PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 170, 182–187 (1982)

Effect of Methylxanthines on the Spontaneous Appetite for NaCl Solutions in Rats[1] (41416)

MELVIN J. FREGLY,[2] ORA E. LOCKLEY,[3] AND KAREN A. CARLBERG

Department of Physiology, Box J274, University of Florida, College of Medicine, Gainesville, Florida 32610

Abstract. Dietary administration of theophylline (2.5 g/kg of food) induced a spontaneous appetite for NaCl solution in female rats given a choice between water and 0.25 *M* NaCl solution to drink. The appetite was accompanied by a reduction in preference threshold to one-third that of controls as determined by the two-bottle choice technique. Dietary administration of 3-isobutyl-1-methylxanthine (IBMX) (2.50 g/kg of food) was also accompanied by a spontaneous appetite for 0.25 *M* NaCl solution when female rats were allowed to choose between it and distilled water to drink. The mechanism by which theophylline and IBMX affect the appetite for NaCl solutions is unknown but could be related to their phosphodiesterase inhibitory activity and/or to their ability to block adenosine receptors.

In a series of studies, Kodama *et al.* (1) and Sakata *et al.* (2) reported that treatment of rats with the methylxanthine, theophylline, attenuated their rejection of quinine-adulterated food. They also showed that treatment with theophylline diminished the acceptance of saccharin-adulterated food when saccharin was present in its "optimal" concentration while diminishing the rejection of the food when saccharin was present in a high concentration. Thus, theophylline-treated rats appeared to have an attenuated gustatory responsiveness to both the sweet and bitter modalities of taste.

It was the initial objective of the studies described below to determine whether the responsiveness of the modality for salt, as represented by sodium chloride, was also attenuated by theophylline. Because the results of these studies revealed an increased responsiveness and a striking appetite for NaCl solutions, an additional methylxanthine compound, 3-isobutyl-1-methylxanthine (IBMX), was also studied.

[1] Supported by Grant AG 639(a) from the American Heart Association, Florida Affiliate, Volusia-Flagler Division.

[2] To whom correspondence should be addressed.

[3] Present address: Department of Biology, Albany State College, Albany, Ga. Supported by Minority Hypertension Summer Development Grant HL 07298.

Materials and Methods. *Experiment 1: Effect of dietary administration of theophylline on spontaneous intake of 0.25 M NaCl solution.* This study used 12 female Blue Spruce Farms (Sprague–Dawley) rats weighing 280 to 320 g. They were maintained in a thermoregulated room (26 ± 1°) which was illuminated from 8 AM to 8 PM. The rats were divided randomly into two equal groups and placed in individual cages. Each rat was given a choice between distilled water and 0.25 *M* NaCl solution to drink. Fluid containers were infant nursing bottles with cast aluminum spouts as described by Lazarow (3). Both groups were fed finely powdered Purina Laboratory Chow. Food containers were spill resistant and have been described in detail (4). Fluids and food were available *ad libitum*. Food and fluid intakes, as well as body weight, of each rat were measured daily for 5 consecutive days. At the end of this time, one group of rats was given the same food into which 2.5 g theophylline/kg was thoroughly mixed. The second group received the same food without theophylline. Daily intakes of food and fluid were again measured for two 5-day periods beginning on the day of treatment.

Experiment 2: Effect of theophylline on the preference threshold for NaCl in rats. The rats used in Experiment 1 were also used in this experiment. One week elapsed between the experiments. During this time

0037-9727/82/060182-06$01.00/0

the treated rats continued to receive theophylline in their food and both groups were given only water to drink.

A preference threshold study was carried out using both groups of rats. Each rat, caged individually, was offered initially a choice between two bottles of distilled water. At 2-day intervals, the concentration of NaCl in one of the bottles was increased while the other bottle contained distilled water. In chronological sequence, the concentrations of NaCl solution used were: 0.010, 0.020, 0.030, 0.050, 0.075, 0.100, 0.200, 0.250, and 0.300 *M*. Each concentration of NaCl solution was made from the same concentrated stock solution by serial dilution. Intakes of water, NaCl solution, and food were measured daily, as was body weight. Preference threshold for each rat was considered to be the concentration at, and above, which NaCl intake was consistently and significantly greater than intake of distilled water offered simultaneously (6).

