Role of nitric oxide and cyclooxygenase products in controlling vascular tone in uterine microvessels of rats

P. R. Saha¹, N. L. Alsip^{1*}, M. K. Henzel³ and E. F. Asher²

¹Center for Applied Microcirculatory Research and Departments of ²Anesthesiology and ³Physiology, University of Louisville, School of Medicine, Louisville, KY 40292, USA

The importance of nitric oxide (NO) and dilator prostaglandins in uterine resistance arterioles was investigated. In pentobarbital anaesthetized rats at dioestrus-2, the uterine microcirculation *in vivo* was transilluminated by a fiberoptic probe and microvessels (circumferential arterioles) viewed by video microscopy. Arteriolar diameters were measured while increasing concentrations of acetylcholine (ACh), serotonin (5-HT), phenylephrine (PE), or angiotensin II (AII) were applied topically (suffused) over the uterus. Agonists were applied alone or with ibuprofen (IBU; cyclooxygenase inhibitor), *N*^ω-nitro-L-arginine (L-NA; nitric oxide synthase inhibitor) or both. Circumferential arterioles were dilated by ACh and 5-HT (10^{-8} – 10^{-4} mol l⁻¹) and constricted by PE (10^{-8} – 10^{-5} mol l⁻¹) and AII (10^{-11} – 10^{-7} mol l⁻¹). Suffusion of L-NA or L-NA with ibuprofen (10^{-4} mol l⁻¹ each) abolished ACh-induced dilation; ibuprofen alone blocked dilation at higher ACh concentrations. Serotonin-induced relaxation was significantly attenuated by L-NA alone or in combination with ibuprofen. Vasoconstriction induced by PE was enhanced by L-NA alone and L-NA with ibuprofen, but ibuprofen alone had no effect. In contrast, AII-induced constriction was enhanced significantly by ibuprofen or L-NA and further enhanced when both ibuprofen and L-NA were present. These results suggest that ACh can release either nitric oxide (NO) or cyclooxygenase products to cause uterine arteriolar dilation and that 5-HT-induced uterine microvascular relaxation is mediated via NO only. They also suggest that PE-induced vasoconstriction is attenuated by the release of NO but not cyclooxygenase products and that constrictor responses evoked by AII are attenuated by both NO and dilator prostaglandin release. Thus, both nitric oxide and dilator prostaglandins are important in the control of uterine microvessels.

Introduction

Mechanisms regulating resistance vessels are often different from those regulating larger conduit vessels. For example, *in vitro*, arginine vasopressin, endothelin, oxytocin and noradrenaline are significantly more potent on the smallest branches of the uterine arterioles (resistance vessels) than on the larger conduit vessels dissected from humans (Ekström *et al.*, 1991). In sheep, catecholamines (noradrenaline, adrenaline, dopamine and isoxsuprine) given *in vivo* showed more pronounced or opposite effects on the middle uterine artery versus its upstream supplier, the common internal iliac artery (Tabsh *et al.*, 1981). Serotonin has different actions depending on the vascular bed and the size of the vessel studied: it vasodilates small arterioles in skeletal muscle (Alsip and Harris, 1991, 1992), pial circulation (Edvinsson *et al.*, 1977), and heart (Lamping *et al.*, 1989), although it acts as a vasoconstrictor in these same organs on larger vessels. Since the smallest branches of the arteries are the resistance vessels,

they are specifically involved in the local regulation of uterine blood flow (Ekström *et al.*, 1991). *In vitro* studies have shown that the arcuate arteries in the uterus but not in the mesentery have an increased sensitivity to phenylephrine in late pregnant compared with virgin rats (D'Angelo and Osol, 1993), suggesting that the uterine vascular bed has a unique responsiveness. Therefore, study of the mediators governing the reactivity of uterine microvessels to various vasodilators and vasoconstrictors is important for understanding their roles in normal circumstances and in pathological conditions and pregnancy.

Endothelium-derived nitric oxide (NO) and prostaglandin products of the cyclooxygenase pathway might play a physiological role in maintaining the blood flow of the uterine circulation (Matsumoto *et al.*, 1992; Kimura *et al.*, 1995). Nitric oxide is synthesized in vascular endothelial cells from L-arginine and released in blood vessels in response to a number of hormones, as well as physiological stimuli, to cause relaxation of smooth muscle by increasing cyclic guanosine monophosphate concentrations. A L-arginine–nitric oxide–cyclic guanosine monophosphate system is present in the uterus of rats and humans (Yallampalli *et al.*, 1993, 1994; Buhimschi *et al.*, 1995). Acetylcholine-induced relaxation of

*Correspondence.

