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Intracerebroventricular Infusions of Angiotensin II Increases Sodium Excretion (41385)

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Abstract. The mechanism by which intracerebroventricular administration of angiotensin II (AII) enhances renal sodium excretion was studied in anesthetized dogs. Intraventricular infusion of AII (6 ng/min) increased sodium excretion independently of changes in renal plasma flow (RPF), glomerular filtration rate (GFR), blood pressure (BP), and plasma concentration of aldosterone. In order to evaluate the intracerebral role of endogenous AII in the control of sodium excretion, the converting enzyme inhibitor, SQ20881 (0.5 μg/min), was infused intraventricularly in another group of dogs. This infusion decreased sodium excretion; in addition, there were no changes in RPF, GFR, BP, and plasma aldosterone concentration. The mechanism of the antinatriuresis remains unclear. However, the fact that SQ20881 administration decreased sodium excretion is consistent with the hypothesis that endogenous AII is tonically active in the brain to stimulate sodium excretion.

Circulating angiotensin II (AII) enhances renal Na retention both by a direct action on the kidney (1) and indirectly by stimulating aldosterone secretion. In contrast, infusion of AII (2–4) or renin (5) into the cerebral ventricles elicits a natriuresis. Centrally administered AII also increases blood pressure, and this led Andersson and associates to hypothesize that the increase in Na excretion results from altered renal hemodynamics secondary to the pressor effect (6, 7).

Many other actions of intraventricularly administered AII have also been well documented. However, it is not clear whether endogenous angiotensin (either plasma borne or generated by the putative brain–renin angiotensin system) is physiologically active within the brain. The present study was designed to test two hypotheses. The first is that the natriuretic effect of centrally administered AII is dependent upon an increase in blood pressure. The second is that endogenously generated AII is tonically active in the CNS in controlling Na excretion.

Methods. Female mongrel dogs weighing 14.8–21.5 kg were anesthetized with sodium pentobarbital, 30 mg/kg iv. Catheters were placed in both femoral arteries for collection of arterial blood samples and measurement of blood pressure with a Statham pressure transducer and Grass polygraph. A femoral vein was cannulated, and both ureters were catheterized with flared polyethylene tubing. The catheters were placed so that tips were at the renal pelvis, and they were regularly checked to verify that urine was free flowing.

The dog was then placed in a stereotaxic apparatus, and a beveled 25-gauge needle connected to PE 50 polyethylene tubing was inserted into the right lateral ventricle (8, 9). By means of a t-connector, pressure in the inflow line was measured with a Statham strain gauge and recorded on the polygraph. CSF pressure ranged from 5 to 10 cm saline relative to the auditory meatus and did not change throughout the experiment. Soon after cannulating the ventricle, an infusion of an artificial CSF was begun at a rate of 30 μl/min. At the end of each experiment, ventricular placement of the needle was verified by infusing blue dye at 30 μl/min for 15 min. Needle placement was considered successful if the dye stained only the ventricular system.

Following surgery, a priming dose of *para*-aminohippurate (PAH) (7 mg/kg) and creatinine (25 mg/kg) was injected iv in approximately 5 ml of isotonic saline. An infusion mixture containing PAH (250 μg/kg·min) and creatinine (500 μg/kg·min) in 0.9% saline was then begun at a rate of 0.52

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ml/min. The renal clearance of PAH was used an an estimate of renal plasma flow (RPF) and the creatinine clearance as an estimate of glomerular filtration rate (GFR). Thereafter, a minimum of 45 min was allowed for equilibration, and the experiment was begun.

The protocol consisted of a 1-hr control period, a 2-hr experimental period, and a 2-hr recovery period. During both the control and recovery periods, the artificial CSF vehicle was infused intraventricularly. In one group of animals, AII (6 ng/min) was infused intraventricularly during the experimental period. In a second group, SQ20881 (0.5 μg/min) was infused. These agents were dissolved in artificial CSF. In a third group of control dogs, artificial CSF alone was administered intraventricularly during the experimental period.

To test whether the effects of SQ20881 were due to an action on the brain and were not secondary to changes produced by the agent after it reached the general circulation by way of the sinus veins, similar doses of SQ20881 (0.2–9.4 μg/min) were infused iv in a final group of dogs.

