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The role of the sympathetic nervous system in oestrogen-induced hypertension in rats

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1 Albino rats of either sex received chronic ethinyl oestradiol (EO) treatment (1.5 mg kg^{-1} daily, i.m.) for 3 weeks. Untreated control rats received arachis oil vehicle alone.

2 Chronic EO treatment resulted in elevation of blood pressure in both sexes. Female rats exhibited significantly greater elevation in blood pressure than males.

3 In chronic EO-treated rats pressor responses to low doses (0.5 $\mu g\,kg^{-1}$) of noradrenaline were significantly increased, while those to angiotensin II, acetylcholine and isoprenaline were unaltered. Chronic EO treatment also sensitized the vascular bed of the rats' hindquarters to noradrenaline.

4 EO-induced hypertension was associated with significant increase in dopamine-β-hydroxylase activity of adrenal glands.

5 Complete bilateral adrenalectomy or chemical sympathectomy prevented the development of EO-induced hypertension.

6 It is suggested that chronic treatment of rats with EO induces and maintains hypertension. The peripheral sympathetic system plays an important role in this phenomenon.

Introduction

Hypertension is one of the most common cardiovascular complications of oral contraceptive (OC) treatment (Kaplan, 1978). The reported incidences of hypertension among OC users varies from 2% to 18% (Woods, 1967). Although OC-induced hypertension has been widely ascribed to a primary influence of oestrogen-mediated modification of the renin-angiotensin-aldosterone system (Laragh *et al.*, 1972), such changes have not provided a complete, satisfactory explanation for the variable effects of OC on arterial blood pressure (Murlow, 1969; Lew, 1980). The mechanism by which OCs elevate blood pressure is controversial. However, it is thought that the oestrogenic component of the pill may be responsible (Wallace, 1971; Weir *et al.*, 1974). Both central and peripheral catecholaminergic neurones have also been implicated in the pathophysiology of hypertension (Doba & Reis, 1974). Elevation of blood pressure and alteration in noradrenaline (NA) concentrations in normotensive and genetically hypertensive rats after oestrogen treatment have been reported (Lew, 1975; 1978). This investigation was undertaken to evaluate the possible role of the sympathetic nervous system in oestrogen-induced hypertension.

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Methods

Oestrogen treatments

Ethinyl oestradiol (EO) which is the common oestrogenic component of widely used OC pills was selected for the study. Albino rats (Haffkine strain) of either sex (250–350 g) were used for this study. The EO-treated group received an intramuscular injection of EO (1.5 mg kg^{-1}) daily for 3 weeks, while the age, sex and weight-matched untreated control group of animals received an intramuscular injection of the vehicle (arachis oil) daily for 3 weeks.

Experiments on ethinyl oestradiol-treated and untreated control rats

Body weight and blood pressure Alterations in body weight and mean blood pressure of both groups of rats were recorded after completion of the treatment. Mean blood pressure of anaesthetized (pentobarbitone sodium: 40 mg kg^{-1} i.p.) rats was recorded directly from the common carotid artery by means of a Statham Pressure Transducer coupled to a calibrated Twin Viso Recorder (Sanborn). The femoral vein was cannulated with a fine polyethylene catheter for

injection of drugs. Agonists, NA (0.5 $\mu g\,kg^{-1}$ and 2.5 $\mu g\,kg^{-1}$), acetylcholine (ACh, 0.1 $\mu g\,kg^{-1}$ and 0.25 $\mu g\,kg^{-1}$), isoprenaline (0.1 $\mu g\,kg^{-1}$ and 0.25 $\mu g\,kg^{-1}$) and angiotensin II (0.1 $\mu g\,kg^{-1}$ and 0.25 $\mu g\,kg^{-1}$), were administered intravenously in a volume of 0.1 ml followed by 0.2 ml of 0.9% sodium chloride solution.

