Effect of the Catecholestrogen 2-Hydroxyestradiol on the Renin-Angiotensin System in the Rat

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Abstract

The effects of the catecholestrogen 2-hydroxyestradiol (250 and 500 μg/day, each for 7 days) on plasma renin substrate (PRS), activity (PRA) and concentration (PRC) were studied in male rats as compared with those of estradiol (250 μg/day, for 7 days) and vehicle alone (for 7 days).

Pre-treatment levels of PRS, PRA, PRC and the PRA/PRC ratio were similar in four groups. After vehicle treatment, PRS, PRA, PRC and the PRA/PRC ratio remained unchanged. Estradiol treatment, however, produced an increase in PRS, an increase in PRA but no change in PRC. The PRA/PRC ratio after estradiol treatment was high. On the other hand, 2-hydroxyestradiol treatment caused no increase in PRS at a daily dose of 250 μg and a slight but significant increase in PRS at a daily dose of 500 μg. This treatment also produced increases in PRA as well as PRC at the two daily doses. These increases in PRA and PRC tended to be higher at a daily dose of 500 μg than at a daily dose of 250 μg. The PRA/PRC ratios after 2-hydroxyestradiol treatment were unaltered at the two daily doses.

It is concluded that, while 2-hydroxyestradiol is less active in increasing PRS than estradiol, the compound is capable of increasing PRC.

Catecholestrogens, which are metabolites of the primary estrogens, have attracted attention because of their biological effects on gonadotropin release and catecholamine metabolism (Ball and Knuppen, 1980). Since it is well known that estrogen treatment induces a consistent increase in plasma renin substrate (PRS), originally reported by Helmer and Griffith (1952), with variable changes in plasma renin activity (PRA) and plasma renin concentration (PRC) both in animals (Menard and Catt, 1973; Menard et al., 1973; Saruta et al., 1975) and in man (McDonald et al., 1977; Pallas et al., 1977; Kaulhausen and Weyand, 1982), it is of interest to see whether catecholestrogens induce any change in the renin-angiotensin system.

The present study was undertaken to examine the effects of the catecholestrogen 2-hydroxyestradiol on PRS, PRA and PRC, and to compare its effects with those of estradiol, a primary estrogen, in male rats.

Materials and Methods

Male rats of the Wistar strain weighing about 180 g at the start of the study were used. The animals were fed a laboratory chow (Oriental Food Co., Tokyo) ad

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libitum and given tap water to drink. They were treated s.c. with various concentrations (250 and 500 μg) of 2-hydroxyestradiol or 250 μg of estradiol dissolved in 0.2 ml propylene glycol containing 0.02% ascorbic acid, daily for 7 days. Control animals were treated with vehicle alone, daily for 7 days. The body weight of all the animals was measured once a day throughout the experimental period. Blood samples for measurements of PRS, PRA and PRC before and after estrogen or vehicle treatment were drawn from the tail vein of the animal without anesthesia by the method of Enta et al. (1968).

PRS, PRA and PRC were determined by the method of Skinner (1967) as described previously (Hirasawa et al., 1968; Morimoto et al., 1975). For PRC determination, plasma specimens were dialyzed to pH 2.8 instead of the original pH 3.3 against a citric acid-phosphate buffer because of a more complete destruction of endogenous renin substrate (Saruta et al., 1975). The PRA/PRC ratio also was estimated to express PRA in relation to PRC.

All results were expressed as the mean±sd. Statistical analysis was performed by Student’s t-test.

Results

Weight gains and changes in PRS, PRA, PRC and the PRA/PRC ratio after estrogen or vehicle treatment for 7 days in rats are shown in Table 1.

Retardations of weight gain were observed in rats treated with 250 μg estradiol (p<0.001) and in rats treated with 500 μg 2-hydroxyestradiol (p<0.05). The retardation of weight gain, however, was more pronounced in the former rats than in the latter rats (p<0.001).

Pre-treatment levels of PRS, PRA, PRC and the PRA/PRC ratio were similar in all four groups. After vehicle treatment, PRS, PRA, PRC and the PRA/PRC ratio remained unchanged. Estradiol treatment, however, produced a significant increase in PRS (p<0.001), a significant increase in PRA (p<0.001) but no change in PRC. The PRA/PRC ratio after estradiol treatment was significantly high (p<0.001). On the other hand, 2-hydroxyestradiol treatment caused no significant increase in PRS at a daily dose of 250 μg and a significant increase in PRS at a daily dose of 500 μg (p<0.001). The increase in PRS in

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<th>No. of rats</th>
<th>Weight gain (g)</th>
<th>PRS (ng/ml)</th>
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<td>Estradiol treatment</td>
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<td>0.73±0.13</td>
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PRS = Plasma renin substrate, PRA = Plasma renin activity, PRC = Plasma renin concentration.

