Adaptive appetites for salted and unsalted food in rats: differential effects of sodium depletion, DOCA, and dehydration

M. J. McKinley
Florey Neuroscience Institutes of Neuroscience and Mental Health, and Department of Physiology, University of Melbourne, Parkville, Victoria, Australia
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McKinley MJ. Adaptive appetites for salted and unsalted food in rats: differential effects of sodium depletion, DOCA, and dehydration. Am J Physiol Regul Integr Comp Physiol 304: R1149–R1160, 2013. First published April 17, 2013; doi:10.1152/ajpregu.00481.2012.—Most ingested sodium is contained in food. The aim was to investigate whether sodium depletion, dehydration, or DOCA alters intakes of salted and unsalted foods by rats given choices of two foods: salted (0.2–0.5% Na) and unsalted food containing either similar or different other dietary components. Diuretic-induced (furosemide or acetazolamide, two treatments on successive days) sodium depletion always caused pronounced falls in intake of unsalted food within 24 h, continuing at least another 2 days (e.g., 20.9 ± 1.6 pretreatment to 14.8 ± 1.2, 10.6 ± 1.5, and 14.3 ± 1.3 g/day for 3 days of depletion). Intake and preference for salted food increased after 24–72 h (e.g., 6.5 ± 1.2 pretreatment to 7.1 ± 1.1, 16.4 ± 2.3, and 17.0 ± 1.5 g/day at 1, 2, and 3 days of depletion). Valsartan (10 mg/day) blocked the increased intake of salted food but not the reduced intake of unsalted food. DOCA (2 mg/day) caused equivalent increase and decrease in intakes of salted and unsalted food, respectively. Water-deprived rats reduced intake (e.g., 14.2 ± 3.1 to 3.2 ± 2.0 g/day) of and preference for salted food (e.g., 56 ± 13% to 21 ± 11%) after 2 days of dehydration but did not consistently reduce intake of unsalted food. Total food ingested/day fell in both sodium-depleted and dehydrated rats. Rats regulate intakes of different foods to balance sodium needs, osmoregulatory homeostasis, and energy requirements. Reduced appetite for unsalted food may be a homeostatic response to sodium depletion, which together with subsequent generation of appetite for salted food, drives animals to ingest sodium-containing food, thereby restoring sodium balance.

Adequate intake of sodium, the major ionic component of extracellular fluid, is essential for the life and health of mammals. Although there are endocrine and renal mechanisms that act to conserve bodily sodium when it is lost in sweat or urine, or from the alimentary tract, such sodium conservation does not bring about the replacement of lost sodium. It is not surprising, therefore, that many mammals manifest an innate propensity to ingest salt when they become sodium depleted (8, 23). This appetite for salt has been studied extensively during the past half century. Indeed, several neuroendocrine, gustatory, neuroanatomical, neurochemical and postigestational factors that influence salt intake have been investigated and elucidated (6, 16, 26, 28, 35, 38). All but a few (1, 5, 13) of the many hundreds of these investigations have focused on mechanisms that drive the selective appetite for solutions of sodium, usually pure aqueous solutions of isotonic or hypertonic NaCl.

For most terrestrial mammals, the natural source of sodium is the salt contained in food, and it is unlikely to be ingested as a pure saline solution outside the laboratory. When ingested in food, salt is tasted and eaten together with many other nutrients and foodstuffs, which may well influence the amount of sodium or food consumed. In this context, there is evidence from some (but not all) investigations that Na depletion is an anorexic influence, which could reduce the intake of Na by Na-depleted animals (5). It is clear, however, from the few studies that have been made on this topic that rats are able to respond to sodium depletion by increasing the intake of salted food (2, 5, 13). In those experiments, rats had the choice of two foods that were identical except for NaCl content, and they exhibited an increased preference for and intake of salted food when they were depleted of sodium by adrenalectomy or by treatment with the diuretic drug furosemide. Again, it is unlikely that rats would ever encounter two foods that were identical but for their sodium content outside the laboratory. Therefore, in the present work, sodium-depleted rats were given choices of foods having compositions that varied not only in sodium content, but also in several other components, in an attempt to bring the experimental conditions closer to those encountered in nature. One food component of particular interest is sucrose. Sodium deprivation has been shown to cause large changes in the electrical activity of neurons in the nucleus tractus solitarius (NTS) that relay signals from primary taste afferent nerves. In particular, sodium depletion inhibits activity of “salt-best” neurons, but increases activity of “sugar-best” neurons in the NTS which, in addition, also become responsive to NaCl (15).

Prior investigations of food preferences in sodium-depleted rats have all utilized the loop diuretic agent furosemide to cause sodium depletion (2, 5). To ensure that effects observed were not specific to furosemide-induced sodium depletion, another natriuretic agent, the carbonic anhydrase inhibitor acetazolamide, was also used to produce sodium depletion in some experiments. The renin-angiotensin system is activated by sodium depletion and is known to play an important role in driving the increased intake of sodium solutions in sodium-depleted animals (16, 19, 29, 41). Consequently, the effect of blocking ANG AT1 receptors on intake of salted food in sodium-depleted rats was tested. Another well-known stimulus for increasing intake of sodium solution in rats is the mineralocorticoid agent DOCA (22, 34, 44). Thus, rats have also been treated with DOCA to determine its effect on their intake of and preference for salted food.
Dehydration that results from depriving animals of water to drink has been reported to increase the intake of hypertonic saline solution before (27, 31, 35) or as soon as (9, 10, 32, 43) dehydrated rats have quenched their thirst with water after its presentation following periods of water deprivation. This may not be surprising because water-deprived animals become sodium-depleted as a result of dehydration-induced natriuresis (18, 33). Although dehydrated rats will ingest a hypertonic saline solution (30, 31, 35), whether such dehydrated animals are manifesting a sodium appetite is debatable because the intake of sodium solution may be an attempt to satisfy thirst. If a sodium appetite does develop in dehydrated rats, testing their preference for salted food may be a means of determining whether they have a drive to ingest sodium without the complicating aspects of ingesting solutions.