Revised manuscript received 24 September 1997.

ring segments of guinea-pig and human uterine arteries is mediated by nitric oxide (Tare *et al.*, 1990; Jovanovic *et al.*, 1994). In skeletal muscle and mesentery of rats, the resistance vessels are less dependent on nitric oxide release to maintain resting tone and for acetylcholine-induced dilation than are larger vessels (Alsip and Harris, 1992; Hester *et al.*, 1993; Adeagbo *et al.*, 1994; Schuschke *et al.*, 1994). Thus, nitric oxide activity in the uterine resistance vessels may not be the same as in larger uterine or other arteries.

Prostaglandin products of the cyclooxygenase pathway are synthesized and released by the uterus (Magness *et al.*, 1992; Magness *et al.*, 1996; Yoshimura *et al.*, 1991) and are thought to be involved in regulating uterine blood flow (Still and Greiss, 1978; Clark *et al.*, 1981; Kimura *et al.*, 1995), although their role in the control of uterine blood flow is still unclear especially in the pregnant state (Magness *et al.*, 1992; Woods, 1993). Much of this work has been done on isolated uterine vessels so the activity of prostaglandins on resistance vessels *in vivo* is still unclear.

Until recently, the results from non-uterine vascular beds or large conduit vessel studies *in vitro* have been extrapolated to the uterine microvascular control mechanisms. There are no studies indicating whether *in vivo* uterine microvascular responses to vasoactive agents are mediated through nitric oxide or products of the cyclooxygenase pathway. The development of a uterine microvascular preparation *in vivo* (Alsip *et al.*, 1996) allows direct measurement of the resistance arterioles, so that it is possible to determine the role of nitric oxide or cyclooxygenase products in mediating resistance vessel responses in this organ. We hypothesize that the responses of the uterine resistance arterioles will differ from that of large uterine vessels and other vascular beds. The purpose of the present study was to determine whether nitric oxide or dilator prostaglandins alone or in combination mediate or modulate the diameter changes of uterine resistance arterioles induced by acetylcholine, 5-HT, phenylephrine and angiotensin II.

Materials and Methods

Female Sprague Dawley rats (Harlan, Indianapolis, IN) were housed individually in a temperature and humidity controlled room of our American Association of Laboratory Animal Care approved animal care facility with a 12 h light:12 h dark cycle. They were fed standard laboratory chow and had access to distilled water *ad libitum*. All protocols were approved by the University of Louisville Institutional Animal Care and Use Committee. Animals arrived at our facility weighing 175–200 g (9–10 weeks old).

A vaginal lavage was performed on each animal once a day to determine the stage of oestrous cycle. Animals were monitored for at least two oestrous cycles and only those animals exhibiting regular 4 day oestrous cycles were used for this study. All experiments were performed on animals at dioestrous day 2. For the acute microvascular experiment, each animal was anaesthetized with an intraperitoneal injection of pentobarbital (50 mg kg⁻¹; Sigma Chemical Co., St Louis, MO) with supplemental doses (1/3 of original dose) given at intervals of 1 h. A carotid artery was cannulated to monitor

arterial blood pressure. A tracheotomy was performed to maintain a patent airway. Body temperature was monitored by a rectal probe and maintained between 37–38°C with a small animal heating pad.

This uterine preparation has been described by Alsip *et al.* (1996). An incision was made in the lower left quadrant of the abdomen and the uterine horn exteriorized. A small hole was made in the mesometrium and a piece of sterile occlusive dressing was inserted through the hole. The dressing was used to elevate the tissue slightly and prevent the uterus from slipping back into the abdominal cavity. Another piece of occlusive dressing, with an opening to expose the uterus, was applied over the entire incision area. This piece anchored the first piece in place and covered the rest of the exposed tissue of the abdomen. The occlusive dressing served three purposes: (1) it isolated the uterus from other abdominal organs, (2) it helped maintain body heat, and (3) it reduced water loss through evaporation from the open body cavity. The uterus was kept moist during surgery by dripping warmed Krebs' solution on the tissue. Care was taken to avoid stretching the uterus during surgery. The blood and nerve supplies to the uterus remained intact.

