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BRIEF COMMUNICATION

Evidence That the Preoptic Region is a Receptive Site for the Dipsogenic Effects of Angiotensin II[1]

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ASSAF, S. Y. AND G. J. MOGENSON. *Evidence that the preoptic region is a receptive site for the dipsogenic effects of angiotensin II.* PHARMAC. BIOCHEM. BEHAV. 5(6) 697–699, 1976. – Drinking elicited by administering angiotensin II (ANG II) to the preoptic region with cannulae passing through the lateral ventricles was attenuated significantly when the ventricles or subfornical organ were pretreated with saralasin acetate (Sar[1]-Ala[8]-angiotensin II). If the cannulae in the preoptic region were angled to bypass the lateral ventricles water intake elicited by ANG II was less and pretreating the cerebral ventricles with saralasin acetate did not reduce the drinking response. The results suggest that the preoptic region may be a receptive site for ANG II in addition to the subfornical organ and/or cerebral ventricles.

Water intake Angiotensin Preoptic region

THERE has been increasing interest during the last few years in the role of the renin-angiotensin system in the regulation of water intake and extracellular fluid volume. Angiotensin II (ANG II), a potent vasoconstrictor, initiates drinking and its dipsogenic effects have been attributed to its action on the CNS. The initial experiments suggested that the preoptic area (POA) and related forebrain structures were angiotensin receptive sites [4]. Subsequently it was suggested that the drinking observed following the injection of ANG II into the POA may be due to ANG II diffusing into the cerebral ventricles and to the subfornical organ (SFO) [13]. The observation that lesions of the SFO disrupted drinking initiated by administering ANG II to the POA supports the proposal that the SFO and not the POA is the receptive site [13]. However, Black *et al.* [2] observed that lesions of the lateral hypothalamus had a greater disruptive effect on drinking elicited by administering ANG II to the POA when the lesions were ipsilateral to the infusion site than when the lesions were contralateral to the infusion site. It is difficult to account for these findings if ANG II diffuses from the POA into the ventricles and acts on the SFO, a midline periventricular structure. Swanson and Sharpe [14], who observed no correlation between distance of the cannulae from the ventricles and the drinking response when small volumes of ANG II were infused into a number of areas of the brain, suggested that the POA is itself an ANG II receptive site. Accordingly it was decided to reinvestigate the POA as a possible site mediating the dipsogenic effects of ANG II using a competitive antagonist of ANG II, saralasin acetate [10], to block the action of ANG II on receptive sites in the ventricles or SFO.

METHOD

Male Wistar rats (Woodlyn Farms, Ontario) weighing 200–350 g were used. They were housed individually in a temperature (22–23°C) and light (on 7:00 a.m.–9:00 p.m.) controlled room with Purina laboratory chow and water available ad lib.

The surgical procedures for implantation of chronic cannulae, behavioral testing, and histological verification of the locus of the cannulae tips were similar to those reported previously [9]. Each rat had cannulae positioned bilaterally into homologous sites of the preoptic region (POA) and a third cannula terminating in the SFO, lateral ventricle or third ventricle. In some of the animals the cannula on one side of the brain penetrated the lateral ventricle to reach the POA and the cannula on the other side was positioned into the POA at an angle to bypass the lateral ventricle.

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[3] Since this paper was submitted Gronan and York (Proc. Soc. Neurosci. 6, 1976, Abstract 426) have reported that neurons in the preoptic region respond to the iontophoretic application of Angiotensin II which is consistent with the findings reported here.

Each animal was tested once every two days for drinking in response to a single infusion of ANG II (100 ng into POA or 500 ng into ventricles) dissolved in 1.0 μl Merlis CSF. If a rat drank at least 3.0 ml of water following each of the last two ANG II infusions it was used in the next phase of the experiment. The ventricle or the SFO were then pretreated with 1-Sar, 8-Ala ANG II to be designated saralasin acetate (2.0 μg in 1.0 μl CSF into ventricles; 1.0 μg dissolved in 0.5 μl CSF into the SFO) followed 5 min later by an infusion of ANG II into the ventricles or the POA. Water intake was measured for 30 min following the ANG II infusion.

