Central losartan blocks natriuretic, vasopressin, and pressor responses to central hypertonic NaCl in sheep

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MANY INVESTIGATORS, studying various mammalian species, have demonstrated that administration of hypertonic saline into the lateral or third cerebral ventricle elicits a large increase in renal sodium excretion (1, 2, 5, 8, 15, 17, 24). This effect has been construed as evidence for a cerebral control of sodium excretion. Intracerebroventricular infusion of hypertonic saline also results in water drinking, vasopressin secretion, and an increase in arterial pressure (1, 2, 6, 14, 15, 24, 29). Natriuretic, dipsogenic, and pressor responses and vasopressin secretion also result from the intracerebroventricular infusion of ANG II (1, 24, 28). There is recent evidence that dipsogenic (6), natriuretic (15, 24), and vasopressin-releasing responses (11, 29) to intracerebroventricular hypertonic NaCl are inhibited by central blockade of ANG II receptors. It is therefore possible that centrally administered hypertonic NaCl and centrally administered ANG II stimulate similar neural pathways.

To investigate this idea in more detail, we have tested the effect of centrally administered losartan on the natriuresis, vasopressin release, and pressor response to both stimuli in conscious sheep. Losartan is an angiotensin AT1 receptor antagonist that has been shown previously to block many of the actions of ANG II, including those to intracerebroventricular ANG II (27). For comparison, and to test the specificity of effects of intracerebroventricular losartan, the effects of centrally administered losartan on the natriuretic, pressor, and vasopressin-releasing responses to intracerebroventricular carbachol have also been tested, because neural pathways subserving pressor and dipsogenic responses to intracerebroventricular carbachol have been dissociated from those mediating responses to intracerebroventricular ANG II (3, 12, 25).

MATERIALS AND METHODS

A total of 8 merino-crossbred ewes (body weight 37–45 kg) were studied. These sheep were housed in individual metabolism cages, fed 0.8 kg of oaten-alalfa chaff each day and given access to drinking water at all times except during an experiment. The Na+ content of the diet was monitored, and sufficient NaCl was added to the food to ensure a daily dietary intake of 100 mmol of Na+. Surgical preparation of the sheep involved ovariotomy to remove influences of the estrous cycle on fluid balance, enclosure of the carotid arteries in bilateral skin loops in the neck, and implantation of guide tubes over each lateral ventricle as described previously (18). These procedures were performed while the sheep were under the influence of general anesthesia induced by intravenous thiopentone sodium (15 mg/kg) and maintained with a Fluothane-oxygen gas mixture. Sheep were allowed at least 2 wk to recover from surgery before experiments were commenced. All surgical and experimental techniques had been approved by the Institute Animal Experimentation Ethics Committee, which adheres to the Australian Code of Practice for the care and use of animals for scientific purposes.

Experimental protocols. On the morning of an experiment, a catheter was inserted into the bladder for the collection of urine. For experiments involving intravenous infusion, a polyethylene cannula was introduced into a jugular vein. A subcutaneous injection of local anesthetic (1% lidocaine; Astra Pharmaceuticals, North Ryde, Australia) was administered for this procedure. Approximately 1 h later, urine collections were commenced. Blood pressure was measured throughout all experiments from a needle fixed in the carotid artery and connected via polyethylene tubing (filled with...
heparinized saline) to a pressure transducer. Arterial pressure was displayed on a chart recorder (Gould).

Effect of intracerebroventricular losartan on responses to intracerebroventricular ANG II. In the first experiment, the effect of intracerebroventricular losartan on responses to intracerebroventricular ANG II was tested in four sheep. An infusion at 1 ml/h of either artificial cerebrospinal fluid (CSF) (see Ref. 22 for composition) or losartan (DuPont-Merck) dissolved in artificial CSF at 1 mg/ml was commenced. After 1 h, these infusions were combined with ANG II (Auspep) at 10 µg/h for 1 h. At the end of the intracerebroventricular infusion of ANG II, the infusions of artificial CSF or losartan continued for a further 90 min. A control experiment in which artificial CSF alone was infused into the lateral ventricle at 1 ml/h for 3 h was also performed in another four sheep. Urine was collected at 15-min intervals throughout the experiment, and its Na⁺ and K⁺ concentrations were measured. A 10-ml sample of blood was obtained from the cannula in the carotid artery immediately before the commencement and again at the end of the intracerebroventricular infusion of ANG II.

