Specific alterations in sodium chloride intake after hypothalamic lesions in the rat

MIGUEL R. COVIAN AND JOSÉ ANTUNES-RODRIGUES
Department of Physiology, School of Medicine, Ribeirão Preto,
São Paulo, Brazil

Covian, Miguel R., and José Antunes-Rodrigues. Specific alterations in sodium chloride intake after hypothalamic lesions in the rat. Am. J. Physiol. 205(5): 922–926, 1963.—Bilateral electrolytic lesions in the hypothalamus of the rat elicited either a decrease or increase in 2% NaCl intake, without a significant change in water ingestion. Lesions placed in the anterior hypothalamus involving supraoptic or paraventricular nuclei, or both, resulted in a conspicuous fall (as much as 93%) of NaCl intake. The decreased consumption remained to the end of the experiments which in some rats lasted 105 days and was accompanied by a decrease in NaCl urinary output. On the contrary, lesions placed in the central hypothalamus determined a specific increase of NaCl intake together with an augmented urinary excretion. The increased ingestion was permanent and lasted to the end of the experiment, attaining in one rat the value of 2 g/day. To account for these results two provisional explanations are advanced, one of them considering the possible existence of two areas of opposite effects regarding NaCl ingestion and the other claiming a neurohumoral mechanism in which oxytocin and aldosterone could be the two responsible hormones.

Of the main features of animal physiology is the constancy of internal conditions due to homeostatic mechanisms, which makes them, in a certain way, independent of changes of the external surroundings. The reaction of the organism as a whole (e.g., ingestion of solids or liquids) in response to a variety of external influences is an example of self-regulation designed to maintain a functionally stable internal environment; this goal is achieved by way of a neurohumoral action in which the autonomic nervous system and the pituitary-adrenocortical system are considered to be of primary importance.

Of all the neural structures involved in the regulation of food and water intake, the hypothalamus has been proved to be of paramount importance. Fisher, Ingram, and Ransom (16) called attention to the role played by the hypothalamus in the regulation of water metabolism. Andersson and McCann (2, 3) and Andersson and colleagues (1) reported for goats an increase and decrease of water intake by hypothalamic influence; Montemurro and Stevenson (15) and Smith and McCann (19) observed adipsia in rats due to hypothalamic lesions regarding liquid intake. All these publications deal with alteration in water intake after hypothalamic stimulations or lesions. Little, if anything, is known with respect to the central regulation of sodium chloride intake. As far as endocrine control of salt ingestion is concerned, the studies by Richter (17) and Covian (6) using the self-selection method, showed the role played by the adrenal glands.

In this laboratory systematic studies were undertaken to determine the effects of bilateral localized lesions of the rat hypothalamus on the free ingestion of a 2% NaCl solution and tap water. In a previous communication Antunes-Rodrigues and Covian (4) related the normal variations in the intake of both fluids and those introduced by the technical procedures preceding the local destruction of the hypothalamus. This paper reports the finding of specific alteration in the ingestion of sodium chloride without significant changes in the water intake following the bilateral destruction of certain hypothalamic areas.

MATERIALS AND METHODS

Thirty-one male rats, 200 to 300-g weight, of the Wistar strain were used. They were kept in individual cages with a food cup filled with a dry mixed diet containing 0.5 g/100 g NaCl and two graduated drinking bottles, filled with NaCl, 2%, and tap water, respectively; these were changed at the same time to avoid difference in the staleness of the two liquids. Daily readings of food and fluid intake were made, as well as of the temperature, relative humidity, and atmospheric pressure of the room in which the rats were housed. Urine was collected daily to determine the Na excretion. Body weight was re-
NaCl INTAKE AFTER HYPOTHALAMIC LESIONS

FIG. 1. Daily z2% NaCl (full line) and water (broken line) intake of one rat in which there was a specific diminution of NaCl ingestion. At arrow: day of bilateral lesions in the anterior hypothalamus (F, 7.0; L, 2.0; H, -3.0). Ordinate: fluid intake in ml. Abscissa: time in days.

Fig. 2. Another example of specific diminution of NaCl intake in one rat in which the observation was prolonged for 105 days. Same references as Fig. 1.

