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Attenuation of Angiotensin-Induced Water Intake in Estrogen-Treated Rats[1]

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FREGLY, M. J. AND T. N. THRASHER. *Attenuation of angiotensin-induced water intake in estrogen-treated rats.* PHARMAC. BIOCHEM. BEHAV. 9(4) 509–514, 1978.—Chronic treatment with either estradiol benzoate (31 and 57 μg/kg/day) or ethynyl estradiol (30 and 72 μg/kg/day) attenuated the drinking responses of female rats to acute administration of either isoproterenol (25 μg/kg, SC) or synthetic angiotensin II (100 and 200 μg/kg, IP). While these studies do not rule out the possibility that attenuation of isoproterenol-induced drink in estrogen-treated rats may be associated with a defect in renin release, attenuation is also apparently associated with a reduced responsiveness to angiotensin II. This may be related to reduced permeability of angiotensin II at the level of the circumventricular organs of the brain; to hyporesponsiveness of receptors for angiotensin II mediating thirst in the central nervous system, or to mechanisms beyond the receptor that are responsible for the conscious behavioral changes which normally accompany angiotensin II-induced thirst.

Thirst Isoproterenol Ethynyl estradiol Estradiol benzoate

ADMINISTRATION of the β-adrenergic agonist, isoproterenol, to rats is accompanied by a striking thirst [8,16]. This response can be inhibited by prior administration of a β_2-adrenergic antagonist [13]. β_2-adrenergic receptors are also known to mediate renin release [1, 3, 11]. Whether these 2 responses are elicited by the same receptors has not been proven conclusively. However, current evidence suggests that the thirst elicited by isoproterenol is mediated by way of renin release with subsequent formation of the putative dipsogen, angiotensin II [8]. This octapeptide is believed to enter the brain and to stimulate thirst receptors located in the subfornical organ [21], preoptic area [19], septum and anterior hypothalamus [7]. Although considerable information is available regarding angiotensin-induced thirst, its contribution to daily water intake is not known with certainty.

Chronic administration of the synthetic estrogen, ethynyl estradiol, to rats is accompanied by a reduction in the responsiveness of the thirst mechanisms to β-adrenergic stimulation [23,24]. The possibility existed that estrogens might attenuate dipsogenesis either at the level of the receptors mediating renin release or at the level of the receptors in the central nervous system mediating the behavioral response to angiotensin II or both. To distinguish between these 2 possibilities, the receptors mediating renin release were bypassed by administering synthetic angiotensin II to estrogen-treated and control rats to determine its effect on thirst. The results of these studies are described below.

METHOD

Experiment 1: Effect of Acute Administration of Graded Doses of Synthetic Angiotensin II on Water Intake of Rats

Twenty-four female rats of the Blue Spruce Farms (Sprague-Dawley) strain weighing from 230 to 260 g were used. They were kept 3 per cage in a room maintained at 25 ± 1°C and illuminated from 0600 to 1800 hr. All rats received tap water and Purina Laboratory Chow ad lib.

At 0900 hr on the day of the experiment, the rats were divided randomly into 4 equal groups and weighed. The first group served as the control group and was administered isotonic saline (0.2 ml) IP. The remaining 3 groups received 50, 100 and 200 μg angiotensin II (Beckman Instruments Inc., Palo Alto, CA)/kg body weight IP. The rats were then placed in individual metabolism cages and given a preweighed water bottle consisting of an infant nursing bottle with a cast aluminum fountain as described by Lazarow [15]. Water intakes were measured hourly for 3 hr thereafter.

Statistical analysis of the data was carried out by linear regression [22]. Significance was set at the 95% confidence limit.

Experiment 2: Effect of Acute Administration of Isoproterenol and Angiotension II on Water Intake of Ethynyl Estradiol-Treated Rats

Twenty-four female rats of the Blue Spruce Farms

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(Sprague-Dawley) strain weighing from 210 to 260 g were used. They were maintained under the same conditions as those described in Experiment 1.

