Pharmacology Biochemistry & Behavior, Vol. 6, pp. 265–267.

Absence of Sodium Appetite in Cyclophosphamide and DOCA Treated House Mice

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(Received 16 August 1976)

PASLEY, J. N., T. I. KOIKE AND H. L. NELDON. *Absence of sodium appetite in cyclophosphamide and DOCA treated house mice.* PHARMAC. BIOCHEM. BEHAV. 6(3) 265–267, 1977. – Intraperitoneal administration of cyclophosphamide 100 mg/kg and 200 mg/kg significantly lowered plasma sodium and significantly increased plasma potassium but did not result in saline preference in a strain of wild-derived house mice given a choice between water and saline (0.15M) to drink. Deoxycorticosterone acetate treatment in dosages up to 1.5 mg for four days also failed to increase salt intake. The data suggest a possible absence of a sodium appetite mechanism in this species.

Cyclophosphamide Deoxycorticosterone acetate Sodium appetite Hyponatremia Mouse

THE BEHAVIORAL phenomenon of a specific hunger for sodium evoked by deficiency of body sodium was first observed by Richter [11]. Sodium appetite can also be elicited in albino rats [12] and rabbits [7] in the apparent absence of sodium deficiency via injections of deoxycorticosterone acetate (DOCA). Cyclophosphamide (CY), a widely used immunosuppressive agent, has been reported to induce a sodium appetite and hyponatremia in the rat [10] and to induce hyponatremia in humans [4].

The purpose of the present study was first to determine whether CY could increase sodium consumption in the mouse and second to determine whether mineralocorticoids play a role in the CY induced salt appetite.

METHOD

Animals

Laboratory-raised male descendants of a wild stock of house mice (*Mus musculus*), 80–90 days old, were housed in individual stainless steel cages in a light (14 hr/day) and temperature (24°C) controlled room. All animals had continuous access to Purina Mouse Chow containing 0.35% sodium throughout the study.

Procedure

All animals were given 10 days to become accustomed to consuming their daily supply of water in one 30 min period which occurred at the same time each day. Immediately after the drinking period on Day 9, the mice were randomly assigned to one of four groups containing 12 mice each (Table 1). One group received 0.15 mg deoxycorticosterone acetate (DOCA) SC dissolved in a sesame oil vehicle in a 0.3 cc volume on Days 9 and 10. On day 10, twelve hr after the group received DOCA, each mouse in the group was administered 100 mg/kg cyclophosphamide (CYTOXAN, Mead Johnson) IP dissolved in isotonic saline in a 0.1 cc volume. A second group received 0.15 mg DOCA on Days 9 and 10 and 12 hr following the Day 10 drinking session received 0.1 cc of 0.15 M NaCl IP. A third group received 0.3 cc sesame oil SC on Days 9 and 10 immediately after the drinking session and 12 hr later on Day 10 received 100 mg/kg CY IP in a 0.1 cc volume. A final group of mice received similar injections of an equivalent volume of sesame oil on Days 9 and 10 and of isotonic saline 12 hr later. On Day 11, two calibrated drinking bottles were placed on the front of each mouse cage during the usual 30 min. drinking session. One bottle containing water the other containing 0.15 M NaCl. The positions of the bottles were randomized for each cage. Consumption was determined to the nearest 0.1 ml for the two solutions. Immediately, after the salt water preference test, all animals were killed by decapitation and their blood collected in heparinized vials. The blood was centrifuged and plasma sodium and potassium concentrations determined by flame photometry. Statistical evaluation of results was carried out using a two way analysis of variance using the statistical package (STATPACK) written by the Western Michigan University Computer Center.

