British Journal of Pharmacology (1998) 125, 455–460
http://www.stockton-press.co.uk/bjp

Enhanced acetylcholine induced relaxation in small mesenteric arteries from pregnant rats: an important role for endothelium-derived hyperpolarizing factor (EDHF)

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1 Small mesenteric arteries from pregnant rats demonstrated greater sensitivity (pEC_{50} : $P<0.001$) and maximum relaxation ($P<0.01$) to acetylcholine (ACh) than those of control non-pregnant animals.

2 Maximum relaxation, but not sensitivity, to ACh remained greater ($P<0.01$) in pregnant animals when evaluated in 25 mM KCl, which prevents relaxation dependent upon hyperpolarization. ACh induced relaxation in the presence of 25 mM KCl was completely inhibited in pregnant and non-pregnant groups by N^{ω}-nitro L-arginine methyl ester (L-NAME, 100 μM), indomethacin (INDO, 10 μM) and oxadiazole quinoxalin (ODQ, 1 μM), suggesting pregnancy associated enhancement of dilator prostanoid and/or nitric oxide (NO) synthesis.

3 ACh induced relaxation in 5 mM KCl was only partially inhibited by a combination of N^{ω}-nitro L-arginine methyl ester (L-NAME, 100 μM), indomethacin (INDO, 10 μM) and oxadiazole quinoxalin (ODQ, 1 μM). The residual relaxation, which was greater in arteries from pregnant rats (maximum relaxation: $P<0.01$), was prevented by 25 mM KCl, indicating pregnancy associated enhanced synthesis/reduced degradation of a hyperpolarizing factor. Residual relaxation to ACh in 5 mM KCl was inhibited by the cytochrome P450 inhibitor, proadifen (1 μM) in the pregnant group ($P<0.001$).

4 Relaxation to spermine NONOate was similar in pregnant and non-pregnant groups and totally inhibited by ODQ (in the presence of L-NAME).

5 This study suggests that, in addition to enhanced endothelium dependent NO/dilator prostanoid synthesis, a hyperpolarizing factor may contribute to the vascular adaptation to pregnancy.

Keywords: Pregnancy; endothelium derived hyperpolarizing factor; nitric oxide; mesenteric artery; endothelium

Introduction

Pregnancy is associated with a fall in vascular resistance, attributed partly to enhanced synthesis of nitric oxide (NO) (Sladek *et al*., 1997). A role for NO is suggested by the exaggerated relaxation to endothelium dependent dilators often reported in the vasculature of pregnant animals and women. The first demonstration by Weiner *et al*. (1989) of enhanced relaxation to acetylcholine (ACh) in carotid and uterine artery of the guinea pig was followed by observations of enhanced relaxation to endothelium dependent vasodilators in smaller, resistance arteries from rats (Davidge & MacLaughlin, 1992; Pascoal *et al*., 1995; Learmont *et al*., 1996) and from pregnant human subjects (Knock *et al*., 1996). However, in common with the non-pregnant state (Garland & McPherson, 1992) small arteries from pregnant rats (Pascoal *et al*., 1995) and pregnant women (McCarthy *et al*., 1994; Knock *et al*., 1996; Pascoal *et al*., 1996) usually show persistent relaxation to ACh and bradykinin when NO and prostacyclin synthesis are inhibited. In non-pregnant animals, this residual relaxation in resistance sized arteries has often been attributed to an endothelium-derived hyperpolarizing factor (EDHF) (Garland *et al*., 1995). Here, we address the possibility that increased relaxation to ACh in small mesenteric arteries of the pregnant Sprague Dawley rat is due, in part, to enhanced EDHF synthesis.

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Methods

Female non-pregnant (NP) and pregnant (P) (19–21 days gestation) Sprague Dawley rats were killed by CO_2 inhalation and cervical dislocation. Small mesenteric resistance arteries (mean internal diameter ± s.e.m NP : 295 ± 9 μm, $n=39$ *v* P : 276 ± 9 μm, $n=34$; p NS) were mounted on a myograph and isometric tension evaluated as described below.

