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# Effects of Chronic Intraventricular Administration of Angiotensin II on Drinking Behavior and Blood Pressure[1]

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GRONAN, R. J. AND D. H. YORK. *Effects of chronic intraventricular administration of angiotensin II on drinking behavior and blood pressure.* **10**(1) 121–126, 1979.—Angiotensin II was continuously infused into the lateral cerebral ventricle of rats, and the effects on daily food and water consumption, urine volume, and aortic blood pressure were studied. AII was infused at a rate of 10 ng/hr for seven days, using subcutaneously implanted osmotic minipumps. An intraventricular (IVT) control group was infused with only the saline vehicle, while a third group received AII subcutaneously. IVT AII rats showed a four-fold increase in water consumption, to a mean of 171 ml/day during Days 2–4 of infusion, whereas water intake of the other groups did not change from preinfusion levels. Urine volume showed a similar pattern to water intake, increasing five-fold in the IVT AII group during Days 2–4. These measures declined during the final three days of AII infusion, but significant tolerance was not observed. Food intake decreased markedly in both saline and IVT AII groups after implantation of the pumps, but the latter resumed normal food intake more slowly than the former, and body weight remained below preinfusion levels throughout the AII period. Aortic blood pressure of the IVT AII rats showed a slight, but progressive, rise during the infusion period, but it did not significantly exceed that of the saline rats. These results indicate that continuous, low-level, intraventricular infusion of AII may markedly increase water intake without significantly increasing fluid retention or blood pressure.

Angiotensin II  Drinking  Feeding  Urine  Blood pressure  Central nervous system

A POSSIBLE role of the renin-angiotensin system in hormonal stimulation of thirst was first suggested by Fitzsimons [6] who demonstrated increased water consumption following manipulations which increased renin release by the kidney. Subsequent investigations showed that systemic or intracranial administration of renin, angiotensin I or angiotensin II (AII) cause increased drinking behavior [2, 8, 15]. AII is a potent dipsogen in a wide variety of species [7]. In the rat, drinking is reliably produced by intracerebroventricular (IVT) injection of doses as low as 10–100 ng [15], although intravenous administration requires much higher doses to be effective [8]. This and other evidence has led to hypotheses that AII acts on receptors in the brain to initiate drinking associated with hypovolemic [7] or hyperosmotic [13] thirst.

Intraventricular administration of AII also elicits an acute rise in blood pressure of conscious animals [15]. This effect does not appear to involve a direct action of AII at peripheral sites, since identical intravenous or intraarterial doses have a much-reduced hypertensive effect [14]. A number of sites bordering the ventricular system have been reported to be involved in this response, including the area postrema [5,21] the subnucleus medialis in the cat [3] and the anterior third ventricle region in the rat [1,10]. Severs *et al.* [15] have presented evidence that, in the rat, the hypertensive effects of IVT AII involve a dual action of central sympathetic stimulation and increased release of antidiuretic hormone (ADH). A number of other reports have confirmed the ADH stimulating effects of IVT AII [17].

Although acute changes following central administration of AII have been described, relatively little is known about the effects of administering AII for longer periods. In rats receiving tap water, continuous IVT infusion of AII for a period of five hours elicited copious drinking only during the first hour [13]. The blood pressure effects of long-term infusion of AII into the cerebral ventricles are of interest not only because such actions might be demonstrable at doses lower than those commonly used in acute studies, but because of a possible relevance to the genesis of certain forms of hypertension [9,20].

The introduction of implantable, osmotically driven, minipumps for drug infusion has facilitated long-term IVT administration of AII in the conscious rat. Two questions were of interest in the present study. First, do the dipsogenic

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effects of AII exhibit tolerance after continuous IVT infusion over a period of many days. Second, does continuous IVT infusion of AII at a dose that is relatively low, but sufficient to induce copious drinking, cause a sustained change in blood pressure over a period of several days.

