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Gender Differences in Sensitivity to Adrenergic Agonists of Forearm Resistance Vasculature

Barry J. Kneale, BA, MRCP,*† Philip J. Chowienczyk, BSc, FRCP,* Sally E. Brett, BN,*
D. John Coltart, MD, FRCP, FACC,† James M. Ritter, D Phil, FRCP*
London, United Kingdom

OBJECTIVES The goal of this study was to investigate the mechanism of reduced vasoconstrictor sensitivity to norepinephrine in women compared with men.

BACKGROUND β_2-adrenergic agonists such as albuterol dilate forearm resistance vessels, partly by activating the L-arginine/nitric oxide pathway. Norepinephrine (which acts as β- as well as α-adrenergic receptors) causes less forearm vasoconstriction in women than it does in men. This could be explained by a greater sensitivity to β_2-receptor stimulation in women than in men.

METHODS Forearm blood flow was measured by venous occlusion plethysmography in healthy women (days 10 to 14 of the menstrual cycle) and in men. Drugs were administered via the brachial artery in three separate protocols: albuterol ± N^G-monomethyl-L-arginine (an inhibitor of nitric oxide synthase); substance P, nitroprusside and verapamil (control vasodilators); norepinephrine (± propranolol, a β-adrenergic receptor antagonist).

RESULTS Vasodilator responses to albuterol were greater in women than they were in men ($p = 0.02$ by analysis of variance). N^G-monomethyl-L-arginine reduced these similarly in men and women. Responses to control vasodilators were less in women than they were in men (each $p < 0.05$). Norepinephrine caused less vasoconstriction in women than it did in men ($p = 0.02$). Propranolol did not influence basal flow in either gender nor responses of men to norepinephrine but increased vasoconstriction to each dose of norepinephrine in women ($p < 0.0001$ for interaction between gender and propranolol). Responses to norepinephrine coinfused with propranolol were similar in men and women.

CONCLUSIONS Stimulation of β_2-adrenergic receptors causes greater forearm vasodilation in premenopausal women, at midmenstrual cycle, than it does in men. This is sufficient to explain why vasoconstriction to brachial artery norepinephrine is attenuated in such women. (J Am Coll Cardiol 2000;36:1233–8) © 2000 by the American College of Cardiology

Resistance vessel tone is an important determinant of arterial blood pressure and regional blood flow. It is controlled by mediators from adrenergic nerves and vascular endothelium. Norepinephrine is the principal peripheral sympathetic neurotransmitter, and it constricts blood vessels through actions on α-adrenergic receptors. It is also a powerful agonist at β_1-adrenergic receptors but is much less potent at β_2-receptors (1). β_2- (but not β_1) agonists administered into the brachial artery dilate forearm resistance vessels, and this action is mediated, in part, through activation of the L-arginine/nitric oxide (NO) pathway (2). Forearm β-adrenergic receptor-mediated vasodilation is impaired, without alteration of norepinephrine spillover, in borderline hypertension (3), suggesting that altered β_2-adrenergic function may have important pathophysiological consequences. We have observed a marked difference between men and women in sensitivity to the vasoconstrictor action of norepinephrine on forearm resistance vessels (4). Women were studied during the follicular phase of the menstrual cycle when endogenous estrogen concentrations are high and unopposed by progesterone. Such women are relatively insensitive to the vasoconstrictor action of norepinephrine infused into the brachial artery. Reduced sensitivity to the vasoconstrictor action of norepinephrine could contribute to gender-related differences in the incidence and outcome of vascular disease (5–7), which are profound and incompletely explained by associations with known cardiovascular risk factors (8–10).