Urine was collected in 15-min periods throughout the experiment. Arterial blood samples were drawn by free flow into chilled plastic tubes every 30 min, at the midpoint of alternate urine collections. All blood samples were replaced immediately with equal volumes of 3% dextran in 0.9% saline injected intraarterially.

PAH, creatinine, and electrolytes were measured in plasma from heparinized blood (8 ml). Blood samples (5 ml) for aldosterone measurement were collected once every hour in EDTA. Plasma was separated into plastic tubes and immediately frozen (−20°) for future analysis. Mean blood pressure, heart rate (HR), CSF pressure, and esophageal temperature were recorded every 15 min at the beginning of each clearance period. Body temperature was maintained at presurgical levels by adjusting the temperature of a heating pad under the animal.

Artificial CSF composition. The composition of the artificial CSF solution (in m*M*) was: Na, 150; Cl, 133; Mg, 2.0; Ca, 2.4; HCO_3, 24.5; PO_4, 0.5; and glucose, 2.5. The CSF was prepared by the University of Michigan Hospital Pharmacy and was sterile and nonpyrogenic.

Analytical procedures. The plasma concentration of aldosterone was measured by radioimmunoassay of methylene dichloride-extracted plasma (Diagnostic Products Corp., Los Angeles, Calif.).

Plasma and urinary concentrations of creatinine were determined by the method of Bonsnes and Taussky (10) and PAH by the method of Smith *et al.* (11). Urinary plasma concentration of sodium was measured by flame photometry.

Statistical evaluation of changes within groups was performed with analysis of variance for repeated measures (12). In the event that there were changes in the time control group, comparison of the differences between groups at each time was tested with one-way analysis of variance and Dunnett's *t* test (12).

Results. The effects of intraventricular infusion of artificial CSF, AII, and CEI on sodium excretion are illustrated in Fig. 1. Although Na excretion increased in time

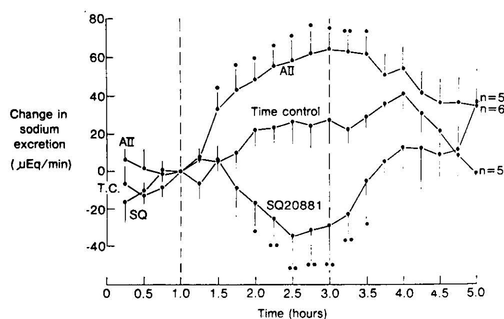

FIG. 1. The effect of intraventricular infusion of angiotensin II (AII), SQ20881, and artificial CSF (time controls) on renal sodium excretion. The data are expressed as the change from the fourth 15-min control period. Points are means ± SEM. AII and SQ20881 were infused (Hours 1–3) during the time bracketed by the dashed parallel lines. * $P < 0.05$, ** $P < 0.01$ when comparisons were made to the time control group at each time. Fourth control period excretion rates (μEq/min) were: for the control group, 69.6 ± 12.4; for the AII group, 62.3 ± 16.4; and for the SQ20881 group, 96.3 ± 22.5. These values are not statistically different ($P < 0.05$) from each other.

control animals ($F = 4.1$, $P < 0.001$), and in dogs which received AII ($F = 11.5$, $P < 0.001$), intraventricular infusion of AII increased the excretion rate of sodium above that exhibited by control dogs. This effect was statistically significant ($P < 0.05$) within 30 min after beginning the AII infusion. In contrast, central CEI infusion decreased the basal Na excretory rate ($F = 1.9$, $P < 0.025$). The decrease was significantly different from control animals within 1 hr of SQ20881 infusion (Fig. 1); however, intravenous infusion of CEI (0.2–9.4 g/min) had no effect on sodium excretion (23 ± 12 μEq/min control; 30 ± 8 μEq/min after 2 hr of iv SQ20881; $n = 4$).

