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Multiple Factors in the Satiation of Salt Appetite

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There is evidence in the literature that has been taken to show that, unlike hunger and thirst, salt appetite cannot be satiated in the absence of salt taste stimulation. The present study showed that repletion of body sodium in the absence of taste stimulation, that is, by gavage, can diminish subsequent saline intake The satiating effects of gavage versus drinking of saline were studied at various intervals after repletion. For the first few hours, gastric loading was constantly less satiating than was drinking. But as the interval between gavage and testing was lengthened beyond 4–8 hr, the satiating effect began to increase until by 16 hr it was equal to that of drinking. The specificity of the satiating effect of saline gavage as a function of time between treatment and testing was also studied. There appeared to be a transient nonspecific blocking effect of solutes on solute intake which had a duration of less than 30 min. The satiating effect of saline gavage became specific after that time. The experiment suggests that there are multiple factors involved in the satiation of salt appetite—a taste factor, a short-latency post ingestional factor, and a long-latency postingestional factor.

This study was initially motivated by experiments that seemed to show that salt delivered directly into the stomach does not satisfy the sodium-deficient rat's craving for salt (DiCara & Wilson, 1974; Nachman & Valentino, 1966). It is well known that taste information is especially important in the regulation of salt intake in the rat, and the above findings have been interpreted to mean that tasting salt during the normal act of ingestion is essential for reducing the drive. But although there are several lines of evidence supporting this notion (e.g., Morrison & Young, 1972; Smith, Holman, & Fortune, 1968), there is also evidence against it (Fitzsimons & Wirth, 1976; Levy & McCutcheon, 1974).

The above interpretation seemed unlikely to us for several reasons. First, the notion that taste input is the sole mechanism for satiation implies an essential difference between salt appetite, on the one hand, and hunger and thirst, on the other, because these drives can be reduced without taste stimulation by gastric loading or parenteral repletion (e.g., Epstein, 1965; Miller & Kessen, 1952; Miller, Sampliner, & Woodrow, 1957). Second, regulatory systems of the body typically involve multiple control mechanisms, and so if taste were the only control of satiation, the salt appetite system would be atypical in this way, too. Third, the statement that taste stimulation is necessary to reduce a salt drive must be qualified in terms of temporal variables. Suppose the body sodium of a deficient rat with a strong salt craving were restored via a nonoral route, bypassing the taste receptors. Even if there were no immediate reduction of the craving, presumably the craving would gradually dissipate—certainly it would not last forever (see Kissileff & Hoefer, 1975, for a relevant study). Fourth, there was, in fact, a distinct

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tendency for a suppression of saline intake following gastric saline loading in the studies of both Nachman and Valentino (1966) and of DiCara and Wilson (1974), but the critical statistical tests did not reach significance.

The main aim of the present series of experiments was to determine the effects of body sodium repletion by gastric infusion of saline on subsequent saline intake in sodium-deficient rats as a function of time between gavage and salt appetite testing. We hoped that a careful analysis of temporal variables would reveal various satiation effects with differing latencies and that such findings would provide fundamental data and clues to guide subsequent investigations of the physiological and neural mechanisms mediating the satiation of the salt drive. In the course of the study, questions arose as to both the generality and the specificity of some of the findings, so additional experiments were added to answer these questions.

General Method

Subjects

Female and male mongrel rats were used. They were randomly interbred from various black, brown, hooded, and albino strains obtained from local distributors and from the breeding colony of the National Institutes of Health. The reason for using rats of mixed strains is that we were interested in elucidating basic mechanisms that are common to the species, and we wanted to minimize the change that any findings were peculiar to inbred laboratory strains. The rats also varied in age from about 60 days to a year, and in body weight from about 150 to 700 g. Within the individual experiments, the weights of the rats generally varied by about 150 g for females and 250 g for males. Presumably these differences in strain and body weight added to the between-subjects variability. We counteracted this in two ways—the first was to use a large number of subjects, and the second was to run each subject in each condition.

Maintenance and Adaptation

The rats were bred and raised in a colony room kept on a reversed 12.12 hr day/night cycle. They were housed in plastic cages in groups of 4 with Purina chow and water ad lib. Different rats were used in each of the experiments of this study except as noted. Before the rats were run in the experiments, they were adapted to the experimental treatments and testing conditions. Each rat was depleted of body sodium at least once and given saline to drink in a test cage. Also, each rat was intubated and given water and/or saline about five times. These procedures are described in detail below.

Sodium Depletion

Groups of 4 rats were transferred from plastic home cages, which had absorbent bedding on the floor, to hanging wire mesh group cages to minimize access to urinary sodium during a 2-day sodium deprivation period. They were given deionized water and a sodium-deficient mash ad lib. The mash was composed of 1 part Kretschmer's wheat germ (3.5 mg of sodium per 100 g), 1 part corn starch, and 0.04 part non-sodium salt mixture (Nutritional Biochemicals Cat. No. 902910) mixed with 2 parts deionized water. At the beginning of the second day of deprivation, the rats were injected with 2.5, 5.0, or 10.0 mg of furosemide to induce a natriuresis. This range of doses produces a moderate-to-strong salt appetite by the end of the second day (see Wolf, 1982, for further details and rationale for the procedures).

