Estradiol increases salt intake in female normotensive and hypertensive rats

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Kensicki, Eric, Gail Dunphy, and Daniel Ely. Estradiol increases salt intake in female normotensive and hypertensive rats. J Appl Physiol 93: 479–483, 2002. First published March 22, 2002; 10.1152/japplphysiol.00554.2001.—The objective of this study was to examine whether or not estradiol (E2) alters sodium intake in hypertensive and normotensive female rats. It was hypothesized that higher doses of E2 would increase sodium consumption and that this response would be greater in spontaneously hypertensive rats (SHR) compared with Wistar Kyoto (WKY) rats. The study involved female SHR and WKY (n = 12/group). All animals were ovariectomized. Six of twelve rats from each strain received three progressively larger doses of β-estradiol propionate (each dose lasting 2 wk), whereas the other six rats from each strain received sham implants. Blood E2 levels were measured by radioimmunoassay after each 2-wk period, allowing a 10-day washout period before the next E2 dose. Rats had access to 0.0, 0.5, 1.0, and 1.5% NaCl solutions to drink throughout the experiment. There was a significant positive correlation between sodium intake and plasma E2 (r = 0.8, P < 0.001). Both strains avoided the 1.5% NaCl, and the increased sodium intake was achieved by an increase in consumption of the 0.5% NaCl. SHR females consumed more sodium than WKY females, which is similar to what has been observed in males of these strains. In conclusion, E2 was positively correlated with sodium intake in both strains of rat, with the hypertensive rats consuming more sodium than the normotensive rats.

METHODS

Four groups (n = 6/group) of virgin female rats (12–18 mo old) were studied. These groups included SHR given E2 implants, SHR given sham implants, WKY given E2 implants, and WKY given sham implants. The rats were housed two per cage (50 × 38 × 20 cm), temperature and humidity were constant, and lighting was on a 12:12-h cycle. Each pair of rats was given four bottles of sodium chloride solution of varying concentrations (0.0, 0.5, 1.0, and 1.5%) and was provided with rat chow (0.3% Na; Teklad, Madison, WI) (1, 10). Total sodium consumed in drinking water and saline concentration preference were determined by weighing each water bottle to the nearest 0.1 g and calculating fluid and sodium consumption. Rats were also weighed to the nearest gram at the beginning, middle, and end of the experiment.

All rats studied were ovariectomized. Rats were sedated with 50 mg/kg Brevital (Eli Lilly, Indianapolis, IN), and their ovaries were then surgically removed. After a recovery period of 2 wk, E2 implants were administered subcutaneously at the base of the neck to one-half of the SHR (n = 6) and one-half of the WKY rats (n = 6). These implants were composed of Silastic tubing (Dow Corning Midland) containing β-estradiol propionate given in three different doses to each rat (6 SHR and 6 WKY). This was accomplished by changing the length of the implant tube. The first implant was 4 mm in length, the second was 7.5 mm in length, and the third was 15 mm in length. The ends were then sealed with Silastic medical-grade silicone adhesive (type A, Dow Corning). Before surgical implantation, the implants were immersed overnight in 5% bovine serum albumin and soaked in 70% ethanol 1 h before surgery (29). Each implant remained in the rat for a period of 2 wk; however, there was a 10-day washout period before a new implant was placed into the rat. This allowed E2 levels to fall back to normal levels. The other six SHR and six WKY rats were given sham implants to provide control for the experiment. The sham implants remained in these rats for the duration of the experiment. Two weeks after each implantation, just before the E2 implant was to be removed, all rats (including sham implants) were ovariectomized. Six of twelve rats from each strain received three progressively larger doses of β-estradiol propionate (each dose lasting 2 wk), whereas the other six rats from each strain received sham implants. Blood E2 levels were measured by radioimmunoassay after each 2-wk period, allowing a 10-day washout period before the next E2 dose. Rats had access to 0.0, 0.5, 1.0, and 1.5% NaCl solutions to drink throughout the experiment. There was a significant positive correlation between sodium intake and plasma E2 (r = 0.8, P < 0.001). Both strains avoided the 1.5% NaCl, and the increased sodium intake was achieved by an increase in consumption of the 0.5% NaCl. SHR females consumed more sodium than WKY females, which is similar to what has been observed in males of these strains. In conclusion, E2 was positively correlated with sodium intake in both strains of rat, with the hypertensive rats consuming more sodium than the normotensive rats.

