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Characterization of receptors mediating contraction of the rat isolated small mesenteric artery and aorta to arginine vasopressin and oxytocin

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1 The exact nature of the receptor subtype(s) involved in the action of arg-vasopressin (AVP) on the rat aorta and small mesenteric artery (SMA) is controversial. Therefore, we have studied the effects of the selective V_{1A} receptor antagonists, OPC 21268 and SR 49059, and the oxytocin (OT) receptor antagonist, atosiban, on the AVP- and OT-induced contractions of the two vessels.

2 AVP and OT displayed similar intrinsic activities in the rat aorta and SMA, but AVP was ~130 fold and ~500 fold more potent than OT, respectively. In the rat aorta, Hill slopes (n_H) were similar for OT and AVP. However, in rat SMA, the OT concentration-effect (E/[A]) curve was significantly steeper than the AVP E/[A] curve (n_H, $= 3.3 \pm 0.20$, 2.3 ± 0.15; $P < 0.001$).

3 In the aorta OPC 21268, SR 49059 and atosiban competitively antagonized the AVP and OT E/[A] curves. Except for atosiban and SR 49059 against AVP, competitive antagonism was also observed in the SMA. Atosiban caused concentration-dependent steepening of the AVP E/[A] curve, whereas SR 49059 decreased the upper asymptote.

4 Schild analysis yielded affinities indicative of V_{1A} receptor involvement in both vessels: pK_B/$pA_2 = 9.20 - 9.48$, $7.56 - 7.71$ and $6.19 - 6.48$ for SR 49059, OPC 21268 and atosiban, respectively.

5 Neither AVP nor OT relaxed U46619 pre-contracted aorta or SMA in the presence of SR 49059, suggesting no interference of a vasodilatory component.

6 Despite predominant involvement of V_{1A} receptors in both vessels, the different Hill slopes of AVP and OT E/[A] curves as well as the steepening of the AVP E/[A] curves by atosiban are indicative of receptor heterogeneity in the rat SMA.

Keywords: Aorta; atosiban; OPC 21268; oxytocin; rat; small mesenteric artery; SR 49059; vasopressin receptors

Introduction

Arg-vasopressin (AVP) is believed to exert its action through binding to two major classes of receptors: V_1 (subdivided in V_{1A} and V_{1B} subtypes) and V_2 receptors (Manning & Sawyer, 1989). In many isolated arteries, including those from human (Lluch *et al*., 1984; Martin De Aguilera *et al*., 1990; Liu *et al*., 1994; Martinez *et al*., 1994a,b; Bax *et al*., 1995; Jovanovic *et al*., 1995; Medina *et al*., 1996; Calo *et al*., 1997), rabbit (Garcia-Villalon *et al*., 1996), dog (Katusic *et al*., 1984; Myers *et al*., 1989) and the rat (Angus *et al*., 1994), vasoconstriction is mediated by the V_{1A} receptor. However, an early study demonstrated that the potency order of vasopressin analogues on the rat mesenteric arterioles differed from that on the rat aorta, suggesting the involvement of distinct receptors (Altura, 1975). This notion seems to be substantiated by the finding that the selective peptide V_1 receptor antagonist, [d$(CH_2)_5$Tyr(Me)[2]]AVP, was ten times more potent on the rat aorta ($pA_2 = 10.84$; Anouar *et al*., 1996) than on the rat small mesenteric artery (SMA; $pK_B = 9.76$); the latter affinity value indicated the involvement of V_{1A} receptor in the rat SMA (Angus *et al*., 1994). Although Burrell and colleagues (1994) reported that the AVP-induced contractions of the rat SMA were also potently antagonized by the non-peptide V_1 receptor antagonist OPC 21268 (Yamamura *et al*., 1991), the displayed antagonism was non-competitive as well as too potent to account for V_1 receptor involvement. These inconsistencies concerning the action of AVP in the rat SMA and aorta might suggest interference by a vasodilator component in the rat SMA (Walker *et al*., 1989; Matinez *et al*., 1994a) and/or the involvement of multiple receptors (Altura, 1975; Angus *et al*., 1994) in the two vessels. In this connection, oxytocin (OT) receptors may also be important, since OT receptors are operative in cardiovascular tissues (Yazawa *et al*., 1996; Gutkowska *et al*., 1997) and AVP and OT can activate each other's primary receptors (Manning & Sawyer, 1984; Jovanovic *et al*., 1995, 1997).

In the present study we aimed to eliminate the inconsistencies concerning the receptor subtype(s) involved in the response to AVP in the rat SMA and aorta. For this purpose, we analysed the mechanisms involved in the contractile action of AVP and OT in these vessels, using the non-peptide V_1 receptor antagonists, OPC 21268 (Yamamura *et al*., 1991) and SR 49059 (Serradeil-Le Gal *et al*., 1993), and the peptide OT receptor antagonist, atosiban (also known as ORF22164, RWJ 22164, or 1-deamino-[D-Tyr(OEt)[2]Thr[4]Orn[8]]OT (dETVT)) (Pettibone *et al*., 1992). A preliminary account of part of these data was presented to the British Pharmacological Society (Stam *et al*., 1996).

