Neural Mechanisms Subserving Central Angiotensinergic Influences on Plasma Renin in Sheep

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Abstract—The mechanisms and brain regions subserving the suppression of plasma renin concentration caused by intracerebroventricular (ICV) infusion of angiotensin II were studied in sodium-depleted sheep. Infusion of angiotensin II (3 μg/h for 1 hour) into the lateral ventricle reduced plasma renin from 4.3±0.4 to 1.6±0.2 pmol angiotensin I/mL per hour at 1 hour after the commencement of infusion. This change persisted for at least another 90 minutes and was blocked by concomitant ICV infusion of the AT_1 antagonist losartan (1 mg/h). Arterial pressure did not change, but plasma vasopressin secretion was increased. ICV infusion of losartan (1 mg/h) significantly increased plasma renin in sodium-depleted sheep. The reduction of plasma renin concentration in response to either ICV angiotensin II or hypertonic NaCl (0.75 mol/L at 1 mL/h) and the increase in response to ICV losartan was prevented in sheep in which the lamina terminalis of the brain had been ablated. Lesions in the median eminence (MEL), which blocked the increased plasma vasopressin levels, did not prevent suppression of plasma renin in response to ICV angiotensin II. However, bilateral renal denervation largely blocked this inhibition of plasma renin concentration but not the increased plasma renin resulting from ICV infusion of losartan in sodium-depleted sheep. The results show that AT_1 receptors, probably located in the lamina terminalis, mediate a central inhibitory influence of angiotensin II on renin secretion. This inhibition of renin release is probably due to a reduction in activity of renal sympathetic nerves innervating the juxtaglomerular apparatus of the kidney. **(*Hypertension*. 2001;37:1375-1381.)**

Key Words: angiotensin ■ renin ■ receptors, angiotensin ■ renal nerves

The secretion of renin by the kidney is influenced by several factors including the activity of the renal nerves,[1] and it is possible that several brain regions may contribute to the initiation of renal sympathetic drive[2] and therefore the control of renin release. However, the brain mechanisms controlling renin secretion are poorly understood. One of the factors that may influence renin secretion is the concentration of angiotensin (Ang) II in the brain.[3–8] Whereas intracerebroventricular (ICV) infusions of Ang II or renin reduce plasma renin concentration (PRC), arterial pressure also increases.[3–8] Thus, the inhibition of renin release may be mediated by renal or arterial baroreflexes secondary to the rise in pressure. ICV infusion of the angiotensin AT_1 receptor antagonist losartan elevates PRC in sodium-depleted sheep, suggesting that an angiotensinergic pathway in the brain may exert a tonic inhibitory influence on renin secretion in these animals.[8]

We have observed that ICV infusion of either Ang II or hypertonic saline suppresses PRC in sodium-depleted sheep without increasing arterial pressure.[8,9] This contrasts with the pressor effects of such ICV infusions in sodium-replete sheep,[6] suggesting that the reduced plasma renin levels are not a reflex response to baroreceptor activation. As well as suppressing PRC, ICV infusion of either Ang II or hypertonic NaCl reduces renal sympathetic nerve activity considerably in conscious sheep,[6] showing that the reduced PRC may have resulted from reduced renal nerve activity, a conclusion reached earlier from studies that used renal denervation.[4]

In regard to the brain regions mediating effects of ICV Ang II on PRC, the lamina terminalis may be crucial. This region of the brain is rich in AT_1 receptors,[10] and transsynaptic neural tracing studies with pseudorabies virus show that the lamina terminalis is neurally linked to the renal nerves.[11] Moreover, high levels of PRC result if the lamina terminalis is ablated,[12,13] suggesting the disruption of an inhibitory influence on renin secretion. The purpose of this study was to characterize more fully the mechanisms by which central Ang II suppresses renin secretion. Sodium-depleted sheep, having elevated PRC, have been studied so that the full extent of central inhibitory influences of Ang II on renin secretion could be observed in the absence of any change in arterial pressure. Our aims were (1) to determine whether an AT_1 receptor mediates the suppression of PRC caused by ICV Ang II, (2) to determine, by studying the effects of brain lesions, whether tissue in the lamina terminalis has a role in this response, (3) to study the role of vasopressin in the suppression of PRC in response to ICV Ang II, and (4) to investigate whether renal nerves have a role in the ICV Ang II–induced reduction of PRC when blood pressure does not rise. Sheep with denervated kidneys were used for this purpose.

