Preoptic-hypothalamic periventricular lesions: thirst deficits and hypernatremia

JAMES BUGGY AND ALAN KIM JOHNSON
Department of Physiology and Biophysics and Cardiovascular Center and Department of Psychology, University of Iowa, Iowa City, Iowa 52242

BUGGY, JAMES, AND ALAN KIM JOHNSON. Preoptic-hypothalamic periventricular lesions: thirst deficits and hypernatremia. Am. J. Physiol. 233(1): R44-R52, 1977 or Am. J. Physiol.: Regulatory, Integrative Comp. Physiol. 2:1: R44-R52, 1977.—To assess the significance of stimulation studies suggesting an anteroventral third ventricle (AV3V) dipsogenic site of action for hyperosmotic and angiotensin thirst stimuli, electrolytic lesions of periventricular tissue surrounding AV3V were produced under ether anesthesia in rats preselected for responsiveness to subcutaneous angiotensin and hypertonic NaCl thirst challenges. Lesions limited to preoptic-hypothalamic periventricular substrates resulted in adipsia; those rats resuming ad lib. drinking after a period of adipsia exhibited persistent drinking deficits to angiotensin and hypertonic NaCl thirst challenges. Reduced drinking following water deprivation, and increased plasma osmolality and sodium. Drinking to polyethylene glycol-induced hypovolemia and feeding after food deprivation did not differ between lesioned and sham-lesioned animals. The disturbances in behavioral control of fluid balance imply that AV3V periventricular tissue normally play a key role in mediating regulatory drinking. It is proposed that these AV3V periventricular lesion-induced effects on drinking behavior are due to destruction of receptors and/or integrative systems monitoring fluid-borne angiotensin and hyperosmotic stimuli.

Adipsia; brain lesions; angiotensin-induced drinking; body fluid homeostasis; hyperosmotic-induced drinking; rat; cerebral ventricles

BEHAVIORAL AND INTERNAL CONTROL systems interact to maintain body water and electrolytes within narrowly defined limits. Physiological mechanisms which modulate urinary water loss in defense of body fluid homeostasis must ultimately be complemented by behavioral actions leading to the seeking out and ingestion of fluids since it is only by addition of water through drinking that absolute deficits in body water are restored. Deficits in body fluid stores must first be detected by the central nervous system for elaboration into thirst; drinking in response to the thirst state then corrects the fluid deficit and thirst subsides. In this report, evidence will be presented indicating that stimuli associated with dehydration, specifically hyperosmolarity and increased circulating titers of angiotensin II (AII), arouse thirst behavior through an action on neural tissue surrounding the third cerebral ventricle at the preoptic-anterior hypothalamic level.

Hyperosmolarity and AII have been proposed to activate the nervous system by direct central action (30, 12), rather than by nervous input from peripheral receptors. Andersson (1) first demonstrated that thirst could be elicited without altering the toxicity of peripheral body fluids by direct application of hyperosmotic solutions to the anteromedial hypothalamus. Similarly, Epstein, Fitzsimons, and Rolls (11) demonstrated that thirst is aroused when AII is injected directly into the brain at doses two to three orders of magnitude lower than necessary to elicit drinking following intravenous delivery. Moreover, Johnson and Schwab (18) reported that thirst aroused by increased circulating levels of angiotensin is attenuated by lower doses of analog antagonist when applied centrally as compared to peripheral administration. These studies implied a central site of action for AII and hyperosmolarity but fail to define the precise anatomical locus.

In an effort to define the central dipsogenic site of action for AII, Johnson and Epstein (17) mapped diencephalic and telencephalic structures and noted that cannula sites bordering cerebral ventricles or cannula trajectories traversing ventricles yielded the highest sensitivity. After varying cannula trajectories to pass through or avoid ventricles and measuring progress of label into cerebrospinal fluid (CSF), they concluded that periventricular tissue mediates drinking to AII.

Employing a technique of regional ventricular obstruction to control spread of intracerebrally injected AII through CSF, Buggy and colleagues (8, 9) concurred that access to AII to periventricular tissue is necessary for drinking to occur. The periventricular site of action for AII was further localized to preoptic-anterior hypothalamic periventricular substrates of the anteroventral third ventricle (AV3V), since following lateral preoptic or intraventricular AII injections drinking resulted only when spread of AII to this region was unimpeded (8, 9, 15). Similarly, periventricular tissue has been implicated in the detection of hyperosmotic stimuli for arousal of thirst; injection of hyperosmotic solutions directly into AV3V rapidly induces drinking in rat (6, 7), goat (2), and sheep (21).

