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Effect of β-Adrenergic Antagonists on the Spontaneous Appetite for NaCl Solution in Rats[1]

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FREGLY, M. J. *Effect of β-adrenergic antagonists on the spontaneous appetite for NaCl solution in rats.* PHARMACOL BIOCHEM BEHAV 21(6) 883–889, 1984.—Chronic dietary administration of the β-adrenergic antagonist, propranolol (1.6–2.0 g/kg), to both male and female rats induced an appetite for 0.25 M NaCl solution when the rats were offered a choice between distilled water and salt solution to drink. Treatment was also accompanied by a significant reduction in both water and food intakes. In addition, increases in intake of NaCl solution during treatment with propranolol were correlated significantly with increases in urinary sodium output. To test for completeness of β-adrenergic blockade, treated and control rats in the first study were administered isoproterenol (25 μg/kg b.w., SC) acutely and given only water to drink. The increased 1 hr water intake characteristically accompanying acute administration of isoproterenol was blocked completely by propranolol. Additional experiments were carried out with butoxamine, a selective β_2-adrenoceptor antagonist, (1.5 and 3.0 g/kg of food) to determine its effect on intake of 0.25 M NaCl solution. Butoxamine failed to produce a significant effect on salt appetite. The results indicate that chronic treatment with propranolol, but not butoxamine, induces an appetite for NaCl solution. They further suggest, but do not prove, that β_1-adrenoceptors may be involved in the appetite for NaCl solutions in rats.

Salt appetite Propranolol Butoxamine β-Adrenergic antagonists Isoproterenol Salbutamol
Fluid intake Salt solution Rats

A number of experimental procedures is now known to stimulate an increase in the spontaneous NaCl intake of rats given a choice between distilled water and NaCl solution to drink. The various conditions under which a salt appetite may be induced suggest that the mechanisms involved in its genesis are complicated and probably multiple. Evidence has accumulated that the renin-angiotensin-aldosterone system may be important in the control of NaCl intake in the rat [3, 6, 8, 10–13, 18, 19, 24]. The present study tested the effect of the β-adrenoceptor antagonist, propranolol, on the initiation of an appetite for NaCl solution in adult rats. Propranolol was used because it has been reported to inhibit β-adrenergic-mediated release of renin, and therefore the formation of angiotensin II, the trophic hormone responsible for the release of aldosterone from the adrenal cortex [1, 4, 14, 21, 23, 25]. On this basis, it would be expected to induce an appetite for NaCl solution. The studies described below were designed to test this possibility. In addition, the selective β_2-adrenoceptor antagonist, butoxamine, was administered to assess the role of β_1- versus β_2-adrenoceptors in induction of an appetite for NaCl solution in rats.

METHOD

Experiment 1: Effect of Dietary Administration of Propranolol on Spontaneous Intake of 0.25 M NaCl Solution

Twelve female rats of the Blue Spruce Farms (Sprague Dawley) strain weighing 280 to 350 g were used. They were maintained in a thermoregulated room (26±1°C) which was illuminated from 7 a.m. to 7 p.m. The rats were separated 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 [16]. Both groups were fed finely powdered Purina Laboratory Chow. Food containers were spill-resistant and have been described in detail [9]. Fluids and food were available ad lib. 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 800 mg d,l-propranolol-HCl/kg was thoroughly mixed. The second group received the same food without propranolol. Daily intakes of food and fluids were then measured for an additional 5 day period.

At the end of this study, the completeness of β-adrenergic blockade was tested. At 9 a.m. on the day of the test, all rats were administered the β-adrenergic agonist, isoproterenol (25 μg/kg, SC), and placed in individual cages. A preweighed bottle of water (26°C) was placed on each cage and water intake and urine output measured hourly for 3 hr.

