Pharmacology Biochemistry and Behavior, Vol. 2, pp. 597–602. ANKHO International Inc., 1974. Printed in the U.S.A.

Antagonism of the Dipsogenic Action of Intraseptal Angiotensin II in the Rat[1]

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(Received 6 May 1974)

PERES, V. L., C. G. GENTIL, F. G. GRAEFF AND M. R. COVIAN. *Antagonism of the dipsogenic action of intraseptal angiotensin II in the rat.* PHARMAC. BIOCHEM. BEHAV. 2(5) 597–602, 1974. – The injection of 0.01 to 2.0 μg of antiotensin II (A II) into the medial septal area of unanesthetized rats in normal water balance caused dose-dependent drinking, during the 60 min period following drug administration. A hyperbolic dose-response curve, rectified by a log dose scale was obtained. Pretreatment with 5 and 10 μg of locally injected haloperidol, 15 min prior to A II (0.3 μg), partially antagonized the dipsogenic effects of A II and a dose of 25 μg of haloperidol completely blocked this effect. A cataleptic-like state followed haloperidol administration. The injection of doses as high as 25 μg of dopamine in the same brain site caused no drinking. Pretreatment with 3 μg of intraseptal Sar[1],Ala[3],Ile[8] – angiotensin I, a competitive antagonist of A II at peripheral receptors, completely antagonized the dipsogenic effect of A II. The same dose (3 μg) of the A II analog alone caused only a mild but significant drinking response. These results suggest that A II acts on specific receptors in the CNS that may be similar to peripheral angiotensin receptors. On the other hand, the role of brain catecholamines in the mediation of A II-induced drinking remains uncertain.

Angiotensin II Intraseptal injection Drinking Haloperidol antagonism
Antagonism by Sar[1],Ala[3],Ile[8]-Angiotensin I

DRINKING behavior has been elicited by electrical stimulation of the lateral hypothalamus [17, 20, 27, 28, 29, 39, 40, 42] and the injection of acetylcholine, carbachol or hypertonic saline into selected areas of the brain in several animal species [1, 2, 3, 9, 21, 22, 23, 26, 35]. Angiotensin II (A II) and related peptides, given intravenously [11,15] or injected directly into the hypothalamus, preoptic region and septum [4, 5, 7, 8, 12, 13, 38] were also reported to cause potent dipsogenic effects. The effect of A II may be due to motivational changes since response rates of water-reinforced operant bar-pressing have shown to increase sharply following the intracerebral injection of A II in the rat [19,33]. The action of angiotensin II on the CNS does not seem to be mediated through muscarinic, alpha or beta adrenergic or tryptaminergic pathways since it has been reported that neither atropine, propranolol, phentolamine nor methysergide block its dipsogenic effect [5, 14, 18, 38]. Conflicting results have been reported on the action of haloperidol, a drug assumed to block central dopamine as well as alpha catecholamine receptors [41,43], upon the dipsogenic effect of A II, since Fitzsimons and Setler [14] reported an inhibitory action, while Swanson *et al.* [38] reported no effect of haloperidol on angiotensin-induced drinking.

Several polypeptide analogs, behaving as competitive antagonists of A II at peripheral receptors [24, 25, 30, 31, 32], failed to block the dipsogenic effect of A II when previously injected into responsive areas of the CNS. On the other hand, many of these analogs caused dipsogenic effects of their own when given at higher than equipotent doses of A II [38].

The present study deals with the antagonistic actions of haloperidol and Sar[1],Ala[3],Ile[8]-angiotensin I (Sar[1], Ala[3], Ile[8]-A I) on the drinking behavior elicited by injections of A II into the medial septal area of water-replete rats. Sar[1],Ala[3],Ile[8]-A I, is a newly synthetized angiotensin analog, devoid of angiotensin-like actions on the isolated rat uterus, guinea-pig ileum and rat blood pressure, but acting as a specific, competitive antagonist of A II, at a low inhibitor : angiotensin ratio (Peña, C. and Stewart, J. M., personal communication).

