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Effect of pregnancy on vasopressin-mediated responses in guinea-pig uterine arteries with intact and denuded endothelium

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Abstract

The effect of pregnancy on vasopressin-induced contraction of guinea-pig uterine arterial rings was investigated. Initially, vasopressin induced contraction ($pD_2 = 9.14$) in pregnant guinea-pig uterine artery with greater potency than in non-pregnant guinea-pig uterine artery ($pD_2 = 8.77$). Removal of the endothelium did not affect vasopressin-induced contractions, regardless of pregnancy status. In all types of preparations, [$d(CH_2)_5$Tyr(Me)^2]vasopressin (10–100 nM) and [$d(CH_2)_5$,D-Ile^2,Ile^4]vasopressin (300 nM–3 μM) produced parallel rightward shifts of the curves for vasopressin. The Schild plots constrained to a slope of unity gave the following $-\log K_B$ values: [$d(CH_2)_5$Tyr(Me)^2]vasopressin vs. [$d(CH_2)_5$,D-Ile^2,Ile^4]vasopressin 8.74 vs. 6.82 and 8.50 vs. 6.72 for non-pregnant guinea-pig uterine artery with intact and denuded endothelium, respectively; 8.38 vs. 6.49 and 8.36 vs. 6.75 for pregnant guinea-pig uterine artery with intact and denuded endothelium, respectively. The pK_A values for vasopressin itself also did not differs between preparations: 6.49 and 6.55 for non-pregnant guinea-pig uterine artery with intact and denuded endothelium, respectively; 6.48 and 6.52 for pregnant guinea-pig uterine artery with intact and denuded endothelium, respectively. The receptor reserve (K_A/EC_{50}) was significantly greater in preparations taken from pregnant than from non-pregnant animals. It is concluded that vasopressin-induced contractions of guinea-pig uterine artery are not modulated by the endothelium, regardless of pregnancy status. The receptor reserve for vasopressin in guinea-pig uterine artery is increased during pregnancy, that is not related to the changes of vasopressin receptor affinity for vasopressin. It is probable that vasopressin receptors involved in vasopressin-induced contraction of all types of vessels studied belong to the V_{1A}-like subtype.

Keywords: Uterine artery; Pregnancy; Vasopressin receptor; Arginine vasopressin; (Guinea-pig)

1. Introduction

The regulation of uterine vascular tone and response is mediated by the interaction of neuronal and circulatory factors (Ekesbo et al., 1991), some of which may be altered by pregnancy. For example, pregnancy alters uterine vascular reactivity to several endogenous constrictor substances (Weiner et al., 1989, 1991, 1992). It has been proposed that this vascular adaptation mediates the increased blood flow to the uterus that is characteristic of pregnancy (Peeters et al., 1980).

In the majority of reports in which the endothelium condition of uterine artery has been verified, it has been found that sensitivity of this artery to various vasoconstrictors is decreased during pregnancy (Weiner et al., 1989, 1991, 1992). On the basis of these results, it has been hypothesized that these pregnancy-associated changes are due to increased production of relaxing factor(s) derived from uterine artery endothelium during pregnancy (Weiner et al., 1991, 1992). This theory is also supported by the fact that endothelial dysfunction and consequently increased sensitivity of uterine artery to various vasoconstrictors may be a cause of preeclampsia and eclampsia (for review see Vokaer, 1992).

It is well established that vasopressin is one of the most important circulating factors in the regulation of uterine blood flow (Ekesbo et al., 1991). In spite of this, the effect of this hormone on the uterine artery has not been studied in detail yet. It is unknown whether uterine artery reactivity to vasopressin is changed during pregnancy. If it is, is it a consequence

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of altered endothelial function, as has been already described for other vasoconstrictors (Weiner et al., 1991, 1992)?

Therefore the purposes of this study were to (1) compare the effect of vasopressin on non-pregnant and pregnant guinea-pig uterine arteries and to (2) determine whether possible changes in uterine artery sensitivity to vasopressin during pregnancy are due to altered vasopressin receptors or/and endothelial function.

2. Materials and methods

Adult female non-pregnant and pregnant (50–60 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 uterine tissue. The right and left uterine arteries, respectively, 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 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 the resting tension (non-pregnant: 4.5 mN; pregnant: 6.2 mN) as has been previously determined (Jovanović et al., 1994). 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 (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 precontracted with prostaglandin $F_{2\alpha}$ (6 μM) were challenged with acetylcholine (10 μM). On the basis of prior study (Jovanović et al., 1994), relaxation greater than 80% 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 prostaglandin $F_{2\alpha}$) was indicative of 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).

Concentration-response curves for vasopressin or 1-desamino-8-D-arginine vasopressin were made by adding increasing concentrations of these compounds 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 ($n = 7$ for each) demonstrated that first and second concentration-response curves for vasopressin 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 prostaglandin $F_{2\alpha}$, addition of acetylcholine, followed by three washes and 30-min equilibration period; (4) concentration-response curve with vasopressin (used as the tissue control) or 1-desamino-8-D-arginine vasopressin, followed by three washes, addition of the antagonist (only when vasopressin was used) and a 15 min equilibration period (Katušić et al., 1984); (5) concentration-response curve with vasopressin.

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

The contraction induced by each concentration of vasopressin was expressed as a percentage of the maximal contraction in response to vasopressin itself and used for constructing the concentration-response curves. The concentration of vasopressin 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 ($-\log$ 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 vasopressin in the presence and absence of an antagonist) at different antagonist concentrations for the different vasopressin/antagonist pairs were calculated for each experiment. Thus, the mean values of concentration ratios for a vasopressin/antagonist pair were plotted as a Schild diagram using regression analysis, and pA_2 was obtained from the intercept of the regression line with the abscissa (Arunlakshana and Schild, 1959). The concentration ratios (the ratio between the EC_{50} value of vasopressin 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) (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 evaluated by t-test and was considered not different from unity if $P > 0.05$.

To analyze the non-competitive antagonism by [1-(β-mercapto-β,β-cyclopentamine propionic acid), 2-(*O*-methyl) tyrosine]arginine-vasopressin ([$d(CH_2)_5$-Tyr(Me)^2]vasopressin) we used the procedure described by Kenakin (1987); equieffective concentrations of vasopressin in the absence [A] and presence of [$d(CH_2)_5$Tyr(Me)^2]vasopressin [A'] were obtained. A plot of $1/[A]$ against $1/[A']$ was constructed. The slope of the regression line and the y-intercept was used to calculate vasopressin (K_A) and [$d(CH_2)_5$Tyr(Me)^2]-vasopressin (K_B) dissociation constants: $K_A = (\text{Slope} - 1)/\text{intercept}$; $K_B = [[d(CH_2)_5\text{Tyr-(Me)}]\text{AVP}]/(\text{Slope} - 1)$. 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 fraction of receptors occupied (RA/RT) was calculating from the following equation: $[RA]/[RT] = [A]/(K_A + [A])$ (Furchgott and Bursztyn, 1967).

