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Lack of effect of a selective vasopressin V_{1A} receptor antagonist, SR 49,059, on potentiation by vasopressin of adrenoceptor-mediated pressor responses in the rat mesenteric arterial bed

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1 The vasopressin receptor subtype involved in the enhancement by vasopressin of adrenoceptor-mediated vasoconstriction was investigated in rat isolated perfused mesenteric arteries.

2 [Arg^8]vasopressin (1–10 nM) dose-dependently increased the perfusion pressure and enhanced the pressor response to the adrenoceptor agonist methoxamine (40 nmol) or electrical stimulation of periarterial nerves (16 Hz), at the concentration of 10 nM of [Arg^8]vasopressin up to 4 and 3 fold, respectively.

3 During prolonged exposure (45 min) the direct vasoconstrictor effect of [Arg^8]vasopressin (10 nM) rapidly declined whereas the potentiation of methoxamine-induced vasoconstriction was maintained.

4 The selective vasopressin V_{1A} receptor antagonist SR 49,059 (1–3 nM) and the non-selective $V_{1A/B}$ and oxytocin receptor antagonist [deamino-Pen^1,Tyr(Me)^2,Arg^8]vasopressin (15–45 nM) inhibited the direct vasoconstrictor action of [Arg^8]vasopressin but had no effect on the enhancement of the pressor response to methoxamine or electrical stimulation.

5 The V_{1B} receptor agonist [deamino-Cys^1,β-(3-pyridyl)-D-Ala^2,Arg^8]vasopressin (100–1000 nM) and the V_2 receptor agonist [deamino-Cys^1,D-Arg^8]vasopressin (1–10 nM) were devoid of any pressor activity and did not potentiate methoxamine-evoked vasoconstriction. In contrast, [1-triglycyl,Lys^8]vasopressin (100–1000 nM) potentiated the methoxamine responses without *per se* inducing vasoconstriction.

6 In arteries precontracted with methoxamine (7.5 μM) pressor responses to [Arg^8]vasopressin (3–10 nM) were not inhibited by a dose of SR 49,059 (3 nM) which abolished the peptide's vasoconstrictor effect under control conditions.

7 These data show that the direct vasoconstrictor effect of [Arg^8]vasopressin is mediated by V_{1A} receptors while the enhancement of adrenoceptor-mediated pressor responses is insensitive to V_{1A}, V_{1B}, and oxytocin receptor antagonists and is not mimicked by selective agonists of V_{1B} and V_2 receptors. In conclusion, an unusual interaction of vasopressin with V_{1A} receptors, or even the existence of a novel receptor subtype, has to be considered.

Keywords: Mesenteric arterial bed; vasoconstriction; methoxamine; vasopressin; vasopressin receptors; oxytocin receptors; electrical stimulation

Introduction

The pituitary hormone vasopressin is among the most potent vasoconstrictor peptides. Although vasopressin plays only a marginal role in maintaining arterial blood pressure under normal conditions (Liard, 1984), it has been implicated in the pathogenesis of arterial hypertension in various experimental models (Liard, 1984; Rossi & Schrier, 1986) and, more substantially, in circulatory adaptation to hypovolaemia or arterial hypotension (Andrews & Brenner, 1981; Zerbe *et al*., 1982; Sander-Jensen *et al*., 1986; Gardiner *et al*., 1989; Claria *et al*., 1991; Sun *et al*., 1991; Carp *et al*., 1994). Vasopressin analogues are also employed therapeutically as vasoconstrictor agents, to minimize bleeding during surgery or to control oesophageal variceal bleeding in cirrhotic patients.

Vasopressin receptor subtypes are currently classified as V_{1A}, V_{1B} and V_2 (Barberis *et al*., 1998). In addition, vasopressin is a potent agonist on oxytocin receptors (Teitelbaum, 1991; Chan *et al*., 1996). The vascular effects of vasopressin are predominantly mediated by V_{1A} receptors, although vasodilator V_2 receptors have also been described in vascular tissue (Hirsch *et al*., 1989; Liard, 1989; Martinez *et al*., 1994). Beside its direct vasoconstrictor action, which is mediated mainly by receptor-coupled activation of phospholipase C and release of Ca^{2+} from intracellular stores *via* the phosphoinositide cascade (Thibonnier, 1992), vasopressin also enhances the sensitivity of the vasculature to other pressor agents (Karmazyn *et al*., 1978; Patel & Schmid, 1988; Noguera *et al*., 1997). In the presence of low vasopressin concentrations, which *per se* do not constrict the vessels to an appreciable extent, pressor responses to several other vasoconstrictors are markedly augmented (Karmazyn *et al*., 1978). However, the receptor subtype which accounts for this vasopressin effect has not been studied in detail.

The present study was hence designed to investigate which vasopressin receptor subtype mediates the enhancement by vasopressin of the vascular sensitivity to adrenoceptor stimulation in the isolated perfused mesenteric arterial bed of the rat.

