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Anteroventral Third Ventricle Site of Action for Angiotensin Induced Thirst

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BUGGY, J. AND A. E. FISHER. *Anteroventral third ventricle site of action for angiotensin induced thirst* PHARMAC. BIOCHEM. BEHAV. 4(6) 651–660, 1976. – The central site of action for angiotensin induced thirst was investigated in rats. Subfornical organ lesions resulted in a temporary abolition of drinking induced by lateral preoptic or lateral ventricle microinjections of angiotensin but drinking to anteroventral third ventricle microinjections of angiotensin (or carbachol) was unaffected. Drinking to elevated systemic levels of angiotensin was attenuated but not abolished by subfornical organ lesions When spread of injected angiotensin via cerebrospinal fluid circulation was controlled by placing plugs at selected locations in the ventricles, drinking was elicited only when intracranial microinjections of angiotensin gained access to anteroventral third ventricle. It was concluded that subfornical organ is not the exclusive dipsogenic receptor for angiotensin, rather angiotensin exerts at least part of its dipsogenic effect by spread through the ventricular system to receptors in the vicinity of the anteroventral third ventricle

Angiotensin induced thirst Periventricular receptors Subfornical organ lesions Ventricular obstruction
Cholinergic thirst Cerebrospinal fluid circulation Chemical brain stimulation Anteroventral third ventricle

THE renin-angiotensin system has been implicated in the thirst resulting from various disturbances of the circulation which reduce or redistribute blood volume without primarily affecting plasma osmolarity [12] Plasma renin activity is increased following several challenges of blood volume or pressure which produce thirst including caval ligation, hypovolemia [23], and isoproterenol-induced hypotension [26], as well as following simple water deprivation [15] When infused intravascularly, angiotensin II (AII) causes rats in normal fluid balance to increase water intake [13]. It is probable that AII exerts this dipsogenic effect through an action on the brain since the threshold dose for elicitation of thirst behavior is greatly reduced by direct intracerebral injection [9]. Furthermore, drinking following peripheral administration of AII is effectively reduced by intracerebral injection of the analog antagonist of AII, saralasin, comparable doses of the analog inhibitor applied peripherally do not attenuate drinking [20]

The drinking response elicited by central injections of AII is one of the clearest examples of a peptide inducing an organized behavioral response and the magnitude of the response is dose-dependent [9] Since the latency to initiate drinking following central microinjection of AII is extremely short, usually 1 min or less, the process which leads to activation of neural thirst mechanisms is presumed to be an interaction of AII with a dipsogenic receptor. Since the role of AII as a hormonal mediator of thirst to extracellular stimuli remains controversial [1,36], localization of the AII dipsogenic receptor is a prerequisite to a more complete understanding of the significance of this hormonal factor in the normal elaboration of thirst.

Previous attempts at localization using the intracerebral chemical stimulation mapping technique have not succeeded in defining a limited critical region [9, 37, 38]. Instead, it was found that AII microinjection into many diverse and often unrelated neural tissue sites elicited drinking Analysis of the generated maps indicated a wide distribution of cannula sites that impinged upon or pierced the lateral or third ventricles. Johnson and Epstein [19] demonstrated that AII injected into most of these sites gained rapid access to the ventricular system, usually by efflux up the cannula shaft, and that direct ventricular injections were as effective as injections into such neural tissue. When cannula trajectories into supposedly sensitive tissue sites such as the lateral preoptic area were angled to avoid passing through ventricles, the investigators could no longer elicit drinking with AII injections Conversely, when cannula trajectories into nonsensitive tissue sites such as caudate were angled to pass through a ventricle, drinking could now be elicited with low doses of AII These results implied that previous mapping studies were confounded by rapid spread of AII into ventricles and suggested, but did not define, a peri-ventricular receptor site for AII.

The subfornical organ (SFO), a circumventricular organ in the dorsal third ventricle near the interventricular foramen, has been proposed as the site housing the dipsogenic receptors for AII since SFO lesions abolished the drinking response to lateral preoptic injections of AII [33] However, other data from our laboratory indicated that a site in the antero-ventral third ventricle seemed exceptionally sensitive to the dipsogenic effects of AII and that blockade with the analog antagonist saralasin was more

effective at this site than at SFO for attenuating drinking induced by lateral preoptic injections of AII (Jim Tarter, unpublished observations).

The experiments to be reported were designed to more clearly identify the site or sites of dipsogenic receptors for AII. The effect of SFO lesions on drinking induced by AII injections into lateral ventricles (LV), lateral preoptic region (LPO), and antero-ventral third ventricle (AV3V) was investigated to determine if sites existed where the dipsogenic response to AII did not vary as a function of SFO lesions. It was also of interest to determine if SFO lesions differentially affected water or saline intake since rats will ingest both water and saline after intracerebral injection of AII [6]. The necessity of ventricular access and transport of AII after intracerebral injection for arousal of thirst was also investigated in a second experiment.

EXPERIMENT 1: SFO LESIONS AND AII INDUCED THIRST

METHOD

Animals

Adult, male rats (Long-Evans and Sprague-Dawley strains) were used. Animals weighed between 225 to 400 g at the time of surgery and were experimentally naive Ambient temperature was maintained at 22°C by air conditioning, a 12 hr light, 12 hr dark cycle was used

Surgery

Animals were anesthetized with Nembutal (50 mg/kg) and positioned in a stereotaxic instrument for implantation of 23 ga stainless steel cannula and obturator assemblies for intracerebral microinjection. A midsaggital incision was made to expose the dorsal surface of the skull, and the tissue was retracted laterally Small jeweler's screws were fixed to the skull to anchor cement Each animal had 2 cannulae implanted, 1 aimed at the AV3V, and the other aimed at the LPO or LV, using coordinates from the Pelligrino and Cushman atlas [28]. A small cork was placed on the skull over the SFO and the cork and cannulae were cemented in place with dental acrylic. Following behavioral screening to determine the drinking response to intracerebral injections at each cannula site, animals were again anesthetized with Nembutal and lesioned under stereotaxic guidance. The cork was drilled away and the skull over the SFO was removed. SFO lesions were made in 3 ways: radiofrequency lesions with a single penetration to the SFO, repeated rotations of a small wire through the SFO region, or electrolytic lesions with three penetrations to SFO as the course of the SFO was followed in the A–P plane and the ventral–dorsal plane. Following lesioning, the skull hole was filled with bone wax and covered with cement.

