Bulletin of the Psychonomic Society
1983, 21 (5), 403-406

Schedule-induced and water-deprivation-induced drinking in rats: Effects of hypertonic saline challenges to homeostatic thirst mechanisms

JAMES J. McDONOUGH and JOSEPH H. PORTER
Virginia Commonwealth University, Richmond, Virginia

Previous studies have produced conflicting results regarding whether or not schedule-induced polydipsia (SIP) drinking is sensitive to homeostatic thirst mechanisms. Prior to SIP test sessions, 10 rats were given hypertonic saline (8.0% NaCl), isotonic saline, or sham intraperitoneal preloads. Rats preloaded with hypertonic saline drank significantly more water during SIP test sessions than those given isotonic saline or sham preloads. A second group of 9 rats on a 23.5-h water-deprivation schedule was given the same three injection test conditions prior to 30-min water-access periods. Water-deprived rats given preloads of hypertonic saline drank significantly more water than they did when given preloads of isotonic saline, but not significantly more than when given sham preloads. The present results support the contention that schedule-induced polydipsia is a nonhomeostatic form of drinking that nevertheless may be sensitive to the state of homeostatic thirst mechanisms (i.e., cellular dehydration).

Since its discovery by Falk (1961) over 20 years ago, schedule-induced polydipsia (SIP) has been reliably demonstrated in rats and a variety of other laboratory animals (see Falk, 1969, 1971). When a rat is reduced to 80% of its normal free-feeding body weight and placed on an intermittent food-delivery schedule, it soon begins drinking large amounts of water. This excessive drinking is characterized by a pattern of behavior in which most of the drinking occurs immediately following the delivery of food. Polydipsic drinking is unusual because it occurs in the absence of any physiological need for large amounts of water.

Under normal conditions, internal body fluid balances exert homeostatic control over an animal's water intake (see Kisseleff, 1973). Previous studies have examined the role of internal fluid balances on the regulation of water intake during schedule-induced drinking by preloading the test animals with fluid to artificially manipulate the internal body fluid balance of the animals, and then examining the effects of this manipulation on schedule-induced drinking. These studies, however, have produced mixed results.

Several studies that have examined the effects of fluid preloads on schedule-induced polydipsia have found little or no effect, suggesting that schedule-induced polydipsia drinking is unaffected by the internal body fluid balances that regulate normal drinking (Cope, Sanger, & Blackman, 1976; Falk, 1969; Porter, Young, & Moeschl, 1978; however, see exception by Chapman & Richardson, 1974). Also, Kenny, Wright, and Reynolds (1976) found that rats with established polydipsia persisted in drinking despite the fact that plasma volumes remained normal during and after the test sessions. They also found that gastric preloads of water reduced, but did not eliminate, polydipsic drinking. Kenny et al. did find, however, that oral infusions of water after each food pellet did completely eliminate polydipsia, and they suggested that schedule-induced polydipsia is mediated by oropharyngeal, rather than hydration (i.e., homeostatic), controls. Stricker and Adair (1966) also examined the state of internal fluid balances in rats with established polydipsia and found no apparent relationship between these balances and polydipsia water intakes. Polydipsic rats continued to consume large amounts of water despite the dilution and overhydration of body fluids and tissues. Stricker and Adair also suggested that postprandial oral factors play a role in schedule-induced polydipsia. These studies seem to suggest that SIP is controlled by nonhomeostatic, rather than homeostatic, mechanisms (see Kisseleff, 1973).

There are, however, several studies that have artificially manipulated the internal body fluid balance and found evidence that schedule-induced polydipsia is responsive to such manipulation. Corfield-Sumner and Bond (1978) allowed rats to preload themselves by consuming approximately 30-35 ml of a glucose/saccharin solution prior to being tested with established schedule-induced polydipsia. They found that such preloading abolished established schedule-induced polydipsia and prevented the acquisition of polydipsia in naive animals. They suggested that previous preloading studies have failed to reduce or eliminate polydipsia because such studies

The study presented in this article was submitted by the first author to Virginia Commonwealth University in partial fulfillment of the requirements for the master's degree. Requests for reprints should be addressed to: Joseph H. Porter, Department of Psychology, Virginia Commonwealth University, Richmond, Virginia 23284.