Mounting of vessels

Small mesenteric arteries were mounted on a small vessel wire myograph as previously described (Mulvany & Halpern, 1977). Arteries were bathed in physiological salt solution (PSS: constituents in mM: NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.16, EDTA 0.026 and glucose 6.0), pH 7.4 at 37°C and gassed with 5% CO_2 in O_2. The passive tension-internal circumference characteristics of the arteries were determined by stretching to achieve an internal circumference equivalent to 90% of that which would be attained when relaxed *in situ* under a transmural pressure of 100 mmHg.

Assessment of vascular function

To confirm viability of the arteries, four contractions (4 min duration) were performed to 5 μM noradrenaline (NA), KPSS (125 mM KCl in PSS) or a combination of both. Arteries failing to produce active tension equivalent to 100 mmHg were rejected. Relaxation to ACh (1 nM–10 μM) was determined in PSS (5 mM KCl) after preconstriction to

noradrenaline (NA, 5 μM). This relaxation was repeated after incubation (20 min) with and in the presence of: indomethacin (INDO, 10 μM), N^{ω}-nitro-L-arginine methyl ester (L-NAME, 100 μM) and the soluble guanylate cyclase inhibitor, oxadiazole quinoxalin (ODQ, 1 μM). To determine the contribution of a hyperpolarizing factor in the relaxation to ACh, responses were also evaluated in the presence of 25 mM KCl (equimolar substitution with NaCl in PSS) in arteries preconstricted with NA (2–4 μM), the concentration of NA being adjusted to evoke similar constrictor tone to that achieved in arteries preconstricted with 5 μM NA alone. Responses to ACh were then evaluated in arteries preconstricted to NA and 25 mM KCl in the presence of INDO, L-NAME and ODQ. A final ACh response was carried out after addition of the cytochrome P450 inhibitor proadifen (1 μM), in normal (5 mM) KCl in the presence of INDO, L-NAME and ODQ. In separate experiments, in order to evaluate NO sensitivity of the vascular smooth muscle, relaxation responses were carried out after preconstriction with 5 μM NA to the NO donor spermine NONOate (1 nM–10 μM), and to evaluate the role of cGMP in spermine NONOate induced relaxation, responses were again evaluated after preincubation (20 min) and in the continued presence of ODQ (1 μM). To obviate any effect of endogenous NO synthesis, L-NAME was also included (100 μM).

Materials

Noradrenaline (Winthrop, Guildford, U.K.); acetylcholine, indomethacin, L-NAME (Sigma, Poole, U.K.); ODQ and spermine NONOate (Alexis Corporation, Nottingham, U.K.); Proadifen (Research Biochemicals; International, Natick, MA, U.S.A.). Others, BDH (Poole, U.K.).

Data analysis

Data is given as mean ± s.e.m. n refers to number of animals. When 2 arteries were used from one animal, values were averaged. Relaxation to ACh was assessed by pEC_{50} ($-\log EC_{50}$) and maximum relaxation (% NA induced tone), and significance by Student's paired and unpaired t-tests. Significance was assumed if $P<0.05$.

Results

Preconstriction to NA was not different between P and NP arteries, or in the presence of any inhibitor.

Acetylcholine induced relaxation; influence of partial depolarization

Sensitivity to ACh and maximum ACh induced relaxation was greater in arteries from P compared with NP rats [pEC_{50} P: 7.73±0.07, $n=18$ *v* NP: 7.01±0.09, $n=21$; $p<0.001$; maximum relaxation (% NA induced tone) P: 95±1%, $n=18$ *v* NP: 86±2%, $n=21$; $P<0.01$]. When the response was repeated in a sub group of arteries in the presence of partially depolarizing PSS (25 mM KCl), sensitivity was no longer significantly different between arteries from P and NP rats (pEC_{50} P: 6.83±0.24, $n=8$ *v* NP: 6.76±0.18, $n=8$; p NS), but maximum relaxation to ACh remained greater in P (maximum relaxation P: 56±6%, $n=8$ *v* NP: 31±6%, $n=8$; $P<0.01$), Figure 1.