Procedure

After surgery for cannulae placement, animals were individually housed in wire rack cages with ad lib rat chow, deionized water, and a 1.8% NaCl solution made with deionized water At least 5 days were allowed for recovery from surgery and adaptation to the drinking fluids. All behavioral testing was done during the dark phase of the lighting cycle.

Angiotensin II (Ciba), carbachol (a cholinergic stimulant), and renin were used to stimulate thirst. Angiotensin was prepared in an isotonic saline vehicle to final concentrations of 100 or 200 ng/μl. Carbachol was also prepared in isotonic saline to a concentration of 1 μg/μl. Renin, in isotonic saline, was in a concentration of 10 units/ml All drugs and solutions were stored under refrigeration, fresh solutions of angiotensin and renin were prepared each month Drugs were allowed to warm to room temperature before injection.

Following recovery from surgery, all animals were screened to determine their drinking responses to angiotensin. Angiotensin was injected through the AV3V and LPO or LV cannula in a counterbalanced order across animals, on separate test days. Several animals were also screened for drinking to carbachol injected into the AV3V site and to intraperitoneal injections of renin (20 U/kg), on separate test days. Intracerebral injections were of 1 μl volume administered over 10 sec through a 30 ga injector cannula inserted inside the 23 ga guide cannula The amount drunk of both water and 1.8% NaCl was recorded to the nearest tenth of a ml. Drinking tests were 1/2 hr for angiotensin, 1 hr for carbachol, and 1–1/2 hr for renin; the length of the test session related to the expected duration of an elevated drinking response following each dipsogenic challenge.

At the end of the screening tests, animals were lesioned as described and returned to their home cages. After lesioning, the rats were retested with the thirst challenges that they had responded to in the screening Post lesion tests with carbachol and renin were conducted 4 and 10 days post lesion, respectively. Post lesion tests with angiotensin were usually conducted on Day 3 post lesion Several animals with initial post lesion deficits in drinking to angiotensin were retested on a later day to determine if recovery of drinking to angiotensin injections would occur.

Histology

At the end of post lesion testing, animals were deeply anesthetized with Nembutal and perfused intracardially with isotonic saline followed by 10% Formalin. Brains were then prepared for histological localization of cannula tips and lesions (40 micron frozen sections, cresyl violet and Weil stains).

RESULTS

Prior to analysis of behavioral data, histological results were used to designate animals with minimal (0–70%) versus substantial (70–100%) SFO damage. Cannula trajectories for LV and AV3V injection sites are shown in Fig. 1. A total of 14 animals with positive responses (3 ml or more fluid ingested) to LV or LPO injections of AII, and 15 animals with positive responses to AV3V injections of AII, sustained minimal SFO lesions. As a group, the animals designated as having minimal SFO damage showed no significant differences in drinking to AII on pre- versus post lesion tests for either water, saline, or total fluid intake measures.

Six animals screened positive to AII in the LV (n = 3) or LPO (n = 3) placement only, and sustained substantial SFO lesions (100% lesion for 4 rats, 90% for 1 rat, 80% for 1 rat). As a group, these animals showed significant deficits in total fluid intake to AII on the post lesion test ($p<0.02$, paired sample t test), intake of both water and saline was reduced. Mean fluid intakes in ml ± SEM were pre-lesion –

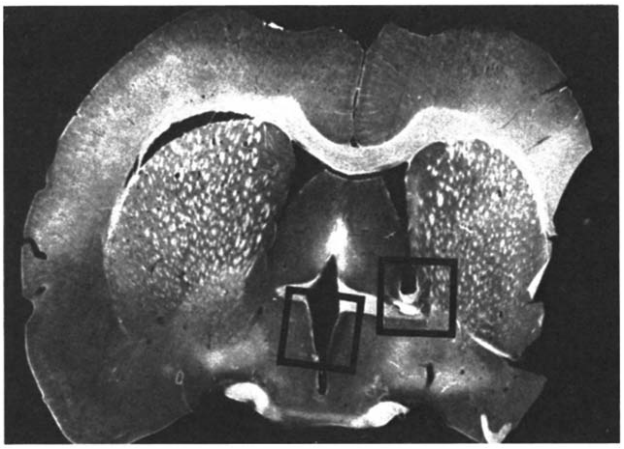

FIG. 1. Typical cannula placements for antero-ventral third ventricle injections and for lateral ventricle injections.

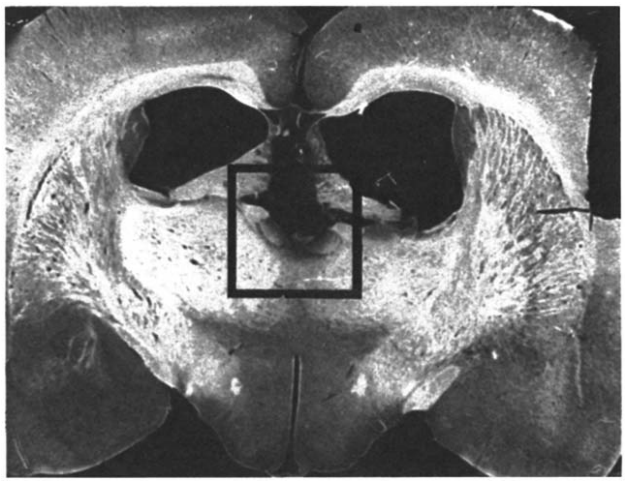

FIG. 2. A complete subfornical organ lesion. Post lesion drinking to angiotensin in this animal was reduced for injections into the lateral ventricle but not for injections into antero-ventral third ventricle Note that the lateral ventricles are dilated suggesting a blockage of cerebrospinal fluid circulation from lateral to third ventricle.

water = 6 7 ± 1.1, 1,8% NaCl = 3.2 ± 0.6, post lesion – water = 1.4 ± 0.9, 1.8% NaCl = 0 7 ± 0.7.