RESULTS

Decrease in NaCl intake. This group of 26 rats was characterized by a specific diminution of NaCl intake of immediate or gradual appearance without any noticeable permanent change in water ingestion. Figure 1 is a good example of the results obtained; during the 34 days preceding the operation the NaCl (full line) and water (broken line) consumption showed the cyclical variations already described (4) which did not keep any correlation with changes in temperature, relative humidity, and atmospheric pressure of the laboratory room. After the bilateral lesion was made at coordinates F, 7.0; L, 2.0; H, -3.0 there was a sharp fall of NaCl intake and this remained at a low level for the 58 days of postoperative observation. Considering the mean values of the intake during both periods, the fall was of 93.8%. The ingestion during the last 15 days before the operation was of 16.1 ml. After the operation, considering the ingestion in three periods of 15 days and one of 10 days, the intake was: 0.40 ml; 0.60 ml; 0.63 ml; and 0.55 ml, respectively. As to the water ingestion there was a transient but sharp fall immediately after the lesion which lasted 2 days; afterwards the preoperative level was regained. The intake during the last 15 days of the preoperative period was 23.2 ml. After the operation and during periods similar to that of NaCl, the consumption was: 19.1 ml; 22.6 ml; 22.3 ml; and 22.2 ml. The cyclical variations in the ingestion of both fluids present in all rats of this
corded each week. No female rats were used in order to eliminate the influence of the sexual cycle in fluid consumption (4). A control period of about 4 weeks preceded the placement of bilateral hypothalamic lesions made by electrolysis under ether anesthesia. A stainless steel electrode 0.36 mm thick and insulated to the tip was mounted in a Krieg-Johnson stereotaxic instrument, and a current of 1-2.5 ma for 5-15 sec was applied. Using the coordinates of de Groot's atlas (8) a survey of the whole hypothalamus was made in steps of 1-mm intervals in the following stereotaxic settings: frontal plane, from 7.4 to 3.8 mm; lateral plane, from 0.5 to 2 mm; and horizontal plane, from -1.5 to -4 mm. After the hypothalamic lesions were made, observations were maintained during a period running from 58 to 105 days, long enough to consider that the alterations found were permanent.

When a specific decrease or increase of NaCl intake was obtained, two groups of rats were submitted to the same stereotaxic settings of rats which presented those alterations, in order to test the constancy of their appearance.

The data of the preoperative and postoperative periods of each rat were compared, utilizing the nonparametric statistical median test of Mood (18). At the end of the experiment the rats were anesthetized with ether and perfused with 4% formaldehyde. Their brains were submitted to routine histological procedures, serially sectioned at 12 µ and every sixth section was stained, using Weigert’s technic modified by Erhart (9).
group persisted after placement of bilateral hypothalamic lesions. Figure 2 is another example with similar results, obtained in a rat in which the stereotaxic coordinates were the same as before, but the observation was extended to 105 days. The fall of NaCl consumption was 79.8%. During the last 15 preoperative days the ingestion of NaCl was 12.2 ml; after the operation, considering the ingestion for periods of 15 days, the values were: 6.9 ml; 0.83 ml; 0.43 ml; 0.44 ml; 0.44 ml; 0.54 ml; 0.42 ml. Regarding water, the intake during the 15 days prior to the operation was 25.2 ml and during the periods of 15 days afterwards it was: 19.0 ml; 23.3 ml; 22.3 ml; 22.0 ml; 23.1 ml; 21.6 ml; 21.1 ml.

These results were similar, with individual variations, in the remaining 24 rats. No permanent change was registered in the ingestion of food after the operation. A significant difference ($P < 0.05$) was proved only for NaCl consumption when preoperative and postoperative intake was compared. Urinary Na excretion was diminished in these rats.

**Localization of hypothalamic lesions.** The lesions were intended to be placed in the anterior hypothalamus at the following stereotaxic coordinates: F, 7.0; L, 2.0; H, −3.

The histological examinations revealed that the lesions were located in the anterior hypothalamus. Combining all the lesions made in the rats, and plotting them on a schematic frontal section through the hypothalamus (Fig. 3) it was observed that they extended from the lateral to the medial part, including the supraoptic and paraventricular nuclei and the fornix. In some rats only one of the nuclei was involved while in others both were destroyed on one side (Fig. 4), as happened in the rat whose intake of NaCl and water is shown in Fig. 1.