All rats were ovariectomized while anesthetized with sodium pentobarbital (40 mg/kg body weight, IP) to avoid any contribution of endogenously-produced ovarian hormones to the observed results. One week after ovariectomy, a Silastic tube containing crystalline ethynyl estradiol was implanted SC between the shoulder blades while the rat was anesthetized with ether. Although it was originally intended that there should be three different doses of ethynyl estradiol, the first 2 rats were implanted in error with the same Silastic tubing (No. 602-281, 0.0315 in. wall thickness; 10 mm long). A second group of 6 rats was also implanted with Silastic tubing (No. 602-231, 0.0095 in. wall thickness; 10 mm long). The remaining 6 rats were implanted with a sealed Silastic tube (No. 602-231) which contained no steroid and served as the control group. Prior to implantation, the tubes containing the steroid were placed in a vacuum desiccator for 48 to 72 hr and then weighed on an analytical balance. The implanted tube in each rat was palpated at weekly intervals to be certain it was still in place.

Dimethylpolysiloxane (Silastic) tubing has been shown to allow diffusion of certain crystalline steroids into various media at a constant rate over relatively long periods of time [6,14]. Previous experience in this laboratory has indicated that this method of steroid administration provides a reliable means of achieving reasonably constant drug release for periods of up to 6 months.

During the seventh week after implantation of the Silastic tubes, each rat was weighed. At 1000 hr, each rat was administered 25 μg dl-isoproterenol/kg body weight SC (Isuprel®, Winthrop Laboratories) and placed in a metabolism cage. Each rat was then given a preweighed water bottle as described in Experiment 1. Water intakes and urine outputs of the rats were measured hourly for 3 hr.

During the eighth week after implantation of the Silastic tubes, each rat was again weighed. At 1000 hr each rat was administered 100 μg angiotensin II/kg IP and placed in a metabolism cage. Each rat was then given a preweighed water bottle as described above and water intakes and urine outputs were measured hourly for 3 hr.

At the end of the 17th week of the experiment, each rat was anesthetized with ether and the tube containing ethynyl estradiol was removed from its subcutaneous site. The tubes never contained fluid inside them. After cleaning each tube of adherent tissue, it was placed in a vacuum desiccator for 72 hr and then weighed on an analytical balance. The group receiving the thicker-walled tube had a mean weight loss of 6.8 $\pm$ 0.7 μg/day while the mean weight loss of the thinner-walled tube was 16.4 $\pm$ 0.5 μg/day. When calculated on the basis of mean body weight during the experiment, weight losses of 30 and 72 μg ethynyl estradiol/kg/day occurred from the tubes.

All data were analyzed statistically by a one-way analysis of variance [22]. Comparison between individual groups was made by Student's t test using the pooled variance from the analysis of variance [10]. Significance was set at the 95% confidence limit.

Experiment 3: Effect of Acute Administration of Isoproterenol and Angiotensin II on Water Intake of Estradiol Benzoate-Treated Rats

Twenty-four female rats of the Blue Spruce Farms (Sprague-Dawley) strain weighing from 280 to 325 g were used. They were maintained under the same conditions as those described in Experiment 1.

All rats were anesthetized with ether and 8 were implanted SC with a sealed, but empty, Silastic tube (No. 602-261, 10 mm long) between the shoulder blades. The remaining 2 groups (8 rats each) were implanted SC between the shoulder blades with the same tubing (10 mm and 20 mm, respectively) containing crystalline estradiol benzoate. Prior to implantation, the tubes containing the steroids were placed in a vacuum desiccator for 72 hr and weighed on an analytical balance. The implanted tube in each rat was palpated weekly to be certain it was still in place.

During the 6th and 8th weeks, experiments identical to those described in Experiment 2 were carried out. In addition to the measurement of water intake and urine output, the urine collected during the 3 hr period in each study was analyzed for sodium and potassium concentrations by flame photometry using lithium as an internal standard. Experiment 3 also differed from Experiment 2 in that a higher dose of angiotensin II (200 μg/kg) was used.