METHOD

Fourty-five male, Holtzman rats weighing 200–300 g at the time of surgery were used. Animals were housed in individual cages with a food cup filled with dry mixed diet and a graduated drinking bottle filled with unfiltered tap

[1] This research was supported by the State of São Paulo Research Foundation (FAPESP).

water; daily readings were made of the intakes. After a few days, a stainless-steel cannula (o.d. 0.71 mm) was stereotaxically implanted into the septal area, under barbiturate anesthesia (sodium pentobarbital, 40 mg/kg, i.p.). The cannulae were positioned 0.7 to 1.0 mm anterior to the bregma, 0.3 mm lateral to the midline and 5.7 to 6.0 mm below the surface of the skull with the incisor bar set at 5 mm above the stereotaxic horizontal plane. Each cannula was provided with a stilette to prevent its obstruction. One week after recovery from the surgical procedure, injections were made through a dental stainless steel cannula (o.d. 0.31 mm) in the unanesthetized, unrestrained rat, which had been maintained with water ad lib. Drug solutions were delivered by a 10 μl microsyringe in a volume of 1.0 μl. For this purpose, a polyethylene plastic tube was connected to the dental cannula which was placed inside the guide cannula and pressed downward to its tip. After the intraseptal injections of A II, the water consumption of rats under usual laboratory living conditions was recorded for 1 hr; this value was compared with the water consumption of rats without any stimulation, or following control injections.

Fifty-three out of 55 rats showed induced drinking after the intraseptal injection of A II (0.3 μg). These animals were divided into 4 groups. The first group received increasing doses (0.01, 0.03, 0.1, 0.5, 1.0 and 2.0 μg) of A II at 48 hr intervals for dose-response curve determination purposes. The second group was injected intraseptally with different doses (5, 10 and 25 μg) of haloperidol, 15 min before the administration of 0.3 μg of A II in the same brain area. Intracerebral injections of either A II alone or 0.2% acetic acid (vehicle for haloperidol solutions) + A II were used as controls. The third group was injected with Sar^1,Ala^3,Ile^8-A I alone, or given 15 min before A II. Dopamine (5, 10 and 25 μg) was injected alone or 15 min prior to A II in the fourth group of animals.

The same rat was tested up to 9 times with at least 2 days between successive tests. At the end of the experiment, animals were anesthetized with sodium pentobarbital and the head perfused with saline (0.9 percent) and Formalin. The brains were removed, sectioned and stained. The injection site was determined by the end of the track left by the implanted cannula.

The following drugs were used: angiotensin II (Asn^1, Val^5,-angiotensin – Hypertensin CIBA), dopamine (Sigma), haloperidol (Johnson and Johnson), Sar^1, Ala^3, Ile^8-angiotensin I was kindly supplied by Prof. John M. Stewart, University of Colorado, Denver, Colorado, USA. Haloperidol was dissolved in 0.2% acetic acid solution, for injection. Other drugs were diluted in isotonic saline.

Statistical analysis was performed using Student's *t*-test for paired observations or analysis of variance.

RESULTS

Determination of the Dose-Response Curve of Intraseptal Angiotensin II on Drinking

The dose-response curve of A II on drinking was determined in 14 rats, in order to find out a suitable dose of the polypeptide to evidence either facilitation or inhibition of the drug effect when combined with the agents to be investigated. There was a dose-dependent effect on water intake during the 60 min following the intraseptal injection of A II. Intake was increased from 2.25 ± 0.72 ml, following 0.01 μg of A II to 10.25 ± 0.93 ml, induced by 2.0 μg of the polypeptide. Analysis of variance of the regression was significant ($p<0.01$). A graphic representation of the dose-response curve is shown in Fig. 1. A linear regression was calculated with log dose data: y = 8.588 ± 3.209 x (Fig 2). The ED 50 of A II was near 0.3 μg and this dose was subsequently used in drug-interaction experiments.

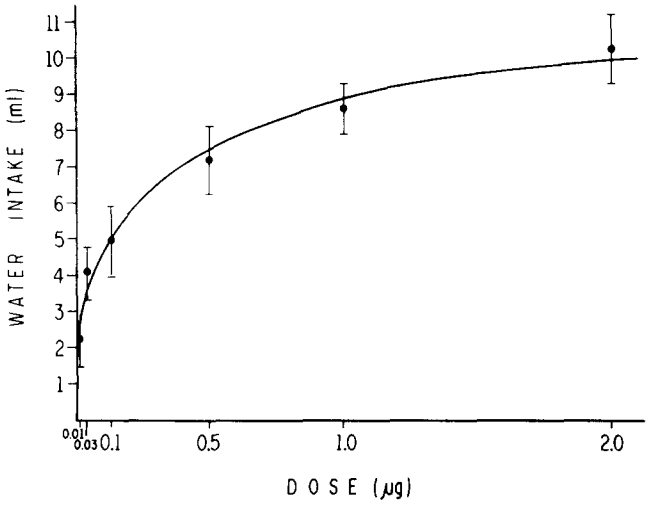

FIG. 1. Dose-response curve of angiotensin II on drinking. Points in the figure represent the mean water intake of 14 rats during the 60 min period following the injection of different doses of A II into the medial septal area of the brain. Bars represent ± S.E.