Experiment 3: Effect of dietary administration of 3-isobutyl-1-methylxanthine on spontaneous intake of 0.25 M NaCl solution. Twelve naive female rats of the Blue Spruce Farms (Sprague–Dawley) strain weighing 230 to 250 g were used. They were maintained under conditions identical to those described for Experiment 1. The rats were divided randomly into two equal groups and caged individually. Each rat was allowed to choose between two bottles, one containing 0.25 *M* NaCl solution and the other containing distilled water. Finely powdered Purina Laboratory Chow was the food provided. Fluid and food were available *ad libitum*. Intakes of fluids and food, as well as body weight, of each rat were measured daily for one week. During the second week, one group received food into which 3-isobutyl-1-methylxanthine (IBMX) (2.50 g/kg) had been mixed thoroughly, while the second group received the same food without the drug. Intakes of food and fluids, as well as body weight, were also measured daily during the second week.

Statistics. For Experiments 1 and 3, the mean intakes of water, NaCl solution, and food for each treated group were compared with those of the appropriate control group by means of a two-tailed two-sample *t* test (5). The same test was used to compare intakes of NaCl solution and distilled water for each group of rats in Experiment 2. Significance was set at the 95% confidence limit.

Results. *Experiment 1.* The effect of dietary administration of theophylline on spontaneous daily intakes of hypertonic (0.25 *M*) NaCl solution, water, and food are shown in Table 1. During the control period (first column), the theophylline-treated group had no drug in its food. Hence, the intakes of 0.25 *M* NaCl solution, water, and food, as well as the ratio of NaCl solution ingested to the total fluid volume ingested were similar for both groups. However, during the first 5 days of treatment (second column), intake of 0.25 *M* NaCl solution increased significantly ($P < 0.05$) in the theophylline-treated group compared to the intake of NaCl solution by the control group. Intakes of distilled water and food, as well as body weight, were unaffected by the addition of theophylline to the food. Intake of theophylline averaged 118 mg/kg/day.

During the second 5-day period of treatment with theophylline, the results were nearly identical to those of the first 5-day period. During the course of this experiment, about 40 to 45% of the total daily fluid intake was ingested from the bottle containing NaCl solution by control rats while theophylline-treated rats ingested about 65% of their total fluid as NaCl solution during each 5-day treatment period.

Experiment 2. Control rats detected the difference between distilled water and NaCl solution offered simultaneously when the concentration of NaCl was 0.030 *M*, while theophylline-treated rats detected the difference at the lowest concentration offered, i.e., 0.010 *M* (Fig. 1). Thus, chronic administration of theophylline decreased the preference threshold for detection of NaCl solution to one-third that of controls. Maximal intake of NaCl solution occurred at the same concentration for both groups, i.e., 0.075 *M* or 75 meq/liter. However, theophylline-treated rats did not reject NaCl solution in favor of water at any con-

TABLE I. EFFECT OF DIETARY ADMINISTRATION OF THEOPHYLLINE (2.5 g/kg FOOD) ON SPONTANEOUS DAILY INTAKES OF HYPERTONIC NaCl SOLUTION, WATER, AND FOOD

Experimental group	Number of rats		Mean fluid and food intakes (ml or g/100 g body wt/day)		
			Control period (5 days)	Treatment period	
				First 5 days	Second 5 days
Control	6	Body weight	303 ± 5[a]	304 ± 7	305 ± 7
		0.25 *M* NaCl	5.2 ± 0.8	4.5 ± 0.6	4.6 ± 0.7
		Distilled water	6.4 ± 1.1	6.6 ± 0.5	7.0 ± 0.8
		Food	4.2 ± 0.3	4.6 ± 0.2	4.8 ± 0.1
		NaCl/total fluid	0.45 ± 0.07	0.40 ± 0.05	0.44 ± 0.07
Theophylline treated	6	Body weight	302 ± 2	303 ± 2	310 ± 3
		0.25 *M* NaCl	6.0 ± 1.3	10.5 ± 2.1*	10.3 ± 2.2*
		Distilled water	6.3 ± 0.7	6.9 ± 1.1	6.5 ± 1.3
		Food	4.1 ± 0.2	4.6 ± 0.2	4.8 ± 0.1
		NaCl/total fluid	0.51 ± 0.07	0.65 ± 0.08	0.66 ± 0.10

[a] One standard error of mean.
* Significantly different from control ($P < 0.05$).