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Methods

Perfused mesenteric arterial bed

Male Sprague-Dawley rats weighing 300–330 g were used. Experiments with isolated perfused mesenteric arterial beds were carried out as originally set up by McGregor (1965) and described in detail by Heinemann *et al.* (1997). Briefly, the superior mesenteric artery was cannulated through a small incision in the abdominal aorta, the mesentery dissected free from the intestine, isolated from the surrounding tissue and then placed on a heated pad (37°C) covered with parafilm. The pad was tilted to facilitate removal of the effluent perfusate. In some experiments the intestine was left intact and the mesentery remained *in situ*, so that the intestinal microvessels and the portal venous bed could also be perfused. In these rats the chest was opened and the thoracic organs were removed. The effluent perfusate, drained *via* the inferior vena cava, was continuously removed from the thoracic cavity. The preparation was perfused at 37°C with oxygenated (95% O_2, 5% CO_2) Krebs solution at a constant rate of 4 ml min^{-1} using a roller pump. The Krebs solution contained (mM): 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 25 $NaHCO_3$, 1.2 KH_2PO_4, 2.5 $CaCl_2$, 11 glucose and 0.026 EDTA calcium, at a pH of 7.4. Perfusion pressure was measured *via* a side arm of the arterial cannula by means of a pressure transducer. Zero pressure was determined at the end of each experiment by perfusing the arterial cannula after removal of the vessel preparation. Drugs were administered to the perfusion system either by intermittent bolus injections, in a volume of 100 μl over 20 s (methoxamine), or by continuous infusion (methoxamine, vasopressin agonists, vasopressin antagonists). For periarterial stimulation of sympathetic nerves bipolar ring-type electrodes made from platinum were put around the mesenteric artery close to its aortal origin. The tissues were stimulated with supramaximal voltage (50 V) and rectangular pulses of 1 ms at a frequency of 16 Hz for 15 s (McGregor, 1965; Kawasaki *et al.*, 1990; Ralevic & Burnstock, 1996).

Experimental protocols

The preparations were allowed to equilibrate for 30–45 min, until pressor responses to bolus injections of methoxamine (40 nmol) became constant. The effect of vasopressin analogues on perfusion pressure was recorded in absolute values (mm Hg) whereas pressor responses to methoxamine were expressed as relative increases (Δ mm Hg) in perfusion pressure above the value measured immediately before administration of methoxamine. In the following five studies, studies 1–4 used the isolated perfused mesenteric arterial preparation, whereas Study 5 employed the *in situ* perfused mesentery.

Study 1 assessed the effect of [Arg^8]vasopressin (AVP) on baseline perfusion pressure and methoxamine-induced pressor responses in isolated perfused mesenteric arteries in the absence or presence of the selective vasopressin V_{1A} receptor antagonists SR 49,059 (Serradeil-Le Gal *et al.*, 1993) and [β-mercapto-β,β-cyclopentamethylene-propionyl,$Tyr(Me)^2$,Arg^8]-vasopressin ($d(CH_2)_5$TyrMeAVP) (Kruszynski *et al.*, 1980), and the non-selective $V_{1A/B}$ and oxytocin receptor antagonist [deamino-Pen^1,$Tyr(Me)^2$,Arg^8] vasopressin (dPTyrMeAVP) (Bankowski *et al.*, 1978). The basic experimental protocol is delineated in Figure 1. Isolated perfused mesenteric arteries were preincubated with vehicle, SR 49,059 (1–3 nM), $d(CH_2)_5$TyrMeAVP (15–45 nM) or dPTyrMeAVP (15–45 nM) for 20 min. Then the infusion of the vehicle of AVP was started and 7 min later the responsiveness to methoxamine (40 nmol) determined by two consecutive bolus injections with a 7 min interval in between. Only the second methoxamine effect was evaluated. This procedure was repeated with infusions of increasing concentrations of AVP (1–10 nM) with adequate washout periods in between, which allowed the perfusion pressure to return to the pre-infusion value (Figure 1). In some preparations the responsiveness to methoxamine was also recorded after the infusion of the highest concentration of AVP had been terminated to test the reversibility of the vasopressin effects. The dose of 40 nmol methoxamine was chosen since it yielded reproducible increases in perfusion pressure but, even in the presence of AVP, did not reach the maximal pressor response, which is 200–250 mm Hg in this preparation (Heinemann & Stauber, 1996).

Study 2 investigated the effect of AVP (1–10 nM) on the pressor responses to electrical stimulation of periarterial nerves in the absence or presence of SR 49,059 (1 nM) or $d(CH_2)_5$TyrMeAVP (15 nM). The same protocol was followed as in Study 1 with methoxamine injections being replaced by electrical stimulation.

Study 3 compared the effects of AVP with its analogues, the V_{1B} receptor-selective agonist [deamino-Cys^1,β-(3-pyridyl)-D-Ala^2,Arg^8]vasopressin (dDPalAVP) (Schwartz *et al.*, 1991), the V_2 receptor-selective agonist [deamino-Cys^1,D-Arg^8]vasopressin (dDAVP) (Manning *et al.*, 1976) and [1-triglycyl,Lys^8]vasopressin (TGLVP), which lacks any direct vasoconstrictor property *in vitro* (Heinemann & Stauber, 1996). In these experiments the same protocol was used as in Study 1 with the exception that the vehicle/antagonist infusion was omitted. The responsiveness to methoxamine (40 nmol) was recorded before and during the infusion of increasing concentrations of vasopressin analogues, AVP (1–10 nM), dDPalAVP (100–1000 nM), dDAVP (1–10 nM) or TGLVP (100–1000 nM). These concentration ranges of the vasopressin analogues were determined in preliminary experiments (TGLVP) or by their