Methods

The rat small mesenteric artery preparation

Male Wistar rats (250–350 g) were anaesthetized (sodium pentobarbitone, 60 mg kg^{-1}, i.p.) and killed by cervical

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dislocation and the mesentery was removed and placed in ice-cold modified Krebs-Henseleit solution (KHS) of the following composition (mM): NaCl 119.0, $NaHCO_3$ 25.0, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 5.5, $CaCl_2$ 2.5. Arterial trees were dissected and cleared from surrounding adipose tissue. From each arterial tree, a ring segment (~2 mm in length) was mounted in a myograph (J.P. Trading, Aarhus, Denmark) with separated 6 ml organ baths (thermostatically controlled at 37°C containing modified KHS and continuously gassed with 95% O_2 and 5% CO_2) as described previously (Mulvany & Halpern, 1977). Tissue responses were measured continuously as changes in isometric force. Following a 30 min stabilization period, the internal diameter of each vessel was set to a tension equivalent to 0.9 times the estimated diameter at 100 mmHg effective transmural pressure (1_{100} = 200–300 μm) according to the standard procedure of Mulvany & Halpern (1977). After a further 30 min stabilization period, a calibration contraction (12.5 ± 0.5 mN, n = 61) was obtained to 100 μM phenylephrine and the presence of the endothelium confirmed. This procedure was followed by 30 min washing.

The rat isolated aortic ring preparation

The rat aorta was removed and placed in ice-cold modified KHS of the same composition as for the SMA, except for, the Ca^{2+} concentration, which was one tenth of that of standard KHS in order to eliminate the spontaneous phasic contractions seen in standard KHS (Martin, 1989). The tissue was mounted as 3 mm ring segments in 15 ml organ baths containing KHS ($CaCl_2$ = 0.25 mM) aerated with 95% O_2 and 5% CO_2 and maintained at 37°C. The ring segments were allowed to equilibrate at a tension of 20 mN for 60 min and were washed every 15 min. After equilibration, a calibration contraction (0.90 ± 0.02 g, n = 58) was obtained to 30 μM 5-hydroxytryptamine (5-HT) and the absence of the endothelium was confirmed. This procedure was followed by 60 min washing. Tissue responses were measured continuously as changes in isometric force with a Harvard isometric transducer.

Table 1 Estimates (means ± s.e.mean) of the upper asymptote (α), midpoint location (pEC_{50}) and Hill slope (n_H) obtained after fitting the individuals AVP and OT E/[A] curves in the rat SMA and aorta to the Hill equation

SMA	α	pEC_{50}	n_H	
AVP	118 ± 3%	9.48 ± 0.04	2.3 ± 0.15	n = 16
OT	126 ± 3%	6.76 ± 0.04*	3.3 ± 0.20*	n = 21
	P > 0.05	P < 0.001	P < 0.001	
Aorta	α	pEC_{50}	n_H	
AVP	73 ± 8%	9.19 ± 0.04	1.9 ± 0.10	n = 13
OT	53 ± 5%	7.07 ± 0.04*	1.8 ± 0.10	n = 9
	P > 0.05	P < 0.001	P > 0.5	

*Significantly different from AVP E/[A] curve.

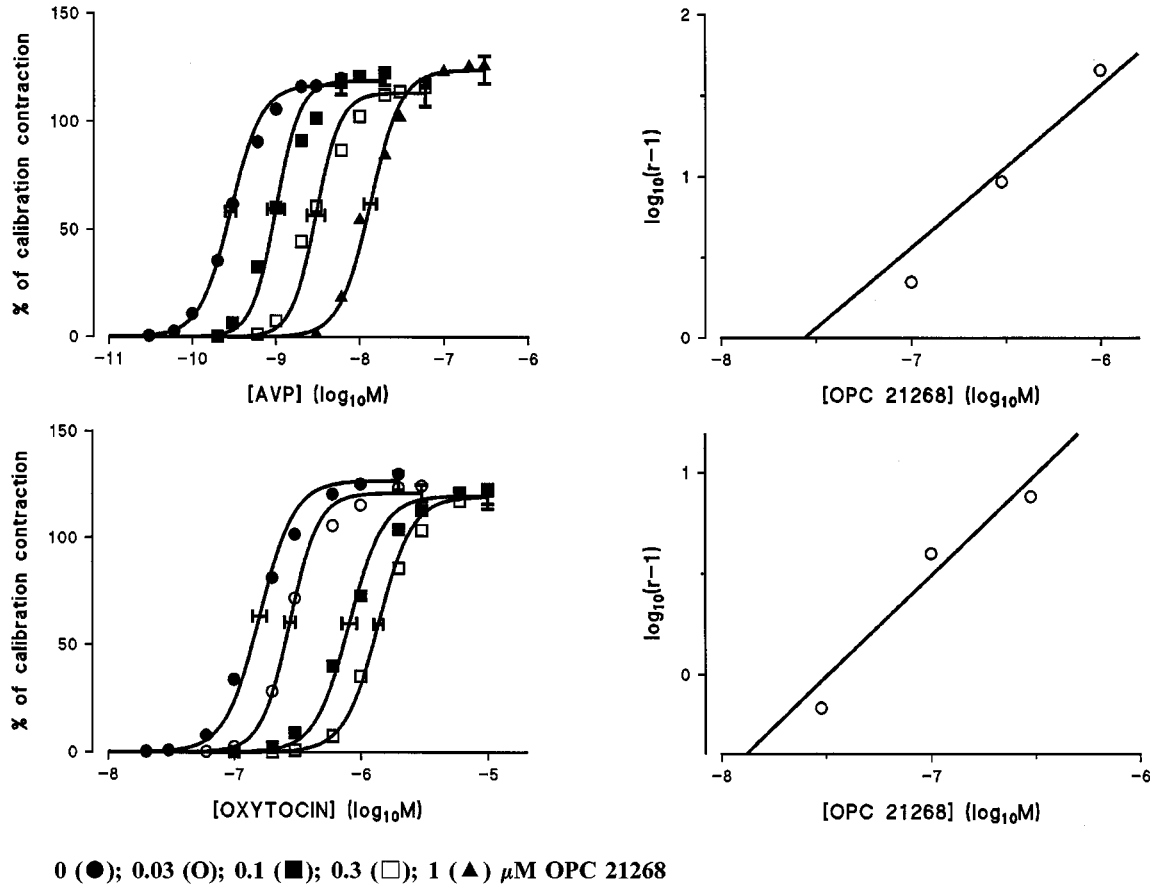

Figure 1 (Left panels) Concentration-effect curves to AVP and OT obtained on the rat SMA in the absence or presence of OPC 21268. The lines superimposed on the mean data points were simulated using the Hill equation. (Right panels) Schild plots for the interaction of OPC 21268 with AVP (upper panel) and OT (lower panel). The solid lines superimposed on mean data points were simulated using the parameters obtained from the constrained model fits.