The mechanism of reduced sensitivity in women to the vasoconstrictor action of norepinephrine in forearm resistance vessels is not known. Evidence from animal studies has been conflicting (11–15). Estrogen replacement therapy reduces vasoconstrictor responses to norepinephrine in perimenopausal women (16), possibly due to increased NO synthesis (17). We hypothesized that reduced sensitivity to the vasoconstrictor action of norepinephrine in women could be a consequence of increased sensitivity to β_2-adrenergic receptor stimulation, offsetting the vasoconstrictor effect of α-adrenergic receptor activation. To test this possibility, we compared the sensitivity of forearm resistance vasculature to the vasodilator action of albuterol (a β_2-adrenergic receptor agonist) in men and women. We also studied substance P, nitroprusside and verapamil, vasodilators that are not β_2-adrenergic agonists and act by diverse mechanisms (18–20) to exclude the possibility that gender-

From the *Department of Clinical Pharmacology, and †Department of Cardiology, Center for Cardiovascular Biology and Medicine, King's College, London, United Kingdom. Supported by the British Heart Foundation.

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Abbreviations and Acronyms
L-NMMA = N^G-monomethyl-L-arginine
NO = nitric oxide

related structural or other differences in this vascular bed could account in a nonspecific way for differences in response to vasodilators. To determine whether β-receptor stimulation was quantitatively sufficient to account for the reduced effect of norepinephrine in women, we studied the effect of coinfusing propranolol (a β-adrenergic receptor antagonist) with norepinephrine.

METHODS

Subjects. Subjects were recruited by advertisement in southeast London. All were white, nonsmoking and receiving no medication (including contraceptive or other hormonal preparations). Other characteristics are summarized in Table 1.

Materials and experimental protocols. Drugs were obtained from: Allen and Hanburys, Uxbridge, United Kingdom: albuterol sulfate; Clinalfa, Laufelfingen, Switzerland: N^G-monomethyl-L-arginine hydrochloride (L-NMMA), substance P; Roche Products, Basel, Switzerland: sodium nitroprusside; Baker Norton, Harlow, United Kingdom: verapamil hydrochloride; Sanofi Winthrop, Guildford, United Kingdom: norepinephrine acid tartrate and Zeneca, Wilmslow, United Kingdom: propranolol hydrochloride.

Protocols were approved by the St. Thomas' Hospital Research Ethics Committee. All subjects provided written informed consent for their participation in the study. All the women had regular menstrual cycles and were studied on day 10 to 14. Studies were performed in the morning after a light breakfast, in a quiet temperature controlled vascular laboratory (24 to 26°C). A 27-gauge unmounted steel needle (Cooper's Needle Works, Birmingham, United Kingdom) sealed with dental wax to an epidural cannula was inserted into the left brachial artery using less than 1 ml of 1% lidocaine hydrochloride to provide local anesthesia. In each protocol subjects rested supine for at least 30 min, and saline (0.9% sodium chloride) was infused by a constant rate pump for at least 12 min before baseline readings were obtained. Throughout each experiment, saline (with or without drug) was infused at a constant rate of 1 ml per minute. Increasing doses of drugs were administered by stepwise increments. Forearm blood flow was measured in both arms using venous occlusion plethysmography (21) with electrically calibrated strain gauges (22). Blood flow to the hands was occluded by wrist cuffs during measurements. The pressure in the upper arm cuffs was 40 mm Hg. Drug effects (at the doses used) reached a plateau within 2 min. Measurements were made from 2 to 4 min. Flows were recorded for approximately 10 s in every 15 s, and the mean of the final five measurements was used for analysis. Three separate protocols were employed:

Sensitivity to albuterol and effect of L-NMMA. Albuterol (0.35, 1, 3.5 and 10 nmol min^{-1}) was infused with a saline vehicle. Saline was then infused alone for 24 min during which blood flow returned to baseline. A second series of baseline measurements was recorded before infusion of L-NMMA (16 μmol min^{-1}). Albuterol (0.35, 1, 3.5 and 10 nmol min^{-1}) was then coinfused with L-NMMA.

