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Characterization of oxytocin actions in guinea-pig isolated uterine artery: The effect of pregnancy

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Abstract

While the contractile effect of oxytocin on uterine artery has been reported, little is known about whether pregnancy affects the responsiveness of this artery to oxytocin. If it does, is it a consequence of changed endothelial function, as has been proposed for some other vasoconstrictors. Furthermore, the receptor subtypes involved in oxytocin action on uterine artery has not been yet determined. Therefore the purposes of this study were to (1) determine the receptor subtypes involved in oxytocin action in non-pregnant and pregnant guinea-pig uterine artery and to (2) determine whether possible changes in uterine artery sensitivity to oxytocin during pregnancy are due to altered endothelial function. Therefore, the effect of oxytocin on non-pregnant and pregnant guinea-pig uterine arterial rings with and without endothelium was investigated. In non-pregnant guinea-pig uterine artery oxytocin induced contraction ($pEC_{50} = 7.63$) with greater potency than in pregnant guinea-pig uterine artery ($pEC_{50} = 7.17$). Removal of the endothelium did not affect oxytocin-induced contractions, regardless of the pregnancy status. The uterine arteries did not respond to [Thr^4, Gly^7]oxytocin. In the preparations studied, [d(CH$_2$)$_5$Tyr(Me)^2]vasopressin and [d(CH$_2$)$_5$, D-Ile^2, Ile^4]vasopressin antagonized oxytocin action with the following pK_B values ([d(CH$_2$)$_5$Tyr(Me)^2]vasopressin versus [d(CH$_2$)$_5$, D-Ile^2, Ile^4]vasopressin): 8.24 versus 7.29 and 8.11 versus 7.17 for non-pregnant guinea-pig uterine artery with and without endothelium, respectively; 8.39 versus 7.25 and 8.35 versus 7.25 for pregnant guinea-pig uterine artery with and without endothelium, respectively. We suggest that, in uterine arteries, oxytocin induces contraction by activation of vasopressin V_{1A} receptors. The potency of oxytocin in uterine artery is decreased during pregnancy and this is not associated with altered endothelial function. © 1998 Elsevier Science B.V.

Keywords: Uterine artery; Oxytocin; Pregnancy; Endothelium; Vasopressin receptor

1. Introduction

Uterine blood flow is increased during pregnancy (Peeters et al., 1980) and this is attributed to an increased sensitivity of the uterine artery to vasodilators, on the one hand and decreased sensitivity to vasoconstrictors on the other (Weiner et al., 1989, 1991). It has been determined that uterine artery smooth muscle and endothelium, in both humans and guinea-pigs, can produce nitric oxide (NO) (Jovanović et al., 1994b,c,d, 1997b). Previous studies with endothelium-intact preparations showed that the pregnancy-associated increase of uterine blood flow is a consequence of increased basal and/or agonist-stimulated production of NO from the uterine artery vascular endothelium (Weiner et al., 1989, 1991, 1992). This hypothesis is not yet unequivocally accepted (Matsumoto et al., 1992; Jovanović et al., 1994a, 1995b,c, 1997b). For example, it has been shown that vasoactive factors derived from uterine artery endothelium do not influence vasopressin-induced contractions (Jovanović et al., 1995c), while NO is not involved in the endothelium-mediated pregnancy-associated decrease in the effects of noradrenaline and prostaglandin $F_{2\alpha}$ on uterine artery (Jovanović et al., 1995a; Grbović and Jovanović, 1996, 1997). However, it should be pointed out that endothelial dysfunction and the resulting increase in the sensitivity of uterine artery to vasoconstrictors may be an important step in the development of pre-eclampsia (for review see Vokaer, 1992). Indeed, inhibitors of NO synthase induce pre-eclampsia in animal

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models (Molnar and Hertelendy, 1992; Buhimschi et al., 1995).

As opposed to some other arteries in which oxytocin evokes relaxation (Katušić et al., 1986; Suzuki et al., 1992), this neurohormone induces contraction in the uterine artery (Ekesbo et al., 1991; Jovanović et al., 1997c). However, although the uterine artery is sensitive to oxytocin, the effect of pregnancy on the actions of oxytocin on this blood vessel has not yet been studied in detail. The underlying receptor mechanism of oxytocin action's in guinea-pig uterine artery is still unknown. Additionally, if pregnancy affects the responsiveness of this artery to oxytocin, is it a consequence of changed endothelial function, as has been suggested for noradrenaline and thromboxane (Weiner et al., 1991, 1992).