The aims of the present investigation were to determine 1) whether salt-depleted rats are able to make the appropriate choice of increasing the intake of a salted food even when it differs quite considerably in overall composition from an unsalted food that they normally prefer, 2) whether the method of sodium depletion (furosemide vs. acetazolamide treatment) is a factor influencing the preference for salted food, 3) whether the sucrose content of food influences intake of salted food, 4) whether ANG II drives an appetite for salted food, 5) whether DOCA treatment stimulates an increased preference for salted food, and 6) whether the preference for salted food increases if a rat is dehydrated, even though dehydration has anorexic influences (9, 18, 40).

MATERIALS AND METHODS

Experiments were performed on 46 male Sprague-Dawley rats of body weight 0.3–0.6 kg. The rats were housed in individual cages comprising a plastic box (25 cm × 40 cm) with a stainless-steel wire mesh top elevated to a height of 25 cm. Either pelleted or biscuit-type food was placed in a recess in the wire mesh, and water was available from two graduated burettes (60 ml). Absorbent animal litter made from paper pulp or wood shavings was used to cover the cage’s floor. Rats were housed in a room at 20–22°C with a 12:12-h light-dark cycle. All experiments were approved by the Animal Ethics Committee of the Florey Neuroscience Institutes, which adheres to the Australian National Health and Medical Research Council Code of Practice for the Care and Use of Animals in Scientific Experiments.

Experimental Procedures

Each rat underwent 2–4 different experimental protocols involving treatment with furosemide, acetazolamide, valsartan, DOCA, vehicles, or water deprivation when they were provided with a number of dietary choices over the course of 2–3 mo. An interval of at least 1 wk was allowed between successive experiments. Treatments were administered in random order, so that rats did not undergo the same sequence of experiments. In most experiments, rats were given a choice of eating two different foods that often varied in NaCl and sucrose content. Approximately equal portions of the two different foods (usually in the form of pellets but sometimes given in biscuit form) were well mixed up and placed randomly on the food recess area of the cage top, thereby ensuring equal access to the different types of food. Changes in the daily intakes of each type of food in response to the various treatments were measured as was daily water intake and body weight. Rats were allowed at least 3 days of access to each combination of foods before administration of treatments, during which time, steady baseline food and water intakes were achieved. The different foods were distinguished from each other by their color or by marking them with food coloring. Cages were checked carefully for any uneaten food particles.

Food types. Three basic food types were used in these studies. The first two used were commercially obtained pelleted rat food, and the third food used was prepared in the laboratory with common ingredients based partially on rat diets of Richter (16).

Salted food. The normal diet fed to rats (Barastoc rat pellets, Ridley Agri Products, Melbourne) contained 0.5% NaCl and 4.6% sucrose and was designated “salted food”. It also contained protein (20%), fat (5%), fiber (5%) and other carbohydrates in the form of cereal grains and legumes (65%). This food was manufactured as pellets and was a light brown color.

Unsalted food. The standard commercially prepared unsalted food (Specialty Feeds, Glen Forrest, Western Australia) fed to rats contained 0.02% Na and 10% sucrose and is designated “unsalted food.” Its basic composition differed from the commercial normal salted diet (above). It consisted of wheat starch (54%), cellulose (5%), casein (20%), canola oil (7%), vitamins (3.5%), minerals (1%), methionine (0.3%), as well as the 10% sucrose and 0.02% NaCl. It was also in the form of hard pellets and was cream-white in color. In one experiment, a variation on the unsalted food was formulated by reducing the low Na/high sugar pellets to a powder and then adding NaCl and water or just to water to make a high-Na/high-sugar food (0.2% Na, 10% sucrose) or low-Na/high-sugar food (0.02% Na, 10% sucrose), respectively. These latter mixtures were baked to constant weight and presented to rats as hard biscuits.

Laboratory-prepared food. To prepare foods with greater variations in sodium and sucrose content, we prepared biscuits with a basic composition of 26–30% rice grain, 26–30% crushed oats, 26–30% plain wheat flour, 0.002% cod liver oil, 5% unsalted butter, and 5% egg protein (% of rice, oats, and flour was adjusted equally to accommodate the addition of sucrose). To this base mixture were added various amounts of NaCl and sucrose to yield several combinations of Na/sucrose content from 0.03 or 0.2% Na and 0 or 10% sucrose. They were mixed with water and then baked to hard biscuits. These foods are designated “lab food” (e.g., high Na-zero sucrose lab food). The variations of this lab food were identical in appearance necessitating the marking of the biscuits with food coloring. This marking was varied from animal to animal within an experiment to prevent any systematic influence it may have had on results.

Diuretic-induced sodium depletion. For most experiments, investigating the effects of sodium depletion on intakes of different foods, the daily intakes of food and water were measured at 0900–1100 on two successive days. On the third day, food and water were removed from cages at 1000, rats were weighed and then injected subcutaneously with furosemide (20 mg/kg; Lasix, Sanofi Aventis), acetazolamide sodium (100 mg/kg; Diamox, Wyeth) in four rats, or isotonic saline (2 ml/kg) as a control. After 2 h, rats were reweighed (to verify diuretic-induced fluid loss of ~15 ml), and food and water were returned to the cage for the subsequent 22 h. This procedure was repeated again on the next day. Daily intakes of food and water were measured for a further 3 days. In some experiments (designated in results), the protocol was similar except that rats were placed in a metabolism cage (to which they had been previously accustomed) between 1000 and 1200 on the days of injection. This allowed collection of urine during the 2 h immediately following diuretic or saline treatment. Rats were weighed before and 2 h after these treatments, then returned to their normal home cage with water and food available. The sodium concentration of urine samples was measured by flame photometry (Clinical Flame Photometer 410C, Corning), and the sodium excreted in urine was calculated as the product of urinary sodium concentration and volume collected during the 2 h.
**Experiment 1. Effect of Sodium Depletion Caused by Furosemide Treatment on Intakes of Unsalted (High Sucrose) and Salted (Low Sucrose) Food**

Rats were given the choice of unsalted (0.02% Na-10% sucrose) or salted (0.5% Na-4.6% sucrose) commercial pellets. Rats were administered furosemide (20 mg/kg sc; n = 7) or isotonic saline (2 ml/kg sc control; n = 9) in random order with at least 1 wk between treatments. Rats were placed in metabolism cages for 2 h following these treatments to enable collection of urine. They were then returned to their home cage for the next 22 h. This procedure was repeated at the same time on the following day. Intakes of food and water were measured for another 4 days.