used preloads that were too small. However, Porter, McDonough, and Young (1982) recently demonstrated that 10-ml intraperitoneal preloads of water, but not isotonic saline, were effective in suppressing the acquisition of SIP. In another experiment, which looked at established polydipsia, they found that the 10-ml water preloads were able to produce a significant decrease in drinking approximately equal to the amount of the preload (10 ml). The finding that water preloads significantly reduced schedule-induced drinking clearly contradicts the previously cited studies (Cope et al., 1976; Falk, 1969; Porter et al., 1978) that had found no such effect. Stricker and Wolf (1967) have reported that water is more effective in reducing osmotic (cellular) thirst than in reducing hypovolemic (extracellular) thirst, whereas isotonic saline is more effective in reducing hypovolemic thirst. The results of the Porter et al. (1982) study suggest that cellular thirst may play a role in the regulation of a schedule-induced polydipsia or may interact with the mechanisms that regulate polydipsia.

The present experiment was designed to test this possibility. Loading an animal with hypertonic saline produces drinking (Adolph, Barker, & Hoy, 1954; Gilman, 1937; Kutscher, 1966a, 1966b). Kutscher (1966b) demonstrated that hypertonic saline loads produce this drinking by triggering cellular-thirst mechanisms. If schedule-induced polydipsia is sensitive to cellular dehydration, then hypertonic saline preloads should increase drinking in a schedule-induced-polydipsia situation. To serve as a control, rats were also given preloads of an isotonic saline solution and sham preloads. Additionally, to serve as an indication of the effectiveness of hypertonic saline preloads on cellular thirst, hypertonic, isotonic, and sham preloads were given to rats maintained on a water-deprivation schedule. Water-deprivation-induced drinking is thought to be primarily under the control of cellular thirst mechanisms (Hall & Blass, 1977). This allowed for a comparison of the effectiveness of hypertonic saline preloads on schedule-induced polydipsia with the effects of hypertonic saline preloads on a form of drinking known to be primarily under the influence of cellular controls, that is, water-deprivation-induced drinking.

METHOD

Animals

Nineteen Sprague-Dawley adult male rats (mean weight = 504.7 g) served as the subjects. All were housed individually in an animal colony room on a 7-a.m.-light/7-p.m.-dark cycle. Access to food and water in the home cage varied according to the experimental group to which the animal was assigned.

Apparatus

Polydipsia test sessions were conducted in five BRS/LVE operant chambers (Model SEC-002) housed in the sound-attenuated cubicles. A lever was mounted on the left side of the intelligence panel, but was not used. A BRS/LVE pellet dispenser (PDC/PPD series) delivered standard-formula 45-mg Noyes food pellets to the food magazine located in the center of the intelligence panel. A water bottle was mounted on the right side of the intelligence panel with the tube protruding 1 cm into the chamber through a hole 3 cm above the chamber floor. Fan motors provided masking noise for each chamber. Standard electromechanical and solid-state programming and recording equipment was located in an adjacent room. All injections were given with a 25-ga, 5/8-in. needle. Water-deprivation measures were made in the home cages.

Procedure

Prior to the beginning of the experiment, a pilot study was conducted to examine the effects of saline preloads on water intake in rats. Rats housed in home cages with ad-lib access to food and water were given preloads of either isotonic (0.9%) saline (N = 4) or hypertonic (8.0%) saline (N = 4), and water intakes were recorded at 20-min intervals for 60 min following the preloads. The rats that received hypertonic saline preloads demonstrated significantly greater water intakes than those receiving isotonic saline preloads during the first 20 min following the preloads. These results paralleled the findings of Kutscher (1966b), and, on the basis of these results, the preloads in the present study were given 5 to 10 min prior to the test sessions in order to obtain the greatest effect on drinking.

The 19 rats were initially maintained on ad-lib food and water and were handled and weighed daily. After stable ad-lib body weights (based on the mean of the last 2 days on ad-lib feeding) were obtained, the animals were assigned randomly to two groups, a schedule-induced-polydipsia group (SIP group) and a water-deprivation group (WD group).