A complementary approach to assessing complicity of AV3V periventricular tissue in mediating thirst is to examine the effect of ablation of this region on drinking behavior. In another report (16), we have described the acute effects of lesions limited to AV3V periventricular tissue on drinking and fluid balance. These effects include adipsia without attendant primary aphagia, loss.
of water in dilute urine, and as a consequence, greatly increased blood osmolality and sodium. Following a period of adipisias, some AV3V lesioned rats regain ad lib. water intake. This report characterizes chronic deficits in control of regulatory drinking behavior which persist following “recovery” from AV3V lesion-induced adipisias. Specifically, the responses to AI, hyperosmotic, and hypovolemic thirst challenges are assessed in sham- and AV3V-lesioned rats; the responses to these standardized, diagnostic dipsogenic tests assess the integrity of neural mechanisms subserving specific systems controlling regulatory water intake. Measures of daily voluntary water intake are reported when food is present and in its absence. Acute ingestive responses to food and water deprivation are also described. Plasma osmolality, sodium, protein, and urea nitrogen measures are presented as further indices of persisting disturbances in body fluid homeostasis resulting from lesions of AV3V periventricular substrates.

METHODS

Animals. Experimentally naive, adult, male albino rats (Sprague-Dawley derived, University of Iowa, Dept. of Psychology Animal Colony) weighing between 250 and 400 g at the start of the experiment were individually housed in hanging cages constructed of hardware cloth (0.75-in. mesh) and sheet metal. Feed (Teklad laboratory pellets) was available on cage floors except during drinking tests and tap water was presented ad lib. from metal drinking spouts entering the front of the cages. Ambient lighting was programmed to turn on at 0800 h and off at 2000 h while colony temperature was maintained at approximately 22°C by air conditioning.

Prelesion screening to thirst challenges. After several days of adaptation to individual housing, presurgery drinking responses to systemic AI (Hypertensin, Ciba) and hypertonic NaCl-induced intracellular dehydration were separately determined for each rat. Food was removed from cages during tests and water intake was measured to the nearest 0.1 ml from graduated burettes before and 1 h after administration of the dipsogens. In random order on separate test days, animals received subcutaneous injections of 0.9% saline (0.67 ml), hypertonic NaCl (0.4 ml of 12%/100 g or 1 ml of 10%), and AI (1.56 mg/kg or 500 μg/rat; AI was dissolved in 0.9% saline). At least one rest day intervened between test days and the AI challenge was administered twice. For screening tests, a drinking criteria was established of greater than 2 ml water intake above control (subcutaneous injection of saline) on both the hypertonic saline and second AI challenge. Animals meeting these response criteria were then randomly assigned at a ratio of 3:1 to receive electrolytic lesions directed at periventricular tissue of the AV3V or to serve as sham-operated controls.

Stereotaxic lesioning of AV3V. Animals were initially anesthetized with ether and positioned in a stereotaxic instrument; anesthesia was maintained with an ether-saturated nose cone for the duration of the lesioning procedure (approx 15-min duration). A mid sagittal incision was made and tissue was retracted laterally to expose the dorsal surface of the skull. After the skull was leveled between lamba and bregma referents, a bone flap centered about lamba about 1.5 mm in diameter was removed. On locating the midsagittal sinus as a midline reference, a lesioning electrode was lowered into the brain on the midline taking care to first incise the dura while retracting the sinus to avoid excessive blood loss. The anterior-posterior placements ranged from +0.3 to −0.7 mm with respect to bregma; one to four electrode penetrations were made within this AP range. In sham-lesion rats, the electrode was lowered vertically 5–7.6 mm from dura (one to four penetrations) but no lesioning current was passed. In animals to be lesioned, the electrode was lowered vertically 7.4–8.0 mm from dura and direct current of from 1.4 to 3.0 mA was passed between the brain electrode and a rectal electrode for 10–30 s. Lesioning electrodes were constructed of Nichrome wire (approx 24 gauge with insulation) or size 0 stainless steel insect pins insulated with Beldenamel except for an exposed, beveled tip of 0.5 mm. Following this lesioning procedure, the skull hole was filled with bone wax and/or Gelfoam and the incision was closed with wound clips.

Postoperative maintenance. Since prior studies demonstrated that periventricular lesions in the AV3V region often resulted in failure to voluntarily ingest water with an attendant dehydration resulting in mortality, intravenous “therapy” was instituted following surgery to increase survival rate. One regime consisted of providing animals with access to 100 ml daily rations of 0.2% sodium saccharin solution as well as food and water for several days before and 9 days after the lesion or sham-lesion procedure. The saccharin solution was offered to induce large fluid intakes on the basis of palatability while contributing to maintenance of fluid balance when drinking of water might otherwise be absent. In other animals, daily voluntary water intake was monitored after surgery and animals failing to ingest at least 10 ml water/day were given 10-ml water supplements by intragastric gavage.