**Experiment 2. Effect of Na Depletion Caused by Acetazolamide Treatment on Intakes of Unsalted (High Sucrose) and Salted (Low Sucrose) Food**

Rats were given the choice of unsalted (0.02% Na-10% sucrose) or salted (0.5% Na-4.6% sucrose) commercial pellets. Rats (n = 4) were administered acetazolamide sodium (100 mg/kg sc). As in experiment 1, rats were placed in metabolism cages for 2 h following this treatment to enable collection of urine. They were then returned to their home cage for the next 22 h. This procedure was repeated at the same time on the following day. Intakes of food and water were measured for another 4 days.

**Experiment 3. Effect of Furosemide-Induced Na Depletion on Intakes of Food Choices That Were Identical Except for a Low- or High-Na Content**

In experiment 3A, two groups of rats were given a choice of unsalted (0.02% Na) and salted (0.2% Na) pellets that had been reconstituted from the commercial pellets that contained 10% sucrose. In this experiment, one group (n = 5) was administered furosemide (20 mg/kg sc), and the other group was administered isotonic saline (2 ml/kg sc) on two successive days. Food and water intakes were measured for 5 days following the first furosemide injection. Urine collections were not made.

In experiment 3B, rats were given a choice of unsalted (0.03% Na) and (0.2% Na) lab biscuits that were otherwise identical and were sucrose-free. One group of rats (n = 5) was treated with furosemide (20 mg/kg sc), while another group was treated with isotonic saline (2 ml/kg sc) on two successive days. Food and water intakes were measured as described above.

**Experiment 4. Effect of Furosemide-Induced Sodium Depletion on Intakes of Salted and Unsalted Food Pellets When Hypertonic 0.5 mol/l NaCl Solution Was Provided in the Cage**

Rats were given the choice of unsalted (0.02% Na-10% sucrose) or salted (0.5% Na-4.6% sucrose) commercial pellets. A group of 5 rats was studied. This protocol was similar to the furosemide treatment in experiment 1, except that 0.5 mol/l NaCl solution was provided to the animals for 3 days prior to, as well as throughout, the period of observations. The choice of foods was between the unsalted (0.02% Na-10% sucrose) and salted (0.5% Na-4.6% sucrose) commercial pellets. No urine collections were made.

**Experiment 5. Effect of the ANG AT1 Receptor Antagonist Valsartan on Intakes of Unsalted (High Sucrose) and Salted (Low Sucrose) Food in Sodium-Depleted Rats**

Rats were given the choice of unsalted (0.02% Na-10% sucrose) or salted (0.5% Na-4.6% sucrose) commercial pellets. After 2 days of baseline measurements, animals (n = 6) were treated with the ANG AT1 receptor antagonist valsartan (7) (Novartis, Basel, Switzerland; 10 mg/kg sc; n = 6) or vehicle (0.1 M Na2CO3, 1 mg/kg; n = 5) on three successive days, and administered furosemide (20 mg/kg sc) on the first 2 days of valsartan or vehicle treatment. In another experiment, valsartan (10 mg/kg sc) only was administered on three successive days. Intakes of food and water were measured for 5 days following the start of all treatments.

**Experiment 6. Effect of DOCA Treatment on Intakes of Unsalted (High Sucrose) and Salted (Low Sucrose) Food**

Rats (n = 5) were given the choice of unsalted (0.02% Na-10% sucrose) or salted (0.5% Na-4.6% sucrose) commercial pellets. After 2 days of baseline measurements, they were given injections of DOCA (2 mg) or peanut oil (vehicle) on three successive days. Measurements were continued for a further 2 days after this treatment ended.

**Experiment 7. Effect of Water Deprivation for Two Days on Intakes of Unsalted (High Sucrose) and Salted (Low Sucrose) Pelleted Food**

Rats were given the choice of unsalted (0.02% Na-10% sucrose) or salted (0.5% Na-4.6% sucrose) commercial pellets. Food and water intakes and body weight of rats (n = 10) were measured each day. In five of the rats, magnetic resonance relaxation analysis was used to measure total body water on an EchoMRI-100/900 Body Composition Analyser (Echo Medical Instruments, Singapore). Rats had become accustomed to entering the EchoMRI instrument during the fortnight prior to the experiment and voluntarily entered the Perspex measurement tube. Measurement was completed within 2 min, and replicate values were averaged for the final value. After 2 days of baseline measurements, rats were deprived of water for 2 days. Food intakes, body weight, and total body water were measured each day, and after 2 days of water deprivation, they were provided with water to drink again, and measurements continued for another 2 days.

**Experiment 8. Effect of Water Deprivation for Two Days on Intakes of Two Foods That Were Identical Except for a Low- or High-Sodium Content**

In experiment 8A, rats were given the choice between unsalted (0.03% Na-10% sucrose) or salted (0.2% Na-10% sucrose) lab biscuits. A high-sucrose content was utilized to ensure approximately similar baseline intake of salted and unsalted food. Daily food and water intakes and body weight were measured each day. After 2 days of baseline measurements, rats (n = 6) were deprived of water for 2 days. Measurements continued for another 2 days following rehydration. Total body water was not measured in this experiment.

In experiment 8B, rats not given a choice of food on the cage. They were allowed access to only one of these foods; either unsalted (0.03% Na-10% sucrose) or salted (0.2% Na-10% sucrose) lab biscuits. After 2 days of baseline measurements, body weight was measured, and rats (n = 5 and 4, respectively) were deprived of water. After 2 days of water deprivation, they were provided with water to drink again, and measurements continued for another 2 days.

