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Sex differences in the relative contributions of nitric oxide and EDHF to agonist-stimulated endothelium-dependent relaxations in the rat isolated mesenteric arterial bed

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1 We have used the isolated, buffer-perfused, superior mesenteric arterial bed of male and female rats to assess the relative contributions of nitric oxide (NO) and the endothelium-derived hyperpolarizing factor (EDHF) to endothelium-dependent relaxations to carbachol.

2 Carbachol caused dose-related relaxations of methoxamine-induced tone in mesenteric vascular beds from male rats described by an $ED_{50(M)}$ of 0.43 ± 0.15 nmol and a maximum relaxation ($R_{max(M)}$) of $89.6 \pm 1.2\%$ ($n=28$) which were not significantly different from those observed in mesenteries from female rats ($ED_{50(F)} = 0.72 \pm 0.19$ nmol and $R_{max(F)} = 90.7 \pm 0.9\%$; $n=22$).

3 In the males, the addition of 100 μM N^G-nitro-L-arginine methyl ester (L-NAME) caused the dose-response curve to carbachol to be significantly ($P<0.001$) shifted to the right 15 fold ($ED_{50(M)} = 6.45 \pm 3.53$ nmol) and significantly ($P<0.01$) reduced $R_{max(M)}$ ($79.7 \pm 2.8\%$, $n=13$). By contrast, L-NAME had no effect on vasorelaxation to carbachol in mesenteries from female rats ($ED_{50(F)} = 0.89 \pm 0.19$ nmol, $R_{max(F)} = 86.9 \pm 2.3\%$, $n=9$).

4 Raising tone with 60 mM KCl significantly reduced the maximum relaxation to carbachol in mesenteries from male rats 2 fold ($R_{max(M)} = 40.3 \pm 9.2\%$, $n=4$; $P<0.001$) and female rats by 1.5 fold ($R_{max(F)} = 55.3 \pm 3.3\%$, $n=6$; $P<0.001$), compared with methoxamine-induced tone. The potency of carbachol was also significantly reduced 1.2 fold in preparations from males ($ED_{50(M)} = 0.87 \pm 0.26$ nmol; $P<0.01$) but not the females ($ED_{50(F)} = 4.04 \pm 1.46$ nmol). In the presence of both 60 mM KCl and L-NAME, the vasorelaxation to carbachol was completely abolished in mesenteries from both groups.

5 The cannabinoid receptor antagonist SR141716A (1 μM), which is also a putative EDHF antagonist, had no significant effect on the responses to carbachol in mesenteries from males or females ($ED_{50(M)} = 1.41 \pm 0.74$ nmol, $R_{max(M)} = 89.4 \pm 2.5\%$, $n=7$; $ED_{50(F)} = 2.17 \pm 0.95$ nmol, $R_{max(F)} = 89.9 \pm 1.8\%$, $n=9$). In mesenteries from male rats, in the presence of 100 μM L-NAME, SR141716A significantly ($P<0.05$) shifted the dose-response curve to carbachol 8 fold further to the right than that seen in the presence of L-NAME alone ($ED_{50(M)} = 53.8 \pm 36.8$ nmol) without affecting $R_{max(M)}$ ($72.4 \pm 4.8\%$, $n=10$). In mesenteries from female rats, the combined presence of L-NAME and SR141716A, significantly ($P<0.01$) shifted the dose-response curve to carbachol 7.5 fold, ($ED_{50(F)} = 6.66 \pm 2.46$ nmol), as compared to L-NAME alone and significantly ($P<0.001$) decreased $R_{max(F)}$ ($70.1 \pm 5.5\%$, $n=8$).

6 Vasorelaxations to the nitric oxide donor sodium nitroprusside (SNP), to the endogenous cannabinoid, anandamide (a putative EDHF) and to the ATP-sensitive potassium channel activator, levcromakalim, did not differ significantly between male and female mesenteric vascular beds.

7 The continuous presence of sodium nitroprusside (SNP; 20–60 nM) had no effect on vasorelaxation to carbachol in mesenteries from either males or females. In the presence of L-NAME, SNP significantly ($P<0.05$) reduced the potency of carbachol 6 fold, without affecting the maximal relaxation in mesenteries from male rats ($ED_{50(M)} = 40.9 \pm 19.6$ nmol, $R_{max(M)} = 79.4 \pm 2.5\%$, $n=11$). Similarly in mesenteries from female rats, the $ED_{50(F)}$ was also significantly ($P<0.01$) increased 7 fold (6.24 ± 2.02 nmol), while the $R_{max(F)}$ was unaffected ($81.9 \pm 11.0\%$; $n=4$).

