Vasoconstrictor sensitivity to noradrenaline and NG\(^6\)-monomethyl-L-arginine in men and women

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(Received 15 May/28 July 1997; accepted 6 August 1997)

1. Nitric oxide has potential anti-atherogenic actions as well as regulating vascular tone. Animal studies suggest that there are sex differences in basal nitric oxide biosynthesis, but it is not known whether such differences exist between men and women.

2. We have investigated this question by measuring forearm blood flow responses, using venous occlusion plethysmography, to brachial artery infusion of NG\(^6\)-monomethyl-L-arginine (an inhibitor of NO biosynthesis) and noradrenaline in 40 healthy subjects (20 men and 20 premenopausal women). Mean arterial blood pressure was 89 ± 10 mmHg (mean ± SD) in men and 87 ± 9 mmHg in women, and mean total cholesterol was 4.25 ± 0.99 mmol/l (mean ± SD) and 4.26 ± 0.80 mmol/l respectively.

3. In men, vasoconstrictor responses to NG\(^6\)-monomethyl-L-arginine, 1-4 pmol/min (15-28% mean reduction in blood flow), were consistently less than responses to noradrenaline, 60-240 pmol/min (26-37%), whereas in women, vasoconstrictor responses to NG\(^6\)-monomethyl-L-arginine (19-30%) were consistently greater than those to noradrenaline (11-17%). The sex difference in relative sensitivity to vasoconstrictors was significant ($P < 0.001$).

4. Our findings are consistent with either greater sensitivity to noradrenaline in men compared with premenopausal women, or a greater basal nitric oxide biosynthesis in premenopausal women compared with men.

INTRODUCTION

Nitric oxide (NO) is synthesized from L-arginine in vascular endothelial cells by a constitutive isoform of NO synthase (ecNOS). Basal release of NO plays a role in the control of resistance vessel tone and arterial blood pressure [1]. NO also has potential anti-atherogenic effects: inhibition of platelet aggregation [2] and adhesion [3], monocyte adhesion [4], and smooth muscle proliferation [5]. Chronic inhibition of NO synthesis in hypercholesterolaemic rabbits promotes atherosclerosis [6]. Release of NO may therefore suppress atherogenesis as well as regulate vascular tone. Reduced basal NO synthesis has been described in forearm vasculature of patients with essential hypertension [7] and vasoconstrictor responses to acetylcholine, mediated in part by stimulation of endothelium-derived NO production, are impaired in hypercholesterolaemia [8-10] which is a risk factor for atherosclerosis.

The prevalence of atherosclerosis is less in premenopausal women than in men [11], and an influence of gender on endothelial NO biosynthesis could contribute to this difference. Gender differences in basal and stimulated NO release have been observed in animal studies [12, 13]. In humans, however, it is not known whether basal NO production in resistance vasculature differs between the sexes. Blood flow responses to intra-arterial infusion of acetylcholine, often used to study the integrity of the L-arginine/NO pathway, are greater in women than in men but this can be explained by the influence of forearm length on responses to this extremely unstable agonist [14].

To investigate the effect of gender on basal NO release in human resistance vessels we measured blood flow responses to brachial artery infusion of the NO synthase inhibitor NG\(^6\)-monomethyl-L-arginine (L-NMMA, 1-4 μmol/min) and noradrenaline in healthy men and premenopausal women. L-NMMA competes with L-arginine, inhibits ecNOS and constricts forearm vasculature [15]. Several studies have shown that L-NMMA (1-4 μmol/min) inhibits vasodilator responses to human forearm vasculature to endothelium-dependent agonists including acetylcholine, bradykinin and substance P [16-19]. Noradrenaline acts at $\alpha$-adrenergic receptors. Comparison of responsiveness to L-NMMA
relative to noradrenaline in the same individual has been used to control for variation between subjects in vascular reactivity unrelated to the L-arginine/NO pathway [7, 20].

