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# The Influence of 17-β-Oestradiol and the Natural Oestrous Cycle on α-Adrenoceptor-mediated Responses of the Cardiovascular System in the Rat

CLARE AUSTIN AND RUSSELL CHESS-WILLIAMS*

*Physiological Laboratory, Crown Street, University of Liverpool, PO Box 147, Liverpool L69 3BX, and *Department of Biomedical Science, University of Sheffield, Western Bank, Sheffield S10 2TN, UK*

## Abstract

The effect of 17β-oestradiol pretreatment and the natural oestrous cycle on responses of rat cardiac and vascular α-adrenoceptors was investigated.

It was found that treatment with the oestrogen had no effect on cardiac adrenoceptor sensitivity, while pretreatment with the hormone caused a significant increase in vascular $\alpha_2$-, but not vascular $\alpha_1$-adrenoceptor responsiveness. Pressor responses of pithed rats to selective $\alpha_1$- or $\alpha_2$-adrenoceptor agonists did not alter, however, during the natural oestrous cycle.

These findings suggest that changes in oestrogen levels are capable of altering α-adrenoceptor-mediated vascular responses but that the relatively small changes in oestrogen which may occur throughout the natural oestrous cycle do not appear to alter the responses of these vascular α-adrenoceptors.

Clinical observation shows a greater predominance of cardiovascular disease, including hypertension, in women than men. This suggests a possible role of sex steroids in the development of the disorder, an involvement further supported by the high incidence of hypertension amongst users of oral contraceptives (Kaplan 1978). The reason for this is unknown but it is thought that the oestrogenic component of the pill is involved (Wallace 1971), and indeed it has been shown that chronic treatment of rats with oestrogen induces and maintains hypertension (Bhatt & Gulati 1986).

Alterations of both myocardial and vascular adrenoceptors have been demonstrated in animal models of hypertension (Kunos et al 1978; Limas & Limas 1979; Borowski & Porter 1984, 1985). It has also been shown that isolated mesenteric arterioles from female rats are more sensitive to catecholamine-induced constriction than arterioles from male rats (Altura 1972) and that sensitivity can be increased by pretreatment with oestrogen (Altura 1975). It is possible, therefore, that oestrogen, by increasing catecholamine-induced vascular-contraction, may be involved in the increased incidence of hypertension in women.

It is now known that vascular smooth muscle contraction may be mediated by both postsynaptic $\alpha_1$- and $\alpha_2$-adrenoceptors. The effect of oestrogen on these adrenoceptor subtypes of the vascular system has not previously been studied. An effect of oestrogen on cardiac adrenoceptors may also contribute to an increased incidence of cardiovascular disease in women, although radioligand-binding studies on cardiac membranes from rats suggest that oestrogen treatment has no effects on the density of α-adrenoceptors (Colucci et al 1984). No functional studies, however, have previously been performed to investigate the effects of this hormone on the sensitivity of the myocardium to α-adrenoceptor stimulation.

The aim of the present study was, therefore, to investigate the effects of oestrogen pretreatment on rat cardiac and vascular α-adrenoceptor responses. The study went on to investigate the effect of the natural oestrous cycle on any changes observed.

Correspondence: C. Austin, Physiological Laboratory, Crown Street, University of Liverpool, PO Box 147, Liverpool L69 3BX, UK.

## Materials and Methods

*Ovariectomy and pretreatment*

Female Wistar rats, 200–250 g, were anaesthetized with Hypnorm (a solution containing 0·315 mg fentanyl citrate and 10 mg fluanisone $mL^{-1}$) (10 mg $kg^{-1}$) and valium (0·5 mg $kg^{-1}$) and ovariectomized. Following the operation, animals were left for a period of four weeks to allow stabilization of hormone levels and full recovery. Ovariectomized animals were divided into two groups; treated and untreated. Treated rats received daily subcutaneous injections of 17β-oestradiol (2·5 mg $kg^{-1}$) made up in ethanol and water (1:3) for seven days while untreated ovariectomized animals received the vehicle alone (Colucci et al 1982). A control non-ovariectomized group of age-matched rats was also examined. In these animals the stage of oestrous was not determined.

