Periventricular preoptic-hypothalamus is vital for thirst and normal water economy

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

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METHODS

Subjects
Male albino rats (Sprague-Dawley derived; Bio Lab Corp., St. Paul, Minn.) weighing 290–410 g at the time of surgery were individually housed in metabolic cages (20.1 cm x 25.1 cm x 16.8 cm; Acme Metal Products). Both Teklad powdered mouse and rat diet (4% fat) and water contained in graduated cylinders and delivered to the cages by metal drinking spouts were available ad libitum except as noted. Lighting in the colony room was programmed to turn on at 800 h and off at 2000 h. Ambient temperature was maintained at approximately 22°C.

Surgery
All surgery was carried out under ether anesthesia (Mallinckrodt). Rats were mounted in a Kopf small animal stereotaxic instrument, the scalp was incised, and the fascia was cleared. With the skull leveled between bregma and lambda, a 2 mm hole was trephined in the skull to permit insertion of a lesioning electrode after incision of the dura. Electrodes were constructed of Nichrome wire insulated with Beldena. baroreceptors; these putative stimuli are considered to act directly on the brain. Based on experiments in which the osmolality, sodium concentration, or ADH concentration in cerebrospinal fluid (CSF) was increased by injection into lateral or third cerebral ventricles, a periventricular site of action has been suggested for these humoral stimuli to effect increased ADH release and thirst. (1, 16, 18). Since other experiments employing compartmentalized (7, 8, 15, 17) or local (1, 5, 22) ventricular stimulation implicated preoptic hypothalamic periventricular sites of action for AV3V. resulted in a marked adipsic syndrome. The following report characterizes the acute postlesion effects produced by ablation of the AV3V region on ingestive behavior and renal water conservation.

WATER BALANCE is the result of coordinated controls of water input and water output. Although some water gain and loss is insensible, the major control of fluid homeostasis is achieved through thirst mechanisms which mediate water intake and through modulation of antidiuretic hormone (ADH) release from the posterior pituitary and subsequent water conservation by the kidney. The site of integration for the elaboration of thirst and the control of ADH secretion is the central nervous system and in particular the preoptic-hypothalamic region. For thirst and ADH mechanisms to maintain water balance, it is necessary that they respond to stimuli which closely reflect the current hydration status of the organism. Increased fluid osmolality (26) or sodium concentration (1) have been proposed as the relevant stimuli reflecting hydration of the intracellular compartment. Angiotensin II (4, 12) and low-pressure baroreceptors (13, 14) have been hypothesized to provide input regarding the state of extracellular volume.

With the exception of nervous input from peripheral baroreceptors, these putative stimuli are considered to act directly on the brain. Based on experiments in which the osmolality, sodium concentration, or ADH concentration in cerebrospinal fluid (CSF) was increased by injection into lateral or third cerebral ventricles, a periventricular site of action has been suggested for these humoral stimuli to effect increased ADH release and thirst. (1, 16, 18). Since other experiments employing compartmentalized (7, 8, 15, 17) or local (1, 5, 22) ventricular stimulation implicated preoptic hypothalamic periventricular sites of action for AV3V. resulted in a marked adipsic syndrome. The following report characterizes the acute postlesion effects produced by ablation of the AV3V region on ingestive behavior and renal water conservation.

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Coated electrodes were approximately 24 gauge and 0.5 mm of insulation was removed at the beveled tip. Lesions of the AV3V region were produced by lowering the electrode on the midline 0.0-0.5 mm caudal to bregma to a depth of 7.2-7.8 mm from the dura and an anodal lesion (rectal cathode) was produced (Stoelting lesioning device) with current parameters of 3 mA for 20 or 25 s duration. In some cases, multiple penetrations were made. Control lesions (3 mA x 25 s duration) placed on the midline at a thalamic site remote from the third ventricle were made through electrodes located 2.8 mm caudal to bregma and 5.1 mm ventral to the dura. Other control animals received sham-lesioning treatments in which all of the above procedures were followed except that the electrode was lowered to a depth of 5.6 mm below the dura and no current passed. As in the case of lesioned animals, multiple penetrations were made in some cases. Surgery was concluded by closing the scalp with wound clips. All animals received an intramuscular prophylactic dose of penicillin G (Pfizerpan AS, 60,000 U/rat).

