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Intracranial Injection Parameters Which Affect Angiotensin II-Induced Drinking[1]

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SELLECK, S. B. AND J. B. SIMPSON. *Intracranial injection parameters which affect angiotensin II-induced drinking.* PHARMAC. BIOCHEM. BEHAV. 13(4) 581–584, 1980.—Three intracranial injection parameters, injectate concentration at equivalent doses, rupture or bypass of the lateral ventricle by cannula in reaching the site, and multiple injections per animal, were studied to assess their effects on the drinking behavior elicited by angiotensin II at the lateral preoptic area (LPOA). Half of the animals were implanted with 23 gauge cannulae which penetrated the lateral ventricle en route to the site. The remaining animals received cannulae that were angled laterally to bypass the ventricle. In ventricular animals, the more concentrated injectate increased the total water intake over a 30 min period and affected the pattern of drinking through time. Animals with cannulae that penetrated the ventricle en route to the LPOA drank significantly more than the animals whose cannulae missed the ventricle. In all cases, no significant difference in drinking response was found between the first and second injections received by each rat. These results indicate that standardized intracranial chemical injection methods are needed for comparison of experiments utilizing this technique.

Intracranial injection Angiotensin II Lateral preoptic area

TO date, numerous and occasionally contradictory reports have appeared describing neural loci which putatively mediate angiotensin II (AII) induced drinking (e.g. [1, 5, 6, 7, 8, 11, 12, 13]. Intracranial injection of AII through chronically implanted cannulae which terminate at a site in question has been one of the principal techniques employed in such investigations. In these studies, the intracranial chemical injection (ICI) parameters that govern delivery of AII, such as the concentration of injectate and the rupture or bypass of the lateral ventricle by cannulae en route to a site, have varied greatly. One reason, then, for the controversy regarding central sites of AII action may be the differing methods used between different experimenters. As previously emphasized [10], the formulation of hypotheses regarding putative sites of AII action should require an understanding of those ICI parameters which affect the elicited behavior.

In order to determine if ICI methodology could affect drinking elicited by AII, we studied the effect of the following parameters on induced drinking behavior: (1) concentration of AII in the injectate (at equivalent doses); (2) rupture or bypass of the lateral ventricle by cannulae along their trajectory to the site; and (3) repeated injections of AII in each animal. We chose the lateral preoptic area (LPOA) as the site of administration since it has been suggested by several investigators that this is one site of the dipsogenic action of AII [5, 6, 8, 13]. Others, however, have failed to obtain data in support of this proposition [7, 11, 12]. The results of our experiments document that the elicited behavior may be affected by modifications in ICI methodology and may provide a resolution to the contradictory reports concerning the sensitivity of the LPOA to AII.

METHOD

Animals

Male Long Evans rats (University of Washington, Department of Psychology Animal Colony) weighing between 250–375 g were individually housed in hanging wire mesh cages. Water and Purina Rat Chow were available ad lib. The vivarium was maintained at 25°C and the lighting controlled to turn on at 0800 hr and off at 2000 hr.

Surgery

In preparation for surgery, animals were given atropine sulfate (0.2 mg/rat) and anesthetized with Equithesin (3.00 cc/kg). The rat was mounted in a Kopf rodent stereotaxic and an incision was made on the top of the head to expose the skull. The periosteum was removed prior to the drilling and insertion of two jewelers screws into the skull bones. A small trephine hole was then drilled through the cranium and a 23 ga guide cannula, containing a 30 ga obturator, was positioned in the brain. Flat-skull coordinates referenced from bregma for animals with ventricular bypass and ventricular rupture were: posterior 0.2 mm, lateral 3.9 mm (angled 14° toward midline), ventral 7.2 mm; and posterior 0.2