Removal of endothelium

It is well known that contractile responses to a number of agonists can be influenced by endothelium-derived factors (Furchgott & Vanhoutte, 1989). Indeed, the contractile responses to AVP show tachyphylaxis in the rat aorta with intact endothelium (Millette & Lamontagne, 1996). Therefore, the endothelium of the aorta was denuded by gently rubbing with a poly-ethylene tube. In contrast, the endothelium of the rat SMA was left intact, since its removal turned out to be technically difficult and was found to be associated with a substantial decrease of the functional reactivity (unpublished observation). Fortunately, the necessity to remove the endothelium in the rat SMA is not that marked, since five repetitive AVP E/[A] curves could be produced without tachyphylaxis (Angus *et al*., 1994).

The integrity of endothelium was checked with acetylcholine (10 μM), which failed to relax rat aorta segments, but produced at least 60% relaxation in all segments of the rat SMA.

Experimental protocol

Tissues were incubated for 60 min with antagonist or vehicle and single agonist concentration-effect (E/[A]) curves were then obtained by cumulative dosing at quarter- or half-log unit concentration increments.

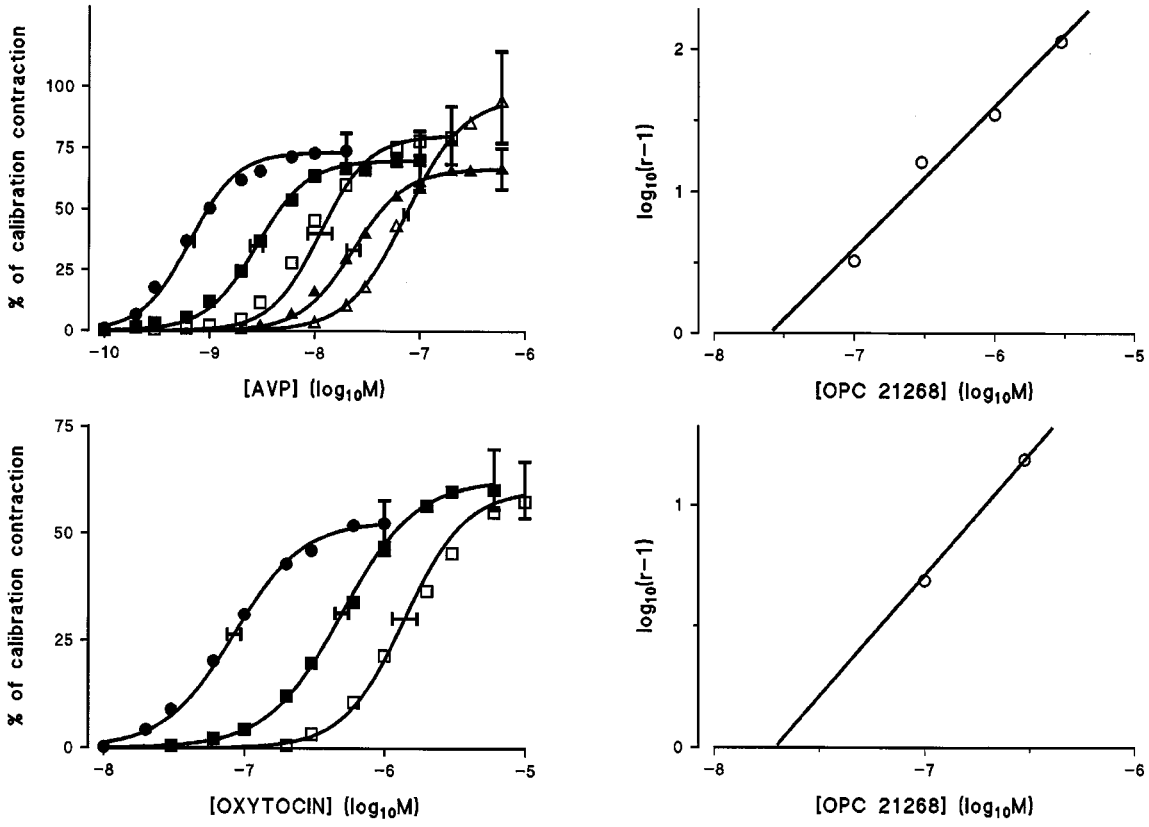

Figure 2 (Left panels) Concentration-effect curves to AVP and OT obtained on the rat aorta in the absence or presence of OPC 21268. The lines superimposed on the mean data points were simulated using the Hill equation. (Right panels) Schild plots for the interaction of OPC 21268 with AVP (upper panel) and OT (lower panel). The solid lines superimposed on mean data points were simulated using the parameters obtained from the constrained model fits.