**Statistical Analysis**

Results are presented as means ± SE. With each treatment, values for each day following treatment were compared with the value obtained on day 2, the last day prior to treatment. A repeated-measures ANOVA (one factor) followed by multiple-comparisons (Dunnett’s test) was used to test whether treatments affected intakes of salted food, unsalted food, total food, and water, as well as preference for salted food (calculated as the percentage of total food intake that salted food comprised).
RESULTS

Experiment 1. Effect of Diuretic-Induced Sodium Depletion on Intakes of Unsalted (High Sucrose) and Salted (Low Sucrose) Food

The aim of this experiment was to investigate the effect of sodium depletion on the intake of salted food when rats were given the choice of foods having compositions that varied not only in sodium content, but in the content of other food components. All rats studied had a strong preference ($P < 0.01$) for the unsalted (0.02% Na-10% sucrose) compared with salted (0.5% Na-4.6% sucrose) pellets prior to diuretic treatment (Fig. 1). Furosemide (20 mg/kg) treatment increased sodium excretion in urine (1.31 ± 0.10 and 1.09 ± 0.18 mmol; $n = 7$, on days 1 and 2, respectively) compared with control injections of saline (0.01 ± 0.01 and 0.02 ± 0.02 mmol; $n = 5$; insufficient urine was obtained for analysis in some rats)

during the initial 2 h following treatment. During the first 24 h of treatment with furosemide, intake of unsalted food fell significantly by ~30%, while there was no significant change in intake of salted food (Fig. 1). However, a significant increase in intake of and preference for salted food was observed on the second and third day of Na depletion, while intake of unsalted food remained at low levels. After this time, intakes of both foods returned to baseline levels as did the preference for salted food. As a consequence of these changes, total mass of food ingested fell significantly on the first day of diuretic treatment (Table 1). Water intake increased significantly during the first 3 days of diuretic treatment (Table 2). Subcutaneous injection of saline (control) had no effect on intakes of either food choice or water (Fig. 1 and Table 2).

Experiment 2. Effect of Na Depletion Caused by Acetazolamide Treatment on Intakes of Unsalted (High Sucrose) and Salted (Low Sucrose) Food

In this experiment, the effect of sodium depletion caused by a natriuretic agent other than furosemide on the intake of salted and unsalted food was determined. Injection of acetazolamide caused a natriuresis. During the 2 h following treatment, the amount of Na excreted in urine was 0.93 ± 0.17 and 0.68 ± 0.19 mmol on days 1 and 2 ($n = 4$), respectively, which was much greater ($P < 0.01$) than that observed in experiment 1 following control injections of isotonic saline but not significantly different from that observed following furosemide injections. Following this treatment, intake of the unsalted food fell significantly for 3 days. After a delay of 24 h, intake of salted food increased so that the preference for salted food was significantly increased on the second and third day following treatment (Table 3). Total food intake decreased for 2 days following acetazolamide treatment (Table 1). Water intake increased significantly (Table 2) for 3 days.

Experiment 3. Effect of Furosemide-Induced Sodium Depletion on Intakes of Food Choices That Were Identical Except for a Low- or High-NaCl Content

In these experiments, the effect of furosemide-induced sodium depletion on intakes of salted and unsalted food was determined when the compositions of the food choices varied only in sodium content. In experiment 3A, the basic composition was that of the commercial pelleted unsalted food that contained a high sucrose content. In experiment 3B, the basic composition of the food was that of lab biscuits that did not contain sucrose so that any influence of sucrose content on intake salted and unsalted food could be determined.

In experiment 3A, rats were given a choice of unsalted (0.02% Na-10% sucrose) and salted (0.2% Na-10% sucrose) reconstituted pellets. There was no significant preference (paired t-test) for unsalted food over salted food prior to treatment. During the first 24 h of furosemide treatment, intake of unsalted food (0.02% Na-10% sucrose) fell significantly by ~50%, while there was no significant change in intake of the salted (0.2% Na-10% sucrose) food (Fig. 2). However, intake of salted food increased significantly on the second and third day after starting furosemide treatment, while intake of unsalted food fell to even lower levels (15% of baseline) during this period (Fig. 2), so that there was a marked increase in the preference for salted food for 3 days after sodium depletion.

![Fig. 1. Preference for salted food (expressed as % of total food intake) (top) and daily intakes of salted (middle) and unsalted (bottom) food of rats that were treated with subcutaneous injections of furosemide (arrows, F/S) (20 mg/kg; o, solid line) or isotonic NaCl (0.15 mol/l; ◦, dashed line) on days 3 and 4. Rats were given a choice of salted (0.5% NaCl-4.6% sucrose) or unsalted (0.02% NaCl-10% sucrose) commercial pellets. Values are expressed as means ± SE. Significant differences from the mean pretreatment value are denoted by *$P < 0.05$ and **$P < 0.01$, using Dunnett’s test.](http://ajpregu.physiology.org/)

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Table 1. Total mass of food ingested each day during experiments

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<th>Experiment and Treatment</th>
<th>Daily Total Mass of Food Ingested, g/day</th>
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Values are expressed as means ± SE. Day 1 and day 2 are values for the two days immediately prior to treatment; day 3 and day 4 (day 5 also for DOCA and valsartan treatments) are values for treatment days. Days 5–7 are posttreatment values. Abbreviations in treatment (Tr) column: A, acetazolamide; D, dehydrated; DS, dehydrated with salted food only; DU, dehydrated with unsalted food only; C, control isotonic saline injection; F, furosemide; V+F, valsartan + furosemide; N+F, Na2CO3 vehicle + furosemide; V, valsartan; O, oil vehicle. Significant difference from value on day prior to treatment (day 2) is denoted by †P < 0.05 and *P < 0.01.

Table 2. Total volume of water ingested each day during experiments

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<th>Experiment and Treatment</th>
<th>Daily Intake of Water, ml/day</th>
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Values are expressed as means ± SE. Day 1 and day 2 are values for the two days immediately prior to treatment; day 3 and day 4 (day 5 also for DOCA and valsartan treatments) are values for treatment days. Days 5–7 are posttreatment values. Abbreviations in treatment (Tr) column: A, acetazolamide; D, dehydrated; DS, dehydrated with salted food only; DU, dehydrated with unsalted food only; C, control isotonic saline injection; F, furosemide; V+F, valsartan + furosemide; N+F, Na2CO3 vehicle + furosemide; V, valsartan; O, oil vehicle. Significant difference from value on day prior to treatment (day 2) is denoted by †P < 0.05 and *P < 0.01.
due entirely to a large reduction in the intake of unsalted food (Fig. 3). Water intake increased significantly following diuretic injections (Table 2). Control injections of isotonic saline did not cause any significant changes in the aforementioned variables (Fig. 3).

