British Journal of Pharmacology (1997) 121, 1468–1474

Effect of oxytocin as a partial agonist at vasoconstrictor vasopressin receptors on the human isolated uterine artery

[1]Aleksandar Jovanović, [2,]*Sofija Jovanović, †Ivan Tulić & Leposava Grbović

Department of Clinical Pharmacology, Pharmacology and Toxicology, Medical School, *Department of Anatomy, Faculty of Veterinary Medicine, Belgrade and †Clinic for Obstetrics and Gynaecology, Medical School, Belgrade, Yugoslavia

1 The effect of oxytocin on endothelium-intact and endothelium-denuded segments of the human uterine artery rings was investigated.

2 In both types of preparation oxytocin induced contraction of human uterine artery with similar potency and efficacy (pEC_{50} values: 6.95 ± 0.05 vs 7.06 ± 0.01; maximal response values: $61 \pm 4.1\%$ vs $63 \pm 5.1\%$ for arteries with and without endothelium, respectively).

3 In contrast, human uterine arteries, both intact and denuded of endothelium, did not respond to the addition of the selective oxytocin receptor agonist, [Thr[4], Gly[7]]oxytocin (10 nM – 1 μM).

4 The vasopressin receptor antagonists, [d(CH_2)$_5$Tyr(Me)]AVP (10 – 100 nM) and [d(CH_2)$_5$,D-Ile[2],Ile[4]]AVP (300 nM – 3 μM) produced parallel rightward shifts of the curves for oxytocin. The Schild plots constrained to a slope of unity gave the following $-\log K_B$ values: [d(CH_2)$_5$Tyr(Me)] AVP vs [d(CH_2)$_5$,D-Ile[2],Ile[4]] AVP 9.24 vs 6.91 and 9.26 vs 6.84 for human uterine artery with intact and those denuded of endothelium, respectively. In contrast, in both types of preparations the oxytocin receptor antagonist, [d(CH_2)$_5$Tyr(OMe), [2]Orn[8]]vasotocin (1 μM), did not significantly affect oxytocin-induced contractions.

5 The calculated pK_A values for oxytocin itself also did not differ between preparations: 6.56 and 6.43 for human uterine artery with and without endothelium, respectively. In both types of preparations, the receptor reserve (K_A/EC_{50}) was close to unity (intact vs denuded: 3.9 vs 3.0).

6 It is concluded that, in human uterine artery, oxytocin induces contractions that are not modulated by the endothelium. It is likely that oxytocin acts as a partial agonist on human uterine artery, regardless of the endothelial condition. On the basis of differential antagonists affinity and affinity of oxytocin itself, it is probable that receptors involved in oxytocin-induced contraction in human uterine arteries belong to the V_{1A} vasopressin receptors.

Keywords: Oxytocin; uterine artery; endothelium; vasopressin receptors

Introduction

It has been shown that oxytocin induces contraction of human uterine artery (Ekesbo *et al*., 1991; Petersen *et al*., 1991), as opposed to some other arteries in which oxytocin evokes relaxation (Katušić *et al*., 1986; Suzuki *et al*., 1992). However, although human uterine artery is sensitive to oxytocin (Ekesbo *et al*., 1991; Petersen *et al*., 1991), the effects of this peptide on this blood vessel have not yet been studied in detail. For example, the underlying receptor mechanism of oxytocin actions in this artery is still unknown. Furthermore, although it has been shown that, in certain arteries, removal of endothelium can potentiate the contractile responses of vascular smooth muscle to different vasoconstrictors (Alosachie & Godfraind, 1988; Randall *et al*., 1988), it is not known whether endothelium modulates contractile responses in human uterine artery induced by oxytocin. Information regarding the influence of endothelium on the responsiveness of uterine artery to oxytocin would be of importance, since it has been suggested that increased sensitivity of this vessel to vasoconstrictors, as a result of endothelial dysfunction in this vascular region, may be a significant component of gynaecological disorders (Hauksson *et al*., 1988; Sarrel *et al*., 1990).

Therefore, taking all this into consideration, the purposes of this study were to (1) examine the influence of endothelium on oxytocin-mediated responses in human uterine artery and (2) clarify the underlying receptor mechanism of the oxytocin action.

[1] Author for correspondence at: Division of Cardiovascular Diseases and Clinical Pharmacology Unit (G-7), Departments of Medicine and Pharmacology, Mayo Clinic and Foundation, Rochester, MN 55905, U.S.A.
[2] Present address as above.