8 The results of the present investigation demonstrate that the relative contributions of agonist-stimulated NO and EDHF to endothelium-dependent relaxations in the rat isolated mesenteric arterial bed, differ between males and females. Specifically, although both NO and EDHF appear to contribute towards endothelium-dependent relaxations in males and females, blockade of NO synthesis alone has no effect in the female. This suggests that EDHF is functionally more important in females; one possible explanation for this is that in the absence of NO, the recently identified ability of EDHF to compensate for the loss of NO, is functionally more important in females than males.

Keywords: Nitric oxide; endothelium-derived hyperpolarizing factor (EDHF); gender; rat mesenteric arterial bed; anandamide; K^+ channels; endothelium-dependent relaxation; sex differences

Introduction

The vascular endothelium plays a central role in vascular regulation by releasing both constricting and relaxing factors. The first relaxing factor to be identified was prostacyclin (Moncada *et al*., 1976). This was followed in 1980 by the discovery of a second substance, the endothelium-derived relaxing factor (EDRF) (Furchgott & Zawadzki, 1980), later identified as nitric oxide (NO) by Ignarro *et al*. (1987) and Palmer *et al*. (1987).

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In addition, a third endothelium-derived relaxant has also been identified, which has been characterized by its ability to activate K^+ channels and induce smooth muscle hyperpolarization (Bolton *et al*., 1984; Chen *et al*., 1988; Taylor *et al*., 1988; Brayden, 1990; Cowan & Cohen, 1991; Garland & McPherson, 1992). This factor, termed the endothelium-derived hyperpolarizing factor (EDHF; Taylor & Weston, 1988) has yet to be conclusively identified, but early findings suggested that it is a cytochrome P450-derived arachidonic acid metabolite, due to the ability of inhibitors of this enzyme system to block EDHF-mediated responses (Bauersachs *et al*., 1994; Hecker *et al*., 1994; Fulton *et al*., 1995). However, the specificity of these agents has been questioned (Murray & Reidy, 1990; Corriu *et al*., 1996; Zygmunt *et al*., 1996) and, indeed, some of these agents have been shown to inhibit K^+ channel activation (Zygmunt *et al*., 1996; Fukao *et al*., 1997; Randall *et al*., 1997). Fukao *et al*. (1997) have also recently demonstrated that acetylcholine-induced hyperpolarizations are unaffected by inhibitors of specific cytochrome P450 isozymes and accordingly the identity of EDHF as a cytochrome P450 metabolite may be invalid. More recently, it has been proposed that an endogenous cannabinoid may represent an EDHF (Randall *et al*., 1996). This novel hypothesis was based on the ability of a specific cannabinoid antagonist to oppose EDHF-mediated relaxations, while the prototype endogenous cannabinoid, anandamide, was shown to cause vasorelaxation through K^+ channel activation.

The vast array of studies concerning endothelium-dependent responses in tissues from male animals dwarf the relatively few studies where tissues from female animals have been used. However, it is clear from these latter studies that there exists sex differences in endothelium-dependent regulation of vascular smooth muscle tone. Endothelium-dependent vasorelaxation responses to acetylcholine in isolated aortae of the rat and rabbit have been demonstrated to be greater in the aortae from females compared to males (Hayashi *et al*., 1992; Kauser & Rubanyi, 1993; 1994; Sánchez *et al*., 1996). Hayashi *et al*. (1992) and Sánchez *et al*. (1996) both observed an enhanced basal and acetylcholine-stimulated nitrite release from the aorta of female rabbits and rats compared to male controls, suggesting a greater release of NO. A reduction in endothelium-dependent vasorelaxation has also been demonstrated in the female human forearm vasculature after ovariectomy, while this impairment may be restored by oestrogen replacement therapy (Pinto *et al*., 1997). It is clear that the response to constrictor substances is attenuated to a greater degree in the female aorta *in vitro* (Kauser & Rubanyi, 1994; Sánchez *et al*., 1996) and the myogenic response is greater in coronary arteries from male or oestrogen-deficient female rats (Wellman *et al*., 1996). These differences are abolished by removal of the endothelium or blockade of NO synthesis, implicating endothelium-derived NO as a mediator of these differences. By contrast, in the rat mesenteric arterial bed, the responses to acetylcholine are similar in tissues from male and female rats (Li & Duckles, 1994). Further, an increase in circulating oestrogen levels (e.g. in pregnancy) does not affect the response to acetylcholine in the rat mesentery (Ralevic & Burnstock, 1996).

