

Male-female differences in response to stomach loads of hypertonic NaCl in rats*

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Adult male, female, and ovariectomized rats were subjected to osmotic stress via hypertonic NaCl stomach loads. Males and ovariectomized females, in addition to increasing water intake, responded to the stressor by decreasing food intake and losing weight 24 h after the load was administered. Female rats did not decrease food intake but did increase water intake. The results were interpreted in terms of female sex hormone activity which affects renal function.

Many investigators (e.g. Gilman, 1937; Novin, 1962; Stricker, 1966) have shown that extracellular fluid (ECF) tonicity and volume increases upon the administration of hypertonic NaCl, and that these changes in ECF parameters are potent stimuli for drinking in adult rats. Drinking, then, is one possibility open to the organism for meeting osmotic stress. Indeed, Fitzsimons (1961) has shown that nephrectomized rats' intake of water is sufficient to dilute the blood to preinjection levels when stressed osmotically.

Another possibility open to the organism in meeting this stress is to excrete solutes in excess of normal operating levels (Adolph et al, 1954; Falk, 1955).

A third way of responding to osmotic stress produced by hypertonic NaCl is to reduce food intake. The eating of dry food increases the tonicity of ECF (Hatton & Almi¹; Novin, 1962); and if the system is already being stressed, the rat decreases its food intake rather than adding to the already present stress. Thus, Adolph (1943) showed that rats decrease their food intake upon voluntary ingestion of seawater and lose weight. Cort (see Brobeck, 1955) reports that, with hypertonic concentrations injected subcutaneously, adult rats decreased their food intake. Similar findings have been reported by Jacobs (1964), Gutman & Krausz (1969), and Schwartzbaum & Ward (1958). These investigators used males or did not specify the sex of their Ss.

The purpose of Experiment 1, therefore, was to determine if a sex difference in response to hypertonic NaCl loading existed.

EXPERIMENT 1 Method

Seven male and four female rats of the Sherman strain, 90 days old, were used. Ss were stomach loaded with a

5.0% body weight (bw) of a 6.0% NaCl solution (wt x volume). The Ss were stomach loaded from a syringe through an orally inserted Fr 8 catheter. Ss were maintained on ad lib food and water. Baseline intakes were measured for 5 days prior to loading and for at least 3 days after loading.

Results and Discussion

The first two columns in Table 1 present data concerning weight, food intake, and water intake in the adult male and female rats. It can be seen that males lose more weight than females ($p < .001$, Fisher test) and decrease their food intake more than females do ($p < .01$, Fisher test).

It should be noted that the amount of sodium and not the volume of the solution determines the weight loss in males. It could be argued that adult males are consistently heavier than females and, therefore, receive larger loads. However, data obtained in the writer's laboratory show that with loads of a fixed size (10.0 cc, 6.0% NaCl) one observes weight losses in males and not in females. Moreover, in males and females of comparable weights, a 3.0% bw load of a 10.0% NaCl solution produces significant weight loss in males but not in females.

These data, therefore, demonstrate

a male-female sex difference in response to ECF stress induced by hypertonic NaCl.

EXPERIMENT 2

Of the many differences between adult male and female rats (size, weight, amount of fat, etc.), an obvious one is the kinds and amounts of sex hormones secreted. At this point a digression is necessary into the physiological mechanisms of the kidney-adrenal feedback loop which controls sodium levels and hence the tonicity of ECF. For excellent treatments of this subject, see Pitts (1968) and Woodbury (1966). We then can consider the way in which gonadal hormones affect this system.

Briefly, hypertonic NaCl increases the tonicity of ECF, and the ADH system becomes active; this system serves to eliminate excess solutes and conserve water. If ECF solute levels are made very high however, the excretion of solutes may obligate a net water loss. There then occurs a secondary hypovolemia (see Blass, 1968). At this point the mechanisms for regulating ECF volume become active; renin, angiotensin, and subsequently aldosterone production increases. Aldosterone is now working at odds with the ADH system to draw sodium back into the ECF. The ADH system, however, predominates as a hypertonic urine is excreted.

Progesterone has been shown to inhibit the sodium-retaining action of aldosterone by a process of competitive inhibition in the renal tubule of the kidney (Sharp & Leaf, 1966; Uete & Venning, 1963). Progesterone also has an inhibitory effect on the synthesis of aldosterone itself (McKerns & Bell, 1960; see also Müller, 1971). Sodium excretion increases with the administration of progesterone (Landau et al, 1955).

The male sex hormone, testosterone, is ineffective as an inhibitor of aldosterone at its

Table 1
Mean Percent Change in Weight, Food and Water Intake from Baseline, and Kidney Function Estimates in Adult Male, Female, and Ovariectomized Female Rats 24 H After Stomach Loading With 6.0% NaCl or Sham Loading

Measure	Male (7)	Female (4)	Ovariectomized Female (8)	Sham Operated Female (2)	Sham-Loaded Ovariectomized Female (6)
Weight Change	-5.0	-0.7	-4.1	-0.6	-0.9
Food Intake Change	-48.0	-8.0	-39.0	-6.0	+0.2
Water Intake Change	+86.0	+50.0	+51.0	+48.0	+8.2
k	.25 ± .03	.14 ± .04	.22 ± .10	.13 ± .04	—

Note—Number in parentheses refers to the number of Ss in each condition.