THE FREE SODIUM INTAKE (ingestion of sodium by animals in neutral or positive sodium balance) of female mammals of most species is greater than that of males of the same species (4, 5, 9, 10, 14, 16, 17, 19–21). Also, we have shown in males that one component driving sodium appetite is the sympathetic nervous system (SNS), because an α-adrenergic blocker (phentolamine), a norepinephrine depletor (reserpine) (1), and clonidine, which blocks central SNS outflow, all reduced sodium consumption in spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats (1, 10). Other mechanisms have been reported that show that ACTH and the renin-angiotensin system can increase salt appetite in pregnancy and during lactation. Therefore, experiments were designed to test the hypothesis that estrogen (E2) increases salt appetite in females and that there would be a greater sodium intake in the hypertensive strain because of higher sympathetic activity than in the normotensive strain, as reported in males (1, 10).
results) were again sedated (50 mg/kg Brevital), and a retroorbital blood sample was taken to determine serum E2 levels. Plasma E2 levels were measured by radioimmunoassay (ICN Pharmaceuticals, Costa Mesa, CA). The E2 kit measured total E2 with the following percentages of cross-reactivity: 17β-estradiol = 100%, estriol = 9%, 17α-estradiol = 7%, and the remainder of the steroids was <0.01%. The percent coefficient of variation for the range that we measured was 5.5% for intra-assay and 10.9% for interassay variation. The E2 plasma levels produced by the varying length of implants were several-fold higher than normal E2 levels in our rats and ranged from 25 to 50 pg/ml over a 4-day cycle. Two-way and three-way ANOVAs were performed by using strain, E2 dose, and NaCl concentration preference with follow-up post hoc analysis if the F-test was significant. Linear regression was performed on E2 dose and sodium consumption. Significance was assumed if \( P < 0.05 \).

RESULTS

E2 levels for rats given sham implants were not measurable. Plasma E2 levels were significantly higher for the 7.5-mm implant compared with the 4-mm implant in both strains (for SHR, \( P < 0.05 \); for WKY, \( P < 0.001 \); Fig. 1). Plasma E2 levels did not change significantly in either strain between the 15-mm implant compared with the 7.5-mm implant.

SHR sodium consumption decreased (50%) with the 4-mm implant compared with its sham control. The 7.5-mm implant produced a 190% increase (\( P < 0.05 \)) in sodium consumption compared with its sham control. The 15-mm implant produced a 218% increase (\( P < 0.05 \)) in sodium consumption compared with its sham control (Fig. 2). The WKY rats showed a similar trend, with the 4-mm implant decreasing sodium consumption by 62% compared with the respective sham control. The 7.5-mm implant and 15-mm implant increased sodium consumption 79% (\( P < 0.01 \)) and 67% (\( P < 0.05 \)), respectively, compared with the respective sham control (Fig. 2).

A significant strain effect was also noted. When given the 4-mm E2 implant, SHR consumed 28% more NaCl than did WKY rats (\( P < 0.05 \)). When given the 7.5-mm E2 implant, SHR consumed 11% more NaCl than did WKY rats (nonsignificant). When given the 15-mm E2 implant, SHR consumed 39% more NaCl than did WKY rats (\( P < 0.05 \)).

There was a strong implant-type sodium preference effect (\( F = 19.8, P < 0.0001 \)). The rats given the 7.5- and 15-mm E2 implants had a significant preference for the 0.5% solution compared with the 0.0% solution (\( P < 0.001 \) for both). The SHR 7.5-mm E2 group drank significantly more 0.5% NaCl than did the 4-mm E2 group (Fig. 3). WKY animals showed a similar implant-type sodium preference effect, with most groups preferring the 0.5% NaCl solution (\( F = 13.9, P < 0.0001 \), Fig. 4).

Table 1 shows that there was a significant strain, E2 implant, and bottle concentration effect (\( P < 0.001 \) for all). Also there were significant interactions due to SHRs consuming more sodium than WKY animals by drinking more of the 0.5% NaCl solution, and, at higher E2 levels, even higher volumes of the 0.5% NaCl were consumed.

There was a significant positive correlation between plasma E2 levels and daily sodium intake (\( r = 0.800, \)
Because rats were housed two per cage, the total plasma E2 level per cage was compared with the total sodium consumed per cage and averaged.

\[ F = 23.149, P < 0.001, \text{Fig. 5}. \]

DISCUSSION

Female mammals of most species consume more sodium than do males of that same species (2, 4, 9, 14, 16, 17). However, other studies have shown that E2 inhibits sodium appetite in the rat (27, 31). The difference in these studies was either that the test period was short, from 8 h to 2 days, which does not allow enough time for hormones to have their full effect, or that the animals were sodium deprived, which could have a significant impact on the sodium intake (31).

