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Characterization of arginine vasopressin actions in human uterine artery: lack of role of the vascular endothelium

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1 The effect of arginine vasopressin (AVP) on human uterine artery rings, both intact and denuded of endothelium, was investigated.

2 Initially, AVP (63 pM – 32 nM) induced concentration-dependent contraction of human uterine artery ($pD_2 = 8.92 \pm 0.01$). Removal of the endothelium did not affect the concentration-response curve for AVP ($pD_2 = 8.83 \pm 0.03$).

3 In contrast, human uterine arteries, both intact and denuded of endothelium, did not respond to the addition of 1-desamino-8-D-arginine vasopressin (dDAVP, 1 nM – 1 μM).

4 In both types of preparations, [d(CH_2)$_5$Tyr(Me)AVP (1 – 10 nM) and [d(CH_2)$_5$,D-Ile^2,Ile^4]AVP (300 nM – 3 μM) produced parallel rightward shifts of the curves for AVP. 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 9.66 vs. 6.69 and 9.61 vs. 6.80 for human uterine artery, intact and denuded of endothelium, respectively.

5 The pK_A values for AVP 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 considerably greater than unity (intact vs. denuded: 228 vs. 244).

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

Keywords: Vasopressin; human uterine artery; endothelium; vasopressin receptors

Introduction

It has been shown that arginine vasopressin (AVP) induces contraction of the human uterine artery (Svane *et al.*, 1990; 1991; Ekesbo *et al.*, 1991; Nelson & Suresh, 1992; Kostrzewska *et al.*, 1993), as opposed to some other arteries in which AVP evokes relaxation (Katušić *et al.*, 1984; Seino *et al.*, 1985; Evora *et al.*, 1993).

However, although human uterine blood flow is regulated by AVP (Ekesbo *et al.*, 1991), the effect of AVP on human uterine artery has not yet been studied in detail. For example, the underlying mechanism of action of AVP in this artery is still entirely unknown. Furthermore, although it has been shown that, in certain arteries, AVP-induced contraction is increased after removal of the vascular endothelium (Randall *et al.*, 1988), in human uterine artery this possibility has not yet been tested.

Taking into consideration that increased sensitivity of the uterine artery to various vasoconstrictors, as a result of endothelial dysfunction in this vascular region, may be an important component of gynaecological disorders (Hauksson *et al.*, 1988; Sarrel *et al.*, 1990; Woolfson & Williams, 1990; Vokaer, 1992), the purposes of this study were to examine the influence of endothelium on AVP-mediated responses in human uterine artery and to clarify the underlying receptor mechanism of the action of AVP.

Methods

Human uterine arteries were obtained from 19 non-pregnant women (mean age ± s.e.mean, 46.8 ± 6.1, range 26 – 59) 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 were anaesthetized 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 organ baths were washed with 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 30 min before experimentation.

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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 for 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 the endothelium, rings precontracted with phenylephrine (10 μM) were challenged with acetylcholine (10 μM). On the basis of prior 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 a vessel with a structurally intact or non-functional endothelium, respectively. The data from a particular tissue were rejected if the relaxation produced by acetylcholine was 20–50%.

Concentration-response curves for AVP (63 pM–32 nM) or 1-desamino-8-D-arginine vasopressin (dDAVP, 1 nM–1 μM) were constructed by adding increasing concentrations of these compounds when the response to the previous concentration had reached equilibrium, or after 10 min if no response was obtained. Experiments followed a multiple curve design since separate experiments in all types of preparations ($n=7$ for each) demonstrated that first and second concentration-response curves for AVP were not significantly different. Therefore, the following protocol was used: (1) contraction in response to potassium-rich Krebs-Ringer-bicarbonate solution followed by three washes and a 30 min equilibration period; (2) contraction in response to potassium-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 AVP (used as the tissue control) or dDAVP, followed by three washes, addition of the antagonist (only when AVP was used) and a 15 min equilibration period; (5) concentration-response curve with AVP.