*Isolated tissue preparations*

Animals were killed by a sharp blow to the head and exsanguination. Left atria and papillary muscles were removed and set up in a Krebs-bicarbonate solution (NaCl 118·4, KCl 4·7, $NaHCO_3$ 25·0, glucose 11·7, $MgSO_4$ 1·2, $KHPO_4$ 1·2 and $CaCl_2$ 1·9 mM) gassed with 95% $O_2$–5% $CO_2$ at 37°C. Tissues were suspended under 0·8 g initial tension and paced at 1 Hz by square-wave pulses (5 ms duration, threshold voltage + 50%), delivered via

bioplar electrodes from Grass S48 stimulators. Isometric tension was recorded via Lectromed UF1 (57 g sensitivity range) force transducers, on a Devices M19 polygraph. Following equilibration, cumulative concentration–response curves were obtained to phenylephrine on all tissues. Experiments were performed in the presence of propranolol (1 $\mu$M), desmethylimipramine (1 $\mu$M) and metanephrine (10 $\mu$M) to antagonize β-adrenoceptors and inhibit amine uptake. A maximum response to the β-agonist isoprenaline was also obtained on each tissue for comparison.

*Pithed rat preparation*

Rats were anaesthetized by intraperitoneal injection (60 mg kg$^{-1}$) of sodium pentobarbitone. The trachea was cannulated and the animals were pithed by inserting a blunt needle (1·5 mm diam.) through the orbit and foramen magnum and into the spinal column. Immediately after pithing, rats were respired artificially with air by means of a respiratory pump (BioScience, Sheerness, UK), operating at 54 cycles min$^{-1}$ at approximately 2·5 mL/100 g. Blood gases were analysed using a Corning 158 pH/blood gas analyser and the volume of the respiration pump was adjusted to maintain blood gases within physiological ranges, i.e. pH 7·0–7·8; $pCO_2$ 18·0–38·0 mmHg; $pO_2 > 70$ mmHg.

The left carotid artery was cannulated and systemic blood pressure was measured via a Bell and Howell type 4-422-0001 transducer and recorded on a Grass model 79D polygraph. A femoral vein was also cannulated for the administration of drugs. All cannulas were filled with heparinized saline. The temperature of each animal, monitored with a rectal probe, was maintained at 37°C.

Following equilibration, responses were obtained to phenylephrine (1–10 $\mu$g kg$^{-1}$) in the presence of propranolol (1 mg kg$^{-1}$) and to UK 14,304 (1–10 $\mu$g kg$^{-1}$) in the presence of prazosin (500 $\mu$g kg$^{-1}$). It was found that pressor responses to UK 14,304 could be significantly reduced ($P < 0·001$) by pretreatment with the $\alpha_2$-antagonist idazoxan (500 $\mu$g kg$^{-1}$) supporting the findings of others that the drug is a selective $\alpha_2$-agonist (Van Meel et al 1981). All drugs were administered intravenously in 0·9% saline in a volume of approximately 1 mL and were flushed in with a further 1 mL of heparinized saline. Antagonists were injected at least 10 min before the addition of agonist. Pressor responses were measured as changes in diastolic blood pressure.

*Determination of state of oestrous*

The oestrous state of each animal was assessed by examination of a vaginal smear. Animals were restrained and samples taken by gently moving a wire loop moistened with saline, around the vaginal wall. The adhering material was smeared onto a glass slide and viewed under a microscope. The four stages of oestrous were identified according to Hafez (1970) and Ham & Cormack (1988). Rats at the appropriate stage of oestrous were pithed and used for examination of α-responsiveness as described above.

*Statistical analysis*

*Isolated tissues.* Increases in developed tension were plotted as a percentage of the maximum developed tension to phenylephrine. Individual EC50 values were determined and geometric mean EC50 values with 95% confidence limits calculated. Student's *t*-tests were performed on logarithmic EC50 values to test for significant differences between groups.