Hindquarter perfusion Female rats (250 g) were anaesthetized with pentobarbitone sodium (40 mg kg^{-1} i.p.), the abdomen was opened by midline incision and the visceral tissues were pulled aside with tissue clamps. After exposing the descending abdominal aorta and the inferior vena cava at the level of bifurcation, both the vessels were cannulated with fine polyethylene catheters to allow the flow of the effluent. The upper part of the body, above the cannulation was then severed from the rest of the body. The abdominal aorta was flushed with warm Krebs-Henseleit solution until the effluent was free from blood; the catheter was connected by rubber tubing to a three-way cannula through which from the opposite end, warm carbogenated (95% O_2 + 5% CO_2) Krebs-Henseleit solution maintained at 37°C (pH 7.4) was continuously perfused at a rate of 4–5 ml per min at an inflow pressure of 50 mmHg. The resistance to perfusion flow through the hindquarter was monitored through one arm of the three-way tube, which was connected to a Statham Pressure Transducer and recordings made on a Twin Viso Recorder (Sanborn). The perfused hindquarter was surrounded by wet cotton soaked with Krebs-Henseleit solution. The rectal temperature of the rat in the hindquarter preparation was maintained at 33–35°C during the experiment by a heating lamp. After a 30 min equilibration period, NA was injected in various doses in volumes of 0.03–0.05 ml with a Hamilton syringe inserted into the rubber tubing connected to the three-way cannula.

Bilateral adrenalectomy This was performed in female rats anaesthetized with ether, by the technique described by De Champlain & Van Ameringen (1972). In sham-operated animals a similar operative procedure was carried out except for the removal of the adrenal glands. All the operated animals received injections of ampicillin (10 mg kg^{-1} daily, i.m.) postoperatively for 5 days and Neosporin-H (polymyxin B sulphate B.P., 5000 u; zinc bacitracin B.P., 400 u; neomycin sulphate I.P., 3400 u and hydrocortisone, I.P., 10 mg g^{-1}) ointment was applied locally to the operative wound. Bilaterally adrenalectomized animals were provided with normal saline (0.9% sodium chloride) for drinking for one week before the 3 week treatments described above. Sham-operated animals also received the same treatment.

Chemical sympathectomy In adult female rats chemical sympathectomy was achieved by use of guanethidine (Johnson & O'Brien, 1976). The EO-treated group of animals first received a daily injection of guanethidine (50 mg kg^{-1}, i.p.), for 2 weeks followed by guanethidine and EO treatment for 3 weeks, while the untreated control group first received a daily injection of guanethidine (50 mg kg^{-1} i.p.) for 2 weeks followed by guanethidine and the vehicle for 3 weeks. The completeness of peripheral sympathectomy was confirmed by the total absence of vasopressor response to tyramine (500 $\mu g\,kg^{-1}$, i.v.). The mean pressor effect of this dose of tyramine in vehicle treated controls was 40 ± 3 mmHg.

Dopamine-β-hydroxylase activity DBH activity with tyramine as substrate (Nagatsu & Udenfriend, 1972) was measured spectrophotometrically in the adrenal glands of female rats after 3 weeks of treatment.

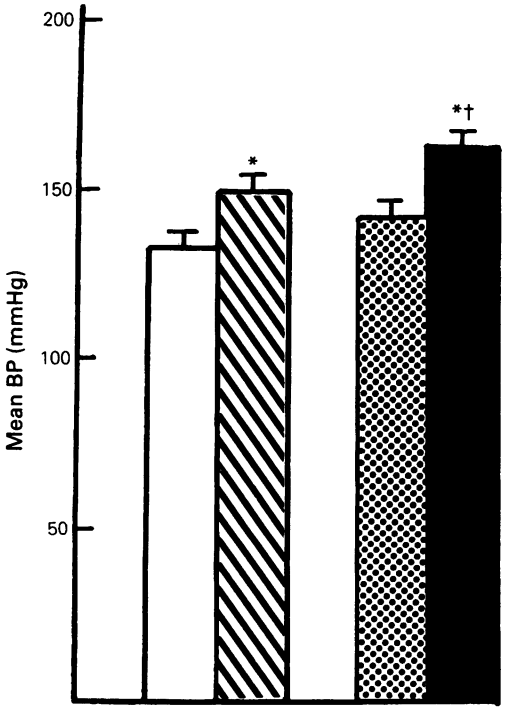

Figure 1 Mean blood pressure (mmHg) of untreated control male (open column) and female (stippled column) and chronic EO-treated male (hatched column) and female (solid column) rats. Vertical lines indicate s.e.mean ($n = 18$ for male and 10 for female rats; $*P < 0.001$ as compared with the corresponding controls; $\dagger P < 0.01$ compared with EO-treated male rats).