Treatment of data and statistics

The contraction induced by each concentration of AVP was expressed as a percentage of the maximal contraction in response to AVP itself and used for constructing the concentration-response curves. The concentration of AVP 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 pD_2 ($pD_2 = -\log EC_{50}$). The pA_2 values ($-\log$ molar concentration of antagonist reducing the agonist response by a factor of two) for vasopressin receptor antagonists were determined from a Schild plot (Arunlakshana & Schild, 1959) using AVP as the agonist. The concentration-ratios (the ratio between the EC_{50} value for AVP in the presence and absence of an antagonist) at different antagonist concentrations for the different AVP/antagonist pairs were calculated for each experiment. Thus, the mean values of concentration ratios for an AVP/antagonist pair were plotted in a Schild diagram using regression analysis, and pA_2 was obtained from the intercept of the regression line with the abscissa scale (Arunlakshana & Schild, 1959). The concentration-ratios (the ratio between the EC_{50} value of AVP in the presence and absence of an antagonist) were also used to calculate a modified Schild plot with a slope of -1, thus giving an estimate of the pK_B value ($-\log$ dissociation constant of antagonist; 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 Student's t test and was considered not different from unity if $P>0.05$.

The AVP dissociation constant was calculated according to the procedure described by Kenakin (1987), using [1-(β-mercapto-β,β-cyclopentamine propionic acid), 2-(O-methyl) tyrosine]arginine-vasopressin ([d(CH_2)$_5$Tyr(Me)]AVP) in a concentration in which it antagonized AVP action in a non-competitive manner. Thus, equieffective concentrations of AVP in the absence [A] and presence of [d(CH_2)$_5$Tyr(Me)] AVP [A'] were obtained. A plot of 1/[A] against 1/[A'] was constructed. The slope of the regression line and the y-intercept were used to calculate AVP (K_A) and [d(CH_2)$_5$Tyr (Me)]AVP (K_B) dissociation constants: K_A = (Slope-1)/intercept; K_B = [d(CH_2)$_5$Tyr(Me)]AVP/(Slope-1). K_A and K_B are presented as pK_A ($-\log K_A$) and pK_B ($-\log K_B$).

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, 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, [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) (Peninsula Laboratories, Belmont, CA, U.S.A.), 1-desamino-8-D-arginine vasopressin (dDAVP) (USV Laboratories, Tarrytown, NY, 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.

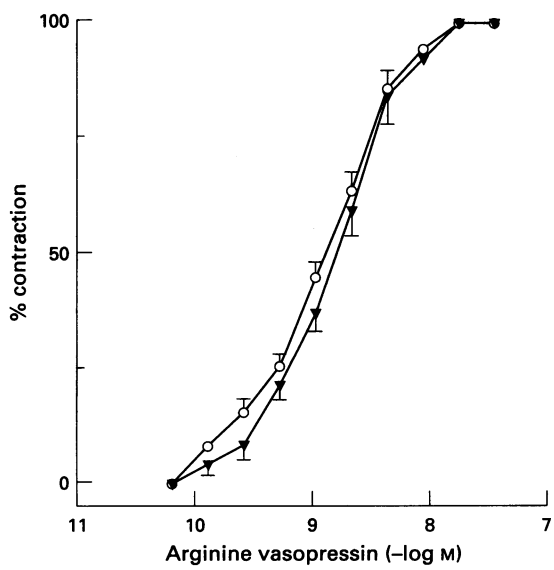

Figure 1 Concentration-response curves for arginine vasopressin in human uterine artery either intact (○) or denuded of endothelium (▼). Each point represents the mean ± s.e.mean ($n=48$). Responses are expressed as a percentage of the maximal contraction induced by arginine vasopressin itself.