Sensitivity to substance P, nitroprusside and verapamil. In separate experiments substance P (0.3, 1, 3 and 10 pmol min^{-1}), nitroprusside (3, 6, 12 and 24 nmol min^{-1}) and verapamil (20, 40, 80 and 160 nmol min^{-1}) were infused sequentially with intervening saline periods (≥24 min) during which blood flow returned to baseline.

Sensitivity to norepinephrine and effect of propranolol. Norepinephrine (60, 120 and 240 pmol min^{-1}) was infused with saline. Saline was then infused alone for at least 18 min during which blood flow returned to baseline. A second series of baseline measurements was recorded before infusion of propranolol (190 nmol min^{-1}). Norepinephrine (60, 120 and 240 pmol min^{-1}) was then coinfused with propranolol (190 nmol min^{-1}).

Statistical analysis. Unless otherwise stated values are expressed as means ± SEM. Vasodilator responses were expressed as increase in blood flow in the infused arm above the immediately preceding baseline period. Vasoconstrictor responses were expressed as percentage reduction in forearm blood flow (infused: control arm) relative to the immediately preceding baseline. Vasodilator and vasoconstrictor responses are most reproducibly expressed in these forms (23). Area under the dose-response curve (expressed in arbitrary units) was used as a summary measure (24). Repeated measures analysis of variance was used to assess differences in blood flow responses across the doses used between men and women and to assess effects of antagonists (L-NMMA or propranolol) on blood flow responses separately within each gender. In addition to these main effects, we sought interactions between gender and antagonist on drug responses. All p values refer to main effects except where it is stated that they refer to tests for an interaction. Differences were considered significant at a value of $p < 0.05$ (two-sided).

Table 1. Characteristics of Subjects*

Characteristics	Women	Men
Age (yr)	27.6 ± 5.3	28.3 ± 7.1
Weight (kg)	66.1 ± 10.6	76.0 ± 11.2†
Height (m)	1.67 ± 0.05	1.80 ± 0.09
Plasma glucose (mmol/L)	4.3 ± 0.4	4.3 ± 0.7
Serum cholesterol (mmol/L)	4.58 ± 1.0	4.35 ± 0.7
Systolic blood pressure (mm Hg)	115 ± 13	123 ± 9
Diastolic blood pressure (mm Hg)	66 ± 9	69 ± 7
Mean arterial pressure (mm Hg)	84 ± 10	88 ± 6
Forearm circumference (mm)	237 ± 13	260 ± 16‡
Forearm length (mm)	244 ± 11	266 ± 12‡
Baseline blood flow (ml min^{-1} 100 ml^{-1})	1.96 ± 0.60	2.63 ± 1.04†

*Plus-minus values are mean ± SD. The three protocols were performed on 8/8, 10/10 and 8/8 (men/women) of these subjects (21 men, 17 women). †$p < 0.05$ compared with values in women; ‡$p < 0.001$ compared with values in women.