## METHOD

### *Animals*

Male, Sprague-Dawley rats weighing 400–600 g were housed individually in metabolism cages which allowed measurement of daily food and water consumption and daily urine volume. Tap water was presented via metal drinking spouts which terminated just outside the cage, and which were accessible to the rat through a hole in the side of the cage. Sodium concentration of the tap water was 1.2 mEq/liter. A spill cup, containing a small amount of mineral oil to prevent evaporation, was positioned under the spout to collect water spilled during drinking by the rat or when changing the water bottle. Powdered lab chow (Purina) was also available ad lib from a metal cup positioned at the end of a narrow extension of the cage, which prevented food from dropping into the urine funnel. Urine was collected in a beaker which also contained a thin layer of mineral oil to prevent evaporation. Ambient lighting was programmed on a 12:12, light:dark cycle (lights off 700–1900 hr) and room temperature was maintained at approximately 22°C.

### *Surgery*

*Intraventricular cannulation.* Under pentobarbital anesthesia (Nembutal, 60 mg/kg, IP) rats were prepared for stereotaxic surgery by incising the shaved scalp and removing the periostium. A hole was drilled into each of the frontal and parietal bones to receive one of four stainless steel anchoring screws. The skull was positioned according to the atlas of Skinner [19] and using Bregma as reference, a 21 ga thin-walled, stainless steel guide cannula was stereotaxically lowered through a burr hole to coordinates AP 0.0, L 1.5, H 3.0. The guide cannula was fashioned from a Becton-Dickinson vacutainer needle, which provides a plastic base firmly attached to the guide cannula, and which is threaded at the top for convenient attachment of a protective polyethylene cap. The guide cannula contained a stainless steel stylet sealed at the top by a collar made of concentric pieces of polyethylene (PE) tubing. The cannula base was fixed to the skull and anchoring screws with methyl methacrylate cement, and the incision was closed.

*Aortic catheter.* In the same surgical session, a chronic polyethylene catheter was implanted into the descending aorta, according to the method described by Weeks and Jones [22] with modifications. The catheter was run subcutaneously to the nape of the neck, where it was exteriorized and stopped with a stylet. Rats received a prophylactic dose of penicillin G (50,000 units) postoperatively. A recovery period of at least one week was allowed after this surgery.

*Minipump implantation.* Under fentanyl/droperidol anesthesia (Innovar, 0.1 cc/kg, IM), an Alzet (Alza Corp.) osmotic minipump, with a nominal reservoir capacity of 170 $\mu$l and pumping rate of 1 $\mu$l/hr, was implanted subcutaneously lateral to the right scapula. The minipumps were filled with AII (Beckman), 10 $\mu$g/ml, in sterile, non-pyrogenic 0.9% sodium chloride solution, which was further purified by passage through a Swinny filter (0.45 $\mu$m). The pumps were weighed before and after this procedure to ensure proper filling. A flow moderator (21 ga stainless steel tubing), connected to PE 60 tubing, was inserted into the pump, and the pumps were then primed for 30 min by placing them in a beaker of saline until a flow of AII solution out of the tube was noted. The minipumps were then implanted subcutaneously through a small incision, and the PE tubing was run to the caudal margin of the acrylic skull cap. Here the tubing was connected to a length of 25 ga stainless steel tubing (prefilled with AII solution), the other end of which was positioned next to the top of the existing IVT guide cannula. This was covered with an additional layer of acrylic. The stylet was removed from the guide cannula and replaced with a 25 ga stainless steel injector cannula, with an attached length of polyethylene tubing. The injector cannula was inserted into the right lateral ventricle 0.5–1.0 mm beyond the tip of the guide cannula, until backflow of CSF could be seen. The polyethylene tubing was looped 180° and joined to the other piece of 25 ga tubing, which led to the pump. Thus, the CSF-filled injector cannula was connected to the AII-filled delivery line without any intervening bubbles. The loop of tubing was protected by a polyethylene screw cap. This arrangement allowed visual inspection of the drug delivery line, and if necessary, the injector cannula, at any time during the experiment.

