Increased Drinking of a Nonpreferred NaCl Solution During Food Deprivation in the C3H/HeJ Mouse

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KUTSCHER, C. L. AND H. STEILEN. Increased drinking of a nonpreferred NaCl solution during food deprivation in the C3H/HeJ mouse. PHYSIOL. BEHAV. 10(1) 29-34, 1973.—When food was given ad lib, C3H/HeJ mice showed a progressive decrease in NaCl solution intake as the concentration increased from 0.5-2.5% under single tube or double tube conditions. Under the latter condition, 1.5% NaCl intake volumes were approximately 20% of water intake volumes. However, when restricted to 1/3 normal food intake, the mice showed significant increase in the intake of 1.5% NaCl and several animals consumed more salt solution than water. Salt intakes declined to predeprivation levels upon return to free feeding. Since feeding of a sodium deficient diet produced no alteration in intake of 1.5% NaCl, the increase during food deprivation was attributed to hypovolemia rather than hyponatremia.

Drinking Food deprivation Polydipsia Sodium deficiency Sodium preference

INTAKE of NaCl in rats has been increased by a variety of manipulations including sodium deficient diet [20], adrenalectomy [5, 24], intraperitoneal dialysis against glucose [6, 7], and injections of deoxycorticosterone acetate [23], aldosterone [30], formalin [26, 31] and polyethylene glycol [32]. There is also one report of increased intake of a highly preferred NaCl solution during partial food deprivation [12].

While studying food deprivation polydipsia in adrenalectomized C3H/HeJ mice (hereafter called C3H), the authors found that, contrary to the findings in rats [5, 24], adrenalectomy resulted in only a slight increase in the intake of the 1.5% NaCl solution provided for postoperative maintenance, but partial food deprivation produced a sharp increase in the drinking of this solution. The following experiment was run to verify and describe this phenomenon in intact mice and to attempt to relate it to sodium deficiency. The latter consideration is important since food deprivation polydipsia has been noted in C3H mice (Kutscher, unpublished observations) and such polydipsia in rabbits [4] and dogs [9] has been linked to sodium deficiency.

EXPERIMENT 1

METHOD

Apparatus

Mice were maintained in individual steel cages (17.8 x 12.7 x 15.2 cm) with 0.6 cm mesh hardware cloth tops and floors. The latter were covered with fine mesh screen wire.

Water and 1.5% NaCl solutions were given in 100 ml eudiometer tubes (graduated in 0.2 ml) or in 100 ml graduated cylinders (graduated in 1.0 ml). Each day two fresh food pellets were placed in the cage and food intake was determined by weighing pellets at the beginning and end of each test day. Only extremely small food particles penetrated the fine mesh cage floor and were lost from measurement. Lights were on for 12 hr/day. Temperature was maintained at 21 ± 1°C and humidity at 50 ± 10%.

Animals

Twenty-four C3H mice, 20 males and 4 females, were obtained from Jackson Laboratory, Bar Harbor, Maine, and were 150-200 days old at the start of the experiment. Mice had been used in a previous experiment in which they had been restricted to 1/3 normal food intake for 4 days, but had not been deprived for at least 60 days before the start of this experiment.

Procedure

This experiment was run in two parts. In the first part, 8 male mice were studied. Two had been adrenalectomized, but the operation was apparently incomplete since the animals quickly regained normal body weight and were able to subsist by drinking only water and not the 1.5% NaCl solution. One mouse had been subjected to a sham adrenalectomy operation and 5 were intact. Intake of food, water and 1.5% NaCl was measured for 4 days with food available ad lib. Next, mice were restricted to 1/3 normal
food intakes for 7–9 days and were then returned to food ad lib. The exact deprivation interval length depended upon the health of the animal. Water and 1.5% NaCl were always available. Position of tubes was alternated every second day. Animals were weighed daily.

In the second part of the experiment, 16 mice, 12 males and 4 females, were assigned to 2 groups, each group containing 6 males and 2 females, and were given initial tests of salt intake in order to give them experience drinking salt solutions and to gather basic data on salt acceptability in this strain of mice. One group was given a single tube test in which a salt solution was the only fluid offered on each test day. The other group was given a double tube test in which the salt solution was always paired with water on each test day. Each salt test day was followed by a nontest day on which only water was given. Salt solutions used were 0, 0.5, 1.0, 1.5, 2.0, and 2.5% NaCl, mixed in tap water. Solutions were given in a randomized order and positions of salt and water tubes were changed for each test day.

