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Mesenteric arterial function in the rat in pregnancy: role of sympathetic and sensory-motor perivascular nerves, endothelium, smooth muscle, nitric oxide and prostaglandins

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1 The effects of pregnancy on mesenteric arterial function were examined in constantly perfused (5 ml min^{-1}) mesenteric arterial beds isolated from 21-day pregnant rats. The function of sympathetic and sensory-motor perivascular nerves, endothelium and smooth muscle was examined. The role of nitric oxide and prostaglandins in vasoconstrictor function was tested by use of N^G-nitro-L-arginine methyl ester (L-NAME; 100 μM) and indomethacin (10 μM), respectively.

2 Electrical field stimulation (EFS; 4–32 Hz, 1 ms, 90 V, 30 s) at basal tone elicited frequency-dependent vasoconstriction which was markedly reduced in preparations from pregnant rats at all frequencies. Vasoconstrictor responses to vasopressin and endothelin were also reduced in pregnancy and there was a trend towards a reduction in maximal responses to noradrenaline (NA). In contrast, there was no difference in vasoconstrictor responses to ATP, 5-hydroxytryptamine (5-HT) or angiotensin II.

3 L-NAME (100 μM) augmented responses to EFS, NA, ATP and vasopressin in control mesenteric arterial preparations. In contrast, L-NAME augmented responses only to EFS in pregnancy, having no significant effect on responses to NA, ATP and vasopressin.

4 Indomethacin (10 μM) attenuated responses to NA and vasopressin, but not to EFS, in controls and in pregnancy. Responses to ATP were attenuated by indomethacin in controls but not in pregnancy.

5 Mesenteric preparations from pregnant rats were resistant to having tone raised by continuous perfusion with methoxamine. Despite an approximately 10 fold greater concentration of methoxamine, there was a significantly smaller increase in tone in preparations from pregnant, 34.27 $\pm$ 4.8 mmHg (n = 11) compared to control, 65.92 $\pm$ 5.4 mmHg (n = 11), rats. EFS (4–12 Hz, 60 V, 0.1 ms, 30 s) in the presence of guanethidine (5 μM) to block sympathetic neurotransmission elicited frequency-dependent vasodilatation due to activation of sensory-motor nerves. Percentage relaxations were similar in preparations from pregnant and non-pregnant rats.

6 Dose-dependent endothelium-dependent vasodilatations to acetylcholine and ATP were similar in preparations from pregnant and non-pregnant rats. Endothelium-independent vasodilatation to sodium nitroprusside and to calcitonin gene-related peptide were also similar between the two groups.

7 There was no significant difference in the basal perfusion pressure of mesenteric arterial beds from control (21.3 $\pm$ 1.0 mmHg, n = 24) and pregnant (20.2 $\pm$ 1.2 mmHg, n = 23) rats. However, a step-wise increase in perfusate flow from 5 to 10, 15, 20 and 24 ml min^{-1} produced smaller increases in perfusion pressure in pregnancy compared to the controls. L-NAME (100 μM) or indomethacin (10 μM) had no significant effect on the relationship between flow and perfusion pressure.

8 The present results show that prejunctional changes are involved in blunted sympathetic vasoconstriction of rat mesenteric arteries in pregnancy. Non-specific postjunctional changes are implicated in the reduced constrictor responses to applied methoxamine, vasopressin and endothelin, but not to ATP. In contrast, sensory-motor nerves and endothelium-dependent and -independent vasodilatation was unchanged. The decrease in receptor-mediated mesenteric arterial constrictor responsiveness in pregnancy does not appear to be due to acute modulation by NO or prostaglandins, but may involve changes in the distensibility of the bed and/or changes in wall thickness.

Keywords: Pregnancy; sympathetic vasoconstriction; sensory-motor vasodilatation; endothelium; rat mesenteric arterial bed

Introduction

Pregnancy is associated with a decrease in systemic vascular resistance which maintains or reduces maternal blood pressure despite the marked increase in blood volume and cardiac output. Characteristically, responses to vasoconstrictors including noradrenaline (NA), angiotensin II (AII), and vasopressin are blunted *in vivo* and *in vitro* (Dogterom & De Jong, 1974; Paller, 1984; Massicotte *et al.*, 1987; Parent *et al.*, 1990; Chu & Beilin, 1993a; D'Angelo & Osol, 1993), although unchanged (Hart *et al.*, 1986) or even increased responses (Jansakul *et al.*, 1990) have also been reported. The mechanism(s) responsible for vasodilatation in pregnancy is not fully understood but is under intense investigation particularly because its malfunction may have a role in pre-eclampsia (Gant *et al.*, 1987).

Since blunted constrictor responses of vessels obtained from pregnant animals have been described *in vitro* in physiological solution this suggests that changes occurring within the blood vessel wall, involving the vascular smooth muscle, endothelium and/or perivascular nerves could be involved. Binding studies have shown that there may be changes in specific receptors in pregnancy (Brown & Venuto, 1986; Parent *et al.*, 1991): however, the non-specific decrease in vasoconstrictor responses to several agonists suggests that a general mechanism is involved. Prostaglandins and en-

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dothelium-derived relaxing factor (EDRF/nitric oxide (NO)) are potent vasodilators which may contribute to attenuated vasoconstriction during pregnancy (Paller, 1984; Conrad & Colpoys, 1986; Gant *et al.*, 1987; Paller *et al.*, 1989; Ahokas *et al.*, 1991; Davidge & McLaughlin, 1992; Chu & Beilin, 1993a,b). Induction of NO synthase (NOS) in early pregnancy and by oestrogen (Goetz *et al.*, 1994; Weiner *et al.*, 1994a,b) supports the concept of a role for NOS in smooth muscle adaptations in pregnancy. Enhanced endothelium-dependent vasodilatation has been described in rat isolated mesenteric arteries in pregnancy (Davidge & McLaughlin, 1992). However, the failure of an inhibitor of NOS to normalize vasoconstriction in these vessels suggested that decreased responsiveness to constrictors is not due to changes in acute modulation by NO (Davidge & McLaughlin, 1992; Chu & Beilin, 1993a,b). It has been suggested that products of the cyclo-oxygenase pathway are not involved in attenuated constriction of rat mesenteric arteries in pregnancy (Davidge & McLaughlin, 1992; Chu & Beilin, 1993b).