The results are expressed as means ± 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. The statistical significance of 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.

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; 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, prostaglandin $F_{2\alpha}$ (Sigma, USA), arginine-8-vasopressin, [1-(β-mercapto-β,β-cyclopentamethylene-propionic acid), 2-(*O*-methyl) tyrosine]arginine-vasopressin ([$d(CH_2)_5$Tyr(Me)^2]vasopressin), [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) (Peninsula Laboratories, USA), 1-desamino-8-D-arginine vasopressin (USV 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 vasopressin

Vasopressin (63 pM–10 nM) induced a concentration-dependent contraction of the non-pregnant and pregnant guinea-pig uterine arterial rings with intact endothelium. The concentration-response curves for vasopressin obtained with pregnant guinea-pig uterine arteries were significantly shifted to the left compared

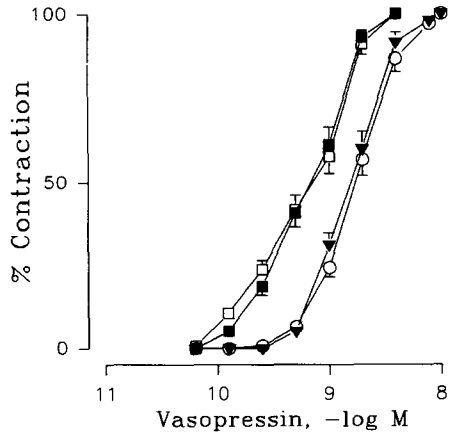

Fig. 1. Concentration-response curves for vasopressin in non-pregnant guinea-pig uterine artery with intact (○) and denuded endothelium (▼), and in pregnant guinea-pig uterine artery with intact (□) and denuded endothelium (■). Each point represents the mean± S.E.M (n = 22–23). Responses are expressed as a percentage of the maximal contraction induced by vasopressin itself.

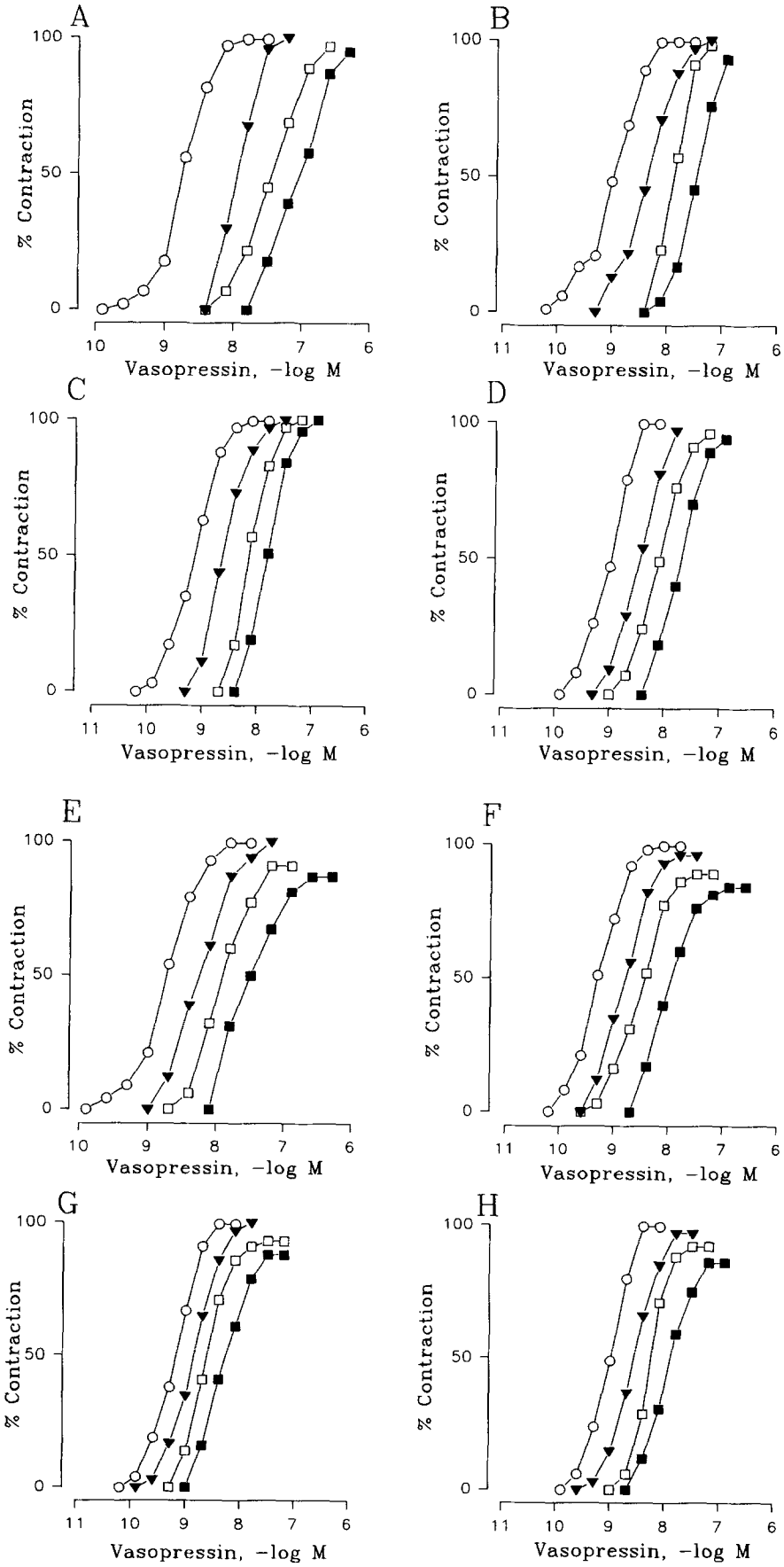
A
B
C
D
E
F
G
H
% Contraction
100
50
0
Vasopressin, −log M
10 9 8 7 6
11 10 9 8 7 6

Table 1
The pD_2 and maximal response values (100% in mN) for vasopressin in the non-pregnant and pregnant guinea-pig uterine arteries with intact (E^+) and denuded endothelium (E^-)

	Non-pregnant		Pregnant	
	E^+	E^-	E^+	E^-
pD_2	8.77±0.01	8.80±0.02	9.14±0.01	9.16±0.01
100% (mN)	6.77±0.71	6.91±0.68	7.31±0.85	7.43±0.88
n	22	23	23	23

The values are expressed as means ± S.E.M.; *n* refers to the number of animals used.

to those obtained with non-pregnant guinea-pig uterine arteries ($P < 0.01$). In both types of preparations removal of the endothelium did not affect contractions in response to vasopressin (Fig. 1, Table 1).