To our knowledge, no studies have been carried out to investigate directly the relative contribution of agonist-stimulated NO and EDHF to endothelium-dependent relaxation in resistance vessels from male and female animals. Thus, the present study was carried out to investigate whether the contributions of NO and EDHF to carbachol-induced endothelium-dependent relaxation differ between the isolated, perfused mesenteric arterial bed of male and female rats. We employed the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) in order to reveal the EDHF-mediated component of vasorelaxation. The functional contribution of EDHF was investigated by use of a raised extracellular K^+ (60 mM KCl; Adeagbo & Triggle, 1993). As EDHF has been suggested to be an endogenous cannabinoid (Randall *et al*., 1996), we also used the cannabinoid receptor antagonist, SR141716A, to probe the EDHF-mediated portion of endothelium-dependent relaxation, in the absence and presence of L-NAME. Responses to exogenous anandamide, a putative EDHF and the NO donor sodium nitroprusside (SNP), were also determined to investigate vascular smooth muscle sensitivity to these mediators. In view of our recent findings that basal NO suppresses EDHF activity (McCulloch *et al*., 1997), we also investigated the effects of exogenous NO (from sodium nitroprusside), in the absence and presence of basal NO, on EDHF-mediated relaxations.

A preliminary account of this study has been presented to the British Pharmacological Society (McCulloch & Randall, 1997).

Methods

Preparation of the isolated buffer-perfused mesenteric arterial bed

Aged-matched male (300–360 g) and female (180–230 g) Wistar rats (Bantin & Kingman, Hull, Humberside) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p., Sagatal, Rhône Mérieux, Harlow, Essex). Females were randomly chosen and vaginal smears were taken on the day of the experiment and examined microscopically to assess the stage of oestrous and to confirm that the females used were evenly distributed throughout the cycle. Following a mid-line incision, the superior mesenteric artery was cannulated and the arterial bed was flushed with Krebs-Henseleit solution before the vasculature was dissected away from the intestines and transferred to a jacketed organ bath (37°C) as described previously by Randall & Hiley (1988). The tissue was perfused at a constant flow rate of 5 ml min^{-1} with gassed (95% O_2/5% CO_2) warmed (37°C) solution (composition, mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2, D-glucose 10 plus 3 μM indomethacin). In experiments involving high K^+, a 60 mM K^+ isotonic Krebs-Henseleit solution was prepared by substituting equimolar concentrations of NaCl with KCl.

Perfusion pressure was continuously monitored by means of a pressure transducer placed close to the inflow cannula, coupled to a MacLab recording system (AD instruments, New South Wales, Australia). Flow was constant and therefore changes in perfusion pressure represented alterations in vascular resistance. At the end of each experiment, the pressure drop across the cannula was measured and subtracted from the recorded basal perfusion pressure in order to ascertain the actual pressure across the bed.

Experimental protocol

Following a 30 min equilibration period, perfusion pressure was raised by the addition of methoxamine (10–50 μM). Once stable tone had been established, bolus doses of the endothelium-dependent vasorelaxant carbachol were administered in random order close-arterially, in volumes less than 100 μl. Vasorelaxation to carbachol was assessed in the

absence and presence of the nitric oxide (NO) synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) to define the NO-mediated component. In the presence of L-NAME, the concentration of methoxamine was reduced to achieve a comparable level of tone (1–6 μM). EDHF acts via the opening of K^+ channels and accordingly we investigated the effects of raising tone with high (60 mM) extracellular potassium (Adeagbo & Triggle, 1993), on the response to carbachol, in the absence and presence of L-NAME.

The effect of increased levels of nitric oxide on vasorelaxation to carbachol was assessed by the addition of an EC_{50} concentration (individually determined for each preparation) of the NO donor sodium nitroprusside (SNP; 20–60 nM) to the Krebs-Henseleit solution. The effect of SNP was also investigated in the presence of L-NAME.

EDHF has recently been suggested to be an endogenous cannabinoid (Randall *et al*., 1996) and, therefore, the effects of the cannabinoid receptor antagonist, SR141716A (1 μM), on vasorelaxation to carbachol were also investigated in the absence and presence of L-NAME, to investigate the role of EDHF.

In addition to determining the relative contributions of EDHF/NO to endothelium-dependent relaxation, the responsiveness of the vascular bed to SNP, anandamide and to the endothelium-independent K_{ATP} channel activator (hyperpolarizing agent), levcromakalim, were also defined.

Data and statistical analysis

The data are presented as mean ± s.e.mean and were compared by analysis of variance (ANOVA) with significant differences between groups being detected by Bonferroni's *post-hoc* test. ED_{50} values for vasorelaxant responses were obtained from the individual dose-response curves as the dose at which the half-maximal relaxation response occurred. The ED_{50} was determined by fitting the data to the logistic equation:

$$R = \frac{R_{max} \cdot A^{n_H}}{ED_{50}^{n_H} + A^{n_H}}$$

where R is the reduction in tone, A the dose of the vasorelaxant, R_{max} the maximum reduction of established tone, n_H the slope function and ED_{50} the dose of vasorelaxant giving half the maximal relaxation. The curve fitting was carried out on KaleidaGraph software (Synergy, Reading, PA, U.S.A.) running on a Macintosh computer. The ED_{50} values were converted to logarithmic values for statistical analysis.