**Increase in NaCl intake.** In five rats, the hypothalamic electrolytic destruction determined a specific increase in NaCl ingestion with no marked variation in water intake. Figure 5 describes the result obtained in one of the rats; after 14 days of observation, bilateral lesions were placed at coordinates F, 6.5; L, 0.5; H, −3. The NaCl ingestion (full line) showed a sharp increase which was maintained with oscillations to the end of the experiment (60 days). Comparing the ingestion during the preoperative days with that of the postoperative period the increase was 290.2%. Water consumption (broken line) showed a transient drop after the operation but it soon regained the previous level. During the last 15 days before the operation the intake of NaCl was 4.8 ml and afterwards, in the periods of 15 days, it was: 10.8 ml; 19.6 ml; 16.3 ml; 16.1 ml. For water and for the same periods, ingestion was 25.7 ml before the operation and 22.1 ml; 22.1 ml; 22.0 ml; 24.8 ml postoperatively. Only NaCl ingestion showed any significance upon statistical analysis. No change was observed either in the cyclical variations of fluid intake or in food consumption.

In these rats the urinary excretion of Na was increased.

**Localization of hypothalamic lesions.** The histological examination of the hypothalamus showed that the lesions were placed in the central hypothalamus involving, in general, the medial and lateral part. No nuclei were destroyed, except the arcuate nucleus in part in one rat and the premammillary in another. The hypothalamic lesion of the rat whose NaCl and water intake is depicted in Fig. 5 was placed in the central medial hypothalamus.
N&l INTAKE AFTER HYPOTHALAMIC LESIONS

The present investigation is the result of experiments designed to evaluate the effect of bilateral hypothalamic lesions on the free ingestion of sodium chloride and water intake. During the course of the work two areas were found which appeared specifically related to the ingestion of NaCl which have not yet been described. Their destruction determined either decrease or increase in salt ingestion without any significant alteration in water and food consumption. As further evidence of the participation of the hypothalamus in sodium chloride metabolism it is well to remember that Lewy and Gassmann (13) reported a transitory increase of NaCl in the blood together with a decrease in urinary output in rats as a result of unilateral destruction of the supraoptic region, while Keeleer (12) in the same species observed an increase of urinary output following bilateral lesions of the paraventricular nuclei.

To account for the facts reported here two explanations can be advanced, one of them claiming a neural mechanism and the other a neurohumoral regulation. The first hypothesis is based on the idea that there could exist in the hypothalamus an arrangement for NaCl intake similar to that of feeding behavior. Hence the salt-drinking behavior would be the result of the interaction between two opposite areas, one of them driving the animal to drink NaCl, and the other restraining it. The destruction of either one would cause an imbalance between the two areas and therefore cause a change in behavior manifested by an increase or decrease in the intake of salt.

The other working hypothesis is based upon the known fact of the hypothalamo-hypophysis interrelationships. It is very likely that through control of the secretion of oxytocin and aldosterone the hypothalamus plays an important role in hydromineral metabolism. As to the hypothesis in discussion it can be said that Olivecrona (16) found a decrease of the oxytocin stored in the neurohypophysis after bilateral lesions of paraventricular nuclei in the rat; Cross (7) reported that electrical stimulation of either supraoptic or paraventricular nuclei, or stimulation of the tuber cinereum induced an increased contractility of the uterus of rabbits, suggesting an augmented secretion of oxytocin. On the other hand, Brooks and Pickford (5) have shown that in dogs, oxytocin favors the excretion of NaCl. Zehallos et al. (20) have shown that this hormone has a diuretic and natriuretic effect in normal rats bloated with water. Regarding the hypothalamic control of aldosterone secretion, Ganong et al. (11) described in dogs a decrease of its secretion as a result of lesions placed in the median eminence and McCann (14) observed in rats with lesions in the same place an absence of response to stress.

In interpreting our results one must take into consideration that the free intake of NaCl is an index of the need of NaCl by the animal, hence an increased ingestion indicates a deficit of salt and, conversely, a diminished intake relates to sodium retention; this is a clear example of the dynamic autoregulation of the organism by means of alimentary behavior. With all these data at hand one can infer that if our lesions determined a decreased production of oxytocin by the hypothalamus and therefore a diminished urinary output on NaCl, then a decrease in salt intake will be the consequence. In our group of rats this behavior was quite evident, suggesting a retention of salt, and confirmed by a diminished urinary excretion. This type of result could also be obtained by an increased liberation of aldosterone, but the anterior localization of the lesions, including the supraoptic and paraventricular nuclei, supports the idea of oxytocin diminution. Of course, aldosterone cannot be ruled out. The increased ingestion of NaCl together with an augmented excretion

---

**FIG. 5.** Daily 2%/ NaCl (full line) and water (broken line) intake of one rat which showed a specific increase of NaCl ingestion. At arrow: day of bilateral lesion in central medial hypothalamus (F, 6.5; L, 6.5; H, 12). Ordinate: fluid intake in ml. Abscissa: time in days.