Results

Effect of AVP

AVP (63 pM–32 nM) induced a concentration-dependent contraction of the human uterine arterial rings with intact endothelium (relaxation in response to 10 μM acetylcholine was 73.4±5.9%, $n=48$) ($pD_2=8.92\pm0.01$, the maximal response was 23.3±2.1 mN, $n=48$). Removal of the endothelium (relaxation in response to 10 μM acetylcholine was 9.3±3.1% of phenylephrine-induced tone, $n=48$) did not significantly affect contractions in response to AVP ($pD_2=8.83\pm0.03$, maximal response = 23.8±1.9 mN, $P>0.05$, $n=48$) (Figure 1).

Effects of vasopressin receptor antagonists

In human uterine artery, either intact or denuded of endothelium, both a selective vasopressin V_1 receptor antagonist, [d(CH_2)$_5$Tyr(Me)] AVP (1–10 nM) and a selective vasopressin V_2 receptor antagonist, [d(CH_2)$_5$,D-Ile^2,Ile^4]AVP (300 nM–3 μM) induced significant shifts to the right in a concentration-dependent manner ($P<0.01$, for both antagonists studied), without suppression of the maximum of the concentration-response curves for AVP ($P>0.05$, for both antagonists studied) (Figure 2). The data from the experiments with vasopressin receptor antagonists were analysed as described by Arunlakshana & Schild (1959). In both types of preparation, 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 antago-