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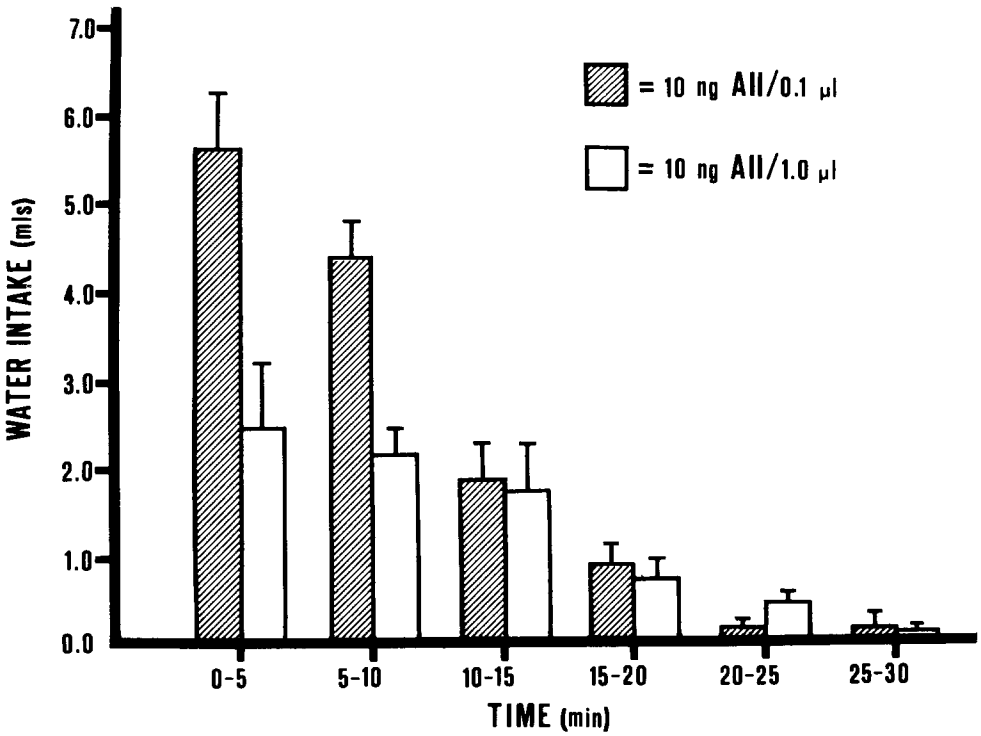

FIG. 1. Mean drinking (±SE) taken at six successive 5 min intervals for animals receiving 10 ng AII/0.1 µl 0.9% NaCl and 10 ng AII/1.0 µl 0.9% NaCl via cannulae that penetrate the lateral ventricle en route to the lateral preoptic area (n=5 for each injectate volume group).

mm, lateral 1.7 mm, ventral 7.4 mm, respectively. The cannula system was fixed to the skull with methyl methacrylate cement. After the incision was closed, the animal was given a prophylactic dose of penicillin (100,000 IU/kg). Intracranial injections were performed six to seven days after surgery.

Experimental Tests

For drinking tests, animals were removed from their cages and restrained by hand while the obturator was removed and the injection cannula inserted. The injector was connected to a 10 µl Hamilton gas chromatography syringe by a length of PE-10 tubing. The injection rate was 0.1 µl/sec. The injector was removed and the obturator replaced 30 sec after injection. The animal was then returned to its home cage. Drinking measurements were taken from a calibrated burette every 5 min for a 30 min time period. Each rat received two intracranial injections, one on each of two consecutive days between 1300 and 1700 hr.

Histology

The brain was removed and placed in 10% Formalin after intracardiac perfusion with 0.9% saline followed by 10% Formalin. Frozen sectioning (40 µm sections) and cresyl violet staining of fixed tissue were performed in order to visualize the cannula track. Scoring of cannula placement for each animal was done without knowledge of the animal's behavior.

EXPERIMENT 1: VENTRICULAR PENETRATION EN ROUTE TO LPOA

The first experiment assessed the effects of injectate concentration and multiple injections on drinking behavior elicited by AII at the LPOA when cannulae penetrated the ventricular ependyma. All intracranial injections contained 10 ng AII in 0.9% NaCl. The volume of injectate, 1.0 or 0.1 µl, was varied between two groups of subjects. Each animal received two injections of the same injectate concentration on subsequent days.