Methods

Human uterine arteries were obtained from 18 non-pregnant women (mean age ± s.e.mean, 43.8 ± 5.1, range 27 – 53) undergoing hysterectomy for benign gynaecological diseases (adenomyosis, endometriosis, fibromyoma). No patients with malignant disease or patients receiving radiological, cytotoxic or antihypertensive therapy were included. During the operation, the patients received anaesthesia with a combination of nitrous oxide, oxygen, thiopentone and fentanyl. Muscle relaxation was induced by suxamethonium and maintained by pancuronium. The vessels were excised at most within 10 min of clamping the blood flow and placed in cold (4°C) Krebs-Ringer-bicarbonate solution. The patients were informed in detail about the purpose of the investigation and had given their consent to the excision of the preparations. After excision, the vessels were immediately transported to the laboratory.

Vascular preparations

The uterine arteries were dissected free from connective tissue. They were cut into 4 mm rings. Care was taken not to damage the endothelium unintentionally. In some rings, the endothelium was removed mechanically by gentle rubbing of the intimal surface with a stainless-steel wire. Ring preparations were mounted between two stainless-steel triangles in an organ bath containing 15 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.

Preparations were allowed to equilibrate for about 1 h in Krebs-Ringer-bicarbonate solution. During this period the bathing solution was exchanged for fresh (37°C) buffer solution every 15 min.

After 60 min, each ring was gradually stretched to the optimal point of its tension (28 mN, Jovanović *et al*., 1994a). Once at their optimal length, the segments were allowed to equilibrate for a further 30 min before experimentation.

Experimental procedure

At the beginning of each experiment the vessel segment was exposed twice to a potassium-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 potassium was equivalent in magnitude to the first (variation less than 10%), was the preparation used for further experimentation. Subsequently, in order to confirm the presence or successful removal of endothelium, the rings precontracted with phenylephrine (10 μM) were challenged with acetylcholine (10 μM). On the basis of previous studies (Jovanović *et al*., 1994a, b), relaxation greater than 50% or less than 20% of maximal relaxation evoked by acetylcholine (maximal relaxation represented complete return to the resting tension from the contraction in response to phenylephrine) was indicative of structurally intact or denuded endothelium, respectively. The data from a particular tissue were rejected if the relaxation produced by acetylcholine was 20–50%.

Concentration-response curves for oxytocin or [Thr^4,Gly^7] oxytocin were constructed by adding increasing concentrations of these compunds when 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 in all types of preparations ($n=4$ for each) demonstrated that 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 with 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 with oxytocin.

In order to determine the dissociation constant of oxytocin we compared the concentration-response curve for oxytocin with the concentration-response curve for vasopressin. Thus, in separate series of experiments 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 with oxytocin and a 30 min equilibration period; (5) concentration-response curve with vasopressin.

Calculations and statistical analysis

The contraction induced by each concentration of oxytocin was expressed as a percentage of the maximal contraction in response to potassium-rich Krebs-Ringer-bicarbonate solution and used for constructing 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 & Schild, 1959) with oxytocin 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 abscissae (Arunlakshana & Schild, 1959). The concentration-ratios (the ratio between the EC_{50} value of oxytocin in the presence and absence of an antagonist) were also used to calculate a modified Schild plot with a slope of -1, thus an estimate of the pK_B value ($-\log$ dissociation constant of antagonist) could be obtained (Tallarida *et al*., 1979). The significance of the Schild plot linearity was tested by analysis of variance (Kenakin, 1987). The closeness of the slope to unity was tested by t test and was considered not different from unity if $P>0.05$.

Since, in human uterine artery, vasopressin and oxytocin acted at a single and same receptor (see Results, Jovanović *et al*., 1995b), the affinity of oxytocin (less efficacious agonist), defined as dissociation constant, was calculated based on the following equation (Kenakin, 1987):

$$1/[A] = [(\varepsilon_A/\varepsilon_{A'})(K_{A'}/K_A)(1/[A'])] + (1/K_A)[(\varepsilon_A/\varepsilon_{A'}) - 1]$$

where [A] and [A′] are equiactive concentrations of two agonists; ε_A and $\varepsilon_{A'}$ intrinsic efficacy of A and A′, respectively; K_A and $K_{A'}$ dissociation constants of A and A′, respectively. Thus, equieffective concentrations of oxytocin [A′] and vasopressin [A] were determined; 1/[A] was plotted against 1/[A′]. The slope of the regression line and the y-intercept were used to calculate the oxytocin dissociation constant ($K_{A'}$): $K_{A'}$ = Slope/intercept. $K_{A'}$ values are presented as $pK_{A'}$ ($pK_{A'} = -\log K_{A'}$).