Sodium Repletion

Repletion of body sodium was accomplished by a gavage of 5 ml of 3% saline or by giving the rats 5 ml of 3% saline to drink. This provides about 2.5 mEq of sodium According to an earlier dose-response study (Wolf, 1982), the total amount of body sodium lost during a 2-day depletion period is as follows for doses of furosemide of 2.5, 5.0, and 10.0 mg per rat—for females weighing between 200 and 300 g, 1.3, 1.8, and 1.9 mEq and for males between 400 and 500 g, 1.8, 2.3, and 2.6 mEq, respectively. In our experience, giving doses on a per-kilogram basis does not substantially reduce between-subjects variability across at least this range of body weights. Also, see Wolf (1982) for sex differences in sodium excretion and intake responses to furosemide. Gavage was performed without anesthesia by passing a 0.3-mm OD French catheter through the mouth and into the stomach while the rat was held in the experimenter's hand. This procedure took about 20 s. For repletion by drinking, the rats were placed in test cages with a small drinking tube containing 5 ml of solution. They generally drank the 5 ml in less than 10 min, but they were given more time if necessary. Rats in other conditions were also put in a test cage but without any saline available so that the treatments were as similar as possible. There was always a water tube in the test cage so that the rats could slake any thirst resulting from the drinking or the gavage of saline. When the interval between repletion and testing was longer than 1 or 2 hr, the rats were returned to the sodium deprivation cages where the sodium-deficient mash as well as water was available until the test time in order to avoid any deficiency other than sodium during the test.

Salt Appetite Test

The test cages were 25 cm long × 9 cm wide and made of wire mesh. Two 15- or 50-ml inverted centrifuge tubes fitted with drinking spouts were attached to the fronts of the cages, one in the upper left quad-

rant and one in the lower right quadrant. The left tube contained a saline solution, and the right tube contained deionized water. The saline solution was either a 0.5% basic (pH = 12% by means of NaOH) NaCl solution or an unadulterated 3% NaCl solution. Unadulterated hypotonic saline solutions are palatable to the rat, but the 0.5% basic saline is unpalatable. The amount of an unpalatable solution that the rat is willing to ingest can be taken as an index of the intensity of its drive (Miller, 1967). The advantage of a weak solution is that it minimizes additional satiating effects of ingestion of the test solution itself. The tests varied in duration from 30 min to 120 min. Intakes of saline and water were measured to the nearest 0.5 ml at 5-, 10-, 20-, or 30-min intervals. Water intakes were analyzed but yielded no relevant data, so they are not presented in the results of the experiments.

Statistical Designs

Except for the first two experiments, the designs were standard analysis of variance designs for repeated measures or independent groups. In the first two experiments, we used less systematic designs, and the rationale and statistical methods are described in Method of Experiment 1.

Experiment 1

In this experiment, we determined the satiating effects of saline gavage as a function of time. The effects of repletion of body sodium by gastric infusion were compared with those of repletion by drinking. Also, urine sodium excretion was measured under each condition to compare intakes and outputs.

Method

There were 11 conditions as follows. Two conditions were run to determine baseline intakes. The first baseline condition was a nondepletion condition. The rats were given the sodium-deficient diet for 2 days but no furosemide treatment (see Wolf, 1982, for evidence that this has no observable effect on salt appetite under the present conditions). The second baseline condition was a nonrepletion condition. The rats were depleted of body sodium (sodium-deficient diets plus 2.5 mg of furosemide) but not repleted prior to salt appetite testing. Half of the rats in the condition were simply intubated 1–2 hr before testing, one fourth were given a gavage of water 1–2 hr before testing, and one fourth were not intubated at all. In three repletion by drinking conditions, the rats were depleted of body sodium as above and repleted by drinking saline 0.5, 2, or 8 hr before testing. In six repletion by gavage conditions, the rats were depleted as above and then repleted by gavage of saline 0.5, 1, 2, 4, 8, or 16 hr before testing.

It should be noted that 2.5 mg of furosemide induces a relatively low level of drive. We reasoned that the chances of finding an effect of repletion by gavage would be maximized if the need were small compared with the amount of sodium loaded. Later experiments demonstrate the phenomenon with higher doses of furosemide.

For the reasons noted in General Method (Salt Appetite Test), we used a 0.5% basic saline solution as the test solution in this experiment. Rats given a 2.5-mg dose of furosemide drink about 5 ml of this solution in the first 10 min of the test. This provides only 0.4 mEq of sodium, whereas the 2-day negative sodium balance is well over 1.0 mEq. In this test, saline intake was measured for 30 min, but the intake of the first 10 min was used as the principal datum for analysis

Because we did not know how much variability we would find in the various conditions, the number of rats to be run in each condition was not set prior to running the experiment. A total of 128 female rats were used. Each rat was run in several of the conditions in a roughly randomized order. No rat was run more than once in any given condition. Tests were run once or twice per week until the *n*s for the nondepletion condition and for the three repletion by drinking conditions were 30 per condition and the *n*s for the nonrepletion condition and for the six repletion by gavage conditions were 60 per condition. This testing schedule resulted in a statistical design in which comparisons among conditions involved some dependent and some independent scores. The methods for statistical analysis of such data are limited, and none seems to be exactly applicable (e.g., Winer, 1971, pp. 49–50). To be conservative in our analysis, we used the Scheffé test for multiple comparisons—one of the least powerful tests in its class (Kirk, 1968, pp. 69–98).

