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Cyclophosphamide-Induced Sodium Appetite and Hyponatremia in the Rat[1]

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MITCHELL, D., L. F. PARKER AND S. C. WOODS. *Cyclophosphamide-induced sodium appetite and hyponatremia in the rat.* PHARMAC. BIOCHEM. BEHAV. 2(5) 627–630, 1974. – Intraperitoneal administration of 100 mg/kg cyclophosphamide resulted in an increase of both sodium preference and consumption by rats given a choice between water and saline (0.15 M) to drink. Measurement of serum sodium concentration revealed that the cyclophosphamide-treated animals had lower values, thus indicating that the cyclophosphamide-elicited sodium appetite was caused by a drug-induced need for sodium.

Cyclophosphamide Hyponatremia Sodium appetite Specific aversions Conditioned taste aversions

THAT the rat can associate the consumption of a novel substance with subsequent illness, even over delays of several hours, has recently become an issue of major theoretical importance [13,15]. A variety of illness-producing drugs has been employed in the course of investigating conditioned taste aversions, and cyclophosphamide, a tumor suppressing agent, has been used extensively [1, 7, 8, 10, 20, 23].

In a series of pilot investigations, we attempted to establish conditioned aversions to either sucrose or sodium solutions by contingently administering cyclophosphamide to rats immediately following their consumption of one of the two solutions. The results of a two-bottle preference test between one of the test solutions and water revealed that, whereas cyclophosphamide produced rather potent conditioned aversions to the sucrose solution, injecting the animals with the drug following saline consumption resulted in a slight increase in saline preference the next day. Although these findings were rather surprising, similar results have been reported by investigators that attempted to condition aversions to saline using injections of the drug Formalin [19], a drug known to be capable of producing conditioned aversions to a variety of other taste solutions [22]. Since Formalin has been reported to induce hyponatremia and elicit sodium appetite [17], these investigators attributed the failure of Formalin to produce aversions to saline to an interaction between the effect of Formalin on sodium balance and the rat's innate response to sodium depletion. That cyclophosphamide has been reported to induce hyponatremia in humans [2] indicates that our failure to obtain conditioned aversions to saline may have likewise been due to an interaction between the rat's response to sodium depletion and the drug's ability to alter sodium balance.

The purpose of the present series of experiments was, therefore, to determine whether cyclophosphamide is capable of eliciting sodium appetite and inducing hyponatremia in the rat.

EXPERIMENT 1

It has been demonstrated that sodium deficient rats manifest a specific increase in sodium consumption and preference, a response termed sodium appetite [3, 9]. The purpose of this experiment was to ascertain if rats treated with cyclophosphamide in the absence of a conditioned taste aversion setting would manifest such a preferential increase in sodium consumption.

Animals

Twenty naive male Long Evans rats approximately 140 days old and weighing 300–350 g were used. They were housed in individual stainless steel cages and had continuous access to Purina Laboratory Chow throughout the experiment.

Procedure

All animals were given 10 days to become accustomed to consuming their daily supply of water in one 30-min session which occurred at the same time each day. Twelve hr following the drinking session on Day 10, the rats were randomly assigned to one of two equal groups. The rats in one group then received intraperitoneal injections of

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cyclophosphamide dissolved in isotonic saline (Cytoxin: 100 mg/kg) while the rats in the other group received similar injections of an equivalent volume (approximately 1.5 ml) of saline (0.15 M). On Day 11, two calibrated drinking bottles were attached to the front of each rat's cage during the usual 30-min drinking session. One bottle contained water and the other contained 0.15 M saline. The relative positions of the two bottles were randomized for each rat. Consumption of each liquid was recorded to the nearest 0.1 ml.

Twelve hr following the two-bottle test session, the animals which had previously received cyclophosphamide received second injections in the same manner as the first. The control animals received the equivalent volume of distilled water intraperitoneally. This was done to assess any effect of water vs. saline as a control solution. A second two-bottle test was administered on Day 12 with water and saline available as before.