A small incision was made on the anti-mesometrial side near the cervical end of the left uterine horn, and the animal was transferred to a Plexiglas board. A fiberoptic probe (2 mm × 2 mm diameter) was inserted through the incision into the lumen of the uterus. A plastic ring, positioned around the uterus and sealed to the occlusive dressing with a stopcock grease, served as a reservoir for the suffusion solution (Krebs' solution). The rats were transferred to the stage of a microscope that was part of a closed circuit television system. The probe was connected to a fiberoptic light source and served as the source of illumination for microscopy.

The uterus was superfused with warmed Krebs' solution (in mmol l⁻¹: 25.5 NaHCO₃, 112.9 NaCl, 4.6 KCl, 2.55 CaCl₂ · 2H₂O, 1.19 KH₂PO₄, 1.19 MgSO₄ · 7H₂O, and 11.6 dextrose). All reagents were purchased from Sigma Chemical Co. (St Louis, MO). Nitrogen and carbon dioxide were bubbled into the solution. The bubbling rate was adjusted to maintain Krebs' solution at pH 7.4 and pO₂ at 40 mm Hg. Temperature of the solution was maintained at 38°C. A light directed towards the Krebs' reservoir was used to maintain the temperature of the solution within it and the uterine tissue. The surface temperature of the uterus was measured with a miniature thermistor and was maintained at 37° ± 1°C.

Vessel selection

Measurements of the circumferential arterioles were taken. The circumferential arterioles run dorsally and ventrally around the uterus between the circular and longitudinal muscle layers and are easily identifiable since they are the only arterioles in the body of the uterus paired with venules. Branches from the circumferential arterioles supply the myometrium and endometrium. All experiments were recorded on videotape for later analysis. Vessel diameters were measured from the video monitor with calipers. The system was calibrated daily with a stage micrometer.

Protocol

Arterial pressure, heart rate (triggered by arterial pressure wave), rectal temperature and uterine surface temperature were monitored constantly. The animals were monitored for 30 min to ensure stable physiological parameters. Experimental protocols were not initiated until all physiological parameters were stable. During this time, vessels were selected for observation. Data from animals that exhibited an unstable or low (mean pressure less than 80 mm Hg) arterial pressure or unstable baseline arteriolar diameters were not included in data analysis. Less than 5% of attempted experiments were excluded by these criteria.

After the stabilization period, a 10 min baseline period was recorded. One of four treatment solutions was then added to the suffusion solution. Saline (control), *N*^o-nitro-L-arginine (L-NA, 100 $\mu\text{mol l}^{-1}$; a nitric oxide synthase inhibitor), ibuprofen (IBU, 100 $\mu\text{mol l}^{-1}$; a cyclooxygenase inhibitor) or both L-NA and IBU (L-NA + IBU, 100 $\mu\text{mol l}^{-1}$ each). The treatments (saline, L-NA, IBU or both L-NA + IBU) were applied for 20 min before starting the concentration–response curve and remained in the suffusion solution during the application of the agonist. After 20 min, arteriolar diameters were recorded for 3 min. Concentration–response curves for acetylcholine (ACh; 10^{-8} – 10^{-4} M), serotonin (5-HT; 10^{-8} – 10^{-5} M), phenylephrine (PE; 10^{-8} – 10^{-4} mol l^{-1}), or angiotensin II (All; 10^{11} – 10^{-7} mol l^{-1}) were then obtained by adding a single agonist to the suffusate in increasing concentrations. Each concentration was suffused over the uterus for 10 min. Only one agonist was tested per animal. All agonists and inhibitors were from Sigma Chemical Co. (St Louis, MO).

The experimental groups were named according to the topical application of agonist (ACh, 5-HT, PE or All) and whether saline (control), L-NA, IBU or both (L-NA + IBU) were present. Concentration–response curves for ACh, 5-HT, PE and All were obtained in the presence of saline (control), L-NA, IBU, or L-NA + IBU. Only one concentration–response curve was obtained per animal. Results presented are from 80 animals.

In all groups, after the last agonist concentration and a washout period, the vasodilator papaverine (200 $\mu\text{mol l}^{-1}$) was added to the suffusate for 20 min to cause maximum dilation of the vessels. Only vessels with maximal diameters at least 50% above baseline diameters were included in data analysis. Vessels without basal tone indicated that the animals were too deeply anaesthetized. It was necessary to add isoproterenol (10^{-7} mol l^{-1}) to the suffusion solution to reduce the uterine muscle contractility caused by acetylcholine. The concentration of isoproterenol used in this study does not change the resting diameters or the response to the agonist tested (data not shown).