RESULTS

The results of Experiment 1 are summarized in Table 1. Analyses disclosed no significant differences in body weights among the four groups of mice. Cyclophosphamide

TABLE 1

EFFECT OF CYCLOPHOSPHAMIDE (CY) AND DOCA TREATMENT ON FLUID INTAKES AND PLASMA SODIUM AND POTASSIUM VALUES IN HOUSE MICE. ALL VALUES ARE MEANS AND THEIR STANDARD ERRORS

Exp. 1	n	Treatment CY	Treatment DOCA	Body Wt. (g)	Total Fluid Intake (ml)	Water Intake (ml)	Saline Intake (ml)	Plasma Sodium (mEq/L)	Plasma Potassium (mEq/L)
Control	12	0	0	25 ± 0.7	2.6 ± 0.2	1.6 ± 0.3	1.1 ± 0.4	146.3 ± 1.0	7.4 ± 0.2
CY	12	2 mg	0	28 ± 1.3	2.2 ± 0.2	2.0 ± 0.2	0.2 ± 0.1	142.2 ± 0.9	8.3 ± 0.3
DOCA+ Saline	12	0	0.15 mg	27 ± 1.2	2.9 ± 0.2	2.0 ± 0.2	0.8 ± 0.2	146.3 ± 1.1	7.0 ± 0.2
DOCA+ CY	12	2 mg	0.15 mg	25 ± 1.9	2.8 ± 0.2	1.9 ± 0.2	0.8 ± 0.2	147.5 ± 1.3	9.0 ± 0.2
Exp. 2									
Control	12	0	0	24 ± 0.9	2.8 ± 0.3	1.7 ± 0.2	1.0 ± 0.4	145.4 ± 0.9	6.4 ± 0.5
CY	12	4 mg	0	26 ± 0.9	2.5 ± 0.2	1.9 ± 0.3	0.6 ± 0.2	140.9 ± 0.8	8.2 ± 0.1
DOCA	12	0	0.3 mg	25 ± 0.8	3.4 ± 0.3	2.2 ± 0.4	1.2 ± 0.4	155.0 ± 4.3	5.2 ± 0.3
DOCA	12	0	1.5 mg	24 ± 0.8	3.2 ± 0.3	1.7 ± 0.3	1.3 ± 0.4	149.1 ± 0.6	6.8 ± 0.3

treatment was without effect on total fluid intake. DOCA treatment alone and in combination with CY, however, resulted in consumption of more total fluids ($p<0.05$) than mice treated with CY alone. Mice that received CY and the oil vehicle consumed less saline ($p<0.05$) than did control mice. No difference was observed after DOCA treatment alone or in combination with CY on saline intake. No differences were observed among the four groups of mice with regard to water intake. Cyclophosphamide treatment lowered plasma sodium values ($p<0.01$) compared to controls. DOCA plus CY treatment increased plasma sodium values ($p<0.03$) compared to DOCA treated, CY treated and control mice. Cyclophosphamide treatment and DOCA plus CY treatment increased plasma potassium levels ($p<0.001$) compared to control and DOCA treated mice.

These results demonstrate that CY apparently does not elicit sodium appetite in this species in contrast to that reported in the rat [10]. Moreover, unlike the rat and the rabbit, DOCA in the dosage used did not induce an increase in sodium consumption in our mice suggesting the possible absence of a salt appetite mechanism in this species.

To examine the effects of DOCA and CY further we designed a second experiment to determine if sodium appetite could be induced by larger doses of these compounds.

Four groups of mice were maintained in Experiment 2 utilizing a similar protocol as in Experiment 1 (Table 1). One group received 0.3 mg DOCA SC on Days 9 and 10; a second received 1.5 mg DOCA SC on Days 7, 8, 9, and 10; a third received sesame oil SC on Days 9 and 10 plus 4 mg CY IP (200 mg/kg) on Day 10; a fourth group acting as our control received sesame oil SC on Days 9 and 10 and saline IP on Day 10. On Day 11 the procedures were identical to Experiment 1.