Thirteen animals that screened positive to AII in both LV and AV3V placements had substantial SFO lesions (100% lesion, n = 6, 90% lesion, n = 4, 80% lesion, n = 1, 70% lesion, n = 2). These rats also drank significantly less to LV injections of AII after SFO lesions. However, these rats showed no decrement in fluid intakes when AII was injected into the AV3V placement. Order of intracerebral injections was counterbalanced so these results do not represent an order effect Results are presented in Table 1. A complete SFO lesion from an animal in this group is shown in Fig. 2.

Twelve rats with initial post lesion deficits in drinking to AII injections into LV were tested again on a subsequent day (4–10 days post lesion) with the same dose of angiotensin. Eight of the 12 rats drank considerably more during the second post lesion test. For the group as a whole, the recovery of drinking (442% on the second test compared to the first test) was significant ($p<0.05$). Histology revealed that the SFO was severely damaged in all of these animals (70% or greater tissue destruction). It is not likely that recovery or enhancement of function of residual SFO tissue can explain the recovery of drinking to angiotensin from the LV site since recovery was noted animals with complete as well as partial SFO lesions. Of the 6 rats with 100% SFO lesions, 5 increased fluid intake on the second test. Results are summarized in Table 2.

TABLE 1

EFFECT OF SFO LESIONS ON DRINKING TO ANGIOTENSIN INJECTED INTO LV AND AV3V

	Prelesion			Postlesion		
	Lateral Ventricle Intake in ML, N=13					
	Water	1 8% NaCl	Total	Water*	1.8% NaCl†	Total‡
Mean	10 5	4 4	14 8	3 7	1 1	4 8
SEM	1 5	1 2	1 9	1 7	0 6	1 5
	Third Ventricle (AV3V) Intake in ML, N=13					
	Water	1 8% NaCl	Total	Water§	1.8% NaCl§	Total§
Mean	9 4	5 6	15 0	11 6	3 1	14 7
SEM	1 3	1 1	1 4	1 5	0 7	1 4

*p less than 0 002 compared to prelesion.
†p less than 0 05 compared to prelesion
‡p less than 0 001 compared to prelesion
§Not significantly different from prelesion value.

TABLE 2

RECOVERY OF DRINKING TO AII INJECTIONS INTO LV AFTER SFO LESIONS

	Mean Fluid Intake in ml ± Standard Error, n=12		
	Prelesion	First Postlesion	Second Postlesion
Water	9 8 ± 1 4	0 7 ± 0 4	4 1 ± 1 1
1 8% NaCl	4 3 ± 1 2	0 4 ± 0 2	0 8 ± 0 6
Total	14 1 ± 1 6	1 1 ± 0 5	4 9 ± 1 5

Seven animals with substantial SFO lesions screened positive to 1 $\mu g/\mu l$ injections of carbachol in the AV3V, and were tested again with carbachol 4 days after the lesion. Mean fluid intakes in ml ± SEM were pre-lesion – water = 17.3 ± 2.1, 1.8% NaCl = 0 6 ± 0.3, post lesion – water = 14.8 ± 3.4, 1 8% NaCl = 1 1 ± 0 7. Note that rats stimulated with carbachol increase water intake dramatically, but drink little or no 1.8% saline Drinking to carbachol injections did not change significantly after SFO lesions, even though post lesion drinking to AII in the LV placement was depressed on Day 3 in 5 of these animals. An additional 5 animals with SFO lesions were tested for drinking to intraperitoneal injections of renin on the 10th day post lesion. Total fluid intake to renin was significantly reduced ($p<0$ 05) after the lesion, but not abolished as 55% of the pre-lesion response remained. Mean fluid intakes in ml ± SEM were· pre-lesion – water = 16.9 ± 3.0, 1 8% NaCl = 6.9 ± 1 7, post lesion – water = 7.0 ± 1.1, 1.8% NaCl = 6 1 ± 2.4

DISCUSSION

Results of this study confirm the observation of

Simpson and Routtenberg [33] that SFO lesions produce a deficit in drinking to AII injections into the LPO and expand it to include LV injection sites Furthermore, this deficit in drinking after SFO lesions extends to the intake of saline as well as water However, since rats with SFO lesions show no decrement in drinking to AII injections into the AV3V (at the same time that LV deficits are apparent), it is not possible to conclude that the SFO is the only site housing dipsogenic receptors for AII

Moreover, it is important to note that 4 animals with SFO lesions did not decrease drinking to AII injected into the LV or LPO In their report [33], Simpson and Routtenberg also indicated that one of the 7 rats with SFO lesions did not decrease drinking to LPO injections of AII

The rapid recovery of drinking to AII injections into LV after SFO lesions is also inconsistent with the hypothesis that SFO contains the exclusive receptors for AII thirst since many of these rats had total SFO destruction Hoffman and Phillips have reported that drinking to LV injections of AII after SFO lesions recovered to 75% of the pre-lesion response by 14 days post lesion [7] What then is the basis for the temporary deficit in drinking to lateral injections of AII usually observed after SFO lesions?

The results suggest that the initial deficit in drinking to LV injections of AII could represent some effect of SFO lesions other than receptor destruction. Analysis of the histology from animals with SFO lesions provided a possible answer to the problem, enlarged lateral ventricles that were observed in several of the lesioned animals suggested that lesion-produced debris or edema had blocked the interventricular foramen and interrupted the normal cerebrospinal fluid (CSF) circulation from lateral to third ventricles This hypothesis of ventricular obstruction would account for the initial deficit in drinking to LV but not AV3V injections of AII if the receptors were located in or near third ventricular sites other than the SFO and downstream from the occlusion. Recovery of drinking to LV injections with the passage of time might then be correlated with re-establishment of ventricular circulation Preliminary evidence with radioactive tracings supports the hypothesis that SFO lesions obstruct CSF circulation and that recovery of drinking to LV injections of AII is correlated with recovery of CSF circulation from lateral to third ventricles [7].