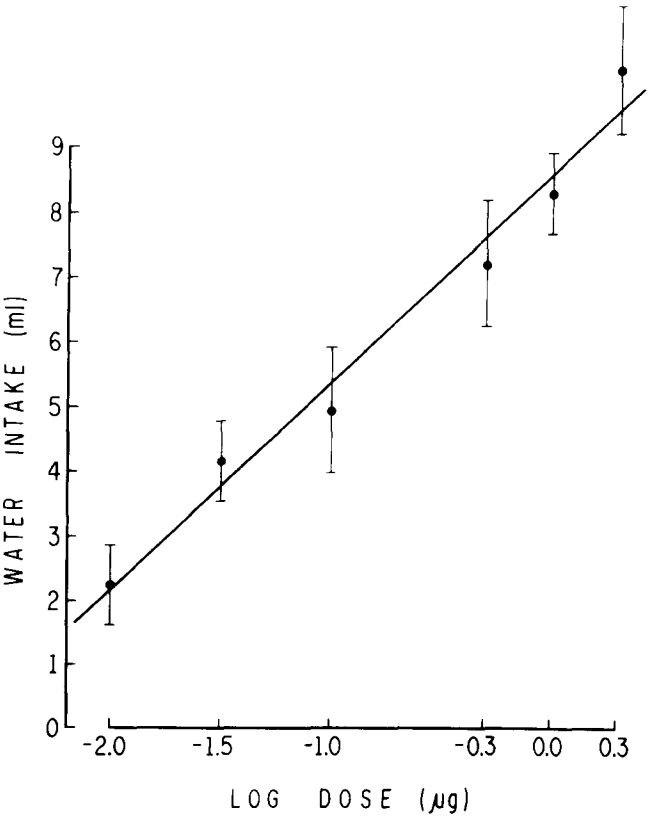

FIG. 2. Log dose-response curve of angiotensin II on drinking. See Fig. 1 for explanation.