Estimates of the receptor reserve were made from $K_{A'}/EC_{50}$ (Ruffolo, 1982; Kenakin, 1987).

The results are expressed as means ± s.e.mean; n refers to the number of experiments. One-way analysis of variance (ANOVA) was used when more than two groups were analysed. Statistical differences between two means were determined by 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.

Drugs and solutions

The Krebs-Ringer-bicarbonate solution had the following composition (in mM): 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, St Louis, MO, U.S.A.); arginine-8-vasopressin, oxytocin, [1-(β-mercapto-β,β-cyclopentamethylene-propionic acid), 2-(O-methyl)tyrosine]arginine-vasopressin ([d(CH$_2$)$_5$Tyr(Me)] AVP), [1-(β-mercapto-β,β-cyclopentamethylene-propionic acid), 2-D-isoleucine, 4-isoleucine] arginine-vasopressin ([d(CH$_2$)$_5$,D-Ile^2,Ile^4]AVP), [Thr^4,Gly^7]oxytocin, [1-(β-mercapto-β,β-cyclopentamethylene-propionic acid), 2-ornitine]vasotocin ([d(CH$_2$)$_5$Tyr(OMe), ^{2}Orn^8]vasotocin) (Peninsula Laboratories, Belmont, CA, U.S.A.). 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.

Results

Effects of oxytocin and [Thr⁴, Gly⁷]oxytocin

Oxytocin (10 nM – 5 μM) induced a concentration-dependent contraction of the human uterine arterial rings with intact endothelium ($pEC_{50} = 6.95 \pm 0.05$, maximal response value was $61 \pm 4.1\%$, $n = 27$). Removal of the endothelium did not significantly affect contractions in response to oxytocin ($pEC_{50} = 7.06 \pm 0.01$, maximal response value was $63 \pm 5.1\%$, $P > 0.05$, $n = 28$) (Figure 1). The human uterine arteries did not respond to the addition of [Thr⁴, Gly⁷]oxytocin (10 nM – 1 μM), a selective oxytocin receptor agonist ($n = 4$ for each) (data not shown), regardless of the presence or absence of the endothelium.

Effect of [d(CH₂)₅Tyr(OMe), ²Orn⁸]vasotocin

[d(CH_2)$_5$Tyr (OMe), ^{2}Orn^8]vasotocin (1 μM), a selective antagonist of oxytocin receptors, did not significantly affect contractions of human uterine arteries evoked by oxytocin, in either endothelium-intact or endothelium-denuded preparations (Figure 2).

Effects of vasopressin receptor antagonists

In human uterine artery, with either intact or denuded endothelium, a selective vasopressin V_1 receptor antagonist, [d(CH_2)$_5$Tyr(Me)]AVP (10 – 100 nM) and a selective vasopressin V_2 receptor antagonist, [d(CH_2)$_5$,D-Ile^2,Ile^4]AVP (300 nM – 3 μM) caused a rightward displacement of the oxytocin concentration-response curve, without alteration of the maximum response (Figure 3). The data from the experiments with vasopressin receptor antagonists were analysed as described by Arunlakshana & Schild (1959). In both types of preparations, the experiments with [d(CH_2)$_5$Tyr(Me)]AVP and [d(CH_2)$_5$,D-Ile^2,Ile^4]AVP yielded straight lines ($P > 0.05$, for both antagonists studied) with 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 endothelium ($P > 0.05$).

