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Effect of Propylthiouracil on Preference Threshold of Rats for NaCl Solutions.*† (30612)

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Hypothyroidism, produced either by thyroidectomy or treatment with antithyroid drugs, induces a spontaneous salt appetite in rats given choice between water and .15 M NaCl solution to drink(1,2). The salt appetite is reminiscent of that commonly observed in adrenalectomized rats by a number of investigators(3,4). Hypothyroid rats also resemble adrenalectomized rats in that they are unable to conserve sodium when the diet is deficient in this element and die (50%) within 3 weeks(5). In addition, intraperitoneal loading with either saline or water results in greater urinary loss of sodium and chloride after 5 hours than is observed for normal controls(6). Since the resemblance of hypothyroid to adrenalectomized rats is so striking, it was deemed worthwhile to test the preference threshold of hypothyroid rats for NaCl solutions to determine if it is reduced as is reported for adrenalectomized rats(4,7).

Methods and results. Two separate experiments were performed. Each utilized male rats of the Carworth CFN strain. The conditions of both experiments were similar in that the rats were kept in individual cages in a windowless room in which temperature was maintained at 26 ± 1°C and illumination was from 8 A.M. to 6 P.M. Each rat was provided with finely ground Rockland Rat Diet in spillproof containers described in detail elsewhere(8). Two containers consisting of cast aluminum fountains and nursing bottles provided drinking fluid(9).

Experiment 1. Six control and 6 propylthiouracil-treated (PTU)[2] rats were used. The PTU-treated rats received the drug in food (1.0 g PTU/kg food) for 8 weeks prior to the experiment and were continued on the drug during the experiment. During the first 2 weeks of the experiment, both bottles contained distilled water. Intakes were measured daily to ascertain that each rat drank roughly equal amounts of water from each bottle. Positions of the bottles on the cage were interchanged daily to avoid habit formation in selection of drinking fluid. During this period, about 20% of the rats were rejected as position drinkers and were replaced by other rats. At the end of the 2-week period, 2-day test periods were begun. During the first period each rat was offered choice between distilled water (bottle A) and .0001 M NaCl solution (bottle B). All NaCl solutions used were made from a concentrated stock NaCl solution by serial dilution. Each dilution was checked for accuracy by determination of chloride concentration (Cotlove Chloridometer). During subsequent 2-day periods, each rat was given choice between distilled water and the following molar NaCl solutions in chronological sequence: .0003, .0004, .0008, .001, .010, .020, .050, .100, .150 and .300. Daily intakes of water and NaCl solution were measured and expressed as ml/100 g BW/day to correct for differences in body weight.

The criterion of preference threshold used was similar to that of Richter(7); namely,

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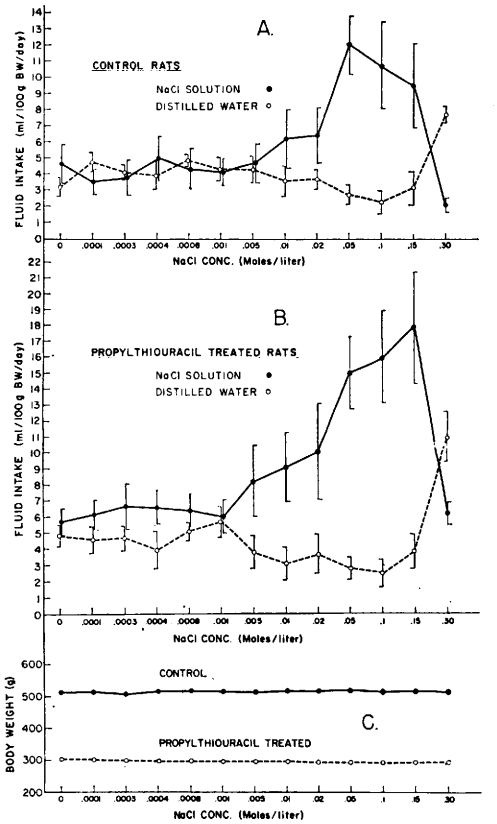

FIG. 1. Spontaneous intakes of NaCl solution (●) and distilled water (○) are shown for control rats (part A) and propylthiouracil-treated rats (part B). Body weight of each group is shown in part C. Means ± 1 standard error are shown.

the concentration of NaCl solution at and above which simultaneous volumes taken from the test bottle exceeded that from the reference (water) bottle and differed significantly from it ($P<.05$). Statistical comparison of the results obtained from the two groups was made by the "t" test(10).