Experiment 4. Effect of Furosemide-Induced Sodium Depletion on Intakes of Salted and Unsalted Food Pellets When Hypertonic 0.5 mol/l NaCl Solution Was Provided

In this experiment, it was determined whether or not furosemide treated rats would still change intakes of salted and unsalted food if NaCl solution was available to drink for the correction of their sodium deficit. These rats had a modest preference for unsalted (0.02% Na-10% sucrose) over salted (0.5% Na-4.6% sucrose) food (commercial pellets) and ingested small volumes of 0.5 mol/l NaCl solution (<1–4 ml/day) during pretreatment observations. The intake of hypertonic saline increased significantly within 24 h of furosemide treatment (Table 3), although this effect did not continue consistently. There was no significant change in the intake of unsalted food, however, there was a significant reduction in the intake of salted food on one day (Table 3). Water intake increased significantly for 2 days with furosemide treatment (Table 2).

Experiment 5. Effect of the Angiotensin AT1 Receptor Antagonist Valsartan on Intakes of Unsalted (High Sucrose) and Salted (Low Sucrose) Food in Sodium-Depleted Rats

In this experiment, a role for ANG II (either from the circulation or centrally generated) in changing the intakes of salted and unsalted food by sodium depleted rats was investigated. Treatment with valsartan completely blocked the increased intake of and preference for salted food (0.5% Na-4.6% sucrose pellets) that was observed with vehicle (Na2CO3) furosemide treatment (Fig. 4) or following furosemide treatment as shown in experiment I (Fig. 1). However, valsartan treatment did not block the reduction in intake of unsalted (0.02% Na-10% sucrose) pellets or the reduced total food intake that follows furosemide treatment (Fig. 4). Water intake increased following valsartan/furosemide treatment, but this only reached significance after valsartan treatment had ended; vehicle/furosemide treatment resulted in increased water intake for 3 days (Table 2). Valsartan treatment alone did not significantly alter intakes of salted or unsalted food (experiment 5C, Table 3).

Experiment 6. Effect of DOCA Treatment on Intakes of Unsalted (High Sucrose) and Salted (Low Sucrose) Food

In this experiment, it was investigated whether DOCA treatment, another known stimulus of sodium appetite that does not involve sodium depletion, would increase intake of salted food. Treatment with DOCA resulted in a significantly increased intake of salted (0.5% Na-4.6% sucrose) pellets on the second and third days of treatment (Fig. 5). Concomitantly, intake of unsalted (0.02% Na-10% sucrose) pellets fell at these times (Fig. 5), but there was no reduction in total mass of food ingested (Table 1). Preference for salted food increased significantly on all 3 days of DOCA treatment. Water intake tended to increase with DOCA treatment but did not reach statistical significance (Table 2). Vehicle treatment had no significant effects (Fig. 5 and Tables 1 and 2).

Experiment 7. Effect of Water Deprivation for Two Days on the Intakes of Unsalted (High Sucrose) and Salted (Low Sucrose) Pelleted Food

In this experiment, it was investigated whether or not dehydrated rats differentially changed intakes of salted and unsalted foods regardless of the other nutritional components that were in these foods. Rats were given a choice of unsalted (0.02% Na-10% sucrose) or salted (0.5% Na-4.6% sucrose) pelleted food. This group of 10 rats did not show a significant preference for unsalted over salted pelleted food prior to dehydration (baseline days). One rat was excluded from analysis because it did not ingest any of the salted food during baseline measurements, showing a 100% preference for unsalted food throughout.

Results are shown as means ± SE. Intake of 0.5 mol/l NaCl for experiment 4 is also included. Day 1 and day 2 are values for the two days immediately prior to treatment. Days 3 and day 4 (day 5 also for valsartan) are values for treatment days. Days 5–7 are posttreatment values. Significant difference from value on day prior to treatment (day 2) is denoted by †P < 0.05 and *P < 0.01.

### Table 3. Intakes of salted and unsalted food, and preference for salted food for experiments 2, 4, and 5C

<table>
<thead>
<tr>
<th>Expt</th>
<th>Variable</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
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</thead>
<tbody>
<tr>
<td>2</td>
<td>SF g/day</td>
<td>4.1 ± 1.0</td>
<td>3.7 ± 0.9</td>
<td>5.2 ± 1.0</td>
<td>9.8 ± 1.0*</td>
<td>8.6 ± 1.5*</td>
<td>4.3 ± 0.8</td>
<td>4.5 ± 0.9</td>
</tr>
<tr>
<td>2</td>
<td>UF g/day</td>
<td>26.3 ± 1.1</td>
<td>25.8 ± 1.6</td>
<td>30.1 ± 3.1</td>
<td>8.1 ± 1.5*</td>
<td>18.32 ± 4.5*</td>
<td>25.5 ± 1.4</td>
<td>24.5 ± 1.1</td>
</tr>
<tr>
<td>2</td>
<td>Pref %</td>
<td>14.3 ± 2.9</td>
<td>18.1 ± 3.7</td>
<td>30.1 ± 3.1</td>
<td>33.3 ± 4.1</td>
<td>38.4 ± 5.8</td>
<td>25 ± 4.3</td>
<td>21.6 ± 3.4</td>
</tr>
<tr>
<td>4</td>
<td>SF g/day</td>
<td>12.6 ± 2.2</td>
<td>11.9 ± 2.7</td>
<td>9.6 ± 2.4</td>
<td>9.5 ± 1.6</td>
<td>8.7 ± 2.2†</td>
<td>10.1 ± 1.9</td>
<td>11.0 ± 2.2</td>
</tr>
<tr>
<td>4</td>
<td>UF g/day</td>
<td>19.5 ± 2.2</td>
<td>17.1 ± 2.1</td>
<td>17.4 ± 2.3</td>
<td>18.0 ± 1.5</td>
<td>21.3 ± 2.2</td>
<td>18.9 ± 1.3</td>
<td>19.1 ± 1.0</td>
</tr>
<tr>
<td>4</td>
<td>Pref %</td>
<td>38.4 ± 7.6</td>
<td>40.4 ± 8.5</td>
<td>35.2 ± 8.4</td>
<td>34.4 ± 5.2</td>
<td>28.6 ± 7.2</td>
<td>34.0 ± 5.5</td>
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</tr>
<tr>
<td>4</td>
<td>Na ml/day</td>
<td>2.4 ± 0.9</td>
<td>2.3 ± 0.8</td>
<td>12.9 ± 3.3*</td>
<td>8.7 ± 2.3</td>
<td>3.3 ± 1.1</td>
<td>3.1 ± 1.2</td>
<td>4.8 ± 1.8</td>
</tr>
<tr>
<td>5C</td>
<td>SF g/day</td>
<td>8.6 ± 0.9</td>
<td>8.4 ± 1.2</td>
<td>8.9 ± 1.3</td>
<td>7.2 ± 1.3</td>
<td>8.6 ± 1.6</td>
<td>9.7 ± 2.0</td>
<td>6.6 ± 2.1</td>
</tr>
<tr>
<td>5C</td>
<td>UF g/day</td>
<td>20.3 ± 2.5</td>
<td>19.6 ± 3.1</td>
<td>18.3 ± 1.3</td>
<td>18.5 ± 0.9</td>
<td>14.9 ± 1.7</td>
<td>17.1 ± 2.2</td>
<td>19.9 ± 1.2</td>
</tr>
<tr>
<td>5C</td>
<td>Pref %</td>
<td>30.6 ± 5.0</td>
<td>31.4 ± 7.3</td>
<td>33.4 ± 5.4</td>
<td>27.5 ± 4.1</td>
<td>37.2 ± 6.5</td>
<td>53.2 ± 7.3</td>
<td>23.8 ± 6.6</td>
</tr>
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</table>