The experiment was carried out under blind conditions. That is, the experimenters recording fluid consumption had no knowledge of group assignments or treatment conditions.

Results and Discussion

The results are summarized in Fig. 1. As depicted, the rats which received cyclophosphamide consumed significantly more saline than did the controls on both test days (Day 11: $t = 4.29$, 18 *df*, $p<0.01$; Day 12: $t = 2.46$, 18 *df*, $p<0.05$). There was no reliable difference in total fluid consumption between the two groups on either test day.

The administration of the different placebo solutions to the control animals resulted in no difference in saline consumption. However, the rats drank significantly more water when 0.15 M saline (1.5 ml) was the placebo relative to when water was used ($t = 4.64$, 18 *df*, $p<0.01$). The reason for this is not clear.

These results demonstrate that the drug, cyclophosphamide, elicits sodium appetite when administered to rats under these conditions. Unlike Formalin, which also elicits sodium appetite [17,22], cyclophosphamide did not elicit thirst, suggesting that a different mechanism may underlie cyclophosphamide-induced sodium appetite.

EXPERIMENT 2

Rats treated with Formalin not only have sodium appetite, but also have a deficit in plasma sodium [17]. The purpose of this experiment was to ascertain if rats treated with cyclophosphamide have a deficit in plasma sodium.

Animals

Ten male Long Evans rats, approximately 140 days old were used. They had previously been used in a lever-pressing experiment, but had received no drugs. All animals were housed in individual stainless steel cages and had continuous access to Purina Laboratory Chow throughout the experiment.

Procedure

A paradigm similar to that of Experiment 1 was employed. The only difference was that on Day 11, at the time of the saline–water preference test in Experiment 1, all animals were killed by decapitation and their blood collected. The blood was centrifuged and sodium and potassium determinations were made on the serum portion by emission spectrophotometry.

A blind procedure was used such that the experimenters that determined sodium and potassium concentrations were unaware of group assignments.

Results and Discussion

The results are summarized in Table 1. Rats which

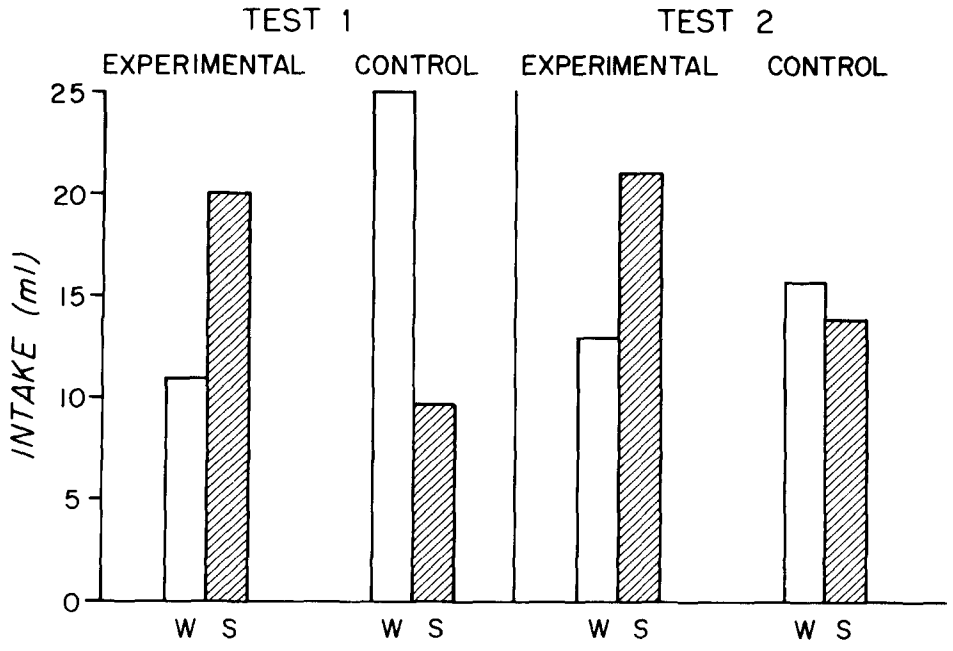

FIG. 1. Mean intake (in ml) of water (W) and 0.15 M saline (S) for the rats in Experiment 1.