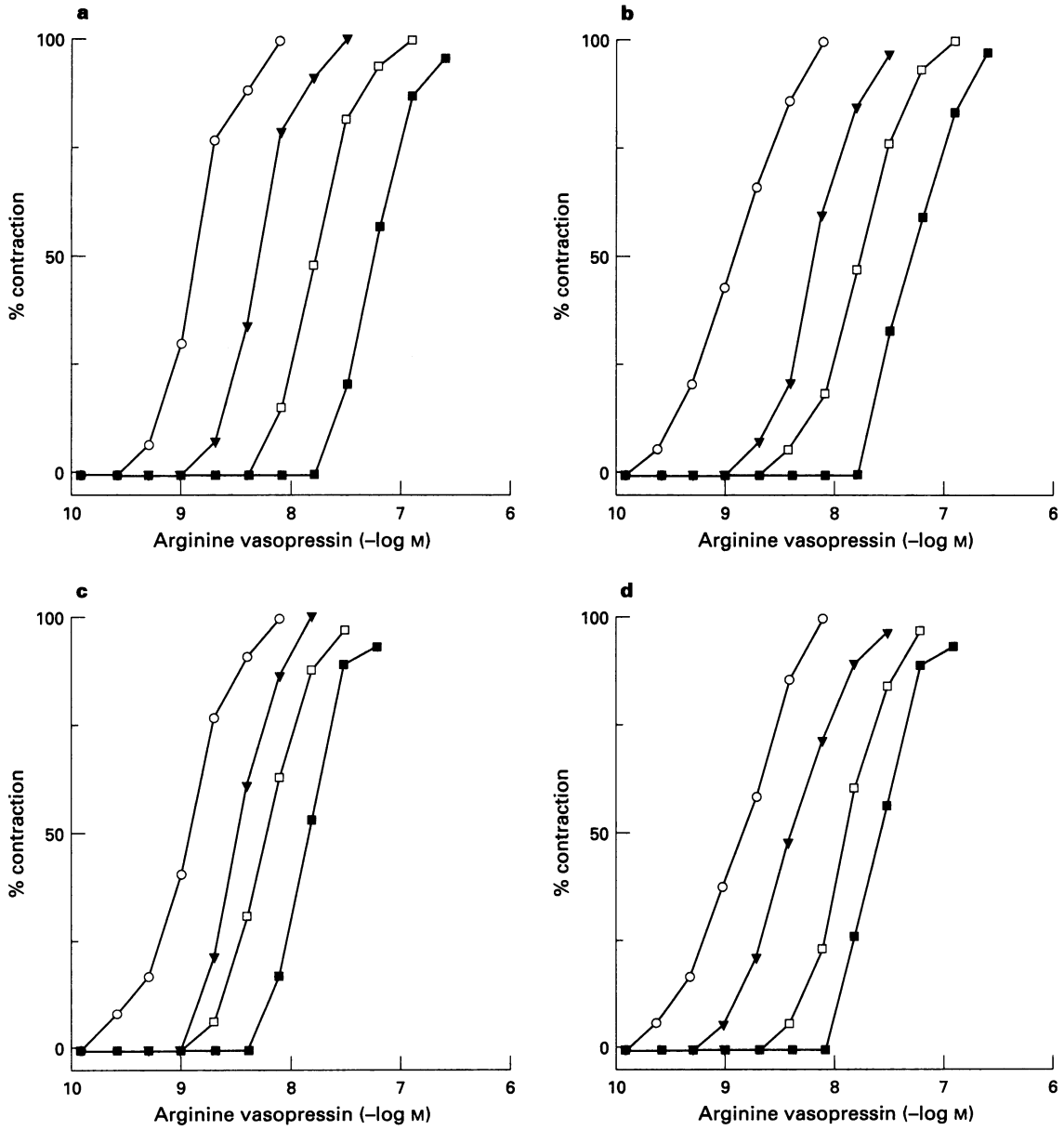

Figure 2 The antagonism of the contractile effects of arginine vasopressin by antagonists of vasopressin receptors. (a,b) Concentration-response curves for arginine vasopressin in human uterine artery with (a) and without endothelium (b) in the absence (○) and presence of 1 nM (▼), 3 nM (□) and 10 nM (■) [d(CH_2)$_5$Tyr(Me)]AVP. Each point represents the mean of 6–23 experiments. Standard errors are excluded for clarity and do not exceed 15% of the mean value for each point. Responses are expressed as percentages of the maximal contraction induced by arginine vasopressin. (c,d) Concentration-response curves for arginine vasopressin in human uterine artery either intact (c) or denuded of endothelium (d) in the absence (○) and presence of 300 nM (▼), 1 μM (□) and 3 μM (■) [d(CH_2)$_5$,D-Ile^2,Ile^4]AVP. Each point represents the mean of 6–22 experiments. Standard errors are excluded for clarity and do not exceed 15% of the mean value for each point. Responses are expressed as percentages of the maximal contraction induced by arginine vasopressin itself.

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, determined by their ability to antagonize arginine vasopressin (AVP)-induced contraction of vascular segments

Endothelium intact	*pA2*	*Slope*	*-log* K_B
$[d(CH_2)_5Tyr(Me)]AVP$	9.67 ± 0.08	0.99 ± 0.07	9.66 ± 0.02
$[d(CH_2)_5,D\text{-}Ile_2Ile_4]AVP$	6.84 ± 0.05	0.81 ± 0.04	6.69 ± 0.06
Endothelium denuded			
$[d(CH_2)_5Tyr(Me)]AVP$	9.61 ± 0.03	1.00 ± 0.03	9.61 ± 0.01
$[d(CH_2)_5,D\text{-}Ile_2Ile_4]AVP$	6.83 ± 0.08	0.96 ± 0.09	6.80 ± 0.03

The values are expressed as means ± s.e.mean ($n = 6-8$).

nists studied) with mean slopes not different from unity (Table 1). The pA_2 and $-\log K_B$ values are shown in Table 1. The $-\log K_B$ values for corresponding antagonists were not significantly different, regardless of the endothelium condition ($P > 0.05$).