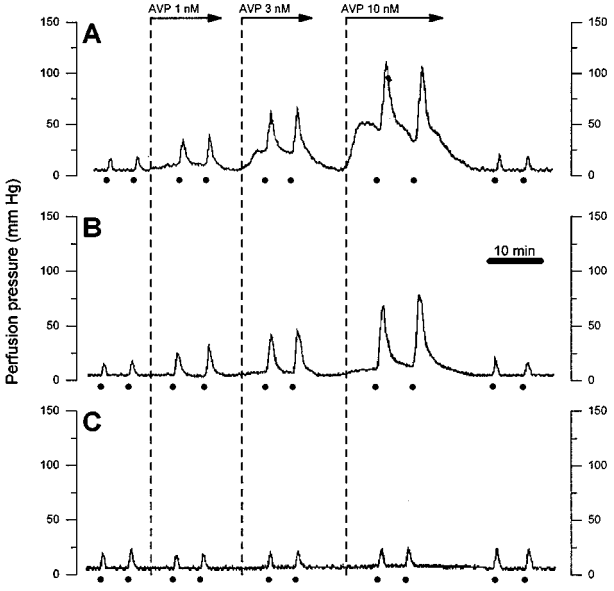

Figure 1 Original tracings of the effects of [Arg^8]vasopressin (AVP) to increase perfusion pressure and enhance methoxamine-evoked pressor responses in isolated perfused mesenteric arteries. AVP was infused at the concentrations indicated whereas methoxamine (40 nmol) was administered by bolus injections which are indicated by dots (•). AVP effects were investigated in the presence of vehicle (A), SR 49,059 (3 nM; B) or [β-mercapto-β,β-cyclopentamethylene-propionyl,$Tyr(Me)^2$, Arg^8] vasopressin ($d(CH_2)_5$TyrMeAVP, 15 nM; C). Note that both SR 49,059 and $d(CH_2)_5$TyrMeAVP abolished the AVP-related increases in perfusion pressure but only $d(CH_2)_5$TyrMeAVP inhibited the potentiation by AVP of methoxamine responses.

potency reported in literature. dDPalAVP has been described as being 36 times less potent than AVP on V_{1B} receptors (Schwartz *et al*., 1991), while dDAVP is at least 2 times more potent than AVP on V_2 receptors (Manning *et al*., 1976).

Study 4 investigated the time course of the effect of AVP (10 nM) to cause direct vasoconstriction *per se* and to potentiate methoxamine-induced vasoconstriction in the isolated perfused mesenteric arterial preparation. Responsiveness to methoxamine (40 nmol), injected as a bolus, was recorded every 7 min before and during infusion of vehicle or AVP (10 nM). Each methoxamine response was evaluated. In an additional group the effect of AVP infusion (10 nM) was investigated without methoxamine stimulation.

In *Study 5* the *in situ* perfused mesentery preparation was used under steady state conditions with continuous methoxamine infusions to mimic physiological conditions more closely. Firstly, the ability of AVP to enhance vascular sensitivity to adrenoceptor stimulation was surveyed. The infusion of AVP (10 nM) was started 30 min before exposing the preparations to increasing concentrations of methoxamine (0.3–100 μM), each for 10 min. Between the individual concentrations of methoxamine washout periods of 10 min were allowed. Pressor responses were expressed as increases above the values taken immediately before the methoxamine infusion. Secondly, the effect of SR 49,059 on vasoconstriction evoked by AVP was investigated under control conditions and in preparations preconstricted with methoxamine (7.5 μM). The infusion of SR 49,059 (3 nM) or its vehicle was started 20 min before exposure to AVP (3–10 nM), with 10 min between the two AVP concentrations. The pressor responses were expressed as increases above the values recorded immediately before the AVP infusion.

Drugs and statistics

[Arg^8]vasopressin (AVP), [deamino-Pen^1,Tyr(Me)^2,Arg^8] vasopressin (dPTyrMeAVP), [β-mercapto-β,β-cyclopentamethylene-propionyl, Tyr (Me)^2, Arg^8] vasopressin (d(CH$_2$)$_5$ TyrMe AVP), [deamino-Cys^1,β-(3-pyridyl)-D-Ala^2,Arg^8]vasopressin (dDPalAVP) and [deamino-Cys^1,D-Arg^8]vasopressin (dDAVP) were purchased from Bachem (Bubendorf, Switzerland) whereas [1-triglycyl,Lys^8]vasopressin (TGLVP) was obtained from Ferring (Malmö, Sweden). The peptides and methoxamine (Sigma; Vienna, Austria) were dissolved in distilled water and were further diluted with saline. A stock solution (10 mM) of SR 49,059 (Sanofi; Toulouse, France), which is (2S) 1-[(2R 3S)-(5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzene -sulfonyl)-3-hydroxy-2,3-dihydro -1H-indole-2-carbonyl] - pyrrolidine - 2-carboxamide, was prepared in dimethyl sulfoxide and diluted with saline, the final concentration of dimethyl sulfoxide in the perfusate being less then 1 : 1,000,000 vol/vol. The respective vehicles were prepared in the same manner as the drug solutions.

Results are presented as means ± s.e.mean. Statistical evaluation of the data was performed with linear regression analysis, the Wilcoxon signed rank test, Mann-Whitney U test, or Kruskal-Wallis H test followed by Dunn's test for multiple comparisons. Probability values of $P<0.05$ were regarded to be statistically significant.