Dissociation constant of the oxytocin-receptor complex

In our previous study we observed that vasopressin, as opposed to oxytocin, induced a maximal contraction in human uterine artery similar to contraction evoked with potassium-rich Kresb-Ringer-bicarbonate solution (Jovanović *et al.*, 1995b). Accordingly, in order to determine dissociation constant of oxytocin-receptor complex, we used the procedure described by Kenakin (1987) (see Methods). In both types of preparation studied, concentration-response curves for vasopressin were significantly shifted to the left (pEC_{50} values for vasopressin vs oxytocin: $pEC_{50} = 8.87 \pm 0.07$ vs 6.92 ± 0.13 for vessels with, and 8.85 ± 0.08 vs 7.16 ± 0.07 for vessels without endothelium, $n = 5$ for each) with significantly greater maximal response value (vasopressin 100% vs oxytocin $54.4 \pm 5.9\%$ for preparations with, and vs $57.2 \pm 6.0\%$ for preparations without endothelium, $n = 5$ for each). The examples of these experiments are presented in Figure 4. The mean $pK_{A'}$ values were: 6.37 ± 0.13 ($n = 5$) and 6.59 ± 0.14 ($n = 5$) for uterine artery with and without endothelium, respectively. These values were not significantly different ($P > 0.05$). The receptor reserve expressed as $K_{A'}/EC_{50}$ was 3.9 ± 0.5 (with endothelium, $n = 5$) and 3.0 ± 0.4 (without endothelium, $n = 5$, $P > 0.05$).

Discussion

In the present study we have confirmed previous findings that, in contrast to some other arteries (Katušić *et al.*, 1986; Suzuki *et al.*, 1992), in which relaxant response to oxytocin has been

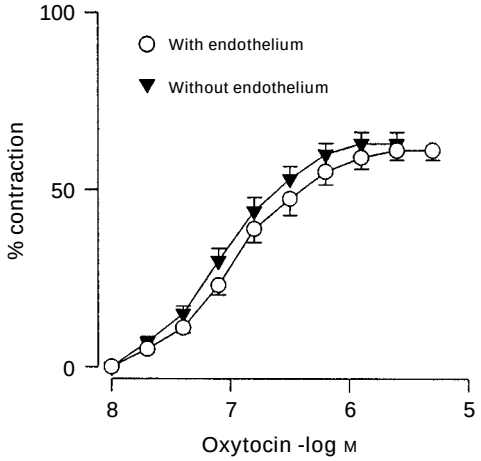

Figure 1 Concentration-response curves for oxytocin in human uterine artery either intact or denuded of endothelium. Each point represents the mean ($n = 27 – 28$); vertical lines show s.e.mean. Responses are expressed as a percentage of the contraction induced by K^+-rich Krebs-Ringer-bicarbonate solution (126 mM KCl, achieved by exchanging the 118.3 mM NaCl with KCl).

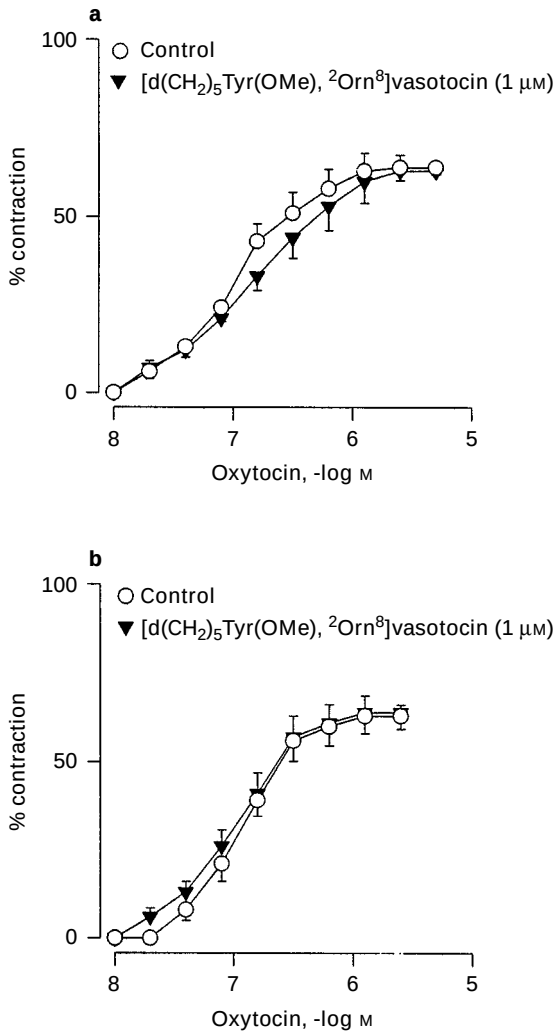

Figure 2 The effect of the selective oxytocin receptor antagonist, [d(CH_2)$_5$Tyr(OMe),^{2}Orn^8]vasotocin, against oxytocin-induced contractions of the human isolated uterine artery with (a) and without (b) endothelium. Responses in the absence and presence of the antagonist are expressed as a percentage of the contraction induced by K^+-rich Krebs-Ringer-bicarbonate solution (126 mM KCl, achieved by exchanging the 118.3 mM NaCl with KCl). Each point represents the mean ($n = 5$) and vertical lines show s.e.mean.

observed, oxytocin induces contraction of uterine arteries (Ekesbo *et al*., 1991; Petersen *et al*., 1991).

