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Effect of An Oral Contraceptive on NaCl Appetite and Preference Threshold in Rats[1]

MELVIN J. FREGLY

Department of Physiology, College of Medicine, University of Florida
Gainesville, Florida 32601

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FREGLY, M. J. *Effect of an oral contraceptive on NaCl appetite and preference threshold in rats.* PHARMAC. BIOCHEM. BEHAV. 1(1) 61–65, 1973.–Dietary administration of the oral contraceptive, Envoid^R, to male rats at 7.5 mg/kg of food for 25 days was accompanied by a spontaneous appetite for salt solution when the rats were given choice between distilled water and 0.15 M. NaCl solution to drink. Administration of the progestational component of Enovid^R, norethynodrel, at 7.5 and 15.0 mg/kg of food for two weeks induced a spontaneous NaCl appetite in other male rats. The estrogenic compound, ethynyl estradiol, administered in food at 1.0 and 2.0 mg/kg for two weeks, was also accompanied by an appetite for NaCl solution in male rats. Thus, the NaCl appetite induced by Enovid^R may be associated with both its estrogenic and progestational components. Other studies have been conducted to determine the effect of chronic administration of Enovid^R at 7.5 mg/kg of food on preference (detection) threshold of female rats for NaCl solution. Drug treatment was accompanied by a significant reduction in preference threshold (0.015 M) compared to controls (0.030 M). The volume of NaCl solution ingested by treated rats was greater than that of control rats at all concentrations tested above threshold, including hypertonic concentrations. Thus, the results suggest that Enovid^R not only induces an appetite for NaCl solution but also reduces the preference threshold for this salt.

Oral contraceptive NaCl appetite Preference threshold

CHRONIC dietary administration of the oral contraceptive, Enovid^R, to ovariectomized rats given a choice between distilled water and NaCl solution to drink was accompanied by a spontaneous NaCl appetite [8]. A preference for the salt solution was present when a concentration of either 0.15 or 0.25 M was offered. An initial objective of these studies was to determine whether Enovid^R would induce a salt appetite in intact male rats. Since Enovid^R is a mixture of both a progestational and an estrogenic agent, a second objective was to determine whether these individual constituents could induce an appetite for NaCl. A number of studies has now shown that a correlation exists between induction of a spontaneous appetite for NaCl solution and reduction in the preference threshold for this salt [5, 10, 11, 18]. Hence, a further objective was to determine whether administration of Enovid^R to rats was accompanied by a reduction in their preference threshold for NaCl solution.

METHOD

Experiment 1

Ten male rats of the Long Evans hooded strain, weighing 350–380 g, were kept in individual cages in a windowless room maintained at 25 ± 1°C and illuminated from 8 a.m. – 6 p.m. Half of the rats were given ground Purina Laboratory Chow into which Enovid^R (each tablet contained 5.0 mg norethynodrel and 0.075 mg mestranol) was mixed at a concentration of 7.5 mg/kg of food throughout the entire experiment. The remaining five rats received Purina Laboratory Chow without the drug. During the first seven days of drug treatment, tap water was given to drink. During the next three days, each rat was given choice between 0.15 M NaCl solution and distilled water to drink. Fluid and food intakes were not measured during these three days but were measured daily during the five following days. At the end of this time, all rats were given tap water to drink. On the 22nd day of drug treatment, each rat was again given choice between 0.15 M NaCl solution and distilled water to drink for three days prior to measurement of food and fluid intakes. During the following five days, fluid and food intakes were measured daily. Fluid containers were infant nursing bottles with cast aluminum caps as described by Lazarow [13]. Food containers have been described in detail by Fregly [4]. A regression analysis of food intake on body weight was performed using the Hewlett Packard Computer Model

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Experiment 2

Thirty male rats of the Long Evans strain, weighing initially from 340–360 g, were maintained in individual cages under the same conditions as in Experiment 1. The rats were divided into five equal groups. Group 1 served as the control group; Group 2 and 3 received ethynyl estradiol at 1.0 and 2.0 mg/kg of ground Purina Laboratory Chow respectively, while Groups 4 and 5 received norethynodrel at 7.5 and 15.0 mg/kg of ground Purina Laboratory Chow respectively. Treatment began two weeks prior to the experiment. During this time all rats received tap water to drink. Three days prior to beginning the experiment, each rat was given choice between 0.15 M NaCl solution and distilled water to drink. On the fifteenth day after beginning treatment, food and fluid intakes were measured for one week.

Experiment 3

Twelve female rats of the Blue Spruce Farms strain, weighing 180 – 210 g were kept in individual cages under the same conditions, including food and fluid containers, as those described in Experiment 1. Two weeks prior to initiation of the experiment half of the rats received food with Enovid^R mixed into it at a concentration of 7.5 mg/kg of ground Purina Laboratory Chow. During this period each rat was allowed to choose its drinking fluid from either of two bottles, each containing distilled water. Observations of the daily fluid intake by each rat indicated that roughly half was ingested from each bottle.

