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Hormonal influence on the release of endothelial nitric oxide: gender-related dimorphic sensitivity of rat aorta for noradrenaline

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Abstract

Male gender shows a higher incidence of vascular disorders and this phenomenon could be explained by sexual dimorphic behaviour of vessels. Both gonadal hormones and endothelial nitric oxide (NO) are involved in the regulation of the vascular reactivity. This study aimed to evaluate a possible sexual dimorphic sensitivity of rat aorta for the catecholamine noradrenaline. To understand the role played by physiological concentrations of sex hormones, the experimental procedures were performed on isolated preparations from intact (sham-operated) and gonadectomized rats of both sexes. In parallel sets of experiments, the biosynthesis of NO was inhibited by N^{ω}-nitro-L-arginine methyl ester (L-NAME) to reveal any potential involvement of the endothelial modulator and its possible link with the endocrinous factor. In aortae from intact male and female rats, noradrenaline induced contractile effects with different potencies (mean±s.d. EC50 values 12.15±5.25 nM and 84.10±18.68 nM, respectively). Gonadectomy resulted in an increased sensitivity for noradrenaline in female vessels and a decreased sensitivity for the agonist in male vessels (EC50 values 25.64±5.04 nM and 21.70±11.13 nM, respectively). In aortae from intact male rats, the inhibition of NO biosynthesis resulted in a weak increase in sensitivity for noradrenaline (EC50 value 6.08±4.53 nM), whereas the increase was higher in vessels from intact female rats (EC50 value 10.38±8.40 nM). After treatment with L-NAME, aortae from gonadectomized male and female rats presented almost equivalent increases in sensitivity for the adrenergic agonist (EC50 values 6.02±3.63 nM and 9.10±9.63 nM, respectively), and no significant difference in sensitivity could be recorded between intact and orchidectomized male rats, or between intact and ovariectomized female rats. It was concluded that rat aorta showed a sexual dimorphic sensitivity for noradrenaline and the female sex was more protected against the adrenergic contractile stimulus because of a higher release of endothelial NO. The gender-related difference in NO release was influenced by gonadal hormones, with the female hormones inducing an increase and the male hormones causing a reduction.

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Introduction

Epidemiological and clinical studies indicate clear differences between the two genders in the development of several cardiovascular disorders in humans. The vessels and heart of pre-menopausal women were more protected against many environmental challenges (Petrofski et al 1975; Frankenhaeuser 1982), and men showed a significant prevalence of coronary diseases (Kannel et al 1976). Sexual

hormones were considered the most important factors promoting gender-related cardiovascular differences. The relationships between sexual hormones and cardiovascular function have been widely investigated. Numerous studies were conducted to understand the effects of acute or long-term estrogen treatments on the vascular function (Vargas et al 1989; Miller & Vanhoutte 1990; Jiang et al 1991; Paredes-Carbajal et al 1995). In several animal species, the protective role of estrogen hormones has been well established (Kushwaha & Hazzard 1981; Adams et al 1990; Holm et al 1995; Sulistiyani et al 1995), and this role has been explained not only by the decrease in the plasma concentrations of cholesterol (Walsh et al 1991), but also by a direct action on the vascular wall (Holm et al 1997). On the contrary, male gender is generally viewed as a deleterious risk factor for the development of different cardiovascular disorders and hypertension. Recent experimental evidence showed that testosterone inhibited the vasorelaxing effects of adenosine in rats (Ceballos et al 1999), and exacerbated hypertension in spontaneously hypertensive rats through a decrease of natriuresis (Reckelhoff et al 1998).

The discovery of the modulatory role of the endothelial layer on the vessel smooth muscle activity could offer a further possible explanation for vascular sexual dimorphic behaviour. Indeed, it was well established that the endothelium-derived relaxing factor, demonstrated to be nitric oxide (NO) by several studies (Ignarro et al 1987; Palmer et al 1987, 1988; Feelisch et al 1994), mediated the relaxing responses to several vasodilators, such as acetylcholine, bradykinin and the calcium ionophore A-23187 (Furchgott and Zawadzki 1980; Furchgott 1984; Gryglewski et al 1986) and prevented the adhesion of monocytes and platelets to the endothelial cells (Radomski et al 1987; Bath et al 1991). Furthermore, NO was found to be responsible for gender-related differences of contractile responses of rat aorta to prostaglandins (Maddox et al 1987) and to vasopressin (Stallone 1993).