Since the results of the test of β-adrenergic responsiveness suggested only moderate attenuation, the concentration of propranolol in food was doubled to 1.6 g/kg. All rats continued to have a choice between distilled water and 0.25 M

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NaCl solution to drink. After receiving propranolol for 2 weeks at the higher dose, a second test of the completeness of β-adrenergic blockade was carried out as described above. The following day, daily intakes of distilled water, NaCl solution, and food were begun for 5 days (fourth week of experiment). At the end of this study, the bottle of NaCl solution was removed from each cage and the rats were allowed to drink water only for one week (fifth week of experiment). During the sixth week of the experiment, daily intakes of water and food were measured in all rats for 5 days. The experiment was terminated at the end of this study.

Experiment 2: Effect of Dietary Administration of Propranolol on Fluid and Food Intakes and Urine Output of Male Rats

Twelve male rats of the Blue Spruce Farms (Sprague Dawley) strain weighing 250 to 320 g were used. They were kept in individual cages and given choice between distilled water and 0.25 M NaCl solution to drink. The food was finely powdered Purina Laboratory Chow. Food and fluid containers were the same as those used in Experiment 1. Urine was collected under light mineral oil to prevent evaporation. The rats were allowed to adapt to the experimental situation for 3 days prior to beginning a 5 day control period. During this time, intakes of water, 0.25 M NaCl solution and food were measured as were urine output and body weight. No measurements were made during the next two days after which d,l-propranolol-HCl (propranolol-HCl was kindly supplied by Ayerst Laboratories, Inc., New York, NY) (2.0 g/kg) was mixed thoroughly into the food. This dose is slightly larger than that used in Experiment 1. It was used as a matter of convenience only. All rats were given propranolol and daily measurement continued for an additional 5 days. No measurements were made during the next 2 days although all rats continued to receive propranolol. Another 5 day period of measurements was begun immediately thereafter with all rats continuing to receive propranolol.

Daily urine output from each rat was analyzed for its sodium and potassium concentrations by flame photometry using lithium as the internal standard.

Statistical comparison of the data collected during each treatment period with the control period was made by a one-way analysis of variance [20]. Comparison of the difference between means was made by means of the *t*-test using the pooled variance from the analysis of variance [15]. Other comparisons were made by means of a linear regression analysis [20].

Experiment 3: Effect of Dietary Administration of Butoxamine on Spontaneous Intake of 0.25 M NaCl Solution

Twelve naive female rats of the Blue Spruce Farms (Sprague Dawley) strain weighing 280 to 320 g were separated into two equal groups. They were maintained under the same conditions described in Experiment 1. The rats were caged individually and given choice between distilled water and 0.25 M NaCl solution to drink. Finely powdered Purina Laboratory Chow was provided as food. The food and fluid containers used were the same as those described in Experiment 1. Two days after allowing choice of drinking fluids, daily intakes of water, NaCl solution and food were measured for 5 days. Two days later, one group was given the selective β_2-adrenergic antagonist, butoxamine HCl (butoxamine hydrochloride was purchased from Burroughs Wellcome Co., Research Triangle Park, NC), mixed into the food at a concentration of 1.5 g/kg. Measurements of food and fluid intakes continued daily for an additional 5 days. At the end of the third week of treatment with butoxamine, completeness of β_2-adrenergic blockade was assessed by the drinking response of the rats to the β_2-adrenergic agonist, salbutamol (100 μg/kg, SC). This study was carried out identically to the drinking test described in Experiment 1 with the exception that salbutamol was used instead of isoproterenol. Since the results of the test of β_2-adrenergic responsiveness suggested only moderate attenuation, all rats were given food without butoxamine for one week. Water and 0.25 M NaCl solution were available to the rats during this time. The rats were then randomized into two groups. Daily intakes of water, 0.25 M NaCl solution and food were measured for 5 days. At the end of this time, one group of rats was given food containing butoxamine at a concentration of 3.0 g/kg. Daily measurements of fluid and food intakes continued for an additional 5 days. The experiment was terminated at this time.