Procedure

Experiment 1. Rats were adapted to metabolic cages for several days. Prior to surgery, body weight, 2 days of base-line food intake (g), and water intake (ml) were recorded. Then surgery was carried out (sham lesions, n = 7; control lesions, i.e., nonperiventricular, n = 3; AV3V lesions, n = 11) between 1000 and 1400 h with sham and lesion operations randomly distributed over the time period. Immediately prior to anesthesia, colonic temperature ± 0.2°F was measured for each animal. After surgery, each animal was returned to its cage and daily body weight, food intake, and water measures resumed and continued for the next 10 days. On days 1 and 3, postsurgery colonic temperatures were determined for all animals.

Experiment 2. Animals were adapted for several days to metabolic cages. After this period, daily determinations of body weight, food intake, water intake, and urine volume were made. Urine was collected under paraffin oil and a sample of each 24-h collection of urine was frozen and retained for subsequent analysis.

Three experimental treatment groups were formed from animals based on the type of surgery performed and the availability of water. The first group of animals, sham lesion–water access group, received a sham lesion (n = 10) (i.e., lowered electrode) and was given ad libitum access to water following surgery. The second group (n = 11), sham lesion–no water access, was comprised of animals that underwent the sham lesion procedure but received no water following surgery. The final group, designated as the lesioned group (n = 28), consisted of animals with lesions aimed to ablate tissue in the AV3V region; water was always available for this group.

For 3 days after surgery, body weight, food and water intakes, and urine volumes were measured and urine samples were saved for subsequent analysis. Three days after surgery, lesioned animals that had 3 days of postlesion adipsia, sham lesion–water access animals, and sham lesion–no water access animals were etherized, laparotomy was performed, and approximately 5 ml of blood for analysis were collected from the inferior vena cava into a heparinized hypodermic syringe. After transcardial perfusion with physiological saline followed by formaldehyde-saline solution, brains were removed for histological analysis.

Blood and Urine Analysis

Plasma proteins were determined by refractometer, plasma and urine osmolalities were determined by freezing point depression (Osmette A, Precision Systems, Inc.), plasma sodium and potassium were determined by flame photometry (IL model 143), and plasma urea nitrogen was measured by an automated colorimetric method (diacetyl monoxime reaction). The highest urea nitrogen value possible with the calibration employed was 100 mg/100 ml. Any reading greater than 100 mg/100 ml was recorded as 100 mg/ml.

Histological Analysis

Rats that survived to the end of the postsurgery observation periods were perfused transcardially with 0.9% saline followed by 10% formaldehyde solution in 0.9% saline. The brain was taken out of the skull and stored for sectioning in formaldehyde-saline solution. Animals that died in the course of the experiment were decapitated and the soft tissue of the head was removed. The calvarium was then stored for several days in formaldehyde-saline before the brain was removed and prepared for sectioning. Frozen sections (40 μm) were cut and the tissue mounted and stained with cresyl violet, Weil, or a luxol fast blue–cresyl violet stain. The extent of the brain damage was then assessed by light microscopy.

Statistical Analysis

Overall statistical analysis was conducted on dependent variables by analysis of variance. In follow-up analyses, t-tests for independent or correlated observations were carried out; group differences with probability of 0.05 or less were considered significant.

RESULTS

Ingestive Behavior

The primary and most profound effect of the destruction of tissue surrounding the AV3V was an immediate cessation of water intake in the majority of lesioned animals. Shown in Fig. 1 are the results from experiment 1 for every animal with AV3V lesions, the sham-lesioned animal showing the most severe postlesion effects on water intake, and representative control-lesioned and sham-lesioned animals. Ten of eleven animals with AV3V region lesions had less than 5 ml of water intake on 1 or more postlesion days (range 1–6 days). No sham- or control-lesion animal was adipsic on any day postsurgery.