A sexual dimorphism in the sensitivity of rat mesenteric arteries for the catecholamines adrenaline and noradrenaline was first demonstrated by Altura (1972), who observed an increased affinity in vessels from female animals. The present study aimed to evaluate a possible sexual dimorphism in the sensitivity of rat aorta for the catecholamine noradrenaline. Because possible differences in muscular tissue, rather than the sensitivity for the contracturant agent, could quantitatively influence a dimorphic development of contractile force, it was decided to evaluate only the potency of noradrenaline. To understand the potential role played by physiological levels of sex hormones in the dimorphic behaviour, the adrenergic agonist noradrenaline was tested on isolated preparations from intact (sham-operated) and gonadectomized rats of both sexes. In parallel sets of experiments, the biosynthesis of NO was inhibited by the arginine analogoue N^{ω}-nitro-L-arginine methyl ester (L-NAME) (Rees et al 1990), to reveal any involvement of the endothelial modulator and its possible link with the endocrinous sexual factor. A preliminary report on the results of this study was presented at the XXVII Congress of the Italian Pharmacological Society (Calderone et al 1994).

Materials and Methods

All the procedures were performed in accordance with the European Community legislation (Directive 86/609) concerning the use of laboratory animals. Adult male and female Wistar rats (200–250 g) underwent bilateral gonadectomy; parallel groups of rats of both sexes were sham-operated. The surgical procedure was performed under ethyl ether anaesthesia and was followed by antibiotic administration. After the surgery, the rats were individually housed with free access to food and water. After two months they were euthanized by cervical dislocation under light ethyl ether anaesthesia and bled. The experimental protocol performed on vessels from intact female rats was carried out without regard for the phase of oestrus cycle.

Drugs

Noradrenaline hydrochloride (Sigma), acetylcholine chloride (Sigma) and L-NAME (Sigma) were dissolved in bi-distilled water. The solutions were freshly prepared immediately before the experiments.

Experimental protocol

The thoracic aorta was immediately isolated, freed from extraneous tissues and cut into multiple-ring preparations as previously described (Calderone et al 1996). The preparations were placed under a resting tension of 2 g in 10-mL organ baths at 37°C and continuously bubbled with a mixture of O_2 (95%) and CO_2 (5%). The bathing fluid was Tyrode saline solution (composition in mM: 136.8 NaCl, 2.95 KCl, 1.80 $CaCl_2$, 1.05 $MgSO_4$, 0.41 NaH_2PO_4, 11.90 $NaHCO_3$ and 5.50 glucose). Wash-out was performed at intervals of approximately 15 min.

The changes of vascular smooth muscle tension were recorded by an isotonic transducer (Basile model 7006), connected to a microdynamometer (Basile model 7050). After 45 min of equilibration, the presence of functional endothelium was confirmed by the ability of an ultramaximal concentration of acetylcholine (10 μM) to relax the aortic preparations, which were pre-contracted by noradrenaline (1 μM). Aortae showing a relaxation $<$ 70% of the contractile responses were discarded. The vessels allowed to equilibrate for 45 min, followed by the cumulative administration of 3-fold increasing concentrations of noradrenaline (1 nM–10 μM). When inhibition of NO biosynthesis was required, a concentration of L-NAME (50 μM), able to ensure an effective inhibition of NO synthase, was added to the organ bath 20 min before noradrenaline administration (Rees et al 1990).

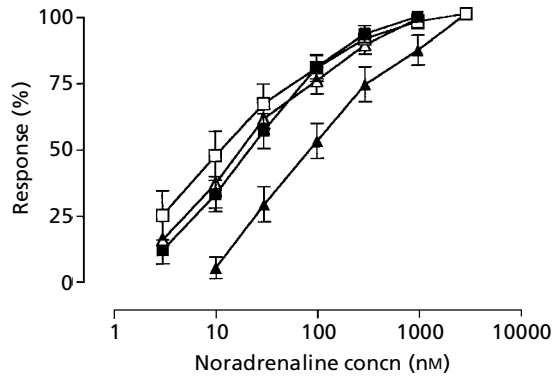

Figure 1 Concentration–response curves for noradrenaline (nM) in aortae from intact male (■) and female (▲) rats and in L-NAME-treated aortae from intact male (□) and female (△) rats. Standard deviation values are shown by vertical bars.