Vessel diameters were measured every 30 s during each 10 min observation period. All of the vessels observed reached a plateau diameter during the 10 min observation period, indicating that the agonist had sufficient time to diffuse into the vessels. The plateau vessel diameter value for each concentration was used for data analysis. Baseline vessel diameter was taken from averaged diameter readings over the last 3 min of the baseline period. Arteriolar diameters were measured for 3 min after saline, L-NA, IBU or both L-NA and IBU had been

suffused over the uterus for 20 min, to determine whether the addition of saline or the inhibitors altered vascular diameters.

Statistical analyses

The mean arterial pressure, heart rate and baseline arteriolar diameters of each group were averaged and compared by a one-way analysis of variance (ANOVA) followed by Newman–Keul's multiple range tests when appropriate. The baseline diameters were analysed by a paired Student's *t*-test. Log concentration–response curves were obtained for all four agonists alone (control) and in the presence of L-NA, IBU or both L-NA and IBU. Results were converted to percentages of the baseline diameter. Differences among treatments at each concentration of each agonist were compared using Newman–Keul's multiple range test after one-way analysis of variance (ANOVA) indicated that there was a significant difference. $P < 0.05$ was taken as the level of significance. Group data are reported as means $\pm$ SEM.

Results

Body weights at the time of experimentation ranged from 195 to 255 g (mean of 221 ± 4.7 g, $n = 70$). Mean arterial blood pressure, heart rate and baseline diameters of arterioles were averaged for each treatment group. The group means for arterial pressure ranged from 100 ± 8.3 to 122 ± 2.7 mm Hg; heart rate of the treatment groups ranged from 326 ± 53.3 to 409 ± 18.6 beats per min; average baseline arteriolar diameters ranged from 39 ± 0.6 to 64 ± 5.6 μm (mean of 51 ± 1.9 μm for all animals). There was no statistical difference between groups (one-way ANOVA) with any of these parameters. Mean arterial pressure and heart rate were not altered by the suffusion of any inhibitor or agonist.

The baseline diameters from all animals receiving L-NA were grouped and compared with the diameters of those same vessels after L-NA suffusion and the baseline diameters from all animals receiving ibuprofen were grouped and compared with the diameters of those same vessels after ibuprofen suffusion. The baseline diameters for all animals receiving L-NA and ibuprofen together were grouped and compared with the diameters of those same vessels after L-NA and ibuprofen suffusion. The baseline diameters were not different between the groups (Fig. 1). Suffusion of L-NA or L-NA with IBU caused significant uterine arteriolar constriction ($P < 0.05$). Suffusion of ibuprofen did not cause a significant change in arteriolar diameters.

Acetylcholine caused a concentration-dependent dilation of circumferential arterioles (Fig. 2) when applied topically (suffused) to the untreated uterus. The application of the nitric oxide synthesis inhibitor, L-NA alone or in combination with the cyclooxygenase inhibitor ibuprofen (Fig. 2) significantly constricted the uterine arterioles (see Fig. 1). Subsequent application of acetylcholine did not result in arteriolar dilation in these groups. Treatment with ibuprofen alone (Fig. 2) inhibited arteriolar dilation at higher (above $1 \mu\text{mol l}^{-1}$) but not lower concentrations of acetylcholine, although ibuprofen itself did not alter the diameters.

Fig. 1. A comparison of the diameters (μm) of circumferential arterioles before (baseline) and 20 min after suffusion of N^o -nitro-L-arginine (L-NA; $\square$), ibuprofen (IBU; $\blacksquare$), or L-NA and IBU together (L-NA + IBU; $\blacksquare$). *Significantly different from corresponding baseline vessels (Paired t -tests, $P < 0.05$). The diameters of the baseline groups were not different when compared by one-way ANOVA. $n = 20$; n refers to the number of animals (one vessel per animal) in each group.