Postlesion assessment of regulatory drinking. After at least 5 days following return of daily ad lib. drinking of water in amounts at least equivalent to prelesion, 1 h drinking responses to the same systemic AI and hypertonic NaCl challenges used in prelesion screening were again determined on separate test days with intervening rest days. Lesioned rats with reduced or absent responses were then retested 1–5 mo postlesion to determine if drinking to the AI and hypertonic NaCl challenges would recover with time. Sham-lesioned rats were always tested at the same time as lesioned rats to ascertain that responsiveness to dipsogenic challenges did not change over time or with repeated testing. Interspersed with the second series of challenge tests were other diagnostic tests for assessing fluid regulatory and behavioral capabilities. These tests included acute ingestive responses in the 1st h following return of food or water after 24 h of deprivation, measures of 24 h ad lib. water intake in the presence or absence of food, and the 10-h drinking response to hypovolemia induced by subcutaneous injection of 1 ml 20% polyethylene glycol/100 g body wt. Food was present during water depriva-
tion but was removed for the 1st h after return of water when drinking was monitored. Water was always available during food deprivation and upon return of food. Food was not present for 10 h following polyethylene glycol injection or during another 10-h period following subcutaneous 0.9% saline injection (this 10-h period following saline injection served as a base-line to compare drinking responses after hypovolemia).

**Histology and blood analysis.** At the end of postlesion testing (3-5 mo postlesion), lesioned rats were deeply anesthetized (with Nembutal, 60 mg/kg, or ether) and perfused intracardially with 0.9% saline followed by 10% formalin in 0.9% saline. In some sham-lesioned and lesioned rats maintained under ether anesthesia, blood was collected into heparinized syringes from the inferior vena cava prior to perfusion. This blood was quickly centrifuged to separate cells and the plasma was frozen until determinations were made of osmolality (freezing point osmometer), sodium (flame photometer), protein (refractometer), and urea nitrogen (colorimetry) concentrations. The hypovolemic thirst challenge was not conducted on rats used for blood analysis to avoid potential confounding of blood measures. Brains were removed after perfusion and stored in jars with formalin. Frozen sections (40 μm) were taken through the extent of the lesion and later stained with cresyl violet, Weil, or luxol fast blue-cresyl violet. The extent of the lesion was then examined under light microscope.

**Statistics.** Group data are expressed as mean ± standard error and were evaluated with the Student t-test. Paired sample analysis was used when possible for pre-versus post-lesion comparisons. Otherwise, the independent t-test was used to compare experimental with sham control groups.

**RESULTS**

Prelesion screening tests for drinking responses to systemic AII and hypertonic NaCl were conducted on 111 rats. Thirty-six rats failed to meet the screening criteria for responsiveness and were removed from further analysis. Of the 75 rats exceeding the screening criteria, 19 were sham lesioned and 56 were lesioned. One sham-lesioned rat and 21 lesioned rats died before the start of postlesion testing; death in lesioned rats was typically preceded by several days of adipsia.

**Grouping of lesioned rats.** On the basis of postlesion drinking responses to systemic AII and hypertonic NaCl, lesioned rats were classified as no-deficit (ND), double-deficit (DD), or hypertonic NaCl deficit (HTSD). To be characterized as a deficit rat (DD or HTSD), the postlesion response to the thirst challenges had to be one-third or less the prelesion value on two or more consecutive tests. ND rats (n = 16) had comparable pre- and postlesion responses on both the AII and hypertonic NaCl tests. DD rats (n = 13) had markedly attenuated postlesion responses to both AII and hypertonic NaCl on at least two consecutive postlesion tests for each challenge. HTSD rats (n = 6) continued drinking to the AII test but failed to drink to the hypertonic NaCl challenge on two or more consecutive tests after the lesion. No lesioned rats failed to respond to AII while maintaining a drinking response to hypertonic NaCl. It must be stressed that no sham-lesioned rats (n = 18) ever qualified for a deficit grouping (i.e., all 18 sham-lesioned rats gave comparable responses pre- and postsurgery).

**Postlesion adipsia.** After surgery, approximately one-fourth of the animals were maintained for a 9-day period with access to saccharin solution. In these animals, no systematic attempt was made to assess daily drinking in the early postoperative period. In the remainder of the animals, however, daily water intake was followed closely. Sham-lesioned rats had a slight depression of water intake on the first day after surgery, but no sham-lesioned rat drank less than 10 ml/day on the 1st day postoperative or on any subsequent day and, consequently, none of these rats required water by gavage. Lesioned rats often failed to consume 10 ml or more of water/day and required supplemental water by stomach tube. The mean period of adipsia (10 ml or less of water/day) in days ± SEM was 0.4 ± 0.1 for ND rats with a range of 0-1 days and 2.6 ± 0.6 for deficit rats with a range of 0-7 days. This difference in length of adipsia for deficit versus no-deficit lesioned rats is significant at the 0.01 level.