Increases in developed tension to phenylephrine were also expressed as % maximum response to isoprenaline. Student's *t*-test was again used to investigate differences between groups. n represents the number of animals used.

*Pithed rats.* Mean changes in diastolic blood pressure were calculated for each group of animals. Differences in responses between groups were compared by the Mann Whitney U-test.

## Results

*Isolated cardiac tissues*

Resting tensions (i.e. tension or contraction developed by the tissues before the addition of any drugs) developed by left atria from control, ovariectomized and 17β-oestradiol-treated ovariectomized animals were all similar being 0·26 ± 0·03, 0·30 ± 0·02 and 0·37 ± 0·18 g, respectively. Initial tensions developed by papillary muscles from control (0·28 ± 0·03 g), ovariectomized (0·36 ± 0·10 g) and oestradiol-treated ovariectomized rats (0·30 ± 0·12 g) again did not differ significantly from each other.

It was found that neither ovariectomy alone nor with oestradiol treatment significantly altered the sensitivity of left atria and papillary muscles to phenylephrine as indicated by similar EC50 values (Fig. 1). The maximum

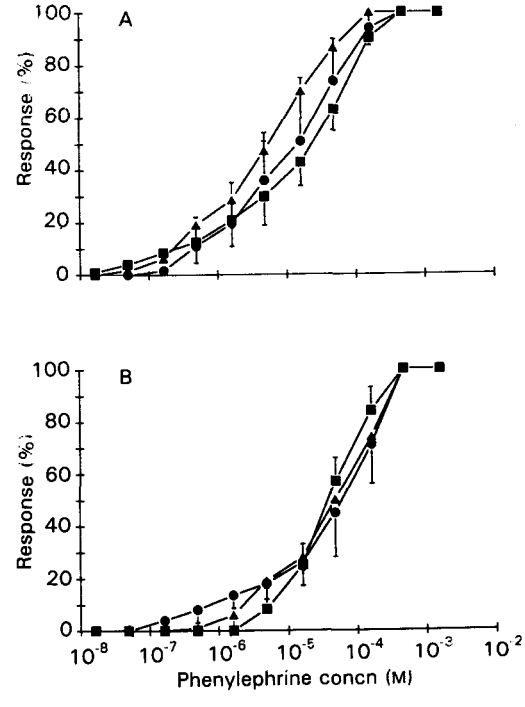


FIG. 1. Concentration–response curves to phenylephrine of left atria (A) and papillary muscles (B) from control (●), ovariectomized (■) and oestradiol-treated ovariectomized (▲) rats.

Table 1. Geometric mean EC50 values (with 95% confidence limits) and maximum response ± s.e.m. (% isoprenaline maximum) to phenylephrine of left atria and papillary muscles from control, ovariectomized, and oestradiol-treated ovariectomized rats.

| | Control | Ovariectomized | Oestradiol-treated |
|---|---|---|---|
| Left atria EC50 ($\mu$M) | 11·41 (1·02–128·00) (n = 4) | 16·29 (1·96–135·28) (n = 3) | 5·22 (1·79–15·28) (n = 3) |
| Maximum developed tension (%) | 56·11 ± 11·76 | 51·4 ± 7·07 | 68·63 ± 4·10 |
| Papillary muscle EC50 ($\mu$M) | 56·00 (12·26–255·78) (n = 4) | 31·79 (11·49–88·00) (n = 4) | 48·85 (17·00–140·70) (n = 4) |
| Maximum developed tension (%) | 34·15 ± 7·35 | 34·15 ± 7·35 | 39·69 ± 4·41 |

responses of cardiac tissues to the α-agonist, as percentage of isoprenaline maximum, were also unaffected (Table 1).

