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Regional differences in the arterial response to vasopressin: role of endothelial nitric oxide

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1 The isometric response to arginine-vasopressin ($10^{-10}-10^{-7}$ M) was studied in 2 mm long rabbit arterial segments isolated from several vascular beds (cutaneous, pial, renal, coronary, muscular, mesenteric and pulmonary).

2 Vasopressin induced contraction in central ear (cutaneous), basilar (pial), renal, coronary and saphenous (muscular) arteries, but had no effect in mesenteric and pulmonary arteries; the order of potency for the contraction was: ear > basilar > renal > coronary > saphenous arteries.

3 Treatment with the blocker of nitric oxide synthesis N^G-nitro-L-arginine methyl ester (L-NAME; $10^{-6}-10^{-4}$ M) increased significantly ($P<0.05$) the contraction to vasopressin in ear (148% of control), basilar (150% of control), renal (304% of control), coronary (437% of control) and saphenous (235% of control) arteries. Removal of the endothelium increased significantly ($P<0.05$) the contraction to vasopressin in basilar (138% of control), renal (253% of control), coronary (637% of control) and saphenous (662% of control) arteries, but not in ear artery. Mesenteric and pulmonary arteries in the presence of L-NAME or after endothelium removal did not respond to vasopressin, as occurred in control conditions.

4 The specific antagonist for V_1 vasopressin receptors d$(CH_2)_5$Tyr(Me)AVP ($3\times10^{-9}-10^{-7}$ M) was more potent ($pA_2=9.3-10.1$) than the antagonist for both V_1 and V_2 vasopressin receptors desGly-d$(CH_2)_5$-D-Tyr(Et)ValAVP ($10^{-7}-10^{-6}$ M) ($pA_2=7.4-8.4$) to block the contraction to vasopressin of ear, basilar, renal and coronary arteries.

5 The specific V_2 vasopressin agonist [deamino-Cys[1], D-Arg[8]]- vasopressin (desmopressin) ($10^{-10}-10^{-7}$ M) did not produce any effect in any of the arteries studied, with or without endothelium.

6 In arteries precontracted with endothelin-1, vasopressin or desmopressin did not produce relaxation.

7 These results suggest: (a) most arterial beds studied (5 of 7) exhibit contraction to vasopressin with different intensity; (b) the vasoconstriction to this peptide is mediated mainly by stimulation of V_1 vasopressin receptors, and (c) endothelial nitric oxide may inhibit the vasoconstriction to this peptide, especially in coronary and renal vasculatures.

Keywords: Vasoconstriction; V_1 vasopressin antagonist; V_1 and V_2 vasopressin antagonist; N^G-nitro-L-arginine methyl ester (L-NAME); rabbit arteries

Introduction

Arginine-vasopressin is a neuropeptide that has been involved in the regulation of cardiovascular function under normal and some pathological conditions (Cowley & Liard, 1987), and may be involved in pathophysiology of vascular abnormalities such as coronary ischaemia (Maturi *et al.*, 1991) and subarachnoid haemorrhage (Mather *et al.*, 1981).

In vitro and *in vivo* experiments indicate that a considerable regional heterogeneity exists in the reactivity of blood vessels to vasopressin, as this peptide produces marked constriction in cutaneous, splanchnic or muscular blood vessels (Schmid *et al.*, 1974; Heydrickx *et al.*, 1976; Liard *et al.*, 1982), dilatation or weak constriction in renal vasculature (Schmid *et al.*, 1974; Heydrickx *et al.*, 1976; Naitoh *et al.*, 1993), no effect or dilatation in pulmonary vasculature (Walker *et al.*, 1989), dilatation in coronary arteries (Turlapaty & Altura, 1982), and both constriction or dilatation in cerebral vasculature (Lluch *et al.*, 1984; Takayasu *et al.*, 1993). The reasons for these differences are as yet unknown, and they may be related to the animal species and the experimental procedures used, as well as to the relative distribution and importance of V_1 and V_2 vasopressin receptors and to the role of the endothelium in vascular reactivity to arginine-vasopressin.

It has been considered that arginine-vasopressin interacts with at least two types of receptors (V_1 and V_2 receptors), and it has been found that in some vascular beds this peptide can produce constriction by activating the V_1 subtype (Martin de Aguilera *et al.*, 1990; Vanner *et al.*, 1990), whereas in other vessels it can produce dilatation by activating the V_2 subtype (Naitoh *et al.*, 1993; Aki *et al.*, 1994). On the other hand, studies in cerebral and coronary arteries indicate that V_1 receptors on the endothelium may mediate inhibition of the contraction (Katusic *et al.*, 1984), and that removal of the endothelium (Bockman *et al.*, 1993) or inhibition of nitric oxide synthesis (Gardiner *et al.*, 1991) can enhance the contraction of some vascular preparations to arginine-vasopressin. Therefore, the vascular actions of arginine-vasopressin and mechanisms involved seem to be complex, and their study remains of interest.