Statistical analysis

Students t test was applied to determine the level of significance. $P < 0.05$ was considered as statistically significant.

Drugs

The following were used: acetylcholine chloride (BDH, London, England), angiotensin II (Ciba-Geigy Pharmaceuticals Co., New Jersey, U.S.A.), ethinyl oestradiol (Cipla, Bombay, India), guanethidine sulphate (Ciba-Geigy of India Ltd., Bombay, India), isoprenaline sulphate (Ward Blenkinsop & Co. Ltd., London, England), noradrenaline bitartrate (Sigma Chemical Co., St. Louis, U.S.A.), octopamine hydrochloride (Sigma Chemical Co., St. Louis, U.S.A.) pargyline hydrochloride (Sigma Chemical Co., St. Louis, U.S.A.). and tyramine hydrochloride (Hoffman-La Roche & Co., Basle, Switzerland). The doses mentioned in the text refer to the salts of the drugs.

Results

Development of hypertension on chronic treatment with ethinyl oestradiol

Chronic EO treatment for 3 weeks produced a small but consistent elevation of mean blood pressure in both male and female rats. The mean blood pressure of EO-treated female rats was significantly ($P < 0.01$) higher than that of EO-treated male rats (Figure 1).

There was a significant reduction in the body weight of both male and female rats after chronic EO treatment (Table 1).

Table 1 Effects of chronic ethinyl oestradiol (EO) treatment on the body weights of female and male rats

	Mean body weight (g) *Before*	*After*	P
(A) Female			
Untreated control	242 ± 7.7	246 ± 21.0	> 0.05
EO-treated	243 ± 7.8	205 ± 1.4	< 0.001
(B) Male			
Untreated control	372 ± 10.5	346 ± 10.5	> 0.05
EO-treated	370 ± 12.6	291 ± 14.5	< 0.001

Values as mean ± s.e.mean ($n = 10$).

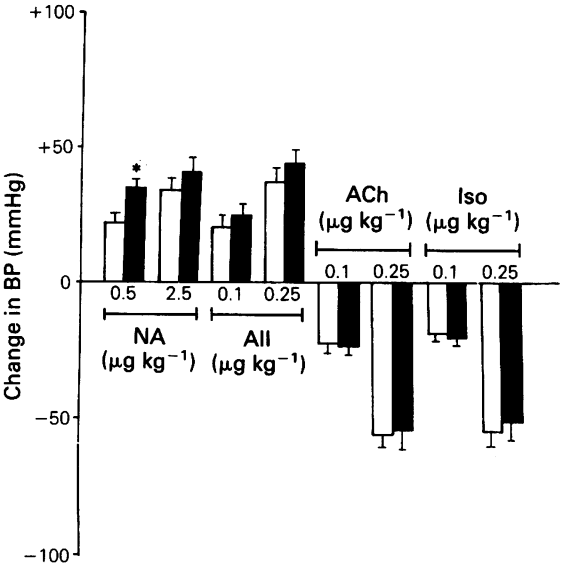

Figure 2 Change in blood pressure (mmHg) produced by intravenous administration of noradrenaline (NA; 0.5 μg kg^{-1} and 2.5 μg kg^{-1}), angiotensin II (AII; 0.1 μg kg^{-1} and 0.25 μg kg^{-1}) acetylcholine (ACh; 0.1 μg kg^{-1} and 0.25 μg kg^{-1}) and isoprenaline (Iso; 0.1 μg kg^{-1} and 0.25 μg kg^{-1}) in untreated control (open columns) and ethinyl oestradiol-treated (solid columns) male rats. Vertical lines indicate s.e.mean ($n = 5$; $^*P < 0.05$ as compared with the corresponding control response).