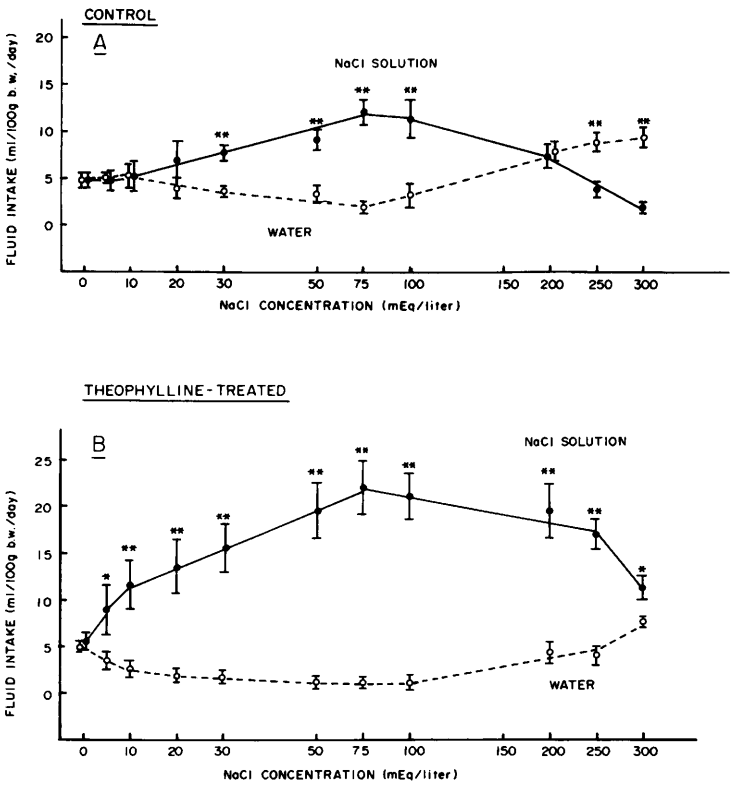

FIG. 1. A preference–aversion curve for control (A) and theophylline-treated (B) rats is shown. Intake of NaCl is represented by the closed circles and intake of distilled water by the open circles. One standard error is set off at each mean. *$P < 0.05$; **$P < 0.01$.

centration of NaCl solution offered whereas control rats first ingested significantly ($P < 0.01$) more water than NaCl solution when the concentration of the NaCl solution was 0.25 *M* or 250 meq/liter (Fig. 1). Treatment with theophylline had no significant effect on food intake, and there were no significant differences in body weight throughout the experiment.

Administration of theophylline was not accompanied by an increase in water intake when choice was allowed between two bottles of distilled water (compare the first set of points in Figs. 1A and B). Total fluid intake increased in the theophylline-treated group only when a choice was offered between distilled water and the first concentration of NaCl.

Experiment 3. Intakes of water and 0.25 *M* NaCl solution were similar for the two groups during the control period prior to drug treatment (Fig. 2). During treatment with IBMX, water intake decreased significantly ($P < 0.01$) while intake of NaCl solution increased significantly ($P < 0.01$) compared with simultaneous intakes by the control group. The ratio of NaCl intake/total fluid intake varied from 20.1 ± 1.5% (SE) during the control period to 46.2 ± 3.4% during the 5-day treatment with IBMX. Treatment with IBMX reduced food intake (4.3 ± 0.2 vs 3.1 ± 0.3 g/100 g body weight/day) and body weight (317 ± 5 vs 292 ± 4) significantly compared to pretreatment control values ($P < 0.01$). Intake of IBMX averaged 78 mg/kg/day.

Discussion. The results of these studies indicate that both theophylline and IBMX induce a spontaneous appetite for NaCl solution in rats at the doses used. Of these compounds, only theophylline was tested to determine its effect on preference threshold for NaCl solution by the two-bottle choice technique. The results of this study showed that theophylline-treated rats could detect the difference between water and NaCl solution when the concentration of the NaCl solution was one-third of that detectable by control rats, i.e., 0.010 *M* versus 0.030 *M*, respectively. Although IBMX was not tested for its effect on preference threshold for NaCl solutions, it is