*Resting values in pithed rats*

*Ovariectomized and oestrogen-treated animals.* Ovariectomy decreased the mean resting blood pressure of animals from 59 ± 6/43 ± 3 mmHg in control to 35 ± 9/29 ± 6 mmHg in ovariectomized animals. This difference was significant ($P < 0·005$) for diastolic blood pressure. Oestradiol-treatment of ovariectomized animals significantly increased both mean systolic ($P < 0·05$) and diastolic ($P < 0·005$) blood pressures to 72 ± 9/44 ± 3 mmHg relative to those untreated ovariectomized, but not control, animals.

Control animals had a mean resting heart rate of 338 ± 14 beats $min^{-1}$. Ovariectomy alone, or with the additional oestrogen treatment, had no effect on mean heart rates, being 352 ± 46 and 336 ± 12 beats $min^{-1}$, respectively.

*Oestrous cycle animals.* The resting blood pressure and mean resting heart rate of the animals used to investigate the state of natural oestrous was 46 ± 6/35 ± 4 mmHg and 320 ± 9 beats $min^{-1}$, respectively. The resting blood pressures and heart rates were similar irrespective of the stage of oestrous the animals were in.

*Responses to α-adrenoceptor agonists in pithed rats*

*Effect of ovariectomy and oestradiol-treatment.* Phenylephrine caused dose-related increases in blood pressure of 7·00 ± 1·64, 11·75 ± 3·15 and 27·50 ± 7·73 mmHg for 1, 3 and 10 $\mu g\,kg^{-1}$ doses, respectively in control animals (n = 6). Similar increases were observed in ovariectomized animals over the dose range studied (n = 6). Oestradiol treatment of ovariectomized animals had no significant effect on responses to the $\alpha_1$-agonist (n = 5) (Table 2).

UK 14,304 produced smaller pressor responses than phenylephrine in all animals studied. Pressor responses to UK 14,304 were reduced in ovariectomized animals relative to controls from 4·75 ± 1·70 to 2·67 ± 0·33 mmHg, 15·33 ± 3·76 to 8·00 ± 1·16 mmHg, and from 27·33 ± 4·34 to 13·67 ± 2·34 mmHg for 1, 3 and 10 $\mu g\,kg^{-1}$, respectively. This change was significant ($P < 0·05$) for the dose of 10 $\mu g\,kg^{-1}$ UK 14,304. Oestrogen treatment of ovariectomized animals significantly increased the elevation of blood pressure in response to the $\alpha_2$-agonist (Table 2). Responses of ovariectomized animals were not significantly different from those of control animals following oestradiol treatment.

Neither phenylephrine nor UK 14,304 had any significant effect on heart rate in any group of animals studied.

Table 2. Diastolic blood pressure (mmHg) of control, ovariectomized and oestrogen-treated ovariectomized pithed rats in response to A. phenylephrine in the presence of propranolol and B. UK 14,304 in the presence of propranolol and prazosin.

| | Mean increase in diastolic blood pressure (mmHg) | | |
|---|---|---|---|
| A | Concn phenylephrine in the presence of propranolol (1 $mg\,kg^{-1}$) | | |
| | 1 $\mu g\,kg^{-1}$ | 3 $\mu g\,kg^{-1}$ | 10 $\mu g\,kg^{-1}$ |
| Control | 7·00 ± 1·64 | 11·75 ± 3·15 | 27·50 ± 7·43 |
| Ovariectomized | 7·58 ± 0·58 | 15·02 ± 0·69 | 24·24 ± 2·91 |
| Oestrogen-treated ovariectomized | 12·54 ± 0·50 | 19·62 ± 3·60 | 39·20 ± 8·01 |
| B | Concn UK 14,304 in the presence of propranolol (1 $mg\,kg^{-1}$) and prazosin (500 $\mu g\,kg^{-1}$) | | |
| | 1 $\mu g\,kg^{-1}$ | 3 $\mu g\,kg^{-1}$ | 10 $\mu g\,kg^{-1}$ |
| Control | 4·75 ± 1·70 | 15·33 ± 3·76 | 27·33 ± 4·34 |
| Ovariectomized | 2·67 ± 0·33 | 8·00 ± 1·16* | 13·67 ± 2·24† |
| Oestrogen-treated ovariectomized | 10·40 ± 0·20*** | 15·03 ± 1·43** | 20·12 ± 1·32 |

† *$P < 0·05$ with respect to control, **$P < 0·005$, ***$P < 0·001$ with respect to ovariectomized animals.