### *Procedure*

Rats were implanted with an intraventricular cannula and intraaortic catheter at least one week prior to the start of drug infusion. Baseline values of food and water consumption and urine volume were taken daily by measuring the difference in weights of the containers. Water consumption was always corrected for spillage. Aortic blood pressure was also measured daily (1700–1800 hr), using a Statham P23Dc transducer and a Grass polygraph. The rats were unanesthetized and lightly restrained during this procedure. These measures were continued for ten days following implantation of the AII-containing minipumps. The nominal infusion rate of the pumps was 10 ng/hr of AII, or approximately 480 ng/kg/day, for seven days (170 hr). At the completion of the experiment, 1 $\mu$l of trypan blue dye was injected into the ventricular cannula. Brains were immediately removed and examined for distribution of dye in the ventricular spaces.

## RESULTS

The dose of 10 ng/hr, IVT, of AII was chosen in a pilot run comparing two rats given this dose with two given 100 ng/hr, IVT. The mean peak water consumption of the latter was 478 ml on the second day, approximately the same as body weight. The lower dose produced a less marked, but still substantial, dipsogenic effect, and was chosen for further study.

In seven rats administered AII, water intake showed a four-fold increase, compared to the preinfusion rate of 42 ml/day, to a mean of 171 ml/day, during Days 2–4 of lateral ventricular infusion (Fig. 1). During the final three days (Days 5–7), mean water intake slowly declined, dropping to 137 ml/day on the seventh day. The pattern of drinking of individual rats resembled this group effect, with water intake of all the rats peaking between Days 2–5, inclusive, and decreasing thereafter. For the entire infusion period, water intake of the IVT AII group significantly exceeded that of the

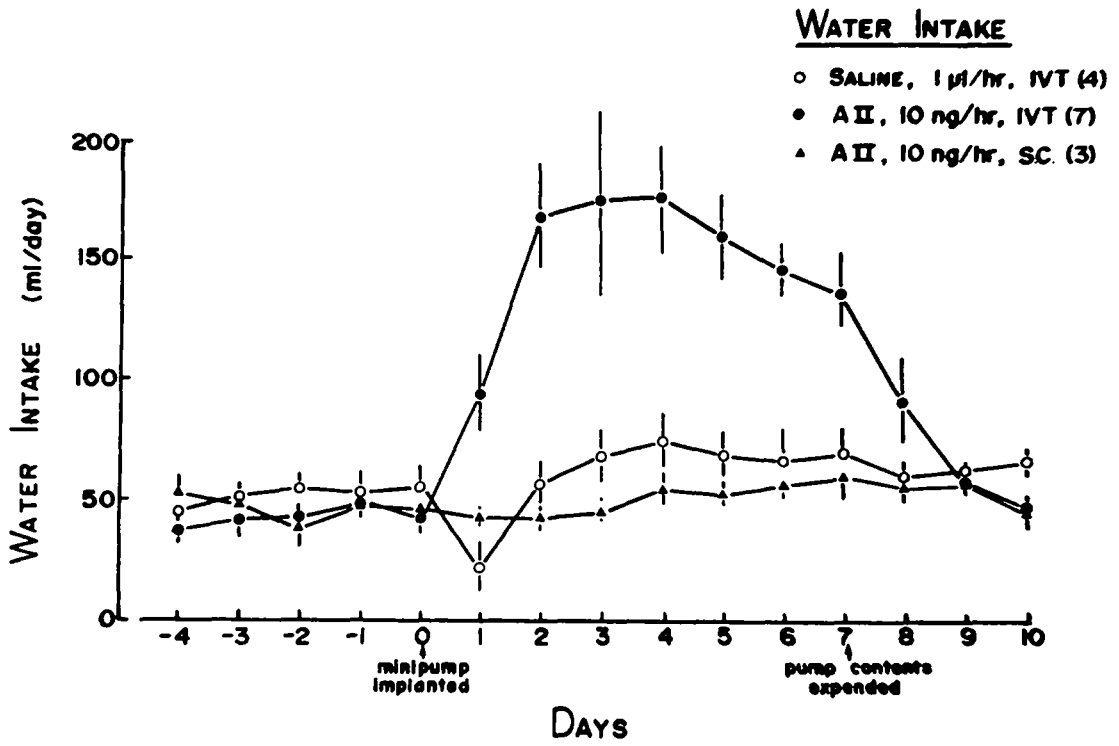


FIG. 1. Mean daily water intake ( ± SE) in rats administered angiotensin II intracerebroventricularly (IVT) or subcutaneously (SC), and in rats infused only with saline vehicle. Infusions began at time zero, and continued for just over seven days (170 hr). Numbers in parentheses indicate group size.

vehicle control (IVT saline) group ($p<0.001$), as determined by least squares analysis of variance.