Therefore, taking all this into consideration, the purposes of this study were (1) to determine the receptor subtype involved in the action of oxytocin in non-pregnant and pregnant guinea-pig uterine artery and (2) to determine whether possible changes in uterine artery sensitivity to oxytocin during pregnancy are due to altered endothelial function.

2. Materials and methods

Adult female non-pregnant and pregnant (50 to 60 days gestation; term, 65–68 days gestation) guinea-pigs (700–900 g) were used in this study. The animals were stunned and decapitated.

2.1. Vascular preparations

The uterine artery, which originates in the pelvis, anastomoses with the uterine branch of the ovarian artery, forming a loop called the arcade artery. Along the arcade several secondary arteries arise that supply the uterine tissue. The right and left uterine arteries were carefully dissected free from surrounding fat and connective tissue and cut into 3-mm long circular segments. All vessel segments were immediately placed in Krebs–Ringer-bicarbonate solution. The endothelium was removed from some rings by gently rubbing the intimal surface with stainless steel wires. Ring preparations were mounted between two stainless-steel triangles in an organ bath containing 10 ml Krebs–Ringer-bicarbonate solution (37°C, pH 7.4), aerated with 95% O_2 and 5% CO_2. One of the triangles was attached to a displacement unit allowing fine adjustment of tension and the other was connected to a force-displacement transducer (Hugo Sachs K30). Isometric tension was recorded on a Hugo Sachs model MC 6621 recorder.

The preparations were allowed to equilibrate for about 1 h in Krebs–Ringer-bicarbonate solution. During this period the buffer in the organ baths was replaced with fresh (37°C) buffer solution every 15 min.

After 60 min, each ring was gradually stretched to the optimal point of the resting tension curve (non-pregnant: 4.5 mN; pregnant: 6.2 mN) as has been previously determined (Jovanović et al., 1994a, 1995b, 1997b). Once at their optimal length, the segments were allowed to equilibrate for 30 min before experimentation.

2.2. Experimental procedure

At the beginning of each experiment the vessel segment was exposed twice to a K^+-rich Krebs–Ringer-bicarbonate solution (126 mM KCl, achieved by exchanging the 118.3 mM NaCl with KCl). Only if the second contractile response to K^+ was equivalent in magnitude to the first response (variation less than 10%) was the preparation used for further experimentation. Subsequently, in order to confirm the presence or successful denudation of endothelium, the rings were precontracted with phenylephrine (80% of the response to K^+-rich Krebs–Ringer-bicarbonate solution, 0.2–0.6 μM) and thereafter challenged with acetylcholine (10 μM). On the basis of prior studies (Jovanović et al., 1994a, 1995b, 1997b), a relaxation greater than 80% or less than 20% of the maximal relaxation evoked by acetylcholine (maximal relaxation represented complete return to the resting tension from the contraction in response to phenylephrine) was indicative of a structurally intact or denuded endothelium, respectively, regardless of pregnancy status. Additionally, at the end of some experiments (10 of each), the condition of the endothelium was verified by Van Gieson's staining with iron hematoxylin and light microscopic examination of the intimal surface (Disbrey and Rack, 1970; Jovanović and Jovanović, 1997). The morphometrical analysis revealed that the outer diameters of non-pregnant and pregnant guinea-pig uterine artery were $9.6 \pm 0.7 \times 10^2$ and $11.9 \pm 0.8 \times 10^2$ μm, as has been already determined (Jovanović et al., 1997a).

Concentration–response curves for oxytocin or [Thr^4, Gly^7]oxytocin were made by adding increasing concentrations of these compounds once the previous concentration had produced its equilibrium response, or after 5 min if no response was obtained. Experiments followed a multiple curve design since separate experiments with all types of preparations ($n = 4$ for each) demonstrated that the first and second concentration–response curves for oxytocin were not significantly different. Therefore, the following protocol was used: (1) contraction in response to K^+-rich Krebs–Ringer-bicarbonate solution followed by three washes and a 30-min equilibration period; (2) contraction in response to K^+-rich Krebs–Ringer-bicarbonate solution followed by three washes and a 30-min equilibration period; (3) contraction in response to phenylephrine, addition of acetylcholine, followed by three washes and 30-min equilibration period; (4) concentration–response curve for oxytocin (used as the tissue control) or [Thr^4, Gly^7]oxytocin, followed by three washes, addition of the antagonist (only when oxytocin was used) and a 15-min equilibration

period (Katušić et al., 1984); (5) concentration–response curve for oxytocin.