3.2. Effects of vasopressin receptor antagonists

In both non-pregnant and pregnant guinea-pig uterine arteries, with either 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 receptor-preferring antagonist, [d(CH$_2$)$_5$,D-Ile^2,Ile^4]vasopressin (300 nM–3 μM), induced a significant shift 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 vasopressin ($P > 0.05$, for both antagonists studied) (Fig. 2). The data from the experiments with vasopressin receptor antagonists were analyzed as described by Arunlakshana and Schild (1959). With 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 mean slope not different from unity (Fig. 3, Table 2). The pA_2 and $-\log K_B$ values are shown in Table 2. The $-\log K_B$ values for corresponding antagonists were not significantly different, regardless of endothelium condition or pregnancy status ($P > 0.05$).

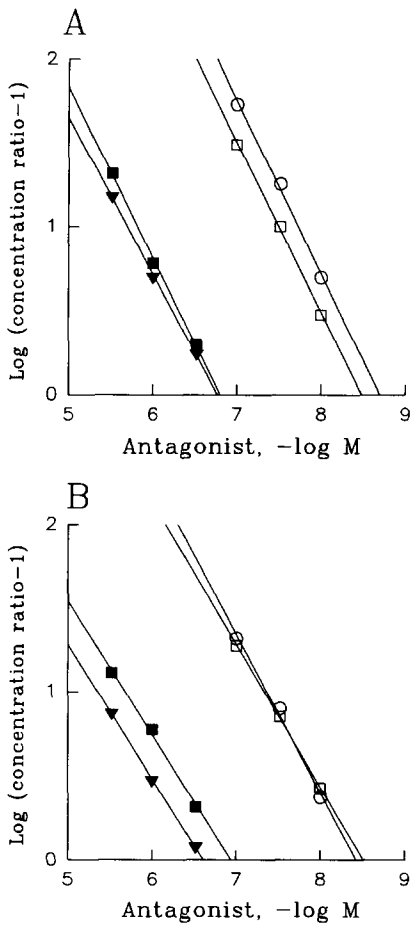

Fig. 3. Schild plots of log (concentration ratio − 1) against − log concentration antagonist for vasopressin-[d(CH$_2$)$_5$Tyr(Me)^2]vasopressin (○ for arteries with intact and □ for arteries with denuded endothelium) and vasopressin-[d(CH$_2$)$_5$,D-Ile^2,Ile^4]-vasopressin antagonism (▼ for arteries with intact and ■ for arteries with denuded endothelium) on non-pregnant (A) and pregnant (B) guinea-pig uterine artery. Each point represents the mean (n = 6–8).

3.3. Dissociation constant of the vasopressin-receptor complex

In order to determine the dissociation constant of the vasopressin-receptor complex, we used the proce-

Fig. 2. The antagonism of the contracting effects of vasopressin by antagonists of vasopressin receptors. (A, B, C, D) Concentration-response curves for vasopressin 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 6–23 experiments. Standard errors are excluded for clarity and did not exceed 15% of the mean value for each point. Responses are expressed as percentages of the maximal contraction induced by vasopressin. (E, F, G, H) Concentration-response curves for vasopressin 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 6–22 experiments. Standard errors are excluded for clarity and did not exceed 15% of the mean value for each point. Responses are expressed as percentages of the maximal contraction induced by vasopressin itself.

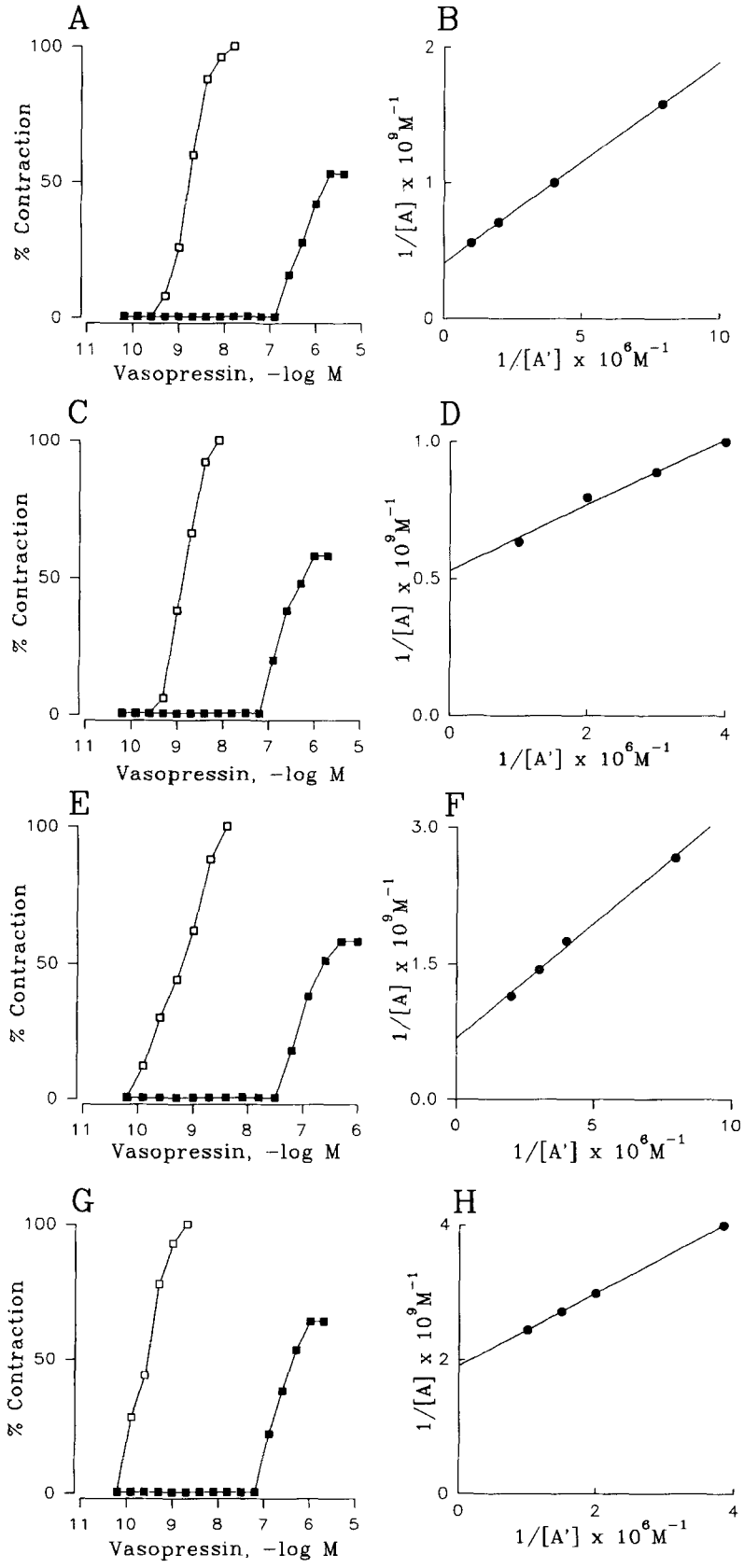
A
% Contraction
Vasopressin, −log M
B
1/[A] x 10^9 M^-1
1/[A'] x 10^6 M^-1
C
% Contraction
Vasopressin, −log M
D
1/[A] x 10^9 M^-1
1/[A'] x 10^6 M^-1
E
% Contraction
Vasopressin, −log M
F
1/[A] x 10^9 M^-1
1/[A'] x 10^6 M^-1
G
% Contraction
Vasopressin, −log M
H
1/[A] x 10^9 M^-1
1/[A'] x 10^6 M^-1