In experiment 2, the lesioned group drank significantly less water each day postsurgery than sham-
FIG. 1. Water intakes, food consumption, body weights, and colonic temperatures, taken over the period of 2 days prior to and 10 days following surgery. Four panels in upper left present the daily measures for 4 control animals and remaining 11 panels the same measures for all animals with lesions in the anterior ventral third ventricle (AV3V) region in the analysis. Rat P-11 was the sham-lesioned animal showing the most severe postsurgery attenuation of water intake within that group. Rats P-2 and 10 were representative sham-lesioned animals and rat P-16 was one of three control-lesioned rats (25-s 3-mA current) all of which showed slight and comparable postlesion effects. Panels displaying measures for animals with lesions in the AV3V region are arranged according to severity of effects of lesions. Days on which animals were alive and on which there was no intake of food or of water are indicated with a 0 on abscissa. Daily food or water intakes which exceeded the limits of the scale on ordinate are indicated at top of appropriate bars.
lesioned rats with access to water. Daily water intakes ± SEM for the 28 rats with lesions in the AV3V region that survived the 3 postoperative days (2 animals died following surgery) were 3.1 ± 1.3, 15.6 ± 3.8, and 25.3 ± 4.4, respectively, for 3 postlesion observation days. For the sake of subsequent analyses on other measures, lesioned animals were classified as adipsic if they drank 10 ml or less per day (10 ml = 6 standard deviations below the mean prelesion daily intake for all animals) and were assigned to subgroups depending on whether they had shown 0, 1, 2, or 3 consecutive days of adipsia following surgery (as in Fig. 2).

In experiment 1, food intake was reduced relatively more in animals with lesions as compared to control-lesioned and sham-lesioned animals. However, this reduction was not as great as the reduction in water intake (see Fig. 1). Nine of ten animals with at least 1 day of adipsia after AV3V lesions showed some food intake while failing to drink. As can be seen from Fig. 1, there was a general tendency for food intake to decline over days as hypodipsia was prolonged.

Shown in Fig. 2 is the food intake for sham lesion–water access, sham lesion–no water access, and lesioned adipsic animals with 1, 2, and 3 consecutive days of adipsia. Both sham lesion–no water access and lesioned adipsic groups showed significantly less food intake as compared to sham lesion–water access animals. However, feeding was comparable in the sham lesion–no water access group and lesioned adipsic groups on each postsurgery day.

Urine Analysis

The urine analysis in experiment 2 indicates a significant effect on urine volume in adipsic animals with AV3V lesions (see Fig. 3). As expected, sham lesion–no water access rats lost significantly less urine each day than the sham lesion–water access group. However, adipsic lesioned rats did not significantly reduce urine output the day after surgery compared to sham lesion–water access rats; on subsequent days lesioned animals did significantly decrease urine output. This later decrease in urine elaboration in adipsic lesioned rats may represent less body water available for renal filtering combined with impaired renal function rather than physiological water conservation (see urine osmolality results below and body fluid analysis results). Compared to sham lesion–no water access rats, adipsic lesioned rats lost significantly more urine on the first 2 days after surgery. Lesioned animals that were adipsic for 3 days lost a total of 24.6 ± 2.51 ml as compared to a total of 13.5 ± 1.79 ml lost by sham lesion–no water access animals (t (18) = 3.7548, P < 0.01). Even taking into account the slight residual water intake of the lesioned animals classified as adipsic (about 1 ml/day), it is apparent that AV3V lesions produce a severe enuresis.

The effects of the treatment conditions on urine osmolality were consistent with the observed changes in urine volume. As shown in Fig. 3, sham lesion–no water access animals had an increasing urine concentration over the 3 days of water deprivation, with the urine osmolality on day 3 significantly exceeding that in sham lesion–water access rats. Adipsic lesioned rats did not manifest a similar increase in urine osmolality; urine osmolality of sham lesion–no water access rats significantly exceeded that of adipsic lesioned rats each day postsurgery. Moreover, urine osmolality of adipsic lesioned rats was actually significantly decreased compared to sham lesion–water access rats on the 1st and 3rd days postsurgery. Thus, while urine volume decreased over days in adipsic lesioned rats, there was not a corresponding increase in urine osmolality.
Body Fluid Analysis

The failure by adipsic lesioned animals to show an appropriate antidiuresis is reflected in the blood measurements of experiment 2 taken 3 days after surgery (Fig. 4). Overall statistical analysis showed a significant treatment effect on plasma protein concentration, plasma osmolality, plasma sodium concentration, and plasma urea nitrogen concentration. There was no significant effect on plasma potassium concentration or plasma osmolality, plasma sodium concentration, and plasma urea nitrogen concentration. There was no significant treatment effect on plasma protein concentration, although there was a tendency for higher values in the lesioned animals that were adipsic for 3 days on which urea nitrogen determinations were made. Values that exceeded the maximum standard (i.e., greater than 100 mg/100 ml) used in the assay. The gross state of dehydration in lesioned animals that were adipsic for 3 days postsurgery was therefore reflected in both cellular and extracellular compartments.