Rat mesenteric arteries are innervated by perivascular sympathetic nerves which mediate constriction, and by sensory-motor nerves which mediate vasodilatation via release of the sensory neuropeptide, calcitonin gene-related peptide (CGRP) (Kawasaki *et al.*, 1988). Although their role is not fully understood a decrease in rat mesenteric arterial sensory-motor nerves has been described in hypertension and aging (Kawasaki *et al.*, 1990; Li & Duckles, 1993).

The aim of this study was to examine whether the decrease in mesenteric vascular resistance known to occur in pregnancy is associated with changes in perivascular neurotransmission and/or an increase in endothelial and smooth muscle NOS or prostaglandins. Responses to electrical field stimulation (EFS) of sympathetic and sensory-motor nerves were examined in mesenteric arterial preparations from pregnant and non-pregnant rats. Responses to exogenous vasoconstrictors and to endothelium-dependent and -independent vasodilators were examined in the absence and presence of an inhibitor of NOS, N^G-nitro-L-arginine methyl ester (L-NAME), and an inhibitor of cyclo-oxygenase, indomethacin. The relationship between flow and perfusion pressure in preparations from control and pregnant rats was also examined.

Methods

Two-to-three-month-old female Sprague-Dawley rats were used in the study. Virgin females and 21-day pregnant rats (term, 22 days) were killed by carbon dioxide asphyxiation. Mesenteric arterial beds were isolated and set up for perfusion by the method of McGregor (1966) essentially as described previously (Ralevic *et al.*, 1993). The abdomen was opened and the superior mesenteric artery exposed and cannulated with a hypodermic needle. The superior mesenteric vein was severed, the gut dissected away and the preparation mounted on a stainless steel grid (7 × 5 cm) in a humid chamber. The preparation was perfused at a constant flow rate of 5 ml min^{-1} using a peristaltic pump (Cole Parmer Instruments). Perfusion was with Krebs solution of the following composition (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, $CaCl_2$ 2.52 and glucose 7.8, gassed with 95% O_2–5% CO_2 and maintained at 37°C. Responses were measured as changes in perfusion pressure (mmHg) with a pressure transducer (model P23, Gould) on a side arm of the perfusion cannula, and recorded on a polygraph (model 7D, Grass). The preparation was allowed to equilibrate for 30 min prior to experimentation.

Stimulation of perivascular nerves was achieved by passing a current between the cannulation needle and the wire grid on which the preparation rested. Sympathetic nerves were activated at basal tone by EFS (90 V, 1 ms, 4–32 Hz for 30 s); the resulting constrictor response was abolished by guanethidine (5 μM), confirming its sympathetic origin. Vasoconstrictor responses to doses (50 μl bolus injections) of NA, ATP and vasopressin were then established, with intervals between doses being 3–20 min depending on the time it took for tone to return to baseline. At the end of each experiment the perfusate flow rate was increased stepwise from 5 to 10, 15, 20 and 24 ml min^{-1}. The perfusion pressure attributable to the system alone was subtracted from that obtained with preparations attached at each flow rate. In each case at least one control and one pregnant preparation were run side-by-side and were treated similarly. In separate preparations the effects of L-NAME (100 μM) or indomethacin (10 μM) were assessed by their addition to the perfusate at the start of equilibration, allowing contact with the tissue for 30 min. These preparations were subject to the experimental protocol described above. Dose-response relationships for 5-HT, vasopressin, AII and endothelian were obtained in separate preparations from pregnant and non-pregnant rats.

In separate experiments the tone of the preparations was raised by the addition of methoxamine to the perfusate to a final concentration of 3–300 μM and vasodilator response-curves to increasing doses of acetylcholine (ACh), ATP and sodium nitroprusside (SNP) were established. Intervals between doses were determined by the time it took for tone to return to its preconstricted level.

In separate preparations, guanethidine (5 μM) was added to the perfusate at basal tone after 20 min equilibration and was present in the perfusate thereafter; the effectiveness of this treatment was confirmed after 10 min by establishing that vasoconstrictor responses to stimulation of sympathetic nerves were abolished. EFS (60 V, 0.1 ms, 1–12 Hz, for 30 s) elicited vasodilator responses due to activation of primary sensory afferents and release of sensory transmitter. These vasodilator responses could be abolished by capsaicin, confirming their sensory origin. Vasodilator dose-response curves to CGRP were also established.

Drugs were injected as bolus doses of 50 μl via an injection port proximal to the tissue. Constrictor responses to potassium chloride (KCl, 0.15 mmol), were established at the end of experiments at basal tone (following washout of methoxamine if included in the protocol) as a measure of receptor-independent contractile function of the vascular smooth muscle.

Drugs

Acetylcholine chloride, sodium nitroprusside, noradrenaline bitartrate, adenosine 5′-triphosphate (disodium salt), 5-hydroxytryptamine (creatinine complex), indomethacin, N^G-nitro-L-arginine methyl ester and methoxamine hydrochloride were obtained from Sigma, Poole, England. Calcitonin gene-related peptide, angiotensin II, arginine vasopressin and endothelin were from CRB U.K. Ltd., Cambridge, England. Guanethidine monosulphate (Ismelin) was from Ciba-Geigy, Horsham, West Sussex. All drugs were made up in distilled water, except for NA which was made up as a 10 mM stock solution in 0.1 mM ascorbic acid.