Table 2
pA_2, $-\log K_B$ values and slopes of Schild plots of the vasopressin receptors V_1 and V_2 antagonists on the non-pregnant and pregnant guinea-pig uterine arteries, determined by their ability to antagonize vasopressin-induced contraction of vascular segments

	pA_2	Slope	$-\log K_B$
Endothelium intact (non-pregnant)			
[$d(CH_2)_5$Tyr(Me)^2]Vasopressin	8.70 ± 0.08	1.03 ± 0.07	8.74 ± 0.02
[$d(CH_2)_5$,D-Ile^2,Ile^4]Vasopressin	6.80 ± 0.05	1.02 ± 0.06	6.82 ± 0.02
Endothelium denuded (non-pregnant)			
[$d(CH_2)_5$Tyr(Me)^2]Vasopressin	8.48 ± 0.05	1.01 ± 0.04	8.50 ± 0.01
[$d(CH_2)_5$,D-Ile^2,Ile^4]Vasopressin	6.77 ± 0.03	0.94 ± 0.03	6.72 ± 0.02
Endothelium intact (pregnant)			
[$d(CH_2)_5$Tyr(Me)^2]Vasopressin	8.43 ± 0.09	0.94 ± 0.08	8.38 ± 0.03
[$d(CH_2)_5$,D-Ile^2,Ile^4]Vasopressin	6.60 ± 0.04	0.85 ± 0.05	6.49 ± 0.06
Endothelium denuded (pregnant)			
[$d(CH_2)_5$Tyr(Me)^2]Vasopressin	8.52 ± 0.03	0.85 ± 0.03	8.36 ± 0.03
[$d(CH_2)_5$,D-Ile^2,Ile^4]Vasopressin	6.94 ± 0.06	0.80 ± 0.05	6.75 ± 0.06

The values are expressed as means ± S.E.M. (n = 6–8).

dure described by Kenakin (1987) (see Materials and methods). [$d(CH_2)_5$Tyr(Me)^2]Vasopressin, applied in a high concentration (300 nM for non-pregnant and 1 μM for pregnant guinea-pig uterine arteries) caused non-competitive inhibition of vasopressin-induced contraction as shown by the depression of the maximum response (control 100% vs. 47.3 ± 4.8%, vs. 54.1 ± 4.9% for non-pregnant and vs. 57.3 ± 6.1%, vs. 61.1 ± 5.7% for pregnant guinea-pig uterine artery with intact and denuded endothelium, respectively, n = 7 for each). The examples of these experiments are presented in Fig. 4. The mean pK_A were: 6.49 ± 0.08 (n = 7) and 6.55 ± 0.09 (n = 7) for non-pregnant guinea-pig uterine artery with intact and denuded endothelium, respectively; 6.48 ± 0.10 (n = 7) and 6.52 ± 0.07 (n = 7) for pregnant guinea-pig uterine artery with intact and denuded endothelium, respectively. The K_A values were not significantly different ($P > 0.05$). The concentration-response relationship with vasopressin (Fig. 1) was replotted as a function of receptor occupancy using calculated mean K_A values (see Materials and methods) and is shown in Fig. 5A. A non-linear stimulus-response relationship was obtained for all types of vessels studied (Fig. 5B–E). The receptor reserve values and percentages of receptor occupancy needed to reach half-maximal and maximal response are presented in Table 3. The difference between receptor reserve, half-maximal and maximal response values was statistically significant regarding pregnancy status ($P < 0.01$). In contrast, removal of the endothelium did not alter these values ($P > 0.05$).

The calculated mean pK_B values for [$d(CH_2)_5$Tyr(Me)^2]vasopressin according to the method of Kenakin (1987) (8.65 ± 0.06, n = 7 and 8.62 ± 0.04, n = 7 for non-pregnant guinea-pig uterine artery with intact and denuded endothelium, respectively; 8.44 ± 0.07, n = 7 and 8.68 ± 0.11, n = 7 for pregnant guinea-pig uterine artery with intact and denuded endothelium, respectively) were similar to these values calculated according to the method of Arunlakshana and Schild (1959) (Table 2).

Table 3
Receptor reserve (K_A/EC_{50}), percentages of receptors occupied by vasopressin (% RA/RT) needed to reach half-maximal (50%) and maximal response (100%) in the non-pregnant and pregnant guinea-pig uterine arteries with intact and denuded endothelium

	Non-pregnant		Pregnant	
	Intact	Denuded	Intact	Denuded
K_A/EC_{50}	188.5 ±21	179.8 ±17	455.5 ±474	33.6 ±41
% RA/RT (50%)	0.66 ± 0.07	0.70 ± 0.06	0.27 ± 0.04	0.30 ± 0.03
% RA/RT (100%)	4.76 ± 0.51	5.33 ± 0.48	1.20 ± 0.15	1.32 ± 0.13

The values are expressed as means ± S.E.M. (n = 7 for each).