In three rats, AII was administered subcutaneously, i.e., the pump was not connected to the IVT cannula. No change in water intake was observed.

Urine volume showed a similar pattern to water intake, increasing five-fold in the IVT AII group during Days 2–4 of infusion, and declining thereafter (Fig. 2). Compared to a preinfusion mean of 24.6 ml/day, urine ouput reached a mean of 133.5 ml/day during Days 2–4, and 115.9 ml/day during Days 5–7. For the entire period of infusion, urine output of the IVT AII group significantly exceeded that of the vehicle control group ($p<0.001$).

In addition, during the first three days of IVT infusion, the difference in volume between water intake and urine output increased sharply in the AII group. The mean difference of 40.8 ml in the AII group significantly exceeded that of 21.5 ml in the saline group during this period ($p<0.01$). This difference decreased to a mean of 30.5 ml during the remaining days of the AII infusion period, which was not different from control.

Food intake decreased markedly following minipump implantation, but returned to baseline levels within 3–4 days (Fig. 3). However, while the initial decline in eating during the first day was similar in both groups, and was probably an after-effect of the surgery or anesthetic, the saline group recovered their previous rate of food intake more quickly than the AII group ($t$-test, $p<0.05$). A difference was also seen in body weight. Mean body weight decreased 18.5 g in saline rats and 17.5 g in IVT AII rats one day after implantation of the minipumps, but whereas the former regained their preinfusion weight by the third day, and continued to gain weight thereafter, the latter remained at this decreased weight level throughout the remaining six days of AII infusion.

Mean aortic blood pressure declined slightly in both the AII and vehicle control groups following minipump implantation, but returned to near preinfusion levels (AII) after four days (Fig. 4). However, no significant difference was found between these groups ($p>0.05$). One rat in each of these groups developed a non-patent blood pressure catheter after the minipumps were implanted, and these are excluded from the data.

The patency of the drug delivery line and injector cannula was checked in two rats during the fifth day of AII infusion. This was done by disconnecting the looped tubing under the head cap, and removing the injector cannula briefly, to check for tissue obstruction. No obstruction was found in either case. In addition, confirmation that the pumps were still working was provided by the movement, over several minutes, of the AII solution in the end of the delivery line.

The two rats checked in this manner had shown the most precipitous declines in water intake, following an initial peak, that was observed in the IVT AII group. Water intake continued to decline after the cannula was checked, indicating that blockage of the delivery system was not the cause.