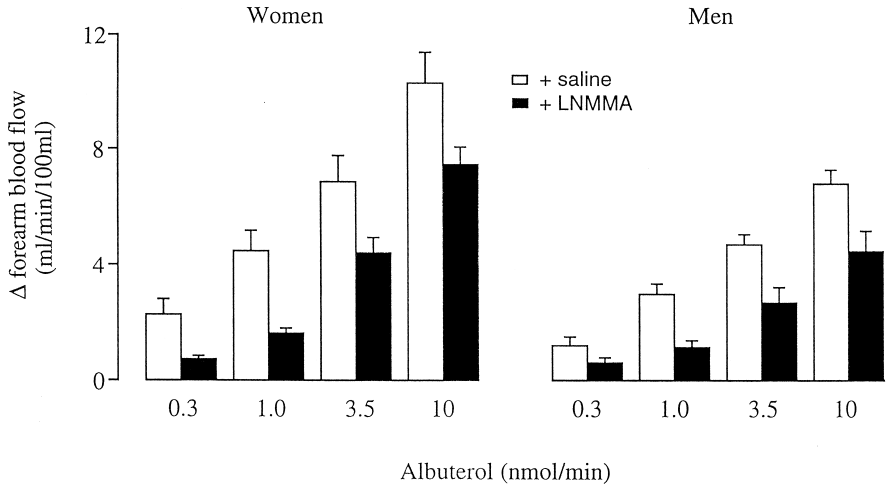

Figure 1. Forearm blood flow responses to albuterol (± L-NMMA) in women and men. **Bars** indicate mean ± SEM increase in blood flow above the immediately preceding baseline. **Open bars** show responses to albuterol coinfused with saline; **closed bars** show responses to albuterol coinfused with L-NMMA (16 μmol/min). Responses to albuterol were greater in women than they were in men (n = 8 for each gender, p = 0.02). N^G-monomethyl-L-arginine inhibited responses to albuterol (p < 0.003). Inhibition by L-NMMA was similar (p = 0.83) in women and men. L-NMMA = N^G-monomethyl-L-arginine.

RESULTS

Forearm blood flow in the noninfused arm did not change significantly during drug infusion in men or women in any protocol.

Sensitivity to albuterol and effect of L-NMMA. Albuterol caused a dose-dependent increase in forearm blood flow in both genders, and vasodilator responses were greater in women than they were in men (n = 16; Fig. 1 and Table 2). N^G-monomethyl-L-arginine caused similar reductions in forearm blood flow, compared with the immediately preceding baseline in men and women: 45 ± 5% in men and 43 ± 6% in women (p = 0.83). Figure 1 shows the effect of L-NMMA on responses to albuterol. N^G-monomethyl-L-arginine inhibited these in women (p = 0.003) and in men (p = 0.002). N^G-monomethyl-L-arginine reduced albuterol area under the curve by 41 ± 5% in women and by 45 ± 9% in men. Gender did not significantly influence inhibition of albuterol by L-NMMA (p = 0.66 for the interaction between gender and inhibition), and responses to albuterol during coinfusion with L-NMMA were greater in women than they were in men (p = 0.02).

Sensitivity to substance P, nitroprusside and verapamil. Substance P, nitroprusside and verapamil each produced a dose-dependent increase in blood flow in both genders. Responses to each of these vasodilators were less in women than they were in men (each p < 0.05, Table 2).

Sensitivity to norepinephrine and effect of propranolol. Each dose of norepinephrine (60, 120 and 240 pmol min^{-1}) reduced forearm blood flow ratio in men. In women there was a tendency toward an increase in blood flow at the lowest dose and reduced blood flow at higher doses (Fig. 2). The difference in responses between men and women was significant (p = 0.02).

Propranolol had no significant effect on baseline blood flow in either gender (p = 0.49 in men, p = 0.66 in women, n = 8 of each gender). Its effects on norepinephrine responses are shown in Figure 2. Propranolol had no significant effect (p = 0.72) on responses to norepinephrine in men but converted the mild increase in forearm blood flow ratio observed at the lowest dose of norepinephrine in women into a vasoconstrictor response and increased the

Table 2. Forearm Blood Flow Responses of Women and Men to Brachial Artery Administration of Vasodilator Agonists