Received September 22, 2000; first decision October 31, 2000; revision accepted November 20, 2000.
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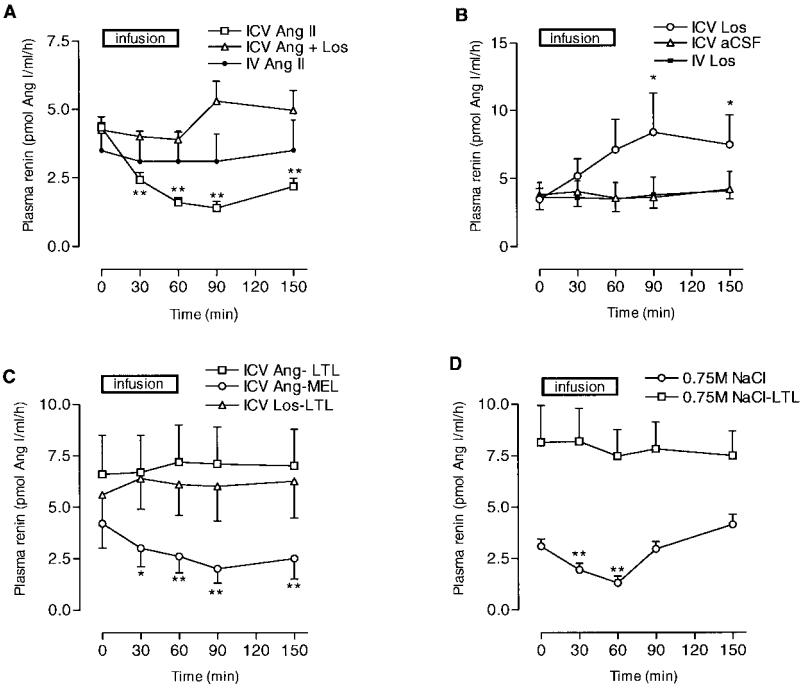

Figure 1. Effects of various ICV or IV infusions on plasma renin concentration of sodium-depleted sheep. A, ICV Ang II at 3 μg/h (n=5), ICV Ang II (3 μg/h) combined with ICV losartan (1 μg/h, n=4), or IV Ang II at 3 μg/h (n=4). B, ICV infusions of either artificial CSF at 1 mL/h (ICV aCSF, n=5), ICV losartan at 1 mg/h (ICV Los, n=5), or IV infusion of losartan at 1 mg/h. C, ICV infusions of Ang II at 1 mg/h for 1 hour into sodium-depleted sheep with lesions in either lamina terminalis (ICV Ang-LTL, n=4) or median eminence (ICV Ang-MEL, n=5). Lack of effect of ICV infusions of losartan at 1 mg/h in sodium-depleted sheep with lesions in lamina terminalis (ICV Los-LTL) is also shown. D, ICV infusions of 0.75 mol/L NaCl artificial CSF at 1 mL/h for 1 hour in sodium-depleted sheep with lesions in lamina terminalis. For comparison, data reported in an earlier publication[13] showing effects of ICV infusion of 0.75 mol/L NaCl are also shown. Mean and SEM are shown. Significant difference from preinfusion value is indicated by $^{*}P<0.05$, $^{**}P<0.01$.

Methods

A total of 24 Merino-Border Leicester crossbreed ewes, 2 to 4 years old, were used. They were housed individually in metabolism cages, provided with water ad libitum, and fed 0.8 kg chaff (sodium content, 90 to 130 mmol/kg) each day. Room temperature was 20°C. Experiments and protocols were approved by the Animal Ethics Committee of the Howard Florey Institute, which adheres to the Australian code of practice for the care and use of animals for scientific purposes.

At least 2 months before experimentation, sheep were given a general anesthetic induced with intravenous (IV) sodium thiopentone (17 mg/kg) and maintained with an isoflurane/oxygen gas mixture. The carotid arteries were surgically enclosed in skin loops in the neck, and the ovaries were extirpated. At a second operation (with the same general anesthesia), a 17-gauge stainless steel guide tube was implanted over each lateral cerebral ventricle as described previously.[14] During the same operation, in 7 of the sheep, a stainless steel electrode (insulated except for 6 to 7 mm at the tip) was implanted along the anterior wall of the third ventricle, and in another 5 sheep, bilateral stainless steel electrodes were implanted over the median eminence. Electrode positions were verified radiographically. Lesions were made by heating these sites to 58° to 60°C for 4 minutes by passage of radiofrequency current with a Grass LM-4 instrument. These methods have been described previously.[14] After ablation of the lamina terminalis, sheep usually showed temporary reductions in daily fluid intake and were maintained with daily ruminal water loads.