**Hypertonic NaCl and AII thirst challenges.** Figure 1 contains pre- and postlesion water intake in the 1-h test period following systemic AII or hypertonic NaCl thirst challenges for sham-lesioned, DD, and HTSD deficit groups. Postlesion measures for sham-lesioned and deficit groups are the mean of two or more tests. Prelesion responses do not differ significantly between sham-lesioned and lesioned rats for either the hypertonic NaCl or AII challenges. Postlesion responses do not differ from prelesion responses for sham-lesioned rats. Lesioned ND rats also had similar pre- and postlesion responses (intake in ml + SEM: hypertonic NaCl-pre = 6.4 ± 0.6, post = 6.6 ± 0.8; AII-pre = 5.4 ± 0.7, post ± 7.8 = 1.9). DD rats had markedly attenuated responses to both systemic AII and hypertonic NaCl (P < 0.001 for each challenge). HTSD rats had severely attenuated responses to the hypertonic NaCl challenge (P < 0.001 compared to prelesion) while the drinking response to systemic AII was significantly increased (P < 0.05) compared to prelesion values. There was no evidence of recovery of drinking to AII or hypertonic NaCl challenges in deficit rats while the drinking responses of sham lesioned rats remained stable over time and repeated tests.

**Hypovolemic thirst challenge.** The net increase in water intake to polyethylene glycol-induced hypovolemia 10 h after injection was 4.9 ± 0.9 ml for 11 shams, 2.9 ± 1.7 for 10 ND, and 3.3 ± 4.1 for 10 DD rats. There are no significant differences between groups reflecting an intact response capability to hypovolemia. Only two HTSD rats were tested with this challenge and both responded with increased water intake (4.4 and 10.9 ml).

**Postlesion responses to food and water deprivation.** Table 1 contains the 1-h food intake following 24 h of food deprivation and the 1-h water intake after 24 h of water deprivation. There are no significant differences in feeding between any of the groups. Likewise, there is no difference in drinking after water deprivation for sham-lesioned and ND rats. However, the drinking re-
response after water deprivation for DD rats is significantly reduced compared to that of sham-lesioned rats, \( P < 0.02 \); the drinking response of HTSD rats is also reduced compared to that of sham-lesioned rats, although this difference is not statistically significant. Moreover, note that the variance for deprivation-induced drinking is greater in the deficit groups. This occurred because some deficit rats responded normally to water deprivation while other rats either completely failed to respond or gave a greatly attenuated response. The lowest water intake after deprivation noted for 34 sham-lesioned or no-deficit rats was 4.8 ml; 8 of 19 lesion-deficit rats drank less than this amount in the 1-h test period. When the deprivation-induced drinking test was repeated in some DD or HTSD rats which had not responded in the initial test, they again failed to show appropriate deprivation-induced drinking.

**Ad lib. water intakes.** Of the 56 lesioned rats, 21 failed to resume ad lib. drinking and died after 1-11 days of adipsia. The remainder of the lesioned rats resumed ad lib. drinking sufficient for survival either immediately postlesion or after several days of adipsia. Presented next are measures of ad lib. intake when stabilized several weeks after surgery. The mean daily ad lib. fluid intake when food was available was \( 45.5 \pm 2.76 \) (SEM) ml for sham-lesioned rats. When food was not available, sham-lesioned rats decreased daily ad lib. fluid intake to \( 18.1 \pm 3.3 \) ml. Daily ad lib. water intakes for lesioned rats were: ND rats— with food, \( n = 16, 55.9 \pm 5.2 \) ml without food, \( n = 10, 37.2 \pm 8.4 \); DD rats— with food, \( n = 12, 76.3 \pm 5.0 \), without food, \( n = 10, 84.4 \pm 8.0 \); HTSD rats— with food, \( n = 6, 51.5 \pm 1.9 \), without food, \( n = 3, 34.7 \pm 6.4 \). Compared to that of sham-lesioned rats, the ad lib. water intakes of DD rats with or without food and ND rats without food were significantly greater \( (P < 0.001, 0.001, 0.05, \) respectively). These results demonstrate that ad lib. water intake in AV3V-lesioned rats is not strictly prandial or feeding related since drinking continues in the absence of food. Moreover, unlike shams, DD rats failed to reduce ad lib. water intake when food was removed.

The variability of ad lib. water measures for lesioned rats is much greater than for sham-lesioned rats; this occurred because some lesioned rats drank amounts comparable to shams while others drank twice as much. There is, however, no correlation between ad lib. water intake and response to thirst challenges in lesioned rats. That is, some deficit rats had ad lib. intakes within the normal range while some no-deficit rats were hyperdipsic. Moreover, most ad lib. drinking occurred at night while drinking during the day, as measured over a 10-h period, was equivalent across lesioned and sham groups. Note that since drinking challenge tests were conducted in the middle of the light period when little or no drinking during baseline tests was observed, it is unlikely that preexisting overhydration could account for reduced drinking responses to challenges in deficit rats.