Table 2 pK_B/pA_2 values (means$\pm$s.e.mean) for SR 49059, OPC 21268 and Atosiban on the rat SMA and aorta against AVP and OT and reported pK_i values for rat liver V_{1A} receptors

	SMA		*Aorta*		
Antagonist	*AVP*	*OT*	*AVP*	*OT*	*pK_i for the rat liver V_{1A} receptor*
SR 49059	9.20$\pm$0.13[a]	9.38$\pm$0.06	9.48$\pm$0.09	9.29$\pm$0.12[a]	9.1[b]
OPC 21268	7.56$\pm$0.11	7.49$\pm$0.08	7.60$\pm$0.07	7.71$\pm$0.08[a]	6.5–7.6[c]
Atosiban	6.48$\pm$0.11[a]	6.34$\pm$0.16	6.19$\pm$0.06	6.30$\pm$0.04	6.7[d]

[a]pA_2. [b]Serradeil-Le Gal *et al*., 1993. [c]Yamamura *et al*., 1991; Pettibone *et al*., 1992; Burrell *et al*., 1993a,b; Serradeil-Le Gal *et al*., 1993, 1994; Hirasawa *et al*., 1994. [d]Pettibone *et al*., 1992.

Analysis

Individual agonist curve data were fitted to the Hill equation using an iterative, least-squares method:

$$E = \frac{\alpha * [A]^{n_H}}{[A]_{50}^{n_H} + [A]^{n_H}}$$

to provide estimates of midpoint slope (n_H), midpoint location ($[A]_{50}$ estimated as a logarithm) and upper asymptote (α). The effect of drug treatment on these parameters was assessed by one-way analysis of variance (ANOVA) or Student's *t*-test, as appropriate. Values of $P<0.05$ were considered to be significant.

When the minimum criteria for competitive antagonism were satisfied, that is the antagonist produced parallel rightward shift of the agonist E/[A] curves with no change in upper asymptote, antagonist affinity estimates were obtained by fitting the individual midpoint location values obtained in the absence ($\log[A]_{50}$) and presence ($\log[A]_{50B}$) of antagonist (B) to the following derivation of the Schild equation as described previously (Black *et al.*, 1985a).

$$\log[A]_{50B} = \log[A]_{50} + \log(1 + [B]^b/10^{logK_B})$$

When the Schild plot slope parameter (b) was not significantly different from unity, then the data were re-fitted with b constrained to unity so that the antagonist dissociation equilibrium constant, K_B, could be estimated as log $K_B \pm$ s.e. (Jenkinson *et al.*, 1995). When one concentration of antagonist was tested or the criteria of competitive antagonism were not completely satisfied, an empirical pA_2 value was estimated using the above equation, with b constrained to unity.

Compounds

Compounds were obtained from the following sources: 5-hydroxytryptamine creatine sulphate, acetylcholine chloride, (−)-phenylephrine hydrochloride, oxytocin, [Arg^8]vasopressin acetate, U46619 (9,11-dideoxy-11α,9α-epoxy-methanoprostglandin $F_{2\alpha}$): Sigma Chemical Company Ltd., The Netherlands; SR 49059 ((2*S*) 1-[(2*R* 3*S*)-5-chloro-3-(2-chlorophenyl)-1-(3,4- dimethoxybenzene - sulphonyl)-3-hydroxy-2,3- dihydro-1*H*-indole-2-carbonyl]-pyrrolidine-2-carboxamide) and OPC 21268 (1-{1-[4-(3-acetylaminopropoxy)benzoyl]-4-piperidyl}-3,4-dihydro-2(1*H*-benzazepine]): a gift from Dr D. Nisato, Sanofi Recherche, Montpellier Cedex, France; Atosiban: a gift from Dr P. Melin, Ferring Pharmaceuticals, Malmö, Sweden. U46619 was dissolved initially in 20% ethanol to give a 1 mM stock solution and further diluted in distilled water. OPC 21268 and SR 49059 were dissolved in dimethylsulphoxide to give a 1 mM stock solution and further diluted in distilled water. All other drugs were dissolved in distilled water.

Results

Contractions to AVP and OT

AVP and OT produced concentration-dependent contractions of the rat SMA and aorta. The individual curves were fitted to