Drugs

All drugs were prepared on the day of the experiment. Methoxamine hydrochloride, carbachol, N^G-nitro-L-arginine methyl ester, sodium nitroprusside, (all from Sigma Chemical Company, Poole, Dorset) were dissolved in saline and diluted in Krebs-Henseleit solution. Levcromakalim, a generous gift from SmithKline Beecham, Surrey, was dissolved in absolute ethanol and diluted in 0.9% saline. SR141716A ((N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride) was obtained from Dr E.A. Boyd (Dept. of Pharmaceutical Sciences, University of Nottingham) and dissolved as a stock solution in ethanol. Anandamide was synthesized from arachidonyl chloride and ethanolamine by Dr E.A. Boyd.

Results

Basal perfusion pressures and established tone

Basal perfusion pressure in the 28 mesenteries from male rats was 17.2 ± 0.9 mmHg and in the vascular bed from female rats was 18.5 ± 1.3 mmHg ($n = 22$). The addition of 10–50 μM methoxamine raised the perfusion pressure by 70.5 ± 2.5 mmHg (males) and 75.2 ± 5.4 mmHg (females). L-NAME had no effect on basal perfusion pressure in mesenteries from either male or female rats. Addition of 2–6 μM methoxamine in the presence of L-NAME resulted in increases in the perfusion pressure of 66.2 ± 4.3 mmHg (males, $n = 13$) and 74.3 ± 9.5 mmHg (females, $n = 9$).

Effects of L-NAME on vasorelaxation to carbachol

Carbachol induced dose-related relaxations of established tone in mesenteries from male rats, described by an $ED_{50(M)}$ of 0.43 ± 0.15 nmol and a maximum relaxation ($R_{max(M)}$) of 89.6 ± 1.2% ($n = 28$; Figure 1a). In the presence of L-NAME, the dose-response curve to carbachol was significantly ($P < 0.001$) shifted to the right 15 fold ($ED_{50(M)} = 6.45 \pm 3.35$ nmol) and the maximum relaxation was significantly ($P < 0.01$) reduced ($R_{max(M)} = 79.7 \pm 2.8\%$; $n = 13$).

In mesenteries from female rats, the dose-response curve to carbachol was described by an $ED_{50(F)}$ of 0.72 ± 0.19 nmol and $R_{max(F)} = 90.7 \pm 0.9\%$ ($n = 22$) and was, therefore, not significantly different from that in males. The presence of L-NAME had no effect on the vasorelaxation to carbachol ($ED_{50(F)} = 0.89 \pm 0.19$ nmol, $R_{max(F)} = 86.9 \pm 2.3\%$; $n = 9$; Figure 1b).

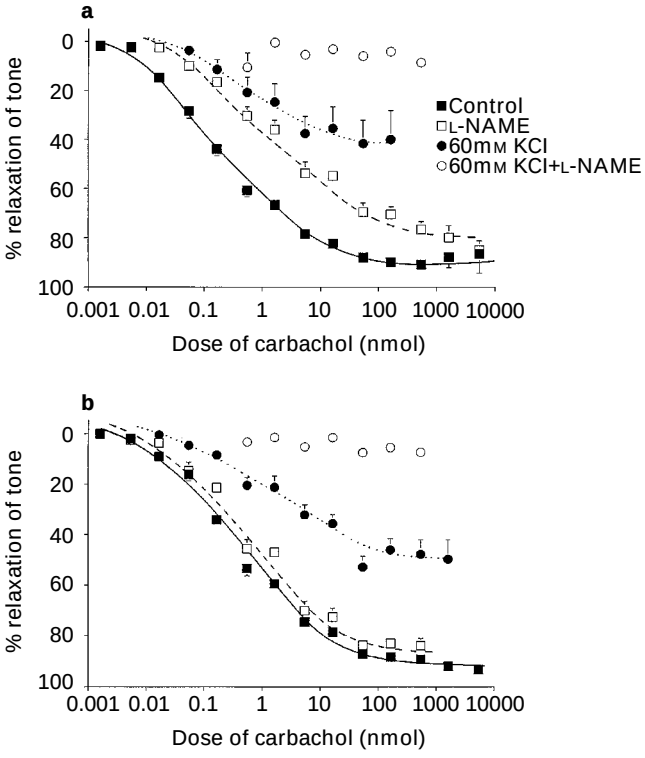

Figure 1 Log dose-response curves for vasorelaxation to carbachol in the (a) male and (b) female rat isolated mesenteric arterial beds in the absence and presence of 100 μM L-NAME (methoxamine-induced tone), when tone was raised by 60 mM KCl and in the presence of both L-NAME and 60 mM KCl. Values are shown as mean and vertical lines indicate s.e.mean.