SIP Group. The rats (N = 10) in the SIP group gradually were reduced to 80% of their ad-lib body weights by adjusting their daily rations of food over a 1-week period. Water remained freely available in the home cages. Body weights were maintained at 80% of ad-lib weight throughout the study. Polydipsia test sessions with two squads of five rats each were conducted daily. Each test session lasted 30 min. Test sessions were conducted in three phases: (1) a 2-day massed-feeding baseline period; (2) a 20-day polydipsia-acquisition period; and (3) an 8-day test period. During the massed-feeding baseline period, 30 food pellets were placed in the food magazine at the beginning of the session. The rat was then placed in the chamber and allowed to feed and drink freely. At the end of the 30-min session, water intake was measured. This allowed baseline measures of water intake in response to eating 30 food pellets, independent of the effects of the injection conditions or the fixed-time 1-min (FT 1-min) food-delivery schedule. Water intake was determined by weighing the water bottle before and after each session (1 g = 1 ml). Beginning with the start of baseline measures, all animals (including those in the WD group) began receiving sham injections at least every other day in order to familiarize them with the injection procedure.

During the 20-day polydipsia-acquisition period, the animal was placed in the test chamber as previously described, but with food pellets delivered on a FT 1-min food-reinforcement schedule. Under the FT 1-min schedule, a food pellet was delivered every minute, independently of the animal's behavior. Each session terminated with the delivery of the 30th pellet. Drinking after each session was measured as previously described. Water intakes during the last 2 days of the 20-day polydipsia-acquisition period (referred to as the baseline acquisition period) were used to determine whether the animals had actually developed polydipsia. The criteria used to establish schedule-induced polydipsia were that the animal consume at least twice its massed-feeding baseline water intake and, since baseline intakes varied, that the animal consume at least 6 ml of water during the test session.

After the animals had acquired stable polydipsia drinking over the 20-day acquisition period, the 8-day test period began. Immediately prior to each test session (within 5 to 10 min), one of three ip injection conditions was given: isotonic saline solution, hypertonic saline solution, or sham injection. The isotonic solution consisted of a 0.9% saline solution. The hyper-

tonic solution consisted of 8.0% saline solution. In the sham injection condition, the needle was inserted into the peritoneal cavity, but no injection was given. Isotonic and hypertonic injection volumes were based on 2 ml per 400 g of body weight (Kutscher, 1966b). Injections were given using a within-subject design counterbalanced to control for possible intrasubject and intrasquad order effects. Over the 8 test days, the first squad of five rats received injections in the following order: sham, hypertonic, sham, isotonic, sham, isotonic, sham, hypertonic. The second squad of five rats received injections in the following order: sham, isotonic, sham, hypertonic, sham, hypertonic, sham, isotonic. Thus, each animal received a total of four sham injections, two hypertonic saline injections, and two isotonic saline injections. Each saline injection day was separated by 1 sham injection day. After each test session, water intake was measured as previously described, and each animal was returned to the home cage.

WD Group. Those animals not selected for polydipsia training were assigned to the water-deprivation group (N = 9). On the same day the massed-feeding baseline period began for the SIP group, the WD group was placed on a 23.5-h water-deprivation schedule. This allowed water intakes to stabilize before the preload test period began. Food remained freely available in the home cages. Water was made available daily between 9:00 a.m. and 9:30 a.m., and intakes were recorded. During the test period, isotonic saline, hypertonic saline, or sham injections were administered immediately prior to the 30-min water-access period. The same number of injections were given in the same manner and in the same order as that described for the SIP group.

RESULTS

Of the 10 rats in the SIP group, 7 developed schedule-induced polydipsia, as defined by our criteria (see Procedure section). Water intakes for these 7 animals averaged 1.6 ml during the massed-feeding baseline condition and 16.1 ml during the last two sessions of the polydipsia-acquisition period. Therefore, in the analysis comparing schedule-induced drinking and water-deprivation-induced drinking, there were 7 animals in the SIP group and 9 animals in the WD group.