*Significantly different from that before estrogen or vehicle treatment (p<0.001).

Significantly different from that before estrogen or vehicle treatment (p<0.001).
rats treated with 500 µg 2-hydroxyestradiol was less pronounced when compared with that in rats treated with 250 µg estradiol (p<0.01). 2-hydroxyestradiol treatment also produced significant increases in PRA (p<0.001) as well as PRC (p<0.001) at the daily doses of 250 and 500 µg. PRA and PRC after 2-hydroxyestradiol treatment tended to be higher at the daily dose of 500 µg than at the daily dose of 250 µg (NS). The PRA/PRC ratios after 2-hydroxyestradiol treatment were unaltered at the two daily doses.

**Discussion**

It is well known that estrogens induce an increase in PRS. Since renin substrate is synthesized by the liver (Freeman and Rostoker, 1972; Nasjletti and Masson, 1972), the ability of estrogens to induce an increase in PRS has been considered to be mediated through their interaction with hepatic receptor proteins (Krakoff and Eisenfeld, 1977; Kneifel and Katzenellenbogen, 1981). However, the effects of estrogens on PRA and PRC vary. For example, Menard and Catt (1973) reported that, when rats were treated for 5 days with 100 µg ethynylestradiol daily, PRA increased transiently for the first 2 days and fell to slightly below normal levels by the 6th day, and PRC fell subnormally by the 2nd day and markedly to less than one-half of the control value by the 6th day. Saruta et al. (1975) found that, during continued treatment with estriol (1.5 mg/kg/day) and stilboestrol (15 mg/kg/day) for 15 days in rats, PRA was consistently increased and PRC remained unchanged. Kaulhausen and Weyand (1982) reported that a single injection of 10 mg estradiol benzoate in ovariectomized subjects resulted in a transient increase in both PRA and PRC. The difference in the variety, amount and/or duration of estrogen treatment may be in part responsible for the variable changes in PRA and PRC (Menard and Catt, 1973; Menard et al., 1973; Saruta et al., 1975; Kaulhausen and Weyand, 1982).

Confirming earlier observations, the present study demonstrated an increase in PRS in rats treated with 250 µg estradiol daily for 7 days. Estradiol treatment also caused a retardation of weight gain, secondary to a reduction of appetite (Glasser, 1954; Benas, 1959). After estradiol treatment, PRA was increased, PRC was unaltered and the PRA/PRC ratio was high. These results suggest that renin secretion may be unaltered by estradiol treatment and that the estradiol-induced increase in PRA is primarily due to an acceleration of the renin-renin substrate reaction. This acceleration of the renin reaction may be explained by both an increase in PRS (Laragh et al., 1967; Skinner et al., 1969; Menard et al., 1973) and an increase in maximal velocity (V max) (McDonald et al., 1977) for the renin reaction.

On the other hand, 2-hydroxyestradiol treatment for 7 days caused no increase in PRS at a daily dose of 250 µg and a slight but significant increase in PRS at a daily dose of 500 µg, demonstrating that the ability of 2-hydroxyestradiol to induce an increase in PRS is less than that of estradiol. Furthermore, 2-hydroxyestradiol treatment produced a slight retardation of weight gain, another well-known effect of estrogens in rats, only at the daily dose of 500 µg. Since the metabolic clearance rate of 2-hydroxyestradiol has been reported to be very high (Kono et al., 1980), whether the inferior ability of this compound to induce an increase in PRS is due to its rapid metabolic clearance or its low hepatic renin substrate production remains unclear from the present study. 2-hydroxyestradiol treatment also produced increases in PRA as well as PRC at the two daily doses of 250 and 500 µg, while the increases in PRA and PRC tended to be higher at the latter dose than at the former dose. The PRA/PRC ratios after 2-hydroxyestradiol treatment were unaltered at the two daily doses. These results suggest that renin secretion may be stimulated by 2-hydroxyestradiol and that the 2-hydroxyestradiol-induced increase in PRA is mainly due to an increase of renin secretion. Although the
mechanism(s) by which 2-hydroxyestradiol caused an increase of renin secretion remains to be determined, the possibility ought to be considered that 2-hydroxyestradiol is a strong competitive inhibitor of the catechol-O-methyltransferase, an enzyme known to inactivate catecholamines, because of its catechol structure (Ball and Knuppen, 1980). Thus, 2-hydroxyestradiol, even if its metabolic clearance is so rapid, could provoke an inhibition of catecholamine degradation leading to a stimulation of renin secretion.

Finally, the present study suggests that the 2-hydroxyestradiol-induced changes in PRS and PRC is attributed to the ortho-hydroxylation at the C-2 of estradiol. It offers a tool for studying the pharmacological mechanism of primary estrogens and their metabolites on the renin-angiotensin system. However, it is unclear whether or not such changes in the renin-angiotensin system occur under physiological or pathophysiological conditions.

References