EXPERIMENT 2: VENTRICULAR BYPASS EN ROUTE TO LPOA

The second study focussed on the same methodological parameters outlined above, but in animals with cannulae that bypassed the ventricle. Each rat received one injection each of 10 ng AII in 0.1 µl and 10 ng AII in 1.0 µl of 0.9% NaCl. The two injection concentrations were presented in counterbalanced order to the two experimental groups.

RESULTS

EXPERIMENT 1: VENTRICULAR PENETRATION EN ROUTE TO LPOA

The mean intake for each 5 min interval for animals receiving 1.0 µl and 0.1 µl injection volumes is presented in Fig. 1. A three-way analysis of variance with two repeated measures was performed to simultaneously test for the effects on water intake of: (1) injectate concentration at equal doses of AII; (2) order of injection; and (3) time course of elicited drinking [3]. This analysis indicated that the more concentrated injection effected an increase in the total water intake $F(1,8)=7.99$, $p<0.025$, and altered the pattern of drinking through time, $F(5,40)=5.44$, $p<0.001$. Figure 2 illustrates the marked increase in total water intake effected by increasing the concentration of AII in the injectate in animals with cannulae that ruptured ventricular ependyma en route

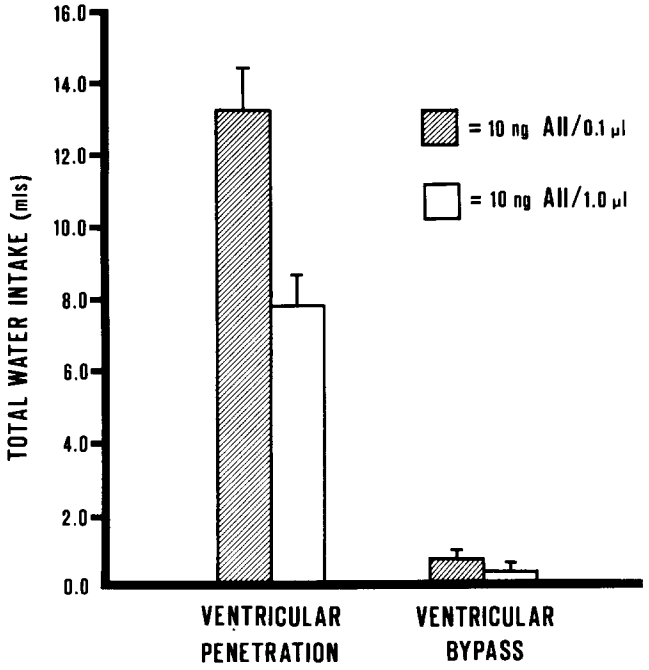

FIG. 2. Total water intake (±SE) over a 30 min period for animals with cannulae that rupture or bypass the lateral ventricle en route to the lateral preoptic area, at both injectate concentrations.

to the LPOA. No significant difference was detected between drinking induced by the first and second injections of AII, $F(1,8)=0.152$, $p>0.25$. In animals with cannulae that penetrated the lateral ventricle en route to the LPOA, then, the increased concentration of AII at a constant dose increased the total water intake and altered the pattern of drinking through time.

EXPERIMENT 2: VENTRICULAR BYPASS EN ROUTE TO LPOA

A two-way analysis of variance contrasting the drinking in response to the first and second injections across time for each injection concentration (Fig. 1) showed that multiple injections had no significant effect on drinking for 0.1 µl injection, $F(1,10)=0.175$, $p>0.25$, and for 1.0 µl injection, $F(1,10)=0.211$, $p>0.25$ [4]. Nor was there a significant effect on the time course of drinking, $F(5,50)=0.053$, $p>0.25$ for 0.1 µl injection and $F(5,50)=0.040$, $p>0.25$, for the 1.0 µl injection. This suggests that the marginal drinking observed was not in response to the AII injection. A paired t-test contrasting total water intake for the two concentrations did not detect a significant difference, $t(11)=0.956$, $p>0.25$, Fig. 2 [14]. Therefore, injectate concentration and multiple injections of AII into the preoptic area had no effect on the drinking behavior of animals with cannulae that bypassed the lateral ventricle.