At the end of the 20th week of the experiment, each rat was anesthetized with ether and the tube containing estradiol benzoate removed from its subcutaneous site. The tubes were cleaned, dried and weighed as described above. The group receiving the longer (20 mm) tube had a mean weight loss of 17.1 $\pm$ 2.3 μg/day while the mean weight loss of the smaller (10 mm) tube was 8.4 $\pm$ 0.7 μg/day. When calculated on the basis of mean body weight during the experiment, weight losses of 31 and 57 μg estradiol benzoate/kg/day occurred from the tubes.

RESULTS

Experiment 1

Administration of graded doses of angiotensin II was accompanied by a direct, linear increase in water intake (Fig. 1). Only the cumulative 3 hr water intake is shown in Fig. 1, although both the first and cumulative 2 hr water intakes were also linearly related to dose of angiotensin II administered. The regression equation for the 3 hr water intake was calculated and is:

$$Y = 0.05\ X + 4.02;\ r = 0.67;\ n = 24;\ p < 0.01$$

where Y represents water intake during 3 hr after administration of angiotensin II (ml/kg body weight) and X is dose of angiotensin II administered (μg/kg body weight).

Experiment 2

Chronic treatment with ethynyl estradiol, at 30 and 72 μg/kg/day attenuated significantly ($p < 0.01$) the drinking response to administration of isoproterenol during the entire 3 hr of the experiment (Table 1). However, urine output during the 3 hr following administration of isoproterenol was not affected significantly by treatment with ethynyl estradiol.

The responsiveness of water intake to angiotensin II (100 μg/kg, IP) was also attenuated significantly ($p < 0.01$) by chronic treatment with ethynyl estradiol during all 3 hr after angiotensin was administered (Table 2). Urine output was also attenuated significantly ($p < 0.05$ to < 0.01) during the 3 hr following treatment with angiotensin II. The single exception to this occurred during the first hour after treatment in the group receiving the higher dose of ethynyl estradiol.

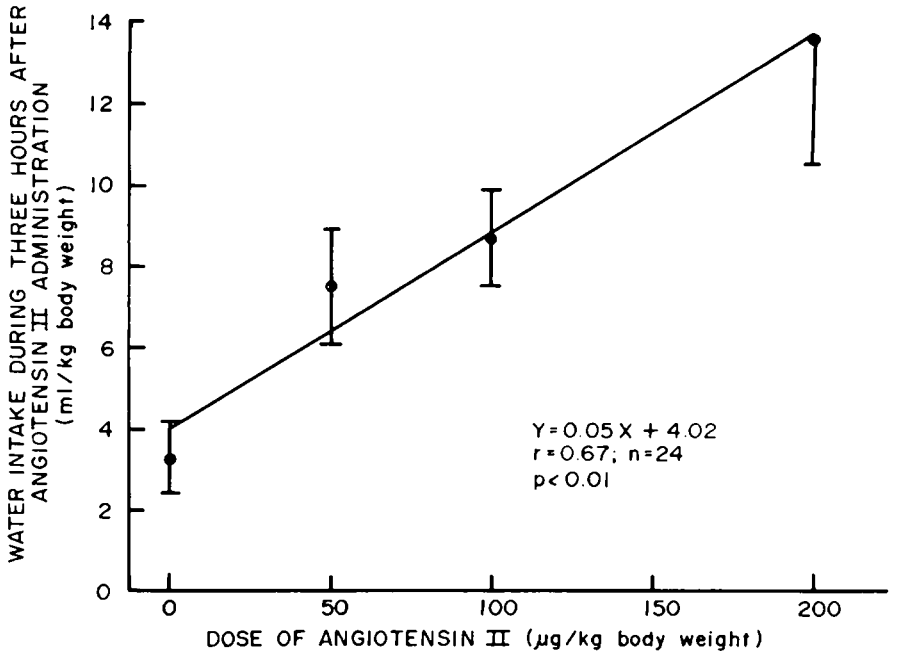

FIG. 1. Dose-response curve relating water intake during 3 hr after administration of angiotensin II (ml/kg body weight) with dose of angiotensin II (μg/kg body weight) administered. One standard error is set off at the means. The regression equation is given in the figure.