Lesion size was a better predictor of the amount of ad lib. drinking than response to thirst challenges. When lesion-deficit rats are grouped on the basis of number of electrode penetrations, rats with two or more electrode penetrations, and consequently larger lesions as confirmed histologically, had daily ad lib. water intakes \( (71.9 \pm 4.6 \) ml) significantly \( (P < 0.001) \) greater than sham-lesioned rats, while lesioned rats with single electrode penetrations and smaller lesions had ad lib. water intakes \( (49.7 \pm 5.36 \) ml) that did not differ from intakes of sham-lesioned rats.

**Blood measures.** Blood samples were taken from six sham-lesioned, six ND, and six lesion-deficit rats 3 mo postlesion. Of the six lesion-deficit rats, three were DD and three were HTSD. Plasma protein concentration, osmolality, and sodium concentration are presented in Fig. 2 for sham-lesioned and lesion-deficit rats. ND rats did not differ from shams on any of these measures. Lesion-deficit rats, on the other hand, had significantly elevated plasma osmolality and sodium, but normal plasma protein concentration, when compared to shams. Plams urea nitrogen values also did not differ between groups (sham, 23.0 \pm 0.83 mg/100 ml; lesion-deficit, 23.6 \pm 1.36; 5 rats in each group).

**Histology of AV3V lesions.** Histological analysis of brain tissue revealed that the common extent of lesions traversed the hypothalamic organum vasculosum laminae terminalis (OVLT) and thalamus. Histological penetrations and smaller lesions had ad lib. water intakes comparable to shams while others drank twice as much. There is, however, no correlation between ad lib. water intake and response to thirst challenges in lesioned rats. That is, some deficit rats had ad lib. intakes within the normal range while some no-deficit rats were hyperdipsic. Moreover, most ad lib. drinking occurred at night while drinking during the day, as measured over a 10-h period, was equivalent across lesioned and sham groups. Note that since drinking challenge tests were conducted in the middle of the light period when little or no drinking during baseline tests was observed, it is unlikely that preexisting overhydration could account for reduced drinking responses to challenges in deficit rats.

**Lesion size was a better predictor of the amount of ad lib. drinking than response to thirst challenges.** When lesion-deficit rats are grouped on the basis of number of electrode penetrations, rats with two or more electrode penetrations, and consequently larger lesions as confirmed histologically, had daily ad lib. water intakes \( (71.9 \pm 4.6 \) ml) significantly \( (P < 0.001) \) greater than sham-lesioned rats, while lesioned rats with single electrode penetrations and smaller lesions had ad lib. water intakes \( (49.7 \pm 5.36 \) ml) that did not differ from intakes of sham-lesioned rats.

**Blood measures.** Blood samples were taken from six sham-lesioned, six ND, and six lesion-deficit rats 3 mo postlesion. Of the six lesion-deficit rats, three were DD and three were HTSD. Plasma protein concentration, osmolality, and sodium concentration are presented in Fig. 2 for sham-lesioned and lesion-deficit rats. ND rats did not differ from shams on any of these measures. Lesion-deficit rats, on the other hand, had significantly elevated plasma osmolality and sodium, but normal plasma protein concentration, when compared to shams. Plams urea nitrogen values also did not differ between groups (sham, 23.0 \pm 0.83 mg/100 ml; lesion-deficit, 23.6 \pm 1.36; 5 rats in each group).

**Histology of AV3V lesions.** Histological analysis of brain tissue revealed that the common extent of lesions

---

**TABLE 1. Feeding and drinking responses (1-h test) after 24-h deprivation for sham- and AV3V-lesioned rats**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>ND</th>
<th>DD</th>
<th>HTSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food deprivation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Feeding, g</td>
<td>6.7</td>
<td>6.6</td>
<td>6.4</td>
<td>5.0</td>
</tr>
<tr>
<td>Water deprivation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>18</td>
<td>16</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>Drinking, ml</td>
<td>11.9</td>
<td>12.8</td>
<td>6.8</td>
<td>8.4</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. * \( P < 0.02 \).
in rats with persisting deficits in drinking responses to AII and hypertonic NaCl is located below the anterior commissure, with bilateral destruction of periventricular tissue from the lamina terminalis through preoptic and anterior hypothalamic levels. Some lesions extended laterally as far as 1 mm from the midline; however, most lesions were localized within 0.5 mm of the midline. Thus, AV3V periventricular strata were consistently ablated while damage to the laterally adjacent nuclear groups, medial preoptic and anterior hypothalamus, was often confined to the most medial aspects of these areas. Figure 3 contains representative coronal sections from lesion-DD rats at the widest lateral extent of the lesion, Fig. 3A shows a lesion of wide lateral extent, while Fig. 3B shows a lesion with a more restricted lateral extent.