Three segments of one vessel were used in experiments designed to examine the effect of an antagonist. Three different concentrations of antagonist were used, but with only one concentration of antagonist per ring.

2.3. Calculations and statistical analysis

Since the maximal contraction achieved with oxytocin and K^+-rich Krebs–Ringer-bicarbonate solution was not significantly different regardless of the presence or absence of the endothelium and pregnancy status (range 5.8–6.5 mN, for each group of experiments $n = 28$), the contraction induced by each concentration of oxytocin was expressed as a percentage of the maximal contraction in response to oxytocin itself and was used to make the concentration–response curves. The concentration of oxytocin eliciting 50% of its own maximum response (EC_{50}) was determined graphically for each curve by linear interpolation. The EC_{50} values are presented as pEC_{50} ($pEC_{50} = -\log EC_{50}$). The pA_2 ($-\log$ molar concentration of antagonist reducing the agonist response by a factor of two) values for vasopressin receptor antagonists were determined from a Schild plot (Arunlakshana and Schild, 1959), using vasopressin as the agonist. The concentration ratios (the ratio between the EC_{50} value for oxytocin in the presence and absence of an antagonist) at different antagonist concentrations for the different oxytocin/antagonist pairs were calculated for each experiment. Thus, the mean values of concentration ratios for a oxytocin/antagonist pair were plotted in a Schild diagram by regression analysis and pA_2 was obtained from the intercept of the regression line with the abscissa (Arunlakshana and Schild, 1959). The concentration ratios were also used to calculate a modified Schild plot with a slope of -1, to obtain an estimate of the pK_B value ($-\log$ dissociation constant of antagonist) (Tallarida et al., 1979). The significance of Schild plot linearity was tested by analysis of variance (Kenakin, 1987). The closeness of the slope to unity was tested by the *t*-test and was not considered different from unity if $P > 0.05$.

The results are expressed as means $\pm$ S.E.M. Unless otherwise stated, the letter *n* represents the number of animals examined. One-way analysis of variance (ANOVA) was used when more than two groups were analyzed. Statistical differences between two means were determined by the Student's *t*-test for paired or unpaired observations where appropriate. A value of $P < 0.05$ was considered to be statistically significant. The least-squares method was used for calculating linear regressions.

2.4. Drugs and solutions

The Krebs–Ringer-bicarbonate solution had the following composition (in mmol/l): NaCl, 118.3; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; CaEDTA, 0.026 and glucose, 11.1. The solution was continuously bubbled with 95% O_2 and 5% CO_2 resulting in pH 7.4 and the temperature was kept at 37°C. The following drugs were used: acetylcholine chloride, phenylephrine hydrochloride (Sigma, USA), oxytocin, [1-(β-mercapto-β,β-cyclopentamethylene-propionic acid), 2-(O-methyl) tyrosine]arginine-vasopressin ([d(CH_2)$_5$Tyr(Me)^2] vasopressin), [Thr^4, Gly^7]oxytocin, [1-(β-mercapto-β,β-cyclopentamethylene-propionic acid), 2-D-isoleucine, 4-D-isoleucine]arginine-vasopressin ([d(CH_2)$_5$, D-Ile^2, Ile^4]vasopressin), [1-(β-mercapto-β,β-cyclopentamethylene-propionic acid) and 2-ornithine]vasotocin ([d(CH_2)$_5$Tyr(OMe)^2, Orn^8]vasotocin) (Peninsula Laboratories, USA). Stock solutions of the drugs were freshly prepared every day. The drugs were dissolved in distilled water. All drugs were added directly to the bath in a volume of 100 μl and the concentrations given are the calculated final concentration in the bath solution.