Results

Isolated perfused mesenteric arterial bed

The vehicles of AVP and methoxamine had no effect on perfusion pressure (data not shown). AVP (1–10 nM) concentration-dependently constricted the mesenteric artery as indicated by an elevation of the perfusion pressure (Figures 1A and 2A). In addition, AVP concentration-dependently potentiated the pressor responses to bolus injection of methoxamine (40 nmol) (Figures 1A and 2B). Both of these vasopressin effects were readily reversible since, 10 min after the peptide infusion had been terminated, baseline perfusion pressure and the responsiveness to methoxamine had declined to the level observed before AVP infusion (Figure 1A). The threshold concentration of AVP for inducing vasoconstriction

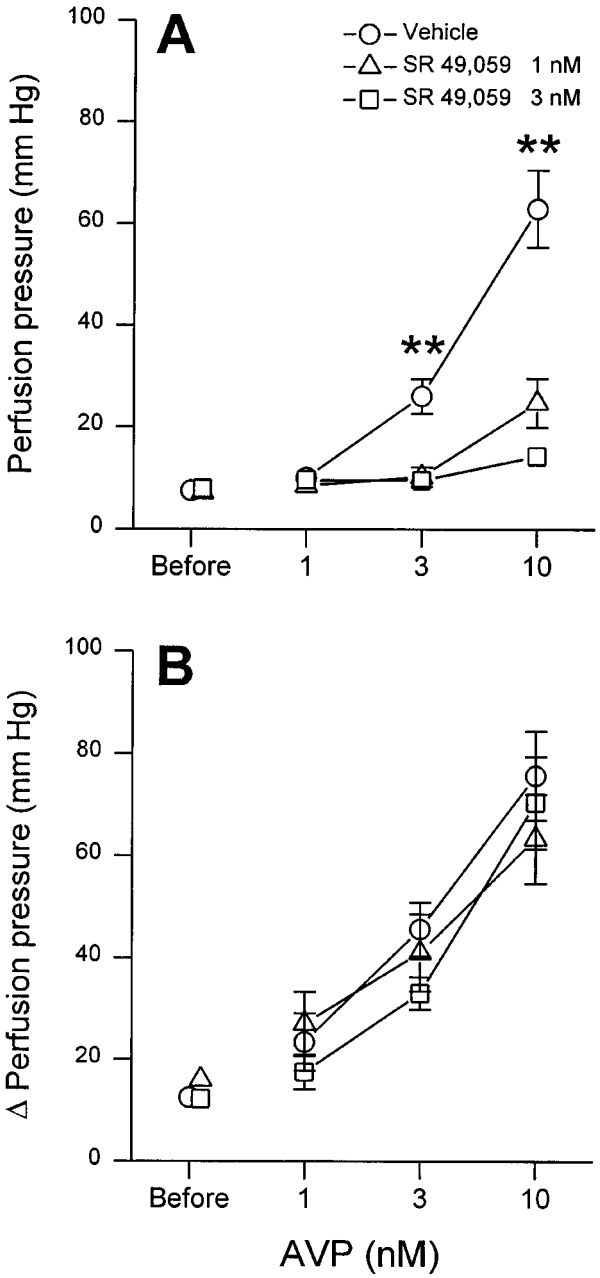

Figure 2 Effect of SR 49,059 on [Arg^8]vasopressin (AVP)-induced increases in perfusion pressure (A) and potentiation of methoxamine-evoked pressor responses (B) in isolated perfused mesenteric arteries. (A) shows the peak values for perfusion pressure in response to infusion of increasing concentrations of AVP whereas (B) depicts the relative increases in perfusion pressure after bolus injections of methoxamine (40 nmol), calculated as peak value minus pre-injection value. Note that SR 49,059 reduced AVP-related increases in perfusion pressure but did not alter the potentiation by AVP of methoxamine responses. Data are shown as means ± s.e.mean, $n=6$–9; $^{**}P<0.005$ vehicle versus both concentrations of SR 49,059.

was about 1 nM (Figures 1A and 2A), while the potentiation of methoxamine responses could be observed at 0.3 nM AVP, given that pressor responses to methoxamine increased from 16.2±3.1 to 21.6±3.7 mm Hg ($P<0.05$, $n=6$). Linear regression analysis revealed that direct vasoconstriction and potentiation of methoxamine responses evoked by AVP (10 nM) were not correlated ($r=0.13$, $n=31$, $P=0.47$).

Preincubation of the preparations with the V_{1A} receptor antagonist SR 49,059 (1–3 nM) *per se* did not alter perfusion pressure but concentration-dependently antagonized the pressor responses to AVP (1–10 nM; Figures 1B and 2A). SR 49,059, however, did not alter the responsiveness to methoxamine (40 nmol) in the absence of AVP and did not significantly inhibit the effect of AVP (1–10 nM) in enhancing pressor responsiveness to methoxamine (Figures 1B and 2B). Almost identical results were obtained with the non-selective V_1 and oxytocin receptor antagonist dPTyrMeAVP (15–45 nM) which *per se* did not alter perfusion pressure but abolished the pressor effect of AVP (1–10 nM; $n=7–13$, data not shown). However, the responsiveness to methoxamine before AVP infusion and the potentiation of methoxamine responses by AVP (1–10 nM) were not modified by dPTyrMeAVP (15–45 nM; $n=7–13$, data not shown). In contrast, preincubation with d$(CH_2)_5$TyrMeAVP (15–45 nM) abolished both the AVP-evoked vasoconstriction and the potentiation of methoxamine responses (Figures 1C, 3A, B).

Vasopressin concentration-dependently potentiated the pressor responses to electrical stimulation of periarterial nerves. As shown in Figure 4B this effect was not significantly altered by SR 49,059 (1 nM) but abolished by d$(CH_2)_5$TyrMeAVP (15 nM).

The V_{1B} receptor-selective agonist dDPalAVP (100–1000 nM) did not mimic the effects of AVP. Even at concentrations 100 fold higher than those used for AVP, dDPalAVP did not increase perfusion pressure (Figure 5A) and did not augment the pressor response to methoxamine to an appreciable extent (Figure 5B). Similarly, the V_2 receptor-selective agonist dDAVP (1–10 nM) failed to alter the perfusion pressure (Figure 5A) and responsiveness to methoxamine stimulation (Figure 5B), and remained inactive at concentrations up to 500 nM ($n=6$, data not shown). TGLVP (100–1000 nM) itself did not induce changes in perfusion pressure (Figure 5A) but mimicked the effect of AVP to enhance methoxamine-evoked vasoconstrictor responses (Figure 5B). The potency of TGLVP, however, was at least 100 times lower than that of AVP.