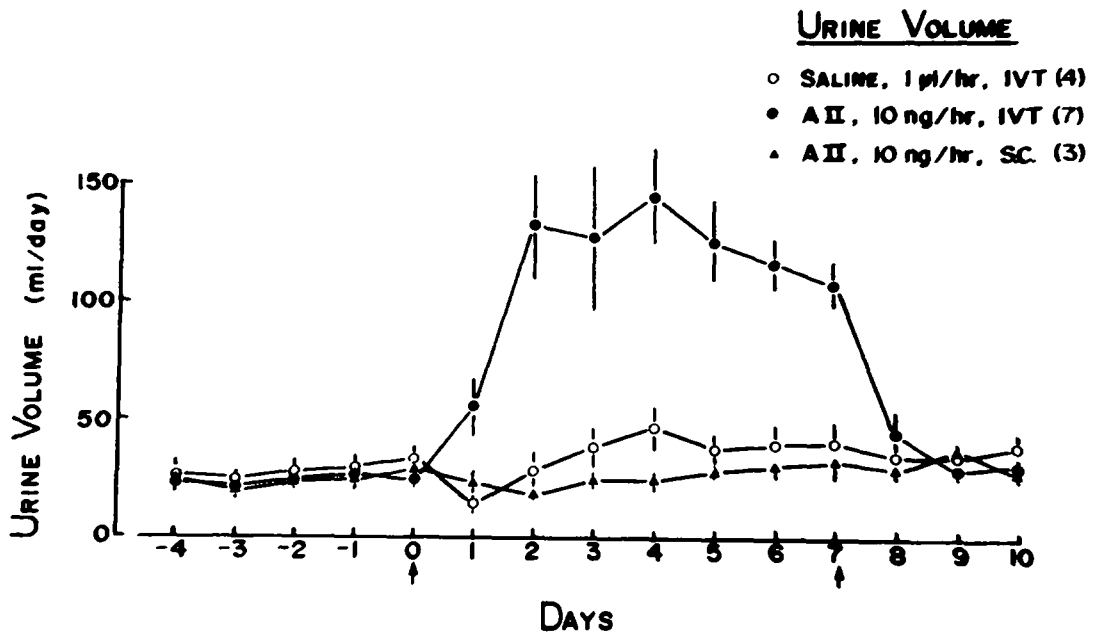


FIG. 2. Mean daily urine output ( ± SE) in rats administered angiotensin II intracerebroventricularly (IVT) or subcutaneously (SC), and in rats infused only with saline vehicle. Infusions began at time zero, and continued for just over seven days (170 hr). Numbers in parentheses indicate group size.