Following salt intake tests, mice were regrouped into 2 groups of 8 each, with 4 mice from each of the 2 salt test conditions placed in each group. Food intake, body weight, and water and 1.5% NaCl intake were measured in all animals for a period of 7 days. For the next 8 days, the deprived group was given 1/3 normal food intake and the control group was maintained on food ad lib. Following deprivation, food was given ad lib for 4 days.

FIG. 1, Daily intake of water and 1.5% NaCl for 3 individual mice. Number 17 and No. 5 are examples of responders which showed increased NaCl intake during food deprivation. Number 16 is an example of nondeprived control animals.

Since there was no basic difference in the data for food deprived mice in both parts of the experiment, the data were combined for analysis. Before food deprivation, mean daily water intake for all 16 mice in the experimental group was 6.6 ml and intake of 1.5% NaCl was 1.3 ml. Thirteen mice responded to deprivation with a substantial increase in NaCl intake and 3 did not respond. For the 13 responders, mean daily NaCl intake was 1.0 ml for the 4 ad lib days preceding deprivation and 7.4 ml for the last 4 days of deprivation. Mean water intake during these 2 time periods was not significantly different (6.7 ml vs. 7.1 ml, respectively). However, the initiation of food deprivation did produce a transient decrease in water intake. Water intake on the first deprivation day (4.8 ml) was significantly less (*P < 4.25, n < 0.01) than on the last ad lib day (7.9 ml).

Figure 1 shows the increase in NaCl intake in 2 mice subjected to food deprivation (No. 17 and 5) and lack of change in salt intake in a nondeprived control (No. 10). Number 17 is an example of a complete reversal during food deprivation of the usual preference for water over 1.5% NaCl. In the case of No. 5, a reversal in preference did not occur, but 1.5% NaCl was accepted in approximately the same volume as water during the latter half of the deprivation interval, even though previously this salt solution was consistently rejected. For all 13 responders intakes of 1.5% NaCl exceeded that of water in 30 out of 135 animal test days during food deprivation; however, salt intake declined to predereprivation levels on the first or second day of refeeding.

For the 8 nondeprived control animals, no increase in NaCl intake was noted during the 17 days of observation. During the 8 days when the experimental animals in Part 2 of the experiment were food deprived, not one single control animal drank more salt than water for even 1 day.

Intake of 1.5% NaCl for the last 4 days of this period (0.7 ml) was not significantly different from that during the 4 days corresponding to the predereprivation period for the experimental animals (0.6 ml).

The intake of 1.5% NaCl seems to be a positive function of the loss in body weight. In Fig. 2 (bottom) mean intakes of 1.5% NaCl are plotted as a function of mean body weight deficit (plotted in 5% units) during food deprivation and refeeding. Because weight change occurred at slightly different rates and not every animal incurred the same weight loss during the deprivation interval, data were not available from every animal for each point on the graph. It does seem clear, however, that for the C3H mouse in a situation of progressive weight loss caused by food deprivation, intake of 1.5% NaCl increases as a positive function of weight loss beginning at some point after that weight loss equals 10%. Diminishing the weight loss to less than 10% by refeeding returns the salt intake to predereprivation levels.

Figure 2 (top) shows that changes in NaCl are independent of changes in water intake. Except for a slight, but significant, dip in water intake at the initiation of deprivation, water intake was not significantly affected by deprivation and refeeding.

The data on the acceptance of various concentrations of NaCl solutions under single tube and double tube test conditions are shown in Fig. 3. It is clear that at all concentrations used here (0.5–2.5%) the nondeprived C3H mice showed no reliable preference for NaCl over water nor...
showed any palatability induced polydipsia. A one-way analysis of variance showed that in the single tube condition, the volume of salt solution ingested decreased significantly as the concentration increased \((F = 8.75, p<0.01)\). It can be seen in Fig. 3 that intake of 1.5% NaCl was approximately half that of water, presumably indicating a mild aversion to this solution since no other drinking solution was available in this test condition. Under the double tube condition also, the volume of NaCl solution ingested declined \((F = 5.88, p<0.01)\) and the water intake increased \((F = 3.05, p<0.05)\) with increasing concentration of NaCl. Figure 3 shows that under the double tube test, little 1.5% NaCl was consumed, but this solution seems to be at the inflection point in the aversion curve, i.e., intakes increased significantly as concentrations were decreased from 1.5%.