Twenty-four-hour urine samples were collected in pans placed under wire mesh cages from pairs of rats in each of the 11 conditions. This 24-hr period spanned the interval between furosemide injection and testing and included urine excreted both before and after repletion. The contents of the pans were collected by rinsing the pans with water and diluting the filtered fluid to 100 ml Samples of the fluid were anlayzed for sodium by flame photometry. There were two sets of 24-hr samples. In the first set there were 5–13 pairs of rats in each of the 11 conditions. The second set of 24-hr urines was collected about 1 year after the first set to check out uncertain results. There were 6–18 pairs of rats in each of the six gavage conditions and in the nonrepletion condition. The urines of these pairs were pooled, so that there were three samples analyzed for sodium in each of the conditions.

We also wanted to get measures of urine volume and sodium concentration after repletion by drinking and repletion by gavage. Therefore, 2-hr urine samples were taken from individual rats in metabolism cages in the 2-hr repletion by drinking condition and the 2-hr repletion by gavage condition during the period between repletion and testing (n = 12 per condition).

Results and Discussion

Figure 1 gives the main results—10-min saline intakes under each of the 11 condi-

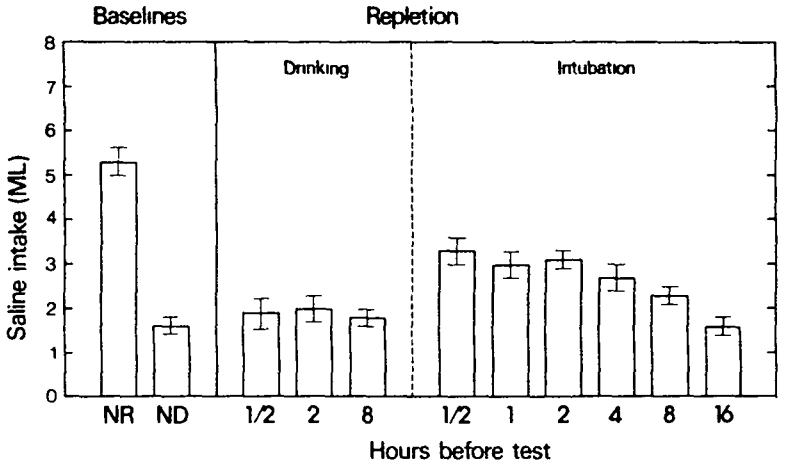

Figure 1 Mean 10-min intake (in ml) of 0.5% basic saline in each condition. (Vertical lines = *SE* NR = nonrepleted condition, ND = nondepleted condition.)

tions. We consider first the answer to the question that motivated the experiment, "Will repletion of body sodium by gavage reduce subsequent saline intake?" The answer is clearly "yes." When the rats were depleted but not repleted (NR condition), they ingested about 5 ml of the saline. (There were no observable differences between water-loaded, sham-loaded, and nonloaded subgroups.) But when the rats were repleted by gavage (RI condition) 0.5–16 hr before the test, they ingested only about 3 ml or less. Saline intakes at each of the six RI intervals were significantly lower than that of the NR conditions ($ps < .01$).

We consider now some additional interesting results. Saline intakes of RI rats (i.e., rats in the RI condition) seem to diminish as a function of time. Although the 16-hr mean was the only one that differed significantly from the others ($p < .05$, compared with 0.5–8-hr mean; $p < .01$, compared with .5–4-hr mean), the trend downward seems to begin at 4–8 hr. It should be noted that the p values reported in the text are lower than would be expected from inspection of the mean differences and standard errors shown in FIgure 1. This is the result of using the conservative Scheffé test.

The repletion by drinking (RD condition) scores are constant at about 2 ml across the 0.5-, 2-, and 8-hr intervals. They are significantly lower than the corresponding RI scores ($p < .05$ for the three intervals combined). This difference was significant for the 0.5-hr interval alone ($p < .05$) but not for the 2- and 8-hr intervals. The intakes at the 16-hr RI interval are diminished to the level of the RD condition—about 2 ml. Mean intake in the nondepletion (ND) condition is also about 2 ml, but this identity is a function of the particular amount of depletion and repletion induced in the rats in this experiment and has no general significance.

Table 1 gives the 30-min saline intake scores. They are from 20% to 35% higher than the 10-min scores, but the general pattern of results is the same. The main difference between the 10- and 30-min patterns is that the mean score of the 8-hr RI interval is relatively less diminished and the mean score of the 2-hr RI interval is relatively elevated at 30 min. We consider these results to be unreliable.

It is of interest to know the relation between saline intake and sodium output scores. The total amounts of sodium lost during the 24 hr between furosemide injection and appetite testing are given in Table 2. The relevant results are as follows. Total sodium excretion in the 0.5-repletion conditions (both by drinking and gavage) ranged around 0.8 mEq. Thus, the differences in saline intake among these conditions cannot be explained in terms of sodium loss. At repletion-testing intervals of 2–16 hr, the total sodium excretion scores were more variable, but a roughly twofold increase over the scores of the NR and the

Table 1
Mean 30-min Saline Intakes at Specified Intervals (in hr) for Each Condition

	Baseline		Drinking			Intubation					
Value	NR	ND	0.5	2	8	0.5	1	2	4	8	16
M	7.4	2.1	2.5	2.7	2.8	4.1	3.8	4.6	3 5	3.5	2.5
SE	0.4	0.3	0 3	0.4	0.3	0.3	0.3	0.4	0.4	0.3	0.2

Note NR = nonrepleted condition; ND = nondepleted condition.

