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Short communication

Raloxifene, an oestrogen–receptor modulator, prevents decreased constitutive nitric oxide and vasoconstriction in ovariectomized rats

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Abstract

Administration of graded doses of [Arg^8]vasopressin (0.06–0.18 μg kg^{-1}, i.v.) induced a dose-dependent increase in arterial blood pressure in the catecholamine-depleted (phentolamine; 10 mg kg^{-1}, i.p.) intact and ovariectomized female rat, with the elevation of blood pressure more marked following ovariectomy. In addition, ovariectomy caused the down-regulation of aortic Ca^{2+}-dependent constitutive nitric oxide synthase (assessed by the citrulline assay). The down-regulation of the Ca^{2+}-dependent constitutive nitric oxide synthase and augmentation of vasopressin-induced blood pressure responses were prevented by the therapy (1 month, p.o.) with the selective oestrogen receptor modulator, raloxifene (0.3–1.0 mg kg^{-1} day^{-1}), or with 17β-oestradiol (0.3 mg kg^{-1} day^{-1}) in ovariectomized rats. Thus, oestrogen deficiency down-regulates vascular constitutive nitric oxide synthase, which appears to be involved in the increased sensitivity of the vasculature to vasopressin, since both effects can be reversed by the exogenous administration of the natural oestrogen 17β-oestradiol or the selective oestrogen–receptor modulator raloxifene.

Keywords: Oestrogen receptor modulator; Oestrogen; Nitric oxide (NO); Blood pressure; Vasopressin; Enzyme regulation

1. Introduction

In the reproductive years, the incidence of cardiovascular disease is lower among women than among men. This gender difference disappears following either natural or surgical menopause, suggesting a protective role of endogenous estrogens in the cardiovascular system (Clarkson et al., 1997).

Under experimental conditions, endogenous estrogen or administration of natural estrogens has been demonstrated to increase Ca^{2+}-dependent constitutive nitric oxide (NO) synthase (cNOS) activity in vascular tissues (Weiner et al., 1994; Morschl et al., 2000). Selective oestrogen–receptor modulators have tissue-specific oestrogen agonist effects, e.g. on bone and lipid metabolism, and antagonist effects, e.g. on breast (Clarkson et al., 1997). In addition, increased NO release has been observed from the isolated rat aorta following the administration of selective oestrogen–receptor modulators such as raloxifene (Rahimian et al., 1997).

It is known that NO, formed continuously by cNOS in the vascular endothelium and neuronal elements, plays a significant protective role in the maintenance of vascular integrity. The inhibition of cNOS provokes blood pressure elevation, platelet aggregation and adhesion of neutrophils to the vascular endothelium (Moncada and Higgs, 1995). Moreover, it has recently been demonstrated that oestrogen can modulate the action of vasopressin on the baroreflex control of sympathetic outflow, and thereby participate in cardiovascular regulation (He et al., 1999).

In the present study, we investigated the vasopressin-induced blood pressure response in catecholamine-depleted ovariectomized rats, by determining the changes of aortic cNOS enzyme activity in conjunction with the vasopressin-provoked blood pressure response following raloxifene or oestrogen supplementation in the oestrogen-deficient state.

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2. Materials and methods

2.1. Experimental protocol

We used sham-operated and ovariectomized female Wistar rats (200–220 g). Operations were performed under transient ether anaesthesia, followed by a 1-month recovery period. In ovariectomized rats, raloxifene (0.3–1 mg kg^{-1}, p.o., once daily) or 17β-oestradiol (0.3 mg kg^{-1}, p.o., once daily) were administered for 1 month. The procedures, doses of compounds, and route of administration have been established in previous studies (Rahimian et al., 1997; Morschl et al., 2000). Control animals received the vehicle at the same time, in the same volume and by the same route.

2.2. Ca^{2+}-dependent aortic nitric oxide synthase enzyme activity

NOS activity was determined as the conversion of L-[^{14}C]arginine monohydrochloride to L-[^{14}C]citrulline based on the method described previously (Salter et al., 1991), with minor modifications aiming to detect the activity of cNOS (Weiner et al, 1994; Garvey et al., 1997). We sacrificed the animals by decapitation, and immediately after autopsy prepared fresh tissues for NOS measurements. Aortic tissues were pooled from the abdominal aorta of two rats. Tissues were homogenized (15 s, Ultra-Turrax homogenizer, 5 mm blade) in buffer (250 mg ml^{-1}, 4°C, 10 mM HEPES, 32 mM sucrose, 1 mM dithiothreitol, 0.1 mM EDTA, 10 μg ml^{-1} soybean trypsin inhibitor, 10 μg ml^{-1} leupeptin, 2 μg ml^{-1} aprotonin, pH 7.4). This was followed by centrifugation (20 min, 10,000 × *g*, 4°C). Samples were mixed with Dowex (AG 50W-8; 200–400, 8% cross-linked, Na^+ form) resin. After further centrifugation (10 min, 10,000 × *g*, 4°C), the supernatant (40 μl) was incubated for 10 min at 37°C in reaction buffer comprising (final concentrations): 50 mM KH_2PO_4, 10 μg ml^{-1} calmodulin, 2.5 mM $CaCI_2$, 50 mM valine, 1 mM dithiothreitol, 15.5 nM L-arginine, 1 mM L-citrulline, 0.3 mM NADPH, 3 μM flavin adenine dinucleotide (FAD), 3 μM flavin mononucleotide (FMN), 3 μM tetrahydrobiopterin and 0.17 μM of [^{14}C]L-arginine. The reaction was terminated by the addition (0.5 ml) of a 1:1 v/v suspension of Dowex:water. After addition of 0.85 ml distilled water and settling for 30 min, the supernatant was removed for scintillation counting. Protein content was estimated by spectrophotometric assay (Bio-Rad Protein Assay), and NOS activity expressed as pmol min^{-1} mg^{-1} protein, with constant linear rates for different amounts of extracts.

