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Dose-Response Analysis of Angiotensin- and Renin-Induced Drinking in the Cat[1]

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BROPHY, P. D. AND R. A. LEVITT. *Dose-response analysis of angiotensin- and renin-induced drinking in the cat.* PHARMAC. BIOCHEM. BEHAV. 2(4) 509–514, 1974. – A dose-response study of drinking elicited by angiotensin-II in the cat found a threshold below 125 ng and a maximally effective dose of 500 to 1000 ng. For renin, the threshold was between 1 and 5 Goldblatt milliunits and the maximally effective dose above 45 Goldblatt milliunits. The dose-response curves for water consumption elicited by these two drugs and the dose-response curves for latency to drink did not differ in the 5 different neural sites examined (septal region, caudate nucleus, preoptic area, lateral hypothalamus and lateral ventricle). Although cats stimulated with angiotensin-II drank at a similar rate to water deprived animals, renin-stimulated cats drank significantly more slowly.

Angiotensin Thirst Chemical brain stimulation Drinking Water ingestion Limbic system Renin Cats Hypothalamus

RENIN is an enzyme released by the kidneys in response to a fluid deficit, signalled to the kidneys by a reduction in blood flow through these organs. In the blood, renin acts on angiotensinogen, an alpha-2-globulin from the liver, to form angiotensin-I (AI), a decapeptide. Angiotensin-I is then cleaved to angiotensin-II (AII), an octapeptide, by converting enzyme [13]. AII has a variety of physiological actions, which may also be shared by renin and AI. These include vasoconstriction, ADH release from the posterior pituitary and aldosterone release from the adrenal cortex [12, 13, 14]. These actions have in common a maintenance of blood pressure and body fluid volume and osmolarity.

In 1969, Fitzsimons and Simons [9] reported that intravenous AII infusion in the rat elicited drinking. It was then discovered that injection of microgram quantities of AII directly into the brain elicited drinking in a variety of mammalian species, including the rat, rhesus monkey, goat and cat [1, 3, 7, 8, 19, 21]. AII is an effective dipsogen when applied in very low doses, down to about 5 ng, and at a variety of hypothalamic and limbic system sites. Renin administration to the brain has also elicited water ingestion; however, it is not clear whether this is a direct action of renin, or due to conversion of angiotensinogen to AI and/or AII [7].

The present paper reports dose-response and latency-to-drink data following the intracranial administration of AII or renin into five different anatomical locations in the cat.

METHOD

Animals

Eight male and 9 female adult mongrel cats weighing between 2.5 and 6.0 kg were used in these two experiments. These 17 animals participated in the AII study and 12 of them were also used in the renin study. The animals were housed individually in 60 x 45 x 75 cm cages with

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dry food (Purina Cat Chow) and tap water available at all times.

Surgery

Animals were anesthetized with Nembutal Sodium (40 mg/kg). Six to eight 23 ga stainless steel guide cannulae were implanted in each cat and cemented to the skull. The cannulae were aimed to rest about 4 mm above the neural structures selected for study. Regions stimulated in these experiments were septum, preoptic area, lateral hypothalamus, caudate nucleus and lateral cerebral ventricle [15]. The number of tested sites in each experiment were: angiotensin-II = septum – 8, preoptic area – 7, caudate nucleus – 6, lateral hypothalamus – 6, lateral ventricle – 5; renin = septum – 6, preoptic area – 5, caudate nucleus – 6, lateral hypothalamus – 4, lateral ventricle – 5. Many of the cats were used in both experiments, and also in a third experiment to be reported separately; however, each site, below each cannula, was only used in one of the studies. A total of 56 sites located within the 5 anatomical structures were examined in the two experiments reported.

Test Procedures

A minimum of 14 days separated surgery from the beginning of chemical testing. Chemical injections were then begun, at 48 hr intervals. Each drug injection was preceded by a 30 min pretest for satiation. The cat was then removed from its cage and held while the appropriate drug injection was made, using aseptic procedures. The drug was injected into the brain, through the previously implanted 23 ga cannula, using a 30 ga needle attached to a Hamilton Company microsyringe. After the injection, the needle was held in place for 5 sec and then replaced by a stylette, to prevent the injected fluid from backing up into the guide shaft. The cat was then observed during a 30 min drinking test. Descriptions of gross behavior were continually recorded. Water intake per 5 min period was also measured to the nearest ml using modified Kimax drinking tubes [22].