Fig. 3. Concentration-response curves for serotonin (5-HT, 5-hydroxytryptamine) in rat uterine resistance arterioles in control animals ($\blacksquare$), and in the presence of N^o -nitro-L-arginine (L-NA 10^{-4} mol l^{-1} ; $\triangle$), ibuprofen (IBU, 10^{-4} mol l^{-1} ; $\diamond$) or both N^o -nitro-L-arginine and ibuprofen ($\bullet$). All agents were suffused over the uterus. Inhibitor: vessel diameters 20 min after suffusion of L-NA, IBU or both L-NA and IBU. Responses are expressed as a percentage of baseline diameter (mean $\pm$ SEM). *Significantly different from control and IBU groups ($P < 0.05$). $n = 5$; n refers to number of animals (one vessel per animal) in each group.

Fig. 2. Concentration-response curves of rat uterine resistance arterioles to acetylcholine in control animals ($\blacksquare$), in the presence of N^o -nitro-L-arginine (L-NA; 10^{-4} mol l^{-1} ; $\triangle$), ibuprofen (IBU; 10^{-4} mol l^{-1} ; $\diamond$) or of both L-NA and IBU ($\bullet$). All agents were suffused over the uterus. Inhibitor: vessel diameters 20 min after suffusion of L-NA, IBU or both L-NA and IBU. Responses are expressed as a percentage of baseline diameters (mean $\pm$ SEM). *Significantly different from control and IBU groups ($P < 0.05$). #Significantly different from control group ($P < 0.05$). $n = 5$ per group; n refers to number of animals (one vessel per animal) in each group.

Like acetylcholine, suffusion of 5-HT onto the surface of the uterus caused a concentration-dependent dilation of the circumferential arterioles in the untreated animals (Fig. 3). The presence of ibuprofen did not alter the arteriolar response to 5-HT, or baseline diameters (Fig. 3). Treatment with L-NA alone or in combination with ibuprofen caused a significant arteriolar constriction and prevented the response to 5-HT (Fig. 3).

Concentration-dependent constriction of circumferential arterioles occurred in response to phenylephrine application (Fig. 4). This constriction was not altered by the presence of

Fig. 4. Concentration-response curves for phenylephrine in rat uterine resistance arterioles in control animals ($\blacksquare$), and in the presence of N^o -nitro-L-arginine (L-NA, 10^{-4} mol l^{-1} ; $\triangle$), ibuprofen (IBU, 10^{-4} mol l^{-1} ; $\diamond$) or L-NA and IBU combined ($\bullet$). All agents were suffused over the uterus. Inhibitor: vessel diameters 20 min after suffusion of L-NA, IBU or both. Responses are expressed as a percentage of baseline diameter (mean $\pm$ SEM). *Significantly different from all other groups ($P < 0.05$). #Significantly different from control group only ($P < 0.05$). $n = 5$; n refers to number of animals (one vessel per animal) in each group.

ibuprofen (Fig. 4). In contrast, when either L-NA or both L-NA and ibuprofen were present, there was a significantly enhanced constriction in response to PE application (Fig. 4).

Like phenylephrine, angiotensin II suffused onto the uterus caused concentration-dependent constriction of circumferential arterioles (Fig. 5). Although ibuprofen itself did not alter the baseline diameters, angiotensin II-induced vasoconstriction of uterine resistance arterioles was significantly increased by

Fig. 5. Concentration-response curves for angiotensin II (AII) in rat uterine resistance arterioles in the absence (■), and presence of *N*^o-nitro-L-arginine (L-NA 10^{-4} mol l^{-1} ; △), ibuprofen (IBU; 10^{-4} mol l^{-1} ; ◇) or both L-NA and ibuprofen (10^{-4} mol l^{-1} each; ●). All agents were suffused over the uterus. Inhibitor: vessel diameters 20 min after suffusion of L-NA, IBU or both L-NA and IBU. Responses are expressed as a percentage of baseline diameters (mean $\pm$ SEM). *L-NA and L-NA + IBU values significantly different from control and IBU values ($P < 0.05$). #Control values significantly different from L-NA and L-NA + IBU values. IBU values significantly different from L-NA + IBU value. **Control and L-NA + IBU values significantly different from each other and from the other two groups. $n = 5$; n refers to the number of animals (one vessel per animal) in each group.

ibuprofen (Fig. 5). The presence of L-NA alone in the suffusion solution (Fig. 5) caused a significant constriction by itself and also enhanced the constrictor response to angiotensin II. The constrictor reactivity was enhanced further when both L-NA and ibuprofen were present in the suffusion solution (Fig. 5).