Data analysis

Vasodilator responses were calculated as a percentage of the methoxamine-induced increase in tone above basal tone. All results are expressed as the mean ± s.e. Data were analysed by analysis of variance with repeated measures, with *post hoc* analysis by Student's *t* test, with Bonferroni correction, to see where the differences lay. $P<0.05$ was taken as significant except where Bonferroni correction was applied, in which case $P<0.025$ was taken as significant.

Results

Animals

Control rats weighed 253.3 ± 3.7 g ($n=36$). The blotted weight of mesenteric arterial preparations from pregnant rats,

6.17±0.3 g (n=16) was significantly greater than that of preparations from the controls, 4.27±0.2 g (n=18).

Basal tone

There was no significant difference in basal perfusion pressure between preparations from non-pregnant (21.3±1.0 mmHg, n=24) and pregnant (20.2±1.2 mmHg, n=23) rats.

Vasoconstrictor responses mediated by sympathetic nerves

EFS (4–32 Hz, 1 ms, 90 V, 30 s) at basal tone elicited frequency-dependent vasoconstrictor responses of the mesenteric arterial beds due to activation of sympathetic nerves. Responses of mesenteric beds from pregnant rats were markedly smaller compared to those from non-pregnant rats at all frequencies (Figure 1a). Maximal constriction was reduced by 32.8%.

Vasoconstrictor responses to noradrenaline, ATP, 5-HT, angiotensin II, vasopressin, endothelin and KCl

There was a trend for maximal responses to NA to be reduced in preparations from pregnant rats although this did not reach statistical significance (Figure 1b). Responses to vasopressin and endothelin were markedly attenuated in pregnancy (Figure

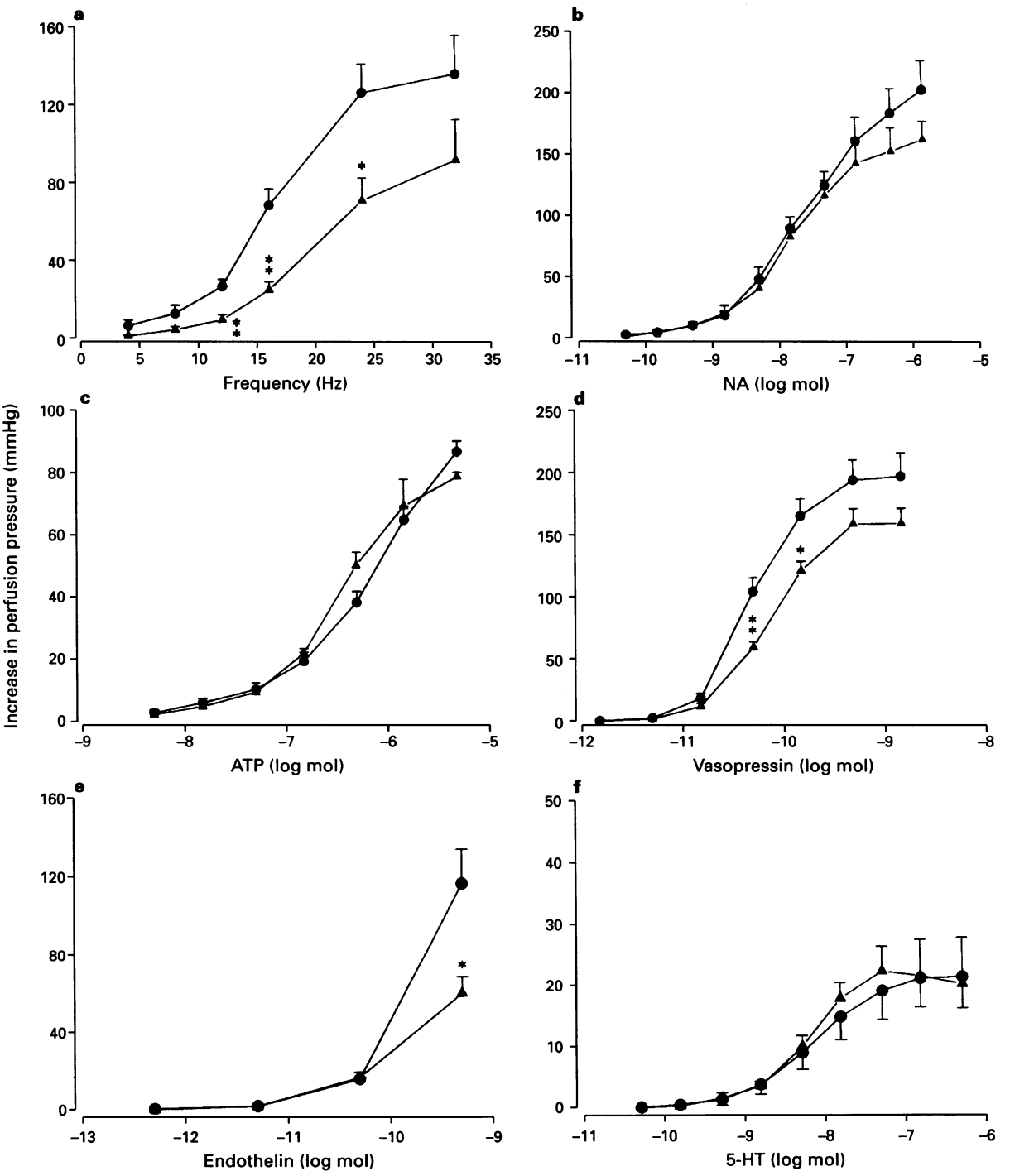

Figure 1 Vasoconstrictor responses of the rat mesenteric arterial bed at basal tone in controls (●, n=7) and pregnancy (▲, n=4) to: (a) electrical field stimulation (4–32 Hz, 90 V, 1 ms, 30 s); (b) noradrenaline (NA); (c) ATP; (d) vasopressin; (e) endothelin; (f) 5-hydroxtryptamine (5-HT). *P<0.05; **P<0.01.