TABLE 1

EFFECT OF ISOPROTERENOL (25 μG/KG BODY WEIGHT, SC) ON WATER INTAKE AND URINE OUTPUT OF OVARIECTOMIZED RATS TREATED CHRONICALLY WITH ETHYNYL ESTRADIOL

Experimental Group	No. Of Rats	Mean Body Weight (g)	Cumulative Mean Water Intake (ml/kg b.w.) During:			Cumulative Mean Urine Output (ml/kg b.w.) During:		
			1st	2nd	3rd hour	1st	2nd	3rd hour
Control	6	282 ± 11*	21.1 ± 3.7	22.5 ± 3.5	23.1 ± 3.9	0	8.5 ± 2.5	23.5 ± 3.9
Ethynyl Estradiol (30 μg/kg/day)	12	224 ± 3‡	5.8 ± 1.2‡	7.0 ± 1.4‡	7.7 ± 1.4‡	0	2.1 ± 1.4	17.8 ± 1.3
Ethynyl Estradiol (72 μg/kg/day)	6	231 ± 14‡	10.1 ± 1.3‡	10.1 ± 1.3‡	13.0 ± 2.3†	0	3.3 ± 2.1	22.0 ± 3.6

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

Mean body weights of the 3 groups treated with ethynyl estradiol were significantly lower ($p<0.01$) than that of controls (Tables 1 and 2). This effect of ethynyl estradiol on body weight has been observed previously [23,24].

Experiment 3

Chronic treatment with estradiol benzoate attenuated significantly ($p<0.05$ to <0.01) the drinking response to administration of isoproterenol during the 3 hr experiment but failed to affect significantly the characteristic antidiuresis following treatment with this catecholamine (Table 3). The output of sodium in urine (mEq/kg/3 hr) was increased significantly ($p<0.01$) in both groups treated chronically with estradiol benzoate. Urinary potassium output was variable in the treated groups and was not affected significantly by treatment.

When the reduction in water intake of the estradiol benzoate-treated groups is considered in conjunction with their unchanged urine output, these animals were in negative fluid balance by the end of the 3 hr experiment. A negative fluid balance was also reported earlier under similar experimental conditions [24]. A negative sodium balance compared with that of control rats, also occurred in estrogen-treated rats (Table 3).

The increase in water intake usually accompanying administration of angiotensin II (200 μg/kg) was attenuated significantly ($p < 0.05$ to 0.01) by chronic treatment with es-

TABLE 2

EFFECT OF ANGIOTENSIN II (100 μG/KG BODY WEIGHT, IP) ON WATER INTAKE AND URINE OUTPUT OF OVARIECTOMIZED FEMALE RATS TREATED CHRONICALLY WITH ETHYNYL ESTRADIOL

Experimental Group	No. Of Rats	Mean Body Weight (g)	Cumulative Water Intake (ml/kg body wt.) During:			Cumulative Urine Output (ml/kg body wt.) During:		
			1	2	3 hours	1	2	3 hours
Control	6	314 ± 14*	13.3 ± 2.2	14.9 ± 2.0	14.9 ± 2.0	9.4 ± 2.6	12.1 ± 1.9	22.2 ± 1.0
Ethynyl Estradiol (30 μg/kg/day)	12	226 ± 3‡	3.7 ± 1.0‡	5.2 ± 1.2‡	6.0 ± 1.3‡	3.4 ± 1.3†	5.9 ± 1.8†	11.7 ± 2.1‡
Ethynyl Estradiol (72 μg/kg/day)	6	224 ± 11‡	4.3 ± 1.3‡	6.0 ± 1.7‡	7.5 ± 2.4†	4.7 ± 1.4	4.7 ± 2.2†	13.3 ± 3.3†

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

TABLE 3

EFFECT OF ISOPROTERENOL (25 μG/KG BODY WEIGHT IP) ON WATER INTAKE AND OUTPUT OF URINE AND ELECTROLYTES IN FEMALE RATS TREATED CHRONICALLY WITH ESTRADIOL-BENZOATE