In general, removal of endothelium can potentiate the responses of vascular smooth muscle to different vasoconstrictors (Alosachie & Godfraind, 1988; Randall *et al*., 1988). It has been shown that the endothelium-dependent modulation of agonist-induced contractile responses is related to release of relaxing factors from the endothelium, which in turn result in a reduction of agonist potency and efficacy (Alosachie & Godfraind, 1986; Pipili-Synetos *et al*., 1991; Adeagbo & Triggle, 1993). However, in the present study removal of the endothelium did not affect the response to oxytocin, suggesting that in human uterine artery oxytocin-mediated responses are not modulated by the vasoactive factors derived from vascular endothelium. This finding provides evidence against the hypothesis that 'basal' release of endothelium-derived relaxing factor(s), which in turn could reduce the responsiveness of underlying smooth muscle to vasoconstrictors, is significant in this blood vessel. This conclusion is supported by previous studies in which human uterine arterial endothelium also did

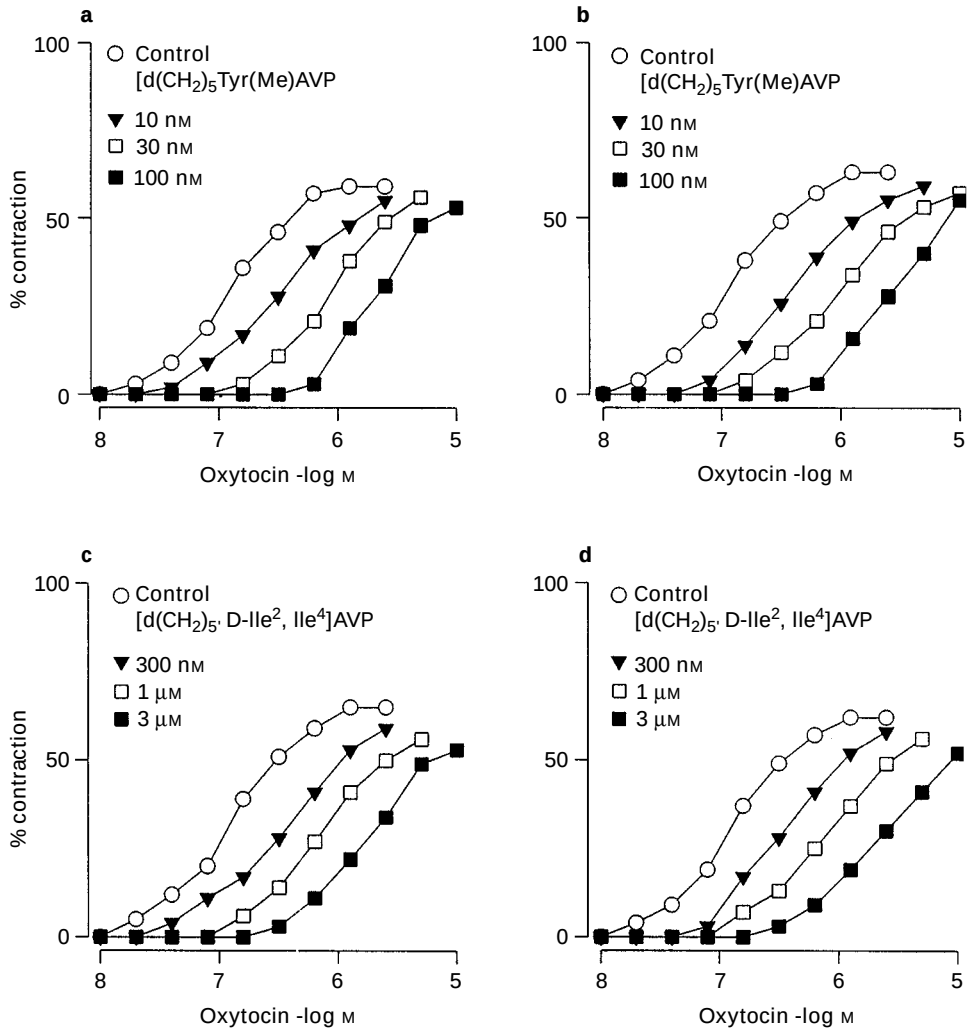

Figure 3 The effects of the selective vasopressin receptor antagonists against oxytocin-induced contractions of the human isolated uterine artery with and without endothelium. Concentration-response curves for oxytocin in human uterine artery with (a and c) and without (b and d) endothelium in the absence and presence of various concentrations of [d(CH_2)$_5$Tyr(Me)]AVP and [d(CH_2)$_5$,D-Ile^2,Ile^4]AVP. Each point represents the mean of 6–18 experiments. Standard errors are excluded for clarity and do not exceed 15% of the mean value for each point. Responses are expressed as a percentage of the contraction induced by K^+-rich Krebs-Ringer-bicarbonate solution (126 mM KCl, achieved by exchanging the 118.3 mM NaCl with KCl).

Table 1 pA_2 (–log K_B) values and slopes of Schild plots of vasopressin V_1 and V_2 receptor antagonists on the vasopressin receptors in the human uterine arteries

	pA2	*Slope*	*–log* K_B
		Endothelium intact	
[d(CH_2)$_5$Tyr(Me)]AVP	9.23±0.07	1.02±0.09	9.24±0.03
[d(CH_2)$_5$,D-Ile$_2$,Ile$_4$]AVP	6.88±0.02	1.03±0.02	6.91±0.01
		Endothelium denuded	
[d(CH_2)$_5$Tyr(Me)]AVP	9.36±0.02	0.89±0.02	9.26±0.03
[d(CH_2)$_5$,D-Ile$_2$,Ile$_4$]AVP	6.81±0.08	1.04±0.09	6.84±0.03

The values are expressed as means±s.e.mean (n=6) and were determined by their ability to antagonize oxytocin-induced contraction of vascular segments.