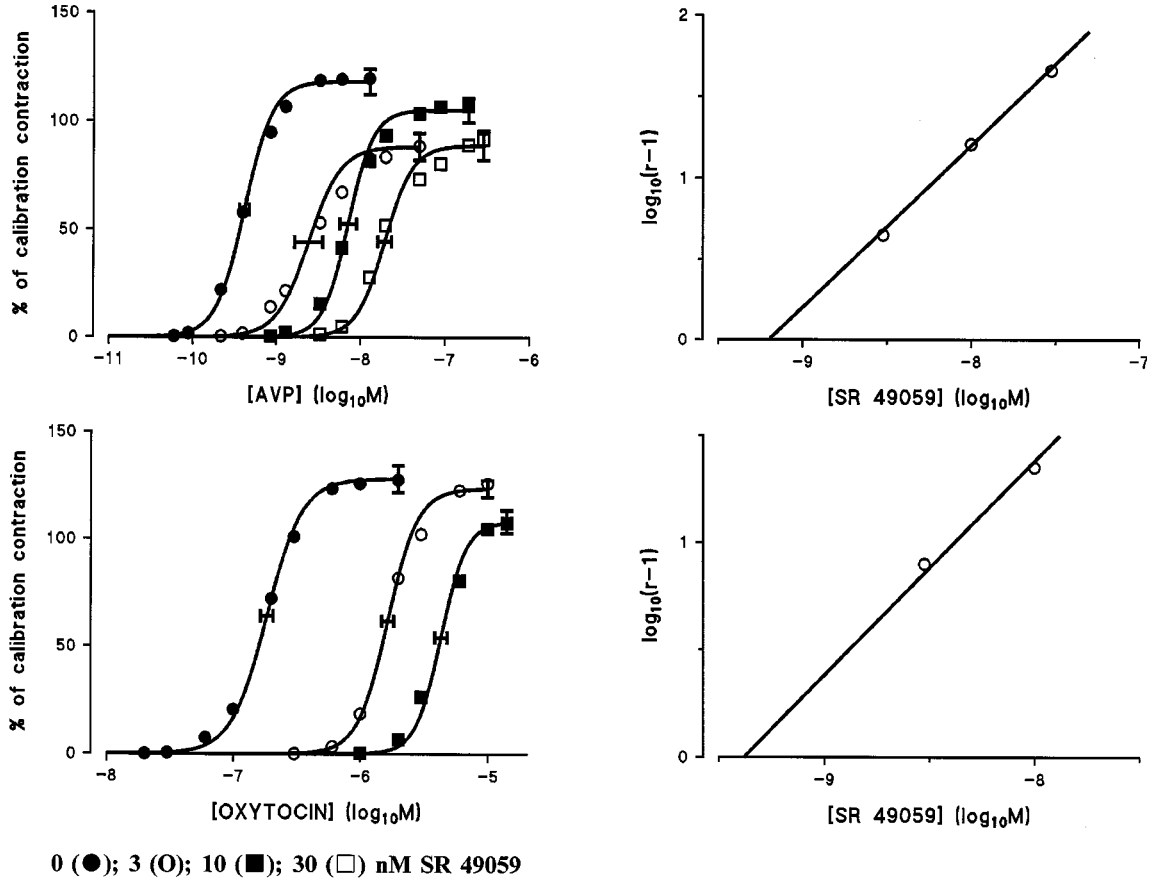

Figure 3 (Left panels) Concentration-effect curves to AVP and OT obtained on the rat SMA in the absence or presence of SR 49059. The lines superimposed on the mean data points were simulated using the Hill equation. (Right panels) Schild plots for the interaction of SR 49059 with AVP (upper panel) and OT (lower panel). The solid lines superimposed on mean data points were simulated using the parameters obtained from the constrained model fits.

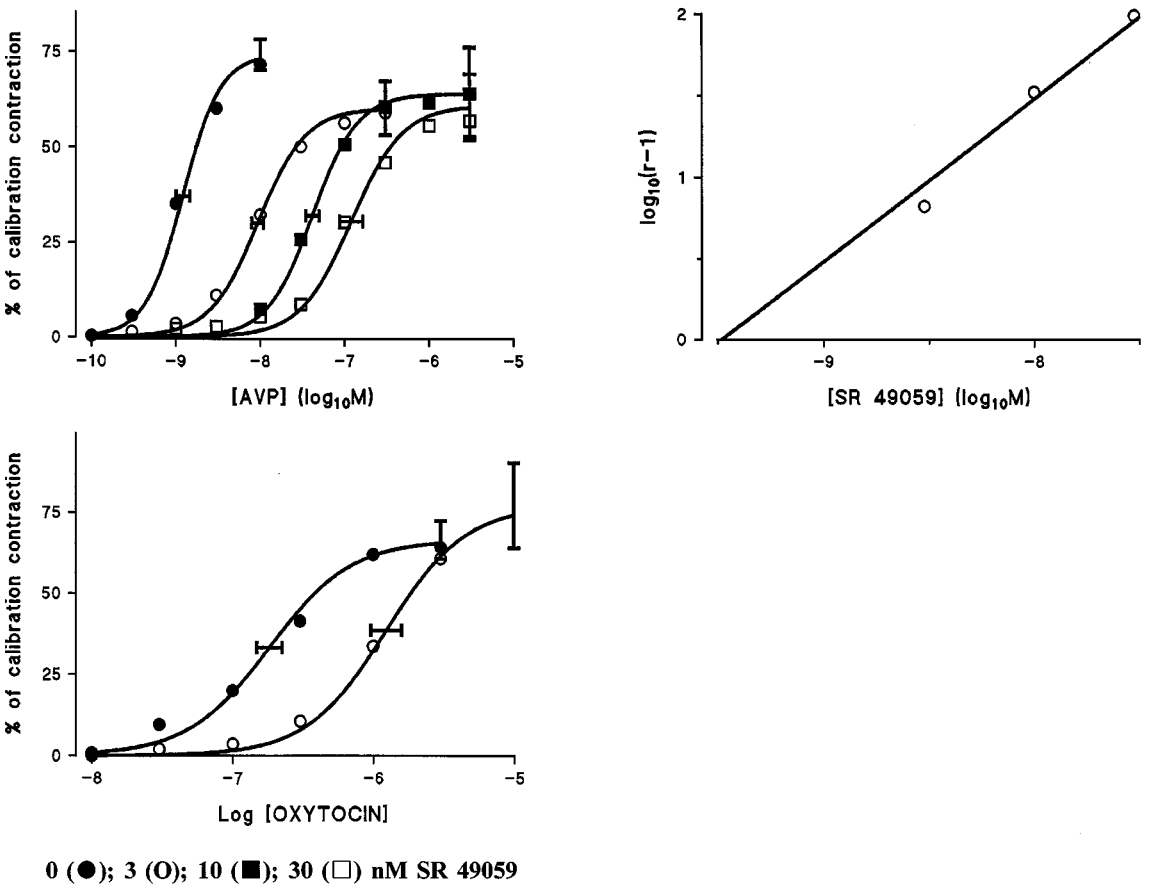

Figure 4 (Left panels) Concentration-effect curves to AVP and OT obtained on the rat aorta in the absence or presence of SR 49059. The lines superimposed on the mean data points were simulated using the Hill equation. (Right panel) Schild plot for the interaction of SR 49059 with AVP. The solid lines superimposed on mean data points was simulated using the parameters obtained from the constrained model fits.

the Hill equation to provide estimates of the midpoint location (pEC_{50}), slope (n_H) and upper asymptote (α) (Table 1). The intrinsic activities of AVP and OT were not significantly different, but AVP was $\sim$500 and $\sim$130 fold more potent than OT in the SMA and aorta, respectively. Interestingly, in the SMA, but not in the aorta, the OT E/[A] curve was significantly steeper than the AVP E/[A] curve.