TABLE 1

SERUM SODIUM AND POTASSIUM VALUES FOR THE RATS IN EXPERIMENT 2. THE NUMBERS REPRESENT THE MEAN ± THE STANDARD ERROR OF THE MEAN AND ARE EXPRESSED IN mEq/l.

	Sodium	Potassium
Cyclophosphamide-injected (n = 5)	132.3 ± 2.5	8.4 ± 0.4
Water-injected (n = 5)	139.3 ± 1.4	7.1 ± 0.2

received cyclophosphamide 12 hr before the blood samples were collected had significantly lower serum sodium levels than the control animals (t = 2.41, 8 df, $p<0.05$). Further, although there was evidence of slight hemolysis in all of the blood samples, there was a significant elevation of serum potassium in animals that had received cyclophosphamide as compared to control animals (t = 2.26, 8 df, $p<0.05$).

These findings demonstrate that cyclophosphamide can cause a deficit in blood sodium, an effect which may provide the basis for the cyclophosphamide-induced sodium appetite observed in Experiment 1. A decrease in blood sodium has also been reported following treatment with cyclophosphamide in man [2]. Those investigators further reported that urine osmolality increased significantly following administration of the drug to humans, thus implying that increased renal sodium excretion might account for both the observed hyponatremia and the increased urine osmolality.

The elevated serum potassium levels observed in cyclophosphamide-treated animals indicate that the drug may damage cell membranes.

GENERAL DISCUSSION

The results of the first experiment clearly demonstrate that the intraperitoneal administration of cyclophosphamide can elicit sodium appetite in the rat. Other procedures that have been reported to produce sodium appetite in the rat include maintenance on sodium-free diets, adrenalectomy, dialysis against glucose, and the administration of various hormones and drugs including mineralocorticoids, antithyroid drugs, diuretics, and oral contraceptives (e.g., [4–6, 11, 14, 16–18, 21]). Since most of these procedures, with the exception of mineralocorticoid, antithyroid, or oral contraceptive drug administration, are known to produce a reduction in total body sodium, sodium appetite may generally be interpreted as an adaptive behavioral response to sodium depletion. That cyclophosphamide was found to induce hyponatremia indicates that the accompanying sodium appetite is yet another example of the rat's remarkable ability to match nutritional intake to dietetic requirements.

It is noteworthy that cyclophosphamide has been used extensively to produce conditioned taste aversions [1, 7, 8, 10, 20, 23]. Investigators employing the drug have generally assumed that its primary symptom is diarrhea [7, 8, 20, 23]. (It is possible that the diarrhea typically caused by this dose of the drug (cf. [20]) may contribute to the hyponatremia.) Perhaps a more important characteristic of cyclophosphamide administration, with respect to conditioned taste aversions, is its ability to reduce specifically body sodium. That is, it has been demonstrated that there exists an interaction between the need state produced by a toxic agent (e.g., Formalin-induced hyponatremia) and the test substance that is poisoned (e.g., saline or sucrose) in determining whether an aversion will be produced by conditioning treatments [19]. Indeed, our failure to obtain an aversion to saline after poisoning with cyclophosphamide, as discussed earlier, probably reflects such an interaction. The constraints that such an interaction may place on learning have been discussed elsewhere [12], and investigators desiring to condition taste aversions, while employing cyclophosphamide as the aversive agent, should consider them. To study learned consummatory behavior in a rat that is sodium deficient can result in constraints on learning that may prove to be confounding [12,19].