Simpson and Routtenberg [32] have also reported that SFO lesions abolish the drinking response of the rat to intracerebral injections of carbachol. In their study, carbachol was injected into the dorsal third ventricle caudal to the SFO, and although other sites such as lateral hypothalamus were analyzed for carbachol-induced drinking in a dose-response study, the effects of SFO lesions on drinking were analyzed only with respect to caudal third ventricle injections. An important consideration, as before, is whether the abolition of drinking is due to destruction of receptors or obstruction of cerebrospinal fluid circulation. Germane to this consideration is the observation in the present study that drinking to AV3V injections of carbachol is not changed following SFO lesions. Block and Fisher (unpublished observations) have also found induction of drinking by carbachol injections into ventral periventricular sites to be unaffected by SFO lesions. Thus, although SFO may be a dipsogenic receptor site for both AII and carbachol, the results do not support the conclusion that it is the only receptor for these dipsogens Indeed, the SFO does not seem to be singularly involved in the elaboration of drinking responses since Simpson and Routtenberg [32] have reported that SFO lesions do not result in altered ad lib drinking beyond Day 1 post lesion and other investigators [29] observed no decrease in drinking to an intracellular or extracellular thirst challenge following SFO lesions.

How can these data be reconciled with Simpson's report [31] of a substantial, but not complete, reduction in the drinking response to 10 μg of AII injected intravenously after SFO lesions and Epstein and Simpson's later finding [10] with SFO lesions that AII induced drinking is absent when near threshold doses are infused intravenously? First, it is important to note that Simpson [31] noted partial recovery over time. In the experiments reported here, drinking to intraperitoneal injections of renin was not abolished by SFO lesions, although the magnitude of the response was decreased Thus, the assessment of the effects of SFO lesions on drinking to high systemic levels of AII depends to some degree on the magnitude of the response In this case, with a robust drinking response to renin, SFO lesions did not abolish, but did decrease, the drinking response. A similar magnitude of reduction would appear as an abolition in animals with a smaller magnitude of response, which could be due in part to non-specific effects of the lesion on the near threshold response

In summary, the results of the studies on drinking following SFO lesions suggest that the SFO is not the exclusive dipsogenic receptor for angiotensin or carbachol Also, the disruption of angiotensin-induced drinking following LPO or LV injection, but not AV3V injection, with SFO lesions suggests that other receptors in the third ventricle may be important for drinking to angiotensin and that, following LV or LPO injection, AII contacts these receptors via spread through CSF. The experiments to be reported next are directed at further analysis of this hypothesis.

EXPERIMENT 2: VENTRICULAR OBSTRUCTION AND AII-INDUCED THIRST

The results of our SFO lesion experiments suggest that one site for dipsogenic AII receptors is in or near the ventral third ventricle. However, it is difficult to localize a receptor site within the ventricular system by conventional chemical stimulation techniques since the drug is rapidly distributed throughout the whole ventricular system by CSF circulation

One of the lines of evidence leading Johnson and Epstein [19] to propose that AII elicits drinking by action on periventricular receptors is that drinking occurred only when radioactivity from intracerebral injections of labelled AII could be recovered in CSF collected from the cisterna magna. However, while the recovery of radioactivity in the CSF is correlated with drinking after intracerebral injection, it is possible that this represents a CSF sink for removal of the intact hormone or labelled fragments after receptor activation rather than an actual means of transport to the receptor Indeed, an alternative hypothesis [33] is that intracerebrally injected AII may be carried by blood vessels from the site of injection to receptors

A more direct method of examining these issues requires that spread of the hormone through the ventricular system be controlled after intracerebral injections of AII. One approach to this problem involves the use of obstructive barriers or plugs (made of Nivea cold cream) placed at

selected sites within the ventricles to control drug spread by acting as physical barriers [17] Ventricular plugs afford a way of restricting an injected substance to specific compartments and attendant periventricular tissue by preventing its passage beyond the cold cream obstruction.

In these experiments, the technique of ventricular obstruction is used to assess the site of action of AII for eliciting drinking after intracerebral injection. Plugs are placed in the interventricular foramen or the third ventricle at the level of the foramen, and the effects of AII injections into lateral sites on drinking are assessed when spread of the drug in CSF from lateral to third ventricle is impeded Third ventricle plugs at the level of the foramen also obstruct communication within the third ventricle, blocking the antero-dorsal region containing the SFO from the antero-ventral region, and the effects of drinking to AV3V injections of AII are assessed when spread to SFO is prevented Finally, plugs are placed in AV3V and the effects on drinking to lateral injections of AII assessed when drug entry via CSF transport into the AV3V is prevented but CSF circulation from lateral to dorsal third ventricles and SFO remains unobstructed

METHOD

Animals

Adult, male rats (Sprague-Dawley strain) weighing between 275–500 g at the time of surgery served as subjects

Surgery

Rats were anesthetized and stereotaxically implanted with 23 ga cannula as described in Experiment 1 All rats were implanted with cannulae aimed for the AV3V and a lateral site (LV or LPO) as illustrated by Fig 1. Depending on the plug site and the areas to be tested for AII sensitivity, some rats were also implanted with cannula aimed for the dorsal third ventricle at the level of the foramen and/or the dorsal third ventricle adjacent to the SFO

Procedure

Following surgery, rats were individually housed with continual access to rat chow, deionized water, and 1 8% NaCl under reverse-cycle lighting conditions (tested during dark phase) except for 1 group of animals housed under normal-cycle lighting (tested during light phase) and without access to saline.

After at least 5 days to allow for recovery from surgery, animals were screened on separate test days for drinking to angiotensin injections into each of the cannula sites. Angiotensin injections were 40 or 100 ng per 1 μl or 60 ng total delivered in 3 μl volume Each animal was tested with the same dose of angiotensin at each implant site, and the same dose was used for pre-plug screening and post-plug testing Fluid intakes were monitored for a half hr period following drug injection.