At the beginning of the experiment during the first two-day test period, each rat was allowed to choose between two bottles of distilled water. During subsequent two-day test periods, each rat was given choice between water and the following molar NaCl solutions in chronological sequence: 0.0006, 0.001, 0.003, 0.006, 0.009, 0.012, 0.015, 0.020, 0.025, 0.030, 0.050, 0.075, 0.100 0.150, 0.200, 0.250, and 0.00 (distilled water). Each concentration was made from a single stock solution by serial dilution, and each dilution was checked for accuracy by determination of sodium concentration by flame photometry using lithium as the internal standard. Positions of the two bottles on each cage were interchanged daily to discourage habit formation in selection of drinking fluids. Daily intakes of water and NaCl solutions were measured and expressed as ml/100 g body weight/day to relate fluid intake to a unit of body weight.

The criterion of preference threshold used was similar to that of Richter [17]; namely, the concentration of NaCl solution at and above which simultaneous mean volume taken from the bottle containing NaCl solution exceeded that taken from the water bottle. A second technique was also used to compare threshold concentrations. Simultaneous intakes of distilled water and NaCl solution were graphed for each rat. The concentration at which NaCl intake increased and remained elevated, while water intake decreased and remained low (cross-over concentration), was then determined [7]. Individual data for rats in each group were averaged and the means were compared statistically using a t test [20].

RESULTS

Experiment 1

Administration of Enovid^R for ten days reduced food intake significantly ($p<0.01$) while increasing intake of NaCl solution in excess of simultaneous water intake in two of five rats. The resultant large variability precluded statistical significance (Table 1). After 25 days of treatment, a significant ($p<0.05$) reduction in food intake and a significant increase ($p<0.01$) in intake of 0.15 M NaCl solution was observed. In neither study was either water intake or body weight affected significantly by treatment.

A regression analysis of body weight (x) in g on food intake (y) in g/day was performed for both groups of rats during the entire experiment (Fig. 1). The slopes of the lines are significantly different ($p<0.01$), but the intercepts do not differ. Thus at a given body weight above 200 g, less food was consumed daily by Enovid^R-treated rats than by controls.

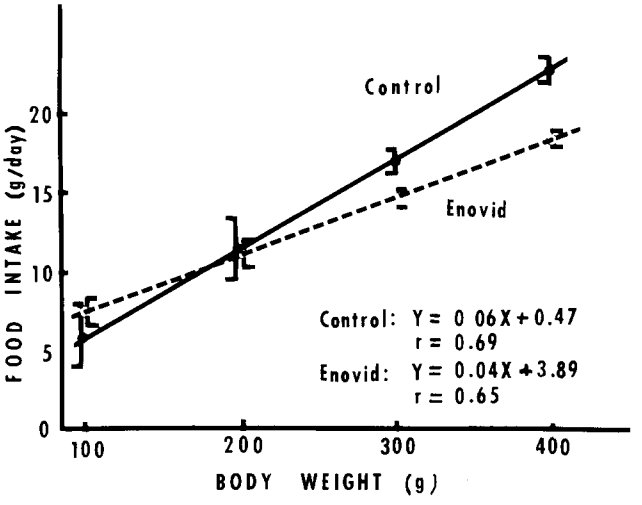

FIG. 1. Regression of food intake (g/day) on body weight (g) is shown for control (solid line) and Enovid^R treated rats (dashed line) in Experiment 1. One standard error is set off at each point. The equation of the regression for control and Enovid^R treated rats is shown in the figure. The correlation coefficient (r) is also given.

Experiment 2

Administration of ethynyl estradiol at both 1.0 and 2.0 mg/kg of food for two weeks prior to the experiment significantly increased ($p<0.05$) intake of 0.15 M NaCl solution and decreased significantly ($p<0.01$) both water intake and food intake (Table 2). Body weight was also significantly ($p<0.01$) reduced. Administration of norethynodrel at 7.5 and 15.0 mg/kg of food for two weeks prior to the experiment also increased ($p<0.01$) intake of 0.15 M NaCl solution and decreased significantly ($p<0.01$) both water intake and body weight (Table 2). Although food intakes of the norethynodrel treated groups were less than that of the control group, the differences were not significant.