Effect of intracerebroventricular losartan on responses to intracerebroventricular hypertonic saline. A solution of 0.75 mol/l NaCl was infused into the lateral ventricle at 66 µl/min for 20 min with or without losartan at 1 mg/h in five sheep. The intracerebroventricular infusion of losartan dissolved in artificial CSF commenced 1 h before the intracerebroventricular infusion of hypertonic NaCl and continued for the duration of the experiment. The infusion of 0.75 mol/l NaCl was kept to 20 min to avoid unwanted effects such as muscle tremors, which we have sometimes observed with more prolonged intracerebroventricular infusions of hypertonic saline. The control experiment utilized a similar protocol except that artificial CSF alone was infused into the lateral ventricle at 1 ml/h before and after the intracerebroventricular infusion of 0.75 mol/l NaCl. Urine collections and arterial pressure recordings were maintained throughout the experiment, and blood samples (10 ml) from the carotid artery were obtained just before and at the end of the infusion of 0.75 mol/l NaCl.

An experiment was also performed to ascertain whether peripheral effects of losartan cleared into the circulation from the brain may have influenced the results. Therefore, the effect of intravenous infusion of losartan (at 1 mg/h, commencing 1 h before the intracerebroventricular infusion of hypertonic NaCl), on responses to intracerebroventricular 0.75 mol/l NaCl at 66 µl/min for 20 min was tested.

Effect of intracerebroventricular losartan on responses to intracerebroventricular carbachol. Carbachol (25 µg infused over 15 min) was infused into the lateral ventricle with or without losartan at 1 mg/h in 5 sheep. The intracerebroventricular infusion of losartan at 1 mg/h or the control infusion of artificial CSF was commenced 1 h before the infusion of carbachol and continued for the remainder of the experiment. The dose of carbachol and its period of infusion were chosen to obtain a similar increase in arterial pressure and sodium excretion as was obtained with intracerebroventricular 0.75 mol/l NaCl and also to avoid unwanted side effects of carbachol (such as continued bleeding) that we had observed in other experiments in sheep using greater doses of carbachol. Urine was collected at 15-min intervals until 1.5 h after the end of the intracerebroventricular infusion of carbachol. Blood samples (10 ml) were drawn from the carotid artery before and at the end of the intracerebroventricular infusion of carbachol.

Analytic methods. Blood samples were centrifuged, and the plasma was split into two samples. One sample was stored at −20°C for future radioimmunoassay. The other, together with the urine samples, was stored at 4°C. The Na⁺ and K⁺ concentrations of urine samples were measured by flame photometry on a Technicon Flame 4 autoanalyzer. 

RESULTS

Effect of intracerebroventricular losartan on responses to intracerebroventricular ANG II. Before the intracerebroventricular infusion of ANG II, plasma AVP concentrations ranged from 0.4 to 1.1 pg/ml. Intracerebroventricular infusion of ANG II at 10 µg/h increased plasma AVP levels to 13.1 ± 5.3 pg/ml (range 4.5–28.5 pg/ml). This increase in plasma AVP concentration induced by intracerebroventricular ANG II was abolished by intracerebroventricular infusion of losartan, with the plasma AVP concentrations being <0.4 pg/ml in three sheep and 1.7 pg/ml in the other at the end of the intracerebroventricular infusion of ANG II with losartan. A large increase in the urinary excretion rate of both Na⁺ and K⁺ also occurred after the intracerebroventricular infusion of ANG II (Fig. 1).

Arterial pressure was recorded in only three of the four sheep due to difficulties in maintaining the arterial cannula in one animal. Mean arterial pressures were 62, 67, and 70 mmHg before the intracerebroventricular infusion of ANG II and increased to 89, 84, and 97 mmHg, respectively, at the end of the 1-h infusion in these sheep. Intracerebroventricular treatment with losartan reduced thesepressor responses to intracerebroventricular ANG II, with the values changing from 65, 72, and 74 to 68, 74, and 85 mmHg, respectively.
when losartan was administered before and during the intracerebroventricular infusion of ANG II.

In four sheep infused with artificial CSF, there was no significant change in arterial pressure or plasma AVP concentration (1.1 ± 0.3 to 1.2 ± 0.4 pg/ml). No significant change in renal sodium or potassium excretion occurred during the intracerebroventricular infusion of artificial CSF (Fig. 1).

No change in plasma Na⁺ or K⁺ concentration occurred as a result of any of these intracerebroventricular infusions (Table 1).

Effect of intracerebroventricular losartan on responses to intracerebroventricular hypertonic saline.