Animals meeting a screening criteria of ingesting at least 4 ml after an injection at a test site were then plugged and retested to determine the effect of ventricular obstruction on drinking to AII injections. Nivea cold cream was utilized for plugging One cc plastic syringes were filled with the cream and connected through a hypodermic needle to a section of PE-50 tubing. The other end of the PE tubing was fed to a 30 ga injector cannula through a connecting sleeve. Plugs were injected through the system described above using the 23 ga implanted cannula as a guide. A microdrive assembly advanced the plunger of the cream filled syringe to slowly exude 8 μl or less of cream over 1 min. Placement of plugs was well tolerated by rats, their activity remained normal, without extreme excitement or depression

Animals were tested for drinking to AII on the same day that the plug was inserted When 2 or more cannula sites were to be tested, the order of injections was counterbalanced across animals with at least 2 hr intervening between tests.

Assessment of Plug Obstruction

On completion of drinking tests, plug placement and effectiveness of ventricular obstruction were verified by dye or radioactive tracing. For dye tracings from more than 1 injection site, separate 2% solutions of acridine orange and eosin bluish were prepared in isotonic saline, india ink was used for dye tracing from single injection site preparations Rats were anesthetized with Nembutal and injected with 1–2 μl of dye in the same sites that had been tested with AII. Within 30 min, animals were perfused intracardially with saline followed by 10% Formalin and the brains were removed and stored in Formalin until sectioning. For dye tracing, brains were blocked and frozen on a microtome stage Diffusion of the dye(s) from the site(s) of injection was then traced as the block was sectioned coronally toward the posterior end or saggitally to the midline If dye was observed beyond the plugged region or if the plug placement obviously did not fill the ventricular lumen, the plug was considered ineffective. If the plug filled the lumen and dye did not penetrate beyond the plug, then the plug was considered effective. Cannula placements were also verified at this time

For radioactive tracing, tritiated DL norepinephrine (New England Nuclear, 1 μc/25 nng/1 μl) was used since it had a high specific activity and norepinephrine has been demonstrated to spread through ventricles rapidly following intracerebral injection [34]. Following post-plug drinking tests for 8 animals with dorsal third ventricle plugs, animals were anesthetized with Nembutal and injected with 1 μl of the labelled compound into the AV3V site Two unplugged rats also had labelled AV3V injections and served as controls Within 5 min, nonperfused brains were removed and two cubes of tissue, about 10–30 cubic mm each, were dissected from each brain in front of and behind the plug site. One piece centered around the AV3V and the other piece centered around the dorsal third ventricle including the SFO The tissue cubes were quickly weighed and homogenized in 2 ml of 0.4 normal perchloric acid to dissolve the norepinephrine. Samples were then centrifuged at 5500 RPM for 1 hr and 0.7 ml aliquots of supernatant were combined with 10 ml of Scintiverse cocktail (Fisher Scientific). All samples and blanks for background determination were then counted on a Beckman LSC 250 for 1 min to a preset 5% error.

RESULTS

Figure 3 is a summary of dye distributions observed with various cannulae and plug placements. Reference will be made to the appropriate sections of this figure as the results for a particular cannula and plug placement are presented.