3. Results

3.1. Effect of oxytocin

Oxytocin (5–320 nM) induced a concentration-dependent contraction of the non-pregnant and pregnant guinea-pig uterine arterial rings with an intact endothelium (Fig. 1). The concentration–response curves for oxytocin obtained with pregnant guinea-pig uterine arteries were significantly shifted to the right compared to those obtained with non-pregnant guinea-pig uterine arteries (non-pregnant: $pEC_{50} = 7.63 \pm 0.02$; $n = 28$; pregnant: $pEC_{50} = 7.17 \pm 0.01$, $n = 28$, $P < 0.01$). In both types of preparations removal of the endothelium did not affect the responses to oxytocin (non-pregnant: $pEC_{50} = 7.67 \pm 0.01$; $n = 28$; pregnant: $pEC_{50} = 7.20 \pm 0.01$, $n = 28$) (Fig. 1).

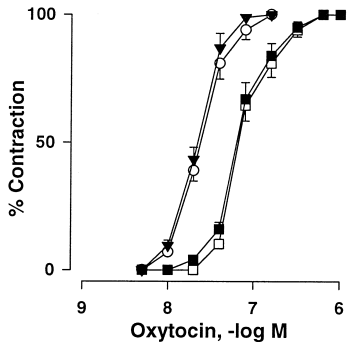

Fig. 1. Concentration–response curves for oxytocin in non-pregnant guinea-pig uterine artery with intact (○) and denuded endothelium (▼) and in pregnant guinea-pig uterine artery with intact (□) and denuded endothelium (■). Each point represents the mean ± S.E.M ($n = 28$). Responses are expressed as a percentage of the maximal contraction induced by oxytocin.