0.5 conditions was evident in most cases (*ps* < .05 in both the first and the second test). (Although the first test indicates a possible diminution in total sodium excretion in the 16-hr RI condition, this effect was not significant and was not replicated in the second test in which the 8-hr score is inexplicably high.) Thus, the diminution in saline intake, which seems to begin at 4–8 hr and becomes statistically significant at 16 hr, is probably not paralleled by a diminution in sodium excretion.

In the first test in Table 2, the sodium excretion in the 2- and 8-hr RD conditions is somewhat lower than in the corresponding RI conditions. However, the 2-hr urine excretion results shown in Table 3 do not confirm this tendency. Total sodium excretion was the same (0.9 mEq) under the RD and RI conditions. The other measures—water intake, urine volume, and urine sodium concentration also did not differ significantly.

Table 2
Sodium Excretion (in mEq/rat) During 24-hr Period Between Furosemide Injection and Salt Appetite Test

	First test			Second test		
Condition	*N*	*M*	*SE*	*N*[a]	*M*	*SE*
Nonrepletion	11	1.05	0 26	4,4,4	0 75	0.11
Nondepletion	10	0 38	0.07			
Repletion by drinking						
0.5	6	0.83	0.15			
2	5	1.20	0.18			
8	13	1.39	0.19			
Repletion by gavage						
0.5	6	0.80	0.24	2,2,2	0.85	0.03
1	8	0.81	0.24	5,5,6	0.75	0 05
2	9	2.09	0.38	3,3,4	1 44	0.32
4	6	1.88	0.53	6,6,6	1.35	0.19
8	9	1.90	0.22	2,2,2	2.45	0.24
16	12	1.31	0.18	4,4,4	1.42	0.20

Note The *N*s represent pairs of rats housed together, but the means represent values for individual rats rather than for pairs (i.e , total sodium output of pairs of rats divided by 2).

[a] The three numbers in each row represent the numbers of pairs of rats in each of the three pooled samples. Each mean and standard error is based on an *N* of 3 and disregards the number of pairs in each pooled sample.

Our main conclusions from this experiment are as follows. First, the taste of salt is not necessary to satiate the craving for salt, although it contributes strongly to satiation. Second, the degree of satiation resulting from infusion of saline is a function of time between gavage and testing. The data suggest a gradual increment in the intensity of the satiation which begins after a 4–8-hr interval. The degree of satiation reaches that of normal ingestion somewhere between 8 and 16 hr after gavage. The theoretical implications of these conclusions are presented in the General Discussion.

Experiment 2

Experiment 1 differed in several procedural details from the experiments of Nachman and Valentino (1966) and of DiCara and Wilson (1974), which failed to find a significant satiating effect of gastric loading of saline, and the difference between our findings and theirs may be due to one or more of these procedural differences. Two procedures of Experiment 1 that seemed most likely to be relevant were the use of female rats and of a hypotonic saline test solution adulterated with NaOH. The two earlier studies used male rats and a hypertonic unadulterated saline test solution. In the present experiment, we compared the effects of repletion by drinking and those by gavage in male rats tested with 3% saline.

Method

Forty-three male rats were run in two or more of four conditions in a mixed design as in Experiment 1

Table 3
Mean 2-hr Water Intake and Urine Excretion Measures After Repletion by Drinking or by Gavage

	Repletion by drinking				Repletion by gavage			
Value	Water intake (in ml)	Urine volume (in ml)	Na (in mEq/liter)	Total Na (in mEq)	Water intake (in ml)	Urine volume (in ml)	Na (in mEq/liter)	Total Na (in mEq)
M	2.5	5 1	181.3	0.9	4.6	5.6	163.7	0.9
SE	0.6	0.5	13.8	0.1	0.9	0.8	15.4	0.2

until there were 30 scores in each of the conditions The four conditions included ND, NR, RD, and RI treatments as in Experiment 1. The interval between repletion and testing was 1 hr The earlier studies used either a 2-hr or a 0 5-hr interval. The dose of furosemide was increased to 5 mg because male rats ingest only half as much saline as females given equal doses of furosemide (Wolf, 1982). The rats were given a 3% saline solution during the 30-min test period. All other procedures were identical to those of Experiment 1.

Results and Discussion

The mean 10-min saline intakes are shown in Figure 2. The overall pattern of results is similar to that of Experiment 1. Most important, the RI condition caused a significant diminution in saline intake in comparison with that caused by the NR condition (4 ml vs. 6 ml; $p < .01$). Again the diminution was not so great as that in the repletion by drinking condition (1.5 ml; $p < .01$). Repletion by drinking did not reduce intake to the ND baseline level as in Experiment 1 possibly because of the greater sodium loss induced in this experiment. The 30-min scores showed the same overall pattern and the same levels of statistical significance and need no further discussion here.

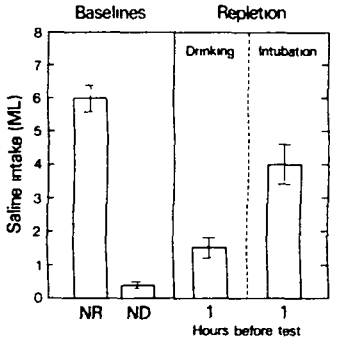

Figure 2 Mean 10-min intake (in ml) of 3% saline in each condition. (Vertical lines = *SE*. NR = nonrepleted condition, ND = nondepleted condition.)