Behavioral and General Effects

On the days immediately after surgery, the demeanor of lesioned animals from both experiments 1 and 2 was indistinguishable from sham- or control-lesioned animals. The lesioned animals groomed well and showed no detectable abnormalities in motor control or posture. All groups of animals in both experiments were comparable in their response to handling.

In experiment 1, AV3V lesions resulted in a significantly reduced body weight compared to the control groups (see Fig. 1). In experiment 2, both sham lesion-no water access and lesioned adipsic groups lost more weight than sham lesioned rats with water access. However, the weight loss (g ± SE) over the 3-day observation period after surgery is significantly greater for lesioned animals adipsic on 3 consecutive days (84 ± 5) compared to sham-lesioned rats water deprived for 3 days (60 ± 3). Some of the increased weight loss of lesioned animals can be accounted for by a greater urine excretion.

In cases of sustained adipsia, lesioned animals would eventually develop signs of motor dysfunction, tremor, and finally ataxia. The transition from a normal appearance (other than signs of dehydration) and vigorous behavioral state to a moribund condition sometimes occurred very rapidly, often within the course of a day or overnight. Animals that reached this terminal condition sometimes would remain in this state for 1 or 2 days prior to death. In experiment 1, the mean day of death for animals that did not resume drinking after lesioning was 5 days. There was no reliable effect of lesions on body temperature (F (2, 16) = 0.4785) (see Fig. 1).

Histological Analysis

The results of histological examination of brains with intended lesions of the AV3V region indicate that these animals sustained damage to tissue immediately surrounding the anterior ventral third ventricle. Lesions were located in periventricular tissue between the anterior commissure and optic chiasm encompassing the lamina terminalis and the organum vasculosum and extending posteriorly to the preoptic periventricular area, the median preoptic nucleus, and the anterior hypothalamic periventricular area (anatomic structures follow the descriptive nomenclature of Christ (10)). The lesions were confined close to the midline and tended to be more extensive in the rostral-caudal as compared to the mediolateral plane. The medial preoptic nuclei, anterior hypothalamic nuclei, and paraventricular nuclei were largely intact and usually sustained little or no apparent damage beyond their periventricular borders. In no case did the ablation of the periventricular tissue reach the level of the supraoptic nuclei, suprachiasmatic nuclei, or the ventromedial nuclei of the hypothalamus or impinge upon the median eminence or the subfornical organ.

One observation noted in lesioned animals that died after several days of adipsia is that regions quite remote from the AV3V had numerous hemorrhages and brain lesions. These appeared for the most part throughout the brain with no consistent pattern or localization. In lesioned animals that recovered drinking, no such hemorrhages were detected. Figure 5A illustrates brain hemorrhages and the lesion in a midsagittal section from rat P-7 (expt 1) which did not recover drinking and died 8 days after lesioning. Shown in Fig. 5, B-D, are sections from three animals from experiment 2 which were adipsic for 3 days and had representative lesions of the AV3V region.

DISCUSSION

The present analyses demonstrate that the destruction of periventricular tissue which surrounds the AV3V produces a primary adipsia and an inappropriate urinary water loss in the face of severe dehydration. One of the most striking aspects of this phenomenon is that the initial adipsia occurs in animals which appear remarkably normal. Behaviorally they are alert, well
In our early observations of adipsic animals with AV3V lesions we noted that many rats died a relatively short time after the lesion. Subjects in experiment 1 with this lesion are representative in that respect since several of the adipsic animals died 2-9 days after the surgery. Since normal rats have been shown to survive up to approximately 2 wk of water deprivation (3) it seemed unlikely that cessation of water intake alone or even adipsia plus general surgical trauma could account for the early death in adipsic lesioned animals. It was the discrepancy in survival time between intact, water-deprived rats and AV3V-lesioned adipsic animals that prompted our experimental consideration of renal water loss after the lesion. It is now apparent that the marked elevation of plasma solute concentration due to a lesion-induced enuresis is probably the primary factor contributing to death after surgery. The major pathology produced by blood hyperosmolality has been shown to be hemorrhagic encephalopathy (24). The brains from moribund adipsic animals were found to have hemorrhagic damage distributed outside of the primary lesion area.