The objective of the present study was to investigate further and compare the effects of arginine-vasopressin in arteries from different vascular beds, analysing the role of the receptor subtypes involved and of the vascular endothelium in these effects. Cutaneous, pial, renal, coronary, muscular, mesenteric and pulmonary arteries from rabbits were prepared for isometric tension recording, and the responses to vasopressin, and

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to the V_2 vasopressin agonist desmopressin, were studied in control conditions, in the absence of the endothelium, during inhibition of nitric oxide synthesis, and during blockade of V_1, or V_1 and V_2 vasopressin receptors.

Methods

Sixty seven New Zealand White rabbits of either sex, weighing 2–2.5 kg, were killed by i.p. injection of sodium pentobarbitone (Sigma, 100 mg kg^{-1}), and the following arteries were dissected: central ear artery (cutaneous), basilar artery (pial), interlobar branches of the renal artery, anterior interventricular coronary artery, branches of the superior mesenteric artery, saphenous artery (muscular) and intrapulmonary arteries. After the arteries had been cleaned of surrounding tissue, they were cut into 2 mm-cylindrical segments. The external diameter of these arteries were in all cases between 0.5 and 1 mm. The arterial segments were then prepared for isometric tension recording in a 6-ml organ bath at 37°C containing modified Krebs-Henseleit solution of the following composition (mM): NaCl 115; KCl 4.6; KH_2PO_4 1.2; $MgSO_4$ 1.2; $CaCl_2$ 2.5; $NaHCO_3$ 25, glucose 11.1. The solution was equilibrated with 95% oxygen and 5% carbon dioxide to give a pH of 7.3–7.4. Two fine, stainless steel pins, 150 μm in diameter, were introduced through the lumen of the vascular segment. One pin was fixed to the organ bath wall, while the other was connected to a strain gauge for isometric recording, thus permitting the application of passive tension in a plane perpendicular to the long axis of the vascular cylinder. The recording system included a Universal Transducing Cell UC3 (Statham Instruments, Inc.), a Statham Microscale Accessory UL5 (Statham Instruments, Inc.) and a Beckman Type RS Recorder (model R-411, Beckman Instruments, Inc.). To determine the optimal passive tension, in preliminary experiments (3 animals) the vessels were stretched to different passive tensions (0.02, 0.1, 0.5, 1, 2 and 5 g) and the contraction to potassium chloride 10^{-1} M was recorded at each tension. For all types of arteries tested, the maximal contraction was observed at a resting tension of 0.5 g, therefore this tension was applied to the tissues in the following experiments, where the vascular segments were allowed to equilibrate for 60–90 min at this passive tension.

To examine the role of the endothelium and nitric oxide in the vascular response to arginine-vasopressin, cumulative concentration-response curves for this peptide (10^{-10}–10^{-7} M) were determined in the arteries under resting conditions in control conditions, in arteries treated with the inhibitor of nitric oxide synthesis N^G-nitro-L-arginine methyl ester (L-NAME, 10^{-6}–10^{-4} M), in the arteries without endothelium and in the arteries without endothelium and treated with L-NAME (10^{-4} M). Removal of the endothelium was accomplished by gently rubbing the vascular lumen with a steel rod, and the adequacy of the procedure was tested by abolition of the relaxing response to acetylcholine (10^{-6} M) in arteries precontracted with endothelin-1 (10^{-9}–10^{-8} M).

The subtype of receptors involved in the contraction to vasopressin was analysed by performing concentration-response curves in the presence of the mixed V_1 and V_2 vasopressin antagonist desGly-d$(CH_2)_5$-D-Tyr(Et)ValAVP (10^{-7}–10^{-6} M) and the V_1 vasopressin specific antagonist d$(CH_2)_5$Tyr(Me)AVP (3×10^{-9}–10^{-7} M). These particular studies were not performed in saphenous, pulmonary and mesenteric arteries, because these arteries showed no contraction to vasopressin, or a contraction too small to obtain reliable EC_{50} values. Also, the effects of the V_2 vasopressin agonist [deamino-Cys[1], D-Arg[8]]-vasopressin (desmopressin) (10^{-10}–10^{-7} M) were studied in ear, basilar, renal, coronary, mesenteric, saphenous and pulmonary arteries, with and without endothelium, and in the presence of L-NAME (10^{-4} M), at basal resting tension.

The experimental protocol was as follows: first, a concentration-response curve to vasopressin or desmopressin was performed in each segment under control conditions, followed by thorough washing. Then a second concentration-response curve was recorded in the same preparation, but in the following conditions: one segment without any treatment, and the other segments pretreated with one of the concentrations used of L-NAME or of the vasopressin antagonists. As it was found that the second concentration-response curve to vasopressin obtained in the vascular segment without any treatment was greater and more consistent than the first one, this second curve in the non-treated vascular segment was considered as the control, and for comparison with that obtained in the segments pretreated with L-NAME or the vasopressin antagonists. L-NAME and the vasopressin antagonists were applied to the organ bath 30–40 min before beginning the second concentration-response curve, and in each arterial segment, only one concentration of L-NAME or vasopressin antagonist was assayed.