RESULTS

Experiment 1

Administration of propranolol at a dose of 800 mg/kg of food (3.2 mg/kg b.w./day) failed to affect significantly intakes of water, 0.25 M NaCl solution and food during the two week treatment. When completeness of the β-adrenergic blockade was tested by administration of isoproterenol and subsequently measuring water intake hourly for 3 hr, a significant ($p<0.05$) reduction in water intake of the propranolol-treated group was observed during the second and third hr (Table 1). No effect of propranolol in attenuating the antidiuretic effect of isoproterenol was observed. These results suggested that a modest but incomplete blockade of β-adrenergic receptors had occurred at this dose of propranolol.

Doubling the dose of propranolol resulted in a significant ($p<0.01$) reduction in water and food intakes but a significant ($p<0.05$) increase in intake of 0.25 M NaCl solution (Fig. 1). There was also a significant ($p<0.01$) increase in the ratio of NaCl intake/total fluid intake.

When the completeness of β-adrenergic blockade was tested in these animals by means of the drinking test mentioned above, water intake of the propranolol-treated group in response to acute administration of isoproterenol was attenuated significantly ($p<0.01$) during all 3 hr of the study (Table 1). Urine output of the propranolol-treated group was reduced significantly ($p<0.01$) during the third hr of the study.

Since the results of the previous study indicated that propranolol mixed into food at a dose of 1.6 g/kg was accompanied by reduced water and food intakes, but an increased intake of NaCl solution, an additional study was carried out in rats given only water to drink. The results of this study confirmed the reductions in water and food intakes accompanying this dose of propranolol (Fig. 2). The ratio of water to food ingested by the control group was 2.24 ± 0.16 (SE) while that for the propranolol-treated group was 1.69 ± 0.14 ($p<0.05$). This suggests that the reduction in water intake was not solely the consequence of the reduction in food intake.

The mean amount of propranolol ingested when the drug was administered at 800 mg/kg of food was 3.2 mg/kg/day while the amount was 6.2 mg/kg/day when 1600 mg/kg was given. Thus, the level of intake required to induce an appe-

TABLE 1

EFFECT OF ACUTE ADMINISTRATION OF ISOPROTERENOL (25 μg/kg, SC) ON WATER INTAKE OF PROPRANOLOL-TREATED RATS

Experimental group	n	Mean Body Wt. (g)	Cumulative water intake (ml/kg b.w.) during: 1	2	3 hr	Cumulative urine output (ml/kg b.w.) during: 1	2	3 hr
			Study 1 Propranolol (800 mg/kg food					
Control	6	270 ±7*	16.9 ±3.2	18.9 ±2.5	19.1 ±2.6	0.0	0.0	8.1 ±2.8
Propranolol-treated	6	280 ±16	9.0 ±2.0	9.8 ±1.5†	9.9 ±1.5†	0.0	2.6 ±1.7	3.7 ±2.7
			Study 2 Propranolol (1.6 g/kg food)					
Control	6	285 ±5.5	17.5 ±3.6	22.1 ±3.8	22.5 ±3.7	0.0	1.1 ±0.7	12.4 ±2.7
Propranolol treated	6	292 ±5.6	2.2 ±0.8‡	2.6 ±0.7‡	3.7 ±0.7‡	0.0	1.5 ±0.6	2.1 ±0.5‡

*One standard error of mean.
†Significantly different from control ($p<0.05$).
‡Significantly different from control ($p<0.01$).