Figure 2 summarizes the changes in BP and renal hemodynamics for the three groups. The increase in sodium excretion resulting from intraventricular AII administration was not accompanied by increased BP. Similarly, BP did not change in dogs which received CEI. C_{Cr} increased slightly in every group (AII group: $F = 8.3$, $P < 0.005$; time control group: $F = 8.6$, $P < 0.001$; and CEI group: $F = 7.4$, $P < 0.005$); however, the increase was not different between the groups. C_{PAH} did not change in any group. It should also be noted that the fractional excretion of Na was increased 5.8 ± 0.8% AII infusion; $P < 0.03$. SQ20881 decreased fractional excretion 2.7 ± 1.3%, $P = 0.06$.

Urine flow rate increased in the time control group (Table I) but did not change with intracerebral infusion of AII or CEI. In Table I are shown the aldosterone values for the three groups. Plasma aldosterone increased with time in dogs which received artificial CSF (time control group) and CEI; however, there were no differences in aldosterone levels between the AII-infused or CEI-infused dogs and the control dogs.

During the initial control period, body temperature averaged 38.3 ± 0.5° in the control dogs, 38.0 ± 0.6° in the AII-infused dogs, and 37.6 ± 0.2° in the CEI group, and did not change in any group. Nor did hematocrit change in any group. The first hourly values were: 42.6 ± 1.9% (time controls), 46.5 ± 1.0% (AII group), and 40.2 ± 2% (CEI group).

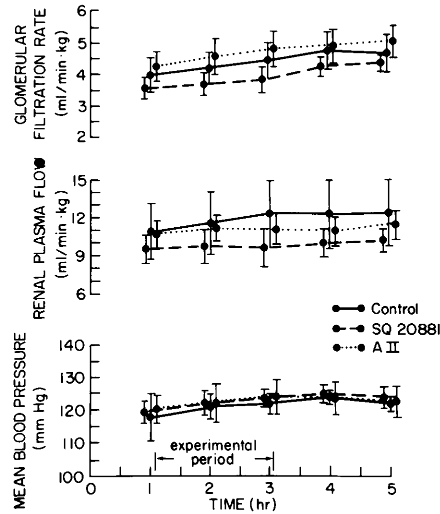

FIG. 2. The effect of intraventricular infusion of angiotensin II (AII) ($n = 4$), SQ20881 ($n = 5$), and artificial CSF (control) ($n = 5$) on renal plasma flow (ml/min·kg body wt), glomerular filtration rate (ml/min·kg body wt), and arterial blood pressure (mm Hg). Points represent mean ± SEM. The 2-hr experimental period (Hours 1–3) is indicated on the abscissa.

Discussion. The present experiments confirm earlier reports that central infusion of AII increases Na excretion (2–6, 7). Andersson and co-workers reported that the natriuretic response to intraventricular AII infusion in the goat correlated with increases in blood pressure. This finding led them to speculate that the increase in sodium excretion may have been secondary to increases in GFR and RPF produced by the pressor response (6, 7). A major finding of the present experiments, however, is that the natriuresis was not associated with measurable changes in BP or in whole kidney GFR or RPF.

The lack of a central AII-induced increase in blood pressure may be explained in part by the low doses of AII used in the present study (6 ng/min). Andersson and associates infused AII in doses of approximately 15–80 ng/min (2, 3, 6, 7), and Severs *et al.* injected a dose (500 ng) in rats which was found to increase blood pressure by about 30 mm Hg (4). Species differences may also exist, however, since higher doses

TABLE I. EFFECT OF INTRAVENTRICULAR INFUSION OF ARTIFICIAL CSF (TIME CONTROLS), AII, AND SQ20881 ON URINE FLOW AND PLASMA ALDOSTERONE CONCENTRATION