Drugs

Drugs were dissolved in an isotonic 5-ion artificial cerebrospinal fluid containing primarily sodium (127.6 mM) and chloride (134.5 mM) with trace amounts of potassium (2.5 mM), calcium (1.3 mM) and magnesium (1.0 mM) ions. For injections into tissue (septum, preoptic area, caudate nucleus and lateral hypothalamus) injection volume was 1 μl. A volume of 16 μl was used when injecting into the lateral ventricle, so as to insure spread of the solution within the ventricular system. To maintain comparable doses, the concentration of the drugs for ventricular injections was 1/16 that used for tissue injections.

Each series of drug injections was made in a randomized order. For Experiment 1 (angiotensin II amide) there were 7 conditions: a dummy injection (D, the lowering of an empty cannula), an injection of the artificial cerebrospinal fluid (CSF) and injections of AII in doses of 125, 250, 500, 1000 and 2000 ng. For Experiment 2 (renin), there were also 7 conditions; D, CSF, 1000 ng of AII and hog renin in doses of 1, 5, 15 and 45 Goldblatt milliunits [11]. The renin was obtained from the Sigma Chemical Company. One μg of the protein, a purified hog kidney extract, is approximately equivalent to one Goldblatt unit. One Goldblatt unit raises the blood pressure of the conscious intact dog by 30 mm of mercury. The injected solutions, containing AII or renin, were approximately isotonic, having a millimolarity between 267 and 280.

Criterion for a Positive Response

If the cat drank 30 ml or more during the 30 min following a brain injection the response was arbitrarily labelled as an induced drinking response. This criterion was based on over 1,000 observations of 30 min control drinking levels, in which this volume or more was consumed on less than 1 percent of the trials. Data are reported in Experiment 1 only for sites at which at least one of the doses of AII elicited at least 30 ml of water intake. For Experiment 2, data are presented only for sites at which the 1000 ng dose of AII elicited at least 30 ml of water consumption.

Histology

Animals were first anesthetized with Nembutal and then 1 μl injections of red ink were made at each of the drug injection sites to be microscopically examined. The cats were then perfused via the innominate artery with saline followed by 10% Formalin. Brains were blocked in the frontal plane, removed from the cranial vault, quick frozen and sectioned at 40 micra. The sections were then stained using a modified Kluver and Barerra technqiue [16]. Data are reported only for sites found histologically to be within the intended structures. The number of correct stimulation sites reported on for each experiment and neural site is listed above in the surgery section. Placements varied between 11.0 and 16 mm AP, 0.0 and 7.0 mm lateral and 8.0 and 18.0 mm in depth, according to the atlas of Jasper and Ajmone-Marsan [15]. A representative photomicrograph has been recently published [21]. Drawings showing all of the actual brain injection sites may be found in the original dissertation [4].

RESULTS

Experimentt 1: Angiotensin

Data reported were obtained from 32 brain injection sites in 17 cats. These data include between 5 and 8 placements in each of the 5 neural sites aimed for. Each placement was categorized as positive for the elicitation of drinking in the cat by AII.

Data on the volume of water consumed following injections into the 5 selected structures for each of the drug doses are shown in Fig. 1. Analyses of variance and post-anova comparisons (Neuman Keuls) showed that average volume of water consumed did not differ across the 5 sites of injections. Volume of water consumed did differ as a function of dose (Fig. 1). The volumes consumed following the lowering of an empty cannula (dummy, D) or the injection of artificial cerebrospinal fluid (CSF) into the brain were the lowest and did not differ from each other. Volume consumed following 125 ng of AII was greater than following the D or CSF injections ($p<0.05$) but less than following the higher doses of AII ($p<0.05$). Volumes consumed following brain injections of 250, 500, 1000 or 2000 ng of AII did not differ from each other.