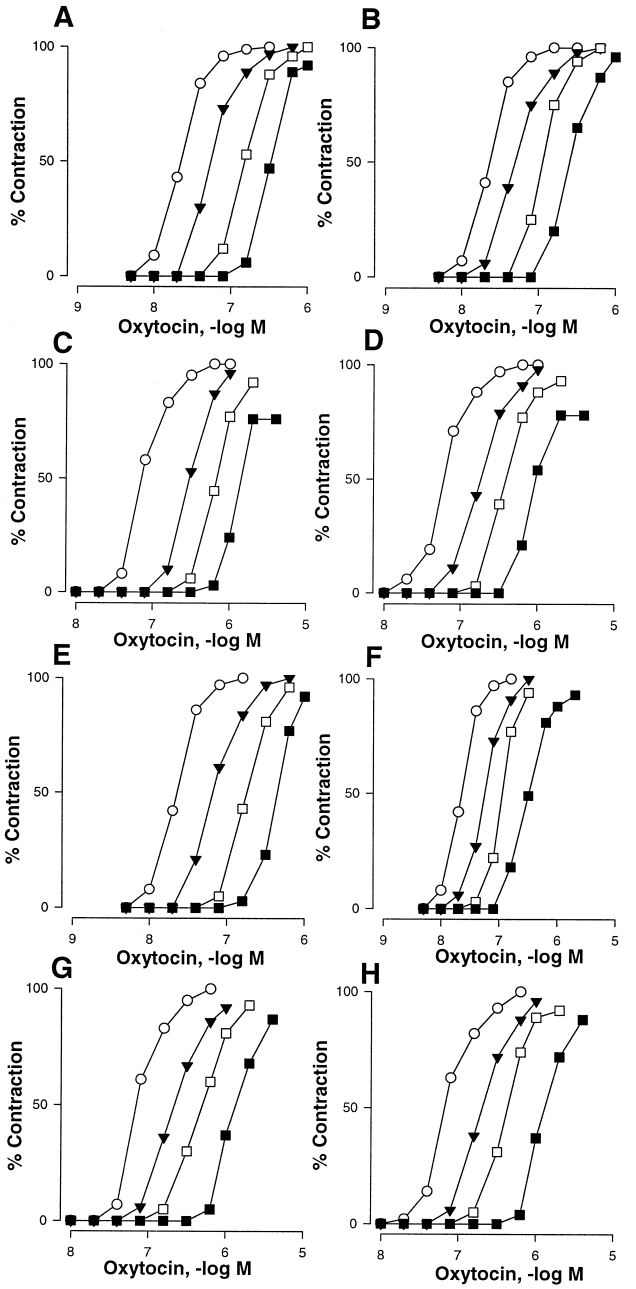

Fig. 2. Antagonism of the contractile effects of oxytocin by antagonists of vasopressin receptors. (A, B, C, D) Concentration–response curves for oxytocin in non-pregnant guinea-pig uterine artery with intact (A) and denuded endothelium (B) and in pregnant guinea-pig uterine artery with intact (C) and denuded endothelium (D) in the absence (○) and presence of 10 nM (▼), 30 nM (□) and 100 nM (■) [d(CH$_2$)$_5$Tyr(Me)^2]vasopressin. Each point represents the mean of 6–18 experiments. Standard errors (8–15% of the mean value for each point) are excluded for clarity. Responses are expressed as percentages of the maximal contraction induced by oxytocin. (E, F, G, H) Concentration–response curves for oxytocin in non-pregnant guinea-pig uterine artery with intact (E) and denuded endothelium (F) and in pregnant guinea-pig uterine artery with intact (G) and denuded endothelium (H) in the absence (○) and presence of 300 nM (▼), 1 μM (□) and 3 μM (■) [d(CH$_2$)$_5$, D-Ile^2, Ile^4]vasopressin. Each point represents the mean of 6–18 experiments. Standard errors (8–15% of the mean value for each point) are excluded for clarity. Responses are expressed as percentages of the maximal contraction induced by oxytocin.

3.2. Effect of [Thr^4, Gly^7]oxytocin

The non-pregnant and pregnant guinea-pig uterine arteries did not respond to the addition of [Thr^4, Gly^7]oxytocin (10 nM–1 μM), an selective oxytocin receptor agonist ($n = 4$ for each) (data not shown), regardless of the presence or absence of the endothelium.

3.3. Effect of [d(CH$_2$)$_5$Tyr(OMe)^2, Orn^8]vasotocin

[d(CH$_2$)$_5$Tyr(OMe)^2, Orn^8]Vasotocin, a selective antagonist of oxytocin receptors, applied in a concentration of 1 μM did not significantly affect contractions of guinea-pig uterine arteries evoked by oxytocin, regardless of the pregnancy status or the presence or absence of the endothelium (non-pregnant, with endothelium: pEC$_{50}$ = 7.70 ± 0.08 in the absence and pEC$_{50}$ = 7.60 ± 0.06 in the presence of [d(CH$_2$)$_5$Tyr(OMe)^2, Orn^8]Vasotocin; non-pregnant, without endothelium: pEC$_{50}$ = 7.61 ± 0.05 in the absence and pEC$_{50}$ = 7.61 ± 0.04 in the presence of [d(CH$_2$)$_5$Tyr(OMe)^2, Orn^8]Vasotocin; pregnant, with endothelium: pEC$_{50}$ = 7.09 ± 0.06 in the absence and pEC$_{50}$ = 7.10 ± 0.07 in the presence of [d(CH$_2$)$_5$Tyr(OMe)^2, Orn^8]Vasotocin; pregnant, without endothelium: pEC$_{50}$ = 7.14 ± 0.08 in the absence and pEC$_{50}$ = 7.18 ± 0.07 in the presence of [d(CH$_2$)$_5$Tyr(OMe)^2, Orn^8]Vasotocin, $n = 5$ for each, $P > 0.05$, data not shown).

3.4. Effects of vasopressin receptor antagonists

In both non-pregnant and pregnant guinea-pig uterine arteries, with either an intact or denuded endothelium, a vasopressin V$_1$ receptor-preferring antagonist, [d(CH$_2$)$_5$ Tyr(Me)^2] vasopressin (10–100 nM) and a vasopressin V$_2$

Table 1
pA$_2$, pK$_B$ values and slopes of Schild plots of the vasopressin receptor V$_1$ and V$_2$ antagonists on non-pregnant and pregnant guinea-pig uterine arteries, determined as their ability to antagonize the oxytocin-induced contraction of vascular segments