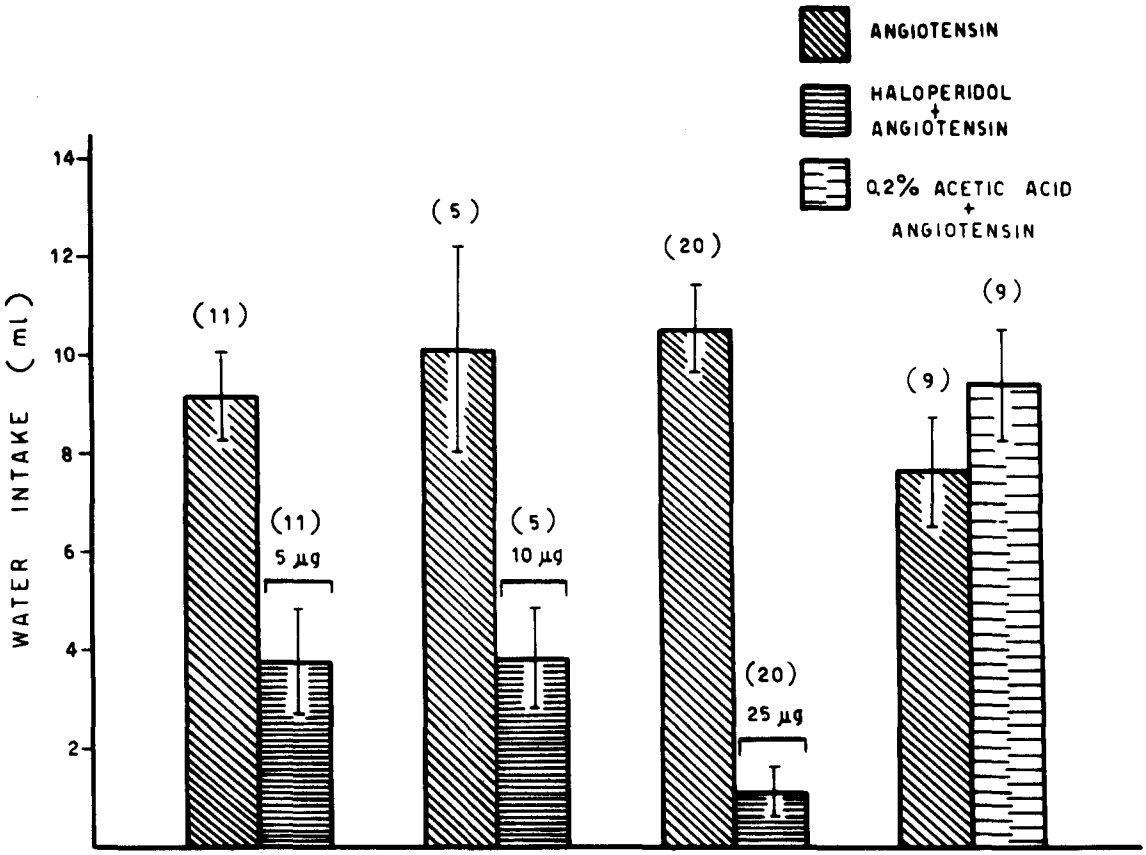

FIG. 3. Effect of haloperidol on angiotensin II-induced drinking. Columns represent mean water intake of water-satiated rats during the 60 min period following the intraseptal injection of A II (0.3 µg in 1 µl). Bars represent ± S.E. Haloperidol was injected into the same brain area, 15 min prior to A II, dissolved in a 0.2% acetic acid solution (1µl). Figures in parentheses indicate number of animals. Figures outside parentheses indicate doses of haloperidol.

Effect of Intraseptal Administration of Haloperidol on Angiotensin II Elicited Drinking

The columns in Fig. 3 represent the mean (± SE) water intake of rats given intraseptal administration of different doses of haloperidol (5, 10 and 25 µg) or 0.2% acetic acid vehicle, 15 min prior to the injection of 0.3 µg A II.

Pretreatment with 5 or 10 µg of haloperdiol caused a marked reduction of the dipsogenic effect of angiotensin (9.13 ± 0.86 to 3.72 ± 1.15 ml/60 min, $p<0.01$ and 10.10 ± 2.10 to 3.80 ± 1.02 ml/60 min, $p<0.01$, respectively), while the dose of 25 µg of haloperidol completely blocked the water intake caused by A II in 15 out of 20 animals. The remaining 5 animals showed a marked reduction in the A II effect after haloperidol. The overall means for these rats were 10.52 ± 0.87 ml and 1.10 ± 0.54 ml/60 min, $p<0.001$ for angiotensin-induced drinking without or after haloperidol, respectively. No effect of 0.2% acetic acid on the drinking response to angiotensin was observed.

Gross-behavioral changes characterized by a cataleptic-like state were induced by the intraseptal injection of haloperidol in all rats even at the lower dose level.

In view of these results with haloperidol it was of special interest to study the effect of intraseptal administration of dopamine on drinking. No effect was obtained after 5, 10 or 25 µg of the catecholamine. Intraseptal administration of dopamine, 15 min prior to A II, did not affect the water intake induced by the polypeptide (11.0 ± 2.01 and 10.07 ± 1.00 ml/60 min, respectively, $p>0.10$).

Effect of Sar^1, Ala^3, Ile^8-Angiotensin I

A dose of 3.0 µg of the polypeptide analog of A II, Sar^1,Ala^3,Ile^8-A I was intraseptally administered, 15 min before the injection A II or saline. The water intake was recorded for 60 minutes after the last injection. As shown in Fig. 4, pretreatment with the angiotensin analog completely blocked the dipsogenic effect of A II (9.11 ± 1.59 to 0.33 ± 0.21 ml/60 min, $p<0.001$). Injection of Sar^1, Ala^3,Ile^8-A I alone induced a water consumption of 1.83 ± 0.47 ml/60 min, that was significantly different ($p<0.05$) from the water intake induced by isotonic saline (0.66 ± 0.20 ml/60 min). One week later, the animals were tested again with A II alone, in order to verify if the angiotensin antagonist had permanently modified neuronal sensitivity to A II. The effect of A II was nevertheless unchanged (7.66 ± 1.00 ml/60 min). Gross-behavioral changes following Sar^1,Ala^3,Ile^8-A I were absent.