Dissociation constant of the AVP-receptor complex

In order to determine the dissociation constant of the AVP-receptor complex, we used the procedure described by Kenakin (1987) (see Methods). $[d(CH_2)_5Tyr(Me)]AVP$, applied in a high concentration (300 nM) caused non-competitive inhibition of AVP-induced contraction as shown by depression of the maximum response (control 100% vs. 18.7 ± 3.8%, vs. 17.1 ± 2.9% for human uterine artery with and without endothelium, respectively, $n = 6$ for each). Examples of these experiments are presented in Figure 3. The mean pK_A values were: 6.56 ± 0.12 ($n = 6$) and 6.43 ± 0.09 ($n = 6$) for uterine artery with and without endothelium, respectively. These values were not significantly different ($P > 0.05$). a non-linear stimulus-response relationship was obtained for both types of vessels studied since the half-maximal response to AVP (calculated from mean K_A values) needed only 0.44 ± 0.07% (endothelium intact, $n = 6$) and 0.40 ± 0.09% (endothelium denuded, $n = 6$, $P > 0.05$) receptor occupancy. The receptor reserves expressed as K_A/EC_{50} were 228 ± 25 (endothelium intact, $n = 6$) and 244 ± 31 (endothelium denuded, $n = 6$, $P > 0.05$).

The mean pK_B values for $[d(CH_2)_5Tyr(Me)]AVP$ calculated according to the method of Kenakin (1987) (9.29 ± 0.09, $n = 6$ and 9.32 ± 0.11, $n = 6$ for uterine artery with and without endothelium, respectively) were similar to these values calculated according to the method of Arunlakshana & Schild (1959) (Table 1).

Effect of dDAVP

Human uterine arteries, both intact and denuded of endothelium, did not respond to the addition of dDAVP (10 nM – 1 μM) ($n = 5$ for each, data not shown).

Discussion

In the present study we confirmed previous findings that, in contrast to some other arteries (Katušić *et al.*, 1984; Seino *et al.*, 1985; Evora *et al.*, 1993), in which relaxant responses to AVP have been observed, AVP induces contraction of uterine arteries (Svane *et al.*, 1990; 1991; Nelson & Suresh, 1992; Kostrzewska *et al.*, 1993). Furthermore, the pD_2 values for AVP in our study were similar to those values obtained for the majority of vessels studied (Katušić *et al.*, 1984; Katušić & Krstić, 1987). It is known that, in certain blood vessels, removal of endothelium can potentiate the responses of vascular smooth muscle to AVP (Katušić & Krstić, 1987; Randall *et al.*, 1988). In addition, it has been shown in numerous blood vessels 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; 1988; Pipili-Synetos *et al.*, 1991; Adeagbo & Triggle, 1993). However, in the present study removal of the endothelium did not affect the response to AVP. Accordingly, it seems that, in this artery, AVP-mediated responses are not modulated by the vascular endothelium, which is similar to previous findings reported for some other arteries (Katušić & Krstić, 1987; Conde *et al.*, 1991).

It is established that AVP induces contraction of isolated arteries through V_1 vasopressin receptors activation (Altura, 1974; Katušić *et al.*, 1984; Gopalakrishnan *et al.*, 1991). However, recently, a vasocontractile effect of AVP, mediated by vasopressin V_2 receptors, has also been reported (Chiba & Tsukada, 1992). The pA_2 value for an antagonist in blocking the response to an agonist should be an accurate indication of its affinity for binding to the receptor site if certain criteria are fulfilled (Furchgott, 1972; Ruffolo, 1982; Kenakin, 1987). In order to establish the contribution of different vasopressin receptor subtypes to the AVP-induced contraction in vessels studied, we used $[d(CH_2)_5Tyr(Me)]AVP$, a selective vasopressin V_1 receptor antagonist (Kruszynski *et al.*, 1980) and $[d(CH_2)_5,D\text{-}Ile^2,Ile^4]AVP$, a selective vasopressin V_2 receptor antagonist (Manning *et al.*, 1983; 1987).