1d,e). There was no significant difference in response to ATP (Figure 1c), 5-HT (Figure 1f) and AII (not shown). AII was a very poor constrictor of the preparations with maximal responses not exceeding approximately 10 mmHg. Vasoconstriction to 0.15 mmol KCl was also not different between the groups: 64.27±5.3 mmHg ($n=11$) and 60.82±7.6 mmHg ($n=11$) in preparations from control and pregnant rats respectively.

Effect of L-NAME on vasoconstrictor responses

L-NAME (100 μM) augmented vasoconstrictor responses to EFS and to NA, ATP and vasopressin in control preparatons (Figures 2, 3, 4 and 5). For NA and vasopressin this was manifested as an increase in the maximum constriction (Figures 3 and 5). In contrast, in pregnancy L-NAME significantly augmented responses only to EFS (Figure 2); L-NAME was without significant effect on responses to NA (except at a single dose of 5 nmol) (Figure 3), ATP (Figure 4) or vasopressin (Figure 5).

Effect of indomethacin on vasoconstrictor responses

Indomethacin (10 μM) significantly attenuated responses to NA (Figure 3) and vasopressin (Figure 5), but not to EFS (Figure 2), in controls and in pregnancy. Indomethacin had no significant effect on responses to ATP in pregnancy (Figure 4).

Raised tone

Mesenteric arterial beds from pregnant rats were refractory to the tone-increasing effects of continuous perfusion with methoxamine. A higher concentration of methoxamine was required in preparations from pregnant rats (0.22±1.8 mM, $n=11$) than in controls (14.5±2.4 μM, $n=11$) to produce a significantly smaller increase in tone: 34.27±4.8 mmHg ($n=11$) and 65.92±5.4 mmHg ($n=11$) in preparations from pregnant and control rats respectively. Because of this difference in tone, responses to sensory-motor nerve stimulation and exogenous vasodilators were expressed as a percentage of the methoxamine-induced increase in tone.

Vasodilator responses mediated by sensory-motor nerves

EFS (1–12 Hz) in raised-tone preparations in the presence of guanethidine (5 μM) elicited frequency-dependent vasodilatation. There was no significant difference in responses in pregnancy compared to control (Figure 6).

Vasodilator responses to acetylcholine, ATP, calcitonin gene-related peptide and sodium nitroprusside

Endothelium-dependent vasodilator responses to ACh and ATP were not significantly different in pregnant and control preparations (Figure 7a,b). Endothelium-independent vasodilator responses to CGRP and SNP were similar in mesenteric arterial preparations from control and pregnant rats (Figure 7c).

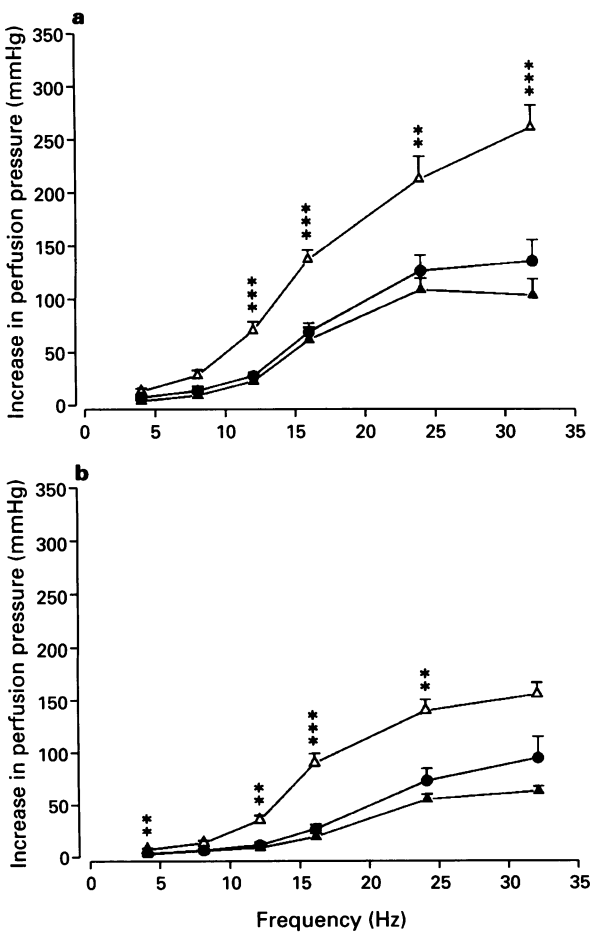

Figure 2 Effect of N^G-nitro-L-arginine methyl ester (△, 100 μM) or indomethacin (▲, 10 μM) on vasoconstrictor responses of the rat mesenteric arterial bed to electrical field stimulation (4–32 Hz, 90 V, 1 ms, 30 s) in (a) controls (●, $n=5-7$) and (b) pregnancy (●, $n=4-7$). $^{**}P<0.01$; $^{***}P<0.001$.