Fig. 1A shows simultaneous intakes of distilled water and NaCl solution for control rats. When both bottles contained distilled water, total fluid intake was 7.9 ml/100 g BW/day. As concentration of NaCl increased in bottle B, control rats gave the first indication of preference for it at 0.01 M but differences between simultaneous intakes of water and NaCl solution were not significant ($P<.01$) until .050 M was given. Differences between intakes of water and NaCl solution remained significant thereafter. In the range of concentrations between .150 M and .300 M, NaCl solution was rejected in favor of water offered simultaneously.

When both bottles contained distilled water, total fluid intake of PTU-treated rats was 10.6 ml/100 g BW/day (Fig. 1B). The increased total fluid intake of PTU-treated rats has been described(11). The first apparent difference between simultaneous intakes of distilled water and NaCl solution occurred when .005 M was given. However, the difference was not significant ($P>.05$). When choice was offered between .010 M NaCl solution and water, the difference between intakes was significant ($P<.05$). Differences between the two fluid intakes were significant thereafter ($P<.01$) for all concentrations of NaCl solution offered. When .30 M NaCl solution was offered, the PTU-treated rats preferred water to the salt solution. The exaggerated intake of NaCl solution by PTU-treated rats is readily apparent by comparison with intakes of control rats in panel A. Control rats ingested maximal amounts of NaCl solution when the concentration was .05 to .10 M while PTU-treated rats ingested maximal amounts of NaCl solution when the concentration was .15 M.

Although all rats were the same age initially, mean body weight of PTU-treated rats was less than that of controls (Fig. 1C). The growth-depressing effect of PTU is well known. Changes in the concentration of NaCl solution had little effect on mean body weight of either group.

Experiment 2 also utilized 12 male Carworth CFN rats different from those used in the previous experiment. The PTU-treated rats received the drug in food (1.0 g/kg food) for 23 weeks prior to the experiment and were continued on the drug during the experiment. This experiment was conducted in the same manner as that described in *Experiment 1* except that the rats were not screened initially to eliminate position drinkers and the test periods were 4 days each. Both changes in procedure were deliberate to determine whether these factors would affect the results obtained. The concentration of the NaCl solution presented to the rats also differed slightly from the previous experiment. During the initial 4-day period, both bottles con-

TABLE I. Effect of Treatment with Propylthiouracil on Preference Threshold of Rats for NaCl Solution.

Treatment	No. of rats	NaCl preference threshold (moles/l)	P*
Control	6	.023 ± .006 †	<.01
PTU-treated (1.0 g/kg food)	6	.004 ± .0004	

* Probability value.
† ± 1 standard error of mean.

tained distilled water. During subsequent 4-day periods each rat was given choice between distilled water and the following molar NaCl solutions in chronological sequence: .0001, .0003, .0004, .0008, .001, .003, .005, .010, .050, .100, .250 and .300. Daily intakes of water and NaCl solution were measured (ml/100 g BW/day).

Simultaneous intakes of distilled water and NaCl solution were graphed for each individual rat. The concentration at which NaCl intake increased and remained elevated, while water intake decreased and remained low, was determined for each rat. In the case of the control rats, the crossover concentration, used as a criterion of preference threshold, varied from .01 to .05 M while crossover concentration for PTU-treated rats varied from .003 to .005 M (Table I). The calculated mean preference threshold values for control and PTU-treated rats were .023 and .004 moles/liter respectively. These preference thresholds agree well with those shown in Fig. 1. Other aspects were similar to those of *Exp. 1* and include a greater initial intake of distilled water by PTU-treated rats (17.5 as compared with 8.5 ml/100 g BW/day) as well as maximal intake of NaCl solution by both groups when the concentration offered was .10 M.