RESULTS

During the 30 min period following the infusion of 0.5 μg of ANG II into the third ventricle, 11 rats drank an average of 10.5 ml of water. As shown in Fig. 1, pretreating the third ventricle with saralasin acetate prior to the infusion of ANG II significantly reduced the volume of water intake (from 10.5 to 2.5 ml; $t \pm 4.29$, $p<0.01$). Pretreating the third ventricle with CSF prior to the infusion of ANG II had no effect. Control infusions of CSF or of saralasin acetate initiated some drinking but in neither case did the intakes differ significantly from spontaneous intake levels. Following the infusion of hypertonic saline into the third ventricles nine rats drank an average of 5.4 ± 0.81 ml in 30 min and when the third ventricle was pretreated with saralasin acetate prior to the infusion of hypertonic saline their water intake was 5.3 ± 0.72 ml in 30 min ($t = 0.11, p>0.10$). These findings provide evidence for the effectiveness and specificity of saralasin acetate in blocking drinking initiated by the administration of ANG II.

The volume of water intake initiated by administering ANG II to the preoptic region was related to whether the cannulae were positioned at an angle to bypass the ventricles or passed through the ventricles. The results for a group of 10 rats are presented in Fig. 2. When the cannulae passed through the ventricles the average water intake was 5.2 ml in 30 min and when the cannulae were angled to bypass the ventricles the average water intake in the same period was 3.2 ml ($t = 4.41$, $p<0.01$). The effects of pretreating the third ventricle (n = 5) or lateral ventricle (n = 5) with saralasin acetate on water intake initiated by administering ANG II to the POA also depended on whether the preoptic cannulae penetrated or bypassed the lateral ventricles ($F = 7.2$, $p<0.05$). Saralasin acetate reduced significantly water intake when the ANG II was administered by means of preoptic cannulae which passed through the lateral ventricles (5.2 ml to 3.6 ml; $p<0.01$) but did not significantly reduce water intake when ANG II was administered by means of cannulae angled into the POA so as to bypass the lateral ventricles (3.2 ml to 3.1 ml; $p>0.05$).

The possibility that ANG II (administered to the POA by means of cannulae which passed through the lateral ventricles) reached the SFO by diffusion through the CSF was investigated by pretreating the SFO with saralasin acetate in a series of 7 rats. Pretreating the SFO with the competitive antagonist reduced water intake initiated by ANG II from 5.1 ml to 2.8 ml ($t = 2.73$, $p<0.05$) but since the drinking response was not blocked it appears that the SFO is not the only receptive site for ANG II.

The locus of each cannula was verified histologically and the data are based on animals in which the cannulae were observed to be in the preoptic region, cerebral ventricles and/or subfornical organ.

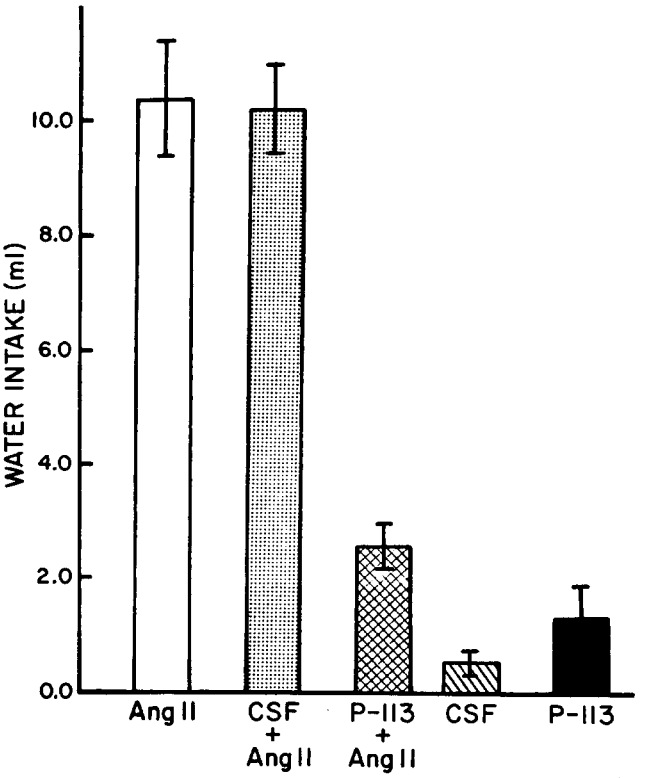

FIG. 1. Water intake during a 30 min period: (a) following the infusion of ANG II (0.5 μg) into the third ventricle, (b) following the infusion of ANG II (0.5 μg) into the third ventricle after the same site had been pretreated 5 min previously with CSF (1.0 μl), (c) following the infusion of ANG II (0.5 μg) into the third ventricle when the same site had been pretreated with saralasin acetate (P-113, 2.0 μg), (d) following a control infusion of CSF into the third ventricle, (e) following a control injection of saralasin acetate (P-113, 2.0 μg) into the third ventricle. The means are based on data from 11 rats and the vertical bars represent ± SEM.

