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BRIEF COMMUNICATION

Drinking Elicited by Intracranial Microinjection of Angiotensin in the Cat[1]

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STURGEON, R. D., P. D. BROPHY, AND R. A. LEVITT. *Drinking elicited by intracranial microinjection of angiotensin in the cat.* PHARMAC. BIOCHEM. BEHAV. 1(3) 353–355, 1973. –When the level of angiotensin II in the brain is augmented by microinjection of this substance the fully-hydrated cat drinks copiously. The magnitude of this response is closely dependent upon the dose of angiotensin II injected. The latency of the drinking response was 1–2 min for each of the doses of angiotensin employed in this study. This is the first report of the induction of thirst in cats by central chemical stimulation and furthers the concept that angiotensin II is a potent dipsogen in vertebrates.

Angiotensin II Intracranial microinjection Drinking Cat

DURING conditions of extracellular fluid depletion (such as from a traumatic loss of blood) as well as during sympathetic nervous system activation (as during stress), a hormone called renin is released from the kidney [6]. Renin then interacts in the blood with its protein substrate angiotensinogen, to form angiotensin I. Angiotensin I is a decapeptide which is converted to angiotensin II (by converting enzyme) in the circulation [1]. Angiotensin II (henceforth referred to simply as angiotensin) is an octapeptide and is the physiologically active form of this hormone. There are several important physiologic effects of angiotensin. First, it has potent vasconstrictor properties. Second, angiotensin stimulates the release of aldosterone from the adrenal cortices (which causes a primary sodium retention and a secondary water retention in the kidneys) [5]. Third, it has been found to cause the release of the antidiuretic hormone (ADH) from the posterior pituitary [2,7]. Fourth, angiotensin has been shown to elicit the ingestion of water [4]. In the latter study it was demonstrated that intravenous infusion of angiotensin in the rat resulted in drinking. It was subsequently found [3] that microinjection of angiotensin in doses as small as 5 nanograms (ng) directly into brain activated drinking in the rat. Setler [8] has also observed drinking to intracranial microinjection of angiotensin in the rhesus monkey. One can readily see how these four effects of angiotensin (increased blood pressure, aldosterone release, ADH release, water ingestion) all would function in consort, in a homeostatic sense, to maintain fluid compartment balances and circulatory system pressure.

Since angiotensin has been found to elicit drinking in both rat and monkey, the possibility exists that this hormone may be involved in normal maintenance of body fluid homeostasis in all vertebrates. We therefore, undertook to investigate the effects of intracranial microinjection of angiotensin in the cat.

METHOD

Nine adult male and female cats that weighed between 2.5 and 5.5 kg were implanted, using strict aseptic techniques under sodium pentobarbital anesthesia (30–50 mg/kg), with four to six cannulae each stereotaxically aimed at cortical and subcortical brain loci. Approximately two weeks recovery time was allowed after surgery before the screening procedure with angiotensin and vehicle solutions was begun. Sites of drug injections were histologically verified subsequent to completion of the screening procedure. Animals were allowed free access to food and water at all times.

Doses of synthetic angiotensin (Hypertensin, Ciba) used in this experiment were 125, 250, 500, 1000 and 2000 ng. Control injections were of two types: empty cannulae and a fluid vehicle (a Ringer-Locke's solution which contained five ions in physiological concentrations). This solution was used as the vehicle for the angiotensin during drug tests.

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The volume of all drug injections was 1 μl. It was found that drinking did not differ among animals to these control (vehicle alone) injections. Water intake was measured during a pretest following which the cats were removed from their home cages and held by hand during injection of a drug solution or lowering of the empty cannula. The cats were then returned to their cages where latency and volume of drinking were measured. All drug injections were given in a completely counterbalanced order.

At the conclusion of this experiment the cats were perfused with saline and fixed with 10% formaldehyde. Brains were removed and frozen prior to sectioning at 40 μ. Sections parallel to cannula tracks were mounted on glass slides and stained with Luxol fast blue and cresyl violet.