In an additional set of experiments the time course of the AVP effects, to cause direct vasoconstriction and potentiate methoxamine responses was investigated (Figure 6). During infusion of the AVP vehicle for 42 min baseline perfusion pressure remained unaltered (Figure 6A). Exposure of the preparations to AVP (10 nM) evoked an immediate increase in perfusion pressure. Despite ongoing AVP infusion a gradual decline of perfusion pressure was observed during the following 20 min reaching a steady state after 30 min (Figure 6A). At this time the AVP-induced increase in perfusion pressure was less than 20% of the initial pressor response. The time-dependent decline of AVP-induced vasoconstriction was not the result of the intermittent stimulation with methoxamine, since it was also observed in preparations which were not subjected to methoxamine injections (Figure 6A). In preparations which were infused with the vehicle of AVP, pressor responses to bolus injections of methoxamine (40 nmol) remained unchanged over the period of 42 min (Figure 6B). Upon infusion of AVP (10 nM) pressor responses to methoxamine (40 nmol) increased 3–4 fold (Figure 6B) and remained constantly augmented for the entire period of AVP infusion (Figure 6B).

In situ *perfused mesentery*

The vasopressin effect to enhance adrenoceptor-mediated vasoconstriction was also observed when methoxamine was administered by continuous infusion to reach steady state concentrations in the vascular tissue. These experiments were performed with the mesenteric vascular bed being left *in situ*

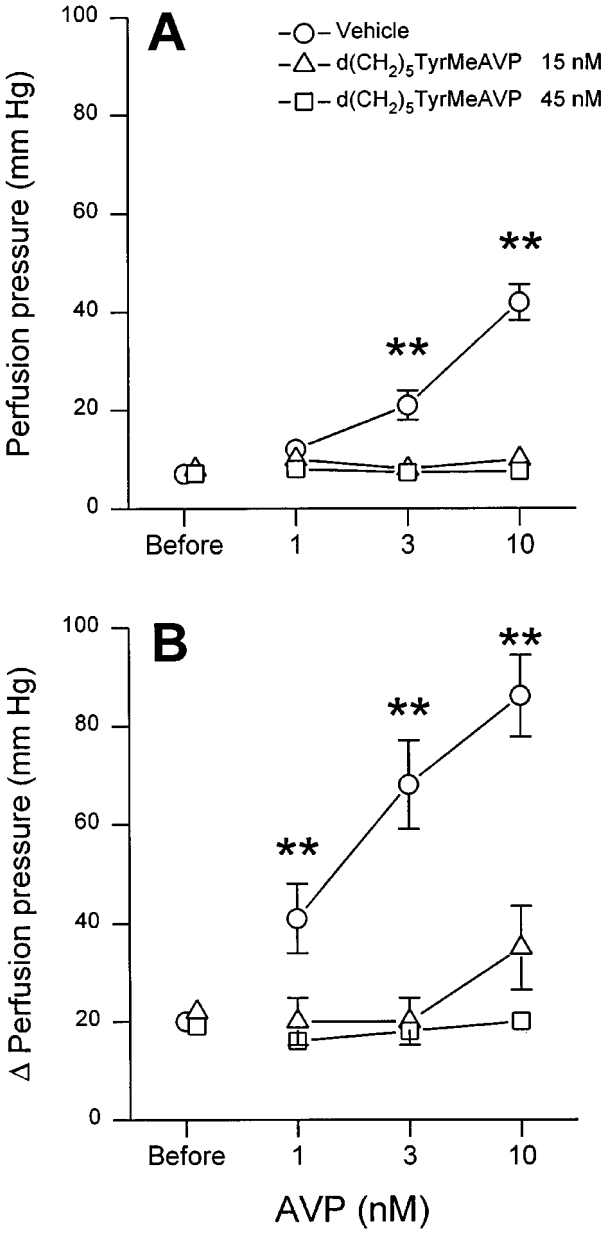

Figure 3 Effect of [β-mercapto-β,β-cyclopentamethylene-propionyl,-Tyr(Me)^2,Arg^8] vasopressin (d$(CH_2)_5$TyrMeAVP) on [Arg^8]vasopressin (AVP)-induced increases in perfusion pressure (A) and potentiation of methoxamine-evoked pressor responses (B) in isolated perfused mesenteric arteries. (A) shows the peak values for perfusion pressure in response to infusions of AVP whereas (B) depicts the relative increases in perfusion pressure after bolus injections of methoxamine (40 nmol), calculated as peak value minus pre-injection value. Note that d$(CH_2)_5$TyrMeAVP inhibited both the AVP-related increases in perfusion pressure and the potentiation by AVP of methoxamine responses. Data are shown as means±s.e.mean, $n=6–7$; $^{**}P<0.005$ vehicle versus both concentrations of d$(CH_2)_5$TyrMeAVP.

and attached to the intestine so that the intestinal microcirculation was also perfused. Methoxamine (3–100 μM) concentration-dependently increased perfusion pressure (Figure 7) in a sustained manner. Preincubation of the preparations for 30 min with AVP (10 nM) markedly augmented the vasoconstrictor effect of methoxamine and decreased the threshold concentration of methoxamine by a factor of 10 (Figure 7). As a result the concentration-response curve to methoxamine was shifted 3–5 fold to the left in the presence of AVP (Figure 7).