**EXPERIMENT 2**

In this experiment, mice were given a diet nutritionally adequate except for sodium in order to separate sodium deficiency from other deficiencies which may result from partial food deprivation.

**METHOD**

Eight mice, 4 from the control group and 4 from the deprived group of the previous experiment, were assigned to the sodium deficient group and were fed the stock sodium deficient diet (Nutritional Biochemicals). The remaining 8 animals served as the control group and were given stock sodium deficient diet with 1.28 g of NaCl added per 100 g of diet so that the sodium content approximated that of Purina Chow. Mice were given Purina Chow for 4 days followed by 20 days on the test diets. Water and 1.5% NaCl were continuously available. Position of water and salt tubes was alternated every second day. Test diets were presented in glass food jars.

**RESULTS**

Mean daily intakes of water and 1.5% NaCl are shown in Table 1. Intake of 1.5% NaCl did not differ significantly between the 2 groups during the 4 days of Purina Chow feeding nor during the 20 days of sodium test diet feeding. Perusal of the raw data failed to reveal a real change in salt intake for any of the individual animals. Thus, feeding a nutritionally adequate diet, deficient only in sodium, did
C3H mice increased salt intake during a prolonged partial food deprivation schedule and 9 out of the 13 showed at least 5 days of reversal of the usual preference for water over food, although this position habit did not produce significantly increased NaCl intake as did food deprivation in Experiment 1. Water intakes did not differ for the 2 groups during Purina Chow feeding or during sodium test diet feeding, although the control group showed a trend of increased water intake when switched from Chow to test diet, with 2 animals doubling mean daily intake. Intake of 1.5% NaCl exceeded water intake on only 3 of 160 animal test days for mice on sodium deficient diet and on 6 of 160 animal test days for mice given sodium deficient diet with salt added.

**DISCUSSION**

The data from Experiment 1 show that 13 out of 16 C3H mice increased salt intake during a prolonged partial food deprivation schedule and 9 out of the 13 showed at least 1 day of reversal of the usual preference for water over 1.5% NaCl. It is not obvious why no increase in salt intake was observed in 3 of the deprived animals, but the records show that their salt intakes were usually only 0.5 ml throughout the tests (equal to the expected tube leakage), thus indicating an almost complete salt aversion. Since the salt and water tubes were alternated every second day, it is probable that these 3 mice at least tasted the salt briefly and did not respond solely on the basis of a learned position habit.

There are some obvious differences between this study and the rat study by Kaunitz et al. [12]. They showed that nondeprived Columbia-Sherman rats clearly prefer 1% NaCl to water and ingest approximately 5 times as much salt as water. During food deprivation, the salt intake approximately doubled and the water intake was unchanged. In the present experiment, the 1.5% NaCl was clearly a nonpreferred solution and even seemed somewhat aversive. The results of Kaunitz et al. were not verified by Hsiao and Kutscher [11] under somewhat different test conditions. Kaunitz et al. [12] also found that rats given only water showed no change in intake during food restriction, a finding in contrast to other rat experiments [11, 13, 14].

but similar to the mouse data reported here. Kaunitz et al. attempted no physiological explanations for increased salt intake, but merely stated that a decrease in one kind of consummatory behavior (eating) resulted in an increase in another (saline drinking).

The major problem raised by the present research is to explain why a mouse strain which shows a low level of salt acceptance when food is given ad lib, under food deprivation drinks large amounts of a slightly hypertonic NaCl solution and even shows a preference for salt over water. There are at least 4 physiological changes which may accompany food deprivation and which are known to affect intake of salt solutions: dehydration, hyponatremia, hypovolemia, and hypersecretion of mineralcorticoids. In view of the extensive literature on salt appetite and the evidence in the present experiment, the first two can be dismissed, but the second two remain as possible causes of the observed increase in salt intake during food deprivation.

Food deprivation produces a state of dehydration in the rat even though water is freely available. Plasma volume and body water volume decline, but no increase in serum osmolality occurs [13, 15]. Bauman [1] has reported an apparent deficiency in renal concentrating ability in the food deprived rat. Mice [16], gerbils and hamsters [14] are known to show polyuria and polydipsia when food deprived, although this polydipsia in gerbils could not be linked to renal insufficiency [17]. However, conditions which increase drinking by inducing dehydration, such as water deprivation [33], salt loading [25], brain lesions producing diabetes insipidus [27], and ingestion of dry food [10] all result in increased intake of water or hypertonic solutions, but no change, or a decrease in the intake of isotonic or hypertonic solutions. The 1.5% NaCl solution in the present experiment is hypertonic to body fluids, and thus intake should be decreased by dehydration, not increased.