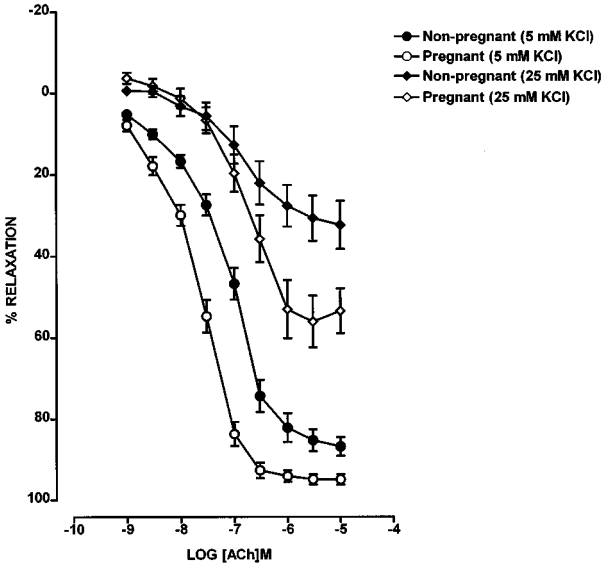

Figure 1 Concentration-response to ACh in mesenteric small arteries from pregnant (P; $n=18$) and non-pregnant (NP; $n=21$) rats in 5 mM KCl and in mesenteric small arteries from pregnant ($n=8$) and non-pregnant ($n=8$) rats in the presence of 25 mM KCl. Sensitivity ($P<0.001$) and maximum relaxation ($P<0.01$) to ACh were significantly greater in (P) *v* (NP) in 5 mM KCl and maximum relaxation significantly greater in (P) *v* (NP) in 25 mM KCl ($P<0.01$).

Acetylcholine induced relaxation; influence of nitric oxide synthase and cyclooxygenase inhibition

In normal PSS (5 mM KCl), ACh induced relaxation was partially inhibited in both groups by a combination of INDO, L-NAME and ODQ leading to a significant shift in the pEC_{50} in the arteries from the pregnant animals (pEC_{50} P: 6.26±0.09, $n=11$ *v* 7.73±0.07 without inhibitors, $n=18$, $P<0.001$) and significant reduction in maximal relaxation in both groups (P: 85±4% $n=11$ *v* 95±1% without inhibitors, $n=18$, $P<0.01$; maximum relaxation NP: 40±8%, $n=12$ *v* 87±2% without inhibitors, $n=21$, $P<0.001$). Calculation of the pEC_{50} for arteries of the non-pregnant animals with inhibitors added was not possible in a minority of vessels as the response became flattened and non-sigmoidal. With the inhibitors present, maximum relaxation to ACh remained greater in arteries from P rats compared with NP ($P<0.01$), Figure 2a. When ACh concentration-responses were carried out in the presence of these inhibitors but also in a partially depolarizing buffer (25 mM KCl in PSS), arteries from P rats showed almost no relaxation and those from NP, partial constriction. (maximum relaxation P: 6±4%, $n=11$ (25 mM KCl) *v* 85±4%, $n=11$ (5 mM KCl); $P<0.001$; NP: −5±4%, $n=6$ *v* 40±8%, $n=12$; $P<0.001$). Responses in 25 mM KCl, INDO, L-NAME and ODQ were not different between the two groups, Figure 2a. A comparison of ACh induced relaxation in the presence of INDO, L-NAME and ODQ (normal 5 mM KCl) *v* ACh induced relaxation in the presence of 25 mM KCl without inhibitors is shown in Figure 3.