Fig. 4. Typical experiments to determine the dissociation constant (K_A) for vasopressin in the non-pregnant and pregnant guinea-pig uterine artery with intact and denuded endothelium. Vasopressin concentration-response curves in non-pregnant guinea-pig uterine artery with intact (A) and denuded endothelium (C), and in pregnant guinea-pig uterine artery with intact (E) and denuded endothelium (G) before (□) and after (■) exposure of the preparation to [$d(CH_2)_5$Tyr(Me)^2]vasopressin (300 nM for non-pregnant and 1 μM for pregnant guinea-pig uterine arteries). Responses are expressed as percentages of the maximal contraction induced by vasopressin. Equieffective concentrations of vasopressin were determined graphically by linear interpolation of the concentration-response curves for vasopressin in the absence and presence of [$d(CH_2)_5$Tyr(Me)^2]vasopressin. (B) Double-reciprocal plot of equieffective concentrations of vasopressin before (ordinate scale, 1/[A]) and after (abscissa scale, 1/[A']) treatment with [$d(CH_2)_5$Tyr(Me)^2]vasopressin, obtained from (A) ($y = 147.5x + 4.2 \times 10^8$, r = 1; pK_A = 6.45, pK_B = 8.69). (D) Double-reciprocal plot of equieffective concentrations of vasopressin before (ordinate scale, 1/[A]) and after (abscissa scale, 1/[A']) treatment with [$d(CH_2)_5$Tyr(Me)^2]vasopressin, obtained from (C) ($y = 119.6x + 5.31 \times 10^8$, r = 0.993; pK_A = 6.65, pK_B = 8.60). (F) Double-reciprocal plot of equieffective concentrations of vasopressin before (ordinate scale, 1/[A]) and after (abscissa scale, 1/[A']) treatment with [$d(CH_2)_5$Tyr(Me)^2]vasopressin, obtained from (E) ($y = 253.5x + 6.8 \times 10^8$, r = 0.998; pK_A = 6.43, pK_B = 8.40). (H) Double-reciprocal plot of equieffective concentrations of vasopressin before (ordinate scale, 1/[A]) and after (abscissa scale, 1/[A']) treatment with [$d(CH_2)_5$Tyr(Me)^2]vasopressin, obtained from (G) ($y = 542.5x + 1.9 \times 10^9$, r = 1; pK_A = 6.55, pK_B = 8.73).

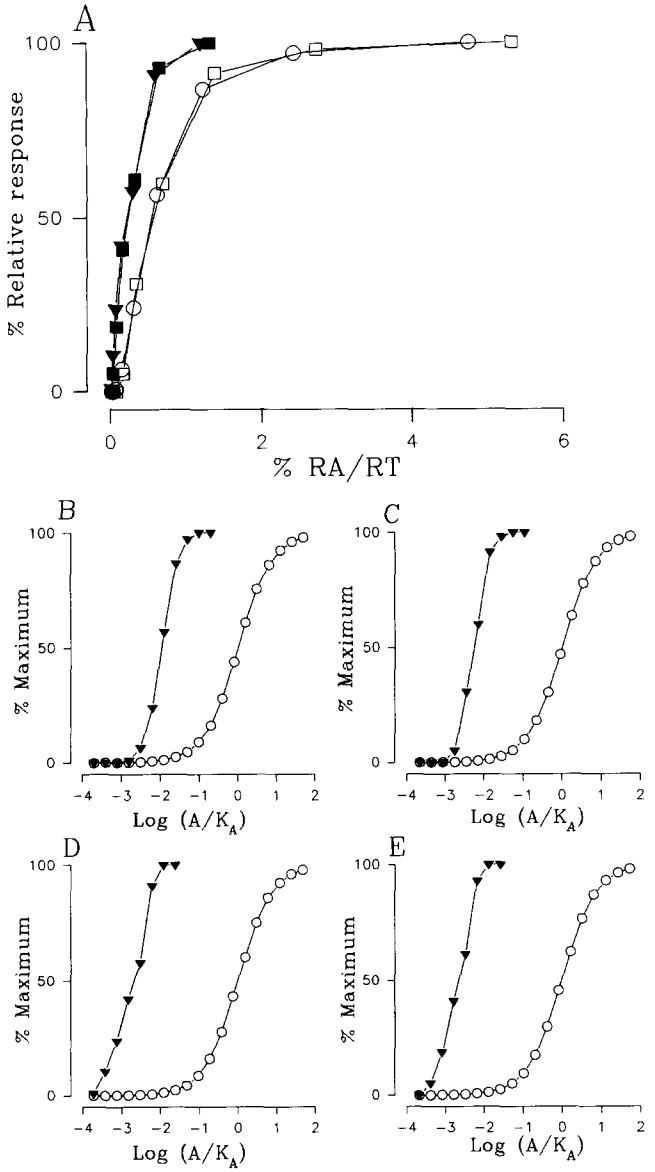

Fig. 5. Relationship of the relative response to the −log concentration and the fraction of receptors occupied (RA/RT) in vasopressin-induced contraction. (A) Replot of data from Fig. 1 showing the relative response to vasopressin in non-pregnant guinea-pig uterine artery with intact (○) and denuded endothelium (□), and in pregnant guinea-pig uterine artery with intact (▼) and denuded endothelium (■) as a function of receptors occupied by vasopressin. The fraction of receptors occupied was calculated employing the average K_A values for vasopressin (for non-pregnant guinea-pig uterine artery with intact endothelium: 323 nM; for non-pregnant guinea-pig uterine artery with denuded endothelium: 282 nM; for pregnant guinea-pig uterine artery with intact endothelium: 331 nM; for pregnant guinea-pig uterine artery with denuded endothelium: 302 nM). (B, C, D, E) The percentage of maximal responses (▼) or receptor occupancy (○) as a function of log concentration vasopressin normalized as fractions of average K_A for non-pregnant guinea-pig uterine artery with intact (B) and denuded endothelium (C), and for pregnant guinea-pig uterine artery with intact (D) and denuded (E) endothelium.

3.4. Effect of 1-desamino-8-D-arginine vasopressin

The non-pregnant and pregnant guinea-pig uterine arteries with both intact and denuded endothelium did not respond to the addition of 1-desamino-8-D-arginine vasopressin (10 nM–1 μM) ($n = 5$ for each) (data not shown).

4. Discussion

In the present study we confirmed previous findings that, in contrast to some other arteries (Katušić et al., 1984; Evora et al., 1993), in uterine arteries, vasopressin induces contraction (Svane et al., 1990; Nelson and Suresh, 1992; Kostrzewska et al., 1993). Furthermore, the pD_2 values for vasopressin in our study were similar to these values obtained in the majority of previous studies (Katušić et al., 1984; Katušić and Krstić, 1987).