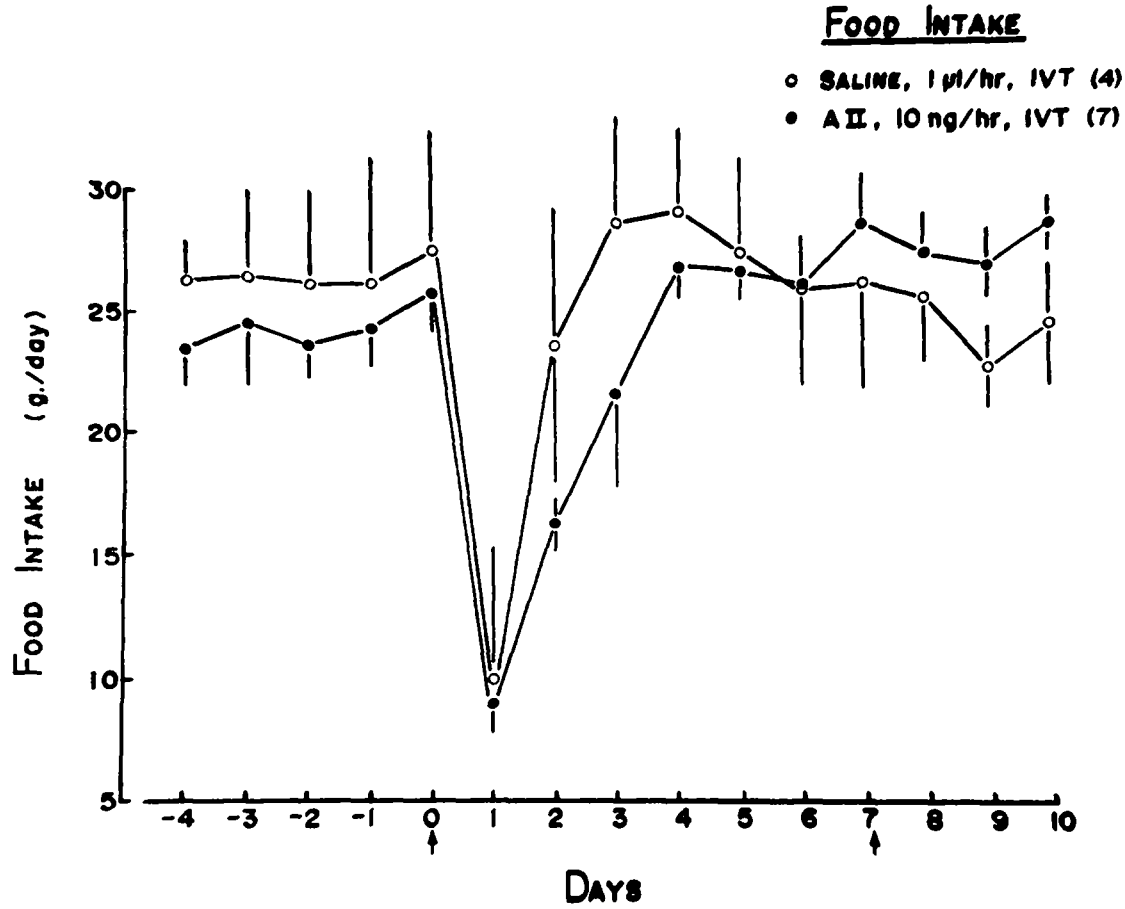


FIG. 3. Mean daily food intake ( ± SE) in rats infused intracerebroventricularly (IVT) with angiotensin II or saline vehicle. Infusions began at time zero, and continued for just over seven days (170 hr). Numbers in parentheses indicate group size.

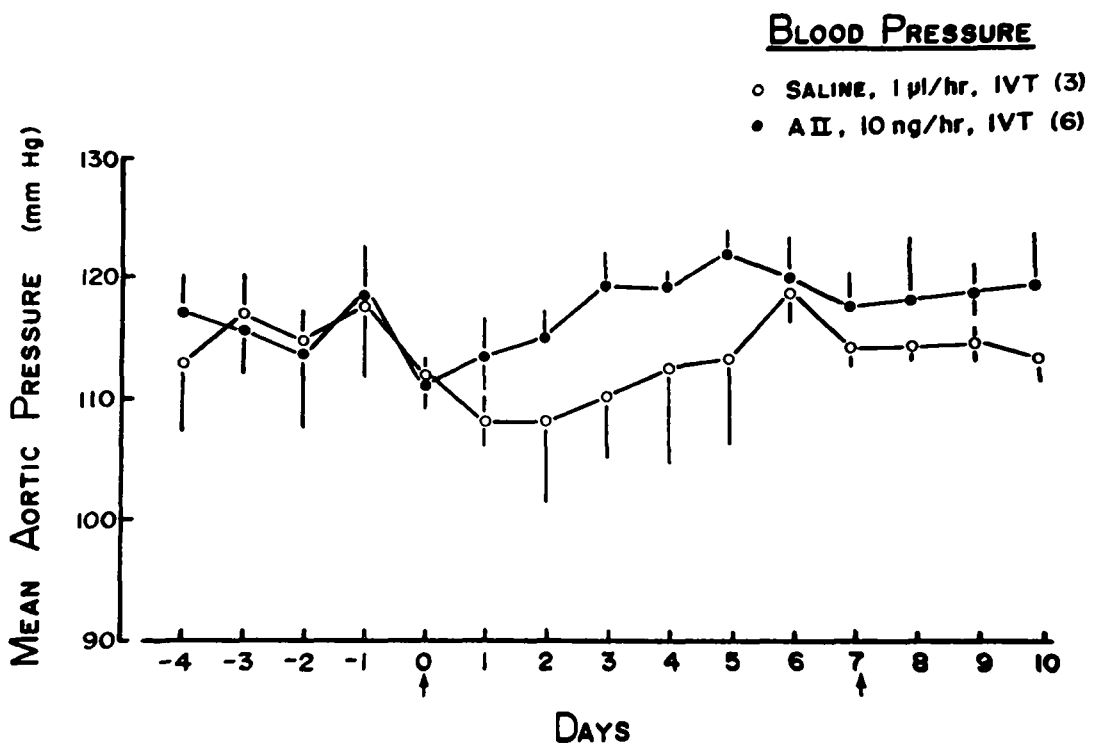


FIG. 4. Mean aortic blood pressure ( ± SE), as measured once daily in rats infused intracerebroventricularly (IVT) with angiotensin II or saline vehicle. Infusions began at time zero, and continued for just over seven days (170 hr). Numbers in parentheses indicate group size.

However, no attempt was made to independently verify the pumping rate specified by the pump manufacturer, which is discussed below.