In another 6 sheep, both kidneys were surgically denervated, as described previously.[15] Briefly, all visible nerves to the kidney were removed, and the renal artery and vein were swabbed with 10% phenol/ethanol after stripping of the adventitia. Catecholamine-containing nerve terminals as shown by formaldehyde-fluorescence histochemistry are totally absent in kidneys for at least 1 month after this procedure.[15] Animals were allowed 1 to 2 weeks to recover.

Experimental Protocols

Sheep were sodium-depleted by loss of saliva from a cannula inserted into a parotid duct (as described previously).[9] After 2 days of loss of saliva, food and water were removed from the cage. A polyethylene cannula filled with heparinized isotonic saline and attached to an 18-gauge needle was then inserted into a carotid artery and fixed there. In some experiments, arterial pressure was recorded by connecting the arterial cannula to a pressure transducer coupled to a polygraph. A 3-way tap enabled blood samples to be drawn from the carotid artery. Two samples of blood were obtained ≈15 minutes apart; a needle was then inserted into the lateral ventricle (as evidenced by withdrawal of a few drops of cerebrospinal fluid [CSF]), and an infusion of test solution (Ang II, losartan, 0.75 mol/L NaCl, or artificial CSF) at 1 mL/h into the lateral ventricle was commenced and continued for 1 hour. Blood samples (10 mL) were obtained at 30 and 60 minutes after the start of the ICV infusion and at 30 and 90 minutes after the ICV infusion was terminated. In some experiments (in which either artificial CSF or 0.75 mol/L NaCl had been infused first into lateral ventricle), a second infusion of a different test substance was then made 2 hours after the completion of the first infusion. Blood samples were again obtained at 30 and 60 minutes after commencement and 30 and 90 minutes after termination of infusions. In some experiments, instead of an ICV infusion, an infusion of test solution was made into a jugular vein at 1 mL/h for 1 hour, with similar time sequence of blood sampling. At least 2 weeks were allowed to elapse between successive experiments, except in sheep with renal denervation. In these animals, a second ICV infusion was made on the day after the first infusion while the sheep were still depleted of sodium, so that experiments were completed within 1 month of the denervation.

Solutions Infused

All solutions infused into the lateral ventricle were prepared in an artificial CSF, based on the composition of sheep CSF. The composition of this artificial CSF was NaCl 150 mmol/L; KCl 2.8 mmol; $CaCl_2$ 1.2 mmol/L; $MgCl_2$ 1.0 mmol/L; and Na_2HPO_4 0.5 mmol/L. Test solutions infused into a lateral ventricle at 1 mL/h for 1 hour were Ang II (Peninsula) at 3 μg/h, losartan (Dupont-Merck) at 1 mg/h, or artificial CSF. Losartan and Ang II were also infused in combination for 1 hour at these doses preceded by ICV infusion of losartan for 15 minutes. Infusion of Ang II (3 μg/h) or losartan (1 mg/h) for 1 hour were also made into the jugular vein.

Analysis of Blood Samples

Blood samples were divided with 2.5 mL going into a tube with EDTA as anticoagulant and 7.5 mL into another tube containing heparin. These tubes were chilled, then centrifuged and the plasma obtained stored at either −20°C (for radioimmunoassays) or 4°C (for ionic analysis). Sodium and potassium concentrations were mea-

TABLE 1. Plasma Sodium and Potassium Concentrations (mmol/L) Before, During, and After ICV Infusions

		Time				
Infusion	Variable	Pre-Inf	+30 min	+60 min	Post 30 min	Post 90 min
ICV artificial CSF	pl[Na]	141±1	143±1	142±1	143±1	142±1
n=5	pl[K]	4.8±0.1	4.5±0.1	4.8±0.1	4.6±0.1	4.8±0.1
ICV Ang II	pl[Na]	141±1	142±1	142±1	141±1	141±1
n=5	pl[K]	4.7±0.2	4.6±0.1	4.6±0.2	4.3±0.1*	4.3±0.1*
ICV Los	pl[Na]	142±1	143±1	143±2	142±1	143±1
n=6	pl[K]	4.6±0.2	4.5±0.2	4.6±0.2	4.5±0.1	4.5±0.1
ICV Ang+Los	pl[Na]	143±1	143±1	142±2	144±2	144±2
n=4	pl[K]	4.5±0.2	4.4±0.2	4.5±0.3	3.9±0.2	4.2±0.2
ICV Ang II−LTL	pl[Na]	145±5	146±6	145±5	147±7	146±6
n=4	pl[K]	4.8±0.3	4.6±0.2	4.7±0.2	4.7±0.1	4.5±0.2
ICV Los−LTL	pl[Na]	147±5	147±7	148±6	148±7	148±6
n=5	pl[K]	4.4±0.3	4.5±0.2	4.5±0.3	4.4±0.3	4.3±0.3
ICV 0.75 mmol/L Na−LTL	pl[Na]	143±7	144±7	143±6	144±7	142±7
n=4	pl[K]	4.5±0.3	4.3±0.3	4.5±0.4	4.3±0.2	4.6±0.4
ICV Ang II−MEL	pl[Na]	139±1	140±2	139±1	139±2	139±2
n=5	pl[K]	4.9±0.2	4.8±0.3	4.9±0.3	4.5±0.3*	4.5±0.3*