DISCUSSION

The results of the present study agree in part with the diffusion hypothesis (see Introduction) in that some of the drinking initiated by administering ANG II to the POA by means of straight cannulae was apparently due to diffusion of ANG II into the ventricles to reach the SFO (see Fig. 2). However, the elicited drinking cannot be accounted for entirely on the basis of diffusion; the POA or neural tissue in its vicinity must also be receptive to ANG II. It may be suggested, therefore, that water intake following infusion of ANG II into the POA consists of two components, drinking due to the diffusion of ANG II into the ventricles and drinking initiated by the action of ANG II on the POA. This suggestion is supported by the following results. First, pretreating the ventricles with saralasin acetate reduced the water intake elicited by infusing ANG II into the POA by approximately one-third. It may be suggested that saralasin acetate eliminated the component of drinking due to diffusion by blocking ANG II receptors in the ventricles or SFO. Second, as shown in Fig. 2, infusing ANG II into the POA by means of cannulae positioned so as not to

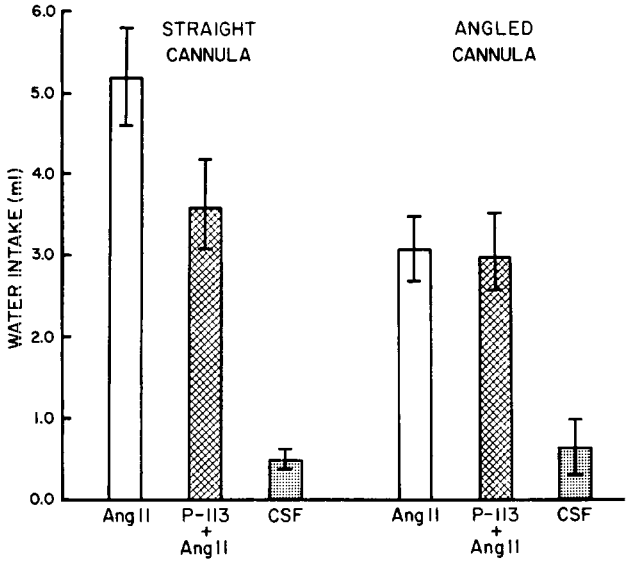

FIG. 2. Water intakes for a period of 30 min following infusion of ANG II (100 ng) into the preoptic region by means of straight cannulae (left) or cannula angled to bypass the lateral ventricles (right). Cross hatched columns show water intakes when the lateral or third ventricles were pretreated 5 min previously with saralasin acetate (P-113, 2.0 μg). Stippled columns show water intakes following the control administration of CSF into the preoptic region. Water intakes are expressed as mean ± SEM for a series of 10 rats.

penetrate the ventricles elicited significantly less drinking as compared to infusing ANG II by means of cannulae that penetrated the ventricles. When the ventricles were pretreated with saralasin acetate only drinking initiated by infusing ANG II through vertically-oriented cannulae was significantly attenuated. It appears that only the component of water intake due to diffusion of ANG II into the ventricles was affected by the intraventricular saralasin acetate. Third, when the SFO was pretreated with the competitive antagonist prior to infusing ANG II into the POA, water intake was reduced by one-third. In addition to supporting the conclusion that some of the drinking was due to diffusion of ANG II into the ventricles this observation suggests that the receptive site is the SFO. Alternatively the saralasin acetate administered to the SFO might have reached the ventricles and blocked a ventricluar site receptive to angiotensin II.

It has been suggested by previous investigators [8] that ANG II might be transported from the POA to the SFO by the cerebral circulation. The attenuation of drinking initiated by infusing ANG II into the POA when the SFO was pretreated with saralasin acetate is consistent with this proposal. However, diffusion through the ventricles is a more likely interpretation since the attenuation of drinking when saralasin acetate was administered to the SFO was no greater than when it was administered into the ventricles. Further doubt is cast on the hypothesis of transport through cerebral circulation by reports that ANG II octapeptide does not readily cross the blod-brain barrier [15].