There is no gross difference in lesion placement for double-deficit versus hypertonic NaCl only-deficit rats, although the extent of periventricular damage appeared more limited in HTSD rats. Figure 4 shows a horizontal section and midsagittal section at the widest extent of the lesion in double-deficit rats. Figure 5 shows a coronal and midsagittal section from HTSD rats at the widest extent of the lesion in those planes.

In deficit animals, portions of the anterior commissure, optic chiasm, suprachiasmatic, septal, and periventricular nuclei were sometimes, but not consistently damaged. Other periventricular structures, including median eminence and subfornical organ, sustained no lesion damage; other areas previously implicated in the mediation of thirst behavior (13), lateral preoptic area, lateral hypothalamic area, ventromedial hypothalamus, zona incerta, and suprachiasmatic nuclei, also sustained no lesion damage. Lesions in ND animals typically spared a substantial portion of AV3V periventricular tissue, with lesions either being centered anterior to the lamina terminalis or being asymmetrical with respect to the midline so that one lateral wall of the AV3V remained intact.

**DISCUSSION**

The results reported here demonstrate that AV3V tissue is essential for the expression of regulatory drinking behavior. Lesions damaging the preoptic-anterior hypothalamic periventricular substrates result in adipsia; those animals that regain ad lib. water drinking after several days of adipsia exhibit a persistent lack of drinking in response to AII and hypertonic NaCl thirst challenges. This failure to respond to these challenges in animals that have resumed ad lib. drinking and that show no deficit in drinking to a hypovolemic challenge is interpreted as reflecting a specific disruption in AII and osmotic controls of drinking. Associated with this impairment in drinking to AII and osmotic challenges are depressed drinking responses to water deprivation and elevated plasma osmolality and sodium concentration despite the return of ad lib. drinking in amounts equal to or greater than intakes of sham-lesioned animals.

The behavioral deficits in fluid regulatory capabilities of rats with AV3V periventricular lesions are not representative of a global motivational deficit or general debilitation since ad lib. feeding responses during adipsia are comparable to water-deprived controls (16) and after recovery from adipsia, deprivation induced feeding is normal. Nor is the ability to engage in the motor act of drinking impaired since animals drink palatable sweet solutions when drinking of water alone is not evident during postlesion adipsia; it is also unlikely that failure to drink to AII and hypertonic NaCl thirst challenges represents a motor problem since ad lib. drinking has resumed. Moreover, temperature (16), blood pressure (5), posture, locomotion, and emotionality are not affected by the lesion. Thus, AV3V lesion-induced deficits are specific to thirst behavior rather than behavior in general.

Given the residual deficits in AII and osmotic controls of drinking in rats with AV3V lesions, it is interesting to consider the nature both of the initial adipsia and the subsequent recovery of ad lib. water intake. Although ad lib. water drinking is considered by some to be secondary, i.e., not based on any apparent internal need for water (13), our results tempt the speculation that AII and osmotic stimuli reflecting the hydration state of body fluid compartments normally play a key role in maintaining ad lib. drinking. Thus, with the disruption of AII and osmotic controls of drinking after AV3V lesions, ad lib. water intake ceases while other behaviors, such as feeding, continue.

Some adipsic animals never regain ad lib. water intake and die, while others resume drinking after several
**FIG. 3.** Coronal sections at the widest lateral extent of AV3V lesions in rats with drinking deficits to AII and hypertonic NaCl thirst challenges. *A* shows a lesion with a wide lateral extent, while *B* shows a lesion with a more restricted lateral extent. Lesioned area is indicated with an arrow. Abbreviations: OC = optic chiasm, AC = anterior commissure, F = fornix, mp = medial preoptic, ah = anterior hypothalamus, sfo = subfornical organ.

**FIG. 4.** *A* shows an AV3V lesion in a horizontal section below the level of the anterior commissure; *B* shows an AV3V lesion in a midsagittal section. These lesions produced drinking response deficits to both AII and hypertonic NaCl thirst challenges. Arrows indicate the lesioned area. Abbreviations: OC = optic chiasm, AC = anterior commissure, 3V = third ventricle, IR = infundibular recess, mp = medial preoptic, ah = anterior hypothalamus, pav = paraventricular nucleus.