The results show that the satiating effect of gastric saline loading is not limited to the conditions of the first experiment which are uncommon in salt appetite research—the use of female rats and of a saline drinking solution with a pH of 12. However, the fact that this experiment used male rats and hypertonic saline solution, as did the two earlier experiments (DiCara & Wilson, 1974; Nachman & Valentino, 1966), should not be interpreted as a nonreplication of the earlier findings. There are several other differences in procedure between this experiment and theirs. But this experiment does provide evidence against one interpretation of their results, namely, under common experimental conditions, and in general, salt taste stimulation is essential for satiation of salt appetite.

Experiment 3

It is possible that direct gavage of hypertonic saline is stressful and that the early diminutions of saline intake observed in the preceding experiments were not due to any specific function of sodium repletion but to a general disruption of behavior. Although a nonspecific stress effect seems unlikely when several hours intervene between gavage and testing, it might play a role when the interval is an hour or less. In other words, even though we found in Experiment 1 that the diminution resulting from a saline gavage given 0.5 hr before testing is about the same in magnitude as one given 2–4 hr before testing, it could be that the former is entirely due to a nonspecific effect of stress whereas the latter is a specific effect of sodium repletion. So there might be no real short-term salt appetite satiating effect of repletion by gavage after all.

The exact roles of stresses or other behavioral disruptions in the early satiating effect of saline load are not easy to determine. We decided that it would be sufficient for the present purposes to merely compare the inhibitory effect on food intake of a saline load administered by gavage with the effect of the same amount voluntarily ingested by the rat. This could at least determine whether our gavage procedure has more of an inhibitory effect on intake than does normal drinking. In order to make the conditions the same as those of the preceding experiments, the sodium depletion and repletion conditions of Experiment 1 were repeated except that a brief period of food deprivation was added to induce a mild hunger. The effect of drinking versus gavage of 5 ml of 3% saline on milk intake was observed.

Method

Forty-eight female rats were used. In addition to the standard adaptation procedures preceding the tests, the rats were given a mixture of 1 part Borden's Magnolia Condensed Milk and 1 part water in a drinking tube in their home cages for several hours per day for 3 days. Sodium depletion was induced by 2 days of dietary sodium deprivation supplemented by an injection of 2.5 mg of furosemide as in Experiment 1. Twelve hours before the test, the food was removed from the cages (water was available ad lib at all times). Thirty minutes before the test, 24 of the rats were given 5 ml of 3% saline to drink, and 24 were given the saline by gavage. For the test, the rats were given the 50% condensed milk and also water for 30 min. Intakes were measured at 10 and 30 min as usual. It should be noted that this experiment uses two independent groups and no repeated measures.

Results and Discussion

The milk intakes of the two groups were nearly identical—7.9 ml at 10 min and 9.1 ml at 30 min for the RD condition and 7.5 and 8.7 ml for the RI condition. The results suggest that the reduction in saline intake induced by saline gavage administered shortly (0.5–1 hr) before salt appetite testing is not due to a nonspecific stress effect. Thus we tentatively interpreted the data of the preceding experiments as indicative of a real short-latency satiating effect of gastric saline loading. The results of the succeeding experiments supported this interpretation.

Experiment 4

The foregoing experiments showed that repletion of body sodium in the absence of normal ingestion and taste stimulation can reduce subsequent saline intake within about 0.5 hr after gastric infusion of saline. It was of interest to us to study in more detail the time-response relations between repletion by gavage and that by saline intake within this period. Also the effect of solutes other than sodium chloride needed to be determined.

Method

Thirty-five female and twelve male rats were used. The male rats were more than 1 year old and weighed an average of 700 g. The depletion procedure was as usual except that the female rats (weighing 150–300 g) were injected with 5 mg of furosemide and the male rats were injected with 10 mg. This resulted in similar levels of saline intake in the female and male rats, and their results were combined for statistical analysis. There were three repletion conditions, and each rat was run once in each condition so that the experiment was a simple repeated measures design with orders of treatment counterbalanced. The three conditions were sham gavage (insertion of empty tube), gavage of 5 ml of 3% saline, and gavage of 5 ml of 34% sucrose (about equal to 3% saline in osmolarity). Gavages were given immediately before placing the rat in the test cage where 3% saline and water were available. Intakes were measured at 5-min intervals for 30 min. Preliminary observations indicated that it would be of interest to extend the testing period to 120 min, and so additional measures were taken at 60, 90, and 120 min.

In this and the subsequent experiments, analyses of variance for repeated measures and Tukey tests as well as Scheffé tests were used to determine the statistical significance of the results

Results and Discussion

Cumulative saline intakes measured at 5-min intervals for 30 min are shown in Figure 3. Both saline and sucrose loads caused a significant diminution in saline intake relative to sham gavage within 5 min ($ps < .01$). The diminution was similar in magnitude, roughly 60% of sham intake, for about the first 10 min of drinking, and then the curves began to diverge. Under the sucrose condition, the rats continued to ingest saline; under the saline condition, intake after 10 min approached asymptote. Analyses of differences between these groups at each measurement period indicated that