Figure 1 presents mean water intakes for the schedule-induced and water-deprivation-induced drinking groups for the baseline acquisition (based on the mean of the last 2 days of polydipsia-acquisition training) and for the sham (based on the mean of the 4 sham test days), isotonic (based on the mean of the 2 isotonic saline test days), and hypertonic (based on the mean of the 2 hypertonic saline test days) injection conditions. Standard deviations for each condition are also indicated. As can be seen in Figure 1, for the SIP group, the mean water intakes for the baseline acquisition, sham injection, and isotonic saline injection conditions (16.1, 17.0, and 17.8 ml, respectively) were essentially the same. Mean water intake for the hypertonic saline injection condition (25.9 ml) was higher than that for the three control conditions. For the WD group, mean water intakes for the baseline acquisition and sham injection conditions were virtually identical (24.8 and 24.7 ml, respectively), with a slight decrease in mean water intake for the isotonic saline injection condition (21.8 ml) and a slight increase in mean water intake for the hypertonic saline injection condition (26.7 ml). Across all four test conditions, the WD group showed a higher mean water intake than that of the SIP group.

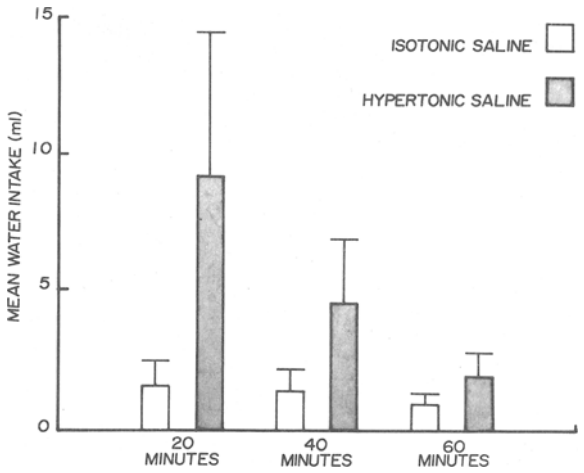

Figure 1. Mean water intakes for the schedule-induced polydipsia and water-deprivation-induced drinking groups are shown for each test condition. Plus one standard deviation is also shown.

A two-factor mixed-design analysis of variance with repeated measures across test conditions was performed on the mean water-intake measures for the two groups and across the four test conditions. The results of the between-groups analysis revealed no significant differences in overall water intakes between the SIP group and the WD group [$F(1,14) = 4.57$, $p > .05$]. Results of the within-subject analysis showed that there was a significant difference in mean water intakes across the four test conditions [$F(3,42) = 19.90$, $p < .001$]. The interaction was also significant [$F(3,42) = 8.13$, $p < .001$]. Post hoc Tukey tests of water-intake measures for the SIP group revealed significantly greater ($p < .01$) water intake during the hypertonic saline injection condition than during the other three test conditions. No significant differences between those three test conditions were found. Post hoc analysis of water-intake measures for the WD group revealed that water intake was significantly ($p < .01$) greater during the hypertonic saline injection condition than during the isotonic saline injection condition.

DISCUSSION

The present study examined the effects of hypertonic saline preloads on schedule-induced (SIP group) and water-deprivation-induced (WD group) drinking. For the SIP group, significantly greater amounts of water were consumed following hypertonic saline preloads than during baseline polydipsia acquisition and during test sessions with sham or isotonic preloads. However, rats in the WD group which were on a 23.5-h water-deprivation schedule, showed less consistent changes in water intakes when given ip preloads of hypertonic saline. The hypertonic saline preloads produced significantly more drinking than did preloads

of isotonic saline, but did not produce significantly more drinking than that seen during baseline acquisition or following sham preloads. These results differed somewhat from those of Kutscher (1966b), who showed that 8.0% NaCl injections reliably increase drinking in rats under water-deprivation conditions (as well as under ad-lib drinking conditions). One possible explanation for the discrepancies between the two studies may be procedural. The rats in the Kutscher study were not adapted to a water-deprivation schedule, whereas the rats in the present study had been. Another possibility is that the degree of cellular dehydration produced by the water-deprivation schedule is greater than that produced by the particular concentration of hypertonic saline used in the present study. One way to test this hypothesis would be to use hypertonic saline preloads with concentrations greater than the 8.0% NaCl used in the present study. This possibility is currently being tested in our laboratory.

