Vascular Action of High Dose Estrogen in Rats

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Abstract

Effects of estradiol hemisuccinate and diethylstilbestrol diphosphate on the cardiovascular system in male rats were examined in vivo and in vitro. In conscious rats, intravenously administered estadiol or diethylstilbestrol inhibited vascular responses to norepinephrine in a dose-dependent manner. Diethylstilbestrol in high doses significantly lowered the blood pressure of conscious rats. In the isolated mesenteric arteries perfused with Krebs bicarbonate solution, estradiol or diethylstilbestrol in the perfusate similarly inhibited vascular reactivity to norepinephrine in a dose-dependent manner. As in the in vivo experiments, a decrease in the basal pressure of the mesenteric vascular bed was observed when diethylstilbestrol was added to the perfusate at high concentrations. The results of these in vivo and in vitro studies strongly suggest that estradiol and diethylstilbestrol act directly on the vascular beds and attenuate vascular response to norepinephrine. It is also suggested that diethylstilbestrol by itself causes vasodilatation and a reduction in blood pressure when present at high concentrations.

Several effects of estrogen on the cardiovascular system have been reported in man and in animals. These results have been, however, quite controversial. Oral contraceptives or estrogen treatment raised blood pressure in man (Laragh et al., 1967; Saruta et al., 1970) and in rats (Saruta et al., 1975), and accelerated the development of hypertension in rats with renovascular or DOCA-salt hypertension (Bunag et al., 1976). Estrogen treatment, however, attenuated the development of hypertension in spontaneous hypertensive rats (Hoeg et al., 1977), and inhibited DOCA-salt hypertension in chickens (Stamler, 1954). Estrogen was also reported to alter the vascular reactivity to vasoactive substances (Altura, 1975). Besides its indirect effect via the renin-angiotensin system and sodium-water balance (Menard and Catt, 1973; Johnson et al., 1970), estrogen directly affects the vascular bed. Estrogen was initially reported to act on the peripheral vascular bed as a vasodilator substance (Reynolds and Foster, 1940; Ueland and Parer, 1966), but this was not confirmed by a later study (Lim et al., 1970).

To examine the direct effect of estrogen upon the vascular bed, estrogen should be administered into the circulation. Diethylstilbestrol diphosphate (Honvan) is a commonly used estrogen which is usually administered intravenously. In this experiment we examined the direct effect of diethylstilbestrol diphosphate, as well as that of estradiol hemisuccinate, on the vascular bed in vivo and in vitro, using conscious rats and isolated mesenteric arteries.

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Materials and Methods

Male (300-350 g) Wistar rats were used in these studies.

In vivo experiments

Arterial blood pressure was recorded directly through a catheter (PE-50) that had been chronically implanted into the right carotid artery. Arterial pressure was recorded continuously in the unanesthetized and unrestrained rats with a Nihon Koden MPU-0.5 pressure transducer coupled to a Nihon Koden RM-25 recorder. Norepinephrine (1-arterenol bitartrate, Sigma) was injected into the jugular vein, and estradiol hemisuccinate (β-estradiol-17β-hemisuccinate, Sigma) and diethylstilbestrol diphasphate (Honvan, Kyorin) were injected into the femoral vein through chronically implanted catheters (PE-10). The arterial and venous catheters were implanted under pentobarbital anesthesia (50 mg/kg, ip) 24 hr prior to the experiment.

At the beginning of the experiment 50, 100 or 200 ng of norepinephrine dissolved in 5% glucose was injected intravenously. These amounts of norepinephrine produced dose-dependent pressor responses. In a preliminary experiment, using 6 rats, we confirmed that pressor responses to norepinephrine were quite reproducible (±5 mmHg). After these initial test doses, estradiol hemisuccinate (1 or 3 mg/kg) dissolved in 5% glucose containing 20% of propylene glycol or diethylstilbestrol diphasphate (10, 30 or 90 mg/kg) dissolved in 5% glucose was injected intravenously. 5% glucose or 5% glucose containing 20% propylene glycol in the amounts used in this experiment (less than 0.3 ml) did not have any effect on vascular reactivity to norepinephrine or blood pressure in conscious rats. Twelve minutes after the injection of estradiol or diethylstilbestrol, 50, 100 and 200 ng of norepinephrine was injected again. Each dose of norepinephrine was injected twice and the pressor responses were averaged for each dose.

