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

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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 fibreoptic 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°-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. Vasocostriction 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 vasocostriction 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 vivo, 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 isoxsuprane) 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 Osof, 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
ring segments of guinea-pig and human uterine arteries is mediated by nitric oxide (Tare et al., 1996; Iovanovic 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 (Magne 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 (Magne 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 oestrus cycle. Animals were monitored for at least two oestrus cycles and only those animals exhibiting regular 4 day oestrus cycles were used for this study. All experiments were performed on animals at dioestrus day 2. For the acute microvascular experiment, each animal was anaesthetized with an intraperitoneal injection of pentobarbitale (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 fibroptic 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 fibroptic 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 NaHCO\(_3\), 112.9 NaCl, 4.6 KCl, 2.55 CaCl\(_2\), 2H\(_2\)O, 1.19 KH\(_2\)PO\(_4\), 1.19 MgSO\(_4\)·7H\(_2\)O, 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 pO\(_2\) 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\textsuperscript{\textcircled{\textit{N}}}-nitro-\textit{l}-arginine (l-NA, 100 µmol l\textsuperscript{-1}; a nitric oxide synthase inhibitor), ibuprofen (IBU, 100 µmol l\textsuperscript{-1}; a cyclooxygenase inhibitor) or both l-NA and IBU (l-NA + IBU, 100 µmol l\textsuperscript{-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\textsuperscript{-10}–10\textsuperscript{-4} M), serotonin (5-HT; 10\textsuperscript{-6}–10\textsuperscript{-5} M), phenylephrine (PE; 10\textsuperscript{-6}–10\textsuperscript{-5} mol l\textsuperscript{-1}), or angiotensin II (All; 10\textsuperscript{-11}–10\textsuperscript{-7} mol l\textsuperscript{-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 µmol l\textsuperscript{-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 anaesthetised. It was necessary to add isoproterenol (10\textsuperscript{-7} mol l\textsuperscript{-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–Keuls’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–Keuls’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 ± 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 ± 33.3 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 IBU with l-NA 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 dilatation 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 dilatation in these groups. Treatment with ibuprofen alone (Fig. 2) inhibited arteriolar dilatation at higher (above 1 µmol l\textsuperscript{-1}) but not lower concentrations of acetylcholine, although ibuprofen itself did not alter the diameters.
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 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...
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^°-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).

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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


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