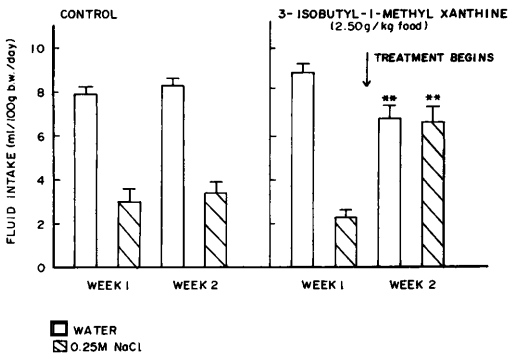

FIG. 2. Mean intakes of water (open bar) and 0.25 *M* NaCl solution (hatched bar) are shown for the control group (left panel) during the first and second week of the experiment. Similar intakes are shown in the right panel for the treated group during Week 1 (prior to treatment) and during Week 2 when IBMX was added to the diet at 2.50 g/kg food. One standard error is set off at each mean. $^{**}P < 0.01$.

likely that the preference threshold would have been reduced. It has been observed in this laboratory that an appetite for NaCl solution is customarily accompanied by an ability to detect the difference between distilled water and NaCl solution at a significantly lower concentration of NaCl than that observed for controls (7–12).

The time course for the development of the appetite for NaCl solution is relatively rapid. The salt appetite developed within 5 days of treatment with theophylline and within 1 week of treatment with IBMX. Thus, the physiologic factors affecting the appetite for salt occur relatively quickly following treatment with these methylxanthines.

A possibility exists that both increased total fluid intake and the appetite for NaCl solution are the result of the diuretic and natriuretic properties of theophylline. However, an increase in water intake was not observed when the treated rats were offered choice between two bottles of distilled water to drink in Experiment 2 (Fig. 1). Hence, the diuretic effect of theophylline, if it existed at the dose administered in this study, did not manifest itself as an increase in water intake. Whether the natriuretic effect of theophylline can account

for the appetite for NaCl solution remains to be studied.

The reduced preference threshold for NaCl solution observed here was a surprising finding in view of the attenuated gustatory responsiveness of theophylline-treated rats for quinine- and saccharin-adulterated foods reported by Kodama *et al.* (1) and Sakata *et al.* (2). No attempt was made in the present study to determine whether the same results would be observed if quinine or saccharin were presented to the rats in solution rather than in food. Such experiments should be performed in theophylline-treated rats to determine whether post-ingestional factors may play a role. If such results are verifiable when saccharin and quinine are presented in solution, they will indicate that theophylline, and perhaps other methylxanthines, increase the sensitivity of the taste modality for NaCl (salt) while decreasing it for quinine (bitter) and saccharin (sweet). On the other hand, the results of this study may also be considered from the point of view of the much higher aversion threshold for NaCl solutions accompanying administration of theophylline (Fig. 1). When this aspect is taken into account, the results appear to agree with those of Kodama *et al.* (1) and Sakata *et al.* (2). Thus, the aversiveness of hypertonic concentrations of NaCl solution was reduced in the presence of theophylline.

With respect to food intake, Merkel *et al.* (13) reported that caffeine, another methylxanthine, may either enhance or decrease food intake, depending on the dose administered acutely to rats. The lowest dose (3.1 mg/kg body wt) enhanced, while the highest dose (100 mg/kg body wt) depressed food intake. In the studies reported here, chronic treatment with theophylline, resulting in a daily intake of 118 mg/kg/day, did not affect food intake significantly (Table I). Since tolerance to methylxanthines develops rapidly, this may account for the lack of effect of theophylline on food intake in the present study (14). However, a daily intake of 78 mg IBMX/kg body weight decreased food intake in the present study.

The mechanism by which the appetite for NaCl solutions is induced by methylxanthines deserves comment. These compounds are known to block adenosine receptors and to inhibit phosphodiesterase, an enzyme which inactivates cyclic AMP by converting it to 5'-AMP (15). Blockade of adenosine receptors can lead to either stimulation or inhibition of cyclic AMP synthesis, depending on the type of cell involved, while inhibition of phosphodiesterase always leads to an increase in cyclic AMP concentration. Whether inhibition of the phosphodiesterase enzyme and/or blocking of adenosine receptors plays a role in the induction of the salt appetite is unknown and awaits additional study.