Influence of cytochrome P450 inhibition

The maximum residual relaxation in the presence of PSS (5 mM KCl), INDO, L-NAME and ODQ was attenuated by proadifen, but this only reached significance in P rats

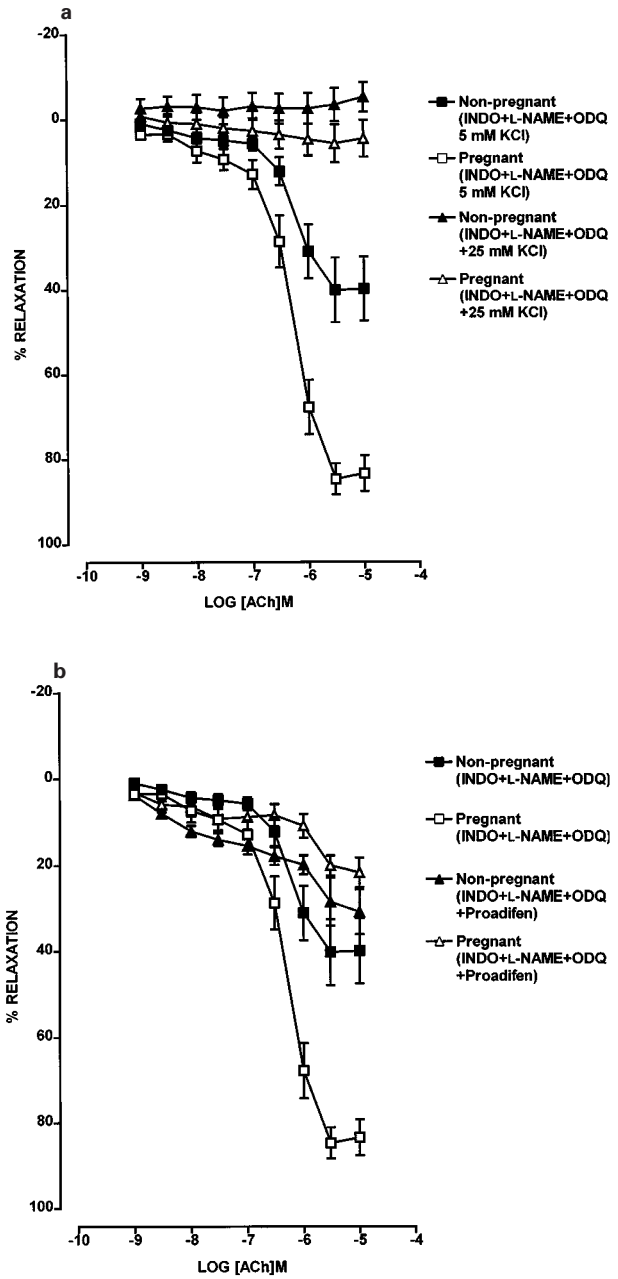

Figure 2 (a) Concentration-response to ACh in mesenteric small arteries from pregnant (P) and non-pregnant (NP) rats in the presence of L-NAME, INDO and ODQ in 5 mM KCl (P: $n=11$; NP: $n=12$) or with L-NAME, INDO and ODQ and 25 mM KCl (P: $n=11$; NP, $n=6$). Maximum relaxation was greater in (P) *v* (NP) in 5 mM KCl ($P<0.01$), but no different in 25 mM KCl. (b) Concentration-response to ACh in mesenteric small arteries from pregnant (P) and non-pregnant (NP) rats in the presence of L-NAME, INDO and ODQ in 5 mM KCl (P: $n=11$; NP: $n=12$) or with L-NAME, INDO, ODQ and proadifen (10 μM) in 5 mM KCl (P: $n=10$; NP: $n=8$). Maximum relaxation was greater in (P) *v* (NP) without proadifen, but with NOS and COX inhibitors ($P<0.01$). Proadifen significantly inhibited ACh induced maximal relaxation only in (P), $P<0.001$.

(maximum relaxation P: $22\pm4\%$, $n=10$ *v* $85\pm4\%$, $n=11$ without proadifen, $P<0.001$), Figure 2b.