In vitro experiments

The isolated superior mesenteric vascular bed was prepared as described by MacGregor (1965). The superior mesenteric artery was cannulated under ether anesthesia and the vascular bed was dissected out and was perfused by a peristaltic flow inducer (Master flex pump, Cole-Parmer) with Krebs bicarbonate buffer (150 mEq sodium, 4.3 mEq potassium, 1 mEq magnesium, 2.5 mEq calcium, 1.7 mEq phosphate, 25 mEq bicarbonate, remaining anion chloride, and 2 g glucose, per liter) at 37°C bubbled with 5% carbon dioxide in oxygen. The pH of the solution was 7.4. The perfusion pressure was recorded via a side arm of the arterial cannula with a Nihon Koden MPU-0.5 transducer, the flow rate being adjusted to give a steady perfusion pressure of 25-30 mmHg. The actual flow rate was around 4 ml/min, which is similar to the expected superior mesenteric artery flow rate in an adult rat. The flow rate was constant during each experiment. Under these conditions there was no significant change in base-line pressure for at least 2 hr. 20 ng of norepinephrine dissolved in 0.05 ml of buffer was injected into the cannula, which produced a transient rise in pressure of approximately 30 mmHg. After three pressor responses of constant amplitude (±5%) to injected norepinephrine had been obtained, progressively increasing concentrations of estradiol hemisuccinate (0.5 to 16 µg/ml) or diethylstilbestrol diphasphate (6.25 to 400 µg/ml) were added to the perfusate. For each concentration perfusion was continued for 12 min at the end of which time the amplitude of the pressor response to norepinephrine was determined. Results are expressed as percentages of the mean initial response. In 6 preliminary experiments, we confirmed that the response to norepinephrine remained constant (±10%) for at least 2 hr when no estradiol or diethylstilbestrol was added to the perfusate. The effect of higher concentrations of diethylstilbestrol (2 and 5 mg/ml) on the basal pressure was also examined. At the end of the experiment, we changed the perfusate to Krebs buffer alone, and confirmed that the vascular response to norepinephrine and the basal pressure returned to the control levels. Estradiol and diethylstilbestrol were originally dissolved in 5% glucose containing 20% of propylene glycol and 5% glucose respectively. The final concentrations of these vehicles in the buffer was less than 0.2% of the volume. These amounts of vehicles alone had no effects on the response to norepinephrine and on the basal pressure.

Six experiments were performed in each study. Results were expressed as mean±SD. Comparisons were made by Student's t-test.

Results

In vivo experiments: Intravenously administered estradiol hemisuccinate or diethylstilbestrol diphasphate attenuated the vascular response to norepinephrine in a dose dependent fashion (Fig. 1). 1 mg/kg of estradiol or 10 mg/kg of diethylstilbestrol significantly reduced the response to norepinephrine (p<0.01, by paired t-test). Estradiol in the amounts used in this experiment did not change the blood pressure of conscious rats. 10 mg/kg of diethylstilbestrol did not change the blood pressure, but
30 and 90 mg/kg of diethylstilbestrol significantly lowered the blood pressure of conscious rats. Changes in blood pressure induced by intravenous administration of 10, 30 and 90 mg/kg of diethylstilbestrol were 2.3 ± 4.9, 26.3 ± 4.9 and 46.4 ± 6.1 mmHg, respectively. (p > 0.05, p < 0.01 and p < 0.001 respectively, by paired t-test) (Fig. 2). The blood pressure remained reduced for at least 30 min.

In vitro experiments: In the isolated mesenteric artery perfused with Krebs bicarbonate solution, estradiol hemisuccinate or diethylstilbestrol diphosphate in the per-
Fig. 2. A typical example of the effect of diethylstilbestrol diphosphate on blood pressure and the response to norepinephrine in conscious rats. The doses of norepinephrine injected are 50 ng (A), 100 ng (B) and 200 ng (C).

Discussion

The results of the present study indicated that intravenously administered estradiol hemisuccinate or diethylstilbestrol diphosphate attenuated the pressor response to norepinephrine. Furthermore, high doses of diethylstilbestrol lowered the blood pressure of conscious rats. Although the effect of estrogen on the heart should be considered (Lim et al., 1970; Stumpf et al., 1977), the similar results we observed in the isolated vascular tissue strongly suggested that both estradiol and diethylstilbestrol also act directly on the vascular beds.

In contrast to our observations, Altura
Fig. 3. Inhibition of pressor response to norepinephrine in the rat mesenteric vascular bed following increasing concentrations of estradiol hemisuccinate. Prior to adding estradiol, three test injections of norepinephrine were given and the mean pressor response was taken as 100%. Pressor responses in the presence of estradiol are expressed as percentages of control ± SD.

