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Effect of Water Temperature on Isoproterenol-Induced Water Intake[1] (40450)

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It is well established that water temperature affects water intake of rats dehydrated for 23.5 hr and then allowed access to water at various temperatures for 0.5 hr (1, 2). When water temperature was 36° water intake was nearly double that observed when water temperature was 12° (1). A similar effect of water temperature on water intake was also observed in rats that had been exposed to air at 5° for 24 hr and abruptly returned to air at 26° (3). Such rats have been shown to become dehydrated during exposure to cold (4, 5).

The objective of the present experiment was to determine whether the thirst induced by the β-adrenergic agonist, isoproterenol, was also affected in the same fashion by changing the temperature of the water offered.

Methods. Twelve female rats of the Blue Spruce Farms (Hooded) strain weighing from 250 to 300 g were used. The rats were kept three per cage in a room maintained at 25 ± 1° and illuminated from 0800 to 1800 hr. Food (Purina Laboratory Chow) and tap water were freely available. On the day of the test the rats were divided randomly into two groups. One group received 25 μg d,l-isoproterenol/kg of body weight[4] s.c., while the second group received an equal volume of the saline vehicle s.c. The rats were placed in individual cages, without food, and given a preweighed water bottle containing distilled water at one of the following mean temperatures, 6.0, 10.5, 25.0, 29.6, 35.8 and 39.3°. Every 20 min preweighed water bottles containing water at the appropriate temperatures were placed on each cage to control changes in the temperature of the water offered to the rats. All studies were carried out at an ambient temperature of 26 ± 1°.

Each water bottle was thermally insulated with a 0.5 in. thick rubber sleeve that completely covered the bottles and left only the tip of the spout exposed. Water bottles consisted of infant nursing bottles with cast aluminum spout as described by Lazarow (6). Pretest measurements showed that the insulation surrounding each bottle allowed an increase in water temperature during a 20-min period of approximately 1.5° when water temperature was 5° but maintained water temperatures successively more constant as they approached the ambient temperature. At the highest temperature tested (40°), a decrease of approximately 1.5° was observed during the 20-min period. To compensate for these changes, water temperatures about 1° higher or lower than the desired temperature of 40 and 5° were used. Control bottles in which water temperature was measured every 5 min served to determine the mean temperature of the water during the 20-min period. These temperatures were averaged and the mean water temperature used here.

Evaporation of water from the drinking spout during the drinking test was determined by means of control bottles. All changes in weight of drinking bottles were corrected for evaporation. To correct for any spillage from the drinking fountains, preweighed squares of absorbent paper were placed beneath the drinking fountain. The rats were observed continuously during the drinking test and any paper square on which water had spilled was weighed immediately to correct for the volume spilled.

Water intakes of the two groups at each water temperature were compared statistically by means of Student's *t* test for the 95% confidence limit (7). A regression analysis of water intake versus temperature of the water offered was also carried out for each group

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and the slopes and intercepts were compared statistically.

Results. The results of this experiment are shown in Fig. 1 for both isoproterenol-treated and control groups. At all temperatures of water tested, isoproterenol-treated rats ingested significantly ($P < 0.01$ to 0.05) more water than controls. In both groups, water intake increased as water temperature increased up to a water temperature of approximately 34°. Beyond this temperature, water intake tended to decrease.

A regression analysis of water intake (Y) versus water temperature (X) measured at the five lowest temperatures used was carried out for each group of rats. The equation for the control group is: $Y = (0.28)(x) - 0.77$; $r = 0.65$; $n = 30$; $P < 0.01$. The equation for the isoproterenol-treated group is: $Y = (0.26)(x) + 13.81$; $r = 0.37$; $n = 30$; $P < 0.05$. The intercepts, but not the slopes, of the two lines differed significantly from one another ($P < 0.01$).

Discussion. Water intake induced by the β-adrenergic agonist, isoproterenol, has a temperature-dependent function (Fig. 1). However, the temperature dependence occurs in controls as well and is not a function of administration of isoproterenol *per se.* A temperature dependence of water intake by normally hydrated rats was also shown previously (3). The results of that earlier study are nearly identical with those shown for control rats in Fig. 1.

Treatment with isoproterenol appeared to increase water intake above that of the control group by approximately the same increment regardless of the temperature of the water offered to the rats. Thus, comparison of the regressions of water temperature for the two groups revealed a significant difference between intercepts but not slopes.

The mechanism by which water temperature affects water intake is incompletely understood. Kapatos and Gold (1) showed a similar temperature dependence in rats dehydrated for 23.5 hr and allowed water for 0.5 hr. They suggested that ingestion of water below body temperature cools the tongue and signals satiation. Although the possibility exists that the temperature of the metal drinking fountain may have influenced water intake by the rats in the present study, this is unlikely

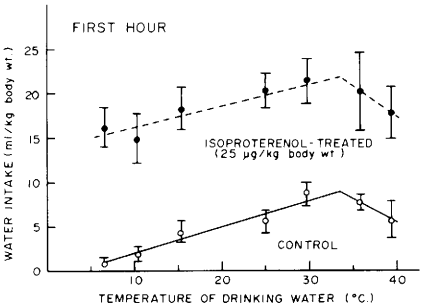

FIG. 1. Water intake (ml/kg of body weight) ± SE as a function of temperature of drinking water offered (°C) for control (○) and isoproterenol-treated (●) rats during the first hour of the experiment.

since Gold *et al.* (2) showed the same temperature dependence of water intake when rats were allowed to drink either from a metal spout or from the middle of a glass bowl located 2.5 cm below the floor of the cage.