The slopes of the Schild plots for both $[d(CH_2)_5Tyr(Me)]AVP$ and $[d(CH_2)_5,D\text{-}Ile^2,Ile^4]AVP$ were not significantly different from unity, indicating that the antagonism is competitive and therefore that the obtained pA_2 value constrained to unity can be taken to be the $-\log K_B$ value (Arunlakshana & Schild, 1959). Additionally, the competitive nature of both antagonists may suggest that AVP-induced contractions of human uterine artery are mediated through a single type of vasopressin 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 estimates 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 AVP-induced contraction of preparations studied was eliminated. Besides, affinity estimates for AVP itself also did not differ between the tissues studied, regardless of endothelial condition.

The $-\log K_B$ values obtained for $[d(CH_2)_5Tyr(Me)]AVP$ in our study (9.61 – 9.66) were higher than those values obtained for vasopressin V_1 receptors in guinea-pig and human submucosal arterioles (8.50 – 9, Vanner *et al.*, 1990), and in the rat vasopressor assay ($pA_2 = 8.62$, Manning *et al.*, 1992), but they were similar to those values obtained for vasopressin V_1 receptors in canine femoral artery ($pA_2 = 9.5$; Katušić *et al.*, 1984), rabbit submucosal arterioles ($-\log K_B = 10 - 10.3$; Vanner *et al.*, 1990) and in human platelets ($-\log K_B = 9.21$; Thibonnier *et al.*, 1993). The reason for this variation in affinity of $[d(CH_2)_5Tyr(Me)]AVP$ is not known, but the possibility of further heterogeneity of vasopressin V_1 receptors exists (Vanner *et al.*, 1990). It has been found that the vasopressin V_1 receptor in the pituitary gland is resistant to antagonism by $[d(CH_2)_5Tyr(Me)^2]AVP$, (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 $[d(CH_2)_5Tyr(Me)]$ AVP for vasopressin receptors in human uterine arteries probably excludes a role for this subtype of vasopressin receptor in AVP-induced contractions. The affinity of $[d(CH_2)_5Tyr(Me)]AVP$ for antagonizing the contractile action of AVP is clearly within the range reported for classical V_1-vasopressin receptor blockade (Katušić *et al.*, 1984; Vanner *et al.*, 1990; Thibonnier *et al.*, 1993), suggesting the presence of contraction-mediating V_1-vasopressin receptors in the preparations studied. In contrast, the $-\log K_B$ values of $[d(CH_2)_5,D\text{-}Ile^2,Ile^4]AVT$ observed at receptors mediating contraction of uterine arteries (6.69 – 6.80) were significantly lower than those values reported for vasopressin V_2 receptors (8.00 – 8.24, Manning *et al.*, 1983; 1984; Sawyer *et al.*, 1988), and correspond to those obtained for V_1 subtypes of vaso-

pressin receptors (6.4–6.9, Manning *et al.*, 1984; Szot *et al.*, 1989). The lack of vasopressin V_2 receptors mediating contraction in human uterine arteries is also supported by the fact that dDAVP, a preferential vasopressin V_2 receptor agonist (Sawyer *et al.*, 1981), had no effect in either type of preparation studied. It should be mentioned that in certain blood vessels dDAVP, in a high concentration, induces contraction by activating vasopressin V_1 receptors (Sawyer *et al.*, 1981; Manning & Sawyer, 1984; Katušić *et al.*, 1984). However, in the present study we did not observe this action.

On the basis of these results, it seems reasonable to suggest that in human uterine artery AVP induces contractions predominantly via activation of classical V_1 (V_{1a}) or V_1-like vasopressin receptors, regardless of endothelial condition.