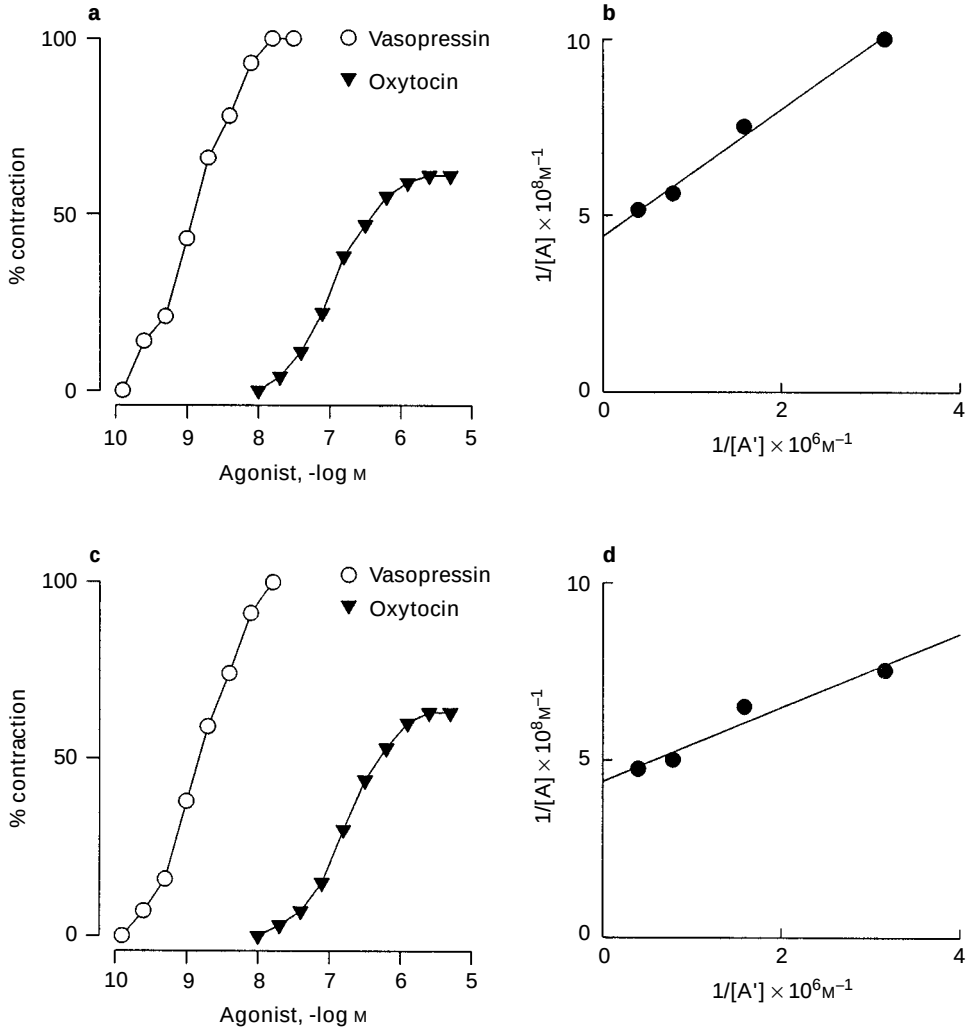

Figure 4 Typical experiments to determine the dissociation constant (K_A) for oxytocin in the human uterine artery with and without endothelium. Concentration-response curves for vasopressin and oxytocin in human uterine artery with (a) and without (c) endothelium. Responses are expressed as percentages of the maximal contraction induced by vasopressin. Equieffective concentrations of vasopressin and oxytocin were determined graphically by linear interpolation of the concentration-response curves for vasopressin and oxytocin. Double-reciprocal plot of equieffective concentrations of vasopressin (ordinate scale, a/[A]) and oxytocin (abscissa scale, 1/[A′], obtained from (a) (b, $Y = 179.3x + 4.4x10^8$, $r = 0.996$; $pK_{A'} = 6.39$) and (c) (d, $Y = 103.9x + 4.4x10^8$, $r = 0.972$; $pK_{A'} = 6.63$).

not modulate the effects of noradrenaline and prostaglandin $F_{2\alpha}$ (Grbović & Jovanović, 1995; Grbović *et al*., 1996). Whether this insignificant role of the vascular endothelium is a specific for human species remains to be established. However, a lack of a role for vascular endothelium in vasopressin- and noradrenaline-mediated responses has also been demonstrated in the guinea-pig uterine artery (Jovanović *et al*., 1995a,b).

The presence of oxytocin receptors has been demonstrated in human vascular smooth muscle cell line (Yazawa *et al*., 1996). Therefore, it was conceivable that oxytocin-induced contraction in human uterine artery may be mediated through oxytocin receptor activation. However, in the present study, [d(CH_2)$_5$Tyr(OMe), ^{2}Orn^8]vasotocin, a selective oxytocin receptor antagonist (Hruby, 1992), applied in a concentration sufficient to block oxytocin receptors (Hong & Moody, 1991), did not significantly affect concentration-response curves for oxytocin, regardless of the presence or absence of endothelium. In