Vasodilator	Dose (nmol/min)	Change in Forearm Blood Flow* (ml/min/100ml) Women (n = 10)	Men (n = 10)	p Value†
Albuterol	0.35	2.3 ± 0.5	1.2 ± 0.3	
	1.0	4.5 ± 0.7	3.0 ± 0.3	
	3.5	6.9 ± 0.9	4.7 ± 0.4	
	10	10.4 ± 1.1	6.8 ± 0.5	
	AUC‡	20.3 ± 2.2	13.5 ± 0.9	0.02
Substance P	0.0003	1.1 ± 0.2	1.8 ± 0.4	
	0.001	2.0 ± 0.3	3.8 ± 0.5	
	0.003	3.5 ± 0.6	6.5 ± 1.2	
	0.01	6.6 ± 0.9	9.8 ± 1.2	
	AUC‡	9.4 ± 1.3	16.2 ± 2.3	0.02
Nitroprusside	3	2.6 ± 0.4	4.1 ± 0.4	
	6	3.5 ± 0.5	5.8 ± 0.7	
	12	4.2 ± 0.5	6.9 ± 0.7	
	24	5.4 ± 0.8	8.5 ± 0.7	
	AUC‡	4.8 ± 0.7	8.4 ± 1.5	0.03
Verapamil	20	3.1 ± 0.3	4.7 ± 0.5	
	40	3.5 ± 0.4	6.1 ± 0.6	
	80	4.8 ± 0.7	7.9 ± 0.8	
	160	6.7 ± 1.0	10.0 ± 1.0	
	AUC‡	6.3 ± 1.0	10.5 ± 1.3	0.04

*Increase in forearm blood flow above immediately preceding baseline; †for difference of AUC between women and men; ‡area under the dose response curve (arbitrary units) ± SEM.
AUC = area under the dose response curve.

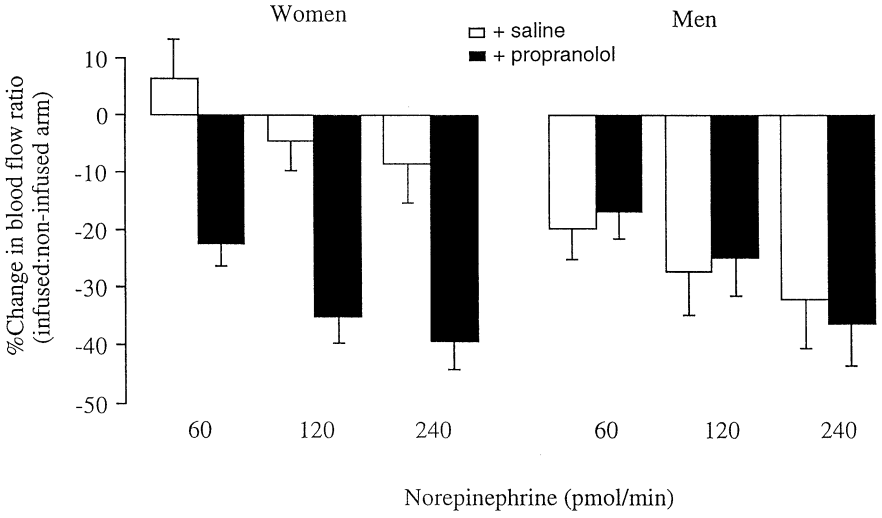

Figure 2. Forearm blood flow responses to norepinephrine (±propranolol) in women and men. **Bars** indicate mean ± SEM percent change in blood flow ratio (infused/noninfused arm). **Open bars** show responses to norepinephrine coinfused with saline; **closed bars** show responses to norepinephrine coinfused with propranolol (190 nmol/min). Reduction in forearm blood flow during norepinephrine infusion was significantly greater in men than it was in women ($n = 8$ for each gender, $p = 0.02$). Propranolol coinfusion significantly increased the response to norepinephrine in women ($p = 0.0004$) but not in men ($p = 0.58$). During infusion of propranolol, responses to norepinephrine were similar ($p = 0.34$) in women and men.

vasoconstriction caused by the two higher doses ($p = 0.0001$ for the interaction of gender with effect of propranolol on norepinephrine response). During infusion of propranolol with norepinephrine, vasoconstriction to norepinephrine in women increased to the extent that responses in women became similar to those in men ($p = 0.34$).