The possibility of arterial relaxation was also explored by recording the effects of arginine-vasopressin (10^{-10}–10^{-7} M) or desmopressin (10^{-10}–10^{-7} M) in the arteries with extrinsic active tone. Ear, basilar, renal, coronary, saphenous and pulmonary arteries were precontracted only with endothelin-1 (10^{-9}–10^{-8} M); mesenteric arteries, in which endothelin-1 alone produced only a weak contraction, were contracted with endothelin-1 (10^{-8} M) plus 5-hydroxytryptamine (10^{-6} M). The effects of arginine-vasopressin in the precontracted arteries were studied in the absence and in the presence of the antagonist of V_1 vasopressin receptors d$(CH_2)_5$Tyr (Me)AVP (10^{-7} M), to eliminate the V_1-mediated component of the contraction.

To assess the contractile ability of the vascular smooth muscle, a contraction to potassium chloride (10^{-1} M) was also determined in each vascular segment.

Data analysis

The contraction produced by vasopressin was expressed as mean ± s.e.mean and the data were evaluated by analysis of variance applied to each group of data, followed by the Dunnet's t test to compare each experimental condition with its control. A probability value of less than 0.05 was considered significant. To quantify the potency of the antagonists for blocking vasopressin receptors, the pA_2 values were calculated from the shift of the dose-response curve produced in each type of artery by the antagonist, as described by Arunlakshana & Schild (1959).

Drugs

Materials used were: [Arg[8]]-vasopressin acetate; [deamino-Cys[1], D-Arg[8]]- vasopressin (desmopressin) acetate; the V_1 and V_2 antagonist des-Gly[9]- (β-mercapto- β, β-cyclopenta- methylenepropionyl[1], O- Et- Tyr[2], Val[4], Arg[8])- vasopressin [desGly-d$(CH_2)_5$-D-Tyr(Et)ValAVP]; the V_1 antagonist (β-mercapto- β, β -cyclopenta- methylenepropionyl[1], O- Me- Tyr[2], Arg[8])-vasopressin [d$(CH_2)_5$Tyr(Me)AVP]; N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME), and acetylcholine chloride, all from Sigma. Endothelin-1 (human, porcine) was purchased from Peninsula Laboratories Europe Ltd.

Results

Response to potassium chloride

In each case, the vascular segments contracted in response to potassium chloride (10^{-1} M), and the magnitude of this contraction in the different arteries is shown in Table 1. The order of potency of the potassium-induced contraction was: saphenous > ear = mesenteric = pulmonary > renal = basilar > coronary. Endothelium removal reduced significantly ($P<0.05$) the contraction to potassium in basilar and saphenous arteries, whereas it did not modify this contraction in ear, renal, coronary, mesenteric and pulmonary arteries.

Response to arginine-vasopressin

Under control conditions, vasopressin ($10^{-10}-10^{-7}$ M) produced concentration-dependent contractions in all the arteries studied with the exception of mesenteric and pulmonary arteries, in which it did not have any effect. The order of potency for the maximal contraction to vasopressin was: ear > basilar > renal > coronary > saphenous. The maximal contractions to vasopressin and EC_{50} values for the different arteries are shown in Table 2.

Application of L-NAME ($10^{-6}-10^{-4}$ M) by itself did not produce any effect in ear, saphenous, mesenteric and pulmonary arteries, whereas it induced contraction in basilar (1.2 ± 0.23 g for L-NAME 10^{-4} M), coronary (0.26 ± 0.12 g for L-NAME 10^{-4} M) and renal (0.14 ± 0.06 g for L-NAME 10^{-4} M) arteries.

In coronary, renal, saphenous and ear arteries, pretreatment with L-NAME increased significantly the contraction to vasopressin and this increase was dependent on the concentration of L-NAME (Figure 1). In the presence of L-NAME 10^{-4} M the vasopressin-induced response was 437% of control in coronary arteries, 304% of control in renal arteries, 235% of control in saphenous arteries and 148% of control in ear arteries. In the basilar artery pretreated with the lowest concentration of L-NAME used (10^{-6} M) the contraction to vasopressin was 150% of control, but pretreatment with higher concentrations of L-NAME ($10^{-5}-10^{-4}$ M) did not significantly modify the response of this artery to vasopressin as compared to control (Figure 1). In mesenteric and pulmonary arteries pretreated with L-NAME, vasopressin did not produce any effect, as in control conditions.