Total NOS activity was defined as citrulline formation abolished by incubation in vitro with N^G-nitro-L-arginine (L-NNA, 1 mM). Basal L-NNA-sensitive activity that was abolished by EGTA was taken as Ca^{2+}-dependent cNOS activity. In addition, Ca^{2+}-independent NOS activity (iNOS) was also determined as the difference between samples containing 1 mM EGTA and samples containing 1 mM L-NNA.

2.3. Blood pressure response to vasopressin

Animals were anaesthetised with urethane (1.25 g kg^{-1}, i.p.), and then pre-treated with phentolamine (10 mg kg^{-1}, i.p.). A single bolus injection of arginine–vasopressin (0.06–0.18 μg kg^{-1}, i.v.) was administered into the tail vein following the stabilisation of blood pressure. The procedure in details has been described previously (László et al., 1991). Briefly, the elevation of blood pressure (expressed as a % maximal increase compared to the basal value) was measured in the right carotid artery through a blood pressure transducer connected to the HAEMOSYS computerised complex haemodynamic analysis system (Experimetria UK, London). The core temperature of rats was maintained at 37°C with a homeothermic control unit (Harvard Instrument, UK).

2.4. Chemicals

L-[^{14}C]arginine monohydrochloride, raloxifene, arginine–vasopressin and phentolamine were purchased from Amersham International (UK), Eli Lilly and Company (USA), Organon OSS (The Netherlands) and Ciba-Geigy (Switzerland), respectively. All non-specified agents were from Sigma.

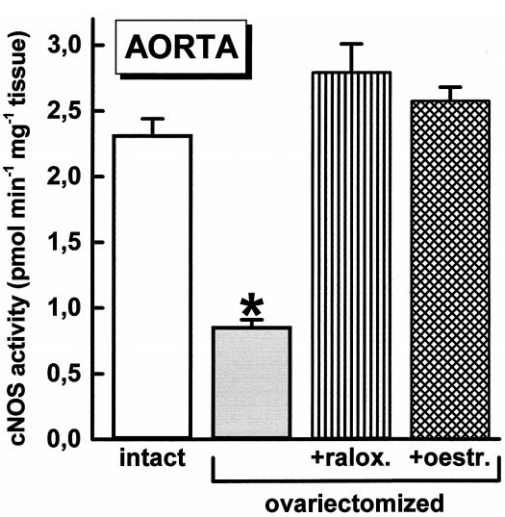

Fig. 1. Ovariectomy-provoked decrease in constitutive aortic nitric oxide synthase (cNOS) enzyme activity (expressed as pmol min^{-1} mg^{-1} tissue). Actions of raloxifene (ralox.; 1 mg kg^{-1}) or 17β-oestradiol (oestr.; 0.3 mg kg^{-1}) supplementation (1 month, p.o.) on cNOS enzyme activity in the aorta of ovariectomized rats. Data are expressed as mean ± S.E.M. of five measurements in each group; $^*P < 0.001$ means significant difference between the intact female and the ovariectomized group.

2.5. Statistics

The data are expressed as the mean ± S.E.M. of (n) rats per experimental group. Data were analyzed by the Tukey–Kramer Multiple Comparisons test, with $P < 0.05$ taken as significant.

3. Results

3.1. Ca^{2+}-dependent aortic nitric oxide synthase enzyme activity

As shown in Fig. 1, ovariectomy significantly decreases aortic cNOS activity, i.e. it falls to 37 ± 2% of the intact rat ($n = 5$; $P < 0.001$). In the aorta of the ovariectomized rat, 17β-oestradiol (0.3 mg kg^{-1}) or raloxifene (1 mg kg^{-1}) supplementation (1 month, p.o.) completely restored cNOS enzyme activity to levels seen in intact females.

In female rats, iNOS activity in the aorta was 0.08 ± 0.05 pmol min^{-1} mg^{-1} protein ($n = 5$). Neither ovariectomy nor administration of 17β-oestradiol or raloxifene changed aortic iNOS activities compared to the intact basal values ($n = 5$, data not shown).