In preparations which were not constricted with methoxamine SR 49,059 (3 nM) abolished the pressor responses to 3 and 10 nM of AVP (Figure 8). Conversely, in vessels preconstricted with methoxamine (7.5 μM), SR 49,059 (3 nM) failed to inhibit the pressor response to 3 nM AVP and only showed an insignificant tendency towards inhibition of 10 nM AVP as compared to vehicle (Figure 8).

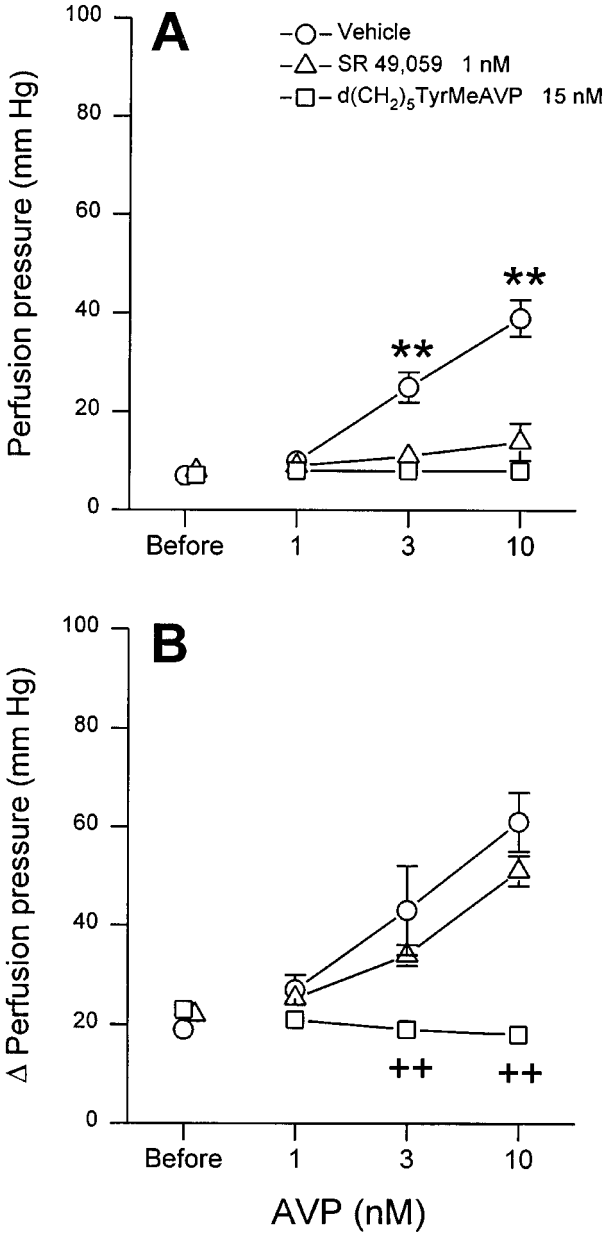

Figure 4 Effects of SR 49,059 and [β-mercapto-β,β-cyclopentamethylene-propionyl,Tyr(Me)^2,Arg^8]vasopressin (d(CH$_2$)$_5$TyrMeAVP) on [Arg^8]vasopressin (AVP)-induced increases in perfusion pressure (A) and potentiation of pressor responses evoked by periarterial nerve stimulation (B) in isolated perfused mesenteric arteries. (A) shows the peak values for perfusion pressure in response to infusions of AVP whereas (B) depicts the relative increases in perfusion pressure after electrical stimulation of periarterial nerves (16 Hz), calculated as peak value minus pre-stimulation value. Note that d(CH$_2$)$_5$TyrMeAVP abolished both the AVP-related increases in perfusion pressure and the potentiation by AVP of responses to electrical stimulation. Conversely, SR 49,059 inhibited the direct pressor effect of AVP only. Data are shown as means ± s.e.mean, $n=6-7$; $^{**}P<0.01$ vehicle versus d(CH$_2$)$_5$TyrMeAVP and SR 49,059; $++P<0.001$ d(CH$_2$)$_5$TyrMeAVP versus vehicle and SR 49,059.

Discussion

It is a well known, although poorly understood, phenomenon that AVP potentiates the vasopressor effect of adrenoceptor stimulation *in vitro*, an action that has been claimed to be mediated by V_1 receptors. This conclusion was based on the ability of d(CH$_2$)$_5$TyrMeAVP, a peptide antagonist selective for the V_{1A} receptor subtype (Kruszynski *et al*., 1980; Antoni *et al*., 1984), to inhibit both the direct vasoconstrictor and potentiating effect of AVP (Patel & Schmid, 1988; Noguera *et*