After endothelium removal, the contraction to vasopressin was higher in saphenous (662% of control), coronary (637% of control), renal (253% of control) and basilar (138% of control) arteries, but it was similar in ear arteries as compared to intact ear arteries (Figure 2). Mesenteric and pulmonary arteries without endothelium did not show any response to vasopressin, as in control conditions.

In ear, basilar, coronary and saphenous arteries without endothelium, pretreatment with L-NAME 10^{-4} M failed to produce any further increase in the response to vasopressin (Figure 2). In renal arteries without endothelium, L-NAME 10^{-4} M did further increase the response to vasopressin, and in these conditions, this response reached a value of 138% of that obtained in arteries without endothelium (Figure 2), although this increment was less than that produced by the same concentration of L-NAME in intact arteries (304% of control). In mesenteric and pulmonary arteries without endothelium and treated with L-NAME no response to vasopressin was observed, as in control arteries.

The antagonist for V_1 and V_2 vasopressin receptors desGly-d$(CH_2)_5$-D-Tyr(Et)ValAVP ($10^{-7}-10^{-6}$ M) and the specific antagonist for V_1 vasopressin receptors d$(CH_2)_5$Tyr(Me)AVP ($3\times10^{-9}-10^{-7}$ M) produced concentration-dependent, parallel rightward shifts of the dose-response curves to vasopressin in ear, basilar, renal and coronary arteries. In each type of artery tested, the V_1 antagonist was more potent than the V_1 and V_2 antagonist at blocking the response to vasopressin. The slope of the Schild plot for both antagonists was not significantly different from 1 in any case. As an example, Figure 3 shows the effects of the V_1 and V_2 antagonist desGly-d$(CH_2)_5$-D-Tyr(Et)ValAVP ($10^{-7}-10^{-6}$ M) on the response of basilar arteries to vasopressin and the corresponding Schild plot. The pA_2 values and the slopes obtained for the two antagonists used in the different arteries are shown in Table 3.

Response to desmopressin

Desmopressin ($10^{-10}-10^{-7}$ M) when added to the arteries at resting tension did not produce contraction or relaxation in any of the arteries studied (ear, basilar, renal, coronary, mesenteric, saphenous and pulmonary), with or without endothelium, or in the presence of L-NAME (10^{-4} M).

Responses of precontracted arteries

Endothelin-1 ($10^{-9}-10^{-8}$ M) produced active contractile tone in ear (1.9 ± 0.11 g), basilar (1.4 ± 0.33 g), renal (1.7 ± 0.29 g), coronary (1.3 ± 0.11 g), saphenous (1.8 ± 0.16 g) and pulmonary (2.1 ± 0.16 g) arteries. Endothelin-1 (10^{-8} M) plus 5-hydroxytryptamine (10^{-6} M) contracted mesenteric arteries (2.1 ± 0.18 g). In the absence of any intervention, this extrinsic active tone remained stable for at least 30 min.

Desmopressin ($10^{-10}-10^{-7}$ M) did not produce any effect in any of the precontracted arteries studied. Arginine-vasopressin ($10^{-10}-10^{-7}$ M), when added to precontracted arteries, produced a further contraction in ear, basilar and renal arteries, whereas it produced no effect on coronary, saphenous, mesenteric and pulmonary arteries. However, in arteries both precontracted and treated with the V_1 vasopressin-antagonist d$(CH_2)_5$Tyr(Me)AVP (10^{-7} M), vasopressin produced no effect in any of the arteries studied.

Table 1 Contractile response (g) of rabbit arteries with (control) and without endothelium to potassium chloride (10^{-1} M)

	Control	*Without endothelium*
Ear	2.45 ± 0.06 (96)	2.79 ± 0.13 (21)
Basilar	1.08 ± 0.04 (86)	0.53 ± 0.04*(17)
Renal	1.31 ± 0.06 (92)	1.25 ± 0.10 (14)
Coronary	0.73 ± 0.04 (89)	0.88 ± 0.09 (17)
Mesenteric	2.14 ± 0.18 (30)	3.00 ± 0.31 (12)
Saphenous	3.26 ± 0.12 (31)	2.07 ± 0.31* (11)
Pulmonary	2.71 ± 0.15 (27)	2.47 ± 0.24 (12)

Values are means±s.e.mean. The number of arterial segments are shown in parenthesis. *Statistically significant difference from the control ($P<0.05$).

Table 2 Maximal contraction and EC_{50} values of rabbit arteries in control conditions to arginine-vasopressin

	Maximal contraction (g)	EC_{50}(M)
Ear ($n=24$)	2.41 ± 0.18	6.3×10^{-10} ($4.2\times10^{-10}-9.5\times10^{-10}$)
Basilar ($n=21$)	0.50 ± 0.07	1.9×10^{-9} ($9.4\times10^{-10}-2.7\times10^{-9}$)
Renal ($n=25$)	0.28 ± 0.08	3.0×10^{-9} ($2.1\times10^{-9}-4.4\times10^{-9}$)
Coronary ($n=26$)	0.051 ± 0.01	2.8×10^{-9} ($2.3\times10^{-9}-3.4\times10^{-9}$)
Saphenous ($n=9$)	0.013 ± 0.007	5.9×10^{-9} ($3.7\times10^{-9}-9.3\times10^{-9}$)

Mesenteric and pulmonary arteries did not show any contraction to arginine vasopressin. Values are means± s.e.mean or 95% confidence interval; n=number of arterial segments.