When 1 ml 0.75 mol/l NaCl was infused into the lateral ventricle at 66 µl/min for 20 min, a large natriuresis and kaliuresis occurred within 20–40 min in four of the five animals studied and arterial pressure increased by 20 mmHg in all five sheep. Plasma AVP levels ranged from 0.4 to 2.4 pg/ml before the intracerebroventricular infusion of 0.75 mol/l NaCl and increased greatly (to 24–65 pg/ml) in response to this infusion in the same four animals that exhibited a natriuresis (Fig. 2). In the four sheep that responded, the intracerebroventricular infusion of losartan abolished the natriuretic response and significantly reduced the plasma AVP levels reached in response to this stimulus (Figs. 2 and 3). A kaliuresis was still observed but of lower magnitude. The total potassium excretion caused by intracerebroventricular hypertonic NaCl during the 90-min observation period

<table>
<thead>
<tr>
<th>Intracerebroventricular Infusion</th>
<th>Plasma [Na]</th>
<th>Plasma [K]</th>
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<tbody>
<tr>
<td>aCSF</td>
<td>Pre: 145.5±0.3</td>
<td>Post: 145.3±0.5</td>
</tr>
<tr>
<td>ANG + aCSF</td>
<td>Pre: 143.5±0.9</td>
<td>Post: 142.3±1.3</td>
</tr>
<tr>
<td>ANG + Los</td>
<td>Pre: 143.0±0.4</td>
<td>Post: 142.5±0.6</td>
</tr>
<tr>
<td>0.75 M Na⁺ + aCSF</td>
<td>Pre: 143.8±0.7</td>
<td>Post: 143.6±0.9</td>
</tr>
<tr>
<td>0.75 M Na⁺ + Los</td>
<td>Pre: 144.6±0.8</td>
<td>Post: 144.6±0.9</td>
</tr>
<tr>
<td>0.75 M Na⁺ + intravenous Los</td>
<td>Pre: 143.4±0.7</td>
<td>Post: 143.6±0.2</td>
</tr>
<tr>
<td>Carbachol + aCSF</td>
<td>Pre: 144.8±1.1</td>
<td>Post: 144.6±0.8</td>
</tr>
<tr>
<td>Carbachol + Los</td>
<td>Pre: 145.6±0.5</td>
<td>Post: 144.8±1.2</td>
</tr>
</tbody>
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Values are means ± SE in mmol/l. Brackets indicate concentration. aCSF, artificial cerebrospinal fluid; Los, losartan.

![Fig. 1](image1.png) Changes in renal sodium and potassium excretion of sheep (n = 4) administered intracerebroventricular (ICV) infusions of ANG II at 10 µg/h for 1 h (indicated by shaded box) combined with intracerebroventricular infusions of either artificial cerebrospinal fluid (aCSF) or losartan (1 mg/h; ○). x, Effect of intracerebroventricular infusion of artificial CSF at 1 ml/h for 3 h in another 4 sheep. Means and SE are shown. Significant difference from corresponding preinfusion value: **P < 0.01.

![Fig. 2](image2.png) Plasma arginine vasopressin (AVP) (n = 4 sheep) and changes in mean arterial pressure (n = 5 sheep) caused by intracerebroventricular hypertonic NaCl during the 90-min observation period.
was 23.3 ± 2.5 mmol, but this was reduced significantly to 14.3 ± 3.5 mmol by intracerebroventricular losartan (P < 0.05, paired t-test). The pressor response to intracerebroventricular hypertonic NaCl was also reduced significantly by intracerebroventricular treatment with losartan (Fig. 2). Intravenous infusion of losartan at 1 mg/h did not significantly reduce the plasma AVP concentrations reached or the natriuretic, kaliuretic, or pressor responses to intracerebroventricular 0.75 mol/l NaCl. Plasma Na⁺ or K⁺ concentration was not affected by these infusions (Table 1).

Effect of intracerebroventricular losartan on responses to intracerebroventricular carbachol. Both mean arterial pressure and plasma AVP levels increased with intracerebroventricular infusion of carbachol. These responses were not significantly affected by intracerebroventricular infusion of losartan (Fig. 4). When combined with a prior infusion of artificial CSF, intracerebroventricular carbachol caused a large increase in renal Na⁺ and K⁺ output lasting at least 1 h. When an intracerebroventricular infusion of losartan was combined with intracerebroventricular carbachol, a significant increase in Na⁺ and K⁺ excretion still occurred (Fig. 5). However, the natriuretic response was less pronounced, with the total amount of Na⁺ in urine excreted during the 90 min after the start of intracerebroventricular infusion of carbachol combined with losartan being 12.5 ± 4.1 mmol, which was significantly less (P < 0.05) than with intracerebroventricular carbachol alone, when 23.5 ± 4.9 mmol of Na⁺ were excreted. Plasma Na⁺ and K⁺ concentrations were not affected by these infusions (Table 1).