	pA_2	Slope	pK_B
Endothelium intact (non-pregnant)			
[d(CH$_2$)$_5$Tyr(Me)^2]vasopressin	8.40 ± 0.11	0.83 ± 0.10	8.24 ± 0.05
[d(CH$_2$)$_5$, D-Ile^2,Ile^4]vasopressin	7.30 ± 0.11	0.98 ± 0.12	7.29 ± 0.04
Endothelium denuded (non-pregnant)			
[d(CH$_2$)$_5$Tyr(Me)^2]vasopressin	8.27 ± 0.03	0.80 ± 0.03	8.11 ± 0.06
[d(CH$_2$)$_5$, D-Ile^2,Ile^4]vasopressin	7.22 ± 0.01	0.94 ± 0.01	7.17 ± 0.02
Endothelium intact (pregnant)			
[d(CH$_2$)$_5$Tyr(Me)^2]vasopressin	8.66 ± 0.09	0.78 ± 0.06	8.39 ± 0.07
[d(CH$_2$)$_5$, D-Ile^2,Ile^4]vasopressin	7.36 ± 0.03	0.88 ± 0.02	7.25 ± 0.03
Endothelium denuded (pregnant)			
[d(CH$_2$)$_5$Tyr(Me)^2]vasopressin	8.58 ± 0.04	0.79 ± 0.03	8.35 ± 0.06
[d(CH$_2$)$_5$, D-Ile^2,Ile^4]vasopressin	7.27 ± 0.01	0.97 ± 0.01	7.25 ± 0.01

The values are expressed as means ± S.E.M ($n = 6$).

receptor-preferring antagonist, [d(CH_2)$_5$, D-Ile^2, Ile^4] vasopressin (300 nM–3 μM), induced a significant shift to the right of the concentration–response curves for oxytocin in a concentration-dependent manner ($P < 0.01$, for both antagonists studied), without suppression of the maximum response ($P > 0.05$, for both antagonists studied) (Fig. 2). The data from the experiments with vasopressin receptor antagonists were analyzed as described by Arunlakshana and Schild (1959). In all types of preparations, the experiments with [d(CH_2)$_5$Tyr(Me)^2]vasopressin and [d(CH_2)$_5$, D-Ile^2, Ile^4]vasopressin yielded straight lines ($P > 0.05$, for both antagonists studied) with a mean slope not different from unity (Table 1). The pA_2 and pK_B values are shown in Table 1. The pK_B values for corresponding antagonists were not significantly different, regardless of the presence or absence of the endothelium or pregnancy status ($P > 0.05$).

4. Discussion

In the present study we confirmed previous findings that, in contrast to some other arteries (Katušić et al., 1986; Suzuki et al., 1992), oxytocin induces contraction of uterine arteries (Ekesbo et al., 1991; Jovanović et al., 1997c).

Many years ago, it was proposed that a decreased sensitivity to vasoconstrictors of the uterine artery during pregnancy mediated the increased blood flow to the uterus (Peeters et al., 1980). Indeed, in certain in vitro studies reduced responses to noradrenaline and thromboxane in uterine arteries during pregnancy have been reported (Weiner et al., 1991, 1992), but later studies did not confirm these findings (Steele et al., 1993; Jovanović et al., 1995a). In contrast, even an increased sensitivity of the uterine artery to α-adrenoceptor agonists and vasopressin in pregnancy has been reported (D'Angelo and Osol, 1993; Jovanović et al., 1995c). In the present study, pregnancy shifted the concentration–response curves for oxytocin to the right, suggesting a decreased sensitivity of the uterine artery to this hormone during pregnancy. In general, it is known that in certain blood vessels removal of the endothelium can potentiate the contractile responses of vascular smooth muscle to different vasoconstrictors (Alosachie and Godfraind, 1988; Randall et al., 1988), as opposed to some other arteries in which a lack of endothelium-dependent modulation has been shown (Katušić and Krstić, 1987; Jovanović et al., 1995c; Grbović et al., 1996). In prior investigations with preparations with an intact endothelium, it has been observed that the sensitivity to some vasoconstrictors is significantly reduced in pregnant guinea-pig uterine artery compared to that in non-pregnant guinea-pig uterine artery (Weiner et al., 1989, 1991, 1992; Grbović and Jovanović, 1996). In these studies, it was reported that removal of the endothelium augmented the contractile responses only in arteries from pregnant animals. On the basis of these results, Weiner et al. (1989, 1991, 1992) suggested that pregnancy-associated changes in endothelial function, such as increased basal production of NO, decrease the contractile response to this agent in uterine arteries. However, the results from other studies did not confirm this hypothesis (Steele et al., 1993; Jovanović et al., 1995a,c). For example, it has been shown that the vascular endothelium does not modulate vasopressin-induced contraction in either non-pregnant or pregnant guinea-pig uterine arteries (Jovanović et al., 1995c). In the present study, removal of the endothelium did not affect the response to oxytocin, regardless of the pregnancy status of animals from which the vessels were taken. This is not consistent with the concept that the production of endothelium-derived relaxing factor(s) is increased in the uterine artery during pregnancy, which in turn would result in a reduction of agonist potency and efficacy (Weiner et al., 1991, 1992). The effect of oxytocin on guinea-pig uterine artery, from this point of view, has not been studied yet, and, consequently, we can not compare our results with those of other studies. In spite of this, if the basal production of endothelium-derived relaxing factor(s) is increased during pregnancy, it seems logical that the oxytocin-mediated contractions would be augmented in pregnant guinea-pig uterine artery after removal of the endothelium. Since this was not the case, it is likely that the endothelium-associated decrease in the contractile response of the uterine artery during pregnancy is related to the agonist used, and that a decreased sensitivity of this vessel to oxytocin is not associated with changes in endothelial function.

