation in rats, the results are generally in agreement with those in sheep in that a substantial drinking response still remained in these rats after water deprivation (3, 18) and the drinking response is reduced in sheep relative to the degree of cellular dehydration. Water deprivation for 24–48 h results in increased expression of c-fos throughout the LT of rats (25), indicating that dehydration results in increased neural activity in the LT. The results show that although the LT has a role in such dehydration-induced drinking, it may be less critical than some other central sites. It also suggests that the neural mechanism mediating the drinking response to an acute rapid increase in systemic tonicity (i.e., over 30 min) may be quite different from that subserving drinking after dehydration involving a much slower increase in plasma osmolality and a concomitant reduction in extracellular volume. Although peripheral sensors detecting these changes in extracellular volume are probably important, it is also possible that osmoreceptors and NaCl sensors in regions other than the LT may have a role.

Another interesting observation made with many of these sheep was the temporary (for 1–25 days) or more prolonged periods of adipsia or hypodipsia after ablation of various parts of the LT. Such effects have also been observed previously in goats and sheep (2, 36, 47). During this time, sheep have to be maintained with gastric water loads, otherwise fatal dehydration would probably ensue as has been observed in rats with AV3V lesions (3). On recovery of the daily water intake to prelesion levels, animals may maintain fluid balance, although the swings in plasma [Na] and osmolality tend to be considerably greater than in normal sheep. In four sheep, adipsia was considered permanent in that it persisted for up to 1 yr. In one sheep a partial recovery occurred after 11 mo. The extent of lesion in the region of the LT that resulted in permanent adipsia tended to have more tissue damage lateral to the midline in comparison to lesions where the adipsic period was temporary.

With regard to the specificity of behavioral effect of lesions in the LT, it should be noted that the sheep appeared in excellent condition and without exception consumed their normal daily ration of food in the customary manner (Fig. 7). Such lesions however do affect other osmoregulatory functions in sheep such as vasopressin secretion, increased sodium excretion, and inhibition of renin secretion that occurs in response to hyperosmolarity (26, 27, 28). In conjunction with the present results, these data indicate that tissue throughout the LT may either detect and/or coordinate the neural mechanisms regulating the responses to acute hypotonicity and to a lesser extent prolonged hypotonicity as occurs in dehydrated animals.

We thank Angela Gibson for analysis of plasma samples, Rod Paterson for radiography, and Craig Thomson for technical assistance and animal care.

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