Mean body weight of control rats was 450 g initially and was maintained relatively constant throughout the experiment. Mean body weight of PTU-treated rats was 280 g initially and was maintained constant throughout the experiment.

Discussion. Preference threshold of PTU-treated rats for NaCl solution was less than that of control rats in both experiments. Preference threshold of control rats in the first experiment lay between .02 and .05 M/liter and in the second experiment between .01 and .05 M/liter. Preference threshold of PTU-treated rats in the first experiment lay between .005 and .01 M and in the second experiment between .003 and .005 M. The agreement between experiments is quite good when it is considered that different rats and different concentrations of salt solutions as well as slightly different experimental conditions were used in the two experiments. Thus, preference threshold of PTU-treated rats was reduced from one-fifth to one-eighth that of controls.

The preference threshold of the control rats observed here is slightly higher than that observed by Weiner and Stellar (.009 M)(12) and Richter (.0094 M)(7) but is within the range observed previously in this laboratory (.009-.018 M)(13) and by Bare (.0103 M)(4). The preference threshold of PTU-treated rats, although lower than that of controls, appears to be higher than that observed either by Richter(7) (.0005 M) or by Bare (4) (.0006 to .006 M) for adrenalectomized rats. Carr(14) reports that the preference threshold of adrenalectomized rats for NaCl solution is somewhat higher (.0020 M). The reduced preference thresholds of both PTU-treated and adrenalectomized rats may be related to the fact that blood levels of aldosterone are reduced or absent in both(15). Administration of either aldosterone of desoxycorticosterone acetate to adrenalectomized rats reduces spontaneous NaCl intake(16,17). Studies are in progress to relate preference threshold for NaCl solution to dose level of mineralocorticoid administered.

Summary. Chronic administration of the antithyroid drug, propylthiouracil (PTU) reduced the NaCl preference threshold of rats from one-fifth to one-eighth that of untreated controls. Preference threshold of normal rats lay between .02 and .05 M while threshold of PTU-treated rats lay between .003 and .01 M. Differences between thresholds were significant. The reduction in preference threshold for NaCl solution adds further to the striking similarities of PTU-treated and adrenalectomized rats.

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Effect of Removal of a Clipped Kidney or Cessation of Renin Administration on Hypertension.* (30613)

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Subcutaneous injections of renin into uninephrectomized rats cause hypertension and hypertensive vascular disease, as well as nephrosclerosis, stimulation of the zona glomerulosa and depletion of renal renin(1). The same changes are seen in rats with unilateral clipping of the renal artery(2). This strongly suggests that renin-induced hypertension is similar, if not identical, to experimental renal hypertension. Additional evidence of this similarity is provided by the present experiments in which we studied the changes in arterial pressure after removal of the "clipped kidney," after cessation of renin administration and the effect of renin injections during remission of renal hypertension.

Methods. Female Sprague-Dawley rats weighing about 150 g were fed Purina fox chow and given tap water to drink. Blood pressure was measured by tail sphygmography once or twice a day. In the first experimental series the effects of renin on blood pressure were studied following removal of a clipped kidney. After a control period, rats were made hypertensive by application of a silver clip on the left renal artery and were kept under observation for a period of 17 days at the end of which only those with pressures around 200 mm Hg were divided into 3 groups. The left kidney was removed in animals of Groups 1 and 2; animals of Group 3 were subjected only to a sham operation. Starting on the same day, animals of Group 2 received subcutaneous injections of crude hog renin 4 times per 24 hours in a daily dose of 45 Goldblatt units, while animals of the other 2 groups received saline injections according to the same schedule. The number of animals per group was 18, 12, and 12 respectively. The experiment was terminated 7 days later. Tissues were removed, weighed and processed for histologic examination.

In the second series we studied the effects of cessation of renin treatment on the course of renin-induced hypertension. Rats were uninephrectomized and divided into 3 groups of 12 animals each, which were placed in metabolism cages. Animals of Groups 1 and 2 were treated with crude hog renin which was administered subcutaneously every 8 hours at a daily dose of 75 Goldblatt units. On

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