pl[Na] indicates plasma sodium concentration; pl[K], plasma potassium concentration; Ang II, 3 μg/h; Los, losartan, 1 mg/h.

Values given average those before commencement of ICV infusion (Pre-Inf), 30 minutes and 60 minutes after start of infusion (+30 min and +60 min, respectively), and 30 minutes (Post 30 min) and 90 minutes (Post 90 min) after ending ICV infusion.

Significant differences from preinfusion values are denoted by *$P<0.01$.

sured by ion-selective electrode on a Beckman Clinical Analyzer. Plasma renin concentration was measured by an antibody capture technique. Briefly, 60 μL of unknown plasma sample, 50 μL of exogenous renin substrate, and 20 μL of Ang I antibody were incubated at 37°C for 60 minutes. At the end of the incubation, 1 mL of [^{125}I]Ang I was added. The Ang I generated was determined by radioimmunoassay and expressed as picomoles of Ang I generated per milliliter of plasma per hour. Intra-assay and interassay coefficients of variation were 4% and 8%, respectively. Arginine vasopressin (AVP) was measured by radioimmunoassay with antiserum raised in rabbits. It had <1% cross-reactivity with oxytocin, the sensitivity of the assay was 0.4 pg/mL, and the intra-assay and interassay coefficients of variation were 3% and 12%.

When experiments were completed in a particular animal, it was killed by injection of IV sodium pentobarbital (100 mg/kg). In sheep with a brain lesion, the head was perfused with saline and fixative, and either coronal or sagittal sections of the hypothalamus were prepared as described previously.[14] The extent of lesions was mapped with a microfilm reader (Zeiss, Jena).

Statistical Treatment of Results

Results are expressed as mean±SEM. For analysis of PRC and plasma sodium, potassium, or protein concentrations, results were subjected to repeated-measures ANOVA. Where a significant effect occurred, values for each time period after the commencement of an ICV or IV infusion were then compared with the value obtained immediately before infusion by Dunnett's test for multiple comparisons.

Results

Effect of ICV Infusion of Losartan on PRC Response to ICV Ang II in Sodium-Depleted Sheep

ICV infusion of Ang II (3 μg/h) caused a marked reduction of PRC within 30 minutes, which persisted for at least 90 minutes after the end of infusion (Figure 1A). No change in PRC occurred during or after ICV infusion of artificial CSF (Figure 1B). Prior ICV infusion of losartan prevented the reduction of PRC caused by ICV infusion of Ang II (Figure 1A). ICV infusion of losartan alone increased PRC above the preinfusion levels at 30 and 90 minutes after infusion (Figure 1B). No change in plasma sodium, potassium, or protein concentrations occurred with these infusions except that plasma potassium concentration fell significantly at 30 and 90 minutes after the end of the ICV infusion of Ang II (Tables 1 and 2). Plasma AVP levels increased from 1.6±0.7 to 13.4±4.6 pg/mL ($P<0.05$, paired *t* test) at the end of the infusion of ICV Ang II but did not change significantly with control infusion of artificial CSF (1.9±1.2 to 2.5±1.4 pg/mL) or ICV infusion of losartan (2.3±1.0 to 1.7±0.8 pg/mL). AVP levels were not measured in the other experiment. Mean arterial pressure (MAP) did not change with ICV infusion of either Ang II or artificial CSF in these sodium-depleted sheep (Table 2).