In the present study, it was predicted that if schedule-induced polydipsia is sensitive to cellular thirst mechanisms, then preloads of hypertonic saline would increase drinking in a schedule-induced polydipsia drinking situation. The present results showed that hypertonic saline preloads did significantly increase drinking in a schedule-induced polydipsia situation. However, these results do not indicate that cellular dehydration produced by the hypertonic saline preloads acted directly on the mechanisms controlling schedule-induced polydipsic drinking. An obvious possibility that must be examined is that the cellular thirst mechanisms stimulated by the hypertonic saline were not directly related to the schedule-induced polydipsic drinking, and that the increases in drinking seen were due to the different, but possibly additive, effects of separate mechanisms controlling cellular thirst and schedule-induced polydipsic thirst. In fact, observation of the rats revealed that following the hypertonic saline injections, the rats drank vigorously for several minutes, apparently in response to the osmotic thirst challenge, and then resumed the typical postpellet polydipsic pattern of drinking. If two separate mechanisms exist, the possibility of an interaction between the two must be considered. Could one system influence or override the other when given a sufficiently strong challenge? The finding that the hypertonic saline preloads seemed to have differential effects on schedule-induced and water-deprivation-induced drinking under the testing conditions of the present study does suggest that two separate mechanisms may be involved and supports the contention that schedule-induced polydipsia is a nonhomeostatic form of drinking.

REFERENCES

ADOLPH, E. F., BARKER, J. P., & HOY, P. A. Multiple factors in thirst. *American Journal of Physiology*, 1954, **178**, 538-562.

CHAPMAN, H. W., & RICHARDSON, H. M. The role of systematic hydration in the acquisition of schedule-induced polydipsia in the rat. *Behavioral Biology*, 1974, **12**, 501-508.

COPE, C. L., SAMGER, D. J., & BLACKMAN, D. E. Intragastric water and the acquisition of schedule-induced drinking. *Behavioral Biology*, 1976, **17**, 267-270.

CORFIELD-SUMNER, P. K., & BOND, N. W. Effects of preloading on the acquisition and maintenance of schedule-induced polydipsia. *Behavioral Biology*, 1978, **23**, 238-242.

FALK, J. L. Production of polydipsia in normal rats by an intermittent food schedule. *Science*, 1961, **133**, 195-196.

FALK, J. L. Conditions producing psychogenic polydipsia in animals. *Annals of the New York Academy of Sciences*, 1969, **157**, 569-589.

FALK, J. L. The nature and determinants of adjunctive behavior. *Physiology & Behavior*, 1971, **6**, 577-588.

GILMAN, A. The relation between blood osmotic pressure, fluid distribution and voluntary water intake. *American Journal of Physiology*, 1937, **120**, 1357-1359.

HALL, W. G., & BLASS, E. M. Orogastric determinants of drinking in rats: Interaction between absorptive and peripheral controls. *Journal of Comparative and Physiological Psychology*, 1977, **91**, 365-373.

KENNY, J. R., WRIGHT, J. W., & REYNOLDS, T. J. Schedule-induced polydipsia: The role of oral and plasma factors. *Physiology & Behavior*, 1976, **17**, 939-945.

KISSELEFF, H. R. Nonhomeostatic controls of drinking. In A. N. Epstein, H. R. Kisseleff, & E. Stellar (Eds.), *The neuropsychology of thirst: New findings and advances in concepts*. New York: Wiley, 1973.

KUTSCHER, C. An osmometric analysis of drinking in salt injected rats. *Physiology & Behavior*, 1966, **1**, 79-83. (a)

KUTSCHER, C. Effects of hypertonic saline injections and water deprivation on drinking, serum osmolality and gut water. *Physiology & Behavior*, 1966, **1**, 259-268. (b)

PORTER, J. H., McDONOUGH, J. J., & YOUNG, R. Intraperitoneal preloads of water, but not isotonic saline, suppress schedule-induced polydipsia in rats. *Physiology & Behavior*, 1982, **29**, 795-801.

PORTER, J. H., YOUNG, R., & MOESCHL, T. P. Effects of water and saline preloads on schedule-induced polydipsia in the rat. *Physiology & Behavior*, 1978, **21**, 333-338.

STRICKER, E. M., & ADAIR, R. R. Body fluid balance, taste, and postprandial factors in schedule-induced polydipsia. *Journal of Comparative and Physiological Psychology*, 1966, **62**, 449-454.

STRICKER, E. M., & WOLF, G. Hypovolemic thirst in comparison with thirst induced by hyperosmolarity. *Physiology & Behavior*, 1967, **2**, 33-37.

(Manuscript received for publication April 22, 1983.)