Table 3. Diastolic blood pressure (mmHg) of pithed rats in the state of oestrous, metoestrous, dioestrous and proestrous to A. phenylephrine in the presence of propranolol, and B. UK 14,304 in the presence of propranolol and prazosin.

| | Mean increase in diastolic blood pressure (mmHg) | | |
|---|---|---|---|
| A | Concn phenylephrine in the presence of propranolol (1 mg kg$^{-1}$) | | |
| | 1 μg kg$^{-1}$ | 3 μg kg$^{-1}$ | 10 μg kg$^{-1}$ |
| Oestrous | 17.31 ± 3.80 | 31.72 ± 7.28 | 61.28 ± 9.92 |
| Metoestrous | 16·62 ± 3.95 | 33.21 ± 3.75 | 63.17 ± 6.94 |
| Dioestrous | 17.61 ± 4.86 | 32.01 ± 9.55 | 64.21 ± 6.19 |
| Proestrous | 12.61 ± 2.25 | 27.42 ± 3.79 | 53.33 ± 5.02 |
| B | Concn UK 14,304 in the presence of propranolol (1 mg kg$^{-1}$) and prazosin (500 μg kg$^{-1}$) | | |
| | 1 μg kg$^{-1}$ | 3 μg kg$^{-1}$ | 10 μg kg$^{-1}$ |
| Oestrous | 8.53 ± 1.68 | 15.02 ± 2.89 | 23.51 ± 5.09 |
| Metoestrous | 7.57 ± 2.31 | 14.51 ± 2.37 | 19.32 ± 5.47 |
| Dioestrous | 7.01 ± 1.16 | 12.27 ± 2.36 | 19.31 ± 4.56 |
| Proestrous | 10.21 ± 1.42 | 13.42 ± 3.09 | 25.71 ± 3.79 |

*Effect of natural oestrous state.* Pressor responses to either phenylephrine or UK 14,304 were similar for all groups of animals studied (Table 3). Responses to both agonists were, therefore, independent of the state of oestrous. Neither agonist significantly altered heart rate.

## Discussion

The results of the present study indicate that neither ovariectomy alone, nor with concomitant 17β-oestradiol treatment, significantly affects the sensitivity of isolated left atria and papillary muscles to phenylephrine. Although functional responses have not previously been examined, binding studies have shown that pretreatment of male rats with 17β-oestradiol for three days does not alter the affinity of cardiac α-adrenoceptors for prazosin, supporting the findings of the present study (Colucci et al 1984). It, therefore, appears that 17β-oestradiol has no effect on the α-adrenoceptors of the rat heart.

In the pithed-rat model used in the present experiments it was found that ovariectomy of rats significantly decreased resting blood pressure, which was subsequently elevated by oestradiol treatment. Although the blood pressure of the pithed animal probably does not reflect that of the whole animal, these findings support those of Bhatt & Gulati (1986) who demonstrated that chronic treatment of rats with oestrogen can result in the elevation of blood pressure and indeed the development of hypertension. This phenomenon was not seen in adrenalectomized or chemically sympathectomized animals, suggesting an involvement of the sympathetic nervous system with this oestrogen-induced effect. Further evidence for this is obtained from the studies of Altura (1972, 1975) who demonstrated not only that terminal mesentric arterioles from female rats were more sensitive to catecholamine constriction than similar preparations from male animals, but that the sensitivity of the latter to catecholamine-induced contractions could be increased by pretreatment with 17β-oestradiol.