TABLE 1

EFFECT OF DIETARY ADMINISTRATION OF AN ORAL CONTRACEPTIVE ON SPONTANEOUS INTAKE OF 0.15 M NaCl SOLUTION BY MALE RATS

Experimental Group	No. of Rats	Number of days given drug prior to expt.	Intakes (ml or g/100g b.w.) of: water	0.15 M NaCl solution	food	Mean body wt (g)
Study 1						
Control	5	--	6.56 ±0.38†	1.49 ±0.68	5.83 ±0.10	360 ± 7
Enovid-treated* (7.5 mg/kg food)	5	10	5.17 ±1.30	5.11 ±2.15	4.58 ±0.32‡	356 ±26
Study 2						
Control	5	--	5.69 ±0.83	2.30 ±1.00	5.74 ±0.25	378 ± 3
Enovid-treated* (7.5 mg/kg food)	5	25	4.60 ±0.99	8.03 ±1.52 §	5.00 ±0.21 §	361 ±24

*Intakes measured for 5 days
†± one standard error of the mean
‡Significantly different from control ($p<0.01$)
§Significantly different from control ($p<0.05$)

TABLE 2

EFFECT OF CHRONIC DIETARY ADMINISTRATION OF ETHYNYL ESTRADIOL AND NORETHYNODREL ON SPONTANEOUS INTAKES OF DISTILLED WATER, 0.15 M NaCl SOLUTION AND FOOD

Treatment	No. of rats	Mean body wt. (g)	Intakes (ml or g/100 g body wt /day) of: Water	0.15 M NaCl solution	Food
Control	6	418±19*	8.2±0.4	2.1±0.5	5.4±0.1
Ethynyl estradiol (1.0 mg/Kg food)	6	329±13‡	2.7±1.1‡	11.7±1.5†	2.9±0.5‡
Ethynyl estradiol (2.0 mg/Kg food)	6	314±11‡	3.2±0.9‡	15.1±3.7‡	3.5±0.4‡
Norethynodrel (7.5 mg/Kg food)	6	352±12‡	4.0±0.4‡	12.1±3.2†	4.7±0.3
Norethynodrel (15.0 mg/Kg food)	6	359±10‡	5.2±0.5‡	7.8±1.0†	5.2±0.2

*One standard error of mean
†Significantly different from control ($p<0.05$)
‡Significantly different from control ($p<0.01$)

Experiment 3

Administration of Enovid^R was accompanied by a significant ($p<0.01$) reduction in preference threshold for NaCl solution (Fig. 2). The treated rats, as a group, detected the difference between distilled water and NaCl solution when the concentration of the latter was 0.015 M while controls detected the difference between the two bottles when the NaCl concentration was 0.030 M. Thus, the preference threshold of the treated group lies between 0.012 and 0.015 M while that of the control group lies between 0.025 and 0.030 M. The cross-over concentration values for the treated and control groups are 0.009 ± 0.004 and 0.030 ± 0.007 (S.E.) respectively ($p<0.01$). Thus, the cross-over concentration of the control group agrees with its preference threshold while that of the Enovid^R treated group is somewhat lower than its preference threshold. However, the preference threshold lies within one standard error of the cross-over concentration and would not appear to be different from it.

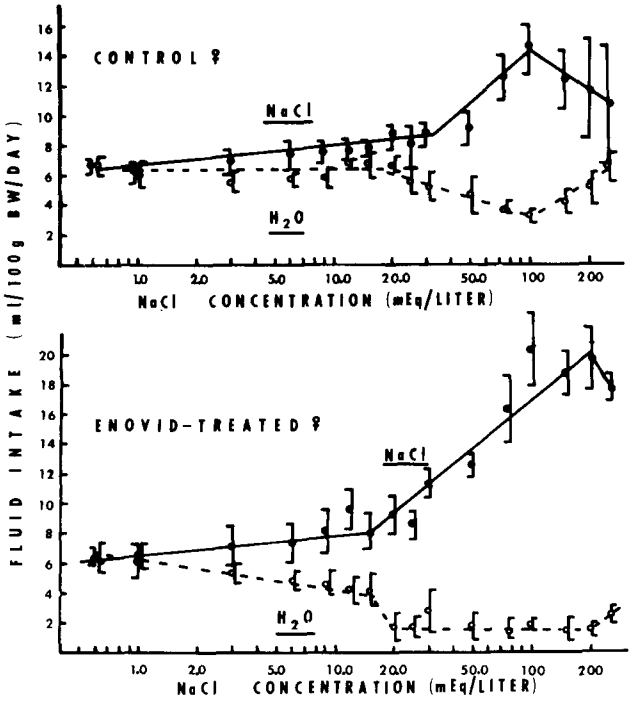

FIG. 2. Spontaneous intakes of NaCl solution (solid line) and distilled water (dashed line) are shown for control rats (upper panel) and Enovid^R treated (lower panel) rats. One standard error is set off at each mean.