Discussion

The present results indicate that the main effect of arginine-vasopressin in arteries from rabbits is to cause constriction, and this peptide failed to induce relaxation of the arteries

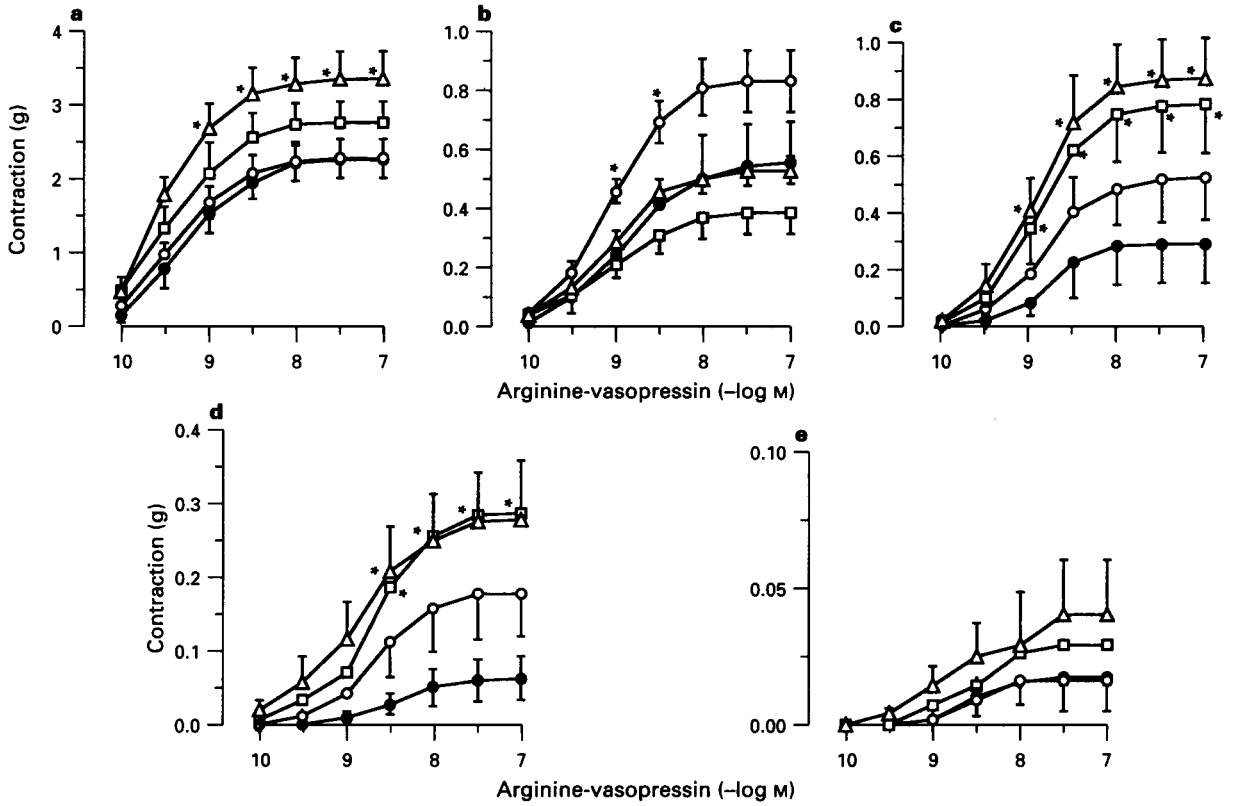

Figure 1 Contraction to arginine-vasopressin of rabbit central ear ($n=7$) (a), basilar ($n=6$) (b), renal ($n=7$) (c), coronary ($n=6$) (d) and saphenous ($n=5$) (e) arteries in the absence (●) and presence of L-NAME (10^{-6} M, ○; 10^{-5} M, □; 10^{-4} M, △). Note the different vertical scale for each type of artery. *n*, number of arterial segments in each condition. Each concentration of L-NAME was assayed in a different arterial segment from the same animal. $^{*}P<0.05$.