Endothelium independent relaxation; role of cyclic GMP

Sensitivity and maximum relaxation to spermine NONOate was similar in pregnant and non-pregnant groups (pEC_{50} P: 5.86 ± 0.22, $n=8$ *v* NP: 5.82 ± 0.20, $n=8$; p NS; maximum relaxation P: $82\pm4\%$, $n=8$ *v* NP: $74\pm3\%$, $n=8$; p NS). However arteries from pregnant animals appeared to display a biphasic response, with significantly greater relaxation at the lower concentrations (1 nM – 30 nM). In both groups, relaxation to spermine NONOate was substantially and similarly inhibited by ODQ, in the presence of L-NAME (maximum relaxation P: $10\pm5\%$, $n=8$ *v* NP: $9\pm1\%$, $n=8$; p NS), Figure 4.

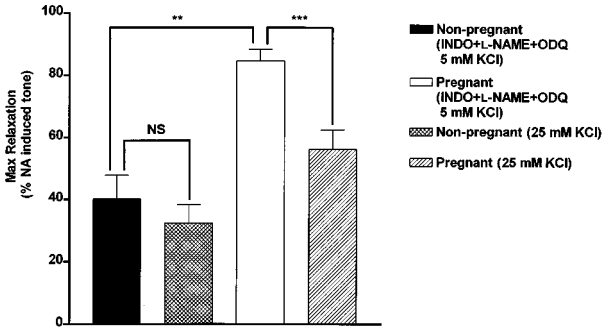

Figure 3 Comparison of maximal relaxation to ACh in arteries from pregnant (P) rats and non-pregnant (NP) rats in the presence of INDO, L-NAME and ODQ or in 25 mM KCl alone. In (NP) there was no significant difference in NO/PGI_2 mediated relaxation (in 25 mM KCl) or in EDHF mediated relaxation (+INDO, L-NAME and ODQ). In (P), EDHF mediated relaxation was greater than the NO/PGI_2 component ($^{***}P<0.001$) and greater than the EDHF component in (NP) ($^{**}P<0.01$).

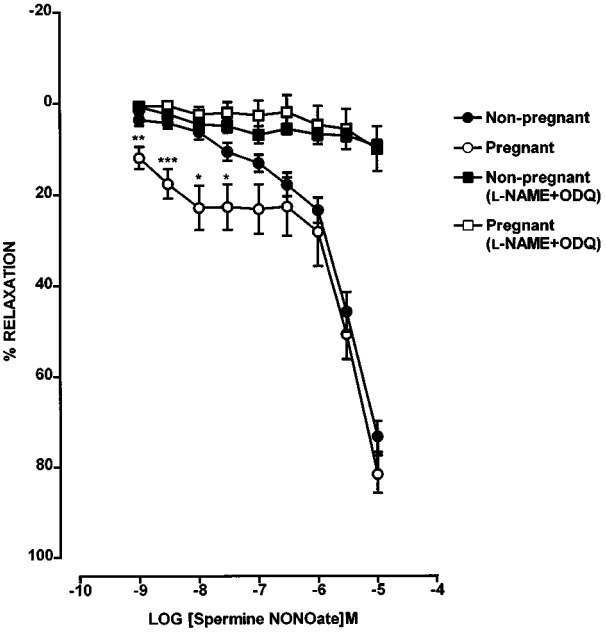

Figure 4 Concentration-response to spermine NONOate in mesenteric small arteries from pregnant (P) and non-pregnant (NP) rats in the absence (P: $n=8$; NP, $n=8$) and presence of L-NAME and ODQ in 5 mM K^+ (P: $n=8$; NP: $n=8$). pEC_{50} and maximal relaxation were similar between (P) and (NP), but significant differences were observed at lower concentrations. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