Data analysis

The parameter of sensitivity for noradrenaline was expressed as EC50, representing the agonist concentration evoking a half-maximal effect (Ariëns and Van Rossum 1957). EC50 values were calculated from the concentration–response curves using the GraphPad Prism computer software. Values of EC50 were expressed as mean$\pm$s.d. Each group was represented by 10 experiments. Only one aortic preparation was obtained from each animal. Data were compared by analysis of variance, followed by the Bonferroni post-test. A value of $P < 0.05$ was considered as statistically significant.

Table 1 EC50 values for noradrenaline (nM) in isolated aortae from intact and gonadectomized rats of both sexes, in the presence or absence of L-NAME.

	EC50 nM	
	Male	Female
Intact	12.15$\pm$5.25	84.10$\pm$18.68
Intact+L-NAME	6.08$\pm$4.53	10.38$\pm$8.40
Gonadectomized	21.70$\pm$11.13	25.64$\pm$15.12
Gonadectomized+L-NAME	6.02$\pm$3.63	9.10$\pm$9.63

Values are expressed as mean$\pm$s.d., for 10 experiments per group.

Results

Intact animals

Noradrenaline induced concentration-dependent contractile effects in aortic preparations from male and female rats. However, the concentration–response curves for noradrenaline were located in different positions: on the left for male rats and on the right for female rats. Therefore, the potency of noradrenaline on aortae from male and female rats was different, with a high degree of statistical significance. EC50 values of noradrenaline in aortae from male and female rats were 12.15$\pm$5.25 nM and 84.10$\pm$18.68 nM, respectively (Figure 1; Table 1).

L-NAME resulted in a left-ward shift of the concentration–response curves for noradrenaline in aortae from both sexes; however the shift was more evident in the vessels from female rats than in those from male rats. Thus, L-NAME induced a weak, but not significant, increase in sensitivity for noradrenaline in aortae from male rats (EC50 6.08$\pm$4.53 nM), and a high and significant increase in sensitivity for noradrenaline in aortae from female rats (EC50 10.38$\pm$8.40 nM), leading to an almost total abolition of the sexual dimorphism (Figure 1; Table 1). The two above values were not statistically different.

Gonadectomized animals

Noradrenaline induced concentration-dependent contractile effects in aortic preparations from gonadectomized male and female rats. Aortae from orchidectomized rats showed a lower, but not signifi-

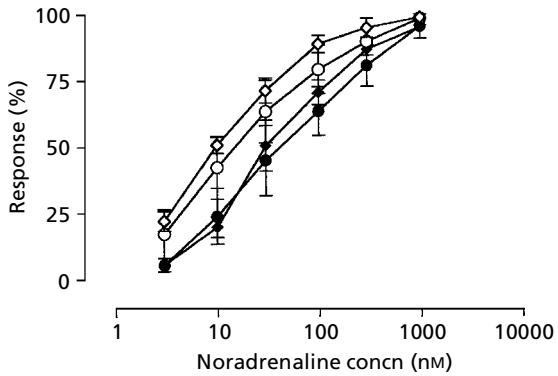

Figure 2 Concentration–response curves for noradrenaline (nM) in aortae from gonadectomized male (◆) and female (●) rats and in L-NAME-treated aortae from gonadectomized male (◇) and female (○) rats. Standard deviation values are shown by vertical bars.

cant, sensitivity for noradrenaline than those from intact male rats. Sensitivity for noradrenaline in vessels from ovariectomized rats was significantly higher than that observed in aortae from intact female rats. No significant difference in the sensitivity for noradrenaline between aortae from gonadectomized male and female rats was observed (EC50 21.70±11.13 nM and 25.64±15.12 nM, respectively) (Figure 2; Table 1).

L-NAME resulted in a left-ward shift of the concentration–response curves and a significant increase in sensitivity for noradrenaline in aortae from gonadectomized male and female rats (EC50 6.02±3.63 nM and 9.10±9.63 nM, respectively) (Figure 2; Table 1). In both sexes, the increases were approximately equivalent in amplitude. The two above values were not statistically different. Moreover, L-NAME-treated aortae from intact and gonadectomized male rats, as well as from intact and gonadectomized female rats, showed equivalent levels of sensitivity for noradrenaline.