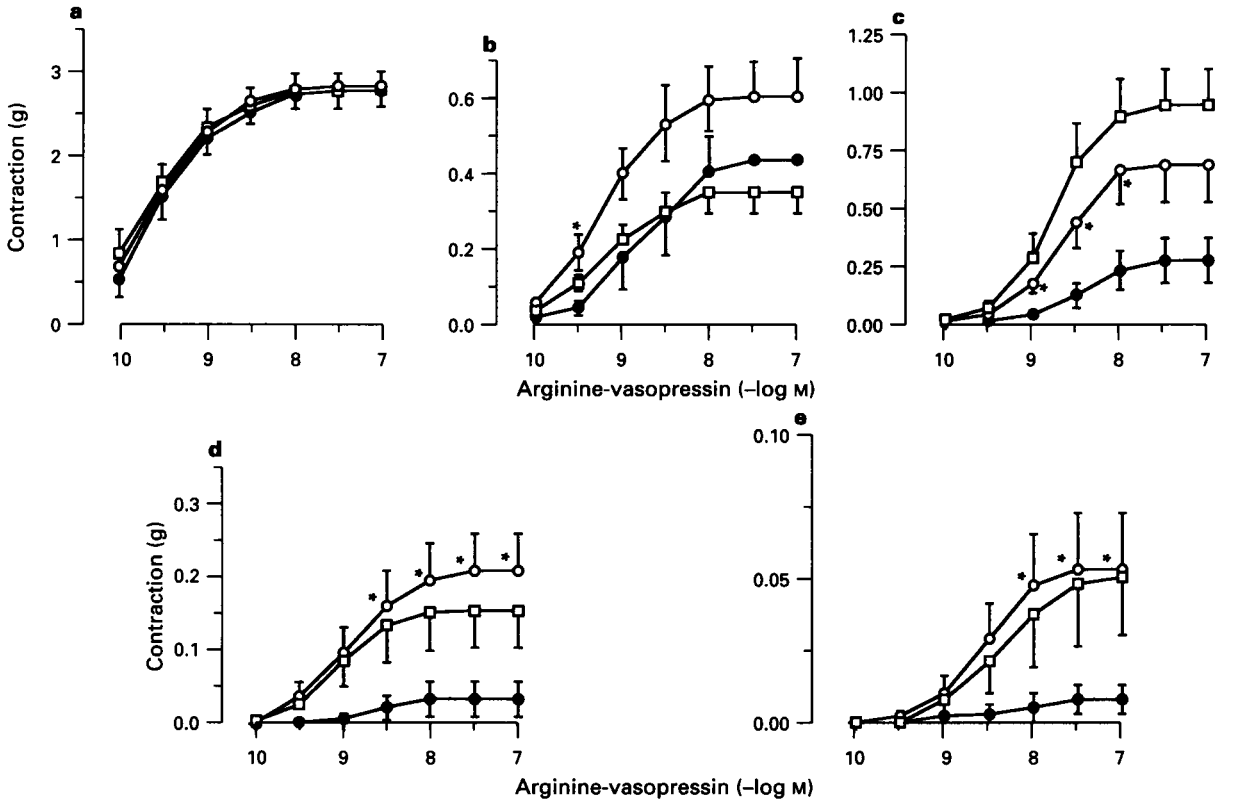

Figure 2 Contraction to arginine-vasopressin of rabbit central ear ($n=5$) (a), basilar ($n=5$) (b), renal ($n=5$) (c), coronary ($n=5$) (d) and saphenous ($n=4$) (e) arteries in control conditions (●), in the arteries without endothelium (○), and in the arteries without endothelium and in the presence of L-NAME (10^{-4} M, □). Note the different vertical scale for each type of artery. *n*, number of arterial segments in each condition. $^{*}P<0.05$.

under resting conditions or with extrinsic tone. Also, our results indicate that the constriction by arginine-vasopressin clearly differs between the vascular beds explored, as in relation to the contraction to potassium chloride it was relatively high in cutaneous arteries (ear artery) (about 100%), intermediate in pial arteries (about 50%), low in renal and coronary arteries (about 21% and 7%, respectively), and very low in muscular arteries (saphenous artery) (about 0.4%); the contraction to arginine-vasopressin was absent in mesenteric and pulmonary arteries. The reason for the observed differences in the vasoconstrictor effect to arginine-vasopressin probably does not lie in the ability of the arterial smooth muscle to contract, as there was not a direct correlation between the arterial response to direct stimulation with potassium chloride and that to vasopressin (see Tables 1 and 2).