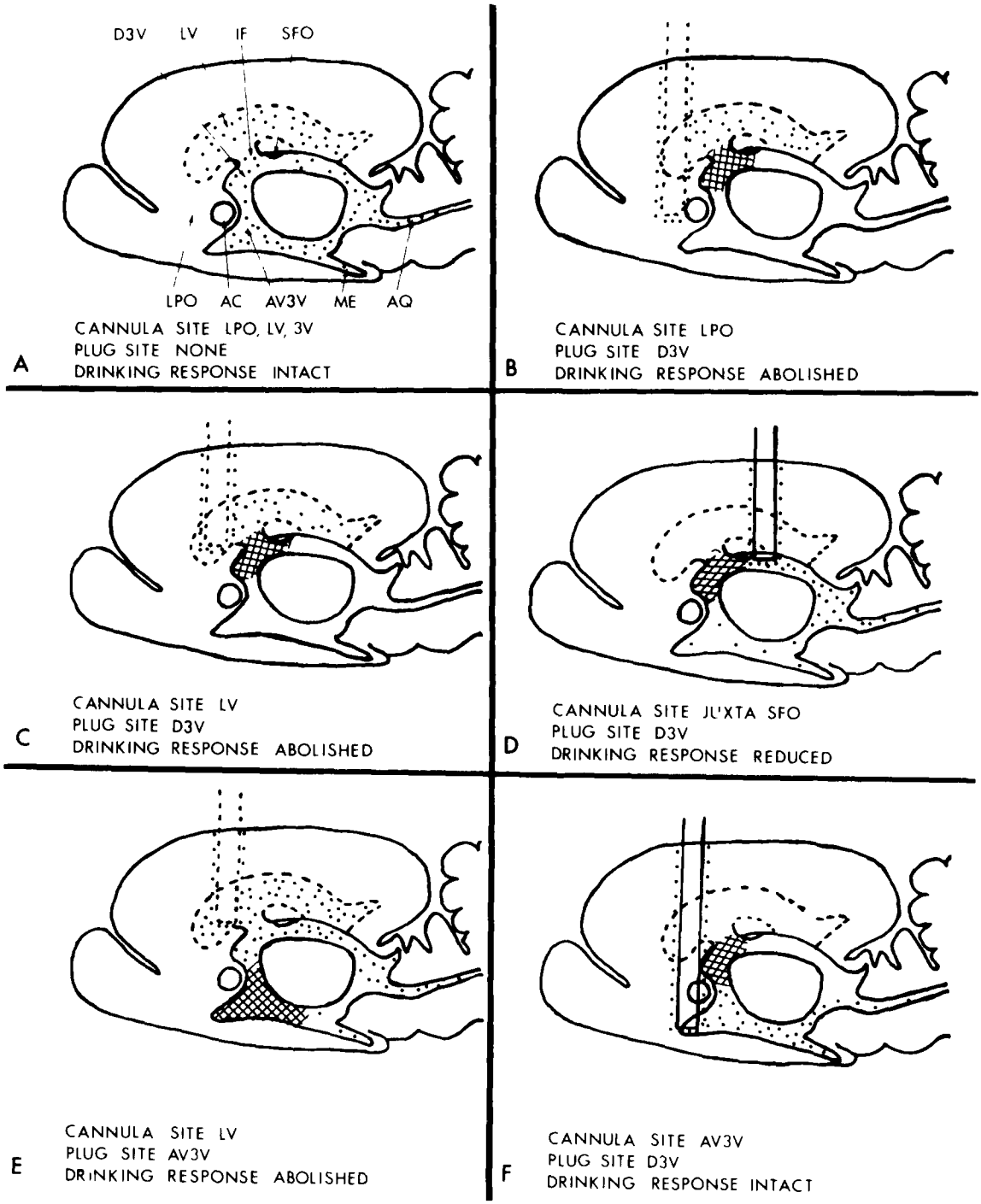

FIG. 3. Areas of distribution for angiotensin after intracerebral injections that communicate with the ventricular spaces in normal (a) and plugged preparations (b–f), and the effects on drinking behavior. Cross hatches indicate plug placement and dots indicate density of distribution following intracerebral injection at the sites indicated by the cannula outlines. Drawings are of a midsaggital section with lateral ventricles and lateral cannula placements depicted by broken lines to indicate their location in a more lateral saggital plane. In a), angiotensin spreads throughout the whole ventricular system following injection into LPO, LV, or third ventricle, and drinking is observed. In drawings b–f), ventricular plugs have interfered with the normal rostral-caudal circulation of CSF, and the resulting area of distribution of angiotensin after intracerebral injections is indicated in each case. The drinking response was greatly reduced when spread of angiotensin to the anterior ventral third ventricle was prevented or impeded as in b, c, d, and e. Only when angiotensin had unrestricted access to the AV3V, as in f, was the post-plug drinking response fully intact. Spread of angiotensin to the dorsal third ventricle region containing the SFO was not fully sufficient for elicitation of an intact drinking response, as in d and e, nor was spread to the SFO region necessary for drinking to occur as in f. Abbreviations D3V = dorsal third ventricle, LV = lateral ventricle, IF = interventricular foramen, SFO = subfornical organ, LPO = lateral preoptic area, AC = anterior commissure; AV3V = antero-ventral third ventricle; ME = median eminence; AQ = cerebral aqueduct.

Dye injections into the LV, third ventricle, or LPO (provided that the cannula trajectory pierces the LV) result in dye distribution throughout the ventricular system as illustrated in Fig. 3a. By inference, AII injections through these same cannulae resulted in a similar pattern of ventricular spread, and drinking was observed in each case. The effects of ventricular plugs on dye distribution from the injection sites and the magnitude of drinking responses elicited by AII injection at these sites before and after plugging will now be presented.

Dorsal Third Ventricle or Foramen Plugs

Ventricular obstruction with cold cream plugs accurately placed in the interventricular foramen or in the third ventricle at the point of entry of the foramen effectively prevented passage of dye into the third ventricle following injection into LV or LPO. Furthermore, with accurately placed dorsal third ventricle plugs, dye injected into the AV3V did not penetrate beyond the plug into the dorsal third ventricle where the SFO was located Indeed, most dorsal third ventricle plugs spread from the injection site and completely covered the SFO as well as filling the interventricular foramen. The plugs were fluid enough to, in most cases, conform to and completely fill the lumen of the ventricle at the plug injection site.

Some plugs did not prevent passage of dye in these cases, the plug cannula was often slightly lateral to the third ventricle or foramen and, consequently, the plug did not completely fill the ventricle. Incomplete plugs which allowed passage of dye did not consistently decrease drinking to AII injections at any site. Results are presented only for animals with complete plugs that prevented dye spread beyond the plug site.

Results of the radioactive tracings also indicated that the plugs acted as effective barriers to spread of injected chemicals. Radioactive counts from the SFO-dorsal third ventricle samples and the AV3V samples were converted to counts per min per g of tissue after subtraction of background counts. A ratio was then made for each animal by dividing SFO counts by AV3V counts. These ratios were then compared in control animals (2 rats without plugs and 1 animal with a plug that was not in the ventricle) and 7 animals with well placed plugs. The mean ratio for the 3 control animals was 2.43 ± 0.8, while the ratio for plugged animals was 0.07 ± 0.02. This difference between controls and animals with well placed plugs was highly significant ($p<0.001$ for independent t). The SFO/AV3V counts ratio for the 7 plugged animals was only 2.9% of the ratio for the controls.

When the plugs were well placed, drinking elicited by AII injections into LPO or LV was virtually abolished. Table 3 presents the fluid intakes induced by LPO injections of AII before and after placement of foramen or third ventricle plugs that prevented passage of dye into the third ventricle. See Fig. 3b for an illustration of this preparation. The magnitude of the post-plug drinking response is only 14% of the pre-plug value; this decrement in drinking to LPO injections of AII is highly significant ($p<0.001$). As a control for possible debilitating effects of the plug preparation which might have prevented drinking from occurring, 10 of the 14 animals studied were tested for drinking to AV3V injections of AII (4 rats had screened negative). In this case, post-plug drinking was 94% of pre-plug values (See Table 3).

TABLE 3

EFFECT OF VENTRICULAR OBSTRUCTION BY FORAMEN OR THIRD VENTRICLE PLUGS ON DRINKING TO ANGIOTENSIN INJECTIONS (40 OR 60 NG)

	Preplug			Postplug		
	Fluid Intake in ML after LPO Injection, N=14					
	Water	1 8% NaCl	Total	Water	1 8% NaCl	Total
Mean	5 8	4 6	10 4	1 4	0 1	1 5
SEM	0 8	1 2	1 4	0 4	0.1	0 4
	Fluid Intake in ML after AV3V Injection, N=10					
	Water	1 8% NaCl	Total	Water	1 8% NaCl	Total
Mean	5 9	1 1	7 0	5 6	1 0	6 6
SEM	0 9	0 5	0 9	0.9	0 5	0 9

As anticipated from the observation that LPO injections spread to the LV, drinking to LV injections of AII is also abolished by plugs in the dorsal third ventricle. Figure 3c illustrates the dye distribution obtained with this preparation. The post-plug intake is only 11% of pre-plug intake, this reduction in drinking is significant at the 0.001 level. Again, it is unlikely that the abolition of drinking to LV injections of AII is due to some nonspecific side effect of the ventricular obstruction, since drinking can still be elicited with angiotensin injections into the AV3V. There are no significant changes in water, saline, or total fluid intake to AII stimulation in the AV3V after dorsal third ventricle plugs. Results are summarized in Table 4.