**FIG. 5.** These sections are from rats with AV3V lesion-induced drinking deficits to hypertonic NaCl but not AII. *A* is coronal and *B* is midsagittal showing AV3V lesions at the widest extent in these planes. Note that these lesions in HTS-rats are somewhat smaller than those in DD rats shown in Figs. 3 and 4. Lesioned area is indicated with an arrow. Abbreviations: OC = optic chiasm, AC = anterior commissure, IR = infundibular recess, mp = medial preoptic, pav = paraventricular nucleus, sfo = subfornical organ, ep = ependyma of third ventricle.
days of adipsia and survive. What events mediate the recovery from initial adipsia? Although it is possible that some residual functional capability in AI1 and osmotic controls of drinking remains after the lesion to mediate recovery of drinking, this seems unlikely when one considers the virtually total refractoriness of recovered lesioned animals to the AI1 and osmotic thirst challenges. Since lesioned rats that have resumed ad lib. drinking will respond to a hypovolemic challenge with increased drinking, it is possible that the volemic control of drinking, presumably baroreceptor mediated and independent of angiotensin mediation (25), may be responsible for the resumption of ad lib. drinking. On the other hand, if volemic control underlies recovery from adipsia, one wonders why adipsia occurred at all. It could be that volemic control was temporarily disrupted but not permanently damaged by the lesion or, alternately, it may reflect on the nature of volemic control, particularly the hypothesized nervous control by peripheral baroreceptors without benefit of angiotensin's dipsogenic mediation, as an emergency mechanism with a high threshold of activation reached only after severe dehydration.

Blood measures during the adipsic period and after recovery of ad lib. drinking are compatible with the hypothesis that recovery from adipsia is mediated by volemic control. During adipsia, plasma sodium, osmolality, and protein concentration are significantly elevated, indicating reductions in both intracellular and intravascular fluid spaces (16). After recovery of ad lib. drinking, plasma protein concentration is normal while plasma osmolality and sodium remain elevated, suggesting that intravascular fluid volume has returned to normal while intracellular fluid volume remains depressed.

Another possible basis for recovery from adipsia involves secondary cues that do not precisely correlate with an internal need for water (i.e., nonhomeostatic) (13). Among these, feeding-associated or prandial drinking does not seem crucial since recovered lesioned rats have ad lib. water intakes at least as great as sham controls whether or not they have access to dry chow. It is apparent that the actual causes of AV3V lesion-induced adipsia and subsequent recovery of ad lib. drinking remain an important unsettled problem requiring further investigation.

The effects of AV3V lesions on fluid regulatory capabilities reported here define more precisely the locus of neural tissue necessary for drinking behavior within a larger, less anatomically specific region previously implicated by adipsic syndromes in dog (32) and goat (3). The specific nuclear groups consistently ablated by effective AV3V lesions include the preoptic periventricular nucleus with its extension into the anterior hypothalamic region and the median preoptic nucleus which lies below the anterior commissure immediately superior to the AV3V. As pointed out by Christ (10), the preoptic-hypothalamic region is a continuum, with division into preoptic and hypothalamic groups arbitrary, so the AV3V lesion may best be described as an ablation of periventricular tissue of the third ventricle at the level of the supraoptic recess. This includes the ventral portion of the anterior wall of the third ventricle with the associated organum vasculosum of the lamina terminalis and the ependymal walls and adjacent periventricular tissue extending posteriorly to the level of the paraventricular nucleus. Periventricular damage never extended posteriorly to the arcuate nucleus–median eminence region, or dorsally to subfornical organ. The paraventricular nucleus was not consistently damaged. In a lateral dimension, medial preoptic nuclei and the posterior extension into anterior hypothalamic nuclei were occasionally damaged on their medial border by lesions, but total destruction to these nuclei is not necessary for the reported effects.

In assessing lesion effects, it is important to distinguish between destruction of cellular processes originating in the lesion site versus destruction of pathways coursing through the lesioned area but with a remote cellular origin. Although AV3V lesions destroyed little tissue beyond the anterior periventricular substrates, afferents from the preoptic regions, fornix, and stria terminalis are acknowledged to enter this region (26). Based on the lesion data alone, it would not be possible to exclude these afferents in favor of intrinsic AV3V cells as mediators of drinking behavior. However, Buggy (6, 7) has previously reported that drinking is rapidly induced by direct microinjection of AI1 or hyperosmotic solutions into the AV3V of sated rats; the low threshold for inducing drinking by AV3V injection (0.1 ng for AI1 and 0.40 osmol for hyperosmotic solutions) indicates exceptional sensitivity. Other investigators have also elicited drinking by injections of hyperosmotic solutions (2, 21) or AI1 (2, 15, 17, 27) into or immediately adjacent to AV3V. Furthermore, in studies where ventricular spread of intracerebral injections of AI1 was controlled by ventricular plugs, drinking occurred to intracerebral AI1 injections only when the peptide reached AV3V periventricular tissue (8, 9, 15). When these ventricular access studies (8, 9, 15) and the studies demonstrating sensitivity of the AV3V region to AI1 and hyperosmotic stimuli (2, 6, 7, 15, 21, 27) are considered along with the results of AV3V lesions reported here, the hypothesis that cellular processes in AV3V periventricular tissue mediate drinking by functioning as dipsogenic receptors for AI1 and hyperosmotic stimuli becomes more compelling.