In the present study, the increased sodium intake associated with higher plasma E2 levels was mostly due to an increase in consumption of 0.5% NaCl rather than an increase in consumption of a higher concentration of NaCl. This was most likely the result of the higher salt concentration being taste aversive. Our results support those of Fregly and Newsome (15), who showed, in female Sprague-Dawley and Long-Evans Hooded rats, that oral contraceptives increased salt appetite within 1 wk of drug administration in a dose-response relationship. The results of this experiment confirm our findings and extend to show that the adrenal gland may play a role in salt appetite because oral contraceptives in adrenalectomized rats failed to have an effect. Also, our data are supported by those of Chow et al. (2), who showed that female rats drank...
more 3% NaCl than did males, both in need-free and need-induced states. They did not manipulate E₂ levels, but they did castrate males at a young age, and, as adults, the males consumed NaCl like females. Also, females given testosterone consumed less need-free NaCl, which suggests that testosterone inhibits sodium intake.

It does not appear that E₂ works through the renin-angiotensin-aldosterone system (8, 12), because E₂ has been shown to actually lower levels of renin. Indeed, Schunkert et al. (28) showed that women replacing E₂ during menopause presented with significantly lower renin levels than those not using such therapy.

Another mechanism for enhanced salt appetite in males is enhanced activation of the SNS (1). The concept of SNS involvement is that, in the SHR model of hypertension, we have shown increased intestinal sodium loss compared with that in normotensive WKY animals, and, as a compensation to sodium loss, the SNS may be activated to help reabsorb sodium from the intestinal tract (30). However, elevated SNS in our SHR females does not seem to be completely responsible for sodium appetite. For example, Li and Duckles (22) have shown that sensitivity to adrenergic nerve stimulation in rat tail arteries was lower in females compared with males. In addition, we have evidence that the SNS is not primarily involved in salt appetite in females. For example, clonidine decreased sodium intake in male SHR and WKY, but not in female WKY, and only partially decreased it in SHR females (10).

The increased sodium appetite in females may be an adaptation for possible pregnancy and the conservation of sodium and other electrolytes (15). Pregnancy implies a challenge to sodium homeostasis, leading to a natural episode of increased sodium need and intake (14, 23, 24, 26). There are many hormonal and physiological changes that occur in pregnancy, many of which can influence sodium homeostasis (18, 24). For instance, sodium is crucial for the developing fetus and for milk production (3). Increases in blood volume and tissue fluid required during pregnancy can be achieved through an increase in maternal water and sodium intake (21).

Experiments using E₂ and progesterone show that hormonal changes alone are adequate to produce an increase in salt appetite. For instance, Covelli et al. (3) injected wild rabbits with E₂ and progesterone for 14 days, which increased sodium intake over twofold, and they remained elevated for 3–5 days after injections ceased. This effect with the use of E₂ and progesterone was >50% than that observed with the same dose of E₂ alone. It was also found that the increased salt appetite effect with the use of 25 μg E₂/day was maximal: a 10-fold increase in E₂ did not result in further enhancement. With regard to E₂ plasma levels in our study, they were high compared with that in control females; however, compared with the sodium intake in the same strain of control females from another study in our laboratory, the sodium intake in WKY control females was 107 mg NaCl·100 g⁻¹·24 h⁻¹ and in control SHR females was 133 mg NaCl·100 g⁻¹·24 h⁻¹ (10). In the present study, the E₂ increased sodium intake in WKY females to 175 mg NaCl·100 g⁻¹·24 h⁻¹ and in SHR females to 200 mg NaCl·100 g⁻¹·24 h⁻¹ with the highest E₂ dose. Therefore, even with high E₂ levels, the percent increase in sodium intake was comparable to that of other studies (2, 3, 15).

In conclusion, increased levels of plasma E₂ caused significantly higher intake of 0.5% NaCl in drinking water and, therefore, higher total sodium intake in WKY and SHR females. However, there was not an increase in preference for a higher NaCl concentration. Our data support the concept that, in SHR females, as in SHR males, there is an increased sodium appetite compared with both sexes of WKY. This finding may have important implications for women during pregnancy when E₂ levels are elevated and for those on E₂ replacement therapy, because increased sodium intake could adversely affect blood pressure and total blood volume by putting an extra load on the heart.

REFERENCES