The presence of oxytocin receptors has been demonstrated in certain vascular smooth muscle cell lines (Yazawa et al., 1996). In the present study, [d(CH_2)$_5$Tyr(OMe)^2, Orn^8]vasotocin, a selective oxytocin receptor antagonist (reviewed by Hruby, 1992), applied in a concentration clearly sufficient to block oxytocin receptors (Hong and Moody, 1991) did not significantly affect concentration–response curves for oxytocin, regardless of the presence or absence of the endothelium and pregnancy status. In addition, [Thr^4, Gly^7]oxytocin, a selective oxytocin receptor agonist (reviewed by Hruby, 1992) did not evoke any response in all types of vessels studied. These findings strongly argue against the possibility that oxytocin receptors are involved in the contractile effect of oxytocin in non-pregnant and pregnant uterine arteries with an intact or denuded endothelium. It has also been reported that in some blood vessels oxytocin induces contraction via activation of vasopressin receptors (Katušić et al., 1986; Briner et al., 1992). In order to examine this possibility we used [d(CH_2)$_5$Tyr(Me)^2]vasopressin, a vasopressin V_1 receptor-preferring antagonist (Kruszynski et al., 1980), and [d(CH_2)$_5$,D-Ile^2, Ile^4]vasopressin, a vasopressin V_2 receptor-preferring antagonist (Manning et al., 1983). The slopes of the Schild plots for [d(CH_2)$_5$Tyr(Me)^2]vasopressin and [d(CH_2)$_5$, D-Ile^2, Ile^4]vasopressin were not signifi-

cantly different from unity, indicating that the antagonism is competitive and therefore that the obtained pA_2 value constrained to unity can be calculated as the pK_B value (Arunlakshana and Schild, 1959). It is known that the K_B value for a specific antagonist acting on the same type of receptor in different preparations should be the same (Furchgott, 1972). In non-pregnant guinea-pig uterine arteries, affinity estimates for both antagonists were not different from the estimates obtained in pregnant guinea-pig uterine arteries, regardless of the presence or absence of the endothelium. Therefore, the possibility that different vasopressin receptor subtypes are involved in the oxytocin-induced contraction of pregnant and non-pregnant uterine arteries with an intact or denuded endothelium was eliminated. The pK_B values for [d(CH$_2$)$_5$Tyr(Me)^2]vasopressin obtained in our study (8.11–8.39) were similar to those values obtained for vasopressin V_1 receptors in guinea-pig and human submucosal arterioles (8.5–9, Vanner et al., 1990) and in a rat vasopressor assay ($pA_2 = 8.62$, Manning et al., 1992). In addition, the pK_B values for [d(CH$_2$)$_5$Tyr(Me)^2]vasopressin reported here are similar to those values obtained in the same vessels when vasopressin was used as agonist ($pK_B = 8.36–8.74$, Jovanović et al., 1995c). It has been found that the vasopressin V_1 receptor in the pituitary gland is resistant to the antagonizing action of [d(CH$_2$)$_5$Tyr(Me)^2]vasopressin (Antoni et al., 1984), and this subtype of vasopressin receptor has been designed as V_{1B} (Jard et al., 1986) or V_3 (Baertschi and Friedli, 1985). The high affinities of [d(CH$_2$)$_5$Tyr(Me)^2]vasopressin for vasopressin receptors in guinea-pig uterine arteries probably excludes a role for the V_{1B} (V_3) subtype of vasopressin receptor in oxytocin-induced contractions. The affinity of [d(CH$_2$)$_5$Tyr(Me)^2] vasopressin for antagonizing the contractile action of oxytocin is clearly within the range reported for classical vasopressin V_1 (V_{1A}) receptor blockade (Vanner et al., 1990; Manning et al., 1992), suggesting the presence of contraction-mediating vasopressin V_{1A} receptors in non-pregnant and pregnant guinea-pig uterine arteries. In contrast, the pK_B values of [d(CH$_2$)$_5$, D-Ile^2, Ile^4]vasopressin observed at receptors mediating contraction of uterine arteries (7.17–7.29) were significantly lower than those values reported for vasopressin V_2 receptors (8–8.24, Manning et al., 1983, 1984; Sawyer et al., 1988) and were much closer to those values obtained for V_{1A} subtypes of vasopressin receptors (6.4–6.9, Manning et al., 1984; Jovanović et al., 1995d). On the basis of these results, it seems reasonable to suggest that in guinea-pig uterine artery oxytocin induces contractions predominantly via activation of V_{1A} vasopressin receptors, regardless of pregnancy status or endothelium condition.