It is known that, in certain blood vessels, removal of endothelium can potentiate the responses of vascular smooth muscle to vasopressin (Katušić and Krstić, 1987; 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; Conde et al., 1991). In prior investigations with verified endothelial condition, it has been observed that sensitivity to a few vasoconstrictors is significantly reduced in pregnant guinea-pig uterine artery with intact endothelium compared to that in non-pregnant guinea-pig uterine artery with the same endothelial status (Weiner et al., 1989, 1991, 1992). Removal of the endothelium affected the responses only in arteries from pregnant animals, and on the basis of these results, Weiner et al. (1989, 1991, 1992 suggested that pregnancy-associated changes in endothelial functions decrease the contractile response to these agents in uterine arteries. In the present study, removal of the endothelium did not affect the response to vasopressin, regardless of the pregnancy status of animals from which vessels were taken. This would not be consistent with the concept that the production of endothelium-derived relaxing factor(s) is increased in uterine artery during pregnancy, which in turn results in a reduction of agonist potency and efficacy (Weiner et al., 1991, 1992). However, the effect of vasopressin on guinea-pig uterine artery, from this point of view, has not been studied yet, and consequently, it is not feasible to compare our results with those previously reported. Nevertheless, if the basal production of endothelium derived relaxing factor(s) is increased during pregnancy it seems logical that the vasopressin-mediated contractions should be reduced in pregnant guinea-pig uterine artery with

intact endothelium compared to other types of vessels studied. Since this was not the case, it seems that the endothelium-associated decrease in contractile response of uterine artery during pregnancy is related to the agonist used. Obviously, contractions of uterine artery evoked by vasopressin are not modulated by vascular endothelium, regardless of pregnancy status.

It has been suggested that the anatomical and histological changes in the uterine artery during pregnancy are estrogen-dependent (Leiberman et al., 1993). Kostrzewska et al. (1993) reported that estrogens reduce contractions of human intramyometrial arteries in response to vasopressin. Our result that pregnancy shifted the concentration-response curves for vasopressin to the left is not in accord with this report. This disagreement could be explained by the fact that the effect of pregnancy on vascular responsiveness is not necessarily identical to the effect of estrogens on vascular responsiveness (Hidaka et al., 1991). Furthermore, it has been reported that vasopressin pressor activity in rats is unaltered during pregnancy (Pan et al., 1990). However, although whole animal pressor responses are blunted during pregnancy, uterine arteries, paradoxically, may become significantly more sensitive to the vasopressor agents (D'Angelo and Osol, 1993). The greater potency of vasopressin in pregnant guinea-pig uterine artery would be in agreement with this point of view, although the possible functional consequences of the increased reactivity of uterine arteries to some vasoconstrictors remain to be established (D'Angelo and Osol, 1993).

It is known that vasopressin induces contraction of isolated arteries through vasopressin V_1 receptor activation (Altura, 1974; Katušić et al., 1984; Gopalakrishnan et al., 1991). However, recently, the vasocontractile effect of vasopressin mediated by vasopressin V_2 receptors has been reported also (Chiba and Tsukada, 1992). In order to compare the contribution of different vasopressin receptor subtypes to the vasopressin-induced contraction between the vessels studied, 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, 1987).

The slopes of the Schild plot for both [$d(CH_2)_5$Tyr-(Me)^2]vasopressin and [$d(CH_2)_5$,D-Ile^2,Ile^4]vasopressin 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 calculated as the $-\log K_B$ 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 endothelium condition. Therefore, the possibility that different vasopressin receptor subtypes are involved in vasopressin-induced contraction of pregnant and non-pregnant uterine arteries with both intact and denuded endothelium was eliminated. Besides, affinity estimates for vasopressin itself also did not differ between the tissues studied, regardless of pregnancy status or endothelial condition.

The $-\log K_B$ values obtained for [$d(CH_2)_5$Tyr-(Me)^2]vasopressin in our study (8.36–8.74) were somewhat lower than the values obtained for vasopressin V_1 receptors in canine femoral artery ($pA_2 = 9.5$, Katušić et al., 1984), rabbit submucosal arteriolas ($-\log K_B =$ 10–10.3, Vanner et al., 1990) and in human platelets ($-\log K_B = 9.21$, Thibonnier et al., 1993), but they were similar to the values obtained for vasopressin V_1 receptors in guinea pig and human submucosal arterioles (8.50–9, Vanner et al., 1990), and rat vasopressor assay ($pA_2 = 8.62$, Manning et al., 1992). The reason for this variation in [$d(CH_2)_5$Tyr(Me)^2] vasopressin affinities is not known, but there is a possibility of further heterogeneity of vasopressin V_1 receptors (Vanner et al., 1990). 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 receptors 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 exclude a role for the V_{1B} (V_3) subtype of vasopressin receptor in vasopressin-induced contractions. The affinities of [$d(CH_2)_5$Tyr(Me)^2]-vasopressin for antagonizing the contractile action of vasopressin are clearly within the range reported for classical vasopressin V_1 receptor blockade (Vanner et al., 1990; Manning et al., 1992), suggesting the presence of contraction-mediating vasopressin V_1 receptors in quiescent preparations. In contrast, the $-\log K_B$ values of [$d(CH_2)_5$,D-Ile^2,Ile^4]vasopressin observed at receptors mediating contraction of uterine arteries (6.49–6.82) were significantly lower than the values reported for vasopressin V_2 receptors (8–8.24, Manning et al., 1983, 1984; Sawyer et al., 1988), and correspond to the values obtained for V_1 subtypes of vasopressin receptor (6.4–6.9, Manning et al., 1984; Szot et al., 1989). The lack of contraction-mediated vasopressin V_2 receptors in guinea-pig uterine arteries is also supported by the fact that 1-desamino-8-D-arginine vasopressin, a preferential vasopressin V_2 receptor agonist (Sawyer et al., 1981), was without any effect in all types of quiescent preparations. On the basis of these results, it seems reasonable to suggest, that in guinea-pig uterine artery, vasopressin induces contractions predominantly via ac-

tivation of V_{1A}-like vasopressin receptors, regardless of pregnancy status.

It is known that the affinity of vasopressin itself for vasopressin V_1 receptors is variable in that it exhibits a high pK_A values for vasopressin V_1 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 adult rat pup (7.27, Szot et al., 1989). We obtained very low affinities for vasopressin at vasopressin receptors mediating contraction of quiescent preparations (pK_A = 6.48–6.55). This difference may be due to profound species-dependent or species-independent differences of vasopressin 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 methods used for calculating pK_A values (binding vs. functional), as has been already described (Pliska, 1991). However, it should be mentioned that the pK_B values (8.44–8.68) for [$d(CH_2)_5$Tyr(Me)^2]-vasopressin obtained according to Kenakin (1987) were similar to those values (8.36–8.74) obtained using the method of Arunlakshana and Schild (1959). This similarity implies that the pK_A values obtained in our study are correct, regarding established criteria (Kenakin, 1987; Yanigasawa et al., 1989). On the basis of these results, it seems logical to conclude that, in guinea-pig uterine artery, vasopressin receptor affinity for vasopressin is not changed during pregnancy.