Effect of Lamina Terminalis Lesions on Renin Responses to ICV Infusions in Sodium-Depleted Sheep

Extent of Brain Tissue Ablated

Of the 7 sheep in which lesions were made in the lamina terminalis, histological examination showed that 5 incurred ablation of >95% of tissue in the lamina terminalis; these are termed lamina terminalis lesion sheep (LTL sheep). In these sheep, the subfornical organ and median preoptic nucleus were completely ablated, whereas there was total ablation of the organum vasculosum of lamina terminalis (OVLT) in 2 of the sheep, and in the other 3 animals the OVLT was largely destroyed with a small amount (≈10%) of the most ventral part

TABLE 2. Plasma Protein Concentration (in g/L) and (in 3 Experiments Only) MAP (in mm Hg) Before, During, and After ICV Infusions in Sodium-Depleted Sheep

		Time				
Infusion	Variable	Pre-Inf	+30 min	+60 min	Post 30 min	Post 90 min
ICV artificial CSF	Plpr	69±1	67±2	66±1	68±1	68±1
	MAP	73±2	74±2	71±2	72±1	73±1
ICV Ang II	Plpr	68±3	64±3	67±2	64±2	65±3
	MAP	73±1	72±1	74±1	70±2	73±2
ICV Los	Plpr	66±1	67±1	68±1	71±2	69±2
	MAP	77±2	79±2	78±3	77±2	76±2
ICV Ang+Los		68±3	64±4	67±4	62±4	67±3
ICV Ang II−LTL		71±7	68±6	69±6	70±6	69±5
ICV Los−LTL		72±6	69±4	72±6	69±5	70±5
ICV 0.75M Na−LTL		73±5	70±6	73±4	71±5	74±5
ICV Ang II−MEL		67±2	63±3*	64±2	61±3†	61±2†

Plpr indicates plasma protein concentration; Los, losartan; number of animals and other abbreviations as in Table 1.

Values given are those before commencement of ICV infusion (Pre-Inf), those at 30 minutes and 60 minutes, (+30 min and +60 min, respectively), and 30 minutes (Post 30 min) and 90 minutes (Post 90 min) after ending ICV infusion.

Significant differences from preinfusion values are denoted by *$P<0.05$, †$P<0.01$.

of the OVLT left intact (Figure 2). In addition to the lamina terminalis, small amounts of tissue close to the midline were damaged ventral to the anterior commissure, and this was mainly the most medial parts of the medial preoptic region, periventricular preoptic nucleus, and vertical limb of the diagonal band. Dorsal to the anterior commissure, lesions extended into the midline part of the medial septal nucleus. The anterior hypothalamus, bed nucleus of the stria terminalis, and the supraoptic, suprachiasmatic, and hypothalamic paraventricular nuclei were not damaged. In the other 2 sheep, the lamina terminalis incurred less damage. The subfornical organ and median preoptic nucleus dorsal to the anterior commissure as well as this fiber tract and the medial septal nucleus were severely damaged (>90% ablated). However, considerable parts (≈50%) of the median preoptic nucleus ventral to the anterior commissure and OVLT were left intact in these sheep (Figure 2).

Changes in Plasma Renin Concentration

Loss of saliva in LTL sheep increased PRC to levels similar to or greater than those observed in intact sheep. No significant change in PRC was observed with ICV infusion of either Ang II or 0.75 mol/L NaCl in sodium-depleted LTL sheep (Figure 1C and 1D). No significant changes in plasma sodium, potassium, or total protein concentrations were observed. Arterial pressure was not measured. In 2 sheep with smaller lesions in the lamina terminalis, after 1 hour of infusion PRC fell from 2.7 to 2.5 and 2.1 to 1.2 pmol Ang I/mL per hour with ICV Ang II and from 2.8 to 1.4 and 2.4 to 1.8 pmol Ang I/mL per hour with ICV infusion of 0.75 mol/l NaCl. When ICV infusions of losartan (1 mg/h) were made into sodium-depleted LTL sheep, there were no significant changes in PRC (Figure 1C) or plasma sodium, potassium, or protein concentrations (Tables 1 and 2).