In principle, since removal of the endothelium did not affect the potency of oxytocin on uterine arteries, the lower potency of oxytocin in pregnant guinea-pig uterine arteries may reflect (a) decreased affinity of receptors for oxytocin; (b) decreased receptor density; (c) decreased efficiency of the oxytocin–receptor complex coupling and (d) changes in the ability of oxytocin to reach its binding site. Since both oxytocin and vasopressin evoke contractions of uterine arteries through the same subtype of vasopressin receptors (Jovanović et al., 1995c), it is logical to expect that the changes in vasopressin receptor density and/or affinity in uterine artery would alter the response of the two agonists in the same manner. However, our previous study has shown that the potency of vasopressin is actually greater in pregnant than in non-pregnant guinea-pig uterine arteries (Jovanović et al., 1995c) and that this is not related to changes in endothelial function or receptor affinity, implying that these possibilities are rather unlikely. Thus, it is possible that oxytocin–V_{1A} vasopressin receptor complex coupling efficiency in guinea-pig uterine artery decreases during pregnancy. It has been already reported that extracellular Ca^{2+} uptake and protein kinase C activity in uterine arterial smooth muscle declines in pregnancy (Farley and Ford, 1992). It is also known that the vasopressin V_{1A} receptor is associated with phosphatidyl inositol turnover/Ca^{2+} pathway (reviewed by Share, 1988). Accordingly, it is possible that an alteration of Ca^{2+} uptake is involved in the decreased responsiveness of uterine artery to oxytocin in pregnancy. However, since both oxytocin and vasopressin act through the same receptor subtype and probably through the same underlying mechanism, this hypothesis can not explain the divergent effects of pregnancy on oxytocin- and vasopressin-mediated responses, and consequently, at this moment, it is not possible to suggest what kind of alterations at the vasopressin V_{1A} coupling level may lead to the opposite effect of pregnancy on the actions of oxytocin and vasopressin in the uterine artery. Nevertheless, the present findings suggest that, in uterine artery, vasopressin V_{1A}-coupling could be altered by pregnancy in such way to modulate the response to oxytocin and vasopressin in opposite manners, which supports the recent hypothesis that the state of the effector may significantly affect the outcome of the stimulus (Terzic et al., 1994; Brady et al., 1996; Jovanović et al., 1996a,b). It should be, however, mentioned that the observed differences in the effect of pregnancy on the actions of vasopressin and oxytocin could be due to differences in the metabolism of the two peptides. For example, oxytocin, but not vasopressin, is a substrate for endopeptidase-24.11 and there is some evidence to suggest that the activity of this enzyme, at least in uterine tissue, may increase in late pregnancy (Ottlecz et al., 1991; Schriefer and Molineaux, 1993). Thus, in principle, this effect of pregnancy may influence the ability of oxytocin to reach its binding site and could be a possible explanation for the differences in the effect of pregnancy on the actions of vasopressin and oxytocin in the uterine artery.

In conclusion, this study has shown that pregnancy decreases oxytocin-induced contractions in guinea-pig uterine artery, an effect which is not related to changes in endothelial function. On the basis of different affinity of

antagonists, we suggest that V_{1A} vasopressin receptors are involved in the oxytocin-induced contraction of both non-pregnant and pregnant guinea-pig uterine artery.

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