In all types of vessels studied, non-competitive antagonism obtained with a high concentration of [$d(CH_2)_5$Tyr(Me)^2]vasopressin revealed a non-linear relationship between contraction and percentage of receptors occupied by vasopressin. Quantification of the receptor reserve from the K_A/EC_{50} ratio expresses the efficiency of coupling (Ruffolo, 1982; Kenakin, 1987). The K_A/EC_{50} ratios for vasopressin are significantly higher than unity, suggesting that vasopressin behaves as a full agonist in guinea-pig uterine artery, regardless of pregnancy status and endothelial condition. However, in pregnant guinea-pig uterine arteries, there was a significantly greater receptor reserve. Since removal of the endothelium did not affect this change of receptor reserve for vasopressin during pregnancy, it is clear that products from vascular endothelium are not involved in these pregnancy-associated alterations. The greater potency of vasopressin in pregnant guinea-pig uterine arteries probably reflects a better coupling of the vasopressin-receptor complex or/and greater vasopressin receptors density in uterine artery during pregnancy (Kenakin, 1987). On the basis of the present results, it is not possible to conclude whether this greater potency of vasopressin during pregnancy is a consequence of better coupling or greater receptor density.

In conclusion, this study has shown that vasopressin-induced contractions in guinea-pig uterine artery are not modulated by vascular endothelium, regardless of pregnancy status. The potency and receptor reserve for vasopressin in guinea-pig uterine artery is greater during pregnancy, which is not related to changes of vasopressin receptor affinity for vasopressin. On the basis of differential antagonist affinity and affinity of vasopressin itself, we suggest that the identical subtype of vasopressin receptors, probably a V_{1A}-like subtype, is involved in the arginine vasopressin-induced contraction of non-pregnant and pregnant guinea-pig uterine artery.

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References

Altura, B.M., 1974, Neurohypophyseal hormones and analogues: rat pressor potency versus contractile potency on rat arterioalas and arteries, Proc. Soc. Exp. Biol. Med., 146, 1054.

Antoni, F.A., M.C. Holmes, G.B. Makara, M. Karteszi and F.A. Laszlo, 1984, Evidence that the effects of arginine-8-vasopressin (AVP) on pituitary corticotropin (ACTH) release are mediated by a novel type of receptor, Peptides 5, 519.

Arunlakshana, O. and H.O. Schild, 1959, Some quantitative uses of drug antagonists, Br. J. Pharmacol. Chemother. 14, 48.

Baertschi, A.J. and M. Friedli, 1985, A novel vasopressin receptor on anterior pituitary corticotrophs, Endocrinology 116, 499.

Chiba, S. and M. Tsukada, 1992, Potent antagonistic action of OPC-31260, a vasopressin V_2 receptor antagonist, on [Arg^8]vasopressin-induced vasoconstriction in isolated simian femoral arteries, Eur. J. Pharmacol. 221, 393.

Conde, M.V., E.J. Marco, M.L. Fraile, J.M. Benito, M.J. Moreno, M.L. Sanz and A.L. Lopez de Pablo, 1991, Different influence of endothelium in the mechanical responses of human and cat isolated cerebral arteries to several agents, J. Pharm. Pharmacol. 43, 255.

D'Angelo, G. and G. Osol, 1993, Regional variation in resistance artery diameter responses to alpha-adrenergic stimulation during pregnancy, Am. J. Physiol. 264, H78.

Disbrey, B.D. and J.H. Rack, 1970, Histological Laboratory Methods (E. and S. Livingstone, Edinburgh, London) p. 111.

Ekesbo, R., P. Alm, P. Ekstrom, L.M. Lundberg and M. Akerlund, 1991, Innervation of the human uterine artery and contractile responses to neuropeptides, Gynecol. Obstet. Invest. 31, 30.

Evora, P.R., P.J. Pearson and H.V. Schaff, 1993, Arginine vasopressin induces endothelium-dependent vasodilatation of the pulmonary artery. V_1-receptor-mediated production of nitric oxide, Chest 103, 1241.

Furchgott, R.F., 1972, The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory, in: Handbook of Experimental Pharmacology, Vol. 33, eds. H. Blaschko and E. Muschol (Springer Verlag, New York) p. 283.

Furchgott, R.F. and P. Bursztyn, 1967, Comparison of dissociation constants and of relative efficacies of selected agonists acting on parasympathetic receptors, Ann. NY Acad. Sci. 144, 882.

Gopalakrishnan, V., Y.J. Xu, P.V. Sulakhe, C.R. Triggle and J.R. McNeill, 1991, Vasopressin (V_1) receptor characteristics in rat aortic smooth muscle cells, Am. J. Physiol. 261, H1927.

Hidaka, A., O. Nakamoto, T. Kitanaka, S. Tomoda and T. Sugawa, 1991, Influence of estrogen and progesterone on the vascular response to angiotensin-II in non-pregnant rabbit vessels-intact or denuded endothelium, Nippon Sanka Fujinka Gakkai Zasshi 43, 281.

Howl, J., T. Ismail, A.J. Strain, C.J. Kirk, D. Anderson and M. Wheatley, 1991, Characterization of the human liver vasopressin receptor. Profound differences between human and rat vasopressin-receptor-mediated responses suggest only a minor role for vasopressin in regulating human hepatic function, Biochem. J. 276, 189.

Jard, S., R.C. Gaillard, G. Guillon, J. Marie, P. Schoenenberg, A.F. Muller, M. Manning and W.H. Sawyer, 1986, Vasopressin antagonists allow demonstration of a novel type of vasopressin receptor in the rat adenohypophysis, Mol. Pharmacol. 30, 171.

Jovanović, A., L. Grbović, D. Drekić and S. Novaković, 1994, Muscarinic receptor function in the guinea-pig uterine artery is not altered during pregnancy, Eur. J. Pharmacol. 258, 185.

Katušić, Z.S. and M.K. Krstić, 1987, Vasopressin causes endothelium-independent contractions of the rat arteries, Pharmacology 35, 264.

Katušić, Z.S., J.T. Shepherd and P.M. Vanhoutte, 1984, Vasopressin causes endothelium-dependent relaxations of the canine basilar artery, Circ. Res. 55, 575.

Kenakin, T.P., 1987, Pharmacologic Analysis of Drug-Receptor Interaction (Raven Press, New York).