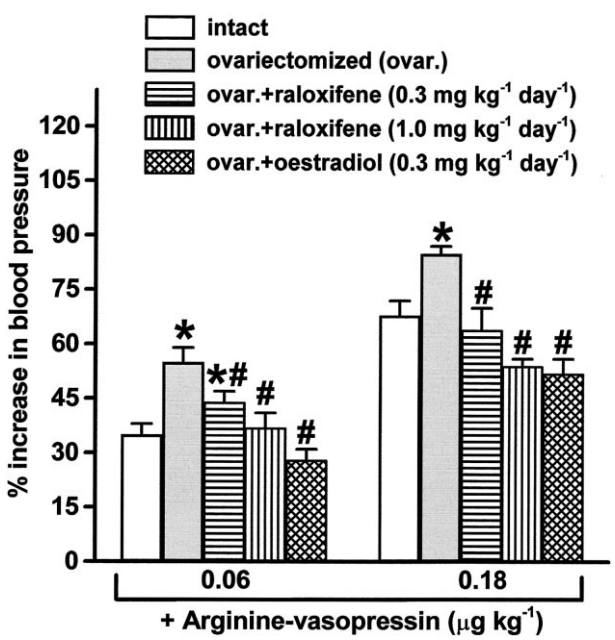

Fig. 2. Increase of arterial blood pressure (expressed as % change, measured in the right carotid artery) by intravenous administration of arginine–vasopressin (0.06–0.18 μg kg^{-1}) in the catecholamine-depleted (phentolamine, 10 mg kg^{-1}, i.p.) sham-operated and ovariectomized (1 month) female rat. Effect of supplementation (1 month, p.o.) with 17β-oestradiol (0.3 mg kg^{-1} day^{-1}) or the selective oestrogen–receptor modulator, raloxifene (0.3–1 mg kg^{-1} day^{-1}) on vasopressin-induced blood pressure elevation in the ovariectomized rat. Data are expressed as mean ± S.E.M., where $n = 8$–12 rats in a group. $^{*}P < 0.05$ means significant blood pressure increase compared to the vasopressin-treated intact female group; $^{\#}P < 0.05$ means significant blood pressure decrease compared to the vasopressin-treated ovariectomized female group.

3.2. Blood pressure response to vasopressin

As shown in Fig. 2, administration of vasopressin caused a dose-dependent increase of arterial blood pressure both in the ovary-intact and ovariectomized female rat. However, in the ovariectomized animals, vasopressin induced a significantly higher elevation of blood pressure than in ovary-intact females. Oestrogen replacement abolished the increased blood pressure response observed, and raloxifene supplementation caused a dose-dependent decrease in blood pressure enhancement provoked by vasopressin in ovariectomized rats.

4. Discussion

The present findings are in agreement with recent observations that vasoconstrictor tone is increased in various vascular beds following ovariectomy, an effect that can be mitigated by oestrogen or raloxifene therapy (He et al., 1999; Zoma et al., 2000). This impaired vasodilatation most likely originates from the down-regulation of cNOS in the oestrogen-deficient state.

Under in vitro circumstances, a reduction of the basal release of NO, as detected by augmented catecholamine-induced contractile response in the presence of an NO synthase inhibitor, was found in aortic rings from ovariectomized animals, an effect blocked by raloxifene or oestradiol treatment (Rahimian et al., 1997). Under in vivo conditions, reduced cNOS activity has been demonstrated in the aortic tissue of ovariectomized rats compared with normal females, and administration of 17β-oestradiol dose-dependently increased aortic cNOS activity in ovariectomized rats to normal female levels (Morschl et al., 2000). Our present findings give further support to the proposition that vascular cNOS activity is regulated by estrogen, and that under in vivo circumstances raloxifene has oestrogen agonist properties with respect to vascular cNOS activity.

Finally, there are pathological circumstances in which release of NO by cNOS from an intact endothelium protects the vascular tissue against the potentially injurious effects of endogenous vasoconstrictors such as vasopressin. For example, in the early compensated phase of endotoxaemia, severe vascular dysfunction can occur following endotoxin challenge when cNOS is inhibited, whereas neither endotoxin nor cNOS inhibitor alone cause dysfunction, and this injury is reversed by a vasopressin antagonist (László and Whittle, 1994). Moreover, in the operating theatre, cNOS has been demonstrated to maintain vascular integrity (László and Whittle, 1999): it effectively counteracts the increase in vascular permeability provoked by vasopressin which is known to be released during major surgical operations (Melville et al., 1985; Pávó et al., 2000). Thus, in the ovariectomized state, impaired expression or/and activity of cNOS may explain

the increased blood pressure response to vasopressin in the absence of oestrogen.

In conclusion, our results suggest that in oestrogen-deficiency aortic cNOS is down-regulated, which strongly suggests its involvement in the increased sensitivity of the vasculature to the vasoconstrictor effect of vasopressin. Both actions can be reversed by the therapy with the natural oestrogen 17β-oestradiol, or the selective oestrogen–receptor modulator raloxifene. Thus, raloxifene behaves as an oestrogen receptor agonist with regards to both regulation of vascular cNOS and vasopressin-provoked increases in blood pressure in vivo.

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