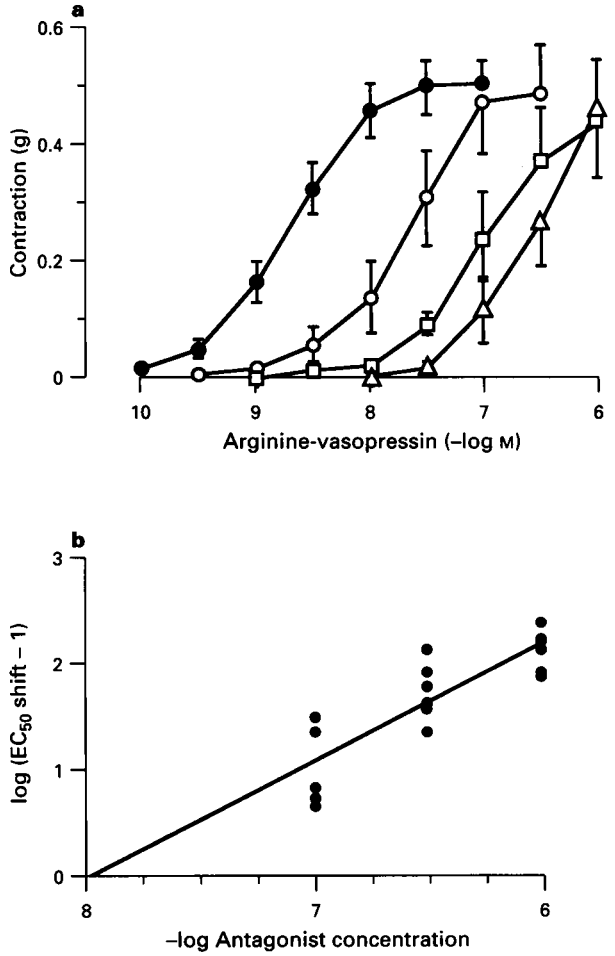

Figure 3 (a) Contraction of rabbit basilar arteries to arginine-vasopressin in the absence (●) and in the presence of the V_1 and V_2 antagonist desGly-d$(CH_2)_5$-D-Tyr(Et)ValAVP (10^{-7} M, ○; 3×10^{-7} M, □; 10^{-6} M △); $n=6$ animals. Each concentration of antagonist was assayed in a different arterial segment from each animal, and compared with a control arterial segment from the same animal. (b) Schild plot corresponding to the antagonism of desGly-d$(CH_2)_5$-D-Tyr(Et)ValAVP on the response of basilar arteries to vasopressin.

Our data suggest that the vasoconstriction to arginine-vasopressin is mediated mainly by V_1 receptors, with little or no participation of V_2 receptors. In the four arteries that showed a reasonable contraction to vasopressin (i.e. ear, basilar, renal and coronary arteries), this contraction was antagonized in a competitive manner by the V_1 antagonist d$(CH_2)_5$Tyr(Me)AVP, and by the mixed V_1 and V_2 antagonist desGly-d$(CH_2)_5$-D-Tyr(Et)ValAVP. The efficacy of the V_1 antagonist was higher than that of the V_1 and V_2 antagonist in the four arteries, and the pA_2 values found for the V_1 antagonist (9.3–10.1) and for the mixed V_1 and V_2 antagonist (7.4–8.4) are consistent with the hypothesis that the vasoconstrictor responses are mediated mainly by the V_1 subtype of vasopressin receptor (Jard *et al.*, 1986, a pA_2 value of 8.2 for the V_1 and V_2 antagonist; Gopalakrishnan *et al.*, 1991, a pK_1 value of 9 for the V_1 antagonist and of 8.2 for the V_1 and V_2 antagonist). In ear arteries, the slope values for the Schild plot were relatively low (0.85) for the V_1 antagonist and relatively high (1.37) for the V_1 and V_2 antagonist, resulting in pA_2 values respectively higher (10.1) and lower (7.4) than expected. This feature could suggest a mixed population of vasopressin receptors in this type of artery; however, as the slope for the two antagonists was not significantly different from unity, we must be cautious on this issue. The observation that the V_2 agonist desmopressin failed to produce contraction or relaxation in any of the arteries studied suggests the presence of a low population and/or efficacy of V_2 receptors in rabbit arteries. Therefore, the observed differences in the vasoconstriction to arginine-vasopressin in the different arteries are probably not related to the type of vasopressin receptors involved, and one possibility is that they are due, at least in part, to different affinities and/or concentrations of vasopressin receptors located in the arterial wall of vascular beds.

Of greater interest may be the results obtained with regard to the role played by the endothelium in the vascular effect by arginine-vasopressin, as it might also account, at least in part, for some of the observed differences in the vascular response to vasopressin. We found that endothelium removal potentiated the contraction to vasopressin, especially in saphenous, coronary and renal arteries, and to a minor degree in basilar arteries; this intervention did not affect the contraction of ear arteries, and did not modify the unresponsiveness of mesenteric and pulmonary arteries to this peptide. This suggests that under normal conditions the endothelium may inhibit the constriction to vasopressin to a different extent in some vascular beds (e.g. coronary, renal, pial and muscular), and it may not be involved in the constriction to this peptide in other blood vessels (e.g. cutaneous).