The results indicate that Enovid^R treated rats could differentiate between NaCl solution and distilled water at approximately one-third to one-half the concentration required by control rats. It should also be noted in Fig. 2 that the volume of NaCl solution ingested by treated rats was greater than that of control rats at all concentrations tested above threshold, including hypertonic concentrations. Mean food intake of treated rats (g/100 g body weight/day; not shown in Fig. 2) was slightly less than that of controls at all periods throughout the experiment although the difference from control value was never significant. Mean body weight of treated rats (not shown in Fig. 2) was also less than that of controls, and the difference from the control group was always significant ($p<0.05$). Mean body weights of the control group at the beginning and end of the experiment were 195 and 236 g respectively while mean body weights of the treated group were 175 and 199 g respectively.

DISCUSSION

Administration of the oral contraceptive, Enovid^R, to male rats for ten days increased their spontaneous intake of 0.15 M NaCl solution. Administration of the drug for 25 days resulted in a greater intake of 0.15 M NaCl solution. These results confirm those of a previous study in which a salt appetite was reported to accompany an eight month administration of Enovid^R to ovariectomized rats [8]. Since the NaCl appetite appears in male rats administered Enovid^R (Table 1) as well as intact female rats (Fig. 1), neither the presence nor absence of ovaries is a prerequisite to induction of a NaCl appetite. The NaCl appetite induced by Enovid^R may be related to both its progestational and estrogenic components since administration of each singly was accompanied by a spontaneous NaCl appetite (Table 2). However, the results with ethynyl estradiol must be accepted with caution because the lowest dose of the compound used here was thirteen times that present in Enovid^R. Norethynodrel was administered at the same (7.5 mg/kg food) and at twice (15.0 mg/kg food) the amount present in Enovid^R.

Both the perference threshold and the cross-over concentration for NaCl solution were reduced by administration of Enovid^R. Thus, treated rats could differentiate between water and NaCl solution at a concentration one-third to one-half that of their controls. The preference threshold of the control rats observed here was similar to values reported earlier from this laboratory (0.029 and 0.023 M) [5, 10] but was higher than that reported by Weiner and Stellar (0.009 M) [23] and Richter (0.0094) [18], both of whom used different strains of rats. The preference threshold concentration of Enovid^R treated rats was similar to that reported earlier for hydrochlorothiazide [5] and propylthiouracil treated [10] rats. It is of interest that all experimental procedures thus far tested inducing a chronic appetite for NaCl solution, viz., adrenalectomy, hypothyroidism and administration of certain hormones and drugs, including desoxycorticosterone acetate, propylthiouracil or hydrochlorothiazide, are also accompanied by a reduced preference threshold for NaCl solution [5, 10, 11, 17, 18]. To this list may now be added treatment with Enovid^R.

The apparent relationship between reduced preference threshold for NaCl solution and NaCl appetite has not been explained satisfactorily, although a number of possibilities exist and have been presented [6]. For example, it has been suggested that these two phenomena may be related directly to the Na/K ratio of the saliva bathing taste receptors and indirectly to blood level of aldosterone, which appears to control salivary Na/K ratio [6]. Blood levels of aldosterone may be altered by oral contraceptives. Thus, increases in renin activity and in blood levels of renin substrate, as well as an increased excretion of aldosterone, are reported for women ingesting oral contraceptives [1–3, 19, 22] and rats administered estradiol [14] or diethylstilbestrol [15, 16]. It is also possible that the salt appetite

and reduced preference threshold are induced by central nervous mechanisms responding to either altered aldosterone or electrolyte concentrations of blood or to altered extracellular fluid volume. Changes in body sodium content resulting from increased renal sodium loss induced at least by norethynodrel may also play a role [12]. It is difficult to state which, if any, of the suggestions mentioned above can explain the spontaneous appetite and reduced preference threshold for NaCl solution. Further studies will be required to separate these possibilities.

Treatment with Enovid^R was accompanied by a reduced gain in body weight. Although food intake of the treated group was always less than that of the control group in Experiment 3, the difference between groups was never significant. These studies provide no clue regarding the mechanism of the weight loss. Wade [21] in a recent review emphasized the central nervous effect of estrogenic compounds in reducing food intake and increasing spontaneous muscular activity. However, the dose of Enovid^R used here reduced spontaneous running activity at least temporarily [8, 9], as did its estrogenic component when tested separately [9]. Thus, it seems unlikely that Enovid^R increased spontaneous activity and that this could explain the reduction in body weight. Other studies have also shown that the food intake of Enovid^R treated rats increases to the same level as that of controls when exposed to air at 6°C [8]. Thus, Enovid^R does not block the cold induced increase in food intake. Further studies are needed to focus attention on the energy exchange of Enovid^R treated rats so that the interrelationship among food intake, muscular activity, and body weight may be better understood.

The dose of Enovid^R used here was chosen because of its use in other studies [9]. A comparison between the daily dose of drug ingested by these rats and that used clinically indicates that the rats received about 2.8 times the daily dose ingested by humans when equated to a unit of body weight. Thus, the applicability of these results to humans cannot be stated with certainty.

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