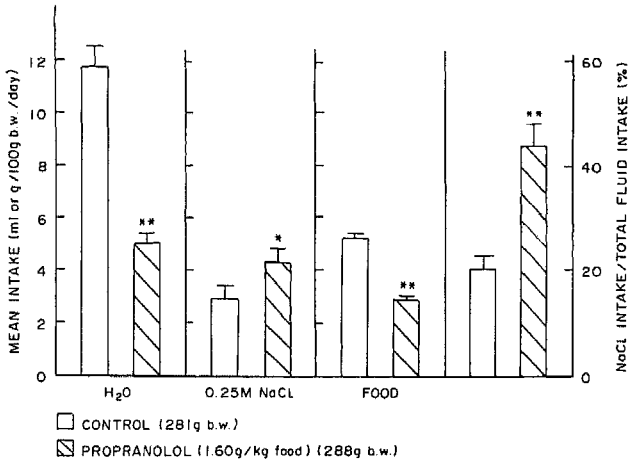

FIG. 1. Mean daily intakes of water (first panel), 0.25 M NaCl solution (second panel) and food (third panel) by control and propranolol-treated rats. The ratio of NaCl intake/total fluid intake for each group is shown in the last panel. The group designations are shown in the figure. Mean body weight of each group is also shown. One standard error is set off at each mean. *$p<0.05$; **$p<0.01$.

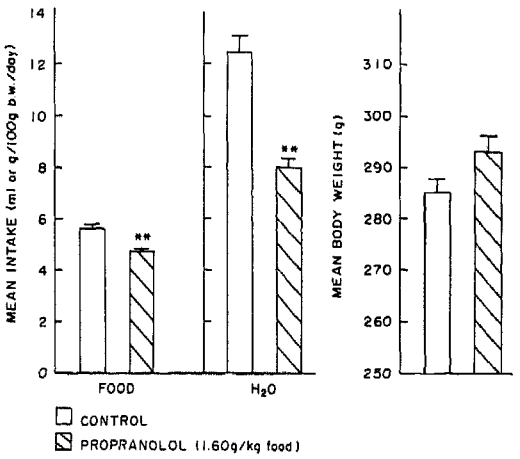

FIG. 2. Mean intakes of food (first panel) and water (second panel) by control and propranolol-treated rats. Mean body weight of each group is shown in the last panel. The group designations are shown in the figure. One standard error is set off at each mean. **$p<0.01$.

tite for NaCl solution lies between these two values. The reduction in food intake shown in Fig. 2 is not reflected in a reduced body weight because the mean body weight of the propranolol-treated group was greater (10 g) than that of the control group at the beginning of the experiment. The control group gained 1 g during the course of the 5 day study while the treated group lost 2 g.

Experiment 2

When choice was offered between distilled water and 0.25 M NaCl solution to drink, male rats drank more than twice as much water as salt solution (Fig. 3A). When treated initially with propranolol, water and food intakes declined significantly ($p<0.01$) from control value while intake of 0.25 M NaCl solution increased, but not significantly (Fig. 3B). During the second week of treatment with propranolol, water intake declined further while intake of NaCl increased significantly ($p<0.05$) (Fig. 3C). Food intake remained significantly ($p<0.01$) depressed during the second week of treatment. Mean body weights of the animals during each week of treatment did not differ significantly ($p<0.01$) from their control value. The percentage of the total daily fluid intake ingested as NaCl solution increased significantly ($p<0.01$) dur-