Localization of Implanted Cannula

The histological examination of the septal area showed

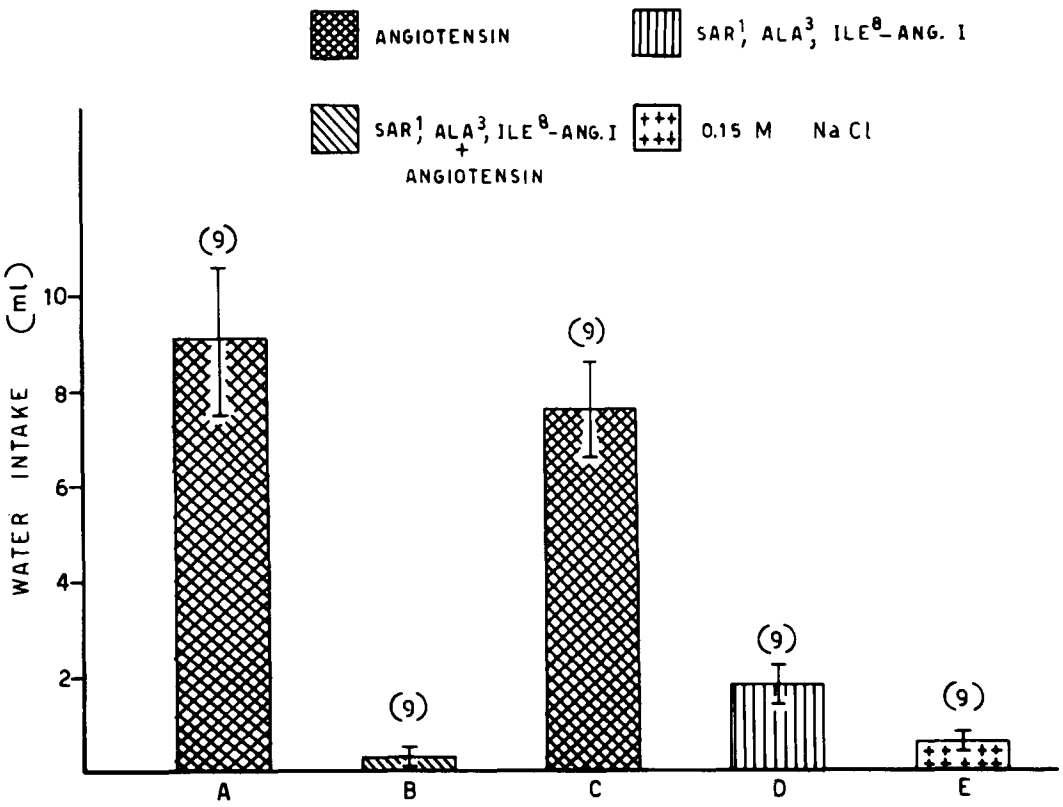

FIG. 4. Effect of Sar^1,Ala^3,Ile^8-angiotensin I on drinking and on angiotensin II-induced drinking. Columns represent mean water intake of water-satiated rats during the 60 min period following the last injection. Bars represent ± S.E. Column A shows the effect of 0.3 μg of A II alone. In B, 3 μg of Sar^1,Ala^3,Ile^8-A I was injected 15 min prior to the same dose of A II. Column C shows the effect of a second dose of A II (0.3 μg) made one week later. D and E show the effect of Sar^1,Ala^3,Ile^8-A I (3 μg), injected 15 min prior to saline or of saline alone, respectively. All injections were made into the medial septal area, in the same group of 9 rats, at intervals of at least 48 hr between successive injections. The effect of Sar^1,Ala^3,Ile^8-A I plus saline was significantly higher than that of saline alone (t-test, $p<0.05$).

that the cannulae tips were positioned dorsally and ventrally, from the medial to the posterior region, and were more medial than lateral. Extensive tissue damage was not observed.

In Fig. 5, the combined sites, plotted on schematic frontal sections through the septal area are presented. Practically the whole septal area was sensitive to A II.

DISCUSSION

The present results show a consistent relationship between dose of intracerebrally injected A II and amount of water ingested by water-replete rats, in agreement with previously reported results [7,38]. The hyperbolic shape of the dose-effect curve, rectified by a log dose scale suggests that a monomolecular interaction takes place between molecules of A II and pharmacological receptors present in the CNS.

The role of brain catecholamines as mediators of A II-induced drinking remains uncertain. Fitzsimons and Setler [14] have suggested that this effect of A II, in the rat is mediated by brain catecholamines, since local pretreatment with haloperidol or destruction of adrenergic neurons by 6-hydroxydopamine blocked the dipsogenic effect of A II. In contrast, the same pretreatments did not affect carbachol-induced drinking. However, results recently reported by Swanson *et al.* [38] failed to confirm the antagonism of the dipsogenic effect of A II by haloperidol.

The present results with haloperidol agree with those of Fitzsimons and Setler [14]. The contrast between our results and the negative findings of Swanson *et al.* [38] is difficult to explain, since the highest and most effective haloperidol : angiotensin ratio (nearly 83:1 on a weight basis) used in the present study was smaller than that (approximately 100:1) used by Swanson *et al.* [38]. Moreover, even the lowest dose (5 μg) of haloperidol used in the present work, caused a significant decrease in the amount of water ingested after 0.3 μg of A II, whereas a similar dose of haloperidol did not antagonize the dipsogenic effect of only 0.05 μg of A II in the study by Swanson *et al.* [38]. In addition, pretreatment time and site of intracerebral injections were comparable in both cases.