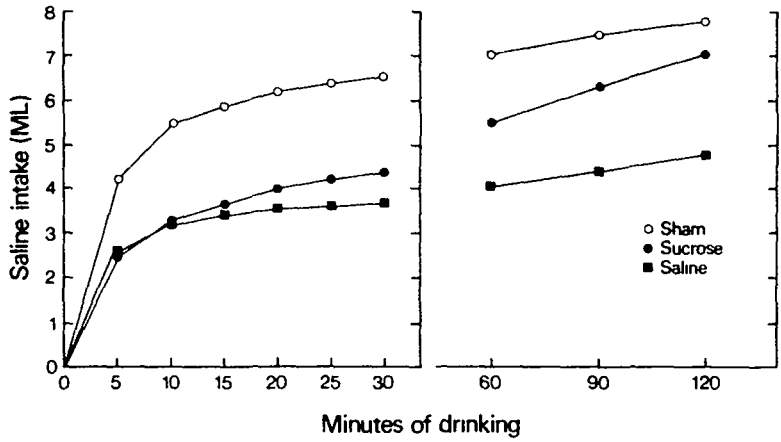

Figure 3 Cumulative mean intake (in ml) of 3% saline after sham, sucrose, and saline gavage. (Left panel: Intake at 5-min intervals during first 30 min of testing. Right panel: Intake at 30-min intervals during subsequent 90 min of testing.)

the first statistically reliable difference occurred at 25 min ($p < .05$). The divergent trends of the sucrose and saline conditions continued through the 120-min period (right-hand panel of Figure 3), so that at the end of the period saline intake in the sucrose condition was closely approaching that in the sham condition (although the 10% difference between the groups was statistically significant, $p < .05$). In contrast, saline intake in the saline load condition remained at 60% of the sham level throughout the entire test period.

The results suggest the following conclusions. For at least the first 10–15 min after gavage, sucrose and saline have identical suppressive effects on saline intake. But thereafter, the rates of intake become increasingly divergent. Saline intake recovers within 15–25 min following sucrose loading but not following saline loading. Therefore, a specific satiating effect of saline loading follows a brief nonspecific inhibitory effect.

The question arises as to the location of the receptors for this satiating effect. There is evidence for receptors in the gastrointestinal tract (Chernigovsky, 1962), the liver (Blake & Lin, 1978), the intravascular compartment (Kaufman, 1983), and the brain (Weisinger et al., 1982). There is evidence that absorption of a hypertonic saline load from the gastrointestinal tract is slow and that only a small proportion is absorbed within 30 min (O'Kelly, Falk, & Flint, 1958). This might be taken to preclude the involvement of receptors outside the gastrointestinal tract in the present effect. However, we do not know the minimal amount of sodium that is effective in stimulating putative extragastrointestinal receptors, and we do not know to what extent the above findings on time course for absorption hold for the present conditions. Thus, it seems best to leave all possibilities open at this time.

Experiment 5

The results of Experiment 4 indicated that a sucrose solution was as effective as saline in suppressing saline intake within a 15–25-min period after gavage. This short-latency inhibitory effect may be due to the effective osmolarity (tonicity) of the solutions, to the solute content independently of osmotic effects, or simply to the volume of fluid deposited in the gastrointestinal tract. (Although we know from Experiment 1 that a water load has no effect on saline intake when there is a delay of 1–2 hr between gavage and testing, it may have an immediate short-lasting effect.) In this experiment, we compared the short-latency effects of gastric infusion of equimolar sucrose and dextrose solutions and of water

on the saline intake of sodium-deficient rats.

Method

There were 12 female and 12 male rats. The female rats were experimentally naive. The male rats were those used in Experiment 4 Furosemide doses for females and males were as in Experiment 4, and again results for females and males were similar and were combined. There were four conditions—sham gavage and gavage of 5 ml of water, 5 ml of 34% sucrose solution, and 5 ml of 18% dextrose solution. Because the *n*s were relatively small in this experiment, each rat was run twice in each condition in a counterbalanced design. The two runs gave similar results and were combined for statistical analysis. The gavage and testing procedures were as described in Experiment 4

Results

Figure 4 shows the cumulative intakes of saline under the various conditions. The four curves differ significantly from each other across the 30-min testing period (*p*s $< .05$ for adjacent curves). Also the relative diminutions in saline intake in the three gavage conditions in comparison with the sham condition tended to be constant at each measurement interval—water about 10%, sucrose about 30%, and dextrose about 40%.

There are two interesting findings—first, the small but significant diminution in saline intake resulting from water loading and, second, the greater effect of dextrose than of sucrose loading in diminishing saline intake. The first finding indicates that there is an inhibitory effect of a gastrointestinal load independent of solute content. The second finding is not so easy to interpret. Certainly it shows that the diminution due to solute content is not attributable to the tonicity of the solution.

It is important to note, however, that tonicity is defined in terms of impermeability of body cells, particularly red blood cells. Neither dextrose nor sucrose crosses the stomach wall in substantial amounts, so that differences in tonicity probably do not play a role at this level. Also, the concentrations of both dextrose and sucrose are monitored in the duodenum, and the rate of gastric emptying is retarded as a function of their concentration or energy value. But, unlike sucrose, dextrose passes

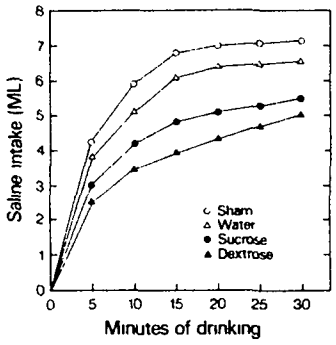

Figure 4 Cumulative mean intake (in ml) of 3% saline after sham, water, sucrose, and dextrose gavage.

freely through the wall of the duodenum by passive diffusion and active transport and so reaches the liver and systemic circulation faster than sucrose does. This may point to a postabsorptive mechanism in the inhibitory effect of these sugars on saline intake. However, other evidence does not support this. In fact, Blake and Lin (1978) found that dextrose infused into the portal circulation *increases* the rat's preference for saline. Weisinger, Denton, and McKinley (1983) found that intravenous infusions of a strong dextrose solution quickly reduce salt appetite in sheep but the effect was less potent than that of mannitol or saline solutions. In view of these considerations, we can offer no adequate account of the finding that dextrose inhibits saline intake more effectively than sucrose does. The finding needs replication and further experimental analyses which are beyond the scope of the present study.