METHODS

Subjects

Healthy volunteers were recruited by local advertisement in Southeast London. All were screened with history, physical examination, creatinine, electrolytes and liver function tests, which revealed no abnormalities. Serum total cholesterol and triacylglycerols were also measured. Blood pressure and heart rate were measured after 10 min supine in a quiet room using an appropriate sized cuff and a Dinamap (Critikon, Ascot, Berks., U.K.) automatic recorder, and the mean of three consecutive readings was recorded. All subjects were white, non-smoking and not taking any medication (in particular, any contraceptive preparation). Other characteristics are summarized in Table 1.

Protocol

The protocol was approved by the St Thomas’ Hospital Research Ethics Committee and all subjects gave written informed consent. Studies were performed in a quiet temperature-controlled laboratory (24–26°C). Women were studied after menstruation within the first 14 days of their menstrual cycle. A 27-gauge unmounted steel needle (Cooper’s Needle Works, Birmingham, U.K.) sealed with dental wax to an epidural cannula was inserted into the left brachial artery using less than 1 ml of 1% lignocaine hydrochloride to provide local anaesthesia. Drugs were dissolved in 0.9% sodium chloride, and saline or drug solution infused at 1 ml/min using a constant infusion pump. Forearm blood flow was measured in both arms simultaneously by venous occlusion plethysmography [21] with electrically calibrated temperature-compensated strain gauges [22]. During measurements, the hands were excluded from the circulation by inflation of wrist cuffs to 180 mmHg. Upper arm collecting cuffs were inflated to 40 mmHg.

Basal blood flow was measured after saline infusion for at least 12 min. This was followed by infusion of noradrenaline (Levophed; Sanofi Winthrop Ltd, Guildford, U.K.) at doses of 60, 120 and 240 pmol/min, each dose for 6 min. Saline was then infused for 18 min and a second series of baseline measurements recorded before infusion of L-NMMA (Clinalfa, Laufelfingen, Switzerland) at doses of 1, 2 and 4 μmol/min, each dose for 6 min. Forearm blood flow was measured for the final 3 min of each infusion period. Flow was recorded for approximately 10 s in every 15 s and the mean of the final five measurements of each recording period used for analysis. Blood flow was expressed as ml of blood per minute per 100 ml of forearm volume.

Statistical analysis

Unless otherwise stated values are expressed as means ± SEM. The ratio of blood flow in the infused forearm to that in the non-infused forearm was calculated for each measurement period. Vasconstrictor responses were expressed as percentage reduction in forearm blood flow (infused: control arm) relative to the immediately preceding baseline [23]. This controls for effects of external influences such as state of arousal (which affects both arms similarly). Repeated measures analysis of variance was used to seek an interaction between drug (L-NMMA and noradrenaline) and gender in determining vascular responses. Effects of potentially confounding factors (age, arterial blood pressure and total cholesterol) were examined by incorporating these as covariates.

The area under the dose–response curve was calculated for each drug [24] as a summary measure of drug response to allow quantitative comparison between men and women. Summary measures and subject characteristics were compared using Student’s t-test. All P values are 2-sided; a P value of less than 0.05 was considered to indicate statistical significance.

RESULTS

In both men and women, forearm blood flow in the non-infused arm did not change significantly during the infusions of noradrenaline or L-NMMA, confirming that these had no systemic effect at the doses used.

In the infused arm L-NMMA and noradrenaline produced a dose-dependent reduction in blood flow in both men and women (Fig. 1). The absolute values of forearm blood flow in the infused arm are shown in Table 2. In women, responses to L-NMMA, 1–4 μmol/min, were greater than responses to noradrenaline, 60–240 pmol/min, whereas in men, responses to L-NMMA were less than to noradrena-
Vasoconstrictor responses in men and women

Fig. 1. Effect on forearm blood flow of intra-arterial infusion of norepinephrine (squares) and L-NMMA (circles) in men (solid symbols, n = 20) and women (open symbols, n = 20). The effect of gender on relative sensitivity to the two agonists was significant (P < 0.001, men compared with women).