Also compatible with the hypothesis localizing receptors mediating drinking to AI1 and hyperosmotic stimuli within the AV3V periventricular substrate are the diverse cell types found in AV3V ependymal and periventricular cell layers. These cells, which include tanyocytes, neurosecretory cells, cells in extra blood-brain-barrier regions (i.e., organum vasculosum of the lamina terminalis), as well as typical neurons provide intimate interconnection of brain, blood, and cerebrospinal fluid with structural capability for hormone reception, secretion, and/or transport (19, 22, 23, 31).

While this analysis places dipsogenic receptors for both AI1 and hyperosmolarity within the same anatomical locus, it does not necessarily support the hypothesis (2) that these dipsogenic stimuli act via a common mechanism, namely an increase in intracellular sodium concentration in key monitor neurons. Rather, the re-
results on lesioned animals (HTSD) that retain drinking responses to AI1 but not hypertonic NaCl injections argue for separate mechanisms. While a significant portion of the populations of receptors sensitive to AI1 and hyperosmotic stimuli may be codistributed in the AV3V, the data suggest that the receptors for each stimulus are independent and separate. Some AV3V lesions might then be expected to have a greater effect on one of the receptor populations than another. Thus, the AV3V-lesioned rats with drinking deficits to hypertonic NaCl but not AI1 may represent cases where receptors for hyperosmotic stimuli sustained sufficient damage to render that system nonfunctional while some portion of all dipogenic receptors survived lesion damage to continue mediation of drinking to angiotensin.

Impaired ADH secretion and hypernatremia without supraoptic or neurohypophysial damage have been described in several recent reports following extensive periventricular lesions in goats (3) or large medial preoptic lesions which probably also destroyed preoptic-anterior hypothalamic periventricular tissue in rats (4, 29). Aside from disruptions of thirst systems, AV3V lesions described in the present study also result in increased urinary water loss and failure to concentrate urine during adipsia (16) and both acute and persistent elevation of plasma osmolality and sodium. Although ADH secretion was not directly measured, these results are consistent with impaired responsiveness of the ADII release system and suggest that the critical neural damage in the above reports may have involved AV3V periventricular tissue or efferents from it.

Lesions limited to AV3V periventricular substrates also reproduce a syndrome of chronic hypernatremia, hypo- or adipsia, and impaired ADH release described in the human clinical literature. This syndrome is characterized by ADH release insufficiency plus inadequate or absent thirst sensation resulting in hypernatremia and dehydration. It should be pointed out that ADH insufficiency alone (as in diabetes insipidus where ADH secretion is virtually absent) does not lead to hypernatremia since when thirst is present increased water intake compensates for increased urinary water loss (24). Rather, lack of thirst sensation in the face of dehydration is necessary for sustained clinical hypernatremia. This syndrome is usually associated with gross hypothalamic damage with anterior pituitary dysfunction (20) but sometimes the neural damage is most conspicuous in periventricular tissue (14, 28); certainly, the clinical literature is compatible with the interpretation that hypernatremia is due to damage to AV3V or its efferents, since AV3V lesions are associated with impaired drinking response to AI1 and osmotic stimuli and impaired regulation of urinary water loss which result in hypernatremia.

The significance of this study is the demonstration that discrete AV3V periventricular lesions result in impaired drinking responses to AI1 and hyperosmotic thirst stimuli, and that this lack of response may be responsible for wide-ranging disturbances in behavioral control of fluid balance as reflected by acute postlesion adipsia, decreased drinking response to water deprivation, and increased plasma osmolality and sodium. It is proposed that these lesion-induced effects are due to destruction of dipogenic receptors and/or integrative systems monitoring fluid-borne AI1 and hyperosmotic stimuli. The more limited anatomical specification of central tissue required for mediation of drinking behavior also provides information needed to assess what role periventricular preoptic-hypothalamic mechanisms sensitive to osmotic and AI1 stimuli play in other aspects of body fluid regulation besides thirst, such as control of ADH secretion and regulation of blood pressure and volume. Our studies demonstrating that AV3V lesions prevent development of renal hypertension (5) suggests that preoptic-hypothalamic periventricular tissue does play a key integrative role in both behavioral and physiological aspects of body fluid homeostasis.

The authors thank M. Housh, S. Boutelle, D. Bert, and W. Packwood for expert technical assistance and Drs. M. Brody, G. Fink, and I. Phillips for advice and colleagueship. This research was supported by grants from the Iowa Medical Research Council, the National Institute of Mental Health (MH-26571-01, R03 MSM and Research Scientist Development Award 1-K02-MH0064-01), and the National Science Foundation (DNS-75-16346).

Present address of J. Buggy: Dept. of Physiology, University of South Carolina School of Medicine, Columbia, S.C. 29208

Received for publication 13 September 1976.

REFERENCES

12. FITZSIMONS, J. T. The renin-angiotensin system in the control of drinking. In: The Hypothalamus, edited by L. Martini, M.