Caffeine has been reported to increase plasma renin activity (by 57%), plasma norepinephrine concentration (by 75%), and plasma epinephrine concentration (by 207%) in humans (16). Caffeine is therefore a potent stimulator of both plasma renin activity and adrenomedullary secretion in humans. It is likely that the concentration of aldosterone would also have been increased under these conditions since angiotensin II is an important stimulator of aldosterone secretion. If a similar response occurs in rats, the appetite for salt may be induced in a number of ways: (a) by a direct effect of angiotensin II at a central site (17), (b) by an effect of aldosterone either centrally or on plasma sodium and potassium concentrations, (c) by an effect on cerebrospinal fluid sodium and/or potassium concentrations, or (d) by an effect on salivary sodium and/or potassium concentrations (12, 18, 19). It is difficult to know which one of these, or other possibilities, can explain the observed appetite for NaCl solution.

We thank Mr. Thomas Conner and Mr. Howard Clark for technical assistance and Mrs. Charlotte Edelstein for graphic illustrations.

1. Kodama J, Fukushima M, Sakata T. Diminished taste reactivity to saccharin following chronic administration of theophylline in rats. Physiol Behav 21:647–652, 1978.

2. Sakata T, Kodama J, Fukushima M. Feeding patterns of theophyllinized rats and effect of dextrose on their food intake. Physiol Behav 17:797–802, 1976.
3. Lazarow A. Methods for quantitative measurement of water intake. Methods Med Res 6:225–229, 1954.
4. Fregly MJ. A simple and accurate feeding device for rats. J Appl Physiol 15:539, 1960.
5. Snedecor GW, Cochran WG. Statistical Methods, 5th ed. Ames, Iowa State College Press, p35, 1956.
6. Richter CP. Salt taste threshold of normal and adrenalectomized rats. Endocrinology 24:367–371, 1939.
7. Fregly MJ, Waters IW. Effect of propylthiouracil on preference threshold of rats for NaCl solutions. Proc Soc Exp Biol Med 120:637–640, 1965.
8. Fregly MJ. Effect of hydrochlorothiazide on preference threshold of rats for NaCl solutions. Proc Soc Exp Biol Med 125:1079–1084, 1967.
9. Fregly MJ. Preference threshold and appetite for NaCl solution as affected by propylthiouracil and desoxycorticosterone acetate in rats. In: Pfaffmann C, ed. Olfaction and Taste III. New York, Rockefeller Univ Press, p554, 1969.
10. Fregly MJ, Newsome DG. Oral contraceptive-induced salt appetite in rats. In: Fregly MJ, Fregly MS, eds. Oral Contraceptives and High Blood Pressure. Gainesville, Fla, Dolphin Press, pp141–158, 1974.
11. Fregly MJ. Effect of the angiotensin converting enzyme inhibitor, captopril, on NaCl appetite of rats. J Pharmacol Exp Ther 215:407–412, 1980.
12. Thrasher TN, Fregly MJ. Factors affecting salivary sodium concentration, NaCl intake, and preference threshold and their interrelationships. In: Kare MR, Fregly MJ, Bernard R, eds. Biological and Behavioral Aspects of Sodium Intake. New York, Academic Press, p145, 1980.
13. Merkel AD, Wayner MJ, Jolicoeur FB, Mintz R. Effects of caffeine administration on food and water consumption under various experimental conditions. Pharmacol Biochem Behav 14:235–240, 1981.
14. Colton T, Gosselin RE, Smith RP. The tolerance of coffee drinkers to caffeine. Clin Pharmacol Ther 9:31–39, 1968.
15. Rall TW. Central nervous stimulants, the xanthines. In: Gilman AG, Goodman LS, Gilman A, eds. The Pharmacological Basis of Therapeutics. New York, Macmillan, p592, 1980.
16. Robertson D, Frolich JC, Carr RK, Watson JT, Hollifield JW, Shand DG, Oates JA. Effects of caffeine on plasma renin activity, catecholamines and blood pressure. N Engl J Med 298:181–186, 1978.
17. Fitzsimons JT. The Physiology of Thirst and Sodium Appetite. Cambridge, Cambridge Univ Press, p458, 1979.
18. Fregly MJ. The role of hormones in regulation of salt intake in rats. In: The Chemical Senses and Nutrition. Baltimore, Johns Hopkins Univ Press, p115, 1967.
19. Fregly MJ, Waters IW. Hormonal regulation of the spontaneous sodium chloride appetite of rats. In: Hayashi T, ed, Olfaction and Taste II. New York, Pergamon, p439, 1967.

Received September 24, 1981. P.S.E.B.M. 1982, Vol. 170.