Experiments in ethinyl oestradiol-treated and untreated control rats

Blood pressure responses Responses to 4 low doses of NA (0.5 μg kg^{-1}) were significantly potentiated while those to higher doses of NA (2.5 μg kg^{-1}) were not altered after chronic EO treatment. The duration of the blood pressure response to NA was not altered after chronic EO treatment. Neither pressor responses to angiotensin II (0.1–0.25 μg kg^{-1}) nor depressor responses to ACh (0.1–0.25 μg kg^{-1}) and isoprenaline (0.1–0.25 μg kg^{-1}) were altered after chronic EO treatment (Figure 2).

Hindquarter perfusion Chronic EO treatment significantly sensitized the vascular bed of the hindquarter of the rat to NA (0.3 μg–50 μg). There was a parallel leftward shift of the dose-response curve to NA as reflected by a significant increase in the perfusion pressure for each dose of NA after chronic EO treatment (Figure 3); however, the duration of the pressor responses was unaltered.

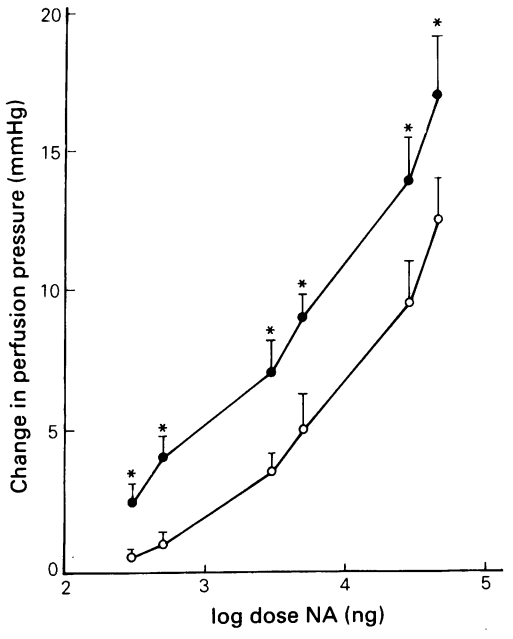

Figure 3 Effects of various doses of noradrenaline (NA; 0.3 μg–50 μg) on changes in perfusion pressure (mmHg) of hindquarter of rats. Abscissa scale represents log dose NA (ng) and ordinate scale the changes in perfusion pressure (mmHg). Shown are responses of untreated control (○) and ethinyl oestradiol-treated (●) female rats. Vertical lines indicate s.e.mean ($n = 6$; * $P < 0.05$ as compared with the corresponding control responses).

Effects of bilateral adrenalectomy and chemical sympathectomy In the sham-operated group, chronic EO treatment produced significant elevation of mean blood pressure (Figure 4). Bilateral adrenalectomy produced significant lowering of the mean blood pressure of the untreated control group and prevented the development of hypertension in the group given chronic EO treatment. The mean blood pressure of the EO-treated bilaterally adrenalectomized group was not significantly different from that of the adrenalectomized control group (Figure 4).

Chemical sympathectomy by guanethidine lowered the mean blood pressure of the untreated control group. Chemical sympathectomy prevented hypertension induced by chronic EO treatment; the mean blood pressure of the chemically sympathectomized EO-treated group was not significantly different from that of the untreated control group (Figure 4).

Table 2 Effects of chronic ethinyl oestradiol (EO treatment on weight and dopamine-β-hydroxylase (DBH) activity of adrenal glands

	Untreated control	*EO-treated*
Adrenal glands (Mean wet weight, mg)	52.2 ± 3.3	65.6 ± 4.7*
DBH activity ($nmol\ min^{-1}\ g^{-1}$)	39.0 ± 6.8	193.0 ± 33.6**

Values are mean ± s.e.mean; $n = 5$. * $P < 0.05$ from untreated control; ** $P < 0.01$ from untreated control.

Dopamine-β-hydroxylase activity of adrenal glands The adrenal glands of almost all the chronic EO-treated rats appeared hypertrophic as compared to those of untreated controls. The hypertrophy was associated with significant increase in weight (Table 2). The DBH activity of adrenal glands after chronic EO treatment was approximately five times higher than that of the untreated control group (Table 2).