Discussion

Acetylcholine releases nitric oxide (NO) in many blood vessels, including the uterine artery, in several species (Altieri *et al.*, 1986; Koga *et al.*, 1989; Tare *et al.*, 1990; Jovanovic *et al.*, 1994). In the present study the concentration-dependent relaxation produced by ACh was abolished by a nitric oxide synthase inhibitor, *N*^o-nitro-L-arginine (L-NA). Surprisingly, in the presence of ibuprofen, arteriolar dilation to ACh in the uterus was normal at lower concentrations, but at higher concentrations the response to ACh was attenuated. These findings suggest that ACh-induced relaxation of uterine microvessels is mediated via both nitric oxide and by formation of the dilator prostaglandins. These findings are in contrast to reports that ACh-induced dilation in humans is mediated by nitric oxide but not by prostaglandins in the isolated uterine artery (Jovanovic *et al.*, 1994). The difference in our findings and this report may be attributed to studying the micro- versus macrovessels or to species differences. In agreement with the findings reported here, in the hamster cremaster muscle, both NO and prostaglandins contribute to ACh-induced dilation of arterioles (de Wit *et al.*, 1993).

Serotonin constricts isolated ring segments of the uterine artery in several species (Weiner *et al.*, 1992). In contrast, in the *in vivo* preparation used in the study reported here, 5-HT produced concentration-dependent dilation of circumferential arterioles of the uterus demonstrating that there are differences in the reactivity of uterine resistance vessels and larger vessels. These results are consistent with data from other microvascular studies in which 5-HT was shown to dilate the precapillary arterioles while constricting larger vessels in the same vascular bed (Edvinsson *et al.*, 1977; Lamping *et al.*, 1989; Alsip and Harris, 1991). L-NA attenuated 5-HT-induced dilation in the present study. Thus, it appears that nitric oxide does play a role in mediating the dilation to 5-HT in this preparation. Although 5-HT releases nitric oxide in many large arteries (Cocks and Angus, 1983; Cohen *et al.*, 1983) dilation of small arterioles in skeletal muscle induced by 5-HT is not mediated by endothelium-derived nitric oxide (Alsip and Harris, 1992). The opposite finding in the present study may reflect a difference in the type of 5-HT receptor present on uterine resistance vessels versus skeletal muscle resistance vessels and large vessels.

When either L-NA or both L-NA and ibuprofen were included in the suffusion solution, the vasoconstriction to phenylephrine was enhanced. In contrast, the presence of ibuprofen alone did not increase the constrictor response to phenylephrine. These results suggest that nitric oxide, but not prostaglandins, play a role in modulating the vasoconstriction to phenylephrine. These findings are in agreement with previous reports that noradrenaline, another α -adrenergic agonist, induces vasoconstriction which is reduced by the release of nitric oxide (Marin and Sanchez-Ferrer, 1990; Shepherd and Katusic, 1991). In isolated rings of uterine artery from non-pregnant guinea-pigs, noradrenaline-induced constriction is enhanced by the nitric oxide synthase inhibitor L-NMMA (Weiner *et al.*, 1991). Thus, as in larger arteries, in the uterine microcirculation, α -adrenergic constriction is modulated by the release of endothelial NO.

In the present study the concentration-dependent constriction of uterine circumferential arterioles in response to angiotensin II is consistent with previous reports that angiotensin II contracts the isolated human uterine artery in a concentration-dependent fashion (Poulsen *et al.*, 1994). The increased angiotensin II-induced constrictions in the presence of ibuprofen observed in this study agree with data collected from other microvascular beds where indomethacin, another cyclooxygenase inhibitor, potentiates angiotensin II-induced constrictions in the nonpregnant rat (Chu and Beilin, 1993). Uterine arteries from gravid sheep release significant amounts of PGI_2 in response to AII application (Maggness *et al.*, 1992).

Angiotensin II-induced constriction was also enhanced by including L-NA in the suffusion solution and the constriction was even greater in the presence of both ibuprofen and L-NA than with either agent alone. This finding suggests a co-release of nitric oxide and prostaglandins in response to angiotensin II in these vessels. In the uterine artery, angiotensin II increased PGI_2 in pregnant but not in nonpregnant sheep while it released nitric oxide in both pregnant and nonpregnant groups (Maggness *et al.*, 1996). These results differ from the present study since our data indicated that angiotensin II releases both nitric oxide and dilator prostaglandins in the nonpregnant rat. This could be a species difference, or it could indicate a

difference in sensitivity between resistance arterioles and larger arteries.