Experiment 6

Another question of specificity arises here. Quite aside from the question of characterizing the properties of solutes that inhibit salt intake, there is the question whether the immediate inhibitory effect of solutes is specific for salt intake or is a general one that will suppress intake of other substances motivated by other drives. The evidence in the literature suggests that the effect is a general one (e.g., McCleary, 1953). As we have seen in the foregoing experiments, the period of observation is a critical factor here. In this experiment, we

looked at milk intake of mildly hungry rats during a 30-min period immediately following various gastric infusions, and we measured intake at 5-min intervals as in Experiments 4 and 5.

Method

There were 8 female and 8 male rats. Four of each sex had been used in Experiment 5, and the other 8 rats were experimentally naive. Before being run in the experiment, the rats were given Carnation evaporated milk in drinking tubes in their home cages for a few hours each day for 3 days and were adapted to a daily 8-hr food deprivation schedule which began at the start of the night cycle. The rats were not deprived of sodium in this experiment because, unlike in Experiment 3, the information that we sought here did not require this manipulation

There were four gavage conditions: sham, 5 ml of water, 5 ml of 3% saline, and 5 ml of 34% sucrose. Gavage was given immediately before putting the rats in the test cages The test cages contained one drinking tube filled with the evaporated milk and one filled with water. Tests were 30 min in duration, with measures taken at 5-min intervals as in the preceding experiments. Each rat was run twice in each condition in a counterbalanced design, and the means of the two trials were used for statistical analysis Results for male and female rats were similar and were combined.

Results and Discussion

Figure 5 shows the cumulative milk intakes over the 30-min drinking period. Compare the sucrose and saline curves in Figure 5 with those in the left-hand panel of Figure 3 (Experiment 4). In both experiments, the sucrose and saline curves are similar in shape and magnitude during the first 10–15 min, and they begin to diverge steadily thereafter. However, in this experiment the saline curve continues to rise, while the sucrose curve approaches asymptote during the remainder of the test. It appears that within a 30-min interval, when the rats are motivated by salt deficiency, saline intake is more depressed by saline than by sucrose load but that when the rats are motivated by hunger, milk intake is more depressed by sucrose than by saline load. However, the difference between these curves did not reach statistical significance at any measurement interval or over the whole 30-min testing period (possibly attributable to the small n in this experiment). On the other hand, the sucrose and saline curves were each significantly lower than either the water or the sham curve ($p < .01$) which did not differ significantly from each other (although there appears to be a tendency for a water load to slightly depress milk intake, as it does saline intake during this period).

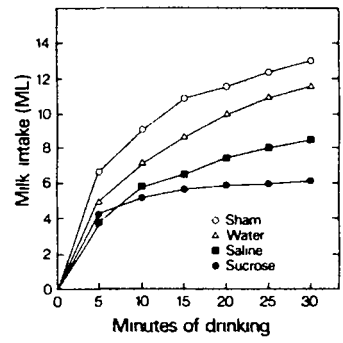

Figure 5 Cumulative mean intake (in ml) of evaporated milk after sham, water, saline, and sucrose gavage.

Although the relevant results did not reach statistical significance, they suggest that the factors involved in short latency suppression of salt appetite and of hunger are the same. And it may reflect a nonspecific inhibitory effect of a solute load on solute intake during the first 10–15 min following gavage. There may also be a transient inhibitory effect of gastric loading independent of solute content on food intake as well as on salt intake.

General Discussion

The experiments showed that body sodium repletion by gastric infusion has a substantial satiating effect on the salt appetite of sodium-deficient rats. The satiating effect had a short-latency onset—apparently between 15 and 25 min. Thus, it appears that the normal ingestion and tasting of salt is not necessary for rapid satiation of salt appetite. But, whereas the satiating effect of gastric infusion was strong and consistent—remaining constant at about 60% of nonrepleted control values between 0.5 and 4 hr following gavage—it was not so pronounced as the satiating effect of drinking during this interval. However, between 4 and 8 hr, the satiating effect

of loading began to increase in magnitude until at 16 hr it equaled the magnitude of the drinking effect.

Consider these results in terms of the concept of multiple factors in the control of alimentary behavior (e.g., Adolph, Barker, & Hoy, 1954). The results suggest the hypothesis that there are two very different types of processes involved in shutting off salt appetite. The first type involves the activation of special satiation mechanisms—a taste mechanism and a postingestional mechanism. These are short-latency mechanisms that monitor the amount of sodium ingested and inhibit further salt intake even though the ingested sodium has not been replenished in all of the body fluid compartments and the receptors for eliciting salt appetite are still registering deficiency. The role of taste in this rapid satiation process has been evident for a long time. We have provided evidence here that there is an additional short-latency postingestional satiation mechanism. But the nature of this mechanism remains to be determined. Although our evidence shows clearly that such a mechanism exists, it does not reveal its characteristics. As we noted in Discussion of Experiment 4, our findings on the latency for the postingestional satiation effect do not put any strong restraints on possible locations of the receptors, and they could be outside the gastrointestinal tract. Therefore, fruitful conjecture about the loci of the receptors and neural pathways mediating postingestional satiation effects must await further experimental analysis.