The inhibitory effect of the endothelium on the vasoconstriction to arginine-vasopressin may be mediated by the release of nitric oxide, as the inhibitor of nitric oxide synthase L-NAME also potentiated the contraction in intact arteries, a feature that did not occur (coronary, basilar, saphenous and ear arteries) or was diminished (renal artery) after endothelium

Table 3 pA_2 and slope values for the Schild analysis of the antagonism of desGly-d$(CH_2)_5$-D-Tyr(Et)Val AVP (V_1 and V_2 antagonist) and d$(CH_2)_5$Tyr(Me)AVP (V_1 antagonist) on the response of rabbit ear, basilar, renal and coronary arteries to arginine-vasopressin

	V_1 and V_2 antagonist		*V_1 antagonist*	
	pA_2	*Slope*	*pA_2*	*Slope*
Ear	7.36	1.37±0.27 ($n=5$)	10.06	0.85±0.16 ($n=6$)
Basilar	7.95	1.11±0.18 ($n=6$)	9.34	1.03±0.14 ($n=5$)
Renal	8.45	0.86±0.22 ($n=5$)	9.43	1.12±0.37 ($n=4$)
Coronary	7.71	1.00±0.22 ($n=5$)	9.61	0.98±0.36 ($n=5$)

Slope values are means±s.e.mean; n, number of animals. None of the slope values was significantly different from unity.

removal. This potentiating effect of L-NAME occurred specifically in coronary and renal arteries, and to a minor degree in saphenous and ear arteries. Thus, it may be hypothesized that the production of nitric oxide is of higher functional importance for affecting the reactivity to arginine-vasopressin in renal and coronary vascular beds than in other blood vessels (e.g. cutaneous). In renal arteries a small potentiating effect with L-NAME was observed in arteries without endothelium, suggesting that an incomplete removal of the endothelium had been achieved or that nitric oxide is also released from non-endothelial tissues (smooth muscle or nerve terminals).

The results obtained when examining vasoconstriction to arginine-vasopressin in the presence of L-NAME or after endothelium removal were parallel in all the vessels studied, except in the ear artery. In this artery, damage to the underlying smooth musculature may have occurred in the arterial segments subjected to the endothelium removal during the procedure used for this, thus masking the possible potentiation produced by elimination of endothelial nitric oxide. The potential damage of the underlying smooth muscle after the manoeuvre used for removing the endothelium is an inconvenience that may occur with some frequency; e.g. the decreased response to potassium chloride found in basilar and saphenous arteries might also be related to this potential damage even though arteries subjected to this procedure exhibited a potentiated response to arginine-vasopressin. This particular finding also suggests that mechanisms involved in the vascular response to potassium chloride and arginine-vasopressin are different, and that nitric oxide could interfere more with the effects of arginine-vasopressin than with those of potassium chloride. In the basilar artery, only the lowest concentration of L-NAME (10^{-6} M) used potentiated the response to vasopressin. This particular observation could be related to the fact that higher concentrations of L-NAME ($10^{-5}-10^{-4}$ M) by themselves produced marked contraction of resting basilar arteries, which could limit further contraction by vasopressin. In the saphenous artery, endothelium removal or inhibition of nitric oxide synthesis also potentiated the contraction to vasopressin, but this response was still relatively low even in these conditions. Thus, in the saphenous artery, although endothelial nitric oxide may also be inhibiting the response to vasopressin, the main cause of the low response of this artery to this peptide is probably related to a low number and/or efficacy of vasopressin receptors. In mesenteric and pulmonary arteries vasopressin did not produce any contraction after endothelium removal or nitric oxide inhibition as occurred in control conditions, suggesting that there are insufficient numbers of receptors in the smooth muscle of these arteries for mediating any effect to arginine-vasopressin.

Data in the literature indicate that vasopressin may stimulate release of nitric oxide by activating V_1 (Katusic, 1992; Takayasu *et al.*, 1993) or V_2 (Aki *et al.*, 1994) receptors. In our study we observed that neither vasopressin nor the V_2 agonist desmopressin produced any relaxation in precontracted arteries, thus suggesting that, in the arteries examined in this study, activation of vasopressin receptors may not stimulate the release of enough nitric oxide to induce vasorelaxation. Consequently, the inhibition of the contraction to vasopressin by endothelial nitric oxide suggested from our results may be related to a basal rather than to a stimulated release of nitric oxide in the arterial endothelium. The observation that L-NAME by itself produced contraction in coronary, renal and basilar arteries with endothelium, but not in those without endothelium, also suggests the presence of a basal vasodilator tone produced by endothelial nitric oxide in these arteries. The present findings agree with those from *in vivo* studies where a vasodilator tone mediated by nitric oxide has been observed in cerebral (Fernández *et al.*, 1993), coronary (García *et al.*, 1992) and renal (Sigmon & Beierwaltes, 1993) circulations.

In summary, the present results suggest that vascular regions differ in their responsiveness to arginine-vasopressin, and that the main arterial response to this peptide is constriction, which may be mediated by the V_1 subtype of vasopressin receptors. Differences in the number and/or efficacy of vasopressin receptors and in the modulatory function played by endothelial nitric oxide may account for the different reactivities of the regional vasculatures to arginine-vasopressin.

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