groomed, and respond normally to handling. Following the lesion, feeding behavior is sustained for a significant period after the onset of adipsia and is equivalent to that seen in intact water-deprived animals. The fact that animals continue to eat dry food while they are adipsic is evidence that the failure to ingest water is not the result of nonspecific motivational or motor disruption produced by the lesion. In other experiments, lesioned adipsic animals given access to a liquid diet or to saccharin solution consumed them avidly. Since the motor patterns involved in the ingestion of these highly palatable liquids are virtually identical to those used for water intake, animals rendered adipsic to water by periventricular lesion apparently are not failing to drink because of motor incapacitation. Also, adipsia produced by lesions in the AV3V region does not appear to be secondary to some more global type of physiological disruption. In the present report, it was shown that body temperature was not consistently altered by the AV3V lesion. There is also no correlation of blood pressure with water intake immediately following AV3V periventricular destruction (6).
Some rats will regain spontaneous drinking following a substantial period of adipsia. The mechanism of the reacquisition of drinking after adipsia poses an interesting recovery of function problem. From the present findings it is impossible to specify whether the reappearance of the water intake is tantamount to traumatized tissue in the vicinity of the lesion regaining functional capacity (e.g., reduction of postlesion edema), to a functional reorganization of remaining neural elements, or to activation of other thirst mechanisms.

The adipsia and the reduction in antidiuresis after AV3V destruction suggests that this region contains elements which function to control drinking behavior and ADH secretion. A precise definition of the nature of these cells is not possible from lesion analyses per se. In particular, a distinction between receptors and fibers of these cells is not possible from lesion analyses per se. CSF-borne findings are relevant to this question. CSF-borne elements which function to control drinking behavior and ADH secretion. A precise definition of the nature of these cells is not possible from lesion analyses per se.

There are several experimental (2, 9, 19, 25, 27) and clinical (20, 24) reports of reduced or absent thirst and attenuated antidiuresis produced by lesions that surround or encroach on the AV3V. The present work shows that lesions sufficient to produce adipsia and failure of renal water conservation severe enough to result in death from dehydration do not have to invade regions remote from tissue immediately around the AV3V. The critical elements appear to be distributed very close to the ventricle, i.e., within one or all of the following structures: the lamina terminalis and associated organum vasculosum, the periventricular nuclei at the preoptic-anterior hypothalamic level, the median preoptic nucleus, and the medial border of the medial preoptic and anterior hypothalamic nuclei. It is not necessary to damage the anterior commissure, the septal area, supraoptic nucleus, or the subfornical organ to produce the phenomena described here.

The present report established that preoptic anterior hypothalamic periventricular lesions produce acute adipsia and deficits in the antidiuretic response to dehydration, thereby leading to altered water and electrolyte balance. Other investigations have examined the AV3V lesion preparation following the recovery of water intake. The chronic effects of AV3V lesions include: chronic hypernatremia (9), depressed drinking responses to hyperosmotic and angiotensin dipsogenic stimuli (6, 9, 17), attenuated pressor responses in conscious rats to intravenous infusions of angiotensin but not norepinephrine (6), and failure to develop one-kidney renal-or steroid-salt hypertension (6, 11). Taken together these findings suggest that the periventricular tissue surrounding the AV3V is a critical focus or nodal point of neural elements involved in the process of humoral-neuronal integration for regulation of body fluid homeostasis.

The authors thank M. Houss, S. Harfst, and W. Packwood for their technical assistance, Drs. M. J. Brody, G. D. Fink, M. I. Phillips, C. C. Wunder, and W. E. Hoffman for their colleagueship, and Dr. R. Hohle for providing the ANOVA computer program.

This research was supported by the National Institute of Mental Health Grants MH 26571-01 and R03 MSM and Research Scientist Development Award 1 K02 MH006 64-01, the National Science Foundation Grant BNS 75-16546, and the Iowa Medical Research Council.

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

Received for publication 2 May 1977.

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