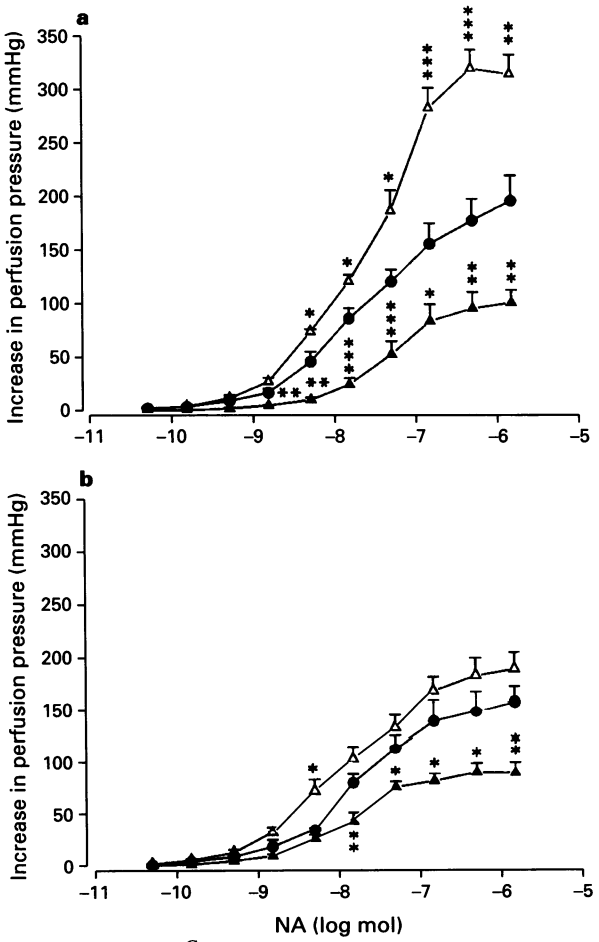

Figure 3 Effect of N^G-nitro-L-arginine methyl ester (△, 100 μM) or indomethacin (▲, 10 μM) on vasoconstrictor responses of the rat mesenteric arterial bed to noradrenaline (NA) in (a) controls (●, $n=5-7$) and (b) pregnancy (●, $n=4-7$). $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

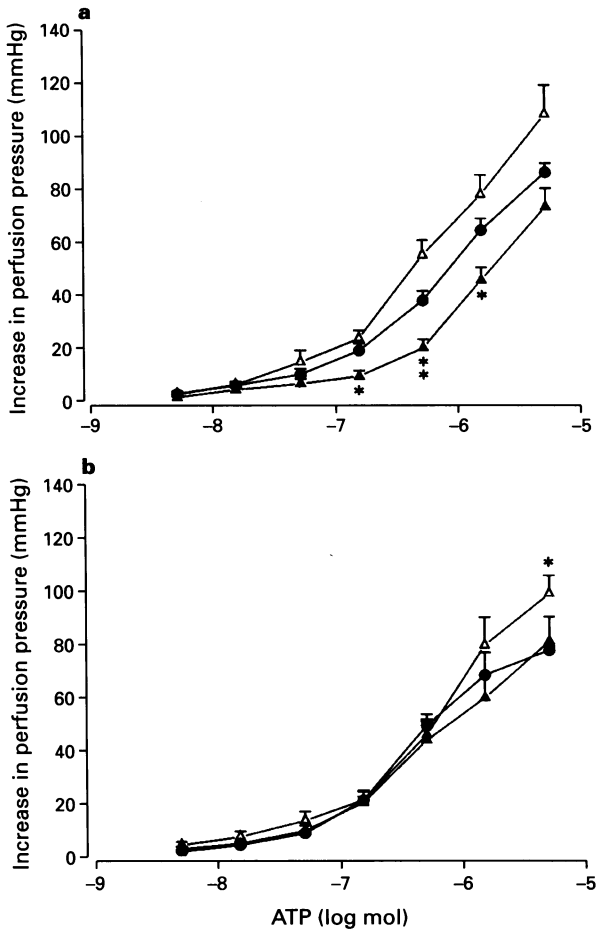

Figure 4 Effect of N^G-nitro-L-arginine methyl ester (△, 100 μM) or indomethacin (▲, 10 μM) on vasoconstrictor responses of the rat mesenteric arterial bed to ATP in (a) controls (●, $n=5-7$) and (b) pregnancy (●, $n=4-7$). $^*P<0.05$; $^{**}P<0.01$.

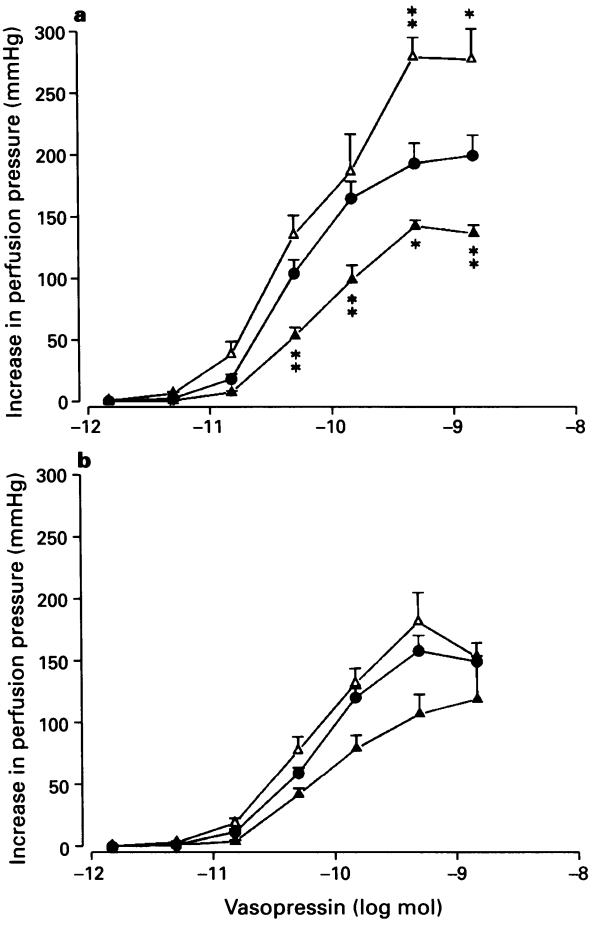

Figure 5 Effect of N^G-nitro-L-arginine methyl ester (△, 100 μM) or indomethacin (▲, 10 μM) on vasoconstrictor responses of the rat mesenteric arterial bed to vasopressin in (a) controls (●, $n=5-7$) and (b) pregnancy (●, $n=4-7$). $^*P<0.05$; $^{**}P<0.01$.