It is known that the affinity of AVP itself for V_1 vasopressin receptors is variable in that it exhibits a high pK_A value for V_1 vasopressin receptors in rat non-pregnant and pregnant mesenteric artery (9.28–9.39, Parent *et al.*, 1991) and human platelets (8.73, Thibonnier *et al.*, 1993), but a low value at vasopressin V_1 receptors in human breast carcinoma cells (7.32, Taylor *et al.*, 1990) and in cingulate gyrus of the rat pup (7.27, Szot *et al.*, 1989). We obtained very low affinities for AVP at the vasopressin receptors mediating contraction of the preparations studied ($pK_A = 6.43–6.56$). This difference may be due to profound species-dependent or species-independent differences of AVP affinity for vasopressin V_1 receptors (Vanner *et al.*, 1990; Howl *et al.*, 1991). The second possibility is that this difference may be due to differences in applied

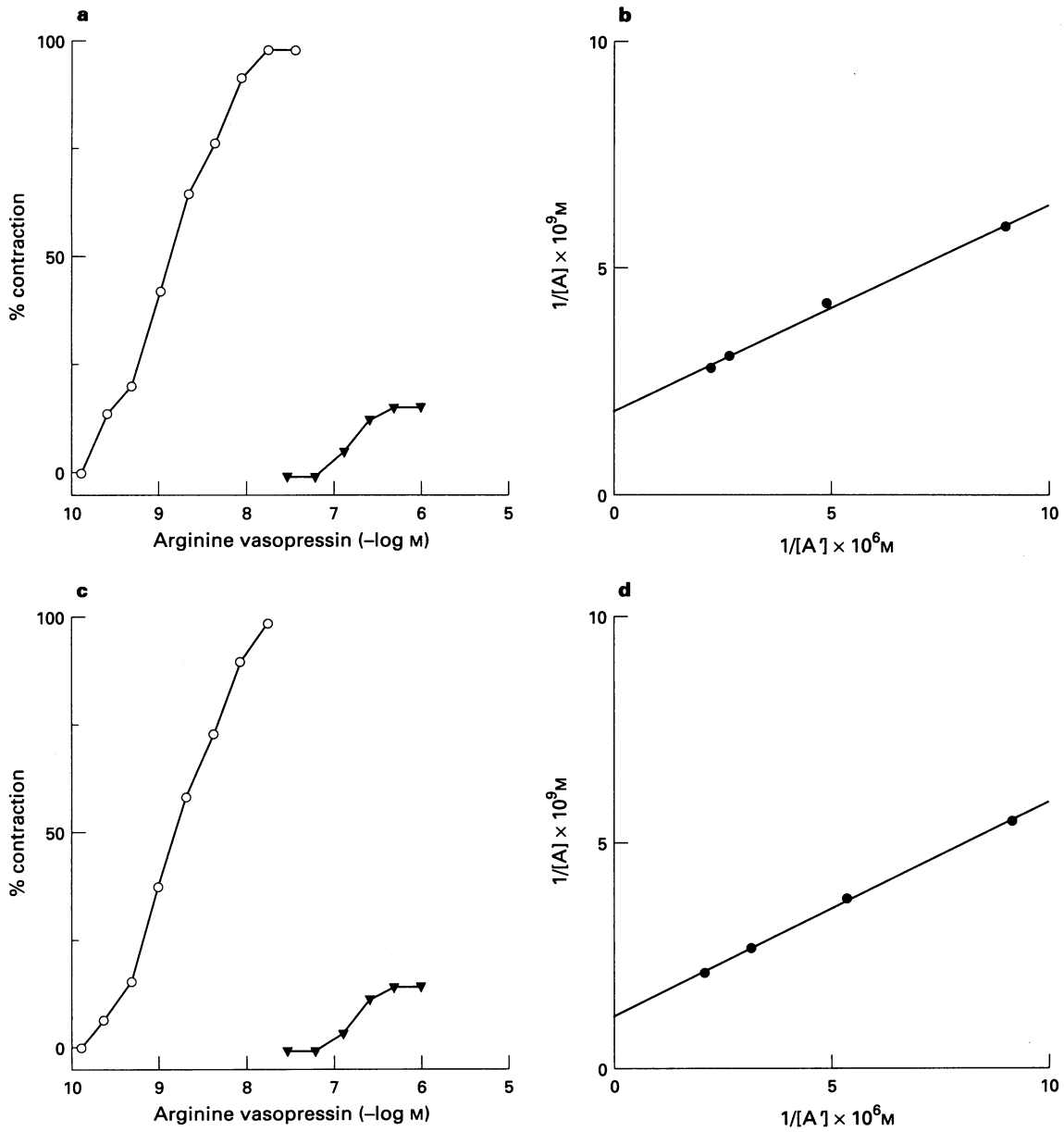

Figure 3 Typical experiments to determine the dissociation constant (K_A) for arginine vasopressin in the human uterine artery with and without endothelium. Arginine vasopressin concentration-response curves in human uterine artery with (a) and without endothelium (c), before (○) and after (▼) exposure of the preparation to [d(CH_2)$_5$Tyr(Me)]AVP (300 nM). Responses are expressed as percentages of the maximal contraction induced by arginine vasopressin. Equieffective concentrations of arginine vasopressin were determined graphically by linear interpolation of the concentration-response curves for arginine vasopressin in the absence and presence of [d(CH_2)$_5$Tyr(Me)]AVP. (b) Double-reciprocal plot of equieffective concentrations of arginine vasopressin before (ordinate scale, 1/[A]) and after (abscissa scale, 1/[A']) treatment with [d(CH_2)$_5$Tyr(Me)]AVP, obtained from (a) ($Y = 481.1x + 1.9 \times 10^9$, $r = 0.997$; $pK_A = 6.60$, $pK_B = 9.20$). (d) Double-reciprocal plot of equieffective concentrations of arginine vasopressin before (ordinate scale, 1/[A]) and after (abscissa scale, 1/[A']) treatment with [d(CH_2)$_5$Tyr(Me)]AVP, obtained from (c) ($Y = 473.4x + 1.2 \times 10^9$, $r = 0.998$; $pK_A = 6.40$, $pK_B = 9.20$).

