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Attenuation of Isoproterenol-Stimulated Plasma Renin Activity by Chronic Estrogen Treatment[1] (41135)

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Abstract. Chronic administration of estradiol benzoate to intact female rats for 12 weeks resulted in a significant reduction in unstimulated plasma renin activity. Acute administration of the β-adrenergic agonist, *dl*-isoproterenol, stimulated plasma renin activity in both control and estrogen-treated animals in a dose-dependent manner. However, chronic administration of estradiol benzoate resulted in a reduced sensitivity to low, but not high, doses of isoproterenol. These results are consistent with those of previous studies in which chronic treatment with an estrogenic agent was shown to reduce the responsiveness of heart rate, tail skin temperature, metabolic rate, and drinking to acute administration of a β-adrenergic agonist.

Previous studies from this laboratory have shown that chronic administration of the estrogenic agents, ethinyl estradiol and estradiol benzoate, to intact female rats reduced their responsiveness to the β-adrenergic agonist, isoproterenol. Thus, the increases in heart rate (1), tail skin temperature (2) and water intake (3, 4) were attenuated in comparison with that of controls. In the latter case, a number of studies suggests that the drinking response to acute administration of isoproterenol can be correlated with its ability to induce renin release (5), assuming that angiotensin II is the ultimate dipsogen under these conditions (6).

The present study was designed to ascertain whether the increase in plasma renin activity (PRA), stimulated by acute administration of isoproterenol, was attenuated by chronic administration of estradiol benzoate (EB) to rats.

Materials and Methods. Female Sprague–Dawley rats from a breeding colony maintained in our animal facility were used in all experiments. The animals were housed in groups of three in stainless-steel cages in a windowless room maintained at 25 ± 1° and lighted from 0600 to 1800 hr. Purina Laboratory Chow and tap water were provided *ad libitum* to all animals.

The animals in each experiment were divided into three groups, a control group and two estrogen-treated groups. The control group in each experiment had a 10-mm length of empty Silastic tubing (0.419-mm wall thickness, No. 602-265, Dow–Corning, Midland, Mich.) implanted between the shoulder blades. The estrogen-treated groups in each experiment had either a 10-mm (EB10) or a 20-mm (EB20) length of similar, but sealed and weighed, Silastic tubing containing crystalline β-estradiol-3-benzoate (Sigma Chemical Co., St. Louis, Mo.) implanted between the shoulder blades. At the end of each experiment the tubes were removed, cleaned of adhering tissue, and placed in a vacuum dessicator for 72 hr. Drug dose was based on mean weight loss of each tube and mean body weight during the period of implantation. Previous studies have indicated that this method of steroid administration provides a reliable means of achieving relatively constant drug release for periods up to 6 months (1–4).

Experiment 1. Effect of chronic treatment with EB on unstimulated PRA. A total of 42 rats weighing 225–325 g were used. The rats were divided randomly into three groups containing 14 rats each. Twelve weeks after implantation of the Silastic tubing, the rats received an injection of isotonic saline (1 ml/kg body wt sc) 10 min

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prior to anesthesia with methoxyflurane (Penthrane, Abbott Laboratories, Chicago, Ill.) and 15 min prior to collection of a 2.0-ml blood sample by cardiac puncture. The increase in PRA associated with this anesthetic is considerably less than with the more commonly used general anesthetics (7). The collection tubes, containing sodium EDTA (1 mg/ml), were immersed in an ice bath during the procedure and, after addition of blood to them, were centrifuged at 1200*g* for 20 min at 4°. Plasma samples were then frozen at −20° until assayed. PRA was determined in duplicate by a modified method of Haber *et al.* (8) using a New England Nuclear angiotensin I (^{125}I) radioimmunoassay kit. Statistical analysis of the data was made by means of a one-way analysis of variance (9). Comparison between individual groups was made by a *t* test using the pooled variance from the analysis of variance (10). Significance was set at the 95% confidence interval.