DISCUSSION

Gender differences in basal and agonist-stimulated forearm blood flow. Women are more sensitive than men to the hypokalemic effect of inhaled β_2-adrenergic receptor agonist (terbutaline) (25), but gender differences in the response of human resistance vasculature to β_2-agonists have not been described previously. The first main finding of this study was that albuterol, a selective β_2-adrenergic receptor agonist, is a more potent forearm vasodilator in women than it is in men. Forearm blood flow is predominantly to striated muscle, which accounts for a substantial fraction of total peripheral vascular resistance. Other specialized vascular beds differ. Blood flow in the skin of the hands is involved in thermoregulation, and finger blood flow responses to another β-adrenergic agonist, isoproterenol, are greater in men than they are in women (26). Vasoconstriction to norepinephrine is potentiated by propranolol in this vascular bed, where β-adrenergic agonists act on arteriovenous shunts (27). These contrasts highlight divergent β-adrenergic effects in vascular beds serving different specialized functions and agree with previously reported divergence between β-adrenergic sensitivity in different kinds of blood vessels in humans in vivo (28).

Gender differences in responsiveness to brachial artery administration of drugs that are unstable in blood (such as acetylcholine) have been reported but are difficult to interpret because they are strongly influenced by basal blood flow and forearm length (29,30), which differ between unselected groups of women and men. However, albuterol is relatively stable in vivo, with an elimination half-life from the circulation after systemic administration in the range of 3 to 5 h (31). Furthermore, we found that women are less sensitive than men to three nonadrenergic vasodilators. These findings were unexpected and have not been reported previously. Since each of these vasodilators acts by a different mechanism, the explanation possibly rests with a structural difference in forearm resistance vessels between women and men. Differences between forearm length, circumference and basal blood flow in women and men studied (Table 1) would be consistent with this. Baseline blood flow was approximately 25% less in women than it was in men, and a similar trend was observed previously (4). The explanation for this is not known but, since blood flow is expressed per 100 ml of forearm volume, it is possible that it relates to differences in forearm composition between the genders. A greater proportion of fat to striated muscle in women could account for the difference since fat is relatively poorly perfused compared with muscle under basal conditions in a warm environment. Despite the gender difference in response to β_2-adrenergic stimulation that we observed, it is not likely that differences between genders in basal blood flow are caused by differences in circulating catecholamines, which are similar in men and women (32). Vasodilator responses to drugs usually vary in parallel with basal flow, possibly as a result of flow-dependent drug elimination in arteries proximal to resistance vessels (30,33), and this may explain the reduced vasodilator response to control agonists in the women in this study. Whatever the explanation of this reduced sensitivity, it makes the increased sensitivity to albuterol in women more striking. Since the gender difference in sensitivity to albuterol is specific, it is possible that

the increased sensitivity of women to albuterol is caused by differences at, or downstream, from β_2-adrenergic receptors in forearm resistance vessels. This contrasts with the nonspecific reduction in sensitivity to isoproterenol in healthy black American men in whom attenuated vasodilator responses are a generalized phenomenon (34).

Vasorelaxation mediated by β_2-adrenergic receptors depends on the presence of intact endothelium in some (35–40), but not all (41–43), blood vessels. Human umbilical vein endothelial cells express β_2-adrenergic receptors, and β_2-adrenergic agonists stimulate adenylyl cyclase in these cells, activating NO synthase (40). Vasodilation to isoproterenol (2,44) and albuterol in forearm resistance vessels is inhibited by L-NMMA in healthy men, whereas vasodilation to verapamil, nitroprusside and prostacyclin are not (2). Vasodilation to isoproterenol and albuterol is only partly inhibited by L-NMMA, suggesting that β_2-adrenergic vasodilation in the forearm is only partly NO-mediated. These findings suggest that β_2-adrenergic vasodilation depends on activation of the L-arginine/NO pathway in addition to the direct effects of β_2-adrenergic agonists on vascular smooth muscle (41–43). The effect of L-NMMA on albuterol responses in women has not been reported previously. This study confirms that L-NMMA reduces basal forearm blood flow similarly in men and women (4). The findings also demonstrate that L-NMMA antagonizes forearm vasodilator responses to albuterol in women by a similar percentage as in men (41 $\pm$ 5% in women and 45 $\pm$ 9% in men). Responses to albuterol during inhibition of NO synthase remain greater in women than they do in men. This suggests that NO-dependent and NO-independent components of the β_2-adrenergic response contribute similarly to the albuterol response in women and men. The simplest explanation for the increased sensitivity to albuterol in women is, thus, an increase in numbers of β_2-adrenergic receptors or increased activity of adenylyl cyclase linked to these receptors. It is not possible to address this directly in human forearm resistance vessels, but it is noteworthy that β_2-adrenergic receptor density and receptor-coupled adenylyl cyclase activity are increased in lymphocytes of women compared with men and varies with the phase of the menstrual cycle (45,46). If similar changes occur in endothelial and vascular smooth muscle cells in forearm resistance vasculature independent of an effect on α-adrenergic receptors, this could account for the increased sensitivity to β_2-adrenergic agonists that we observed in women.