Discussion

The principal aim of the present study was to evaluate the potential role of an endothelium-derived hyperpolarizing factor (EDHF) in the adaptation of the vasculature to the state of

pregnancy. This was prompted by the observation that relaxation to ACh in arteries from pregnant rats (Pascoal *et al*., 1995; Bobadilla *et al*., 1997) and from pregnant women (McCarthy *et al*., 1994; Pascoal & Umans, 1996) is incompletely inhibited or totally unaffected by nitric oxide synthase (NOS) and cyclooxygenase (COX) inhibitors. For example, Pascoal *et al*. (1995) observed 38% of the relaxation to ACh to in pregnant rat mesenteric small arteries to be unaffected by the NOS inhibitor, L-NNA and we have reported that 60% of ACh relaxation remains in subcutaneous resistance arteries from normotensive pregnant women in the presence of NOS and COX inhibition, compared with 37% in arteries from non-gravid women (McCarthy *et al*., 1994). Using small omental microvessels Pascoal and colleagues, (1996; 1998) found all relaxation to ACh and bradykinin (BK) to be unaffected by NOS and COX inhibition in arteries from non-pregnant and normotensive pregnant women. In arteries from non-pregnant animals, a large literature describes how a similar lack of inhibition of endothelium dependent relaxation may reflect synthesis of an EDHF (Garland *et al*., 1995). Many of these studies have been carried out in isolated small mesenteric arteries mounted on a wire myograph, an identical preparation to that used in this study (Garland & McPherson, 1992). The role of an EDHF has been confirmed by simultaneous direct measurements of both endothelium dependent relaxation and smooth muscle membrane potential (Garland & McPherson, 1992). Participation of EDHF can also be evaluated by determining relaxation to endothelium dependent vasodilators in the presence and absence of partial depolarization (to prevent hyperpolarization) of the preparation. This method, used in this study, negates the action of an EDHF whilst unaltering endothelial production of other vasodilators (Parsons *et al*., 1994; Adeagbo & Triggle, 1993).

The increased sensitivity to ACh observed in the small mesenteric arteries of the pregnant rats agrees with an earlier report in Sprague Dawley rats (Pascoal *et al*., 1995). Increased sensitivity to endothelium dependent dilators has previously been observed in studies of resistance sized arteries in human pregnancy (Knock *et al*., 1996) and in the rat (Davidge & MacLaughlin, 1992; Pascoal *et al*., 1995; Learmont *et al*., 1996). In a previous study from our laboratory enhanced maximum relaxation to bradykinin was observed in small mesenteric arteries from the pregnant Wistar rat (Learmont *et al*., 1996), but sensitivity and maximum relaxation to ACh was not significantly increased. The difference between that and the present study could reflect the difference in rat strain or, more likely, the greater variability in the response to ACh observed in the Wistar rat compared with the Sprague Dawley used in this study. Increased sensitivity to ACh has however, also been observed in large arteries including the mesenteric (Kim *et al*., 1994) and carotid arteries from the pregnant guinea-pig (Weiner *et al*., 1989) and appears, generally, to be characteristic of arteries in pregnancy.

In the presence of COX and NOS inhibition, maximal relaxation to ACh remained significantly greater in arteries from the pregnant animals than from non-pregnant animals, as we have observed previously in arteries from pregnant and non-pregnant normotensive women (McCarthy *et al*., 1994). Elimination of this residual relaxation in arteries from both pregnant and non-pregnant animals by partial depolarization to 25 mM KPSS strongly suggests the involvement of a hyperpolarizing factor, the synthesis or half life of which is increased in pregnancy. One previous study in small human omental arteries has suggested indirectly that an EDHF is synthesized in arteries from pregnant women. Pascoal and Umans, (1996) showed that BK induced relaxation was unaffected in both pregnant and non-pregnant women by NOS or COX blockade but inhibited when arteries were preconstricted by 125 mM potassium gluconate. Relaxation to BK and inhibition to potassium gluconate was similar in arteries from pregnant and non-pregnant women, but variable sensitivity to inhibitors of K_{Ca} channels was observed, leading to the speculation that a novel endothelium dependent factor, potentially a hyperpolarizing factor was synthesized in pregnancy. A recent study in rat thoracic and abdominal aorta has proposed that a hyperpolarizing factor may increase in pregnancy, as residual relaxation to ACh was greater in arteries from the pregnant animals in the face of NOS and COX inhibitions (Bobadilla *et al*., 1997). However, these authors did not demonstrate equivocally the presence of an EDHF, as no attempt was made to partially depolarize the preparation or to measure membrane potential.