Experimental Group	No. Of Rats	Mean Body Weight (g)	Cumulative Water Intake (ml/kg body wt.) During:			Cumulative Urine Output (ml/kg body wt.) During:			Urine Na Output (mEq/kg 3 hr)	Urine K Output (mEq/kg 3 hr)
			1	2	3 hours	1	2	3 hours		
Control	8	306 ± 7*	11.9 ± 1.8	13.6 ± 1.4	14.4 ± 1.6	0.5 ± 0.2	9.5 ± 3.1	16.5 ± 3.5	0.19 ± 0.04	0.45 ± 0.06
Estradiol Benzoate (31 μg/kg/day)	8	314 ± 12	6.6 ± 1.3†	7.1 ± 1.2‡	7.2 ± 1.3‡	0.7 ± 0.7	7.5 ± 1.2	15.8 ± 2.1	0.63 ± 0.11†	0.87 ± 0.23
Estradiol Benzoate (57 μg/kg/day)	8	320 ± 6	7.6 ± 1.1‡	8.0 ± 1.1‡	8.3 ± 1.1‡	1.9 ± 0.9	8.4 ± 2.1	15.7 ± 2.1	0.53 ± 0.08‡	0.74 ± 0.13

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

TABLE 4

EFFECT OF ANGIOTENSIN II (200 μG/KG BODY WEIGHT IP) ON WATER INTAKE AND OUTPUT OF URINE AND ELECTROLYTES IN FEMALE RATS TREATED CHRONICALLY WITH ESTRADIOL-BENZOATE

Experimental Group	No. Of Rats	Mean Body Weight (g)	Cumulative Water Intake (ml/kg body wt.) During:			Cumulative Urine Output (ml/kg body wt.) During:			Urine Na Output (mEq/kg 3 hr)	Urine K Output (mEq/kg 3 hr)
			1	2	3 hours	1	2	3 hours		
Control	8	312 ± 7*	11.5 ± 1.9	12.1 ± 2.2	13.1 ± 3.4	2.4 ± 0.5	8.3 ± 1.8	10.6 ± 2.4	0.35 ± 0.06	0.63 ± 0.12
Estradiol Benzoate (31 μg/kg/day)	8	311 ± 12	5.5 ± 1.4‡	6.5 ± 1.6‡	7.5 ± 1.7	6.4 ± 1.9	12.5 ± 3.0	18.2 ± 2.6	1.05 ± 0.20‡	0.93 ± 0.12
Estradiol Benzoate (57 μg/kg/day)	8	313 ± 8	4.0 ± 1.4‡	4.8 ± 1.9‡	5.6 ± 1.9‡	3.6 ± 1.5	8.9 ± 2.3	12.3 ± 2.0	0.90 ± 0.11‡	0.67 ± 0.08

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

tradiol benzoate while urine output (ml/kg/3 hr) was unaffected (Table 4). However, urinary sodium output (mEq/kg/3 hr) urinary Na/K ratio (not shown in Table 4) were significantly ($p<0.05$ to 0.01) increased in the groups treated with estradiol benzoate but urinary potassium output (mEq/kg/3 hr) was not (Table 4).

Mean body weights of the rats treated with estradiol benzoate were not significantly different from the control group. The effect of estradiol benzoate on body weight is in sharp contrast to that of ethynyl estradiol.

DISCUSSION

The results of the present experiments are the first to show that an ester of the naturally occurring estrogen, estradiol, inhibits the isoproterenol-induced drink (Table 3). Previous studies used the synthetic estrogens, ethynyl estradiol and mestranol [23,24]. An inverse linear relationship between the logarithm of the dose of ethynyl estradiol administered and water intake was established earlier for rats administered isoproterenol acutely [24]. The lack of an apparent dose-response relationship between dose of ethynyl estradiol administered and water intake following acute administration of isoproterenol in the present study (Table 1) is related to the fact that the 2 doses of ethynyl estradiol used were in the range of maximal attenuation of water intake, as judged by the dose-response relationship observed earlier [24].