Effects of 60 mM KCl and L-NAME on vasorelaxation to carbachol

Raising extracellular K^+ to 60 mM reduced the potency of carbachol 1.2 fold ($ED_{50(M)} = 0.87 \pm 0.26$ nmol, $P < 0.01$) and maximal relaxation ($R_{max(M)} = 40.3 \pm 9.2\%$, $n = 4$; $P < 0.001$) to carbachol in mesenteries from male rats. In the females, maximum relaxation was significantly ($P < 0.001$) reduced 1.6 fold ($R_{max(F)} = 55.3 \pm 3.3\%$; $n = 6$), with $ED_{50(F)}$ being unaffected (4.04 ± 1.46 nmol). The presence of 60 mM extracellular KCl and L-NAME abolished vasorelaxation to carbachol in mesenteries from both male and female rats (Figure 1a and b).

Effects of SR141716A and L-NAME on vasorelaxation to carbachol

The selective cannabinoid receptor antagonist, SR141716A (1 μM), had no significant effect on vasorelaxation to carbachol in mesenteries from male or female rats ($ED_{50(M)} = 1.41 \pm 0.74$ nmol, $R_{max(M)} = 89.4 \pm 2.5\%$, $n = 7$; $ED_{50(F)} = 2.17 \pm 0.95$ nmol, $R_{max(F)} = 89.9 \pm 1.8\%$, $n = 9$; Figure 2a and b).

However, in the presence of 100 μM L-NAME, SR141716A significantly ($P < 0.05$) decreased the potency of carbachol ($ED_{50(M)} = 53.8 \pm 36.8$ nmol) 8 fold compared with L-NAME alone in mesenteries from male rats, without affecting maximal relaxation ($R_{max(M)} = 72.4 \pm 4.8\%$, $n = 10$).

In mesenteries from female rats, the presence of both L-NAME and SR141716A shifted the dose-response curve to carbachol 7.5 fold ($P < 0.01$) compared with L-NAME alone ($ED_{50(F)} = 6.66 \pm 2.46$ nmol). Maximum relaxation was also significantly ($P < 0.001$) reduced 1.2 fold compared with L-NAME alone ($R_{max(F)} = 70.1 \pm 5.5\%$, $n = 8$).

Effects of sex on vasorelaxation to sodium nitroprusside

The vasorelaxant responses to SNP did not differ significantly between mesenteries from male and female rats ($ED_{50(M)} = 1.40 \pm 0.80$ nmol, $R_{max(M)} = 83.7 \pm 3.1\%$, $n = 8$; $ED_{50(F)} = 1.10 \pm 0.39$ nmol, $R_{max(F)} = 87.4 \pm 2.5\%$, $n = 7$; Figure 3a).

Effect of sex on vasorelaxation to anandamide

The vasorelaxant effects of anandamide were found to be similar in mesenteries from both male and female rats ($ED_{50(M)}$

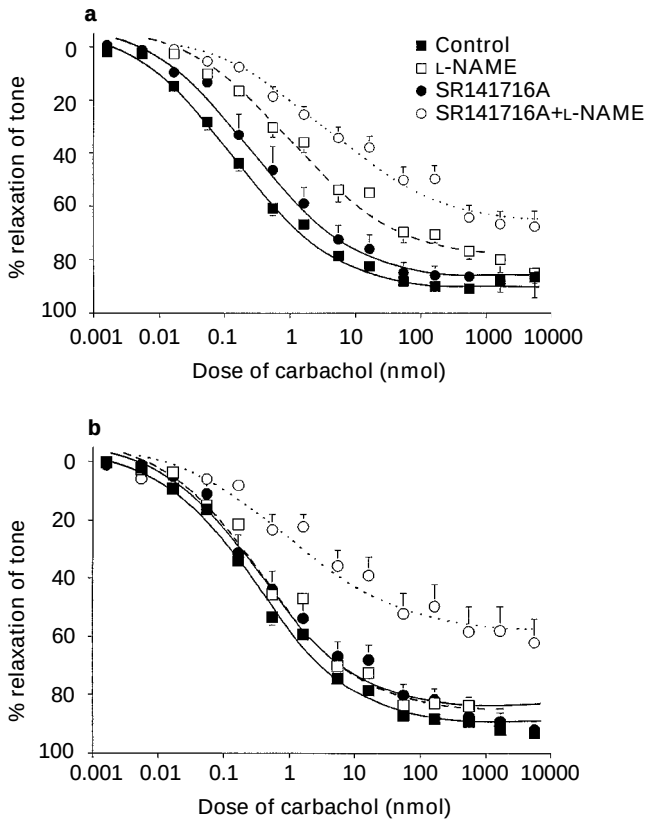

Figure 2 Log dose-response curves for the vasorelaxation of methoxamine-induced tone in the (a) male and (b) female rat isolated mesenteric arterial beds to carbachol in the absence and presence of L-NAME (100 μM), in the presence of SR141716A (1 μM), and in the combined presence of L-NAME and SR141716A. Values are shown as mean and vertical lines indicate s.e.mean.