The observation that maximal ACh induced relaxation was greater in arteries from the pregnant animals in the presence of 25 mM KCl, but in the absence of COX and NOS inhibition, would suggest that enhanced synthesis/efficacy of EDHF cannot alone explain the difference in relaxation to ACh observed in the 5 mM KCl. The combination of NOS and COX inhibitors completely blocked this dilatation, suggesting that increased NO or PGI_2 synthesis may play a role in the pregnant rats. There is considerable evidence that PGI_2 synthesis is enhanced in pregnancy, but that it is confined to discrete circulations particularly the uterine circulation (Poston *et al*., 1995). To our knowledge, there is no evidence that PGI_2 synthesis is increased in the rat mesenteric circulation. NO, on the other hand is considered to contribute substantially to the increase in endothelium dependent vasodilatation of pregnancy, the evidence for which has recently been reviewed (Sladek *et al*., 1997). In support of raised NO synthesis in the rat mesenteric circulation in pregnancy, we have shown enhanced, L-NAME sensitive, flow mediated dilatation in isolated perfused small arteries from pregnant rats (Learmont *et al*., 1996) and enhanced eNOS protein expression has been reported in the same arteries (Xu *et al*., 1996). We have also observed enhanced flow induced vasodilatation in small subcutaneous arteries from pregnant women, also inhibitable by L-NAME (Cockell *et al*., 1997). Whilst, there is substantial evidence for increased synthesis of NO in the pregnant rat mesenteric circulation, enhanced sensitivity to ACh could also arise from increased sensitivity of the smooth muscle to NO. Previous studies have shown no difference in sensitivity to sodium nitroprusside between arteries from pregnant and non-pregnant rats (Pascoal *et al*., 1995; Learmont *et al*., 1996; Ni *et al*., 1997) and between arteries from pregnant and non-pregnant women (McCarthy *et al*., 1994; Knock *et al*., 1996), but the use of sodium nitroprusside has been criticized as it leads to production of nitrovasodilators other than NO (Feelisch *et al*., 1991). In the present study no difference in EC_{50} or maximal response was observed to spermine NONOate, considered to be a pure NO donor, releasing NO on an almost equimolar basis (Ramamurthi & Lewis, 1997). However, the response in the arteries from the pregnant animals appeared bimodal and the significantly greater relaxation which occurred at the lower concentrations of spermine NONOate could reflect an element of enhanced sensitivity to low NO concentrations.

There is still some controversy as to whether EDHF is a distinct entity from NO, since in other vascular beds NO may hyperpolarize vascular smooth muscle either by direct activation of potassium channels (Bolotina *et al*., 1994) or

through cyclic GMP dependent pathways (Archer *et al*., 1994). In this study, in which guanylate cyclase was inhibited, it is theoretically possible that if NOS were to be ineffectively inhibited by L-NAME, residual NO could mediate cyclic GMP-independent hyperpolarization. This, rather than EDHF, could then account for the difference in responses to ACh in pregnant and non-pregnant rats in the presence of NOS and COX blockade. This possibility deserves attention in light of a recent report in the rabbit carotid artery indicating that L-NAME may be an inefficient inhibitor of NOS and that successful inhibition is achieved only upon addition of a second NOS inhibitor (Cohen *et al*., 1997). In that study, all residual relaxation to ACh in the presence of L-NAME was accounted for by residual synthesis of NO and NO-mediated hyperpolarization, and an EDHF was not implicated. This hyperpolarization could, however, have been cyclic GMP dependent as no attempt was made, as in the present study, to inhibit guanylate cyclase. Moreover, the investigation was carried out in rabbit carotid artery, whereas in the rat mesenteric circulation NO-mediated hyperpolarization appears to play little role in ACh induced relaxation, or in the relaxation to exogenous NO gas in noradrenaline preconstricted arteries (Garland & McPherson, 1992). Furthermore, unlike the rabbit carotid artery, the mesenteric circulation of the non-pregnant rat seems more responsive to NOS inhibition, as the addition of a second inhibitor has no additional inhibitory effect on endothelium dependent relaxation (Plane & Garland, 1996). In the present study, relaxation to spermine NONOate was completely inhibited by ODQ, indicating complete cyclic GMP dependence of NO mediated relaxation. This, together with the lack of NO-mediated hyperpolarization observed by Garland & McPherson (1992) in this vascular bed renders a role for cyclic GMP independent hyperpolarization by NO extremely unlikely. The efficacy of ODQ to inhibit spermine NONOate induced relaxation is strikingly different from an observation in small mesenteric arteries using SIN-1 as an NO donor, in which complete insensitivity to ODQ was observed (Plane *et al*., 1996). SIN-1 is known, however, to produce peroxynitrite and oxygen free radicals which in turn may evoke cyclic GMP independent relaxation (Feelisch *et al*., 1991). This could potentially explain the difference between NO-mediated relaxation in that and the present study.