Figure 2 illustrates the data on the latency to onset of drinking at each of the 5 sites of injection as a function of brain injection condition. An analysis of variance and post-anova comparisons showed a significant difference in latency to drink across structures. This, however, was the

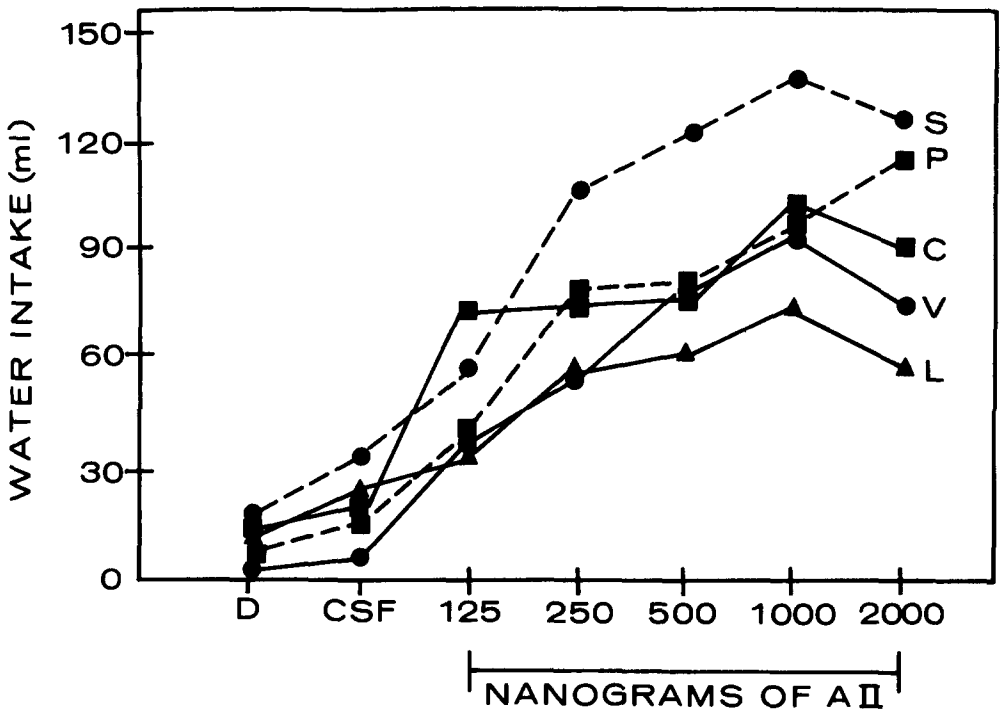

FIG. 1. Angiotensin-induced drinking: volume of water ingested during a 30 min test (D = dummy injection, CSF = artificial cerebrospinal fluid, AII = angiotensin, S = septum, P = preoptic area, C = caudate nucleus, V = lateral cerebral ventricle, L = lateral hypothalamus; see text for details).

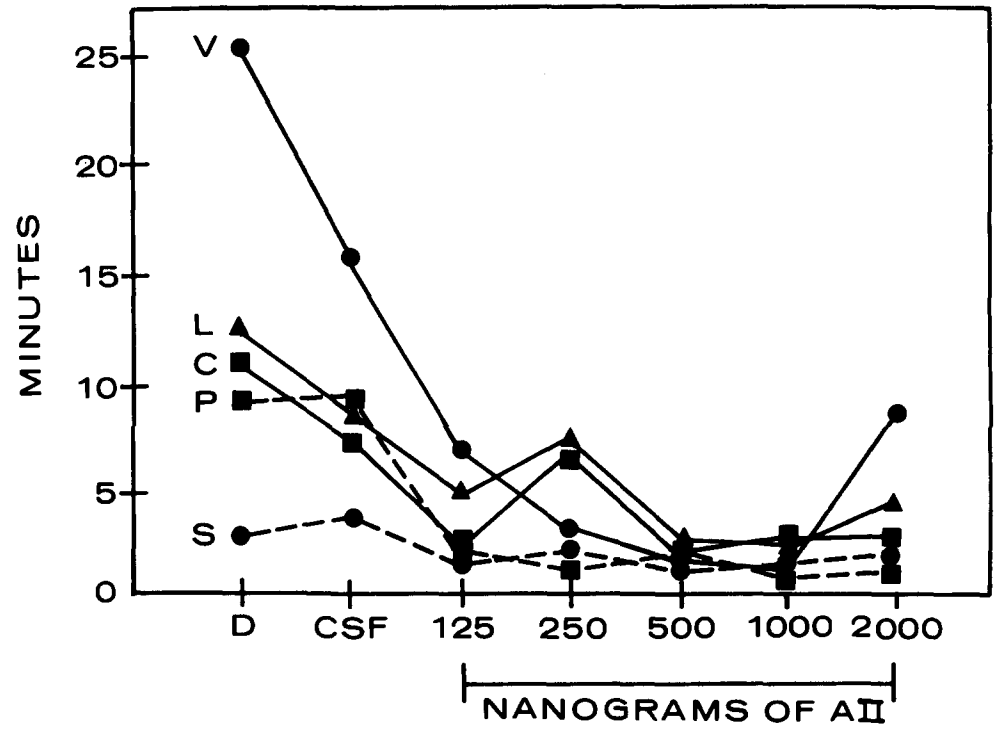

FIG. 2. Angiotensin-induced drinking: latency to begin drinking (see legend to Fig. 1 and text).