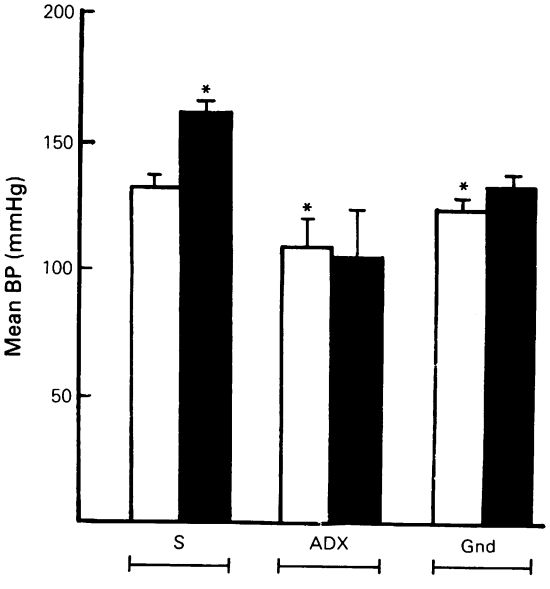

Figure 4 Effects of ethinyl oestradiol (EO) treatment on the mean blood pressure (mmHg) of sham (S) operated controls, bilaterally adrenalectomized (ADX) and chemically sympathectomized (Gnd) rats. Vertical lines indicate s.e.mean ($n = 5$; open columns = untreated control and solid columns = EO-treated; * $P < 0.05$ as compared with the blood pressure of sham-operated controls).

Discussion

In agreement with the results of Saruta *et al.* (1972, 1975), chronic administration of EO induced hypertension in rats. There was a decrease in the body weight in all the animals, as reported previously by other workers (Meites, 1949; Glasser, 1954; Saruta *et al.*, 1975; Lew, 1975). It is suggested that the decrease in body weight of the rats after chronic EO treatment may be due either to (a) anorectic and/or (b) antianabolic actions of EO (Meites, 1949; Glasser, 1954).

The blood pressure after chronic EO treatment was significantly higher in female rats than in male rats. The difference could be due to the presence of androgenic hormones in the male rats which may tend to inhibit the peripheral vascular action of catecholamines and neurohypophyseal hormones (Altura, 1973; Greenberg *et al.*, 1973). In addition, it has been reported that mesenteric arterioles of female rats are more sensitive than those of males to the vasoconstrictor action of catecholamines and neurohypophyseal hormones (Altura, 1975).

The increased vascular reactivity observed in most forms of experimental hypertension and in human hypertension has been interpreted as an indirect indication that the sympathetic system might be involved in the etiology and maintenance of hypertension (De Champlain, 1972; Takeda & Bunag, 1980). In chronic hypertension in both man and experimental animals, an increase in total peripheral resistance and an enhanced response to pressor agents are found (Bohr, 1974). An initial increase in blood pressure, probably due to altered sympathetic control leads to an increased wall: lumen ratio (Folkow, 1971; 1978) or altered adrenergic innervation (Bevan *et al.*, 1975) and results in enhanced responsiveness to pressor agents and maintenance of a stable hypertensive state. In the present study the pressor response to a low dose of NA was potentiated and that to a higher dose was unaltered while blood pressure responses to ACh, isoprenaline and angiotension II were not modified in chronic EO-treated hypertensive rats. Therefore, it seems likely that EO-induced hypertension may be associated with a selective increase in responsiveness to α-adrenoceptor agonists.

Inhibition of the enzymes monoamine oxidase and catechol-*O*-methyltransferase enhances the contractile responses of vascular smooth muscles to sympathomimetics both in terms of duration and amplitude (Kalsner, 1971; Trendelenburg, 1974). Since chronic EO treatment did not prolong the blood pressure response to NA, the altered metabolic degradation of NA may not be the cause of potentiation.

In agreement with the above observations, increased vascular sensitivity to NA of perfused hindquarters was observed after chronic EO treatment of rats. It is also interesting to note that in EO-treated animals, the blood pressure response to a higher dose of NA was not altered while vasoconstrictor responses in the hindquarter experiments to all doses of NA were potentiated. This could be explained on the premise that in a whole animal the presence of cardiovascular reflexes may attenuate the blood pressure response to a higher dose of NA, while in an isolated perfused hindquarter preparation such reflexes are not present.