Effect of flow

There was no significant difference in basal perfusion pressure between preparations from controls 21.3 ± 1.0 mmHg ($n=24$) and pregnant rats, 20.2 ± 1.2 mmHg ($n=23$). However, increases in perfusion pressure with increases in flow up to 24 ml min^{-1} were smaller in preparations from pregnant rats than in those from controls (Figure 8). Neither L-NAME (100 μM) nor indomethacin (10 μM) had any effect on the relationship between perfusion pressure and flow in either of the two groups (Figure 8).

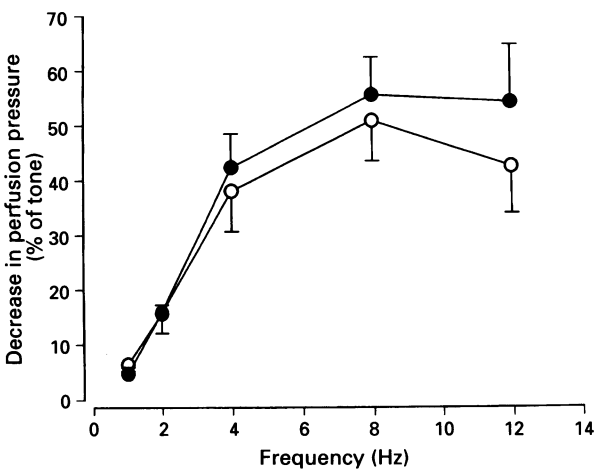

Figure 6 Vasodilator responses of the rat mesenteric arterial bed to electrical field stimulation of sensory-motor nerves (1–12 Hz, 60 V, 0.1 ms, 30 s) at rasised tone and in the presence of guanethidine (5 μM): (●) controls ($n=6$); (○) pregnancy ($n=6$).

Discussion

The main aim of this study was to see if there is a role for perivascular nerves, the vascular endothelium, smooth muscle, NO and/or prostaglandins in the decrease in peripheral vascular resistance and blunted constrictor responses which occur in pregnancy.

Changes in perivascular innervation in pregnancy have been described in the uterine artery of the guinea-pig, involving a decrease in NA-, and an increase in CGRP-, substance P-, vasoactive intestinal peptide- and neuropeptide Y-containing nerves (Bell & Malcolm, 1978; Tare *et al.*, 1988; Mione *et al.*, 1993). A decrease in sympathetic responses to EFS and in endogenous NA levels, and an increase in neuronally-mediated dilatation (mediated in part by NO) has been reported in pregnant human uterine arteries (Nelson *et al.*, 1995). However, neurogenic control of blood vessels other than uterine vessels has not been extensively investigated. In the present study there were no differences in the vasodilator function of sensory-motor nerves in control and pregnant rat mesenteric arteries. In contrast, constrictor responses mediated by sympathetic nerves were significantly blunted. Since responses to applied NA were not significantly different, prejunctional