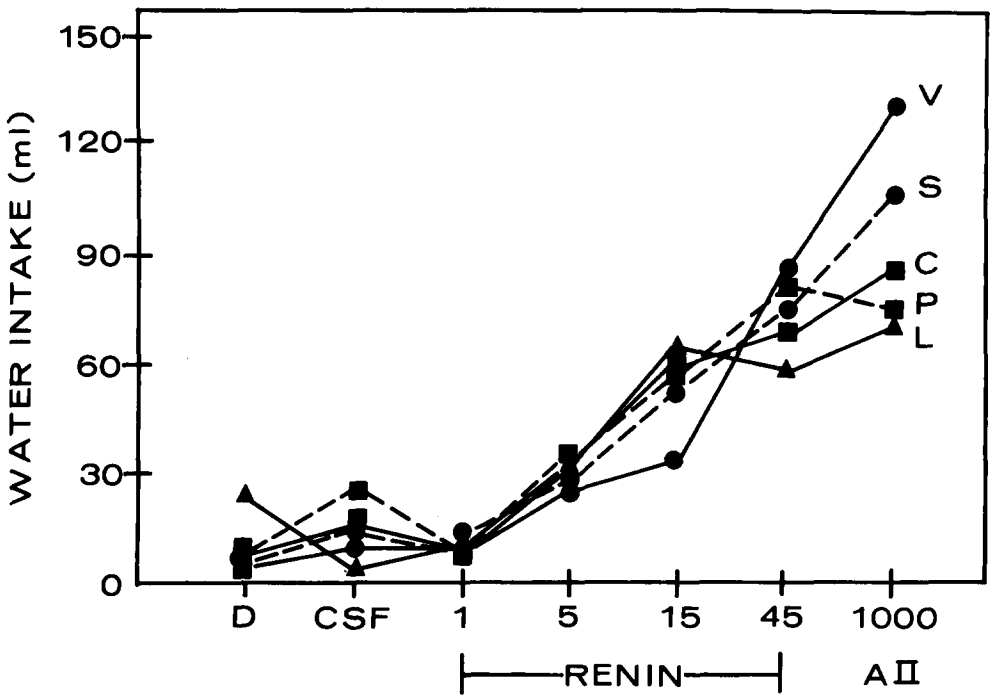

FIG. 3. Renin-induced drinking: volume of water ingested during a 30 minute test (doses of renin – 1, 5, 15 and 45 are in Goldblatt milliunits; dose of AII is 1000 ng; see legend to Fig. 1 and text).

result of some uncontrolled source of variability involving primarily the ventricular injections. The D and CSF injections for the ventricular sites were accompanied by higher drinking latencies than for the other structures of injection. For the conditions when a dose of AII was actually injected into one of the sites, no difference in drinking latency was found between structures. The relatively large variability for latency between all injection site groups during the D and CSF conditions appear to have been neutralized by the potent dipsogenic action of the AII.

A reliable effect of drug injection condition on latency to drink was also found. However, post-anova comparisons showed simply that latencies were higher following the D or CSF injections ($p<0.05$) than following any of the doses of AII, which did not differ from each other.

Following the intracranial injection of the effective doses of AII the cats would typically begin to drink within 2 min. They drank rapidly in periodic bursts during the next 15 to 20 min and would then sit quietly in their cages grooming. No obvious changes in emotional reactivity or general demeanor were observed.

Experiment 2: Renin

Data reported were obtained from 26 brain injection sites in 12 cats. These data include between 4 and 6 placements in each of the 5 neural sites aimed for. Each was categorized as positive based on intake to the 1000 ng dose of AII.