## DISCUSSION

In this study, an increase in water intake was observed every day of a continuous seven-day infusion of AII into the lateral ventricle of the rat. At this relatively low dose, significant development of tolerance to the dipsogenic action was not seen. However, a progressive decrease in water intake was observed during the final three days of infusion. This may have been due to a decrease in the pumping rate during the course of the infusion period. Data provided by the pump manufacturer shows that, under conditions of subcutaneous implantation in rats, the pumping rate declines approximately 17% (from the nominal rate of 1 µl/hr) after the third day of operation. A decrease of this magnitude could account for the decline in drinking observed during the latter part of the infusion period. This would appear to place limitations on the usefulness of these pumps in estimating development of tolerance. Thus, an answer to the question of tolerance to the dipsogenic effects of low level intraventricular infusion of AII may await the development of osmotic pumps which display constant pumping rates over a longer period of time.

These results demonstrate that continued drinking of water was necessary to achieve, or maintain, satiation of the AII-induced thirst. It has previously been reported [13] that intraventricular infusion of AII for a period of 5 hr elicits drinking of water only during the first hour, whereas drinking of isotonic saline continues unabated. This was interpreted to indicate that water intake lowered plasma osmolality and satisfied an osmotic thirst drive induced by AII. Such a finding is not necessarily inconsistent with those reported here, since we measured total intake, but not the pattern of water intake, over the course of a day. Indeed, casual observation revealed that rats did generally drink in bouts. Thus, the possibility that drinking followed a cyclical pattern, with oscillations in the state of hydration of the animal, requires further investigation.

Comparison of water intake and urine output gives some indication of the state of hydration induced by AII. During the first three days of AII infusion, the difference between water volume and urine volume increased markedly over preinfusion levels. This difference might be due to an increase in insensible water loss, or considering the larger volumes involved, increased evaporation from the collecting funnel. On the other hand, evidence of angiotensin's dipsogenic and ADH stimulating actions [16] suggests the possibility of water retention. Examination of body weights does not appear to support this latter possibility, however. While both groups lost weight immediately after the minipump surgery, saline animals, but not those receiving intraventricular AII, regained weight quickly. This appears to be due to the observed depression of food intake accompanying AII-induced drinking, a phenomenon that has been reported in acute studies [4]. Fluid retention due to AII might be expected to reduce or reverse the difference in body weights between these groups. Thus, these results do not point to significant fluid retention induced by continuous low level infusion of AII.

Rats to which AII was administered subcutaneously did not increase water intake. This is not surprising, since the nominal infusion rate of 0.166 ng/min in this study is several orders of magnitude less than the dose of AII required to elicit drinking after acute intravenous administration [8] AII has also been shown to have dipsogenic effects after subcutaneous administration of comparably high doses [11]. Considering the low dose used in this study, it is not likely that systemic absorption of subcutaneously administered AII would have been significantly impeded by local tissue vasoconstriction. Therefore, these findings would appear to be in accordance with studies, previously cited, which have established a central site(s) of AII's dipsogenic actions.

Aortic blood pressure did not change significantly at this level of AII infusion. This does not rule out the possibility that AII may have induced a transient hypertensive effect immediately after the infusion began, a possibility suggested by reported increases in blood pressure caused by acute IVT doses of AII as low as 1 ng [15]. Rather, starting with the first blood pressure observation almost 24 hr after pump implantation, no substantial hypertensive effect was seen. A slight hypotensive effect resulting from surgery or vehicle infusion is suggested in saline rats, but does not appear to be present in AII animals.

The blood pressure effects of chronic IVT AII have not previously been reported in the scientific literature. However, a graph, recently distributed with promotional literature for the osmotic pumps by Alza Corp., shows a 40 mm Hg rise in systolic blood pressure during the second week of IVT infusion of AII to rats. This longer infusion period was accomplished by replacing the pump after one week with a new one. The rate of AII infusion, 6 $\mu$g/hr, was 600 times greater than that used in the present study, and the drinking fluid was isotonic saline. The larger dose and longer infusion period, in particular, could account for the differences with the results reported here.

AII was also reported to induce hypertension in dogs after chronic intravenous infusion at a rate approximately 20 times (10 $\mu$g/day) that used in the present study, if the blood pressure testing was done in a busy laboratory environment [12]. Little change in blood pressure was noted if testing was performed under conditions of minimal environmental disturbance, as was done in our study. This suggests that the blood pressure effects of continuous AII administration may interact with other factors that influence sympathetic tone. The osmotic pump delivery system employed here appears to offer great opportunity for further investigation of such interactive effects.

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