AJP-Regul Integr Comp Physiol • doi:10.1152/ajpregu.00481.2012 • www.ajpregu.org
duction in the preference for salted food at this time (Fig. 6). Interestingly, with rehydration, there was a reversal of this effect within 1 day, so that while the intake of unsalted food often remained at a reduced level, intake of salted food reverted to predeprivation levels. The total mass of food ingested fell significantly on both days of water deprivation, so that by day 2, it was only half that of baseline days (Table 1). It then returned to baseline on rehydration. Water intake is shown in Table 2.

Experiment 8. Effect of Water Deprivation for Two Days on Intakes of Two Foods That Were Identical Except for a Low- or High-NaCl Content, Both Having the Same High-Sucrose Content

The aim of this experiment was to determine whether the reduced intake of salted food observed in dehydrated rats in experiment 7 was due to its NaCl content or to another component(s) of the salted food. In experiment 8A, rats were given a choice of salted (0.2% Na-10% sucrose) and unsalted (0.03% Na-10% sucrose) lab biscuits, identical except for NaCl content and with a high sucrose content. There was no clear preference for either food during the baseline period. When deprived of water, body weight fell from 510 ± 46 to 493 ± 45 g after 1 day and to 482 ± 44 g after 2 days of water deprivation. Rats significantly reduced the intake of salted food without changing intake of unsalted food (Fig. 7). A large, significant reduction in preference for salted food was observed on both days of water deprivation. This effect did not persist on rehydration. Total food intake was significantly reduced on both days of water deprivation (Table 1). Water intakes are shown in Table 2.

In experiment 8B, rats were given only one type of food; either salted (0.2% Na-10% sucrose) or unsalted (0.03% Na-10% sucrose lab food (no choice)). The aim of this experiment was to investigate whether dehydrated rats would reduce the intake of unsalted food to the same extent as salted food, if unsalted food were the only choice available to eat. When
deprived of water, rats that ate only salted food showed body weight loss from 441 \(\pm\) 10 to 423 \(\pm\) 10 g and 409 g after 1 and 2 days, respectively, and from 470 \(\pm\) 26 to 458 \(\pm\) 26 and 447 \(\pm\) 26 g in those eating only unsalted food. When there was no dietary choice, rats significantly reduced the intake of salted or unsalted food on both days of water deprivation (Table 1). This effect was similar with either type of food. However, with rehydration, while food intakes reverted back to baseline in both groups, intake of salted pellets was significantly greater than baseline on the second day following rehydration (Table 1). Water intakes are shown in Table 2.

**DISCUSSION**

**Sodium Depletion**

The results show that sodium-depleted rats are able to increase their preference for and intake of salted food regardless of other nutrient and gustatory influences that they may encounter in food. This effect is independent of the type of diuretic agent (either furosemide or acetazolamide) used to produce sodium depletion. Rats that were depleted of sodium and given the choice of two foods that varied not only in sodium content, but also in the content of many other nutrients, increased the intake of and preference for salted food after a 24-h delay (Fig. 1, Table 3). The increase in intake and preference for salted food continued for another 1–2 more days until normal sodium balance (as calculated from urinary losses and intakes of salted food) would have been restored. At this time, the rats once again returned to preferring unsalted food.

Could the observed responses have been due to development of an appetite for another nutrient that had been excreted following diuretic injection? This possibility is unlikely because experiments 3A and 3B showed that rats still increased the intake of and preference for salted food even if the foods were identical in every way except for NaCl content. This

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Could the observed responses have been due to development of an appetite for another nutrient that had been excreted following diuretic injection? This possibility is unlikely because experiments 3A and 3B showed that rats still increased the intake of and preference for salted food even if the foods were identical in every way except for NaCl content. This
result is consistent with previous findings (2, 5). Also, rats that restored sodium balance following furosemide treatment in experiment 4 by drinking hypertonic saline did not alter their intake of salted or unsalted food, showing that if sodium balance were achieved by intake of NaCl solution, then need for other nutrients is not a consideration. All in all, these data show that the increased intake of salted food following diuretic-induced sodium depletion almost certainly reflects an appetite for NaCl in food rather than that for another nutrient, and that rats are able to correctly choose sodium-rich foods from others regardless of varying compositions and other tastes.

An interesting aspect of this investigation was the delay of at least 24 h that elapsed before rats increased their intake of salted food following diuretic treatment, whereas intake of 0.5 M NaCl solution increases within 24 h as shown in experiment 4. This latter observation, together with numerous previous studies (e.g., 16, 19, 43), shows that an appetite for sodium develops in rats within 24 h of them being depleted of sodium. A possible reason for the lack of increased intake of salted food during the first 24 h of sodium depletion could be that rats had already ingested most of their daily caloric intake in food during the first 12 h of the period of sodium depletion. This food intake may have occurred prior to the development of a sodium appetite, the onset of which may be delayed by up to 10–12 h (8). Postigestational satiety signals could have inhibited further food intake, including salted food, during the remainder of that first day of sodium depletion.