Experiment 2. Effect of acute administration of isoproterenol on PRA of EB-treated rats. A total of 96 rats weighing 220−320 g were used. The control group consisted of 36 rats and each EB-treated group consisted of 30 rats. Twelve weeks after implantation of the Silastic tubing, each rat was administered either 0.1, 1, 3, 10, or 30 μg *dl*-isoproterenol (Isuprel, Winthrop Laboratories, New York, N.Y.)/kg body wt sc. Each dose of isoproterenol was administered to 6 rats in each group, and an additional 6 rats from the control group received 0.3 μg *dl*-isoproterenol/kg body wt sc. A 2.0-ml blood sample was collected from each animal after anesthesia with methoxyflurane and PRA determined, as described in Experiment 1. Since the variances from the statistical analyses of PRA at the various doses of isoproterenol were not homogenous, but were approximately proportional to the square of the means, a logarithmic transformation was performed to stabilize the variances and to analyze the data statistically. Therefore, a two-way analysis of variance was performed using the natural logarithm of PRA, the two factors being dose of isoproterenol and level of estrogen treatment (11). The presence of a significant two-way interaction required further analysis. A one-way analysis of variance was performed at each of the five doses (9). A Duncan's multiple range test was then performed at those doses at which there was a significant effect of treatment (12).

Experiment 3. Effect of chronic treatment with EB on serum concentrations of estradiol. A total of 18 rats weighing 220−280 g were divided randomly into three groups containing 6 rats each. Twelve weeks after implantation of Silastic tubing, the animals were anesthetized with methoxyflurane, sacrificed by decapitation, and the trunk blood was collected. The serum was separated and stored at −20°. Serum levels of estradiol were determined by radioimmunoassay (13).

Results. *Experiment 1.* The loss of EB from the tubes of the group receiving the lower dose of estradiol (EB10) was calculated to be 7.7 ± 0.6 (SE) μg/day or 33 ± 4 μg/kg body wt/day and from the tubes of the group receiving the higher dose of estradiol (EB20) to be 12.6 ± 1.3 μg/day or 61 ± 5 μg/kg/day. Chronic treatment with either dose of EB significantly reduced the unstimulated PRA below that of controls ($P < 0.01$) (Fig. 1). One animal in the EB10 group died during treatment; there was no known cause.

Experiment 2. The loss of EB from the tubes of the EB10 group was 7.6 ± 0.5 μg/

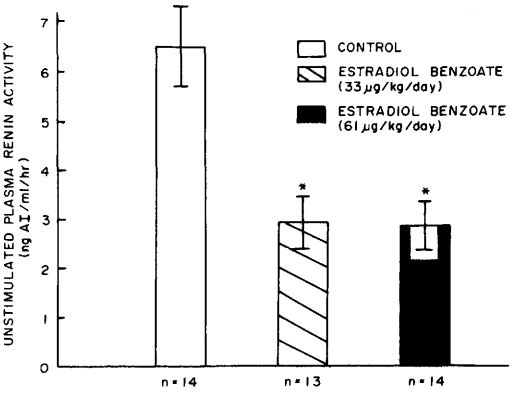

FIG. 1. Unstimulated plasma renin activity of control and estradiol benzoate-treated groups. The groups are designated in the figure. (*) Significantly different from control ($P < 0.01$). One standard error is set off at each mean.

day or 31 ± 3 μg/kg/day and from those of the EB20 group was 14.9 ± 0.7 μg/day or 62 ± 3 μg/kg/day. The effect of acute administration of graded doses of isoproterenol on PRA of the three groups of animals is shown in Fig. 2. Analysis of the data indicated a significant two-way interaction ($P = 0.02$), estrogen treatment × dose of isoproterenol. There was no significant difference between PRA of control and EB-treated animals after administration of 0.1 μg isoproterenol/kg body wt. However, after administration of 1 μg isoproterenol/kg body wt, PRA of the control group was significantly greater ($P < 0.001$) than those of the EB-treated groups. PRA, stimulated by higher doses of isoproterenol (3, 10, and 30 μg/kg body wt), did not differ among the groups. The ED_{50} values for isoproterenol were approximately 1.0 μg/kg body wt for the control group and 3.3 and 3.0 μg/kg body wt for the EB10 and EB20 groups, respectively. The responses of both groups of EB-treated animals to all doses of isoproterenol did not differ significantly from each other.