(1975) reported that pretreatment of male rats with estrogen resulted in an enhancement of the vasoconstrictor actions of catecholamines. His observations, however, might not reflect the direct effect of estrogen on the vascular beds, because in his experiments estradiol was administered subcutaneously 18 to 24 hr before observation. Estrogen when it is administered in vivo changes hormonal balances, especially the renin-angiotensin system (Menard and Catt, 1973) and promotes sodium and water retention (Johnson et al., 1970). These changes occur rather rapidly and might exert secondary effects upon vascular reactivity. In our study the vascular responses to norepinephrine were examined 12 minutes after the administration of estradiol or diethylstilbestrol into the circulation. This means, in addition to the observations in the mesenteric artery, that the inhibitory effect of estradiol or diethylstilbestrol on the pressor response to norepinephrine is attributable to its direct action on the vascular beds, not mediated by the change in hormonal or sodium balance.

In this study intravenous administration of 3 mg/kg of estradiol hemisuccinate did not change the blood pressure of conscious rats. We were not able to examine the effect of higher doses of estradiol, because of the low solubility of this substance. But 30 mg/kg or 90 mg/kg of diethylstilbestrol diphosphate significantly reduced the blood pressure. Since high concentrations of diethylstilbestrol decreased the basal pressure in the isolated mesenteric artery, the decrease in blood pressure we observed in...
vivo is likely to be attributable to the dilation of peripheral vessel. Estrogen has been reported to be a vasodilator substance in the peripheral vascular beds. Estrogen dilated the capillaries in the ear of the rabbit (Reynolds and Foster, 1940) and uterine vessels in man (Borell et al., 1953), and decreased peripheral resistance in the sheep (Ueland and Parer, 1966). But Lim et al., (1970) reported that an intravenous injection of 20 mg of estrone did not change the limb vascular resistance in man. These results seem to indicate that only huge amounts of estrogen can dilate the vessels.

Clark et al., (1978) reported that vasodilation of rat uterine vessels induced by estradiol was prevented by pretreatment with α-adrenergic blocking agents, suggesting a specific interrelationship between catecholamines and estrogen. Krall et al., (1977) also reported that estrogen reduces the α-adrenergic receptor concentration in myometrium. Although the changes in blood pressure and vascular reactivity to norepinephrine are not always parallel (Doyle and Fraser, 1961; Ichikawa et al. 1978; Kondo et al. 1980), our finding that vascular response to norepinephrine was attenuated by estrogen suggests that estrogen may reduce the sympathetic tone in vivo, and thereby dilates the peripheral vessels. However our preliminary experiments (unpublished data) showed that estradiol attenuated the vascular response of rat mesenteric artery to angiotensin II, potassium chloride or vasopressin. Further studies on the vascular effects of estrogen are necessary to elucidate the mechanism of its action.

The chronic administration of estrogen increased the blood pressure in man (Laragh et al., 1967; Saruta et al., 1970) and in rats (Saruta et al., 1975), and accelerated the development of hypertension with renovascular or DOCA-salt hypertension in rats (Bunag, 1976). These changes in the cardiovascular system may be due to stimulation of the renin-angiotensin system or the sodium and water retention induced by estrogen (Saruta et al., 1970 and 1975). In contrast to these findings, estrogen treatment attenuated the development of hypertension in spontaneous hypertensive rats (Hoeg et al., 1977) and inhibited DOCA-salt hypertension in chickens (Stamler, 1954). The inhibitory effect of estrogen on the development of hypertension could possibly be explained by the direct effect of estrogen on the vascular beds such as observed in our present study. It should thus be stressed that the direct effect of estrogen, as well as the chronic changes in the hormonal and sodium balance induced by estrogen, should be considered when the effect of estrogen on blood pressure is discussed.

Diethylstilbestrol diphosphate is a commonly used estrogen which is usually administered intravenously. This substance was reported to have slight estrogen-like activity before it is hydrolyzed by phosphatase in the tissue (Druckrey and Raabe, 1952). In the present experiments the inhibitory effect of estradiol on the vascular response to norepinephrine was 10–50 times higher than that of diethylstilbestrol diphosphate. But the doses of diethylstilbestrol diphosphate we used in this experiment were comparable to those in clinical use (Band et al., 1973; Smith, 1975). Our results seem to indicate a cardiovascular side effect of this drug when it is administered intravenously.
References