The volume of water consumed during the 30 min test as a function of structure of stimulation and drug of injection is shown in Fig. 3. Water intake did not vary as a function of stimulation site, but did vary as a function of the drug injected into the brain. The D, CSF and 1 and 5 Goldblatt milliunits of renin conditions were followed by the lowest volumes of water ingestion, and did not differ from each other. The injections of 15 or 45 Goldblatt milliunits of renin or 1000 ng of AII produced progressively larger volumes of water ingestion. These 3 conditions each differed from the previous 4 as well as from each other (Neuman Keuls; each significant $p<0.05$). Therefore, statistically, in terms of increasing volumes of drinking, the conditions can be grouped as follows: D, CSF, 1, 5 < 15 < 45 < 1000..

Figure 4 depicts the data on the latency to begin drinking for this experiment. Again, structure of injection had no significant effect on latency to drink. The drug injected, however, did affect drinking latency. In general, drinking latencies progressively decreased with the highest latencies for the D and 1 conditions and latencies decreasing through the CSF, 5, 15, 45 and 1000 conditions. The following analysis depicts this finding. Conditions underlined by a common line were not significantly different from each other. Conditions not underlined by a common line were different from each other (Neuman Keuls; each significant difference, $p<0.05$).

D 1 CSF 5 15 45 1000

In order to compare the drinking rates for AII and renin to that of water deprived cats, 12 of the cats were twice water deprived for 24 hr (at one week intervals) and then their water ingestion was measured every 5 min for 30 min. Figure 5 illustrates the drinking rates for 24 hour water deprived cats, compared to drinking rates for these same cats

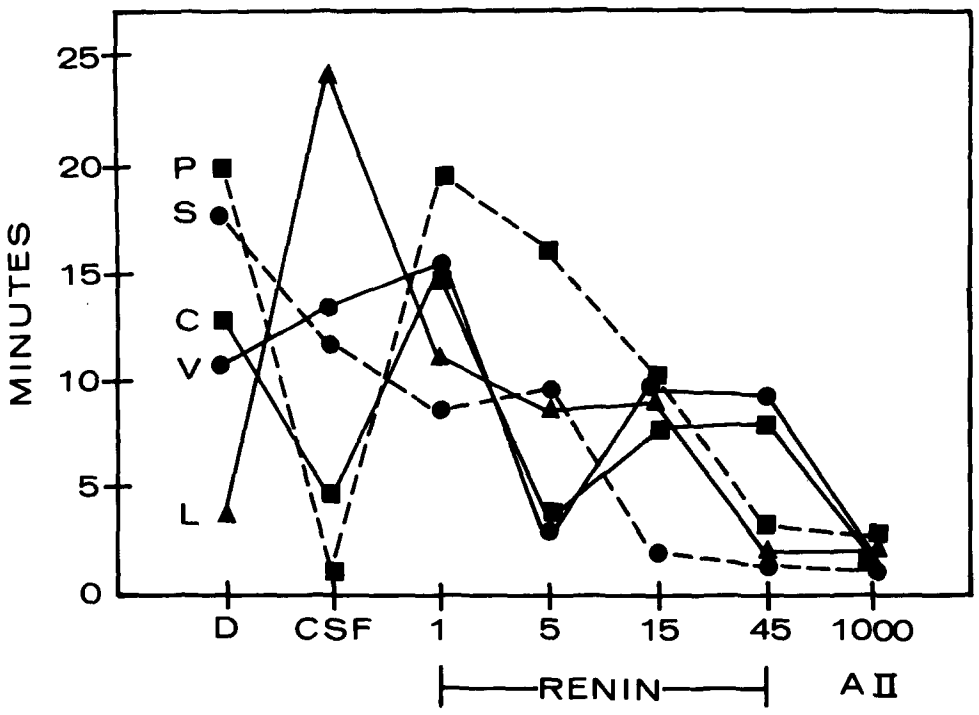

FIG. 4. Renin-induced drinking: latency to begin drinking (see legends to Figs. 1 and 3, and text).