Dorsal third ventricle plugs also attenuated the drinking induced by AII injections into the third ventricle adjacent to the SFO (the cannula placement was lateral to SFO to avoid destroying the structure). Figure 3d illustrates this cannula and plug arrangement. In 6 rats, post-plug drinking to AII injections near the SFO was only 35% of pre-plug values, this decrement was significant at the 0.02 level. Again, drinking to AV3V injections of AII in these animals was not affected by the plugs Table 5 presents the results

Dorsal third ventricle plugs at the level of the foramen were not effective at blocking drinking induced by AV3V injections of AII in any of the above experiments. In total, 17 rats with dorsal third ventricle plugs were tested for drinking following AII injections into the AV3V. Figure 3f illustrates this arrangement. For these animals, no significant changes in water, saline, or total intake were observed, with 40 or 100 ng AII injections. Mean intakes and standard errors were as follows: pre-plug – water = 9.6 ± 1.2, 1.8% NaCl = 2.8 ± 1 2, post-plug – water = 8 4 ± 1.0, 1 8% NaCl = 3.6 ± 1.2.

Anterior Ventral Third Ventricle Plugs

Eight rats were tested for drinking to 100 ng injections of AII into the LV before and after AV3V plugs. Figure 3e illustrates this preparation. Mean pre-plug intakes ± standard deviation for these rats were 7.28 ± 2.1 ml, post-plug intakes were 0.3 ± .09 ml (only water was available). The decrement in post-plug response compared to pre-plug response is significant at the 0 001 level. Examination of the dye diffusion patterns and plug sites after LV injection revealed that the plugs extended to the interventricular foramen in 4 animals, and consequently, dye was not observed anywhere in the third ventricle. In the other 4

TABLE 4

EFFECT OF VENTRICULAR OBSTRUCTION BY DORSAL THIRD VENTRICLE PLUGS ON DRINKING TO ANGIOTENSIN INJECTIONS (40 OR 100 NG)

	Preplug			Postplug		
	Fluid Intake in ML after LV Injection, N=9					
	Water	1.8% NaCl	Total	Water	1.8% NaCl	Total
Mean	6.2	1.1	7.4	0.7	0.1	0.8
SEM	1.0	0.4	1.0	0.4	0.1	0.4
	Fluid Intake in ML after AV3V Injection, N=9					
	Water	1.8% NaCl	Total	Water	1.8% NaCl	Total
Mean	11.2	3.5	14.7	7.8	5.7	13.5
SEM	2.1	2.0	3.2	1.4	2.0	3.5

TABLE 5

EFFECT OF VENTRICULAR OBSTRUCTION BY DORSAL THIRD VENTRICLE PLUGS ON DRINKING TO AII INJECTIONS (40 OR 100 NG) INTO AV3V OR THIRD VENTRICLE NEAR SFO

	Preplug			Postplug		
	Fluid Intake in ML after SFO Injection, N=6					
	Water	1.8% NaCl	Total	Water	1.8% NaCl	Total
Mean	11.0	2.4	13.4	4.3	0.3	4.7
SEM	1.6	1.9	2.3	0.8	0.3	0.8
	Fluid Intake in ML after AV3V Injection, N=5					
	Water	1.8% NaCl	Total	Water	1.8% NaCl	Total
Mean	8.8	2.7	11.5	10.9	1.3	12.3
SEM	0.9	2.1	1.9	1.8	0.9	2.6

rats, the plugs did not spread to the foramen from the AV3V injection site and dye from the LV was observed in the dorsal third ventricle (including the SFO) extending into the cerebral aqueduct and fourth ventricle, but not in the plugged ventral third ventricle Drinking after LV injection of AII was reduced in these 4 animals despite the fact that a channel for drug spread via CSF between LV and SFO remained open (Pre-plug intake. 8.4 ± 2.0 ml, Post-plug intake 0.7 ± 1.3 ml) This decrement in drinking to LV injections of AII when plugs allowed hormone access to SFO but not AV3V is significant at the 0.02 level.

DISCUSSION

The experiments with ventricular obstruction indicate that plugs are an effective method for limiting spread of injected chemicals through the ventricular system. When plugs block access of CSF flow from lateral ventricles to third ventricle through the interventricular foramen, LV injections of AII are no longer effective in eliciting drinking. This result implies that AII injected into LV normally induces thirst by spread to third ventricle Thus, these data support the hypothesis that one or more tissue sites in or near the third ventricle contain the central dipsogenic receptors for AII.

Plugs which block access from LV to third ventricle also abolish the drinking normally observed after LPO injection of AII. This result implies that AII injected into LPO elicits drinking by spread to lateral and then third ventricle. The hypothesis that AII may be transported by blood from LPO to a distant site of action is not supported by these data. The data also do not support the hypothesis that LPO contains dipsogenic receptors for AII [4], since LPO injections of AII are ineffective when drug spread via CSF to third ventricle is retarded.

Results of the ventricular obstruction studies do suggest that the primary site of action for intracerebral injections of AII is in the anterior-ventral third ventricle. With dorsal third ventricle plugs, AII injected into the AV3V does not spread in significant amounts to the SFO (radioactive tracing indicates that with dorsal third ventricle plugs, access to the SFO region is only 2.9% of its normal value). Since the drinking response to AV3V injections of AII did not change after dorsal plugs, it is unlikely that SFO was involved in the mediation of drinking. If the SFO was involved in mediating drinking to AV3V injections of AII despite the presence of dorsal third ventricle plugs, then a 97% reduction in the amount of drug reaching the SFO should result in a greatly attenuated response since drinking to direct SFO injection is dose-dependent [33]. However, it is possible that while blood transport from LV or LPO is not apparent, significant blood transport from AV3V to receptors did occur If so, it seems doubtful that blood transport to SFO is the essential event, since SFO lesions do not abolish drinking to AV3V injections of AII

These results imply that drinking to intraventricular injections of AII is not dependent on access to the SFO When AII is injected into the third ventricle lateral to the body of the SFO, drinking is severely attenuated by a dorsal third ventricle plug However, it must be noted that AII induced drinking was not completely abolished in this case as 35% of the pre-plug response remained This result does not make it possible to rule out an action of CSF-borne AII on the SFO to elicit drinking. Thus, the action of AII on SFO may be sufficient to cause drinking but not necessary, as other receptors in the distribution of injections into AV3V are also sufficient, independent of SFO mediation.