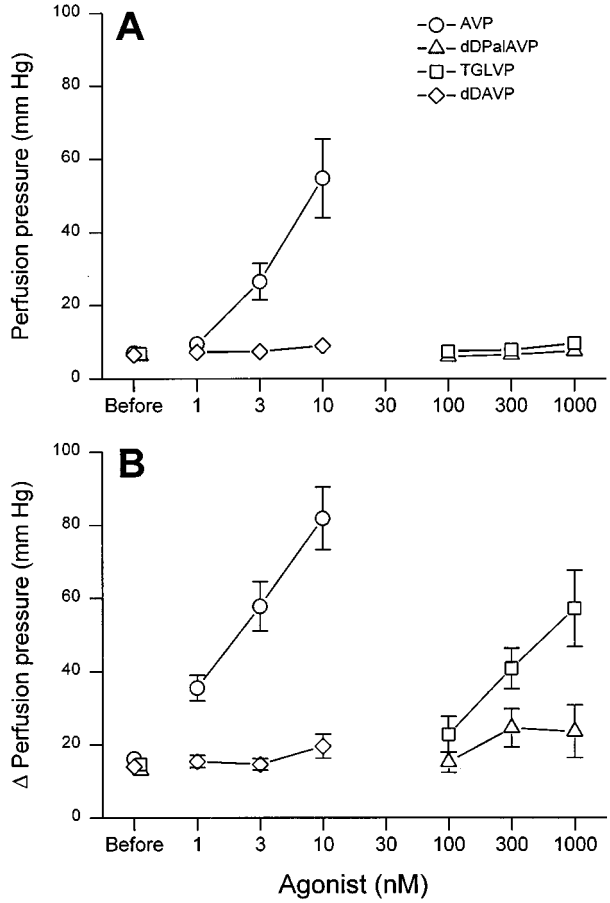

Figure 5 Effects of different vasopressin receptor agonists, [Arg^8]vasopressin (AVP), [deamino-Cys^1,β-(3-pyridyl)-D-Ala^2,Arg^8]vasopressin (dDPalAVP), [deamino-Cys^1,D-Arg^8]vasopressin (dDAVP) and [1-triglycyl,Lys^8]vasopressin (TGLVP), on perfusion pressure (A) and methoxamine-evoked pressor responses (B) in isolated perfused mesenteric arteries. (A) shows the peak values for perfusion pressure in response to infusions of AVP whereas (B) depicts the relative increases in perfusion pressure after bolus injections of methoxamine (40 nmol), calculated as peak value minus pre-injection value, in the presence of the vasopressin analogues. Note that dDPalAVP and dDAVP neither increased perfusion pressure nor enhanced methoxamine responses whereas TGLVP potentiated the pressor responses to methoxamine without *per se* raising perfusion pressure. Data are shown as means ± s.e.mean, $n=6-10$.

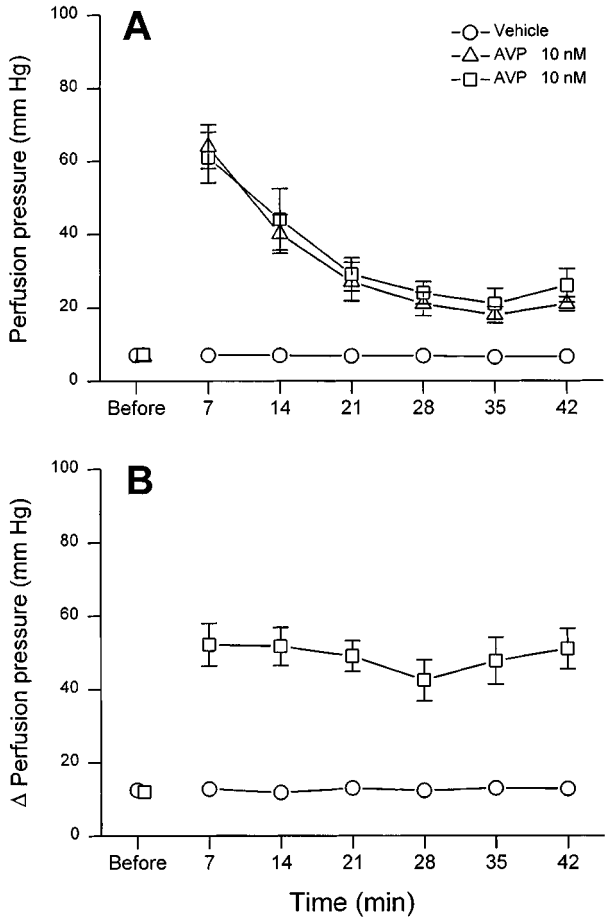

Figure 6 Time course of the effects of [Arg^8]vasopressin (AVP) to increase perfusion pressure (A) and enhance methoxamine-evoked pressor responses (B) in isolated perfused mesenteric arteries. (A) Shows the values for perfusion pressure during infusion of vehicle or AVP. Some of the preparations exposed to AVP were not subjected to intermittent bolus injections of methoxamine and are shown in a separate group (open triangles). (B) Depicts relative increases in perfusion pressure after bolus injections of methoxamine (40 nmol), calculated as peak value minus pre-injection value, in the presence of vehicle or AVP. Note that the direct pressor response to AVP time-dependently declined whereas the enhancement by AVP of methoxamine-induced vasoconstriction was maintained over the whole experimental period. Data are shown as means±s.e.mean, $n=6-9$.

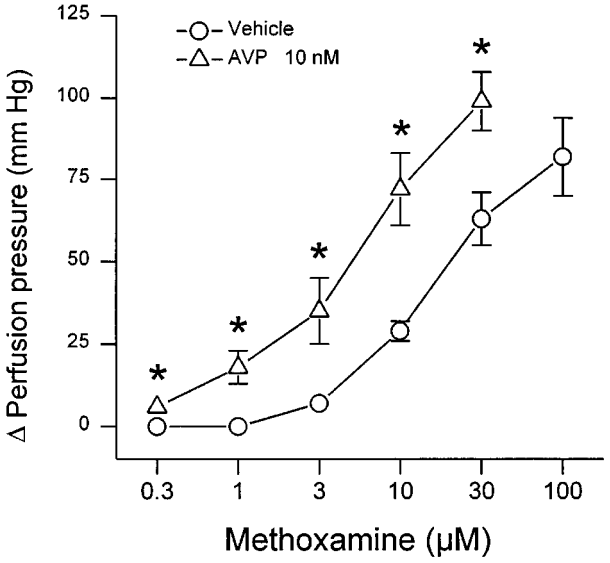

Figure 7 Effect of [Arg^8]vasopressin (AVP) on methoxamine-evoked vasoconstriction in the *in situ* perfused mesenteric preparation. Following preincubation with AVP or its vehicle for 30 min by infusion, which was continued throughout the whole experiment, the preparations were exposed to infusions of incremental concentrations of methoxamine. The diagram shows relative increases in perfusion pressure in response to methoxamine above pre-infusion value. Note that AVP not only markedly augmented responsiveness to methoxamine but also decreased the threshold concentration of methoxamine. Data are shown as means±s.e.mean, $n=7$; $^*P<0.01$ AVP versus vehicle.