Experiment 3. The loss of EB from the tubes of the EB10 group was 8.9 ± 0.8 μg/day or 37 ± 3 μg/kg/day and from the tubes of the EB20 group was 17.4 ± 0.9 μg/day or 73 ± 4 μg/kg/day. The serum concentrations of estradiol, as measured by radioimmunoassay, were 22 ± 7 pg/ml in the control group, 174 ± 10 pg/ml in the EB10 group, and 340 ± 37 pg/ml in the EB20 group.

Discussion. The administration of EB by Silastic tubing is an effective mechanism for elevating serum concentrations of estradiol. The levels of estradiol achieved in rats following implantation of 10- and 20-mm lengths of Silastic tubing containing EB are approximately three and six times, respec-

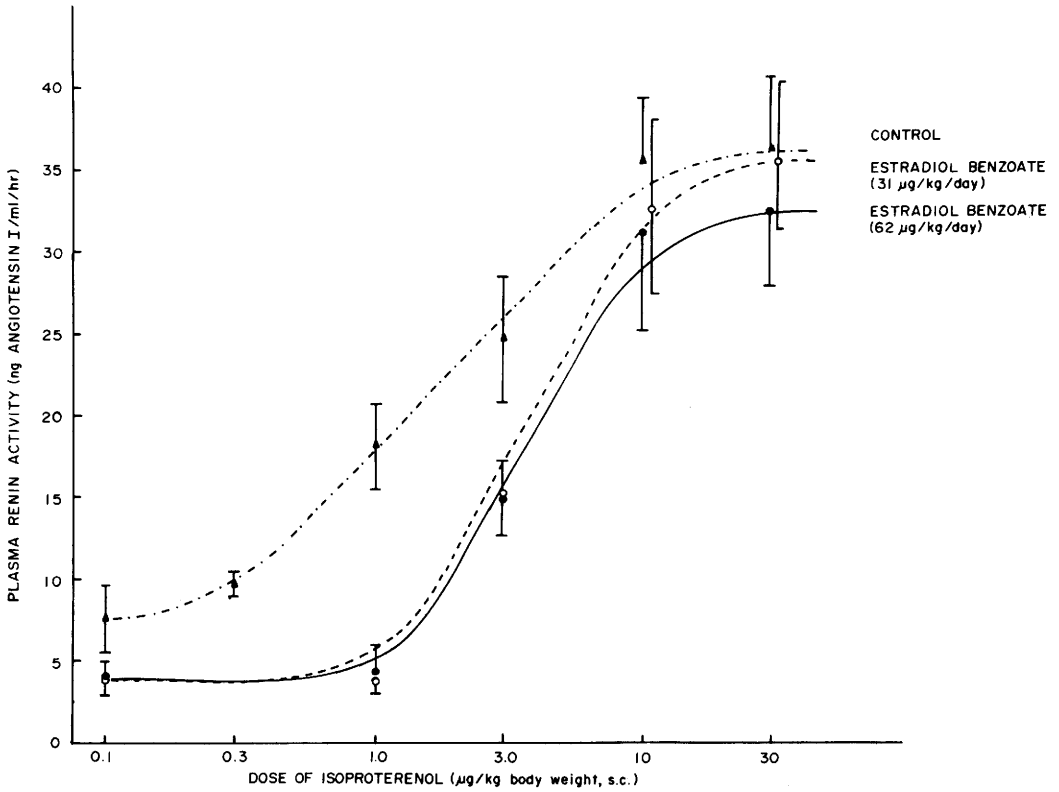

FIG. 2. The effect of chronic administration of estradiol benzoate on isoproterenol-stimulated plasma renin activity. The groups are designated in the figure. One standard error is set off at each mean.

tively, the estradiol concentrations of cycling female rats during proestrus (13). The high serum levels of estradiol achieved are likely responsible for the lack of difference between the effects of the two treatment regimens.