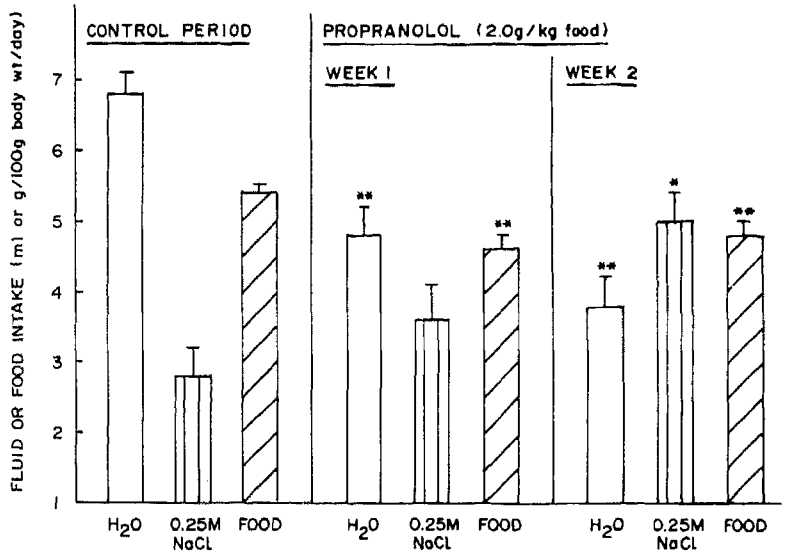

FIG. 3. Mean daily intakes of water, 0.25 M NaCl solution and food during a 1 week control period (first panel) and during 2 weeks of treatment with propranolol (second and third panels). The same rats were used throughout. One standard error is set off at each mean. *$p<0.05$; **$p<0.01$.

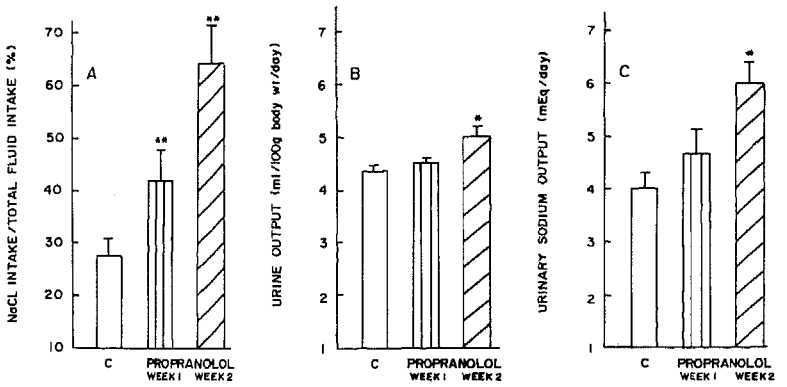

FIG. 4. The rates of NaCl intake/total fluid intake (A), urine output (B), and urine sodium output (C) of the same rats in Fig. 3 are shown for the control period and each week of treatment with propranolol. One standard error is set off at each mean. *$p<0.05$; **$p<0.01$.

ing each week of treatment (Fig. 4A). Urine output did not increase significantly during the first week of treatment but did so during the second week (Fig. 4B). Urine sodium output increased progressively during each week of treatment with the last week being significantly greater ($p<0.05$) (Fig. 4C). In contrast, urine potassium output was not significantly affected by treatment with propranolol. Potassium outputs during the control period and the two treatment periods were 4.4±0.1 (S.E.), 4.2±0.1 and 4.4±0.2 mEq/day, respectively.

By means of a linear regression analysis, the correlation between urinary sodium output (X, mEq/day) and intake of 0.25 M NaCl solution (Y, ml/100 g body weight/day) by each rat was determined during the control period and during each week of treatment with propranolol. The calculated equations for the control period, week 1 and week 2 of treatment with propranolol, respectively, are given below.

Control: $Y=1.16X-1.84$; $r=0.94$, $n=12$; $p<0.01$
Week 1: $Y=1.22X-2.06$; $r=0.96$, $n=12$; $p<0.01$
Week 2: $Y=1.31X-2.77$; $r=0.95$, $n=12$; $p<0.01$

There were no significant differences between either slopes or intercepts of any two equations. Thus, treatment with propranolol failed to affect the fundamental relationship between urinary sodium output and intake of 0.25 M NaCl solution observed during the control period.

A regression analysis of intake of 0.25 M NaCl solution (X, ml/100 g b.w./day) versus simultaneous intake of water (Y, ml/100 g b.w./day) was also carried out for data obtained during the control and treatment periods. The equations are given below.