The lower dose of ethynyl estradiol used here was approximately 10 to 20 times the therapeutic dose used for contraception in humans. However, the plasma half-life of ethynyl estradiol in the rat is most likely shorter than in the human. Studies of others have shown a plasma half-life of 40 to 60 hr for radioactive ethynyl estradiol in humans as compared with 2 hr in rabbits [2]. Hence, the doses of ethynyl estradiol used in the present studies may be less pharmacologic than they appear when the half-life of the hormone is taken into consideration. While doses of ethynyl estradiol less than 30 μg/kg were not used in the present study, estradiol benzoate was. This estrogenic agent has 1/40 to 1/200th the biological activity of ethynyl estradiol [5]. The effects of both estrogenic substances on either isoproterenol-induced or angiotensin II-induced thirst were the same in spite of the differences in their potency. The results shown in Table 3 actually suggest that even 31 μg estradiol benzoate/kg/day may have induced maximal attenuation of isoproterenol-induced thirst.

Thirst induced by other experimental procedures may also be affected by chronic treatment with estrogenic agents. Other studies from this laboratory have shown that chronic treatment with an estrogenic agent attenuates the drinking response to an IP load of hypertonic saline (1 M NaCl, 1% of body weight) but does not affect the drinking response to a 24 hr period of dehydration [23]. A reduced drinking response to intragastric hypertonic sodium chloride loading in food-deprived rabbits treated acutely with approximately 40, 80 and 160μg estradiol/kg of body weight was also reported earlier by Nocenti and Cizek [20].

The possibility exists that attenuation of isoproterenol-induced drinking by estrogenic agents might occur primarily at the level of the receptors for renin release. While this was not studied here, bypassing these receptors by administration of synthetic angiotensin II, the putative dipsogen, was carried out in rats treated with ethynyl estradiol or estradiol benzoate. The results of both experiments were identical in that attenuation of the dipsogenic response to acute administration of angiotensin II occurred in all groups treated chronically with estrogenic agents. These same groups also had an attenuated drinking response to administration of isoproterenol. A possibility exists that the administered angiotensin failed to enter the brain in sufficient amount in estrogen-treated animals to stimulate thirst receptors in the subfornical organ and preoptic area of the brain [19,21]. An additional possibility is that the receptors for thirst in these areas are less responsive in estrogen-treated rats than are those of controls. Additionally, the possibility that a block may occur beyond the receptor must be considered. The present experiments do not distinguish among these possibilities.

The lack of effect of estradiol benzoate on body weight at the doses used is particularly interesting. Ethynyl estradiol may affect body weight by reducing food intake as a result of effects at the level of the central nervous system [25]. If so, it would appear that estradiol benzoate does not share this effect at the doses employed. Differences between these studies and those of others [25] may be related to the fact that Silastic tubing allows continuous release of hormone throughout the day as opposed to the cyclic increase in blood levels following single daily injections. Further, the caloric value of the vehicle used to dissolve the drug must be considered when estrogens are administered chronically and either body weight or food intake is the measured variable. Since body weight, and presumably food intake, did not differ among the groups in Experiment 3, it is unlikely that these factors play a role in the reduced water intake of the estradiol benzoate-treated rats administered either isoproterenol or angiotensin II. In addition, the serum osmolalities of estrogen-treated and control rats have been measured and shown to be similar [9]. Hence, changes in serum osmolality cannot account for the difference in dipsogenic responsiveness between estrogen-treated and control rats.

The effect of acute administration of angiotensin II in increasing urine sodium output in estradiol benzoate-treated rats was an unexpected finding. Estrogenic agents are known to induce an increase in plasma renin activity with a subsequent increase in aldosterone secretion [17,18]. This would be expected to reduce urinary sodium loss. Furthermore, estrogens alone are reported to have sodium retaining activity independent of aldosterone [4,12]. This observation merits additional study.

Whether the ovaries were present or absent appeared to have little effect on the interpretation of the experimental results. However, none of the studies reported here compared simultaneously water intakes of ovariectomized with intact rats in response to administration of either isoproterenol or angiotensin II. The results of such studies await additional experimentation.

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