The interpretation of the haloperidol antagonism of A II-induced drinking is less clear. Haloperidol has been reported to block both alpha catecholamine receptors as well as would be specific dopamine receptors [41,43]. Therefore, haloperidol antagonism suggests participation of either alpha receptor stimulation or dopamine receptor

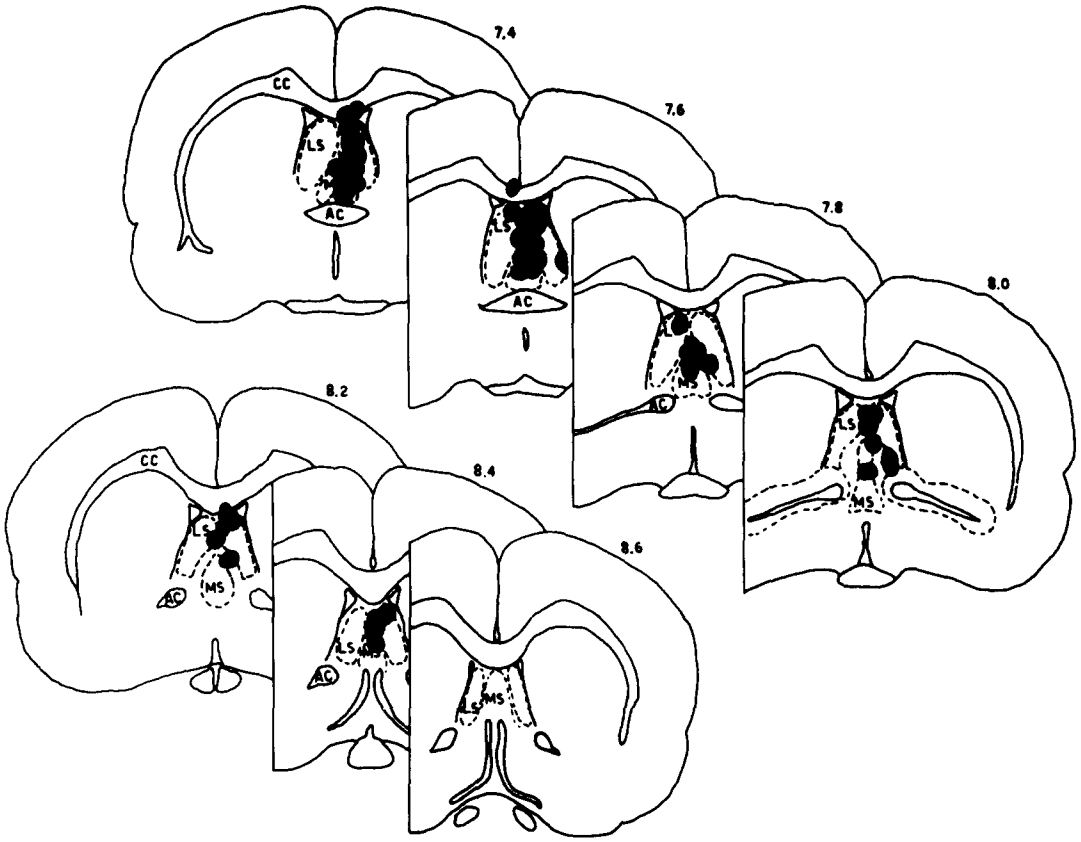

FIG. 5. Graphical representation of superimposed injection sites where a significant drinking response to intracerebral angiotensin II was observed. Figures represent anterior coordinates of De Groot's [6] rat brain atlas. AC: anterior commissure; CC: corpus callosum; LS: lateral septal area; MS: medial septal area.

stimulation, or both, in the mediation of A II-induced drinking. Nevertheless our results have shown that doses up to 25 μg of dopamine did not cuase any drinking effect, when injected at the same brain site where A II was highly effective. In addition, it has been reported that phentolamine, a more specific and more potent alpha-blocking agent than haloperidol, did not prevent drinking caused by intracerebrally administered A II [5].

Another question raised by the present observation is that even the lowest intraseptal dose of haloperidol (5 μg), caused cataleptic-like gross-behavioral changes that could unspecifically interfere with A II-induced drinking. Nevertheless the problem remains controversial because, as previously mentioned, Fitzsimons and Setler [14] observed that carbachol-induced drinking was not affected by doses of haloperidol that blocked the effect of A II. In addition to the unspecificity of haloperidol as a pharmacological tool, the existence of adrenergic neurotransmission in the septal area has not been definitively established.