In the study of Bertino and Tordoff (2), no observation of appetite for salted food was made during the first 24 h of sodium depletion. However, as in the present experiments, those investigators observed a much greater intake of sodium when it was ingested as a hypertonic NaCl solution than as salted food at 24–48 h after furosemide treatment (2). It was suggested that either the competing tastes in food or greater postigestational inhibitory signals from the gastrointestinal tract related to caloric intake and gastric motility may explain the lesser intake of sodium when it is ingested as food rather than as solution (2). Unlike these investigators, a reduction in

![Fig. 6. Top: preference for salted food (expressed as % of total food intake; n = 9). Middle: daily intakes of salted (×, solid line) and unsalted food (open circles, dashed). Bottom: total body water (n = 5 rats) in rats that were deprived of water for 2 days. Rats were given a choice of salted (0.5% NaCl-4.6% sucrose) or unsalted (0.02% NaCl-10% sucrose) commercial pellets. Values are expressed as means ± SE. Significant differences from the final predehydration value are denoted by *P < 0.05 and **P < 0.01, using Dunnett’s test.](http://ajpregu.physiology.org/)

![Fig. 7. Preference for salted food (expressed as % of total food intake) and daily intakes of salted and unsalted food of rats that were deprived of water for 2 days. Rats were given a choice of salted (0.2% NaCl) or unsalted (0.03% NaCl) lab biscuits that were otherwise identical and contained 10% sucrose. Values are expressed as means ± SE. Significant differences from the final predehydration values are denoted by **P < 0.01, using Dunnett’s test.](http://ajpregu.physiology.org/)
overall food and energy intake with the onset of sodium depletion was observed in the present study (Table 1). It is possible that this anorexic effect of sodium depletion could be exerting a “brake” on the ingestion of salted food that would delay and diminish its intake after diuretic treatment. Nevertheless, intake of salted food does eventually increase from 24 to 72 h after diuretic treatment despite a continuation of the anorexic influence of sodium depletion that is manifested particularly in the large reduction in intake of unsalted food. As well, it is likely that the increased intake of salted food during the 72 h after diuretic treatment is precisely regulated so that normal sodium balance is restored. This contrasts with apparent excessive intake of NaCl when it is provided as a hypertonic solution to sodium-depleted rats that was observed here and by others (2).

The pronounced reduction in intake in unsalted food (Figs. 1 and 2) was the initial behavioral response to sodium depletion. It occurred regardless of the other components of the unsalted food, including sucrose, or of the type of diuretic used, and despite the fact that rats normally had a much greater preference for unsalted food compared with salted food. This response was more rapid than the increased intake of salted food, occurring within 24 h of diuretic treatment, and it lasted for up to 72 h. It also resulted in an overall reduction in total mass of ingested food and energy intake (Table 1), despite the eventual increase in intake of salted food. Others have also observed reduced intake of unsalted food during a 2–4 h test period following 48 h of sodium depletion that was concomitant with increased intake of salted food so that there was either unchanged or reduced overall food intake (2, 5). It was suggested that reduced intake of unsalted food in sodium-depleted rats is merely a “trade-off” for the increase in intake of salted food, so that increased energy intake does not occur (2). However, because the reduction in intake of unsalted food occurred during the first 24 h of sodium depletion, well before any increase in intake of salted food, other factors are likely to be involved as well.

Rats normally prefer food with a lower content of sodium (1, 25). In the present experiments (experiments 1 and 2), the greater sucrose content (10%) of unsalted food compared with salted food (4.6%) is also likely to be a factor contributing to the preference of sodium-replete rats for the pelleted unsalted food, because when 10% sucrose was included in salted, as well as unsalted food (experiments 3A, 8A), no clear preference was observed. While an anorexic effect of sodium depletion will contribute to the reduction in intake of unsalted food, the change in preference away from the sucrose-rich unsalted food in those experiments probably reflects a marked reduction in reward associated with sucrose-containing food when rats are sodium deficient. Studies of preference for salted or sweetened solutions have shown that preference for sucrose solution diminishes with the onset of sodium depletion when rats were adrenalectomized (12). In addition, taste sensitivity of nerve fibers in the chorda tympani or neurons within the nucleus tractus solitarius receiving primary afferent signals from sweet and salt receptors is changed by Na depletion (6, 15). From the point of view of alleviation of sodium deficiency, if the rewarding effect of sucrose in food that contains little sodium (but provides necessary caloric intake) is not diminished in sodium-depleted animals, then there may be little incentive for such animals to seek out other rewarding nutrients, such as salt-containing food (that will also provide calories) to replenish their Na deficit. Other factors beside the reward aspects of sucrose also play a role, because a marked reduction in intake of unsalted food still occurred with Na depletion in experiment 3B, even though it did not contain any sucrose.

It is possible that a conditioned taste aversion (CTA) may be playing a role in the altered food preferences that were observed following furosemide treatment, because there is evidence of CTA at a 10-mg (equivalent to ~40 mg/kg) dose of furosemide (37). A CTA is unlikely to explain the large reduction in intake of unsalted food following furosemide treatment because 1) a similar reduction of intake of unsalted food with Na depletion was observed when acetazolamide was used to cause sodium depletion; 2) this effect occurred on the initial treatments with diuretics, and 3) the dose of furosemide that was used was only one-half of the dose causing CTA (37), and CTA has not been observed with doses of furosemide (2, 3) lower than this.

Most rats studied underwent more than one episode of Na depletion. Repeated episodes of sodium depletion result in a potentiation of intake of hypertonic NaCl solution (28). However, it has been shown by other workers that there is no potentiation of intake of salted food with repeated episodes of sodium depletion and repletion (2), suggesting that it is unlikely that enhancement of sodium appetite by prior episodes of sodium depletion is influencing results here.

It is generally accepted that the renin-angiotensin system plays a crucial role in the generation of salt appetite in sodium-depleted animals (16, 19, 29, 41), in part, by actions of circulating ANG II on the subfornical organ (42) and in synergy with mineralocorticoid action (11, 16, 29). Effective blockade of the renin-angiotensin system in rats has been shown to severely inhibit the intake of saline solutions in sodium-depleted rats (19, 41). In the present study, treatment with the ANG AT1 receptor antagonist valsartan (7) completely inhibited the appetite for salted food during sodium depletion (Fig. 4). This result shows that there is probably a common ANG II-mediated pathway that drives the intakes of saline solution and salted food by sodium-depleted rats. Interestingly, valsartan treatment did not inhibit the reduced intake of unsalted food in sodium-depleted rats, showing that it is independent of ANG II mechanisms.