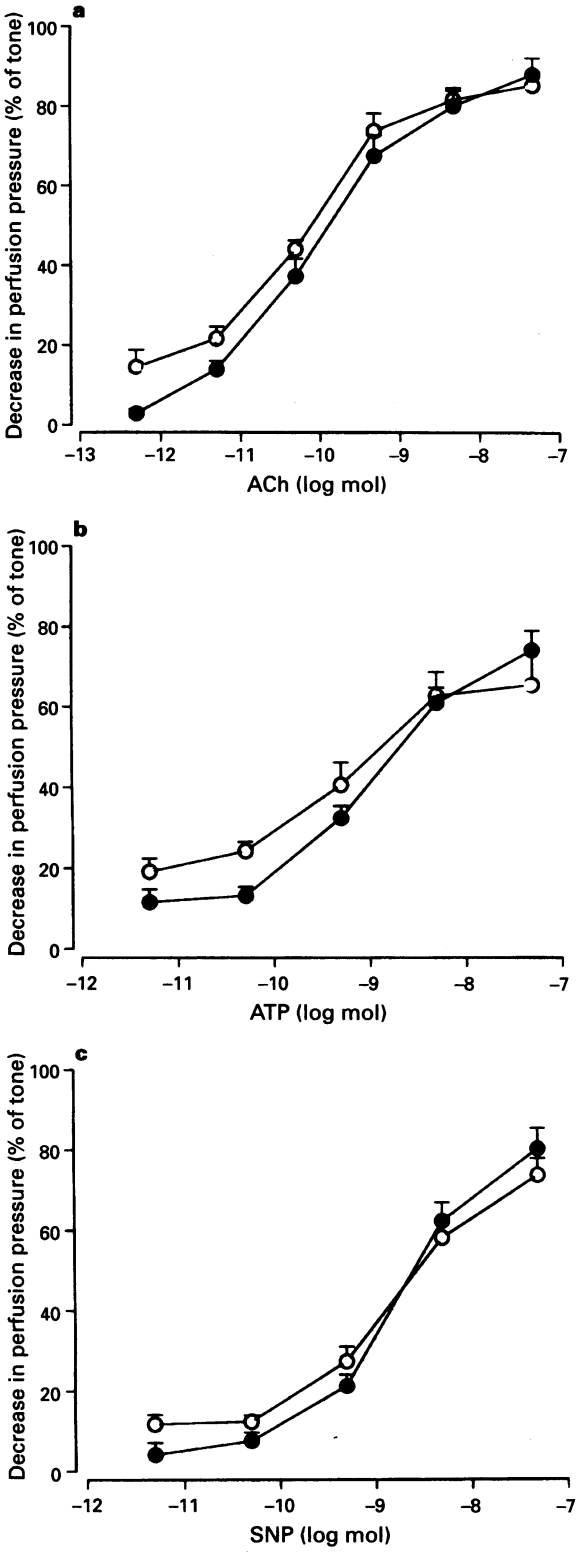

Figure 7 Vasodilator response of the rat mesenteric arterial bed to (a) acetylcholine (ACh), (b) adenosine 5'-triphosphate (ATP); (c) sodium nitroprusside (SNP); (●), controls ($n=6$); (○) pregnancy ($n=6$).

changes are likely to be involved. This may involve alterations in sympathetic transmitter content, release, uptake or degradation. It is also possible that the density of sympathetic innervation decreases relative to the increase in size of the mesenteric beds in pregnancy. A decrease in sympathetic neurotransmission in pregnancy has also been shown in rat mesenteric veins (Hohmann *et al.*, 1990). However, no change

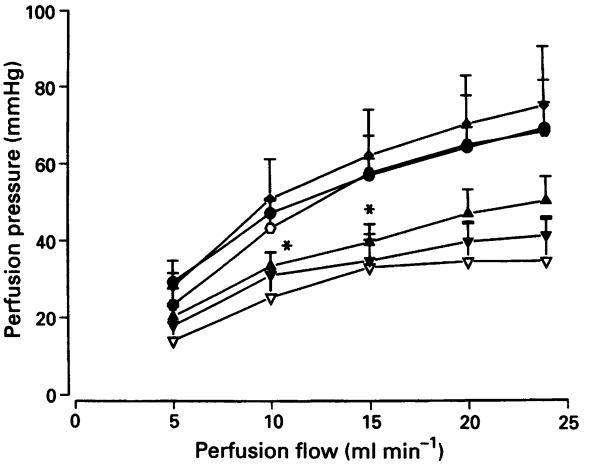

Figure 8 Relationship between increase in perfusate flow (ml min^{-1}) and perfusion pressure in mesenteric arterial beds from control ($n=5-7$) and pregnant ($n=4-7$) rats (values corrected for pressure attributable to the system alone). Curves were constructed in the absence of agents: (●) control ($n=7$); (▲) pregnant ($n=4$); in the presence of N^G-nitro-L-arginine methyl ester (100 μM): (◆) control ($n=6$); (▼) pregnant ($n=7$); in the presence of indomethacin (10 μM), (◇) control ($n=5$), (▽) pregnant ($n=5$). Differences between mesenteric arterial preparations from non-pregnant and pregnant rats are indicated by $^*P<0.05$.

in sympathetic responses, NA content and tyramine-induced contraction has been reported in rat isolated mesenteric arteries (Hart *et al.*, 1986; Yong *et al.*, 1992). Increased neuronal uptake of catecholamines in mesenteric arteries from late pregnant rats has been described (Crandall *et al.*, 1990).

Supersensitivity to NA as a consequence of reduced sympathetic innervation could explain the relative lack of blunting of responses to exogenous NA in pregnancy, in contrast to the substantially reduced responses observed to other similarly efficacious vasoconstrictors (vasopressin and endothelin). Concurrent supersensitivity to exogenous NA and depression of contractile responses mediated by electrical stimulation of sympathetic nerves has previously been reported in isolated capacitance-size rat mesenteric veins in pregnancy (Hohmann *et al.*, 1990).

Attenuation of responses to vasopressin and endothelin and the trend towards blunted maximal responses to NA, suggests that this phenomenon is not specific for a particular receptor, consistent with the findings of other workers (Massicotte *et al.*, 1987; Chu & Beilin, 1993a,b). The lack of differences between control and pregnant preparations to 5-HT and AII may be related to the fact that these were not particularly efficacious, maximal responses not exceeding 30 mmHg; differences between the control and pregnant preparations were more apparent when responses involved greater increases in perfusion pressure. However, this cannot account for the lack of attenuation of responses to ATP in pregnancy. ATP is a sympathetic cotransmitter and it is possible that, as discussed for NA, supersensitivity to ATP masks a blunting of the responses. However, more likely is the possibility that this is related to the mechanism of constrictor action of ATP. ATP elicits vasoconstriction via P_{2X}-purinoceptors on the smooth muscle which allow non-selective entry to cations and membrane depolarization. D'Angelo & Osol (1993) reported that in pressurized rat mesenteric arterial segments in pregnancy there was no change in constriction following depolarization with KCl, despite a reduced sensitivity of responses to NA. In the present study responses to KCl (as well as those to ATP) were also unaffected by pregnancy. Hence, our results are broadly in agreement with the conclusion of these workers that changes in responsiveness in pregnancy are 'specific for receptor-mediated contraction and not for a receptor-independent mechanism such as depolarization' (D'Angelo & Osol, 1993). However,

our findings suggest that this statement should be modified to take into account the fact that some receptors cause membrane depolarization. These findings are not consistent with the observation that rat mesenteric arteries are hyperpolarized in pregnancy and show reduced responsiveness to receptor-independent stimuli (K^+) (Meyer *et al.*, 1993).