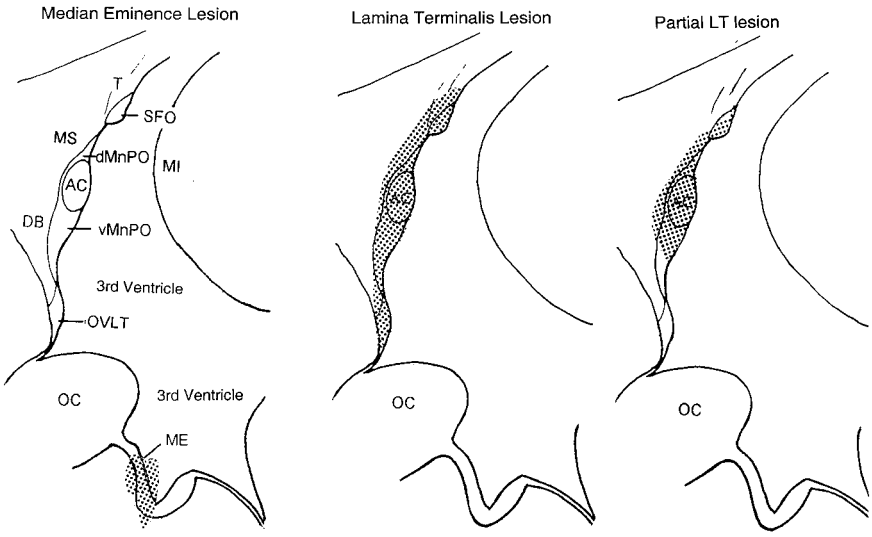

Figure 2. Diagrams of lesions made in either median eminence or lamina terminalis (LT) of sheep brain. Diagram of parasagittal midline is shown; area of lesion common to each sheep in a particular group is indicated by stippled area. AC indicates anterior commissure; DB, diagonal band of Broca; dMnPO; dorsal part of the median preoptic nucleus; ME, median eminence; MI, massa intermedia; MS, medial septal nucleus; OC, optic chiasm; OVLT, organum vasculosum of lamina terminalis; SFO, subfornical organ; T, septal triangularis nucleus; and vMnPO, ventral median preoptic nucleus.

TABLE 3. Effects of ICV Infusion of Ang II (3 μg/h, n=6) or Losartan (1 mg/h, n=5) for 1 Hour on PRC, Plasma Sodium, Potassium, Protein, and AVP Concentrations, and MAP of Sodium-Depleted Sheep With Kidneys Denervated

		Time				
Variable	Infusion	Pre-Inf	+30 min	+60 min	Post 30 min	Post 90 min
PRC, pmol Ang I/mL per hour	Ang	6.2±1.4	5.8±1.3	5.5±1.0	4.5±0.8*	4.4±0.9*
	Los	6.7±1.5	7.3±1.6	7.6±1.5	8.2±1.4*	8.8±1.9†
Pl[Na], mmol/L	Ang	138.7±0.5	139.0±0.9	139.0±0.6	138.8±0.6	138.5±0.7
	Los	139.0±1.0	139.0±1.0	139.0±1.0	139.0±1.0	139.0±1.0
pl[K], mmol/L	Ang	4.6±0.1	4.8±0.3	4.7±0.1	4.6±0.1	4.4±0.1
	Los	4.3±0.2	4.3±0.2	4.3±0.2	4.1±0.2*	4.2±0.2
Plpr, g/L	Ang	70±2	68±2	68±2	65±2*	66±2*
	Los	66±2	66±1	68±1	66±2	69±1*
Plasma AVP, pg/mL	Ang	2.1±1.3	NM	7.4±2.3†	NM	3.9±1.6
	Los	0.8±0.2	NM	0.9±0.2	NM	1.1±0.3
MAP, mm Hg	Ang	76±5	78±6	78±7	78±7	73±5
	Los	69±2	68±2	68±2	67±3	66±1

NM indicates not measured; other abbreviations as in Tables 1 and 2.
Values are mean±SEM. Significant difference from preinfusion values are denoted by *$P<0.05$, †$P<0.01$.

Effect of ICV Infusion of Ang II in Sodium-Depleted Sheep With Median Eminence Ablated

Loss of saliva for 2 days caused PRC to increase to levels similar to those observed in sodium-depleted intact sheep. ICV infusion of Ang II (3 μg/h for 1 hour) caused PRC to fall significantly by the end of the infusion (Figure 1C) and remain at low levels for the duration of the experiment.

Plasma AVP concentration was measured before and at the end of the ICV infusion of Ang II. It was below the level of detectability (<0.4 pg/mL) and remained so in 3 of the sheep, whereas in the other 2 sheep, small increases to 0.6 and 0.8 pg/mL were measured. No significant change in plasma sodium or potassium concentration occurred, but a significant reduction of plasma protein was observed after the ICV Ang II infusion (Tables 1 and 2). Arterial pressure was not measured.