It is well known that postjunctional adrenoceptor-mediated pressor responses are mediated by two types of α-adrenoceptor; $\alpha_1$ and $\alpha_2$. The results of the present study confirm the importance of oestrogen in mediating vascular adrenoceptor responses; however, it was found that only the postjunctional $\alpha_2$-adrenoceptor-, but not the $\alpha_1$-adrenoceptor mediated-responses are influenced by the hormone, responses to UK 14,304 being depressed by ovariectomy and raised to near control levels by oestradiol pretreatment, while responses to phenylephrine were unchanged. Although the effect of oestrogen on vascular adrenoceptors has not previously been investigated, a similar sub-type selectivity has been reported in the uterus where binding studies have shown that oestrogen treatment of rabbits causes a significant increase in $\alpha_2$-adrenoceptor density, without affecting the number of $\alpha_1$-adrenoceptors (Hoffman et al 1981). In isolated femoral artery preparations, however, it has been found that oestrogen treatment depresses post-junctional $\alpha_2$- but not $\alpha_1$-adrenoceptor-mediated responses (Giscard et al 1987). Although the study indicated a selective regulation of $\alpha_2$-adrenoceptors by oestrogen, the change is clearly in the opposite direction to that found in the present study, i.e. depression rather than elevation. No effect, however, was found in the same study on isolated saphenous vein preparations. This suggests that the effects of oestrogen on α-adrenoceptors may vary throughout the vascular system. This may explain the increased phenylephrine-induced contractions of mesentric arterioles from oestrogen-treated animals demonstrated by Altura (1975), which is in contrast to the unchanged blood pressure observed in the present experiment. In addition, results may be influenced by varying doses, or by the duration of dosing with oestrogen and indeed on the type of oestrogen used as suggested by Shackleford et al (1988). It does seem therefore that although in the pithed rat oestrogen treatment leads to a net enhancement of pressor response to UK 14,304, but not to phenylephrine, it cannot be assumed that all blood vessels are affected in a similar way by the treatment.

The way in which oestradiol selectively alters $\alpha_2$-adrenoceptor-mediated responses is unknown; however it is

interesting to note that, in some blood vessels at least, $\alpha_2$-adrenoceptor agonists stimulate both adrenoceptors on vascular smooth muscle (tending to contract the tissue) and endothelial adrenoceptors causing the release of endothelium-derived relaxing factor (EDRF) (tending to cause vasodilation), with the net vascular response representing a balance between these two counteracting pathways (Angus et al 1986). It is possible, therefore, that the selective effect of ovariectomy/oestradiol treatment on $\alpha_2$-mediated responses may be due to a suppressant effect of the hormone on stimulation of the endothelial $\alpha_2$-adrenoceptors; however in other tissues, the presence of an endothelium has been shown to influence responses to both $\alpha_1$- and $\alpha_2$-adrenoceptor agonists (MacLean et al 1993).

In the present study it was found that ovariectomy decreased responses to $\alpha_2$-adrenoceptor stimulation. This suggests that endogenously circulating ovarian hormones are of a sufficiently high level to influence $\alpha_2$-adrenoceptor status. It was, therefore, of value to determine whether the cyclic changes in hormone/oestrogen levels throughout the natural oestrous cycle influence responsiveness to $\alpha_2$-adrenoceptor stimulation. It was found, however, that pressor responses were similar to all stages of oestrous. This suggests that either the natural fluctuations of oestrogens are not large enough to affect vascular $\alpha_2$-adrenoceptor-mediated responses, or that the maximal effect of oestrogen occurs at basal plasma levels such that increases above this level during the cycle will have no further effect.

The results of the present study, therefore, indicate that the treatment of female rats with 17β-oestradiol increases pressor responses to post-junctional $\alpha_2$-adrenoceptors while having no effect on vascular and cardiac $\alpha_1$-adrenoceptors. Although the levels of oestrogen fluctuate throughout the natural oestrous cycle they do not appear to alter $\alpha_2$-adrenoceptor status. This clearly simplifies the study of vascular α-adrenoceptors in female rats.

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