RESULTS

Data are presented for 24 anatomical sites for which each of the doses of angiotensin and the control injections were completed and histological verification was obtained. Data are also presented for 14 sites where histological loci of stimulation were verified but each dose of angiotensin or both of the control injections were not tested. The dose-response relationship for the volume of water ingested to the various levels of angiotensin employed in this study, and also the pretest, empty cannula and a 5-ion control ingestion data are presented in Fig. 1. It was found that pretest, empty cannula and 5-ion control data did not differ reliably (Newman-Keuls), although it was found that the 5-ion solution had a dipsogenic effect at angiotensin-positive sites compared to the effect of that solution at negative sites (t 22 df = 2.44, $p<0.05$). In this figure drinking to the different doses of the drug at positive and negative drinking sites is also compared. The criterion used for placing an animal in the positive drinking response category was that it drank more than 30 ml of water to at least two of the doses of angiotensin. This criterion was established from data that cats consumed less than 30 ml of water during 95% of over 1000 pretest sessions, empty cannula and control injections. Using this criterion 12 sites were classified as negative (no drinking) and 12 sited were classified as positive for the elicitation of drinking subsequent to intracranial injection of at least two of the doses of angiotensin. It was learned that these sites (positive vs negative) differed reliably in terms of volumes of water ingested to angiotensin (F 1, 176=173., $p<0.001$). There was a reliable difference among the doses of angiotensin in relation to the volume of water ingested (F 7, 176=14., $p<0.001$).

In Table 1 a summarization is presented of the brain sites for which histological data are available. This includes the 24 sites for which a complete set of dose-response data is available and also 14 sites for which only partial data were obtained. The criterion for classifying a brain site as positive (drinking) or negative (nondrinking) again was that the drinking of 30 ml of water was obtained at that site to at least two of the doses of angiotensin. It can be seen from these data that drinking to intracranial injection of angiotensin was obtained at diffuse brain loci.

Figure 2 is representative histology obtained from cat No. 10 where the site of stimulation was found to lie in the lateral septal nucleus. The volume of water ingested at this site subsequent to injection of the drugs used in this study were: 2000 ng=264 ml; 1000 ng=257 ml; 500 ng=212 ml; 250 ng=143 ml; 125 ng =110 ml; vehicle=27 ml; empty cannula=14 ml. Drinking is elicited at neocrotical and subcortical stimulation sites, and angiotensin-effective sites include limbic as well as nonlimbic loci. One may question the role of the ventricles in this phenomenon. It was noted, however, that positive as well as negative results were found with stimulation sites that appeared to lie within the ventricles themselves. Also, positive and negative drinking sites were found to be juxtaposed and positive sites were not alway found to lie closer to the ventricle than did negative sites.

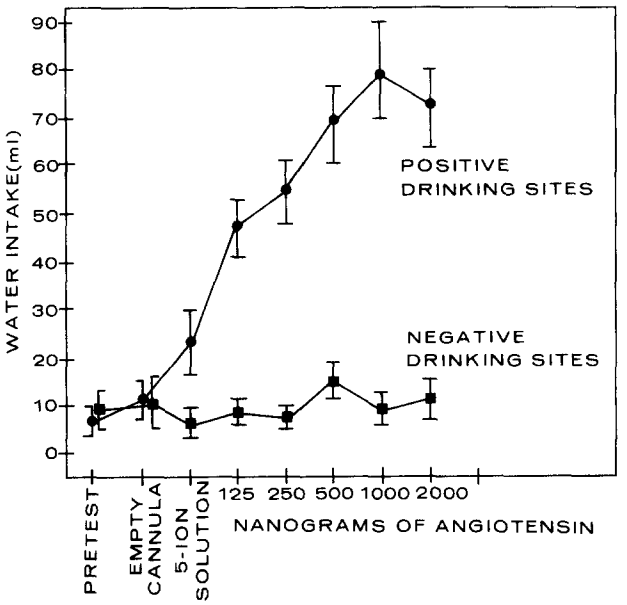

FIG. 1. Drinking elicited by intracranial angiotensin in the cat (means and standard errors for 12 positive and 12 negative drinking sites). Drug treatments are shown on the abscissa. Water intake is measured by the ordinate. All measures are of water intake within 30 min after the drug treatment.