Effect of Renal Denervation on Renin Response to ICV Infusion of Either Ang II or Losartan

Plasma renin levels in sheep with denervated kidneys increased to levels similar to those that occur in sodium-depleted sheep with intact renal nerves. However, with ICV infusion of Ang II (3 μg/h for 1 hour), no significant reduction in PRC was observed until 30 and 90 minutes after the cessation of infusion. Thus, in comparison to sheep with intact renal nerves, reduction in PRC induced by ICV Ang II was delayed by at least 1 hour (Table 3). Plasma AVP levels increased with ICV Ang II infusion in these sheep (Table 3). Plasma electrolyte concentrations did not change. Plasma protein levels fell at 30 and 90 minutes after the conclusion of the ICV Ang II infusion.

In 5 sheep with kidneys denervated, ICV infusion of the AT_1 receptor antagonist losartan caused a significant increase in PRC at 30 and 90 minutes after the termination of the ICV infusion. No changes in MAP or plasma sodium concentration were observed (Table 3). Plasma protein concentration increased significantly 90 minutes after the completion of the infusion.

Effect of IV Infusions of Either Ang II or Losartan on PRC of Sodium-Depleted Intact Sheep

IV infusion of either Ang II at 3 μg/h or losartan at 1 mg/h for 1 hour did not significantly change PRC (Figure 1, A and B) or plasma sodium or potassium (not shown) of intact sodium-depleted sheep.

Discussion

Several investigators have previously shown that ICV administration of either renin or Ang II in sodium-replete animals reduces PRC. This is probably a consequence of reduced renin secretion by the kidney.[3–7] Our studies show that this central effect of Ang II is blocked by ICV losartan, a potent AT_1 receptor antagonist, indicating that it is mediated by a central AT_1 receptor. The increase in PRC in response to ICV but not IV infusion of losartan suggests that angiotensin that is endogenous to the brain may have tonic inhibitory influences on renin secretion in sodium-depleted animals. The lack of effect of systemic infusions of either Ang II at 3 μg/h or losartan at 1 mg/h on PRC confirms that the effects seen with ICV infusions were not due to peripheral actions of Ang II or losartan that had been cleared from the brain.

Ablation of the lamina terminalis of sheep prevented the changes in PRC that occur in response to ICV infusion of either Ang II or losartan, suggesting that this region of the brain plays an important role in the central influences of Ang II on renin secretion. The lamina terminalis, which comprises the subfornical organ, median preoptic nucleus, and OVLT, is rich in angiotensin receptors in all mammalian species studied, including sheep.[10] In addition, ICV infusion of Ang II has been shown to result in the activation of neurons (as indicated

by Fos production) throughout the lamina terminalis of the rat.[16] Therefore, it seems likely that the suppression of PRC resulting from ICV infusion of Ang II is due to activation of neural pathways that originate in the lamina terminalis. The observation in 2 sheep with partial lesions, in which some of the ventral lamina terminalis was left intact, that ICV Ang II or hypertonic saline still caused some albeit less inhibition of PRC emphasizes the importance of the ventral lamina terminalis in the response. Weekley[7] found that ablation of the medial basal forebrain of rats (producing relatively larger lesions than those made in the present study in sheep) prevented the inhibition of renin secretion in response to ICV Ang II in anesthetized rats. The data in sheep are consistent with this result because the lamina terminalis was included in the region of medial basal forebrain ablated in the rat.

The observation that ablation of the lamina terminalis also disrupted the inhibition of PRC in response to ICV hypertonic NaCl was not unexpected because this inhibitory response is probably mediated by a central angiotensinergic pathway,[9] which as discussed above, is also disrupted by such a brain lesion. Previous work in a number of species has shown the importance of the ventral lamina terminalis in other centrally mediated responses to Ang II or hypertonic saline such as drinking, vasopressin secretion, and natriuresis.[12–14,16,17]

Ablation of more caudal midline tissue in the median eminence, which interrupts the AVP-containing axons of the hypothalamo-neurohypophysial tract, almost abolished the increase in plasma AVP concentration resulting from ICV infusion of Ang II. However, this infusion still reduced PRC considerably in these sheep with MEL, suggesting that increased AVP levels do not mediate the central influence of Ang II on PRC, as suggested previously.[5]

It is possible that the reduced renin secretion in response to ICV Ang II that was reported previously[3–7] is due to reduced renal sympathetic nerve activity resulting from baroreceptor activation caused by the pressor effect of ICV Ang II. Arterial pressure did not increase with ICV infusion of Ang II in sodium-depleted sheep, showing that the observed changes in PRC were not secondary to alterations in arterial pressure and baroreceptor activity. ICV infusions of Ang II increase arterial pressure in sodium-replete sheep[6]; therefore, it seems likely that this centrally mediated pressor response is dependent on the animals' sodium status, analogous to the pressor action of systemically administered Ang II being inhibited by sodium depletion.[18,19] Studies performed with an inhibitor of vascular nitric oxide (NO) production in sodium-depleted sheep showed that NO had a role in the reduced pressor responsiveness to peripheral Ang II.[19] We speculate that increased vascular NO may also have a role in the lack of a pressor response to ICV Ang II in sodium-depleted sheep.