It seems unlikely that the change in responsiveness to NA is solely due to alteration in the structural component of the resistant vessels as has been previously suggested (McGregor & Smirk, 1970; Folkow & Hallback, 1977), since one would expect quantitatively similar changes for the other agonists, if this were the case.

Since supersensitivity to NA was demonstrable in both, whole animal and perfused hindquarter, the underlying mechanisms for the increase in the reactivity to NA seem to be located in the blood vessels themselves.

After oestrogen treatment increase in the number of α-adrenoceptors has been demonstrated in extravascular smooth muscles without change in their affinity (William & Lefkowtiz, 1977; Robert *et al.*, 1977). However, Colucci *et al.* (1982) using a radioligand technique, proposed that oestrogen-induced increase in sensitivity to catecholamines of the mesenteric vascular bed, may be partly, mediated through an increase in α-adrenoceptor affinity. In the present study, no attempt was made to study affinity of the vascular bed to NA or 'upgrading' of α-adrenoceptors.

The contribution of the adrenal medulla to the regulation of blood pressure in normotensive and DOCA-salt hypertensive rats (De Champlain *et al.*, 1976; 1977) as well as in SHRs (Aoki, 1963; Ozaki, 1966) has been demonstrated. Bilateral adrenalectomy produces a greater blood pressure fall in DOCA-salt hypertensive rats than in normotensive rats; however, blood pressure remained at hypertensive levels suggesting an active but partial role of the adrenal medulla (De Champlain, 1977). Also the role of the adrenal medulla in rats made hypertensive after the application of a clip on the renal artery for 3 weeks (De Champlain & Van Ameringen, 1972), in androgen-induced hypertension (De Champlain, 1977) and in neurogenic hypertension (De Quattro *et al.*, 1969) has been suggested. In all the above studies, bilateral adrenalectomy either produced significant lowering of blood pressure or prevented the development of hypertension. In the present study, bilateral adrenalectomy prevented EO-induced hypertension in rats, suggesting an important role for the adrenal glands in the development of hypertension after chronic EO treatment. This conclusion is further supported by the significant increase in DBH activity in the adrenal glands of the EO-treated animals. It seems possible that the increase in DBH activity in adrenal medulla

after chronic EO treatment may increase the synthesis of catecholamines and subsequently release more catecholamines into the general circulation producing elevation in blood pressure. Increases in NA levels in hypothalamic regions and in the adrenal medulla have been previously reported (Lew, 1982) after higher doses of mestranol, whereas decreases have been described in young rats receiving lower doses of mestranol (Lew, 1975; 1978;). Women taking OCs have also been shown to have an increase in serum DBH activity (Rockson *et al.*, 1975) and increases in DBH activity of the mesenteric vessel wall of young SHRs has been reported (Trajkov *et al.*, 1974; Nagatsu *et al.*, 1976). Belmer *et al.* (1979) reported an increase in DBH activity and distribution of noradrenergic vesicle markers in rabbit oviduct after progesterone treatment.

Leonara & Crane (1970) reported that long term administration of EO and 3-methyl EO caused a significant increase in pituitary and adrenal gland size with hyperplasia of the zona fasciculata. The significant increases in weight of adrenal glands observed after EO treatment, are also in agreement with previous findings with diethylstilboestrol (Lew, 1975).

Chemical sympathectomy has been employed in animals for the evaluation of the relative contribution of the peripheral sympathetic function and of the adrenal medulla in the regulation of blood pressure (De Champlain & Van Ameringen, 1972; Gauthier *et al.*, 1972). Administration of guanethidine to adult rats has been shown to produce a marked and permanent destruction of peripheral sympathetic nerve endings without a significant cytotoxic action on either the adrenal medulla or central noradrenergic neurones (Johnson & O'Brien, 1976). In the present study, chemical sympathectomy produced by guanethidine prevented the EO-induced hypertension in rats, suggesting an active role of the peripheral sympathetic system in this phenomenon. These observations also lend further support to the observed supersensitivity of vascular beds to NA in EO-induced hypertension in rats.

In conclusion the present observations strongly suggest that the main mechanism responsible for the induction and maintenance of EO-induced hypertension may be hyperactivity of both the peripheral sympathetic fibres and the adrenal medulla.

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