Effect of OPC 21268 on the response to AVP and OT

The selective V_1 receptor antagonist OPC 21268 (0.1–3 μM, $n=4-11$) behaved as a competitive antagonist of AVP- and OT-induced contractions of the rat SMA (Figure 1) as well as aorta (Figure 2). The Schild slope parameters (b) for the antagonism of OPC 21268 against AVP and OT in the SMA ($b=1.27\pm0.15$ and 0.84 ± 0.14, respectively) and aorta ($b=0.82\pm0.10$ and 1.04 ± 0.48, respectively) were not significantly different from unity, allowing for the estimation of pK_B values (Table 2).

Effect of SR 49059 on the response to AVP and OT

In the rat SMA (Figure 3), the other selective V_1 receptor antagonist SR 49059 (3 and 10 nM, $n=5$) behaved as a competitive antagonist of OT ($b=0.86\pm0.15$; $pK_B=9.38\pm0.06$; Table 2). In contrast, however, SR 49059 produced a small non-concentration related depression of the maximum response to AVP. Notwithstanding this complex behaviour of SR 49059, the data were fitted to the Schild equation. The Schild slope parameter was not significantly different from unity ($b=0.97\pm0.17$) and the estimated pA_2 value was 9.20 ± 0.13 (Table 2).

In the rat aorta (Figure 4), SR 49059 (3–30 nM, $n=3-5$) produced parallel rightward shifts of the AVP and OT E/[A] curves. Schild analysis yielded a slope parameter not significantly different from unity ($b=1.15\pm0.1$) for the antagonism of the AVP response ($pK_B=9.48\pm0.09$; Table 2). pA_2 value for SR 49059 against OT, obtained after fitting the data to the Schild equation with b constrained to unity, was 9.29 ± 0.12 (Table 2).

Effect of atosiban on the response to AVP and OT

In the rat SMA (Figure 5; Table 2), atosiban (0.3–3 μM, $n=4-5$) behaved as a competitive antagonist of the OT E/[A] curves. Again, however, the AVP E/[A] curve in the rat SMA was not displaced in a parallel manner, since atosiban (1–10 μM, $n=4$) produced a significant concentration-dependent steepening (Hill slopes: 1.96 ± 0.01, 2.14 ± 0.10, 2.40 ± 0.16 and 2.70 ± 0.11 for 0, 1, 3 and 10 μM atosiban, respectively, $P<0.05$). Notwithstanding this complex behaviour, the data were fitted to the Schild equation to obtain values of b (1.06 ± 0.15) and pA_2 (6.48 ± 0.11; Table 2).

In the rat aorta (Figure 6), atosiban (0.3–10 μM, $n=5-7$) produced parallel rightward shifts of the AVP and OT E/[A] curves. Schild analysis yielded slope parameters not significantly different from unity ($b=0.82\pm0.10$ and 0.81 ± 0.14) and