The gender difference in the sensitivity to norepinephrine was significant (Fig. 2: P < 0.001 men compared with women) whereas sensitivity to L-NMMA was not significantly different in men and women (Fig. 2: P = 0.40). Results of the statistical analysis were not influenced by the inclusion of blood pressure, age, serum total cholesterol or triacylglycerols as covariates.

DISCUSSION

Abnormal basal NO biosynthesis may be more important in the pathogenesis of arterial disease than agonist-stimulated NO production, and we therefore sought to investigate the influence of gender on basal NO biosynthesis. Evidence for the production of NO under basal conditions in humans comes from experiments in which L-NMMA was used to inhibit the L-arginine/NO pathway and was shown to constrict forearm vasculature [15] and to increase total peripheral vascular resistance [25]. We measured vasoconstrictor responses to L-NMMA to compare the fraction of basal vasodilator tone attributable to the L-arginine/NO pathway in forearm resistance vessels between the sexes. Using a comparative vasoconstrictor as an internal control allows responsiveness to L-NMMA to be assessed in a manner in which the forearm blood flow technique is at its most powerful, controlling for inter-individual differences in baseline blood flow and in resistance vessel structure [7, 20, 26, 27]. In the present study we used norepinephrine, reasoning that α1-mediated vasoconstriction would be unlikely to differ between men and women.

Unexpectedly, we found responses to norepinephrine to be strikingly different between the sexes with men being more sensitive to norepinephrine than women (P < 0.001). This observation has important implications for forearm studies in which norepinephrine is used as a comparative vasoconstrictor, and emphasizes the need for balanced sex ratios in control and experimental groups in such studies. Responses to L-NMMA are similar in men and women (P = 0.40) although we cannot exclude a small difference. The findings of a greater sensitivity to norepinephrine in men could be due to an effect of gender upon sensitivity to α1-adrenergic receptor stimulation. Such an effect has not been reported in humans while the evidence from animal studies is inconclusive: in contrast to the present findings, α1-adrenergic receptor affinity and vascular catecholamine sensitivity are increased in segments of small mesenteric arteries of female and oestrogen-treated male rats compared with male rats [28], whereas sensitivity to norepinephrine and phenylephrine in rat tail artery segments shows no gender difference [29].
Noradrenaline acts on \( \alpha_2 \) as well as \( \alpha_1 \) adrenergic receptors. Some post-junctional \( \alpha_2 \) adrenergic vascular actions lead to vasoconstriction, but in some species and vascular beds endothelial \( \alpha_2 \) receptors initiate endothelium-dependent relaxation [30, 31]. Endothelium-dependent \( \alpha_2 \)-adrenergic receptor-mediated relaxation of pig epicardial and coronary resistance vessels is completely inhibited by \( l \)-NMMA [32, 33], and pig aortic endothelial cells express \( \alpha_2 \mathrm{C} \) subtype receptors that initiate endothelium-dependent relaxation [34]. In rat mesenteric artery, endothelial \( \alpha_2 \mathrm{D} \) receptors initiate NO-mediated relaxation [35]. It is not known whether endothelial cells in human forearm vasculature express \( \alpha_2 \) adrenergic receptors, and if so, what their subtype is and whether they initiate NO release. If this was the case, a gender difference in \( \alpha_2 \) adrenergic receptor densities could account for the different sensitivity to noradrenaline in men and women observed in the present study. 17\( \beta \)-Oestradiol depresses the responsiveness of isolated blood vessels from ovariectomized rabbits to \( \alpha \)-adrenergic agonists [36]. This effect of oestrogen can also be demonstrated in pregnancy and is inhibited by \( l \)-NMMA [37], suggesting that increased NO availability is the mechanism of reduced vasoconstriction in this setting. Similarly, gender related differences in the response of rat aorta to the vasoconstrictor prostaglandin \( F_{2\alpha} \) are endothelium-dependent [38]. Oestrogen replacement therapy reduces vasoconstrictor responses to noradrenaline in perimenopausal women [39], but this may be due to increased NO synthesis [40].