The results of Experiment 2 are summarized in Table 1. As in Experiment 1, there was no difference in total fluid consumption between control mice and the other three groups. In contrast to results in Experiment 1, saline consumption and water consumption was not different among the four groups of mice. Cyclophosphamide treatment, however, lowered plasma sodium levels ($p<0.01$) and enhanced plasma potassium levels ($p<0.001$) compared to controls and DOCA treated mice in agreement with the first experiment. DOCA treatment of 0.3 mg increased plasma sodium levels ($p<0.03$) and lowered plasma potassium values ($p<0.001$) compared to 1.5 mg DOCA treatment and CY treated mice and control values.

DISCUSSION

In a number of animal species, natural or experimental sodium depletion results in a marked increase in sodium appetite that may even be associated with selection of what is normally unpalatable hypertonic solutions [5]. In rats, cyclophosphamide treatment, apparently resulting in depletion of body sodium stores, is also associated with an increase in sodium appetite [10]. In addition to deficits in sodium, hormonal factors are known to influence the salt appetite mechanism [6]. Thus, the administration of DOCA to rats [3] or to rabbits [7] as well as adrenocorticotrophic hormone (ACTH) to rabbits [1] will increase salt ingestion in the absence of sodium deficiency.

Cyclophosphamide or DOCA administration in the dosages used in the present study failed to increase salt ingestion in mice. These results might conceivably be interpreted to suggest that a sodium appetite mechanism is lacking in mice or that the hormonal mechanisms effecting sodium conservation in this species differ from those in the rat, rabbit or sheep. Such a conclusion, however, is tentative and somewhat premature as relatively little is known of the mechanisms that maintain sodium homeostasis in this species. There are similarities between the house mouse and desert rodents such as the kangaroo rat in that house mice can survive without drinking and can tolerate extremely high salt concentrations in their drinking water [8,9]. Salber and Zucker [12] have reported that in the hamster, also a rodent of desert ancestry, salt appetite is not apparent after adrenalectomy or DOCA-treatment. Moreover, the hamster can maintain sodium balance apparently independent of adrenal steroids. Denton [6] has suggested that a reduction in sodium intake causally related to a deficiency in environmental sodium favored the concurrent evolutionary emergence of linked salt appetite and salt conservation mechanisms. Thus, it is conceivable

that a relatively high sodium content of the environment in which our mice evolved provided for an adequate dietary intake of sodium and greatly reduced the selection pressure favoring the emergence of salt appetite or conservation mechanisms.

In the first experiment CY treated house mice consumed significantly less saline than did controls suggesting either illness or perhaps enhanced neophobia to saline induced by this compound. The suggestion of neophobia in our wild-derived mice is not unreasonable since it is apparently more profound in wild species than in the domesticated animals [2]. In the second experiment the lack of difference in saline intake between CY treated and control mice is difficult to explain since one would expect illness or neophobia to be enhanced by higher dosages of this compound. Plasma sodium values were reduced and plasma potassium levels were increased, however, in both experiments after CY treatment in agreement with work by Mitchell *et al.* [10] in rats.

The effects of DOCA in Experiment 2 on sodium and potassium were not dose dependent. The phenomenon of mineralocorticoid escape characterized by initially increased potassium excretion followed by either potassium balance or retention has been described in humans [3]. Perhaps the apparent lesser effect after four days treatment of the 1.5 mg dosage of DOCA compared to the 0.3 mg dose is indicative of the escape phenomenon in our mice.

In conclusion, while CY treatment lowered plasma sodium levels and increased plasma potassium levels in this strain of wild-derived house mice an increase in sodium appetite was not observed. Moreover, DOCA treatment of up to 1.5 mg for four days failed to increase salt intake. Further studies designed to examine the mechanisms of salt balance are needed in order to relate the apparent absence of sodium appetite in this species to its phylogenetic significance.

ACKNOWLEDGEMENT

We wish to thank Dr. Louis Pryor and Dr. Robert Walls for their expert assistance with the statistical analysis of the data.

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