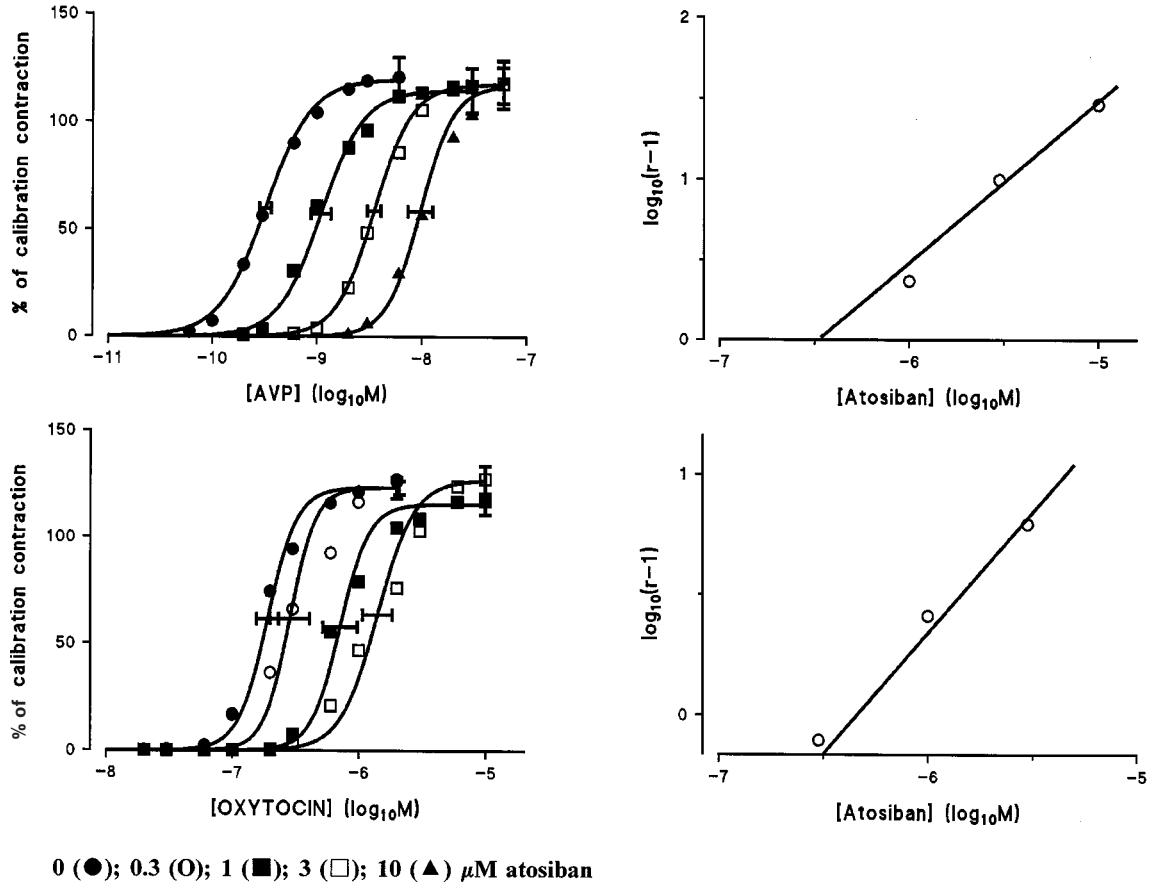

Figure 5 (Left panels) Concentration-effect curves to AVP and OT obtained on the rat SMA in the absence or presence of atosiban. The lines superimposed on mean data points were simulated using the Hill equation. (Right panels) Schild plots for the interaction of atosiban with AVP (upper panel) and OT (lower panel). The solid lines superimposed on mean data points were simulated using the parameters obtained from the constrained model fits.

pK_B values of 6.19 ± 0.06 and 6.30 ± 0.04 against AVP and OT, respectively (Table 2).

Relaxant responses to AVP and OT

In order to study whether AVP and OT displayed a non-V_{1A} receptor-mediated vasodilator response, the rat SMAs and aortae were pre-contracted with 100–200 nM and 10–30 nM U46619, respectively, after selective V_{1A} receptor blockade by 30 min pre-incubation with the SR 49059 (10 nM). After the contractile response had stabilized ($78 \pm 11\%$ and $73 \pm 11\%$ of the calibration contraction, for the rat SMA and aorta, respectively) AVP or OT E/[A] curves were obtained. No relaxation to AVP and OT was observed in either tissue ($n = 4-5$, data not shown). In fact, a slight further contraction was seen.

Discussion

To date the receptor subtype involved in the AVP-induced contraction of the rat SMA has been controversial. The peptide V_1 receptor antagonist [d$(CH_2)_5$Tyr(Me)^2]AVP defined the receptor involved as V_1 (Angus *et al.*, 1994). However, the data obtained with OPC 21268 in the rat SMA were inconsistent with the involvement of a V_1 receptor (Burrell *et al.*, 1994). Furthermore, the potencies of AVP receptor agonist as well as antagonist peptides differed for the rat aorta and mesenteric resistance arteries (Altura, 1975; Angus *et al.*, 1994; Anouar *et al.*, 1996). This suggests regional differences in the receptor subtype(s) involved in the response to AVP.

In the present study, AVP and OT produced concentration-dependent contractions of the rat SMA and aorta, with AVP being about 500 and 130 times, respectively, more potent than OT. The estimated antagonist affinities of OPC 21268 (7.49–7.71), SR 49059 (9.2–9.5) and atosiban (6.19–6.48) were similar with respect to the agonists (AVP and OT) and vessels (SMA and aorta) studied. Since these affinity values are in accordance with the reported binding affinities for V_{1A} receptors on the rat liver membranes (Table 2) and SR 49059 displays only a 10^{-7} M affinity for the OT receptor (Serradeil-Le Gal *et al.*, 1993), it is tempting to conclude that the functional responses to both AVP and OT in the rat SMA and aorta are mediated *via* a single receptor that can be classified as V_{1A}. However, the analysis of the action of AVP suggests a more complex situation in the rat SMA. The Hill slopes of the AVP and OT E/[A] curves ($n_H = 2.3$, 3.3, respectively) differed significantly. In case of a homogeneous receptor population, different Hill slopes would be expected only if the intrinsic activities of the agonists were different (Black *et al.*, 1985b). This was not the case as the upper asymptotes of the AVP and OT E/[A] curves in the rat SMA were similar (see Table 1).

Studying α_1-adrenoceptor responses in the rat aorta, Van der Graaf and colleagues have modelled that the differences in Hill slope values of agonists with similar intrinsic activity are best accounted by assuming multiple receptors (Van der