An alternative explanation of the findings is that differences in observed noradrenaline sensitivity occur as a result of structural differences in forearm geometry and/or vasculature. Such an influence of resistance vessel structure on vasoconstrictor response to noradrenaline (due to changes in wall thickness/lumen diameter ratio) is observed in animal models of hypertension independent of changes in actual drug sensitivity [41]. If sensitivity to noradrenaline is a true reflection of between-subject differences in such structural characteristics, the relatively greater sensitivity of women to \( l \)-NMMA than to noradrenaline implies increased sensitivity of women to the vasoconstrictor action of \( l \)-NMMA in forearm resistance vasculature. This could be due to greater basal NO biosynthesis in this vascular bed in women than in men (in whom responses to noradrenaline are greater than to \( l \)-NMMA). Increased expression of ecNOS occurs in pregnant guinea pigs and in oestradiol-treated male and non-pregnant female animals [42]. Reduced basal NO release occurs in mice lacking the gene for the nuclear oestrogen receptor, whereas NO-mediated responses are normal in such animals [43]. Oestrogen receptors have been demonstrated in human coronary artery and umbilical vein endothelial cells [44] and 17\( \beta \)-oestradiol increases ecNOS mRNA expression in aortic endothelial cells [45]. The present finding that in premenopausal women responses to \( l \)-NMMA are greater than to noradrenaline, whereas in men responses to \( l \)-NMMA are less than to noradrenaline, accords with these animal studies.

Functional gender differences in vascular responsiveness could occur through multiple mechanisms. Differences in circulating oestrogen between men and premenopausal women are a strong candidate to explain differences in basal NO biosynthesis. Oestrogen replacement therapy in postmenopausal women increases circulating nitrite/nitrate concentrations consistent with increased NO biosynthesis [46], supporting the possibility that reduced responses to noradrenaline relative to \( l \)-NMMA in women reflect increased basal NO biosynthesis. An alternative explanation is that oestrogen reduces NO breakdown in resistance vessels, but the observation that sensitivity to the NO donor nitroprusside is similar in men and women argues against this [47]. Oestrogen has antioxidant effects, including inhibition of oxidative modification of low-density lipoproteins [48] which influence the L-arginine/NO pathway [49, 50]. Superoxide anion combines with NO to form peroxynitrite anion and inhibits basal but not acetylcholine-stimulated NO synthesis in rat aorta [51]. Antioxidant effects of oestrogen could reduce superoxide anion availability in premenopausal women thereby potentiating effects of basal NO. It is beyond the scope of the present study to resolve the contributions of these possible mechanisms. However, irrespective of the underlying mechanism, increased basal activity of the L-arginine/NO pathway could have important physiological and pathophysiological consequences for women. Pharmacological inhibition of NO synthase increases blood pressure [25] and mutant mice lacking ecNOS activity are hypertensive compared with wild-type litter mates [52]. It is possible that increased basal NO biosynthesis contributes to the lower blood pressure observed in epidemiological studies in women from 15 to 50 years of age compared with men [53]. Chronic inhibition of NO synthesis accelerates atherogenesis in cholesterol-fed rabbits [6], and the present results suggest that protection from atherosclerosis in premenopausal women could relate to increased basal NO biosynthesis.

In conclusion, we have observed that premenopausal women have reduced vasoconstrictor responses to noradrenaline compared with men, but similar responses to \( l \)-NMMA. This is consistent with a direct effect of gender on \( \alpha \)-adrenergic responsiveness or, if noradrenaline sensitivity is dependent on gender effects on vascular structure, a higher basal NO biosynthesis in the forearm vasculature of healthy premenopausal women compared with men. The latter could be due either to oestrogen-induced increased expression of ecNOS in women or to reduced NO inactivation by reactive species as a result of the antioxidant effect of oestrogen. Decreased vasoconstrictor responses to noradrenaline or increased basal NO biosynthesis/action could have important consequences in pro-
tecting premenopausal women from cardiovascular disease.

ACKNOWLEDGMENT
This project is supported by the British Heart Foundation. B.K.J. is a British Heart Foundation Junior Research Fellow.

REFERENCES