Control: $Y=-0.43X+7.96$; $r=0.61$, $n=12$; $p<0.05$
Week 1: $Y=-0.57X+6.83$; $r=0.87$; $n=12$; $p<0.01$
Week 2: $Y=-0.31X+5.30$; $r=0.69$; $n=12$; $p<0.05$

TABLE 2

EFFECT OF CHRONIC ADMINISTRATION OF BUTOXAMINE ON INTAKES OF WATER, NaCl SOLUTION AND FOOD BY FEMALE RATS

Experimental Group	No. of Rats	Mean Body Weight (g)	Control Period (ml or g/100 b.w./day) Water	0.25 M NaCl Solution	Food	Mean Body Weight (g)	Treatment Period (ml or g/100 b.w./day) Water	0.25 M NaCl Solution	Food
				Butoxamine (1.5 g/kg food)					
Control	6	295 ± 2*	9.0 ± 0.6	5.0 ± 0.5	5.0 ± 0.1	294 ± 2	8.8 ± 0.5	5.6 ± 0.8	4.8 ± 0.2
Treated	6	307 ± 3	6.6 ± 0.8	7.1 ± 0.6	4.7 ± 0.1	308 ± 3	6.6 ± 0.6	7.3 ± 0.6	4.9 ± 0.1
				Butoxamine (3.0 g/kg food)					
Control	6	330 ± 11	8.3 ± 1.0	5.3 ± 1.4	4.2 ± 0.1	315 ± 8	7.1 ± 1.8	4.1 ± 0.8	4.2 ± 0.3
Treated	6	328 ± 11	9.0 ± 1.3	5.4 ± 1.4	5.0 ± 0.5	308 ± 8	7.7 ± 1.9	4.2 ± 0.9	4.4 ± 0.2

*One standard error of mean.

There were no significant differences between the slopes of any two equations but the intercepts of equations for weeks 1 and 2 differed significantly from that of the control period ($p<0.05$ and <0.01 respectively). Hence, treatment with propranolol for 2 weeks failed to affect the slope of the relationship observed during the control period but reduced the intercept significantly. Of additional interest is the fact that the slopes of the relationship are negative. This indicates that increasing intakes of the hypertonic 0.25 M NaCl solution were accompanied by decreasing intakes of water. This relationship has been reported previously [12].

Experiment 3

Dietary administration of butoxamine at either 1.5 or 3.0 g/kg of food had no significant effect on the intake of 0.25 M NaCl solution, water or food (Table 2). Partial blockade of β_2-adrenoceptors was accomplished by the lower dose of butoxamine as assessed by attenuation of the drinking response to salbutamol (Fig. 5).

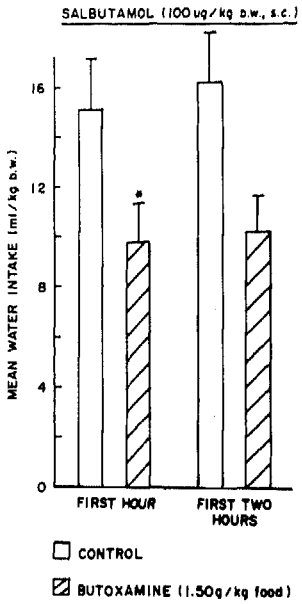

FIG. 5. Mean cumulative water intakes by control and butoxamine-treated rats during the first and second hr after treatment with salbutamol.

DISCUSSION

When choice was allowed between a hypertonic saline solution (0.25 M NaCl) and distilled water to drink, rats treated chronically with the β_1- and β_2-adrenoceptor blocker, propranolol, manifested an appetite for the NaCl solution (Figs. 1 and 4). Approximately two weeks of treatment were required for the appetite to develop. The results also suggest that β_1- but not β_2-adrenoceptors may mediate the salt appetite since butoxamine, the selective β_2-adrenoceptor antagonist, failed to induce an appetite for salt (Table 2). Blockade of β_2-adrenoceptors was shown by the reduced drinking response to administration of salbutamol, the β_2-adrenoceptor agonist.