REFERENCES

1. Bolles, R. C., A. L. Riley and B. Laskowski. A further demonstration of the learned safety effect in food-aversion learning. *Bull. Psychon. Soc.* **1**: 190–192, 1973.
2. DeFronzo, R., M. Colvin, H. Braine and P. J. Davis. Cyclophosphamide-induced hyponatremia. *Clin. Res.* **20**: 891, 1973.
3. Denton, D. A. Salt appetite. In: *Handbook of Physiology*, Sec. 6, Vol. 1. Alimentary Canal, edited by C. F. Code. Washington, D.C.: American Physiological Society, 1967, 433–459.
4. Fregly, M. J. Effect of hydrochlorothiazide on preference threshold of rats for NaCl solutions. *Proc. Soc. exp. Biol. Med.* **125**: 1079–1084, 1967.
5. Fregly, M. J. Preference threshold and appetite for NaCl solution as affected by propylthiouracil and desoxycorticosterone acetate in rats. In: *Olfaction and Taste*, edited by C. Pfaffmann. New York: Rockefeller University Press, 1969, 554–561.
6. Fregly, M. J. Effect of an oral contraceptive on NaCl appetite and preference threshold in rats. *Pharmac. Biochem. Behav.* **1**: 61–65, 1973.
7. Garcia, J., F. R. Ervin and R. A. Koelling. Bait-shyness: A test for toxicity with n = 2. *Psychon. Sci.* **7**: 245–246, 1967.
8. Garcia, J., W. G. Hankins, J. H. Robinson and J. L. Vogt. Bait-shyness: Tests of CS-US mediation. *Physiol. Behav.* **8**: 807–810, 1972.
9. Handal, P. J. Immediate acceptance of sodium salts by sodium deficient rats. *Psychon. Sci.* **3**: 315–316, 1965.
10. Lorden, J. F., M. Kenfield and J. J. Braun. Response suppression to odors paired with toxicosis. *Learn. Motiv.* **1**: 391–400, 1970.
11. Nachman, M. Taste preferences for sodium salts by adrenalectomized rats. *J. comp. physiol. Psychol.* **55**: 1124–1129, 1962.
12. Parker, L., A. Failor and K. Weidman. Conditioned preferences in the rat with an unnatural need state: Morphine withdrawal. *J. comp. physiol. Psychol.* **82**: 294–300, 1973.
13. Revusky, S. and J. Garcia. Learned associations over long delays. In: *Psychology of Learning and Motivation*, edited by G. Bower and J. T. Spence. New York: Academic Press, 1970, 1–83.
14. Richter, C. P. Increased salt appetite in adrenalectomized rats. *Am. J. Physiol.* **115**: 367–371, 1939.

15. Rozin, P. and J. Kalat. Specific hungers and poison avoidance as adaptive specializations of learning. *Psychol. Rev.* **78:** 459–486, 1971.
16. Smith, M., G. L. Holman, Jr. and K. H. Fortune. Sodium need and sodium consumption. *J. comp. physiol. Psychol.* **65:** 33–37, 1968.
17. Stricker, E. M. and G. Wolf. Blood volume and tonicity in relation to sodium appetite. *J. comp. physiol. Psychol.* **62:** 275–279, 1966.
18. Weisinger, R. S. and S. C. Woods. Aldosterone-elicited sodium appetite. *Endocrinology* **89:** 538–544, 1971.
19. Weisinger, R. S., L. F. Parker and J. D. Skorupski. Conditioned taste aversions and specific need states in the rat. *J. comp. physiol. Psychol.*, in press.
20. Wilcoxin, H. C., W. B. Dragoin and P. A. Kral. Illness-induced aversions in rat and quail: Relative salience of visual and gustatory cues. *Science* **171:** 826–828, 1971.
21. Wolf, G. Sodium appetite elicited by aldosterone. *Psychon. Sci.* **1:** 211–212, 1964.
22. Woods, S. C., R. S. Weisinger and B. A. Wald. Conditioned aversions produced by subcutaneous injections of Formalin in rats. *J. comp. physiol. Psychol.* **77:** 410–415, 1971.
23. Wright, W. E., D. P. Foshee and G. E. McCleary. Comparisons of taste aversion with various delays and cyclophosphamide dose levels. *Psychon. Sci.* **22:** 55–56, 1971.