methods for calculating pK_A values (binding vs. functional), as have been previously described (Pliska, 1991). However, it should be mentioned that the pK_B values (9.29–9.32) for [d(CH_2)$_5$Tyr(Me)]AVP obtained in quiescent preparations using the method of Kenakin (1987) were similar to those values (9.61–9.66) obtained using the method according to Arunlakshana & Schild (1959). This similarity implies that the pK_A values obtained in our study are correct, according to the established criteria (Kenakin, 1987; Yanigasawa *et al.*, 1989).

In both types of vessel studied, noncompetitive antagonism with [d(CH_2)$_5$Tyr(Me)]AVP revealed a non-linear relationship between contraction and the percentage of receptors occupied by AVP. Quantification of the receptor reserve by the ratio K_A/EC_{50} expresses the efficiency of coupling (Ruffolo, 1982; Kenakin, 1987). The K_A/EC_{50} ratios for AVP are significantly higher than unity, and a considerable receptor reserve exists in these tissues for the half-maximal and maximal responses for AVP. These results strongly suggest that AVP behaves as a full agonist in human uterine artery, regardless of endothelial condition, as opposed to some other arteries in which a partial agonist action of AVP has been shown (Katušić & Krstić, 1987).

In conclusion, this study has shown that AVP induces contraction of human uterine artery. Removal of the endothelium did not affect AVP-induced contraction of human uterine artery, suggesting a lack of endothelium-dependent modulation of AVP effects in this vessel. Noncompetitive antagonism with [d(CH_2)$_5$Tyr(Me)]AVP revealed a non-linear relationship between contraction and percentage receptors occupied by AVP implying that, in these preparations, AVP acts as a full agonist, regardless of endothelial condition. On the basis of differential antagonist affinity and the affinity of AVP itself, we suggest that an identical subtype of vasopressin receptor is involved in AVP-induced contraction of human uterine artery, either intact or denuded of endothelium. It is probable that the vasopressin receptors involved belong to the V_{1a} or V_{1a}-like subtype.

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