The mechanism by which an appetite for NaCl solution is induced is not clearly known. It is likely that the renin-angiotensin-aldosterone system is involved. Thus, adrenalectomy increases the preference for NaCl solutions and this can be reduced to the level of intact rats by administration of low doses of mineralocorticoid hormones (aldosterone, deoxycorticosterone and 9-α-flurocortisol [10, 18, 19, 24], but not by glucocorticoid hormones (cortisone and corticosterone) [10,13]. The renin-angiotensin aldosterone system may initiate an appetite for NaCl in a number of ways: (a) by a direct effect of angiotensin II at a central site [7]; (b) by an effect of aldosterone on either urinary excretion of sodium or on plasma concentrations of sodium and potassium; (c) by an effect on salivary sodium or potassium concentration or both [10, 11, 22]; (d) by an effect on cerebrospinal fluid sodium or potassium concentration, or both. It is difficult to know which one of these or other possibilities is primarily responsible for the observed appetite for NaCl solution.

There is experimental evidence that administration of angiotensin II intracerebroventricularly (IVT) can induce an increase in spontaneous intake of NaCl solution in rats [5–8]. It seems unlikely that central levels of angiotensin II would be increased by propranolol since an important effect of this compound peripherally is to decrease angiotensin II concentration of the plasma [1, 4, 14, 21, 23, 25]. However, a clear

relationship between central and peripheral angiotensin II concentrations remains to be established under this and other experimental conditions.

The results of the present studies reveal a direct linear relationship between intake of 0.25 M NaCl solution and urinary output of sodium. The relationship was unaffected by treatment with propranolol. Thus, although propranolol induced an increase in intake of NaCl solution, it was accompanied by an increase in urinary sodium excretion without affecting the fundamental relationship. In spite of the excellent correlation between these two variables, it has not been possible to separate cause from effect because the data were collected only at 24 hr intervals. Thus, it is not possible to suggest that treatment with propranolol first induced an increase in NaCl intake which was later accompanied by an increase in urinary sodium output or vice versa. Additional studies will need to be designed to determine this. It is possible to speculate, however, that a decrease in aldosterone concentration of blood induced by propranolol could have been responsible for both the increased NaCl intake and urinary sodium loss [1, 4, 14, 21, 23, 25].

Recent studies from this laboratory have shown that salivariectomized rats infused via the submaxillary duct to the mouth with an artificial saliva ingested decreasing volumes of 0.15 M NaCl solution and increasing volumes of water, offered simultaneously, when the concentration of sodium in the artificial saliva was increased [22]. The potassium concentration of the artificial saliva was maintained constant. Maximal reduction in intake of 0.15 M NaCl solution occurred when the sodium concentration of the artificial saliva was 75 mEq/liter. This suggests that intake of NaCl solution by rats can be changed by changing the concentration of sodium in saliva. A similar conclusion was reached by both O'Mahony [17] and Bartoshuk [2] for humans. Thus, the appetite for NaCl solution induced by propranolol could have been mediated by a change in salivary chloride (and sodium) concentration. Additional studies will be required to confirm this.

The inverse linear relationship between intake of NaCl solution and water is of interest because it appears at first glance to be opposite of expectation. However, rearrangement of the equation suggests that the constant is a function of total fluid intake (thus, water intake + NaCl intake=total fluid intake) and explains why the slope should be negative when water intake is Y and NaCl intake is X.

These studies provide no information as to whether cerebrospinal fluid sodium and potassium concentrations were affected by treatment with propranolol. There are thus a number of possibilities to explain the appetite for NaCl solution induced by propranolol. Insufficient information is available to determine which, if any, is correct. However, the present results are consistent with the possibility that propranolol may induce an appetite for NaCl solution in rats by way of an effect on salivary concentration of sodium resulting from a reduction in plasma aldosterone concentration. Additional studies will be required to establish firmly this possibility for propranolol as well as for other experimental interventions that induce an appetite for NaCl solution.

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