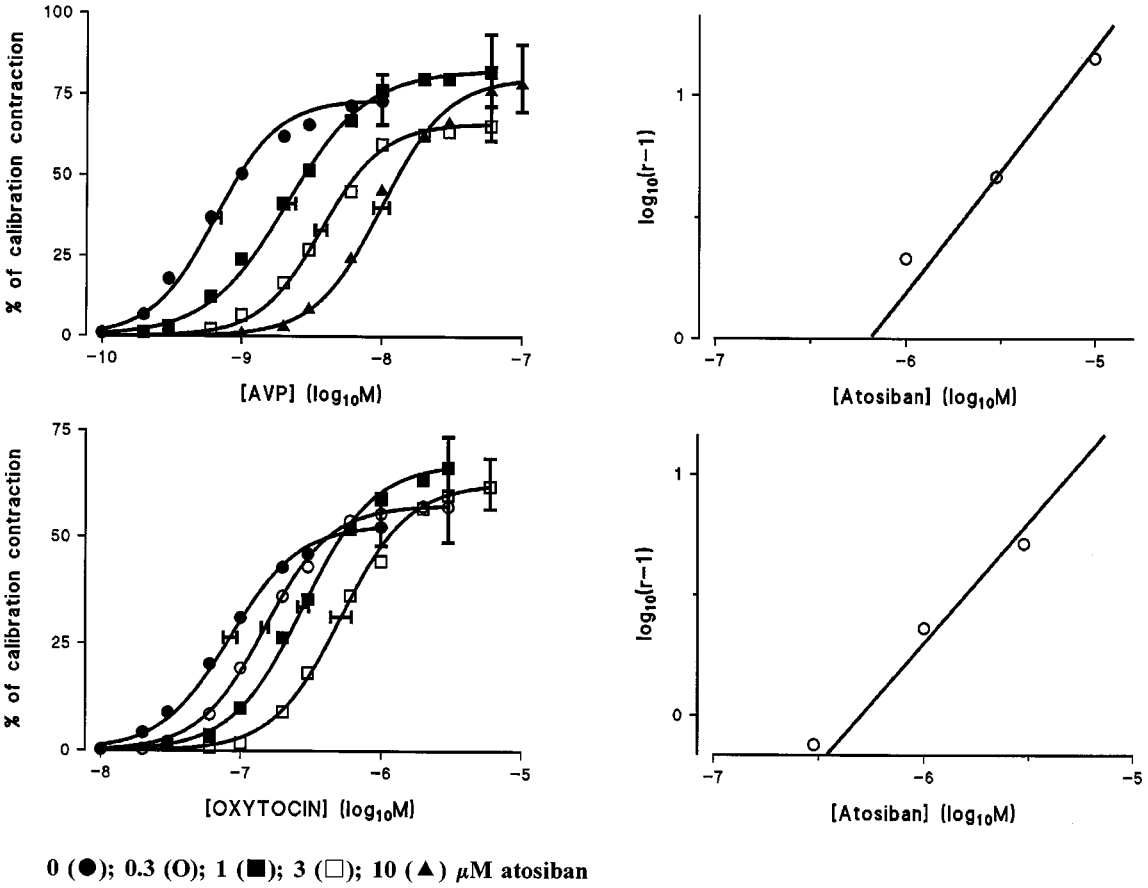

Figure 6 (Left panels) Concentration-effect curves to AVP and OT obtained on the rat aorta in the absence or presence of atosiban. The lines superimposed on the mean data points were simulated using the Hill equation. (Right panels) Schild plots for the interaction of atosiban with AVP (upper panel) and OT (lower panel). The solid lines superimposed on the mean data points were simulated using the parameters obtained from the constrained model fits.

Graaf *et al*., 1995). The concentration-dependent steepening of the AVP E/[A] curve by atosiban in the rat SMA substantiates the significance of the difference in Hill slope parameter between OT and AVP E/[A] curves. Interestingly, atosiban caused the Hill slope parameter of the AVP E/[A] curve to shift towards that of the OT E/[A] curve (see Table 1). In other cases also, the antagonist-induced changes of the Hill slope parameter proved to be a more sensitive indicator of receptor heterogeneity than the Schild plot slope parameter (Van der Graaf *et al*., 1996; Prentice & Hourani, 1997). Thus, in the present study, the contraction of the SMA by AVP is likely to involve a heterogeneous (V_{1A} and non-V_{1A}) receptor population. Receptor heterogeneity does not readily explain the failure of SR 49059 to satisfy the criteria for competitive antagonism of the AVP-induced contraction in the SMA. The compound exhibits slow dissociation kinetics due to its high affinity (D. Nisato, personal communication). Indeed, incubation of the rat SMAs with SR 49059 decreased the E_{max} of the AVP E/[A] curve (Figure 3). However, the decrease in E_{max} was small and independent of the concentration used. A similar small decrease in AVP E_{max} in the rat SMA has also been observed with peptide antagonists (Angus *et al*., 1994).

Interestingly, in contrast to the non-competitive nature of SR 49059 and atosiban in the rat SMA with intact endothelium, both compounds behaved as competitive antagonists in the rat aorta, where endothelium had been removed (see Methods). Thus, it is possible that vasodilator responses elicited by AVP due to a release of endothelium-derived factors (Katusic *et al*., 1984; Myers *et al*., 1989; Russ & Walker, 1992; Martinez *et al*., 1994b; Suzuki *et al*., 1994) may interfere with its contractile responses in the rat SMA. Since, in addition, AVP can also elicit endothelium-independent vasodilatation (Martinez *et al*., 1994a,b), we studied the effects of AVP as well as OT on both vessels after pre-contraction with the thromboxane-mimetic agent, U46619 in the presence of SR 49059. Both agonists, however, failed to relax either the rat SMA or the rat aorta. The lack of vasodilator responses with AVP and OT strengthens the notion that the AVP-induced contraction of the rat SMA seems to involve heterogeneous receptors.

We would like to point out that our results with respect to the competitive antagonism displayed by OPC 21268 in the rat SMA ($pA_2 = 7.56$) differ from those reported in an earlier study (Burrell *et al*., 1994). Burrell and colleagues (1994) demonstrated that OPC 21268, at a concentration of only 10 nM, almost completely blocked the AVP-induced contraction of the rat SMA. Although the authors did not discuss this observation, the antagonism of OPC 21268 suggested either a non-competitive action or the co-existence of an underlying relaxant response (not observed in the present study). We cannot explain the discrepancy. However, the pA_2 values obtained by us are in agreement with the reported pK_i values in the rat liver (see Table 2). Moreover, a parallel rightward shift of the AVP-induced pressor response in the rat by OPC 21268 (Yamamura *et al*., 1991) is also in accordance with our findings in the rat SMA, which is generally believed to represent a resistance vessel (Fenger-Gron *et al*., 1997).

In summary, the results of the present study show that AVP and OT contract the rat aorta and SMA and, according to most criteria, the data are consistent with the response being predominantly mediated by a V_{1A} receptor. However, the non-competitive antagonism of the AVP-induced contraction of the rat SMA by atosiban and SR 49059 as well as the Hill slope difference between AVP and OT E/[A] curves indicate receptor heterogeneity in the rat SMA. In this respect, it is of interest to note that Heinemann *et al*. (1998) have suggested the involvement of a novel AVP receptor in the pressor response of the rat perfused mesentery. Overall, therefore, the existence of another atypical receptor in the rat SMA cannot be excluded.

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