DOCA treatment. The mineralocorticoid agent DOCA has long been known to be a powerful stimulus of salt appetite (22, 23, 34, 44) and stimulation of mineralocorticoid receptors in the brain may drive increased intake of NaCl solutions (11). The present study shows that this stimulation of salt appetite by DOCA extends to increased intake of salted food. However, unlike the condition of sodium depletion, where a large reduction in intake of unsalted food occurs prior to the onset of salt appetite, with DOCA treatment, the intake of unsalted food while being reduced, only balanced the increase in intake of salted food (Table 1). Thus, there was no overall change in the total mass of food ingested and the reduction in intake of unsalted food with DOCA treatment may have been a caloric “trade-off” for increased intake of salted food.

Dehydration. In contrast to the increased preference for salted food observed in salt-depleted rats, loss of 6–10% of body water caused by water deprivation resulted in a pronounced reduction in preference for and intake of salted food. This response to dehydration was observed whether or not
components of the food choices other than sodium also differed (as in experiment 7) or were identical (experiment 8A), showing that sodium preference was diminished by dehydration. While it has been shown previously that a sodium appetite follows a period of water deprivation once thirst has been quenched upon rehydration (9, 10, 32, 43), the present results show that sodium appetite is inhibited in dehydrated rats. Indeed, it could be considered that an inhibition of salt appetite may be beneficial for water-deprived animals in that a reduced intake of sodium would lessen the hypertonic load being imposed on their body fluids. In this regard, such a reduction in intake of salted food would act in harmony with the natriuresis that occurs in dehydrated animals (26) to ameliorate an inevitable increase in their plasma osmolality. Compared with sodium-depleted rats, dehydrated rats have been shown to have a reduced preference for 0.5 mol/l NaCl solution vs. 0.4 mol/l sucrose (38). Consistent with reduced preference for salted food in dehydrated rats is the aversion to hypertonic saline solution that develops when mice become dehydrated (20), a response mediated by Na sensors in the subfornical organ (20). In the dehydrated rat, it is likely that there are opposing influences on sodium appetite. Reduced extracellular fluid volume, body sodium deficit (due to dehydration-induced natriuresis), and increased activation of the renin-angiotensin system provide stimuli that should drive sodium appetite in dehydrated rats. However, increased plasma sodium concentration and intracellular dehydration are strong inhibitory signals to sodium intake (8) in dehydrated animals. These inhibitory influences may prevail as dehydration progresses. However, on rehydration, they will be rapidly dissipated, leaving stimulatory influences on sodium intake in the ascendancy, because rehydrated animals are still depleted of sodium. As a result, the renin-angiotensin system will still be activated, and extracellular volume will be depleted until enough sodium has been ingested in food to correct the deficit.

Interestingly, there was a rebound in preference for salted food in the immediate 24 h following rehydration, sometimes intake of salted food reaching a level greater than that in the predehydration period (Table 1). This observation is consistent with the idea expressed above that animals suppress a salt appetite as they deplete themselves of sodium when they become dehydrated, and this suppression of salt appetite is dissipated upon rehydration. There is evidence that increased circulating or central levels of oxytocin, which result from dehydration in rats, have an inhibitory influence on salt appetite (36, 39). Therefore, it is possible that a suppression of sodium appetite in dehydrated rats is extinguished upon rehydration because elevated oxytocin levels fall with rehydration.

Like many others previously (10, 18, 32, 33, 40), we observe an overall reduction in total food and caloric intake in dehydrated rats. In experiment 8A, this reduction of caloric intake was achieved entirely by a reduction in the intake of salted food, with no change in intake of unsalted food. Yet in experiment 8B, where only one food was provided at a time, similar reduction in intakes of salted or unsalted food was observed during the period of water deprivation (Table 1). This result shows that caloric intake is tightly regulated in dehydrated animals and that sodium intake is regulated within the context of the requirement for energy balance.

**Perspectives and Significance**

The overall impression gained from these results was the adeptness with which rats regulate the intakes of different foods of varying composition so that sodium needs and osmoregulatory homeostasis are achieved and balanced with energy requirements. The pronounced reduction in intake of unsalted food by sodium-depleted rats before they increased their appetite for sodium was unexpected. A centrally mediated mechanism causing an inhibition of intake of normally rewarding unsalted food may be an important homeostatic response to sodium depletion. This response, together with subsequent generation of a salt appetite, will lead animals to ingest salted food causing the restoration of bodily sodium balance. Studies showing that both opiate and dopamine antagonists reduce the intake of sodium solution in salt-depleted animals (4, 14, 17, 26) suggest that central reward mechanisms are stimulated by the intake of sodium in these animals. We propose that the reward value of salted food increases, while that of unsalted food decreases in sodium-depleted rats. Conversely, salted food appears to become aversive in dehydrated animals.

A reduction in overall food intake in both sodium-depleted and dehydrated rats was consistently observed. While an anorexic response may have benefits in regard to reducing the osmotic load of dehydrated animals, the benefit to be gained in Na-depleted rats is not immediately evident. Oxytocin may have a role in the signaling mechanisms associated with dehydration-induced anorexia (21, 24). However, it is unlikely that oxytocin mediates the anorexia associated with sodium depletion because oxytocin has an inhibitory influence on sodium intake (36, 39). Such an influence would be inappropriate in a sodium-depleted animal. Other factors that could influence intakes of salted and unsalted food in dehydrated, as well as sodium-depleted rats, are nervous and humoral signals arising from changes in cardiovascular function related to depletion of the extracellular volume and sympathetic nerve activation. These factors have not been investigated in the present study. The signaling mechanisms that underpin the anorexic effects of sodium depletion and dehydration have yet to be fully elucidated and may differ for each of these metabolic states.

In conclusion, while the temporal resolution of appetitive responses in these studies of sodium intake in food is limited, they do allow investigations of some inhibitory influences on sodium intake that may not be possible when investigating intake of NaCl as solution. They also provide insights into the changes in appetite for unsalted food and may be an experimental model that resembles more closely the sodium intake that occurs in nature.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

Author contributions: M.J.M. conception and design of research; M.J.M. performed experiments; M.J.M. analyzed data; M.J.M. interpreted results of experiments; M.J.M. prepared figures; M.J.M. drafted manuscript; M.J.M. edited and revised manuscript; M.J.M. approved final version of manuscript.

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