Basally-related NO is an important modulator of vascular tone as shown in control preparations by potentiation of constrictor responses with L-NAME. However, L-NAME significantly augmented responses only to EFS, but not to NA, vasopressin or ATP in pregnancy. Thus, acute modulation by NO (smooth muscle or endothelial) cannot account for reduced responses to constrictors in pregnancy. This is in agreement with a report showing that an inhibitor of NO did not normalize responses to all constrictors in rat isolated mesenteric arteries in pregnancy, suggested to be inconsistent with an acute modulatory effect of EDRF (Davidge & McLaughlin, 1992; Chu & Beilin, 1993b). Paradoxically, the general lack of effect of L-NAME in the current study points towards a decrease rather than an increase in NOS in pregnancy. A similar conclusion has been drawn by Griggs *et al.* (1993) in their study of rat renal interlobal arteries based on the smaller potentiation of responses to phenylephrine by NOS inhibition in pregnancy. In the current study, since the effect of L-NAME in controls was manifest primarily as an increase in the maximal response, it is possible that the attenuated maximal response in pregnancy presents an unsurmountable barrier to potentiation by L-NAME.

Acute modulation by prostaglandins also does not appear to play a significant role in blunted constrictor responses in pregnancy since there was a similar attenuation of responses by indomethacin in control and pregnant preparations. Indomethacin has previously been shown to attenuate responses to vasoconstrictors including NA and vasopressin in the non-pregnant rat isolated mesenteric arterial bed (Manku & Horrobin, 1976; Kondo *et al.*, 1980). Prostaglandin E_2 (PGE_2) reversed the effects of indomethacin and appeared to be the primary endogenous prostaglandin required to ensure full vasoconstrictor responses. Paradoxically, increased blood PGE_2 has been described in pregnancy (Chaudhuri *et al.*, 1982).

It has been suggested that vascular remodelling may contribute to the decrease in peripheral vascular resistance and blunted pressor responses in pregnancy. That changes in the mesenteric vasculature do occur in pregnancy is indicated by the greater wet weight of preparations from pregnant compared to non-pregnant rats. The shallower slope of the pressure-flow curves in pregnancy suggests that there may be an increase in the distensibility of the mesenteric arterial vasculature, which could contribute to the blunting of constrictor responses, although this may also be a reflection of the greater size of the beds. This is consistent with reports of an increase in the distensibility of isolated segments of rat mesenteric arteries in pregnancy, associated with a decrease in wall stiffness and decrease in collagen and elastin (McLaughlin & Keve, 1986; Mackey *et al.*, 1992). Neither L-NAME nor indomethacin affected the relationship between pressure and flow. Changes in distensibility are independent of wall thickness. Folkow *et al.* (1992) have suggested that a decrease in maximal constrictor responsiveness of perfused rat mesenteric arteries is a consequence of reduced wall thickness. The maximal constrictor response of the mesenteric arterial beds obtained in pregnancy (approximately 180 mmHg, in the presence of L-NAME) was markedly less than that of controls (approximately 320 mmHg, also in the presence of L-NAME). Thus, the implication of the current results is that mesenteric arteries of pregnant rats are thinner than those of non-pregnant rats.

In summary, based on the results at basal tone, acute modulation of mesenteric vasoconstrictor responses by NO and prostaglandins does not appear to account for blunting of these responses in pregnancy. An increase in distensibility and decrease in wall thickness may account for blunting of responses and reduced maximal vasoconstrictor responsiveness. However, an additional factor must explain the differential reduction in responses to vasopressin and endothelin but not to ATP, which may involve their ability to cause membrane depolarization.

At raised tone there was no augmentation of vasodilatation of the mesenteric arterial bed to the endothelium-dependent vasodilators ACh and ATP in pregnancy. This indicates a lack of change in receptor-stimulated EDRF/NO and endothelium-derived hyperpolarizing factor (Hwa *et al.*, 1994; Waldron & Garland, 1994). Receptor-stimulated release of NO is distinct from the basal release of NO from smooth muscle and endothelial cells, the role of which we tested using L-NAME at basal tone. Our findings are in contrast with results showing pregnancy-associated potentiation of endothelium-dependent relaxation of isolated rat mesenteric resistance vessels (Davidge & McLaughlin, 1992), and ring segments of guinea-pig isolated mesenteric arteries (Kim *et al.*, 1994). The reason for the discrepancy between these results is not clear.

The question of whether EDRF/NO is crucially involved in the decrease in systemic vascular resistance in pregnancy is still unresolved. Further, it is possible that the contribution of NO is heterogeneous in different organs. Enhanced endothelium-dependent relaxation to ACh has been shown in pregnancy (Weiner *et al.*, 1989; Davidge & McLaughlin, 1992; Goetz *et al.*, 1994; Kim *et al.*, 1994), and there is an increase in plasma levels and urinary excretion of the stable NO metabolite, nitrate, and of cyclic 3',5'-guanine monophosphate (the second messenger of EDRF-mediated vasodilatation) (Conrad & Vernier, 1989; Conrad *et al.*, 1993). Induction of constitutive NOS has been shown in pregnancy and with oestrogen in the rat and guinea-pig (Goetz *et al.*, 1994; Weiner *et al.*, 1994a,b). While our results at basal and at raised tone are not indicative of an acute role for NO, a trophic role for NO, and prostaglandins, in vascular remodelling in pregnancy cannot be excluded.

In conclusion, pregnancy causes blunted responses to constrictors in the perfused mesenteric arterial bed of the rat which cannot be explained by acute modulation by NO or prostaglandins. Sympathetic constriction is reduced and this involves a prejunctional mechanism. In contrast, there is no difference in sensory-motor or endothelium-dependent vasodilatation. Vascular remodelling, involving an increase in distensibility and reduced wall thickness in pregnancy may contribute to the decrease in constrictor responses in pregnancy.

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