Our results must be interpreted with caution since they were conducted in anaesthetised animals. Assali *et al.* (1974) found that responses to angiotensin II (uterine blood flow and uterine vascular resistance) in pregnant sheep were different in anaesthetized (pentobarbital) versus chronically instrumented animals. These authors acknowledged that the chronically instrumented animal is not feasible in many situations. At this time, we have no other way of determining the vascular activity at this level of the circulation, *in vivo*. Thus, although our animals were anaesthetized, these data do yield valuable information about the uterine microcirculation.

In conclusion, both nitric oxide and dilator prostaglandins appear to play a major role in mediating the responses of uterine resistance vessels to vasoactive substances.

References

- Adeago ASO, Tabrizchi R and Triggie CR (1994) The effects of perfusion rate and N^G -nitro-L-arginine methyl ester on cirazoline and KCl-induced responses in the perfused mesenteric arterial bed of rats *British Journal of Pharmacology* **111** 13–20
- Alsip NL and Harris PD (1991) Receptor mediation of microvascular responses to serotonin in striated muscle *American Journal of Physiology* **261** H1525–H1533
- Alsip NL and Harris PD (1992) Serotonin-induced dilation of small arterioles is not mediated via endothelium-derived relaxing factor in skeletal muscle *European Journal of Pharmacology* **229** 117–124
- Alsip NL, Hornung JW, Saha PR, Hill JB and Asher EF (1996) A new technique for studying the uterine microvasculature in the rat *American Journal of Obstetrics and Gynecology* **175** 388–395
- Altieri RJ, Kiritsy-Roy JA and Catravas JD (1986) Acetylcholine induced contractions in isolated rabbit pulmonary arteries: role of thromboxane A_2 *Journal of Pharmacology and Experimental Therapeutics* **236** 535–541
- Assali NS, Brinkman CR, III and Nuwayhid B (1974) Comparison of maternal fetal cardiovascular functions in acute and chronic experiments in sheep *American Journal of Obstetrics and Gynecology* **120** 411–425
- Buhimschi I, Yallampalli C, Dong Y-L and Garfield RE (1995) Involvement of a nitric-oxide-cyclic guanosine monophosphate pathway in control of human uterine contractility during pregnancy *American Journal of Obstetrics and Gynecology* **172** 1577–1584
- Chu ZM and Beilin LJ (1993) Mechanisms of vasodilation in pregnancy: studies of the role of prostaglandins and nitric oxide in changes of vascular reactivity in the *in situ* blood perfused mesentery of pregnant rats *British Journal of Pharmacology* **109** 322–329
- Clark KE, Austin JE and Stys SJ (1981) Effects of bisenoic prostaglandins on the uterine vasculature of nonpregnant sheep *Prostaglandins* **22** 333–348
- Cocks TM and Angus JA (1983) Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin *Nature* **305** 627–630
- Cohen RA, Shepherd JT and Vanhoutte PM (1983) 5-Hydroxytryptamine can mediate endothelium-dependent relaxation of coronary arteries *American Journal of Physiology* **245** H1077–H1080
- D'Angelo L and Osol G (1993) Regional variation in resistance artery diameter responses to α -adrenergic stimulation during pregnancy *American Journal of Physiology* **264** H78–H85
- De Wit C, von Bismarck P and Pohl U (1993) Mediator role of prostaglandins in acetylcholine-induced vasodilation and control of resting vascular diameter in the hamster cremaster microcirculation *in vivo*. *Journal of Vascular Research* **30** 272–278
- Edvinsson L, Hardebo JE, Mackenzie ET and Stewart M (1977) Dual action of serotonin on pial arterioles *in situ* and the effect of propranolol on the response *Blood Vessels* **14** 366–371
- Ekström P, Lam P and Akerlund M (1991) Differences in vasomotor responses between main stem and smaller branches of the human uterine artery *Acta Obstetrica et Gynecologica, Scandinavica* **70** 429–433
- Hester RL, Eraslan A and Saito Y (1993) Differences in EDNO contribution to arteriolar diameters at rest and during functional dilation in striated muscle *American Journal of Physiology* **265** H146–H151