DISCUSSION

These results show clearly that centrally administered losartan inhibits natriuretic, kaliuretic, pressor,
and AVP-releasing responses to intracerebroventricular infusion of hypertonic NaCl as well as to ANG II in conscious sheep. Peripherally administered losartan did not inhibit these responses to intracerebroventricular hypertonic NaCl, indicating that losartan was acting at a central site to block these effects. Most of the responses to intracerebroventricular carbachol were not reduced by centrally administered losartan, suggesting that they are largely independent of central ANG II.

These data complement other recent results demonstrating that intracerebroventricular losartan also blocks drinking, natriuretic, or vasopressin release in response to intracerebroventricular hypertonic saline in a number of mammals (6, 11, 15, 23, 24). The results also show clearly that intracerebroventricular losartan inhibits the pressor response to intracerebroventricular hypertonic saline, a point that was not evident in earlier studies in the rat. Such results are indicative that at least some, if not all, of the neural pathways mediating responses to intracerebroventricular ANG II. A possible explanation of these results is that intracerebroventricular infusion of hypertonic saline stimulates the release of endogenous ANG II utilized as a transmitter in neural pathways in the brain involved in the regulation of arterial pressure, vasopressin secretion, and sodium excretion. ANG II has been identified immunohistochemically in vesicles adjacent to synapses at several sites within the central nervous system (CNS), including the hypothalamic paraventricular nucleus, subfornical organ, and other parts of the lamina terminalis (13, 19), that have been implicated in centrally mediated pressor, natriuretic, and AVP-releasing responses to intracerebroventricular hypertonic saline (4, 22).

High-affinity receptor binding sites for ANG II in these regions (16) are also consistent with a neurotransmitter role for ANG II in the CNS. Of particular relevance to the present study are observations in the rat that hypertonicity results in the release of both ANG II and ANG III in the region of the paraventricular nucleus (10, 22).

Recently, it has been reported that losartan administered into the subfornical organ of rats blocks natriuretic responses to intracerebroventricular hypertonic NaCl (24). This suggests that the subfornical organ may be the site at which intracerebroventricular losartan is exerting its inhibitory effect on ANG II action. Because the subfornical organ lacks a blood-brain barrier and is influenced by blood-borne ANG II, it is feasible that the effects of losartan on responses to intracerebroventricular hypertonic NaCl are due to blockade of ANG II (from the circulation) acting on the subfornical organ if circulating ANG II increased in response to intracerebroventricular hypertonic NaCl. However, it has been shown previously that intracerebroventricular hypertonic NaCl causes a reduction in circulating renin levels (9, 15), and therefore probably a reduction in blood-borne ANG II levels, making it unlikely that circulating ANG II acting on the subfornical organ would have a role in responses to intracerebroventricular hypertonic NaCl. Rather, a more likely explanation is that intracerebroventricular hypertonic NaCl activates central neural pathways that utilize ANG II as a synaptic transmitter.

It has been reported that microinjection of the subfornical organ in rats with carbachol (7) resulted in similar effects on blood pressure and electrolyte excretion as those seen for intracerebroventricular infusion of carbachol in sheep, indicating that these effects are probably caused by activation of the same brain regions as hypertonic saline and ANG II. Ablation of the anterior wall of the third ventricle greatly attenuated responses to carbachol, suggesting that there is a hierarchical structure to the physiological mechanisms that mediate the increase in blood pressure and electrolyte excretion. Sladek and colleagues (26) have suggested that AVP release in response to hyperosmotic challenge could also be mediated by parallel angiotensinergic and cholinergic pathways. These pathways appeared to be independent, because saralasin did not block AVP release induced by acetylcholine or nicotine. That there is a coordinated response of discrete brain regions.
regions that mediate the changes in blood pressure, electrolyte excretion, and AVP release is compatible with histological studies that have mapped angiotensinergic connections both within the lamina terminalis and those projecting to the hypothalamus and brain stem (13, 19). However, such connectivity studies have not been performed to the same extent for cholinergic pathways.