It was also suggested by Deaux and Engstrom (8) that a decrease in body temperature accompanying the ingestion of cold water by rats might be responsible for reducing water intake when water was offered at temperatures lower than body temperature. Subsequent experiments failed to support this suggestion (9). Deaux (9) also suggested that the rate of movement of cold water from stomach to intestine was slower than that of warm water and that the gastric distention known to inhibit water intake (10, 11) might therefore account for the smaller volume of water ingested when water temperature was 5° as opposed to 35°. However, Sawchenko *et al.* (12) recently showed the opposite to be the case; that is, water at 5° passed through the stomach faster than warm (35°) water. The increased rate of passage of cold water from stomach to intestine may facilitate absorption of water into plasma with subsequent reduction in plasma osmolality. The more rapid reduction in plasma osmolality by ingestion of cold water could be an important factor in the reduced water intake of dehydrated rats.

Sawchenko *et al.* (12) also showed that severing the two gastric branches of the vagus nerve in rats resulted in a reduction in the intake of warm (35°) water compared to that observed for cold (5°) water during the first 20 minutes water was available following a 23.6-hr dehydration. No effects of vagotomy

on the level of intake of cold (5°) water were observed. Thus, disruption of nervous input from temperature receptors in the stomach results in abolition of temperature-dependent thirst in dehydrated rats. This suggests that the graded increases in water intake with graded increases in water temperature are related to graded changes in neural input from gastric temperature receptors. It may be assumed that central interpretation of the changes in gastric neural receptor activity as water temperature is decreased is a reduction in thirst drive.

The mechanism by which thirst is induced following administration of isoproterenol is not understood completely. Isoproterenol is well known to increase the rate of secretion of renin which reacts with renin substrate in plasma to form, ultimately, angiotensin II (13, 14). The latter is disputed by others who have shown that the concentration of angiotensin II in plasma that must be reached to induce thirst is unphysiological (15, 16). Although the mechanism by which isoproterenol induces thirst is disputed, the fact that it can induce a thirst in a variety of animal species is undisputed (17, 18). Several types of physiologically induceable thirst have been described (19). The first type is associated with reductions in both extracellular and intracellular fluid volumes and an increase in extracellular sodium concentration. The receptors believed to mediate thirst under these conditions are osmoreceptors. A second type of thirst is associated with an increase in intracellular fluid volume; a decrease in extracellular fluid volume and a decrease in plasma sodium concentration. These changes are accompanied by an increased rate of secretion of renin with subsequent formation of angiotensin II. Thirst is stimulated when angiotensin II enters the brain via the circumventricular organs and activates receptors in the brain (20–23). Administration of isoproterenol and the subsequent formation of angiotensin II appear to mimic activation of the mechanisms stimulating the second type of thirst described above. This is the first report that this type of thirst also has a water temperature-dependent component. Previous studies have been concerned with the first type of thirst; that is, dehydration-induced thirst (1, 2, 8, 9).

It seems important here to point out that water temperature-dependent drinking does not require the induction of either type of thirst described above. It occurred in normally hydrated rats (Fig. 1) (3). Dehydration, exposure to cold or administration of isoproterenol appear to amplify the basic response. Further, since isoproterenol-induced thirst is not initiated by changes in plasma osmolality, the difference in water intake when water is cold (6°) as compared to warm (30°) must be attributed to factors other than changes in plasma osmolality. It seems more likely that preabsorptive factors influence water intake (24). Whether these factors include temperature receptors in the stomach which influence behavior via nerve impluses in the vagus nerve remains for further investigation.

A number of investigators have reported that maximal rates of water intake appear to occur when water at near body temperature is offered to either dehydrated or cold-treated rats (1, 3). Similar results are reported here (Fig. 1). An adequate explanation for this is not yet available but it may be related to the possibility that ingestion of water warmer than body temperature induces aversive, perhaps painful, stimuli in rats. The importance of this observation relates to the rate at which rehydration is achieved following dehydration and may be of special significance to those species (e.g. man, rat) requiring 1–2 hr to rehydrate. The question also arises as to whether the preference–aversion relationship between water temperature and water intake in those animals (e.g. dog, burro) that rehydrate quickly after dehydration is different from those that do not. This remains for further study.

Summary. When female rats were administered the β-adrenergic agonist, isoproterenol (25 μg/kg body weight, s.c.), and allowed access to water at 6.0, 10.5, 25.0, 29.6, 35.8 and 39.3°, to drink, increasing amounts of water were ingested during the 1 hr test period as water temperature increased up to 29.6°. Water intake decreased when water at temperatures higher than this was offered. A similar increase in water intake with increasing water temperature was also observed in untreated control rats. Thus, the effect of water temperature on water intake was not a function of the thirst induced by administra-

tion of isoproterenol but is a characteristic of normally hydrated rats.

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