- Jovanovic A, Grbovic L and Tulic I (1994) Predominant role for nitric oxide in relaxation induced by acetylcholine in human uterine artery *Human Reproduction* **9** 387–393
- Kimura T, Okamura T, Yoshida Y and Toda N (1995) Relaxant responses to prostaglandins $\text{F}_{2\alpha}$ and E_2 of isolated human uterine arteries *Journal of Cardiovascular Pharmacology* **26** 333–338
- Koga T, Takata Y, Kobayoshi K, Taskishita S, Yamashita Y and Fujishima M (1989) Age and hypertension promote endothelium-dependent contractions to acetylcholine in the aorta of the rat *Hypertension* **14** 542–548
- Lamping K, Kanatsuka H, Eastham CL, Chilian W and Marcus M (1989) Non-uniform vasomotor responses of the coronary microcirculation to serotonin and vasopressin *Circulation Research* **52** 343–351
- Magness RR, Rosenfeld CR, Faucher DJ and Mitchell MD (1992) Uterine prostaglandin production in ovine pregnancy: effects of angiotensin II and indomethacin *American Journal of Physiology* **263** H188–H197
- Magness RR, Rosenfeld CR, Hassan A and Shaul PW (1996) Endothelial vasodilator production by uterine and systemic arteries. I. Effects of ANG II on PGI_2 and NO in pregnancy *American Journal of Physiology* **270** H1914–H1923
- Marin J and Sanchez-Ferrer CF (1990) Role of endothelium-formed nitric oxide on vascular responses *General Pharmacology* **21** 575–587
- Matsumoto T, Kanamaru K, Sugiyama Y and Murata Y (1992) Endothelium-derived relaxation of the pregnant and nonpregnant canine uterine artery *Journal of Reproduction Medicine* **37** 529–533
- Poulsen H, Sjöberg N-O, Stjernquist M and Zia E (1994) Atrial natriuretic peptide antagonizes the contractile effect of angiotensin II in the human uterine artery *Human Reproduction* **9** 1939–1943
- Schuschke DA, Miller FN, Lominadze DG and Feldhoff RC (1994) L-arginine restores cholesterol-attenuated microvascular responses in the rat cremaster *International Journal of Microcirculation* **14** 204–211
- Shepherd JT and Katusic ZS (1991) Endothelium-derived vasoactive factors: I. Endothelium-derived relaxation *Hypertension* **18** (Supplement III) III-76–III-85
- Still JG and Greiss FC (1978) The effect of prostaglandins and other vasoactive substances on uterine blood flow and myometrial activity *American Journal of Obstetrics and Gynecology* **130** 1–8
- Tabsh K, Nuwayhid B, Erkkola R, Zugaib M, Lieb S, Ushioda E, Brinkman CR, III and Assali NS (1981) Hemodynamic responses of the pelvic vascular bed to vasoactive stimuli in pregnant sheep *Biology of Neonate* **39** 52–60
- Tare M, Parkinson HC, Coleman HA, Neild TO and Dusting GJ (1990) Hyperpolarization and relaxation of arterial smooth muscle caused by nitric oxide derived from endothelium *Nature* **346** 69–71
- Weiner CP, Liu K-Z, Thompson LP, Herrig JE and Chestnut D (1991) Effect of pregnancy on endothelium and smooth muscle: their role in reduced adrenergic sensitivity *American Journal of Physiology* **261** H1275–H1283
- Weiner CP, Thompson LP, Liu K-Z and Herrig JE (1992) Pregnancy reduces serotonin-induced contraction of guinea-pig uterine and carotid arteries *American Journal of Physiology* **263** H1764–H1769
- Woods LL (1993) Role of angiotensin II and prostaglandins in the regulation of uteroplacental blood flow *American Journal of Physiology* **264** R584–R590
- Yallampalli C, Izumi H, Byam-Smith M and Garfield RE (1993) An L-arginine-nitric oxide-cyclic guanosine monophosphate system exist in the uterus and inhibits contractility during pregnancy *American Journal of Obstetrics and Gynecology* **170** 175–185
- Yallampalli C, Byam-Smith M, Nelson SO and Garfield RE (1994) Steroid hormones modulate the production of nitric oxide and cGMP in the rat uterus *Endocrinology* **134** 1971–1974
- Yoshimura T, Rosenfeld CR and Magness RR (1991) Angiotensin II and α -agonist. III. *In vitro* fetal-maternal placental prostaglandins *American Journal of Physiology* **260** E8–E13