Several A II polypeptide analogs have been described as specific and competitive antagonists of A II at peripheral organs or systems, in vitro as well as in vivo [24, 25, 31, 32]. Four of those A II analogs have been recently injected into the rat brain, in areas where A II caused drinking in satiated animals [38]. Though less potent that A II, all these peptides induced drinking responses. However, when given before A II none of them antagonized the drinking effect of A II. On the basis of these results, Swanson *et al.* [38] suggested that central A II receptors might be somewhat different from those at the periphery. The present results nevertheless show that Sar^1,Ala^3,Ile^8-A I, a newly synthetized polypeptide behaving as a specific and competitive antagonist of A II in the isolated rat uterus, guinea pig ileum and rat blood pressure and being practically devoid of A II-like actions (Peña, C. and Stewart, J. M., personal communication), completely blocked the effect of a ten times lower dose of A II on drinking, when locally applied into the brain, prior to the agonist. Only a mild agonistic action was observed when the same dose of Sar^1, Ala^3,Ile^8-A I that antagonized A II was given alone. These results clearly suggest that specific angiotensin receptors exist in the CNS that might be similar to peripheral receptors. Therefore, A II seems to act directly on specific receptors of brain neurons in order to elicit drinking behavior, as has already been suggested [5,38].

Since A I and A II [10] as well as renin-like enzyme activity [10,16] and A I – converting enzyme activity [34,44] have been reported to occur in brain tissues, present evidence on the existence of specific angiotensin receptors in the CNS gives further support to the view that locally released A II plays a role in the central regulation of thirst.