The long-latency effect of saline loading found in Experiment 1 and also several other facts about body sodium regulation suggest that the second type of process, which shuts off salt appetite, is a slow process of restoration of normal sodium levels in the various body fluid compartments. This results in the deactivation of the receptor systems that elicit salt appetite. So, in addition to the activation of satiation mechanisms, which is an initial, fast-acting process for shutting off salt appetite, there is the second, slow process of deactivation of elicitation mechanisms, which constitutes the final termination of the episode.

There are some facts about body sodium regulation that suggest the hypothesis that a long-latency elicitation mechanism is associated with levels of sodium in one or more of the body's sodium reservoirs. Although an earlier detailed formulation of a "reservoir" hypothesis of salt appetite proved inadequate to account for subsequent findings (Denton, 1982; Stricker & Wolf, 1969; Wolf & Stricker, 1967), the general notion remains quite plausible, especially in the context of multiple factors in sodium regulation. Some of the facts that support this notion are that under natural conditions sodium deficiency develops gradually over weeks of dietary deprivation. The sodium levels of the extracellular compartments have to be defended during this time, and normal levels are maintained by sodium supplied from body sodium reservoirs. This constitutes the "slowly exchangeable pool of body sodium," so-called because this component of total body sodium enters and leaves the reservoirs very slowly. Furthermore, salt appetite is not closely correlated with sodium levels in the extracellular compartments, and in the absence of priming by dietary sodium deprivation or mineralocorticoid treatment, there is a latency of several hours between acute depletion of body sodium and the emergence of salt appetite (Stricker & Wolf, 1969).

Considering the above facts, the hypothesis that a sodium reservoir might have the function of monitoring sodium homeostasis as well as supplying sodium to body fluid compartments in which normal sodium levels must be closely guarded is plausible. With regard to the present study, the hypothesis accounts for the increasing satiating effect of saline loading which begins only several hours after the gavage. Specifically, as the reservoir begins to be repleted during the course of several hours, its receptors stop registering deficiency, and, as a result, the craving for salt dissipates.

We now begin to put together a picture of the overall process by which salt appetite is suppressed and finally terminated. According to our view of the factors involved in satiation, the function of the short-latency satiation mechanisms is to monitor

sodium intake and suppress further ingestion when the amount ingested is sufficient to restore homeostasis. This suppression takes place long before the ingested sodium is distributed to all of the relevant body fluid compartments. In other words, the short-latency mechanisms inhibit salt intake until the slow process of restoration of normal body sodium distribution is completed and the long-latency elicitation mechanisms are shut off.

The signals from the short-latency receptors must have a long-lasting suppressive effect because the redistribution of sodium to the critical fluid compartments takes several hours and saline intake is inhibited during this time. What can be said about the roles of taste and of postingestional factors in this long-lasting suppression, and what kinds of neural and chemical mechanisms might be involved? To begin with, the salt signals from taste receptors presumably cease when salt intake ceases. Yet the satiating effect of the taste signal lasts a much longer time. The results of Experiment 1 provided strong evidence for this. When the rats were given the salt appetite test up to 8 hr after sodium repletion, those in the repletion by drinking condition took less saline than did those in the repletion by gavage condition. Thus, taste signals of about 5-min duration (the average time for drinking the 5 ml of hypertonic saline) can have a satiating effect that lasts at least 8 hr. This suggests that the taste signals activate central reverberating circuits that sustain the satiating effect after peripheral input ceases.

But there are other data that must be considered here. Postingestional signals that normally follow the ingestive act are necessary for the satiating effect of taste signals to persist after taste input has ceased (Mook, 1969). In any case, on the basis of existing data, it seems reasonable to hypothesize that the short-latency satiation signal from both taste and postingestional receptor systems functions by blocking the effect of reservoir receptors on the salt-craving system of the brain. This blocking effect persists until the excitatory input from the reservoir is quelled by restoration of normal reservoir sodium levels and the current episode of salt appetite is permanently terminated. But there is good evidence for other mechanisms in regulating salt intake which we have not yet taken into account. The roles of aldosterone and angiotensin in the elicitation of salt appetite must be considered. Aldosterone circulates in the blood stream at elevated levels for many hours after hypersecretion has ceased, so it could stimulate salt appetite for a significant period of time after salt ingestion. It is also relevant to note that the natrorexigenic effect of aldosterone has a long latency of action and that although angiotensin injected into the brain has a natrorexigenic effect (Avrith & Fitzsimons, 1980; Bryant, Epstein, Fitzsimons, & Fluharty, 1980; but see also Fluharty & Manaker, 1983), that effect appears to depend on pretreatment with a mineralocorticoid (Fluharty & Epstein, 1983). It seems that the short-latency satiation mechanisms in addition to blocking persisting natrorexigenic signals from depleted reservoirs must also block the natrorexigenic effect of circulating aldosterone until the levels return to normal. Denton and his colleagues have also found various sorts of evidence that point to the existence of short-latency mechanisms for elicitation as well as for satiation of salt appetite in the brain of sheep (Denton, 1982). Our results suggest, however, that these short-latency mechanisms are inadequate to account for all the data and that one must think in terms of multiple factors in the elicitation and satiation of salt appetite.

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