The lack of any significant reduction of PRC during the hour of ICV infusion of Ang II in sodium-depleted sheep with denervated kidneys suggests that reduced renal nerve activity may a play a role in the inhibitory influence of ICV Ang II on renin secretion in conscious sodium-deplete sheep, consistent with earlier results in anesthetized cats.[4] It has been shown previously that ICV infusion of Ang II causes a pronounced and long-lasting inhibition of renal sympathetic nerve activity in sodium-replete sheep, which is partly independent of baroreceptor activation.[6] This effect of ICV Ang II on renal nerve activity, like that on renin secretion, is also prevented by ablation of the lamina terminalis.[20] Therefore, we propose that ICV infusion of Ang II acts on neurons in the lamina terminalis to activate polysynaptic neural pathways leading to the eventual inhibition of renal sympathetic nerve activity and renin secretion. We have recently provided a neuroanatomic basis for such a proposal by showing that the neurotropic virus pseudorabies is transported retrogradely from injection sites in the kidney of the rat to the lamina terminalis through at least 3 synaptic passages.[11] Because Ang II almost invariably has a direct stimulatory influence on neurons in the lamina terminalis,[21] an inhibitory synapse must be involved at one point in the polysynaptic neural pathway for the observed inhibition of renal nerve activity and renin secretion to occur.

Although renal denervation severely disrupted the inhibitory effect of ICV Ang II on renin release, the increase of PRC resulting from ICV infusion of the angiotensin antagonist losartan was still evident in sheep with denervated kidneys. This suggests that an additional factor to renal nerve activity may also have a role in mediating central angiotensinergic influences on renin secretion. Presumably, this effect of ICV losartan is humorally mediated, because no change in arterial pressure occurs with ICV losartan in sodium-depleted sheep. Involvement of such a humoral factor may explain the slowly occurring reduction of PRC that was observed at 30 and 90 minutes after the start of the ICV infusion of Ang II in sheep with denervated kidneys. Plasma AVP levels were elevated after ICV infusion of Ang II in sheep with denervated kidneys, and AVP could have a role in this slow reduction of PRC. It should be noted that a significant fall in plasma protein concentration occurred after the end of the ICV infusion of Ang II in these sheep, indicating a probable increase in blood volume at these times, which may also have influenced PRC.

It is not clear at present why blood volume should increase after ICV infusion of Ang II. It is unlikely that this effect is due to the increased plasma AVP levels causing marked fluid retention by the kidneys because plasma protein concentration also fell in sheep with an MEL, which prevented plasma AVP levels from increasing in response to ICV Ang II. We have observed that ICV infusion of Ang II reduces the copious saliva flow from the parotid gland of the sheep (M.J. McKinley and M.L. Mathai, unpublished observations). This could result initially in less fluid leaving the bloodstream during ICV infusion of Ang II. Thus, blood volume could increase before fluid reabsorption from the gastrointestinal tract was adjusted.

Conclusion

These data suggest that as well as a direct influence on the kidney of circulating Ang II to inhibit renin secretion,[18] there may also be an inhibitory influence of brain angiotensin on renal renin secretion, which is probably signaled to the kidney as a reduction in renal sympathetic nerve activity. Angiotensin-receptive neurons in the lamina terminalis may initiate these changes; however, the polysynaptic neural pathway from lamina terminalis to kidney, with an inhibitory synapse, remains to be determined. We

speculate that angiotensin may have actions on neurons in the lamina terminalis that connect to spinally projecting neurons of the paraventricular nucleus, to influence preganglionic neurons linked to the renal nerves to inhibit renal nerve activity and renin secretion. Because parts of the lamina terminalis lack a blood-brain barrier, it may be influenced by increases in endogenous circulating Ang II. Angiotensinergic nerve terminals may innervate the median preoptic nucleus,[17] which cannot be accessed by circulating Ang II. Therefore, it is possible that either peripherally or centrally generated Ang II could have a role in the central regulation of renin secretion by the kidney.

Acknowledgments

This work was supported by Institute Block Grant 983001 from the NHMRC of Australia. We thank Angela Gibson, Elizabeth Cooper, and Craig Thomson for their skilled assistance.

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