In regard to the likelihood of nonspecific depressant influences on neuronal function, the lack of effect of intracerebroventricular losartan on pressor responses or AVP secretion following administration of a different agonist (intracerebroventricular carbachol) argues against this possibility. However, in regard to the natriuretic response to intracerebroventricular carbachol, intracerebroventricular losartan did cause a partial inhibition of the response. One possibility is that an angiotensinergic pathway may have a partial role in natriuretic responses to intracerebroventricular carbachol. In this regard, there is some evidence obtained from studies in the rat which have mapped neuronal activation in the brain (by immunohistochemical detection of Fos protein) in response to intracerebroventricular injections of carbachol or ANG II in combination with losartan. Both ANG II and carbachol activated neurons in the lamina terminalis and the hypothalamic paraventricular and supraoptic nuclei (25). However, whereas AT1 receptor blockade abolished ANG II-induced Fos expression in these brain regions, this abolition was found only in the median preoptic nucleus for carbachol-induced Fos expression (25). This evidence may explain the selective blockade of the carbachol-induced natriuresis and the comparative lack of effect on the increase in blood pressure and AVP secretion. Thus our results present functional evidence for an angiotensinergic influence on cholinergic neuronal pathways subserving sodium excretion release, but not blood pressure or AVP release, where these mechanisms appear to be independent.

Although losartan is thought to be a specific blocker of AT1 receptors (27), there remains the possibility that it could be nonspecifically depressing neuronal function or also be blocking another, as yet unspecified, agonist. Regarding the likelihood of some nonspecific influence of losartan on natriuretic responses via other receptors, there is a lack of evidence in the literature to assess this proposition. However, it may seem more than coincidence that not only does intracerebroventricular losartan block responses to intracerebroventricular hypertonic saline as well as to intracerebroventricular ANG II, but the agonist effects of intracerebroventricular ANG II are also quite similar to those of intracerebroventricular hypertonic saline, suggestive that the latter responses specifically involve an angiotensinergic-mediated pathway.

In the present experiments, the concentrations of NaCl and osmolality that would result in ventricular CSF as a result of the intracerebroventricular infusions of hypertonic saline would be expected to be supraphysiological. This conclusion is based on earlier measurements of CSF Na⁺ concentration resulting from intracerebroventricular infusion of 0.45 mol/l NaCl at 1 ml/h (14). This raises the question of the physiological significance of the responses to the intracerebroventricular hypertonic saline and their relationship to the responses that result from physiological increases in plasma tonicity and Na⁺ concentration that occur, for example, in dehydrated animals. Although the increases in CSF Na⁺ may be supraphysiological, the change in Na concentration and osmolality in periventricular tissue could be much less at quite small distances in from the ventricular surface (18). If the sensors (osmo- and/or Na⁺ sensors) that are stimulated by hypertonic saline are at just a small distance from the ventricular surface as has been proposed (1, 2, 14), then physiologically relevant pathways may have been stimulated. Regardless of whether the responses to intracerebroventricular hypertonic saline are of a physiological or pharmacological nature, the present results demonstrate that it is likely that central angiotensinergic pathways are activated by intracerebroventricular hypertonic saline. This could provide a useful experimental tool to study the endogenous release of ANG II in these pathways.

In the present study, we observed that systemic infusion of losartan appeared to cause a moderate reduction in the magnitude of the AVP secretory response (this reduction was not statistically significant) to hypertonic saline and delay the onset of the natriuresis (although not affecting the magnitude of the response). These inhibitory effects of systemically infused losartan were muted compared with intracerebroventricular infusion and are probably best explained by autoradiographic evidence that losartan can cross the blood-brain barrier and block central AT1 receptors (30). Similarly, it has been shown that dipsogenic responses to central ANG II infusion displayed a delayed inhibition when losartan was infused systemically, indicating that a time interval was required for the antagonist to cross over to the brain (21).

These experiments do not allow conclusions to be drawn regarding the effector mechanism between brain and kidney whereby central stimulation with angiotensin or hypertonic NaCl increases sodium excretion. There is evidence from studies in rats that, as well as a yet-to-be-identified humoral agent, the pressor response and increased vasopressin blood levels are contributing factors (5, 17, 28). However, it is unlikely that increased vasopressin secretion is a major factor in the centrally mediated natriuretic response in sheep because it has previously been shown that much higher blood levels of vasopressin are needed to increase Na⁺ excretion in the sheep than those observed to occur in response to intracerebroventricular infusion of hypertonic ANG II, carbachol, or 0.75 mol/l NaCl (20).

In summary, we observed that centrally infused losartan inhibited several of the responses elicited by both intracerebroventricular hypertonic saline and intracerebroventricular ANG II, suggesting that common neural pathways are involved in the responses to these two stimuli. We propose that intracerebroventricular infusion of hypertonic saline activates angiotensinergic pathways.
pathways in the CNS subserving the regulation of fluid and electrolyte balance and arterial pressure.

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