Kostrzewska, A., T. Laudanski and S. Batra, 1993), Effect of ovarian steroids and diethylstilbestrol on the contractile responses of the human myometrium and intramyometrial arteries, Eur. J. Pharmacol. 233, 127.

Kruszynski, M., B. Lammek, M. Manning, J. Seto, H. Haldar and W.H. Sawyer, 1980, [1-(β-Mercapto-β,β-cyclopentamine propionic acid), 2-(*O*-methyl) tyrosine]arginine-vasopressin and [1-(β-mercapto-β,β-cyclopentaminehylene-propionic acid)]arginine-vasopressin, two highly potent antagonists of the vasopressor response to the arginine-vasopressin, J. Med. Chem. 23, 364.

Leiberman, J.R., A. Wiznitzer, M. Glezerman, B. Feldman, J. Levy and Y. Sharoni, 1993, Estrogen and progesterone receptors in the uterine artery of rats during and after pregnancy, Eur. J. Obstet. Gynecol. Reprod. Biol. 51, 35.

Manning, M., A. Olma, W.A. Klis, J. Seto and W.H. Sawyer, 1983, Potent antagonistic of the antidiuretic response to arginine-vasopressin based on modifications of [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-D-phenylalanine, 4-valine]-arginine-vasopressin at position 4, J. Med. Chem. 26, 1607.

Manning, M., E. Nawrocka, A. Misicka, A. Olma, W.A. Klis, J. Seto and W.H. Sawyer, 1984, Potent and selective antagonists of the antidiuretic response to arginine-vasopressin based on modifications of [1-(β-mercapto-β,β-pentamethylene-propionic acid), 2-D-isoleucine, 4-valine]arginine-vasopressin at position 4, J. Med. Chem. 27, 423.

Manning, M., K. Bankowski and W.H. Sawyer, 1987, Selective agonist and antagonists of vasopressin, in: Vasopressin, eds. D.M. Gash and G.J. Boer (Plenum Press, New York) p. 335.

Manning, M., S. Stoev, K. Bankowski, A. Misicka, B. Lammek, N.C. Wo and W.H. Sawyer, 1992, Synthesis and some pharmacological properties of potent and selective antagonists of the vasopressor (V_1-receptor) response to arginine-vasopressin, J. Med. Chem. 35, 382.

Nelson, S.H. and M.S. Suresh, 1992, Lack of reactivity of uterine arteries from patients with obstetric hemorrhage, Am. J. Obstet. Gynecol. 166, 1436.

Pan, Z.R., M.D. Lindheimer, J. Bailin and W.M. Barron, 1990, Regulation of blood pressure in pregnancy: pressor system blockade and stimulation, Am. J. Physiol. 258, H1559.

Parent, A., E.L. Schiffrin and J. St. Louis, 1991, Receptors for Arg^8-vasopressin, angiotensin II, and atrial natriuretic peptide in the mesenteric vasculature of pregnant rats, Can. J. Physiol. Pharmacol. 69, 1337.

Peeters, L.L.H., G. Grutters and C.R. Martin, 1980, Distribution of cardiac output in the unstressed pregnant guinea pig, Am. J. Obstet. Gynecol. 138, 1177.

Pliska, V., 1991, Pharmacological versus binding analysis of receptor systems: how do they interplay? Myometrial cell receptors for oxytocin as a paradigm, Experientia 47, 216.

Randall, M.D., A.P. Kay and C.R. Hiley, 1988, Endothelium-dependent modulation of the pressor activity of arginine vasopressin in the isolated superior mesenteric arterial bed of the rat, Br. J. Pharmacol. 95, 646.

Ruffolo Jr., R.R., 1982, Review: Important concepts of receptor theory, J. Auton. Pharmacol. 2, 277.

Sawyer, W.H., Z. Grzonka and M. Manning, 1981, Neurohypophysial peptides design of tissue-specific agonists and antagonists, Mol. Cell. Endocrinol. 22, 117.

Sawyer, W.H., K. Bankowski, A. Misicka, E. Nawrocka, M. Kruszynski, S. Stoev, W.A. Klis, J.P. Przybylski and M. Manning, 1988, Potent V_2 vasopressin antagonists with structural changes at their C-terminals, Peptides 9, 157.

Svane, D., S. Lundin, K.E. Andersson and A. Forman, 1990, Regional differences in vascular responses in the human uterus, Br. J. Obstet. Gynaecol. 97, 1033.

Szot, P., K.M. Myers, M. Swank and D.M. Dorsa, 1989, Characterization of a ^{3}H-arginine-vasopressin binding site in the cingulate gyrus of the rat pup, Peptides 10, 1231.

Tallarida, R.J., A. Cowan and M.W. Adler, 1979, pA_2 and receptor differentiation: a statistical analysis of competitive antagonism, Life Sci. 25, 637.

Taylor, A.H., V.T. Ang, J.S. Jenkins, J.J. Silverlight, R.C. Coombes and Y.A. Luqmani, 1990, Interaction of vasopressin and oxytocin with human breast carcinoma cells, Cancer Res. 50, 7882.

Thibonnier, M., A.L. Bayer and Z. Leng, 1993, Cytoplasmic and nuclear signaling pathways of V_1-vascular vasopressin receptors, Regul. Pept. 45, 79.

Vanner, S., M.M. Jiang, V.L. Brooks and A. Surprenant, 1990, Characterization of vasopressin actions in isolated submucosal arterioles of the intestinal microcirculation, Circ. Res. 67, 1017.

Vokaer, A., 1992, Biological prediction of pre-eclampsia: the vasoactive substances, J. Gynecol. Obstet. Biol. Reprod. (Paris) 21, 305.

Yanigasawa, T., H. Hashimoto and N. Taira, 1989, Interaction of potassium channel openers and blockers in canine atrial smooth muscle, Br. J. Pharmacol. 97, 753.

Weiner, C.P, E. Martinez, C.H. Chestnut and A. Ghodsi, 1989, Effect of pregnancy on uterine and carotid artery response to norepinephrine, epinephrine, and phenylephrine in vessels with documented functional endothelium, Am. J. Obstet. Gynecol. 161, 1605.

Weiner, C., L.K. Zhu, L. Thompson, J. Herrig and D. Chestnut, 1991, Effect of pregnancy on endothelium and smooth muscle: their role in reduced adrenergic sensitivity, Am. J. Physiol. 261, H1275.

Weiner, C.P., L.P. Thompson, K.Z. Liu and J.E. Herrig, 1992, Endothelium-derived relaxing factor and indomethacin-sensitive contracting factor alter arterial contractile responses to thromboxane during pregnancy, Am. J. Obstet. Gynecol. 166, 1171.