Discussion

Gender-related dimorphic behaviours of the cardiovascular system are widely described in literature. Male Dahl salt-sensitive rats and male spontaneously hypertensive rats, as well as male desoxycorticosterone-NaCl hypertensive rats, developed a more rapid and severe hypertension than female rats (Dahl et al 1975; Iam & Wexler 1977, 1979; Cambotti et al 1984; Ouchi et al 1988; Ganten et al 1989). In the female rat, vasopressin played a major role in the blood pressure compensation after haemorrhage (Crofton & Share 1990). Endothelium was demonstrated to play an essential function in the relaxing responses of vessels to several endogenous and exogenous vasodilators, but also in the modulation of the responses to the contractile agonists (Martin et al 1986). Endothelium removal increased the sensitivity of vessels for the catecholamines noradrenaline and adrenaline (Ruffolo et al 1982), and the contractile responses evoked by the α_1-adrenergic agonist, methoxamine, were enhanced by treatment with detergent, determining an endothelial ablation (Randall & Hiley 1988). Furthermore, endothelium played a crucial role for the development of sexual dimorphic profiles in the prostaglandin receptors (Karanian et al 1980) and in the responses of rat aorta to vasopressin (Stallone 1993) and prostaglandin $F_{2\alpha}$ (Maddox et al 1987). In the present study, a significant sexual dimorphism was demonstrated in rat aorta, with a lower sensitivity for noradrenaline exhibited by female vessels and a higher sensitivity shown by male vascular preparations. The contrary pattern observed in rat mesenteric arteries (Altura 1972), showing a higher sensitivity for the catecholamines in vessels from female animals, could be reasonably explained by vascular district differences. Treatment with L-NAME abolished the gender dimorphism, resulting in a strong increase in the sensitivity for the adrenergic agonist in female aortae and a weak or no increase in male vessels, clearly indicating that differential NO release (higher in female and lower in male animals) was the cause of such a gender-related dimorphism. A similar observation was also proposed in rabbits (Hayashi et al 1992).

Gonadectomy also abolished the sexual dimorphism, leading to almost equivalent values of sensitivity for noradrenaline in both sexes, with an increase of the original value in ovariectomized rats and a decrease in orchidectomized rats, with respect to the dimorphic values recorded in intact animals.

A direct effect of estrogens on the density and/or affinity for catecholamines of α-adrenergic receptors has been proposed (Collucci et al 1982; Larson et al 1984; Bento & De Moraes 1992). However, a recent study demonstrated a reduction of the vasocontractile effects of noradrenaline, serotonin, calcium and potassium in aortic rings from ovariectomized rats previously treated with a long-term administration of exogenous 17-β-estradiol, suggesting the involvement of an estrogen-induced increase of NO release (Andersen et al 1999). The above hypothesis was confirmed by other experimental observations, demonstrating an estrogen-induced up-regulation of the NO synthase, in human endothelial cells (Hishikawa et al 1995) and in rat arterioles (Huang et al 1997). As regards human

male gender, a comparative study between control adult men and patients that had undergone bilateral orchidectomy (as a surgical treatment of neoplastic diseases), showed that the withdrawal of gonadal male hormones could be associated with an increase of the endothelium-mediated vasorelaxing responses of brachial artery (Herman et al 1997).

In the present study, in female rats, the strong NO-mediated vasoprotection against noradrenaline was reduced by the abolition of the physiological concentrations of female hormones (ovariectomy). A different phenomenon was observed in male rats, because orchidectomy resulted in an increase of the protective release of NO, which was very weak in intact animals. Therefore, a relation between sexual hormones and NO function could be hypothesized. The changes in sensitivity for noradrenaline could be explained by different amounts of released endothelial NO. Furthermore, the presence or absence of physiological amounts of sexual hormones could not cause differential effects when the biosynthesis of NO was abolished, because in L-NAME-treated vessels, no difference in sensitivity for noradrenaline was recorded between intact and gonadectomized female rats, or between intact and orchidectomized male animals. This evidence probably excluded a parallel and independent action of both sexual hormones and NO, suggesting a possible sequential mechanism linking sexual hormones and endothelial NO, which could be viewed as the final target in the hormonal influence on vascular function.

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