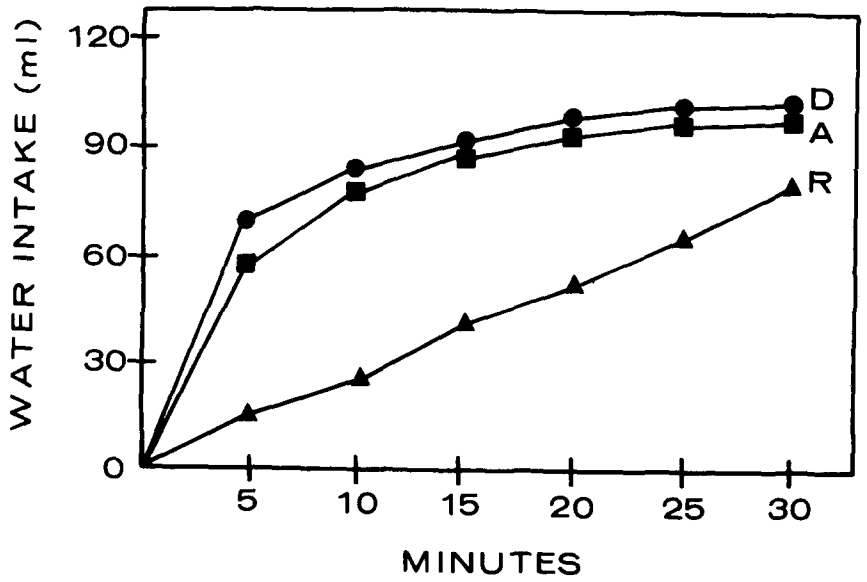

FIG. 5. Cumulative drinking curves following angiotensin, renin and deprivation (D = 24 hours of water deprivation, A = 1000 ng of angiotensin II, R = 45 Goldblatt milliunits of renin; see text for details).

when injected with the most effective doses of AII (1000 ng) and renin (45 Goldblatt milliunits). The AII and renin data illustrated in Fig. 5 were each obtained from one site in each of these same 12 cats that generated the deprivation data. Analyses of these data showed that the drinking curves for 24 hr of water deprivation and for AII were not different from each other, but that the drinking curve for renin was different from these other two. It is not simply that the renin causes less drinking during the 30 min tests than the other two stimuli, but rather that the slope of this curve is different from the other two. Stimulation with renin is accompanied by the drinking of a lower percentage

of the eventual 30 min volume during the first 15 min (50%), and a higher percentage during the last 15 min (50%), when compared to the deprivation and AII conditions (90% and 10%, respectively, for the comparable time periods; each significant $p<0.05$). Furthermore, while the volume of water consumed during the first 5 min is less for renin than for the AII and deprivation conditions (each $p<0.001$), by the end of the 30 min the total volumes are not significantly different from each other.

DISCUSSION

The drinking of water can be elicited in cats by intracranial microinjection of either AII or renin. No difference in volume consumed or latency to drink for AII or renin was found as a function of the 5 injection sites used in this experiment. The dose-response functions for volume and latency also did not differ across structures. For AII, a maximally effective dose, in terms of volume and latency was found to be between 250 and 1000 ng. Threshold would appear to be below 125 ng. For renin, the threshold was between 1 and 5 Goldblatt milliunits. However, the peak effective dose was not reached. Volume was still rising and latency decreasing at the maximum dose tested (45 Goldblatt milliunits).

One question of interest is whether renin itself is dipsogenic. Water ingestion has been elicited by intracranial microinjection in the cat in the present study, and also in rats in a previous study [7]. The enzymes responsible for the conversion from renin to AI to AII have been found present in the brain (in dogs) [10]. Furthermore, an AII antiserum was found in the rat to block the elicitation of drinking by AII or angiotensinogen, suggesting the necessity of conversion of this precursor to AII for dipsogenic activity [6]. These findings in conjunction with the current finding of a slower drinking rate for renin than for AII or water deprivation, suggest a significant role of conversion to AII in the drinking that follows injection of renin.

Another question concerns the distribution of neural sites sensitive to AII and subserving its dipsogenic function. The possibility of ventricular or circulatory diffusion from many dipsogenic sites to a single or a few actual sites has been suggested [2, 17, 18, 23]. Evidence for a central role for the subfornical organ in AII-elicited drinking has also been found [20]. Furthermore, radioassay studies with peripherally administered AII find localization only along the walls of the cerebral ventricles, with little or no penetration into the substance of the brain [24]. However, the presence of AII-forming enzymes in the brain itself [10] suggests the possibility of AII endogenous to the brain having a role in the neural mediation of thirst, in addition to the role of peripherally-formed AII in drinking behavior. This suggestion has also been made elsewhere [5,21].

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