Experiment 2 showed that dietary sodium restriction alone did not produce significant alternation in NaCl intake. Nachman and Pfaffman [21] did produce an increased NaCl intake in rats following 20 days on a sodium deficient diet, but only when intake was measured over a brief (15 min) test period. In Experiment 1, NaCl intake was elevated during food deprivation even when measured on a 24-hr basis. It seems unlikely that mice which showed a sharp increase in NaCl intake after only 2-5 days on partial food deprivation were actually hyponatremic. It is often difficult to deplete animals of sodium by dietary means because of their ability to conserve this electrolyte by stringent reabsorption in renal tubules [17] and because of substantial sodium stores in the bone [22].

There seem to be two physiological conditions which produce increased salt intake independent of hyponatremia, i.e., hypovolemia and increased level of mineralcorticoids. The suggestion is made here that the increased salt intake may be due to either one, or both, of these factors. Hypovolemia produced by polyethylene glycol injections produces increased sodium intake even in adrenalectomized animals [32]. The injections presumably produce osmotic withdrawal of isotonic extracellular fluid into the injection site, producing hypovolemia without hyponatremia. In the rat, plasma volume losses during food deprivation are disproportionalty greater than body weight losses [13, 15], even though deprived rats seem to drink more water than is required for their water needs (relative polydipsia) and excrete copious urine [18]. Although some mice in the

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**TABLE 1**

**DAILY INTAKE OF WATER AND 1.5% NaCl (MEAN±SD) WHEN PURINA CHOW AND TEST DIETS WERE GIVEN**

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>Sodium Deficient Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>7.3±1.5 ml</td>
<td>7.0±1.0</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>1.5% NaCl</td>
<td>2.2±2.0</td>
<td>1.4±1.3</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

**Test Diets (20 Days)**

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>Sodium Deficient Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>10.0±4.4</td>
<td>7.0±1.1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>1.5% NaCl</td>
<td>2.6±1.8</td>
<td>2.3±1.5</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>
present experiment showed increased NaCl intake on the second day of deprivation, most salt drinking occurred in the latter half of the 7-9 day observation period when body weight losses were approximately 20% or more and plasma volume losses were probably substantial. It is also possible that food deprivation may produce increased salt intake by means of hypersecretion of mineralcorticoids from the adrenal cortex. It has long been known that deoxycorticosterone acetate injections can produce increased salt appetite [23], even though this manipulation produces no sodium deficiency [29] and may even result in sodium retention. Wolf and Handal [30] showed that d-aldosterone acetate injected in doses small enough to be in the normal physiological range produced significant increases in NaCl intake in the rat. Evidence has been presented showing increased level of adrenal activity in rats [19] and mice [3] during the stress of food deprivation. Kutscher (unpublished observations) found that rats food deprived for 4 days showed stringent sodium retention, even though urine volumes were substantially above ad lib baselines. On the first day of refeeding, the urine volumes were normal but the salt retention continued, indicating possible mineralcorticoid activity throughout food deprivation and the first day of refeeding. Although all of this evidence indicating a hypersecretion of mineralcorticoids during food deprivation is circumstantial rather than direct, it does seem somewhat convincing, particularly in view of the fact that hypovolemia is a potent stimulus to aldosterone secretion [8]. Thus, both hypovolemia and hypersecretion of aldosterone are known to increase salt intake and may be possible causes for the increased NaCl intake obtained in the present experiment. From the evidence at hand there is no way to assess the relative importance of each.

Food deprivation should be added to the list of procedures which produce significant alteration in sodium chloride intakes. This may require the reinterpretation of some previous experiments. For example, Vance [28] found increased salt intake in rats following desalivation and speculated that this result might be explained by the alterations in oral chemistry produced by the operation which changed functions of taste receptors. Taste receptor function in humans is known to be influenced by the chemistry of the solution bathing and adapting the receptors [1]; however, it is also possible that the increased salt intake Vance noted may be due to food deprivation since his animals lost weight following the operation, presumably due to the difficulty encountered in masticating and swallowing food.

REFERENCES