addition, [Thr^4, Gly^7]oxytocin, a selective oxytocin receptor agonist (Hruby, 1992), did not elicit a contraction in either the presence or absence of the endothelium. These findings suggest that oxytocin receptors are not involved in the contractile effect of oxytocin in human uterine arteries with and without endothelium. On the other hand, it has been found that in some blood vessels oxytocin induces contraction through activation of vasopressin receptors (Katušić *et al*., 1986; Briner *et al*., 1992). In order to establish the contribution of vasopressin receptors to the oxytocin-induced contraction in vessels studied, we used [d(CH_2)$_5$Tyr(Me)]AVP, a selective vasopressin V_1 receptor antagonist (Kruszynski *et al*., 1980), and [d(CH_2)$_5$,D-Ile^2,Ile^4]AVP, a selective vasopressin V_2 receptor antagonist (Manning *et al*., 1983). The slopes of the Schild plots for both [d(CH_2)$_5$Tyr(Me)]AVP and [d(CH_2)$_5$,D-Ile^2, Ile^4]AVP were not significantly different from unity, indicating that the antagonism is competitive and therefore that the pA_2 value obtained constrained to unity can be taken to be the $-\log K_B$ value (Arunlakshana & Schild, 1959; Tallarida *et al*., 1979; Kenakin, 1987). Additionally, the competitive nature of both antagonists may suggest that oxytocin-induced contractions of human uterine artery are mediated through a single type of receptor population (Kenakin, 1987). 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 human uterine artery with intact endothelium, affinity estimate for both antagonists were not different from those obtained in the human uterine artery denuded of endothelium. Therefore, the possibility that different vasopressin receptor subtypes are involved in oxytocin-induced contraction of preparations studied was eliminated. Besides, affinity estimates for oxytocin itself also did not differ between the tissues studied, regardless of the presence of the