REFERENCES

1. Andersson, B. and S. M. McCann. The effect of hypothalamic lesions on the water intake of the dog. *Acta physiol. scand.*, **35:** 312–320, 1956.
2. Antunes-Rodrigues, J. and M. R. Covian. Water and sodium chloride intake following microinjection of carbachol into the septal area of the rat brain. *Experientia* **27:** 784–785, 1971.
3. Antunes-Rodrigues, J. and S. M. McCann. Water, sodium chloride and food intake induced by injections of cholinergic and adrenergic drugs into the third ventricle of the rat brain. *Proc. Soc. exp. Biol. Med.* **133:** 1464–1470, 1970.
4. Booth, D. A. Mechanism of action of norepinephrine in eliciting an eating response on injection into the rat hypothalamus. *J. Pharmac. exp. Ther.* **160:** 336–348, 1968.
5. Covian, M. R., C. G. Gentil and J. Antunes-Rodrigues. Water and sodium chloride intake following microinjections of angiotensin II into the septal area of the rat brain. *Physiol. Behav.* **9:** 373–377, 1972.
6. De Groot, J. The rat forebrain in stereotaxic coordinates. *Trans. r. neth. Acad. Sci.* **52:** 1–140, 1959.
7. Epstein, A. N., 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.
8. Epstein, A. N., J. T. Fitzsimons and B. J. Simons. Drinking caused by the intracranial injection of angiotensin into the rat. *J. Physiol.* **200:** 98P–100P, 1968.
9. Fisher, A. E. and J. N. Coury. Chlorinergic tracing of a central neural circuit underlying the thirst drive. *Science* **138:** 691–693, 1962.
10. Fisher-Ferraro, C., V. E. Nahmod, D. J. Goldstein and S. Finkielman. Angiotensin and renin in rat and dog brain. *J. exp. Med.* **133:** 353–361, 1971.
11. Fitzsimons, J. T. The kidney as a thirst receptor. *J. Physiol.* **191:** 128–129, 1967.
12. Fitzsimons, J. T. The role of a renal thirst factor in drinking induced by extracellular stimuli. *J. Physiol.* **201:** 349–368, 1969.
13. Fitzsimons, J. T. Interactions of intracranially administered renin or angiotensin and other thirst stimuli on drinking. *J. Physiol.* **210:** 152–153, 1970.
14. Fitzsimons, J. T. and P. E. Setler. Catecholaminergic mechanisms in angiotensin-induced drinking. *J. Physiol.* **218:** 43P–44P, 1971.
15. Fitzsimons, J. T. and B. J. Simons. The effect on drinking in the rat of intravenous infusion of angiotensin given alone or in combination with other stimuli of thirst. *J. Physiol.* **203:** 45–57, 1969.
16. Ganten, D., J. L. Minnich, P. Granger, K. Hayduk, M. H. Brecht, A. Barbeau, R. Boucher and J. Genest. Angiotensin-forming enzyme in brain tissue. *Science* **173:** 64–65, 1971.
17. Gentil, C. G., G. J. Mogenson and J. A. F. Stevenson. Effects of scopolamine on drinking elicited by hypothalamic stimulation. *Physiol. Behav.* **7:** 639–641, 1971.
18. Giardina, A. R. and A. E. Fisher. Effect of atropine on drinking induced by carbachol, angiotensin and isoproterenol. *Physiol. Behav.* **7:** 653–655, 1971.
19. Graeff, F. G., C. G. Gentil, V. L. Peres and M. R. Covian. Lever-pressing behavior caused by intraseptal angiotensin II in water satiated rats. *Pharmac. Biochem. Behav.* **1:** 357–359, 1973.
20. Greer, M. A. Suggestive evidence of a primary "drinking center" in hypothalamus of rat. *Proc. Soc. exp. Biol. Med.* **89:** 59–62, 1955.
21. Grossman, S. P. Eating and drinking elicited by direct adrenergic and cholinergic stimulation of hypothalamus. *Science* **132:** 301–302, 1960.
22. Grossman, S. P. Direct adrenergic and cholinergic stimulation of hypothalamic mechanisms. *Am. J. Physiol.* **202:** 872–882, 1962.
23. Grossman, S. P. Effects of adrenergic and cholinergic blocking agents on hypothalamic mechanisms. *Am. J. Physiol.* **202:** 1230–1236, 1962.
24. Khrairallah, P. A., A. Toth and F. M. Bumpus. Analogs of angiotensin II. Mechanism of receptor interaction. *J. med. Chem.* **13:** 181–184, 1970.
25. Marshall, G. R., W. Vine and P. Needleman. A specific competitive inhibitor of angiotensin II. *Proc. natn. Acad. Sci. U.S.A.* **67:** 1624–1630, 1970.
26. Miller, N. E., S. Gottesman and N. Emery. Dose response to carbachol and norepinephrine in rat hypothalamus. *Am. J. Physiol.* **206:** 1384–1388, 1964.
27. Mogenson, G. J. and J. A. F. Stevenson. Drinking and self-stimulation with electrical stimulation of the lateral hypothalamus. *Physiol. Behav.* **1:** 151–154, 1966.
28. Mogenson, G. J. and J. A. F. Stevenson. Drinking induced by electrical stimulation of the lateral hypothalamus. *Expl Neurol.* **17:** 119–127, 1967.
29. Mogenson, G. J., C. G. Gentil and J. A. F. Stevenson. Feeding and drinking elicited by low and high frequencies of hypothalamic stimulation. *Brain Res.* **33:** 127–137, 1971.
30. Pals, D. T., F. D. Masucci. F. Sipos and G. S. Denning. A specific competitive antagonist of the vascular action of angiotensin II. *Circulation Res.* **29:** 664–672, 1971.
31. Rioux, F., W. K. Park and D. Regoli. I. Pharmacology of angiotensin antagonists. *Can. J. Physiol. Pharmac.* **51:** 108–113, 1973.
32. Regoli, D., W. K. Park and F. Rioux. II.Pharmacology of angiotensin antagonists. *Can. J. Physiol. Pharmac.* **51:** 114–121, 1973.
33. Rolls, B. J. and B. P. Jones. Cessation of drinking following intracranial injections of angiotensin in the rat. *J. comp. physiol. Psychol.* **80:** 26–29, 1972.
34. Roth, M., A. F. Weitzman and Y. Piquilloud. Converting enzyme content of different tissues of the rat. *Experientia* **25:** 1247, 1969.
35. Routtenberg, A. Drinking induced by cabachol: thirst circuit or ventricular modification. *Science* **157:** 838–839, 1967.
36. Swanson, L. W., V. J. Perez and L. G. Sharpe. Accurate and reliable intracerebral delivery of minute volumes of drug solutions. *J. appl. Physiol.* **33:** 247–251, 1972.
37. Swanson, L. W., L. G. Sharpe and D. Griffin. Drinking to intracerebral angiotensin II and carbachol: Dose-response relationships and ionic involvement. *Physiol. Behav.* **10:** 595–600, 1973.
38. Swanson, L. W., G. R. Marshall, P. Needleman and L. G. Sharpe. Characterization of central angiotensin II receptors involved in the elicitation of drinking in the rat. *Brain Res.* **49:** 441–446, 1973.
39. Valenstein, E. S., V. C. Cox and J. W. Kakolewski. Modification of motivated behavior elicited by electrical stimulation of the hypothalamus. *Science* **159:** 119–1121, 1968.
40. Valenstein, E. S., V. C. Cox and J. W. Kakolewski. Hypothalamic motivational systems: Fixed or plastic neural circuits. *Science* **163:** 1084, 1969.
41. Van Rossum, J. M. Different types of sympathomimetic receptors. *J. Pharm. Pharmac.* **17:** 202–216, 1965.
42. Wise. R. A. Hypothalamic motivation system: Fixed or plastic neural circuits. *Science* **162:** 377–379, 1968.
43. Woodruff, G. N. Dopamine receptors: a review. *Comp. gen. Pharmac.* **2:** 439–455, 1971.
44. Yang, H. Y. T. and N. H. Distribution and properties of angiotensin converting enzyme of rat brain. *J. Neurochem.* **19:** 2443–2450, 1972.