**FIG. 6.** Schematic frontal section through hypothalamus of rat whose fluid consumption is shown in Fig. 5 (specific increase of NaCl ingestion). Black area represents regions destroyed in median medial hypothalamus, near the median eminence, destroying part of the arcuate nucleus on both sides (Fig 6)

**Constancy of results.** When the lesions were made on the aforementioned zones, a specific decrease or increase in NaCl ingestion was always obtained. Lesions in other places failed to produce any significant or permanent change in salt intake. On the contrary, specific changes in water consumption were sometimes observed and destruction of some areas resulted in total adipsia. These results will be reported later.

**DISCUSSION**

The present investigation is the result of experiments designed to evaluate the effect of bilateral hypothalamic lesions on the free ingestion of sodium chloride and water intake. During the course of the work two areas were found which appeared specifically related to the ingestion of NaCl which have not yet been described. Their destruction determined either decrease or increase in salt ingestion without any significant alteration in water and food consumption. As further evidence of the participation of the hypothalamus in sodium chloride metabolism it is well to remember that Lewy and Gassmann (13) reported a transitory increase of NaCl in the blood together with a decrease in urinary output in rats as a result of unilateral destruction of the supraoptic region, while Keeleer (12) in the same species observed an increase of urinary output following bilateral lesions of the paraventricular nuclei.

To account for the facts reported here two explanations can be advanced, one of them claiming a neural mechanism and the other a neurohumoral regulation. The first hypothesis is based on the idea that there could exist in the hypothalamus an arrangement for NaCl intake similar to that of feeding behavior. Hence the salt-drinking behavior would be the result of the interaction between two opposite areas, one of them driving the animal to drink NaCl, and the other restraining it. The destruction of either one would cause an imbalance between the two areas and therefore cause a change in behavior manifested by an increase or decrease in the intake of salt.

The other working hypothesis is based upon the known fact of the hypothalamo-hypophysis interrelationships. It is very likely that through control of the secretion of oxytocin and aldosterone the hypothalamus plays an important role in hydromineral metabolism. As to the hypothesis in discussion it can be said that Olivecrona (16) found a decrease of the oxytocin stored in the neurohypophysis after bilateral lesions of paraventricular nuclei in the rat; Cross (7) reported that electrical stimulation of either supraoptic or paraventricular nuclei, or stimulation of the tuber cinereum induced an increased contractility of the uterus of rabbits, suggesting an augmented secretion of oxytocin. On the other hand, Brooks and Pickford (5) have shown that in dogs, oxytocin favors the excretion of NaCl. Zehallos et al. (20) have shown that this hormone has a diuretic and natriuretic effect in normal rats bloated with water. Regarding the hypothalamic control of aldosterone secretion, Ganong et al. (11) described in dogs a decrease of its secretion as a result of lesions placed in the median eminence and McCann (14) observed in rats with lesions in the same place an absence of response to stress.

In interpreting our results one must take into consideration that the free intake of NaCl is an index of the need of NaCl by the animal, hence an increased ingestion indicates a deficit of salt and, conversely, a diminished intake relates to sodium retention; this is a clear example of the dynamic autoregulation of the organism by means of alimentary behavior. With all these data at hand one can infer that if our lesions determined a decreased production of oxytocin by the hypothalamus and therefore a diminished urinary output on NaCl, then a decrease in salt intake will be the consequence. In our group of rats this behavior was quite evident, suggesting a retention of salt, and confirmed by a diminished urinary excretion. This type of result could also be obtained by an increased liberation of aldosterone, but the anterior localization of the lesions, including the supraoptic and paraventricular nuclei, supports the idea of oxytocin diminution. Of course, aldosterone cannot be ruled out. The increased ingestion of NaCl together with an augmented excretion
of Na, which is typical of the second group, can be explained either as a decreased aldosterone or as an increased oxytocin secretion. The localization of the lesions in the central hypothalamus and the aforementioned results of Ganong and McCann favors the idea of aldosterone interference.

We may conclude that hypothalamic lesions have different effects on the intake of NaCl, showing that the hypothalamus participates in controlling the hydro mineral metabolism. The histological studies do not enable us to draw any conclusion as to whether the regions destroyed represent a "center" or a pathway for fibers coming down from structures more cephalically situated. Regarding the mechanism of action it can be one or two, working in parallel, that can be selectively separated by appropriate lesions.

REFERENCES