The effects of short-term, high-dose estrogen treatment on plasma renin parameters have been reported previously (14). PRA rose initially and subsequently fell to values below those of control's during the 5-day treatment. In the present, more prolonged study, lower-dose administration of estrogen resulted in a significant reduction in PRA. It has been reported that plasma renin substrate and angiotensin II are elevated by estrogen treatment in the rat (14), and that increased blood levels of angiotensin II inhibit renin secretion under conditions of basal and stimulated renin release in the dog (15). It remains to be elucidated whether animals treated with EB for 12 weeks have elevated plasma angiotensin II levels. It is also possible that EB-treated rats in the present study responded differently from those reported in the earlier studies of others because of the anesthetic used. Methoxyflurane is the anesthetic of choice for measurement of PRA because it increases PRA to a considerably lesser extent than do the more commonly used anesthetics (7). Therefore, a reduction in either methoxyflurane-induced catecholamine release or in the sensitivity to catecholamines in rats treated with EB might contribute to a reduced basal PRA.

Systemic administration of isoproterenol has been shown to stimulate renin release in the rat both by its hypotensive action (5), and by direct stimulation of β-adrenergic receptors in renal slices *in vitro* (16). The results reported here suggest that the increases in PRA accompanying acute administration of isoproterenol are attenuated in EB-treated rats. This may be a result of an enhanced negative feedback of angiotensin II on renin release or a reduced response to the anesthetic as mentioned above. However, administration of an estrogen has been shown to have general effects on the responsiveness of unanesthetized rats to β-adrenergic agonists. The increases in heart rate, water intake, and tail skin temperature in response to acute administration of isoproterenol are reduced by chronic treatment with estrogenic agents (1–4), as are the increases in isoproterenol-induced accumulation of cyclic adenosine 3′, 5′-monophosphate in rat cerebral cortical slices (17). The latter study revealed that the reduction in responsiveness to isoproterenol was at least partially due to a reduction in the number of β-adrenergic receptors. The possibility that the reduced response of the heart and kidney to isoproterenol is mediated by a reduction in the number and/or affinity of β-adrenergic receptors is presently under investigation.

It is possible that either increased metabolism or increased excretion of isoproterenol by rats treated chronically with estrogen might contribute to the reduced responsiveness to isoproterenol. However, this seems unlikely as isoproterenol is metabolized very rapidly in extraneuronal sites in the rat (18) and estrogen has been shown to inhibit extraneuronal uptake of catecholamines (19). Therefore, treatment with estrogen might actually increase the half-life of isoproterenol in the rat.

There is evidence that β-adrenergic-induced drinking in the rat is mediated by way of the renin-angiotensin system (6, 20). Estrogen treatment attenuates normal water intake (21) and water intake stimulated by 25 μg isoproterenol/kg body wt (4). Since the commercially available radioimmunoassay measures PRA as angiotensin I, the present study suggests that the reduced drinking by estrogen-treated rats to administration of isoproterenol is not due to a deficit in the formation of angiotensin I. A reduction in the activity of angiotensin I converting enzyme by chronic estrogen treatment can be ruled out, as this has been shown actually to be enhanced under these conditions (22). It is most likely that attenuation of the drinking response to acute administration of isoproterenol observed in estrogen-treated rats is related to a reduced responsiveness to angiotensin II at the level of the central nervous system, as suggested by earlier studies from this laboratory (4).

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