TABLE 1

LOCATIONS OF POSITIVE AND NEGATIVE DRINKING SITES (38 PLACEMENTS IN 9 CATS)

	Positive Sites	Negative Sites
Neocortex	4	2
Corpus Callosum	1	1
Caudate Nucleus	5	0
Putamen	0	1
Pulvinar	0	1
Thalamic Nuclei	7	3
Septal Region	2	1
Hippocampus	1	2
Amygdala	0	1
Internal Capsule	0	2
Optic Tract	0	1
Lateral Ventricle	2	1

FIC. 2. Brain section from cat No. 10 with site of stimulation in the lateral septal nucleus. Brain coordinates [9] are 16.0 mm anterior to the interaural line, 0.0 mm horizontal and 1.5 mm lateral to the midline.

The latency of the drinking response after intracranial microinjection of angiotensin was approximately 1–2 min. Drinking was copious with most of the water being ingested within 15–20 min. During this period cats often ingested 80–90 ml of water. The drinking appeared to be normal with no seizures or other after-effects. The observation that the 1000 ng (1.0 μg) dose elicited somewhat more drinking than the 2000 ng dose of angiotensin is suggestive that the highest dose was not physiological and was in fact inhibiting the neural system mediating drinking relative to that mediated by the lower doses.

DISCUSSION

This is the first report of a primary dipsogenic effect of exogenous drug injections into CNS tissue of the cat. Microinjection of angiotensin into brain loci of cats maintained in normal water balance is a potent stimulus for the elicitation of drinking. This response is repeatable over test sessions with several dose levels of the drug. It appears there are a number of nonlimbic and limbic brain loci at which angiotensin is effective in the cat for elicitation of drinking. The renin-angiotensin system appears to be functional during conditions of extracellular fluid depletion in all mammalian species thus far studied.

Our data suggest a diffuse distribution of neurological sites at which angiotensin is an effective dipsogen. Receptors sensitive to blood-borne substances such as hormones, sodium chloride and glucose have characteristically been found localized in particular regions of the hypothalamus. In contrast, receptors sensitive to chemical synaptic transmitter agents such as acetylcholine and norepinephrine have been found widely distributed into limbic system circuits. These data lead us to suggest the possibility that angiotensin may be a synaptic transmitter agent (manufactured and released in the brain) as well as a hormone. This state of affairs is, of course, known to exist for norepinephrine.

REFERENCES

1. Biron, P. and C. G. Huggins. Pulmonary activation of synthetic angiotensin I. *Life Sci.* **7**: 965–970, 1968.
2. Bonjour, J. P. and R. L. Malvin. Stimulation of ADH release by the renin-angiotensin system. *Am. J. Physiol.* **218**: 1555–1559, 1970.
3. Epstein, A. M., J. T. Fitzsimons and B. J. Rolls. Drinking induced by injection of angiotensin into the brain of the rat. *J. Physiol.* **210**: 457–474, 1970.
4. Fitzsimons, J. T. and B. J. Simons. The effect of drinking in the rat to intravenous infusion of angiotensin given alone or in combination with other stimuli of thirst. *J. Physiol.* **203**: 45–47, 1969.
5. Goodman, L. S. and Gilman. *The Pharmacological Basis of Therapeutics.* New York: The Macmillan Co., 1970.
6. Guyton, A. *Textbook of Medical Physiology* 4th Ed. Philadelphia: W. B. Saunders Co., 1966.
7. Mouw, D., J. P. Bonjour, R. L. Malvin and A. Vander. Central action of angiotensin in stimulating ADH release. *Am. J. Physiol.* **220**: 239–242, 1971.
8. Setler, P. Drinking induced by injection of angiotensin II into the hypothalamus of the rhesus monkey. *J. Physiol.* **217**: 59P, 1971.
9. Jasper, H. H. and Ajmone-Marsan, C. Stereotaxic atlases: B. The diencephalon of the cat. In: *Electrical Stimulation of the Brain,* edited by D. E. Sheer. Austin: University of Texas Press, 1961, pp. 203–231.