Effect of propranolol on forearm responses to norepinephrine in men and women. We originally sought to determine whether there is increased sensitivity in women to β_2-adrenergic receptor stimulation in forearm resistance vessels to explore the hypothesis that this could explain the blunted vasoconstriction to norepinephrine observed in women during the follicular phase of the menstrual cycle (4). The observations discussed above indicate that women are, indeed, more sensitive to brachial artery infusion of albuterol than men. This raises the question of whether this difference is sufficient to account for the observed blunting of vasoconstriction to norepinephrine in women. We addressed this by coinfusion of propranolol with norepinephrine, arguing that, if the difference in sensitivity to norepinephrine between men and women was caused by differences in β-adrenergic receptor activation, this difference should be abolished by blockade of β-adrenergic receptors.

Brachial artery administration of this dose of propranolol in healthy men has been reported to have no effect on basal forearm blood flow (47). The present findings confirm this and extend it to healthy women. In addition, we observed that propranolol has no significant effect on vasoconstrictor responses to norepinephrine in men, indicating that there is little or no β_2-adrenergic receptor activation by these doses of norepinephrine in men. In contrast, propranolol markedly potentiates forearm vasoconstriction to norepinephrine in women, and the second main finding of this study was that propranolol completely abolished the gender difference in sensitivity to norepinephrine. Thus, increased sensitivity to β_2-adrenergic receptor stimulation is quantitatively sufficient to account for the observed attenuation of norepinephrine-mediated vasoconstriction in women. Consequently, it is not necessary to invoke differences in α-adrenergic responsiveness to explain the observed gender difference in responsiveness to norepinephrine in forearm vasculature, in contrast with the cutaneous circulation where there is a gender difference in adrenergic response (27) that appears to be due to tonically increased sympathetic tone with consequent down-regulation of α-adrenergic receptors.

Increased sensitivity of resistance vessels in striated muscle to β_2-adrenergic stimuli could represent an important control mechanism in premenopausal women, and responsiveness to adrenergic receptor stimulation may contribute to the pathogenesis of vascular disease. Forearm blood flow responses to isoproterenol are attenuated in black men, and it has been suggested that blunted vasodilator responses mediated by β_2-adrenergic receptors may play a part in the pathogenesis of hypertension in this ethnic group (48). In this study all subjects were white, and the findings suggest the possibility that increased responses to β-adrenergic stimuli could influence vascular function and contribute to gender-related differences in the incidence or progression of vascular disease in premenopausal women.

We concluded that a previously unrecognized gender difference in forearm resistance vessel sensitivity to β_2-adrenergic receptor stimulation underlies reduced responsiveness of this vascular bed to norepinephrine in premenopausal women.

Reprint requests and correspondence: Dr. J. M. Ritter, Department of Clinical Pharmacology, St. Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, United Kingdom. E-mail: james.ritter@kcl.ac.uk.

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