As previously reported [7], traversing the lateral ventricle by cannulae en route to LPOA has dramatic effects on AII elicited drinking (Fig. 2). A two-way analysis of variance comparing ventricular and ventricular bypass animals at both injectate concentrations revealed a significant difference, $F(1,16)=73.62$, $p<0.001$ [14].

DISCUSSION

Our results indicate that variations in ICI parameters may considerably alter behavior induced by an intracranially injected compound. More concentrated injections, containing the same dose of AII, increased drinking in animals with cannulae that traversed the lateral ventricle en route to the LPOA. In support of previous work, penetration of the ventricle by cannulae was necessary for drinking induced by 10 ng AII at this site [7]. Our data confirm this important observation.

These data also indicate that it is likely that more concentrated AII injections increase diffusion and hence flux of AII across distant receptors, thereby increasing the evoked drinking. This hypothesis is supported by previous work that suggested diffusion of substances injected into the brain is a function of concentration and osmolality [2]. In our study, however, the osmolality of the injectate per se did not provoke drinking at the LPOA, since animals with cannulae which bypassed the ventricle did not drink in response to the more concentrated injection of AII.

The possibility that increased injectate concentration effects greater diffusion indicates that the problem of chemical spread is not circumvented simply by using smaller, more concentrated injections, as many investigators have assumed [9,13]. Indeed, the counter-intuitive enhancement of spread observed in small volumes but more concentrated AII injections is predictable after consideration of Fick's First and Second Laws. It is therefore naive to assume that decreasing the injectate volume, thereby increasing the concentration at equivalent doses, localizes the injectate to the region immediately surrounding the cannula tip. Parameters governing ICI methodology should receive careful attention in future research.

REFERENCES

1. Abdelaal, A. E., S. Y. Assaf, J. Kucharczyk and G. J. Mogenson. Effect of ablation of the subfornical organ on water intake elicited by systemically administered angiotensin II. *Can. J. Physiol. Pharmac.* **52:** 362–363, 1974.
2. Bondareff, W., A. Routtenberg, R. Narotzky and D. G. McLone. Intrastriatal spreading of biogenic amines. *Expl. Neurol.* **28:** 213–229, 1970.
3. Bruning, J. L. and B. L. Kintz. *Computational Handbook of Statistics*. Glenview, Illinois: Scott, Foresman and Co., 1968, p. 269.
4. Edwards, A. L. *Experimental Design in Psychological Research* (4th edition). New York: Holt, Rinehart and Winston Inc., 1972, p. 488.
5. 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.
6. Fitzsimons, J. T. and J. Kucharczyk. Drinking and haemodynamic changes induced in the dog by intracranial injection of components of the renin-angiotensin system. *J. Physiol., Lond.* **276:** 419–434, 1978.
7. Johnson, A. K. and A. N. Epstein. The cerebral ventricles as the avenue for the dipsogenic action of intracranial angiotensin. *Brain Res.* **86:** 399–418, 1975.
8. 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.

9. Mangiapane, M. L. and J. B. Simpson. Subfornical organ: Forebrain site of pressor and dipsogenic action of angiotensin II. *Am. J. Physiol.*, in press.
10. Routtenberg, A. Intracranial chemical injection and behavior: a critical review. *Behav. Biol.* **7:** 601–642, 1972.
11. Simpson, J. B. and A. Routtenberg. Subfornical organ: site of drinking elicitation by angiotensin II. *Science* **181:** 1172–1175, 1973.
12. Simpson, J. B. and A. Routtenberg. Subfornical organ lesions reduce intravenous angiotensin-induced drinking. *Brain Res.* **88:** 154–161, 1975.
13. Swanson, L. W., J. Kucharczyk and G. J. Mogenson. Autoradiographic evidence for pathways from the medial preoptic area to the midbrain involved in the drinking response to angiotensin II. *J. comp. Neurol.* **178:** 645–660, 1978.
14. Zar, J. H. *Biostatistical Analysis.* Englewood Cliffs, NJ: Prentice-Hall Inc., 1974, p. 620.