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production site (McKerns & Bell, 1960), or at the level of the kidney, as it has no appreciable effect on the renal excretion of electrolytes (Thorn & Engel, 1938).

The above physiological considerations suggest that adult female rats, because of their hormonal chemistry (i.e., the presence of gonadal hormones which inhibit the sodium-retaining action of aldosterone), should be able to excrete a salt load more rapidly than males can. If ECF tonicity is high, then, food intake will impose an additional osmotic stress (eating dry food increases ECF tonicity; Novin, 1962). An animal which can dispose of an imposed osmotic imbalance rapidly (here, the adult female) will then be able to eat without incurring a further osmotic overload. In males, which lack significant amounts of female sex hormones, the longer lasting osmotic deficit may impose a persisting reluctance to accept the intensified osmotic imbalance that feeding would entail.

This explanation implies that the persistence of feeding and maintenance of body weight in the adult female depend upon ovarian secretions. With ovarian removal, the female rat should decrease its food intake and lose weight after osmotic loads, as does the adult male. Therefore, this experiment compared the response to such loads of ovariectomized rats to intact females and males.

Method

Ss used in this experiment were Sherman rats, 14 ovariectomized females and two sham-ovariectomized females. All Ss were on ad lib food and water and housed in constant light. Ss were stomach loaded with a 5.0% bw of a 6.0% NaCl solution (wt x volume). Weight, water, and food intake were measured for 5 days prior to loading and were used as baseline intake. Ovariectomies and sham operations were performed under ether anesthesia following the procedure outlined by Zarrow et al. (1964). Of the 14 females that were ovariectomized, 8 received stomach loads and 6 received sham loads (i.e., the stomach tube was inserted but no load was injected). Ovariectomized Ss were tested 10-15 days after ovariectomy. These Ss had shown the weight gains one typically observes with ovariectomy (Rodier, 1969).

RESULTS AND DISCUSSION

Table 1 shows percent change from baseline in weight, water intake, and food intake after stomach loads of hypertonic solution and sham loads. It can be seen from Table 1 that ovariectomized females lose more weight than normal females ($p < .01$,

Fisher test) but less than adult males, but not significantly so. Sham-operated and ovariectomized sham-loaded females show no substantial decrease in food intake or body weight.

Ovariectomized females decrease their food intake more than intact females ($p < .02$, Fisher test). Intact, ovariectomized sham-loaded, and sham-operated females show only small changes in food intake. Water intake is increased in all animals.

These data can also be used to provide an estimate of kidney function. This transformation should serve to clarify the effects that sex hormones have on the contribution of the kidney to osmotic regulation.

Corbit (1969) has shown that drinking by the rat when exposed to osmotic stress induced by hypertonic NaCl loads can be described by the following equation: $D = k[(n/\alpha) - v]$, where D is the amount drunk, k is the contribution of the kidney to osmotic regulation and is a function of the time after loading, n is the number of millimoles of effective osmotic solute, v is the amount of water in milliliters in which the solute is dissolved, and α is the effective osmotic solute dissolved in the body fluids of the S in water balance. For the rat, $\alpha = 0.15$ M.

Assuming that the rat on ad lib access to food and water is in osmotic balance, one can solve the equation for k to determine the contribution of the kidney to osmotic regulation. A k of 1.0 means no kidney function (a nephrectomized animal, where the rat drinks precisely the volume of water required to dilute the load to isotonicity); k = 0.0 means total and immediate elimination of excess effective solute. Any value between 0.0 and 1.0 is interpreted as follows: if k = 0.25, it means that 75% of the load is excreted in the time it takes the S to drink 25% of the water necessary to dilute the load to isonicity. The k values for the Ss in Experiments 1 and 2 are also presented in Table 1.

The groups of primary importance to this report are males, females, and ovariectomized females. Males have higher k values than females ($p = .014$, Fisher test). These data are interpreted as follows: the female kidney is more "responsive" to osmotic loads as 86% of the load is excreted in the time it takes males to excrete 75% of the load. The ovariectomized female's k value is also significantly greater than the intact female ($p = .03$, Fisher test) but is not different from the male k value. This means that the ovariectomized female rat's kidney is also not as "responsive" to osmotic loads as the intact female's kidney.

These results, then, support the hypothesis that the sex hormones of

the female, probably through their action on the kidney, allow it to "tolerate" an osmotic stress so that it does not have to decrease its food intake as does the male.

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NOTE
1. Hatton, G. I., & Almlı, C. R. Plasma osmotic pressure and volume changes as determinants of drinking threshold. Unpublished manuscript, 1969.