	Hours						
	0	1	2	3	4	5	
Time controls (n = 5)							
Urine flow (ml/min)		0.49 ± 0.03	0.62 ± 0.05	0.75 ± 0.07	0.80 ± 0.08	0.69 ± 0.10	$F = 8.6$, $P < 0.001$
Plasma aldosterone concentration (ng/dl)	28 ± 8	34 ± 9	48 ± 12	48 ± 11	47 ± 11	57 ± 12	$F = 8.1$, $P < 0.001$
AII group (n = 5)							
Urine flow (ml/min)		0.62 ± 0.11	0.69 ± 0.09	0.77 ± 0.07	0.77 ± 0.04	0.75 ± 0.07	
Plasma aldosterone concentration (ng/dl)	35 ± 3	35 ± 3	37 ± 5	38 ± 5	38 ± 7	40 ± 4	
SQ20881 group (n = 5)							
Urine flow (ml/min)		0.67 ± 0.13	0.70 ± 0.16	0.62 ± 0.10	0.73 ± 0.10	0.73 ± 0.08	
Plasma aldosterone concentration (ng/dl)	24 ± 7	26 ± 9	28 ± 8	38 ± 11	38 ± 12	36 ± 12	$F = 3.3$, $P < 0.05$

Note. Values represent mean ± SEM. The experimental period includes Hours 2 and 3.

of AII (100–1000 ng) injected into the ventricular system of conscious dogs failed to elicit a pressor response (13).

Although these experiments rule out a pressor-induced increase in GFR or RPF as the factor which mediated the natriuresis, the possibility that a shift in renal blood flow distribution affected sodium excretion cannot be excluded. Other efferent pathways may have been involved. Central AII administration decreases renal nerve activity (14). Since renal nerve activation promotes sodium retention (15), an alternative hypothesis may be that decreased renal nerve activity contributed to the natriuretic state.

Of the possible humoral agents affecting sodium excretion, aldosterone is an unlikely candidate since plasma levels did not change during intraventricular AII infusion and the onset of response was too short to be mediated by aldosterone (16). This finding is in conflict with a previous study of ours which demonstrated that intraventricular AII infusion inhibits aldosterone secretion (8); however, the animals in that study were Na deprived. Hence, the ability of central AII administration to affect aldosterone secretion appears to depend on the sodium balance of the animal.

Intraventricular infusion of the converting enzyme inhibitor reduced renal Na excretion. Again, the changes in excretory rate could not be related to changes in BP, GFR, or RPF, or plasma aldosterone levels. Hence, the mechanism which mediated the Na retention following central administration of SQ20881 remains to be identified. Possibilities that cannot be ruled out by these experiments are: (1) a redistribution of renal blood flow and (2) a decrease in the level of a natriuretic hormone.

The ability of intraventricular CEI infusion to suppress renal Na excretion, an effect opposite to the natriuretic effect of central AII administration, implies that endogenous angiotensin acts in the brain to stimulate the kidney to excrete Na. Clearly, the effect of SQ20881 is due to a direct action on the brain since intravenous infusion had no effect on Na excretion. This observation is in agreement with the report that intrarenal infusion of SQ20881 (2 μg/min) did not affect Na excretion in animals fed a normal Na diet. Indeed, SQ20881 is natriuretic in animals in which the renin–angiotensin system is activated (17, 18).

The fact that the action of CEI was limited to the brain suggests that the inhibitor blocked intracerebral conversion of AI to AII. This possibility is supported by reports that converting enzyme activity is present in the brain (11, 19). Whether AI is plasma generated or produced by the putative brain renin–angiotensin system is not revealed by the present studies. However, the conclusion that intracerebral infusion of CEI acts by interference with endogenous generation of AII must remain tentative due to its other known effects. The ability of SQ20881 to potentiate the action of bradykinin in peripheral plasma has been well documented (20, 21). Since bradykinin has been localized in brain (20), it is possible that the effect of SQ20881 to reduce Na excretion was mediated through increases in bradykinin concentration. Nonetheless, the fact that the converting enzyme inhibitor produced an effect opposite to the natriuretic action of intracerebral AII, favors the hypothesis that the converting enzyme inhibitor interfered with CNS AII formation. It should be pointed out that these experiments were acute ones carried out in anesthetized dogs in which the renin–angiotensin system was undoubtedly elevated. In spite of this both AII and CEI caused changes in Na excretion. The change in Na excretion was independent of changes in BP, RPF, or GFR, and was apparently unrelated to plasma aldosterone levels (the effect was seen too rapidly to be a result of undetected changes in aldosterone). These data are consistent with the hypothesis that endogenously generated AII may affect Na excretion by action on a central receptor. The efferent path of this response, however, remains to be characterized.

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