There is a growing body of evidence that the SFO is a receptive site for ANG II as indicated, for example, by the response of neurons of the SFO to the iontophoretic application of ANG II [5,11] and the sensitivity of the SFO to low doses of ANG II for the initiation of drinking [3]. The hypothesis that the SFO is a receptive site for ANG II is particularly appealing since the SFO does not have a blood-brain barrier [12] and there is some doubt about how readily ANG II crosses the blood-brain barrier [15]. However, following lesions of the SFO, drinking to the systemic infusion of ANG II is attenuated, not blocked [1], so there is a possibility that some ANG II of systemic origin reaches and acts on the POA. An alternative suggestion [9] is that ANG II of systemic origin acts on the SFO whereas ANG II from the renin-angiotensin system, intrinsic to the brain [6,7], acts on the POA.

Further evidence, although of an indirect nature, has been obtained recently in support of the suggestion that there are multiple receptor sites for ANG II. Lesions of the medial aspect of the lateral hypothalamus have been shown to disrupt drinking initiated by administering ANG II to the POA but not drinking initiated by administering ANG II to the SFO or ventricles [9].

REFERENCES

1. Abdelaal, A. E., S. Y. Assaf, J. Kucharczyk and G. J. Mogenson. Affect of ablation of the subfornical organ on water intake elicited by systematically administered angiotensin-II. *Can. J. Physiol. Pharmac.* **52:** 1217–1220, 1974.
2. Black, S. L., J. Kucharczyk and G. J. Mogenson. Disruption of drinking to intracranial angiotensin by a lateral hypothalamic lesion. *Pharmac. Biochem. Behav.* **2:** 515–522, 1974.
3. Epstein, A. N. The physiology of thirst. Fourth J. A. F. Stevenson Lecture. *Can. J. Physiol. Pharmac.* **54:** 639–649, 1976.
4. Epstein, A. N., J. T. Fitzsimons and B. J. Rolls. Drinking induced by injection of angiotensin into the brain of the rat. *J. Physiol., Lond.* **210:** 457–474, 1970.
5. Felix, D. and K. Akert. The effect of angiotensin-II on neurons of the cat subfornical organ. *Brain Res.* **76:** 350–353, 1974.
6. Fischer-Ferraro, C., V. E. Nahmod, D. J. Goldstein and S. Finkelman. Angiotensin and renin in rat and dog brain. *J. exp. Med.* **133:** 353–361, 1971.
7. Ganten, D., J. L. Minnich, P. Granger, K. Hayduk, H. M. Brecht, A. Barbeau, R. Boucher and J. Genest. Angiotensin-forming enzyme in brain tissue. *Science* **173:** 64–65, 1971.
8. Johnson, A. K. and A. N. Epstein. The cerebral ventricles as the avenue for the dipsogenic action of intracranial angiotensin. *Brain Res.* **86:** 394–418, 1975.
9. Kucharczyk, J., S. Y. Assaf and G. J. Mogenson. Differential effects of brain lesions on thirst induced by the administration of angiotensin-II to the preoptic region, subfornical organ and anterior third ventricle. *Brain Res.* **108:** 327–337, 1976.
10. Pals, D. T., F. D. Masucci, F. Sipos and A. S. Denning. A specific competitive antagonist of the vascular action of angiotensin-II. *Circulation Res.* **24:** 664–672, 1971.
11. Phillips, M. I. and D. Felix. Specific angiotensin-II receptive neurons in the cat subfornical organ. *Brain Res.* **109:** 531–540, 1976.
12. Phillips, M. I., L. Balhorn, M. Levitt and W. Hoffman. Scanning electron microscope study of the rat subfornical organ. *Brain Res.* **80:** 95–110, 1974.
13. Simpson, J. B. and A. Routtenberg. Subfornical organ: site of drinking elicitation by angiotensin II. *Science* **181:** 1172–1174, 1973.
14. Swanson, L. W. and L. G. Sharpe. Centrally induced drinking: comparison of angiotensin-II and carbachol-sensitive sites in rats. *Am. J. Physiol.* **225:** 556–573, 1973.
15. Volicer, L. and C. G. Loew. Penetration of angiotensin-II into the brain. *Neuropharmacology* **10:** 631–636, 1971.