The present study has suggested that NO/PGI_2 and EDHF are increased in the arteries from the pregnant rat. However, the sum total of the EDHF and NO/PGI_2 components of ACh induced relaxation, as determined independently in the arteries from the pregnant rat, by inhibition of NO/PGI_2 or by partial depolarization was greater than the maximal relaxation to ACh without inhibitors. This was not the case for the non-pregnant arteries. It appears therefore that in the pregnant rat, when NO/PGI_2 is inhibited, EDHF synthesis increases and *vice versa* (for comparison of maximal responses in the presence of inhibitors or 25 mM KCl, see Figure 3). Despite this apparent interplay, the EDHF component of ACh induced relaxation was enhanced compared to that of NO/PGI_2. It is of interest that Bauersachs *et al*. (1996) have previously reported a functional interaction between NO and EDHF in non-pregnant rabbit carotid and porcine coronary arteries which was substantiated by measurement of the membrane potential. Likewise, McCulloch *et al*. (1997) observed an increase in NO-independent vasorelaxation in the presence of NO blockade in the perfused rat mesenteric bed.

EDHF may be a product of the cytochrome P450 pathway (Chen *et al*., 1996). The observation that the cytochrome P450 inhibitor, proadifen, significantly reduced cyclooxygenase and NOS-independent relaxation to ACh in the pregnant group, suggested that endothelium dependent hyperpolarization in the arteries from the pregnant animals could be due to the action of a cytochrome P450 derivative. This agrees with a recent study in pregnant rat aorta in which clotrimazole (a cytochrome P450 inhibitor) abolished any residual relaxation both in pregnant and non-pregnant aortas (Bobadilla *et al*., 1997). However, proadifen and clotrimazole are weak inhibitors of K^+ channels (Edwards *et al*., 1996) and may directly prevent relaxation through K^+ channel inhibition, rather than by blockade of EDHF synthesis. In relation to the suggestion that EDHF may be a fatty acid derivative, it is notable that pregnancy is associated with increased plasma concentrations of many fatty acids (Wang *et al*., 1991) which may provide potential for enhanced synthesis of EDHF. Moreover, the fatty acid profile is abnormal in preeclampsia (Wang *et al*., 1991), a condition associated with reduced relaxation to ACh (McCarthy *et al*., 1993; Pascoal *et al*., 1998).

In conclusion, EDHF synthesis/reduced degradation could play a role in lowering of vascular tone in the mesenteric circulation of pregnant rats. Investigations in other vascular beds, including those of pregnant women, together with direct measurement of the membrane potential are required to determine the relative importance of EDHF in relation to other vasodilators in the adaptation of the cardiovascular system to the state of pregnancy.

The authors would like to thank the Special Trustees of St. Thomas' Hospital, London, U.K. and Tommy's Campaign, London, U.K. for supporting this work.

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(Received May 11, 1998
Revised June 24, 1998
Accepted July 1, 1998).