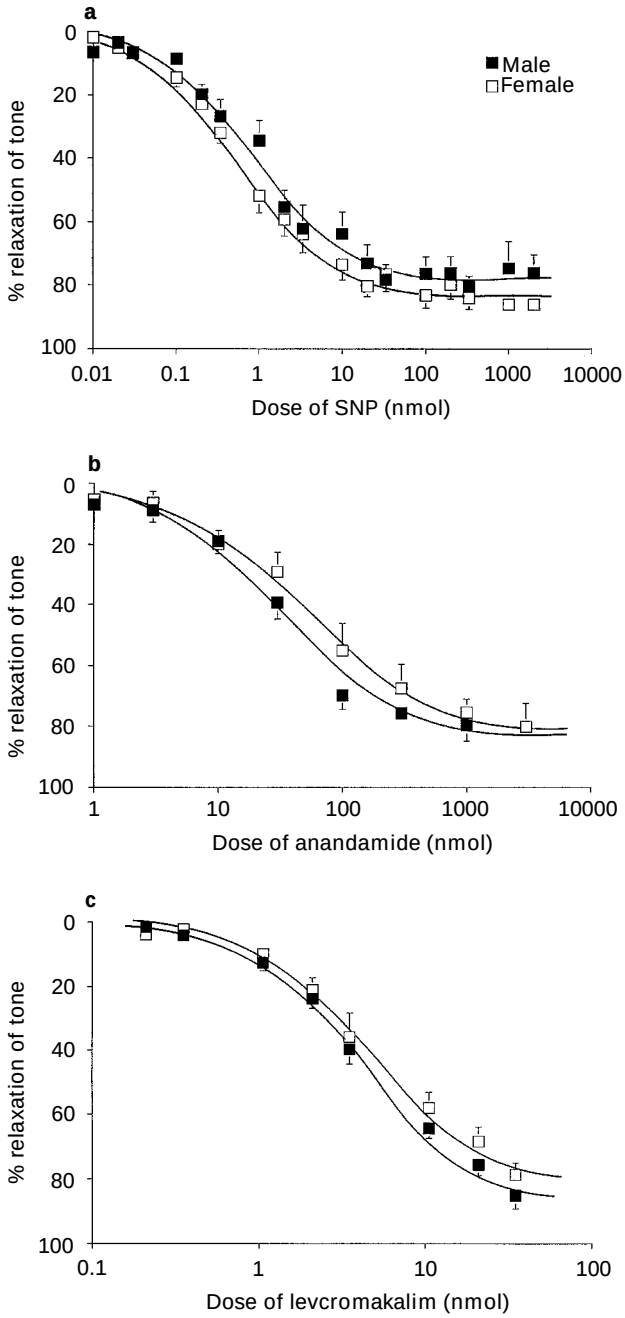

Figure 3 Log dose-response curves for the vasorelaxation of methoxamine-induced tone in the male and female rat isolated mesenteric arterial bed to (a) sodium nitroprusside, (b) anandamide and (c) levcromakalim. Values are shown as mean and vertical lines indicate s.e.mean.

$=52.5\pm15.8$ nmol, $R_{max(M)}=84.5\pm3.4\%$, $n=7$; $ED_{50(F)}=69.2\pm18.2$ nmol, $R_{max(F)}=81.7\pm6.4\%$, $n=7$; Figure 3b).

Effect of sex on vasorelaxation to levcromakalim

The dose-response curves to levcromakalim were not significantly different between mesenteries from male and female rats ($ED_{50(M)}=4.38\pm0.65$ nmol, $R_{max(M)}=85.7\pm3.9\%$, $n=7$; $ED_{50(F)}=5.34\pm0.84$ nmol, $R_{max(F)}=84.2\pm2.0\%$, $n=6$; Figure 3c).