endothelium. The pK_B values obtained for [d$(CH_2)_5$Tyr (Me)]AVP in our study (9.24–9.26) were close to those values obtained for vasopressin V_1 receptors in guinea-pig and human submucosal arterioles (8.50–9, Vanner *et al*., 1990), canine femoral artery (pA_2 = 9.5, Katušić *et al*., 1984) and in human platelets ($-\log K_B$ = 9.21, Thibonnier *et al*., 1993). In addition, the pK_B values for [d$(CH_2)_5$Tyr(Me)^2]AVP found in the present study are similar to those values obtained at the same vessels when vasopressin was used as agonist ($-\log K_B$ = 9.61–9.66, Jovanović *et al*., 1995b, c). Thus, the affinities of [d $(CH_2)_5$Tyr(Me)]AVP for antagonizing the contractile reaction of oxytocin is clearly within the range found for blockade V_1 (V_{1A})-vasopressin receptors, suggesting that the oxytocin action is mediated through V_{1A}-vasopressin receptors in the preparations studied. In contrast, the pK_B values of [d$(CH_2)_5$,D-Ile^2,Ile^4]AVP observed at receptors mediating contraction of uterine arteries (6.84–6.91) were significantly lower than those values obtained for vasopresin V_2 receptors (8–8.24, Manning *et al*., 1983; 1984; Sawyer *et al*., 1988), and corresponded to those obtained for V_1 subtypes of vasopressin receptors (6.4–6.9, Manning *et al*., 19984; Szot *et al*., 1989). It should be mentioned that the vasopressin V_1 receptor in the pituitary gland is resistant to antagonism by [d$(CH_2)_5$Tyr (Me)^2]AVP (Antoni *et al*., 1984), and this subtype of vasopressin receptor has been designed as V_{1b} (Jard *et al*., 1986) or V_3 (Baertschi & Friedli, 1985). The high affinities of [d$(CH_2)_5$Tyr(Me)]AVP for vasopressin receptors in human uterine arteries probably excludes the role for this subtype of vasopressin receptor in oxytocin-induced contractions. On the basis of these results, we suggest that in human uterine artery oxytocin induces contractions predominantly via activation of classical V_1(V_{1A}) vasopressin receptors, regardless of endothelial condition. In addition, this conclusion is also supported by the finding that pK_A values (6.37–6.59) estimated for oxytocin were significantly lower than those values obtained for oxytocin receptors (pK_A = 10.22, Di Scala-Guenot & Strosser, 1992; pK_A = 8.52, Sheldrick *et al*., 1993; pK_A = 8.78, Yazawa *et al*., 1996), and much closer to the pK_A values obtained for the oxytocin-vasopressin receptor complex (7.29, Taylor *et al*., 1990; 7.00, Yazawa *et al*., 1996).

In both types of vessels studied the maximal response achieved with oxytocin was significantly lower than the maximal response obtained with vasopressin, suggesting that in this artery oxytocin acts as a partial agonist. The ratio K_A/EC_{50} expresses efficiency of coupling and/or receptor density (Kenakin, 1987). It is known that this ratio close to unity is typical for partial agonism (Kenakin, 1987). In the present study the ratio K_A/EC_{50} was close to the value 1, confirming that oxytocin is a partial agonist on human uterine artery, regardless of the endothelial condition.

In conclusion, this study has shown that oxytocin induces contraction of human uterine artery. Removal of the endothelium did not affect oxytocin-induced contraction of human uterine artery, suggesting the lack of an endothelium-dependent modulation of the effect of oxytocin. The maximal responses obtained with oxytocin were significantly lower than those obtained with vasopressin and a linear stimulus-response relationship for oxytocin was obtained in vessels studied, implying that in these preparations oxytocin acts as a partial agonist, regardless of endothelial condition. On the basis of differential antagonist affinity and affinity of oxytocin itself, we suggest that an identical subtype of receptor is involved in oxytocin-induced contraction of human uterine artery in both intact and endothelium-denuded preparations. It is probable that the receptors involved belong to the vasopressin V_{1A} subtype.

We thank Dr Stanko Stojiljkovic for providing vasopressin, [d$(CH_2)_5$Tyr(Me)]AVP, [d$(CH_2)_5$,D-Ile^2,Ile^4]AVP, [Thr^4, Gly^7] oxytocin, oxytocin and [d$(CH_2)_5$Tyr(OMe), ^{2}Orn^8]vasotocin. A.J. is a recipient of Merck Sharp & Dohme International Fellowship in Clinical Pharmacology.

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(Received December 6, 1996
Revised April 2, 1997
Accepted April 23, 1997)