With this hypothesis, the severe attenuation of drinking to AII injections into the vicinity of the SFO when dorsal third ventricle plugs are in place is perplexing. It is possible that access to SFO of injections just lateral to it was impaired by plugs which often spread to the SFO region This could account for the attenuation noted On the other hand, it is possible that spread of AII to AV3V in rats with dorsal third ventricle plugs is greater after injections near the SFO than it is after LPO or LV injections, since the SFO injection site is closer to the plug. Results from the study with anterior-ventral third ventricle plugs suggest that the latter interpretation may be more appropriate. In that study, drinking to LV injections of AII was greatly reduced even when the plugs permitted spread via CSF from LV to dorsal third ventricle and SFO. Thus, these data indicate that the drinking induced by intraventricular injections of AII critically depends on access of the hormone to a particular region of periventricular tissue, the anterior-ventral third ventricle. With this view, access of the hormone via CSF to the SFO is neither necessary nor sufficient to arouse drinking.

It must be noted however, that these results do not necessarily imply that SFO is devoid of dipsogenic receptors for AII. It may be that SFO contains dipsogenic

receptors for AII that primarily are affected by blood-borne, as opposed to CSF-borne AII. In this regard, Simpson has reported that AII injections into the body of the SFO elicit drinking at a lower threshold than injections just outside the body of the SFO, into the dorsal third ventricle [31].

In subsequent use of the ventricular plugging technique, Hoffman and Phillips [18] have also concluded that drinking following intraventricular injection of AII depends on access of the hormone to the anterior-ventral third ventricle, and not SFO Furthermore, they reported that the pressor response observed after intraventricular injections of AII depends on access of the hormone to anterior-ventral third ventricle as well.

Finally, the dorsal third ventricle plug artificially simulates the postulated effect of SFO lesions that results in temporary decreases in drinking to LV or LPO (but not AV3V) injections of AII, that is, blockage of the foramen and CSF circulation by lesion produced edema or debris. In this light, it is interesting to note that qualitatively equivalent effects on drinking to intracerebral injections of AII are produced by dorsal third ventricle or foramen plugs and SFO lesions.

GENERAL DISCUSSION

The results of the experiments reported here indicate that the drinking response elicited by intracerebral injections of AII depends on spread of the hormone in CSF to anterior ventral third ventricle receptors. The issue of the location of receptors responsible for drinking following systemic infusions of AII was not directly addressed by these experiments, through an exclusive role for the SFO in mediating this response was questioned since SFO lesions reduced but didn't abolish drinking to intraperitoneal injections of renin. Thus, SFO may contain dipsogenic receptors sensitive to blood levels of AII but it appears unlikely that these are the only blood-faced AII receptors At present, there is no evidence which demonstrates that intact AII of peripheral origin gains entry to the brain or CSF, though there is pharmacological evidence implicating central periventricular receptors in drinking to elevated systemic levels of AII [20]. Our data suggest that the AV3V region may mediate, at least in part, drinking following systemic, as well as intraventricular, injections of AII.

Several other lines of evidence point to the importance of the AV3V region as a receptor site mediating thirst behavior Andersson [2] has provided data implicating this region as a receptor site for hyperosmotic stimuli, which he believes involves an increase in sodium concentrations in sensitive neurons. Another group [25] and one of us [5] have also reported elicitation of thirst following hyperosmotic injections into third ventricle, although neither of these reports could concur that increased sodium concentration rather than increased osmolarity was the crucial stimulus arousing thirst. Thus, it is possible that the AV3V contains dipsogenic receptors sensitive to hyperosmotic stimuli as well as AII.

Lesions of anterior-ventral periventricular zones at the level of the anterior hypothalamus and preoptic areas in dogs have been reported to produce adipsia without aphagia lasting up to 14 days post lesion [3]. In rats, medial preoptic lesions impaired operant responding for water but not food reinforcement [8]. These data support the hypothesis that the AV3V region is importantly involved in the mediation of thirst behavior.

Neuroendocrinologists interested in endocrine feedback mechanisms for control of pituitary secretion have also been impressed with the possibility of ventricular mediation of hormone reception, secretion, or transport [21,35] There are several distinguishing features of the anatomy of the ventral third ventricle region which deserve consideration. Atypical ventricular ependyma (tanycytes) with long basal processes extending from CSF to neuronal and capillary endings are especially numerous in this region [27,30]. Two extra blood-brain-barrier structures, the median eminence and the organum vasculosum of the lamina terminalis (OVLT), are also located in the AV3V region. The ultrastructural and biochemical features of these circumventricular organs are consistent with a neurosecretory function [22,39]. Also, note that OVLT and SFO seem to share a common innervation. Both have been implicated in the cholinergic limbic circuit of Lewis and Shute [24], and both receive afferents from the median preoptic nucleus [16] Since the plug technique does not permit precise localization of receptors within the AV3V, the periventricular nuclear groups and circumventricular organs within the region must be considered as potential receptors for AII; the possibility of transport to receptors somewhat removed from the ventricles by tanycyte processes can not be dismissed either.

It must also be noted that none of the procedures in the present studies succeeded in separating water from saline intake after AII-induced drinking [6]. Although these studies were not directly addressed to whether water and saline intake induced by AII are due to an action on anatomically separate receptors, the results do not lend support to such a hypothesis

The precise conditions or mechanisms which mediate drinking following AII injections remain unelucidated It is also unclear what the significance of the endogenous brain-based renin-angiotensin system is for thirst [11,14]. Whether angiotensin of renal or brain origin is actually involved in the mediation of extracellular thirst, either crucially or as a seldom used safety factor, remains unanswered [1,36]. However, the definition of a necessary substrate (AV3V) for the dipsogenic effect of the hormone provides an important first step needed to investigate these remaining problems concerning the significance of angiotensin-induced thirst.

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