Effects of sodium nitroprusside and L-NAME on vasorelaxation to carbachol

The presence of an EC_{50} concentration of sodium nitroprusside (SNP) had no effect on vasorelaxation to carbachol in mesenteries from male or female rats ($ED_{50(M)}=0.32\pm0.08$ nmol, $R_{max(M)}=89.3\pm3.2\%$, $n=5$; $ED_{50(F)}=0.97\pm0.35$ nmol, $R_{max(F)}=83.5\pm2.5\%$, $n=6$; Figure 4a and b) compared with control values. However, in the presence of L-NAME, SNP induced a significant 6 fold increase in the ED_{50} value for vasorelaxation to carbachol in mesenteries from males ($ED_{50(M)}=40.9\pm19.6$ nmol; $P<0.05$) and 7 fold increase in mesenteries from females ($ED_{50(F)}=6.24\pm2.20$ nmol; $P<0.01$) compared to L-NAME alone. Maximum relaxation was unaffected by this treatment ($R_{max(M)}=79.4\pm2.5\%$; $R_{max(F)}=81.9\pm11.0\%$).

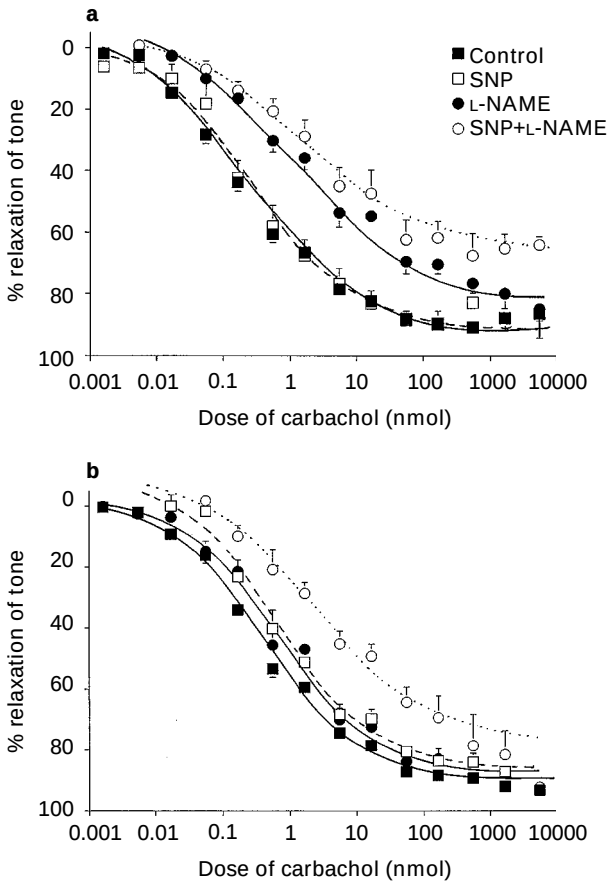

Figure 4 Log dose-response curves for the vasorelaxation of methoxamine-induced tone in the (a) male and (b) female rat isolated mesenteric arterial bed by carbachol in the absence and presence of SNP (20–60 nM), in the presence of 100 μM L-NAME and in the combined presence of SNP and L-NAME. Values are shown as mean and vertical lines indicate s.e.mean.

Discussion

The results of the present study clearly indicate that there are differences between male and female rats in the relative contributions made by agonist-stimulated NO and EDHF to endothelium-dependent relaxations in the mesenteric arterial bed. Specifically, NO appears functionally more important in males and EDHF appears to be of greater importance in females. These findings may result from two possibilities. First, EDHF may be functionally more important under normal conditions in females than males or, alternatively, EDHF may be better able to compensate for the loss of NO (McCulloch *et al*., 1997) in females than in males.

Our first finding was that there were no differences in the endothelium-dependent responses to carbachol between mesenteries from male and female rats. This is in agreement with the findings of Li & Duckles (1994). However, in the mesenteric arterial bed from male rats, it was found that inhibition of NO synthase with 100 μM L-NAME reduced the potency of carbachol 15 fold, suggesting an involvement of NO in mediating the vasorelaxation to carbachol. Furthermore, a substantial L-NAME-insensitive component of vasorelaxation was also revealed. Similar observations have been made in other arterial beds and isolated artery segments, including human brachial artery *in vivo* (Chowienczyk *et al*., 1993) and *in vitro* (pial arteries, Petersson *et al*., 1995). This NO-independent pathway of vasorelaxation is thought to be due to EDHF, which acts by increasing membrane K^+ conductance, inducing hyperpolarization and thus relaxation (Taylor & Weston, 1988). The L-NAME insensitive component was inhibited in the present study, by raising extracellular K^+ (60 mM), which dissipates the gradient for K^+ movement and thus prevents hyperpolarization and relaxation (Adeagbo & Triggle, 1993). In the presence of L-NAME, high K^+ abolished the residual relaxation, indicating that this NO-independent pathway in mesenteries from male rats may be mediated by EDHF.

Assessment of vaginal smears revealed that there was no apparent change in the activity of agonist-stimulated NO or EDHF throughout the cycle. This is in accordance with the findings of Li & Duckles (1994). Possible differences in the basal release of NO were not assessed in this investigation.

In the mesenteric arterial bed from female rats, L-NAME had no effect on the vasorelaxation to carbachol. This suggests that in the female rats, NO and EDHF contribute differently to endothelium-dependent relaxation, as compared to male. Superficially, the most obvious suggestion from this is that NO does not contribute to carbachol-induced endothelium-dependent relaxations in the mesenteric arterial bed from females and that vasorelaxation is mediated entirely by EDHF. However, in the present study, when EDHF-mediated responses were abolished by raising extracellular K^+ in mesenteries from the females, there was clearly a residual relaxation, which was sensitive to NO synthase inhibition, suggesting that NO does, indeed, contribute towards the relaxation in females. The possibility exists, therefore, that under normal conditions, NO does mediate the relaxation to carbachol, but when NO synthesis is blocked, EDHF activity is up-regulated to compensate and thus mediates the vasorelaxation. This has previously been suggested by Kilpatrick & Cocks (1994), Hatake *et al*. (1995), Kemp *et al*. (1995), and in a earlier study, where we demonstrated that the 'cross-talk' between NO and EDHF may be mediated by guanosine 3′:5′-cyclic monophosphate (cyclic GMP; McCulloch *et al*., 1997). A potential explanation for the lack of effect of L-NAME could be that on inhibition of NO, EDHF is up-

regulated to compensate for this loss and that this mechanism is sufficient to overcome the effects of L-NAME.

It has recently been suggested that EDHF may be an endogenous cannabinoid (Randall *et al*., 1996). We have therefore investigated the EDHF-mediated component of relaxation by use of the selective cannabinoid antagonist, SR141716A. In the male rat isolated mesentery, SR141716A alone had no effect on vasorelaxation to carbachol. However, in the presence of L-NAME, SR141716A shifted the dose-response curve to carbachol 8 fold further to the right. This indicates an involvement of an endogenous cannabinoid in endothelium-dependent relaxation to carbachol. It also indicates that under normal conditions, i.e. when NO is present, EDHF has little, if any, role to play in endothelium-dependent relaxation, but that an EDHF-component of vasorelaxation is 'unmasked' when NO is blocked. In female rat isolated mesenteries, the presence of SR141716A alone also had no effect on vasorelaxation to carbachol and when L-NAME and the cannabinoid antagonist were both present, the dose-response curve to carbachol was significantly shifted 7.5 fold to the right. This further suggests that NO is involved in endothelium-dependent relaxation in females, but that EDHF is able to compensate in its absence. In the case of the females, SR141716A induced a relatively greater decrease in the response to carbachol, consistent with an enhanced role for EDHF in females when NO is blocked. It should be noted that we have only investigated the effects of one cannabinoid antagonist in these studies, due to the limited availability of adequate research tools. However, the results clearly show that the cannabinoid receptor antagonist is only effective against the effects of agonist-stimulated EDHF in the absence of basal NO release, indicating an interaction between these systems.

Another possible explanation for the gender differences could relate to the sensitivity of the vascular smooth muscle to NO and EDHF. However, evidence that there is no difference in the vasorelaxant response of mesenteries from males and females to either the putative EDHF, anandamide or exogenous NO, or to the hyperpolarizing agent, levcromakalim, suggests that this is not the case.

The presence of increased levels of exogenous NO (via continuous perfusion with SNP) had no effect on vasorelaxation to carbachol in mesenteries from either males or females. In the absence of basal NO, SNP induced a significant rightward shift in the dose-response curve to carbachol in both the males (6 fold) and females (7 fold). These observations suggest that the presence of basal NO may modulate the release of or responses to, EDHF, such that in the absence of basal NO, EDHF is up-regulated and that the subsequent addition of exogenous NO is able to reduce the contribution made by EDHF to vasorelaxation to carbachol. However, maximum relaxation was unaffected by this treatment, and therefore, NO may reduce the sensitivity of the vascular smooth muscle to EDHF, rather than its release *per se*. These findings are in accordance with a previous study (McCulloch *et al*., 1997), where we demonstrated in the male rat mesentery, that NO may modulate the activity of EDHF, via cyclic GMP.

In summary, we have demonstrated a difference in the relative contributions of NO and EDHF to endothelium-dependent relaxations in the mesenteric arterial bed of male and female rats. EDHF appears to be functionally more important in females in the absence of NO. The explanation for this difference may relate to an enhanced ability of EDHF to compensate for the loss of NO in females. These findings may be relevant to the apparent protection in females against cardiovascular disease.

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