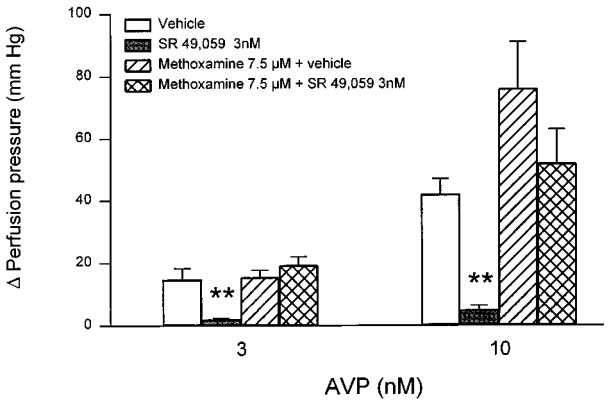

Figure 8 Effect of SR 49,059 on [Arg^8]vasopressin (AVP)-induced increases in perfusion pressure in the *in situ* perfused mesenteric preparation under control conditions and after preconstricting the vessels with methoxamine. The diagram shows relative increases in perfusion pressure in response to AVP, calculated as peak minus pre-infusion value. Note that SR 49,059 markedly inhibited AVP responses under control conditions while pressor responses to AVP were left largely unaltered by SR 49,059 in methoxamine-precontracted vessels. Data are shown as means±s.e.mean, $n=6-9$; $^{**}P<0.005$ vehicle versus SR 49,059.

al., 1997). It was also these two vasopressin effects that were analysed in the present study. Firstly, AVP concentration-dependently increased perfusion pressure in the absence of methoxamine, which reflects the direct vasoconstrictor action of AVP. Secondly, vasoconstrictor responses to the selective α_1-adrenoceptor agonist methoxamine were concentration-dependently enhanced by AVP. The significance of this vasopressin effect was demonstrated by a 3–5 fold increase in vascular sensitivity to methoxamine. AVP potentiated the vasoconstrictor responses not only to bolus injections but also to steady state concentrations of methoxamine in the tissue, suggesting that enhanced permeability of the adrenoceptor agonist through the endothelial barrier was not the underlying mechanism. On the other hand, the potentiating effect of vasopressin was also seen in the intact mesenteric circulation or when electrical stimulation of periarterial nerves was used as the vasoconstrictor stimulus. Since the pressor effect of electrical stimulation of perarterial nerves in the mesenteric circulation is abolished by the α-adrenoceptor antagonist prazosin (Kawasaki *et al*., 1990) it is inferred that potentiation of adrenoceptor-induced vasoconstriction was the mechanism underlying the enhancement by AVP of responses to electrical stimulation. It is hence tempting to speculate that facilitation of adrenoceptor-induced vasoconstriction is the primary action of vasopressin, since it was observed at concentrations of AVP which *per se* did not induce vasoconstriction.

The AVP effects to cause direct vasoconstriction and potentiate the pressor responses to methoxamine and electrical stimulation were abolished by d(CH_2)$_5$TyrMeAVP, which is in agreement with previous studies (Patel & Schmid, 1988; Noguera *et al*., 1997). In contrast, the non-peptide compound SR 49,059, a novel and highly selective V_{1A} receptor antagonist (Serradeil-Le Gal *et al*., 1993), inhibited the direct vasoconstriction (i.e. the increase in perfusion pressure) evoked by AVP, but did not alter the action of AVP to enhance methoxamine- or electrical stimulation-induced pressor responses. Similarly, the peptide dPTyrMeAVP, a non-selective vasopressin antagonist on $V_{1A/B}$ and oxytocin receptors (Bankowski *et al*., 1978), abolished the AVP-induced increases in perfusion pressure without reducing the potentiation of methoxamine responses. These data not only confirm previous findings that the direct vasoconstrictor effect of vasopressin in the mesenteric artery, like in most other vascular beds, is mediated by V_{1A} receptors (Ohlstein & Berkowitz, 1986; Vanner *et al*., 1990) but also clearly demonstrate that the enhancement of adrenoceptor-mediated vasoconstriction by vasopressin involves a mechanism distinct from those triggered by classical activation of V_{1A} receptors. Therefore, the inhibitory effect of d(CH_2)$_5$TyrMeAVP, which was seen at concentrations more than five times above its pA_2 (Kruszynski *et al*., 1980), might be explained by a loss of selectivity at higher concentrations of the antagonist.

Noguera *et al*. (1997) reported that the potentiating effect of AVP was abolished by the calcium channel blocker nifedipine while the peptide's direct vasoconstrictor effect was left unaffected. The conclusion that direct vasoconstriction and potentiation of methoxamine-induced pressor responses by AVP involve completely different mechanisms is further supported by the rapid decline of the direct vasoconstrictor effect of AVP despite continuous peptide infusion while the enhancement of methoxamine responses was maintained. This phenomenon is most probably consistent with desensitization of V_{1A} receptors (Caramelo *et al*., 1991) which, however, does not affect the potentiation of methoxamine responses by AVP.

The V_{1B} receptor-selective agonist dDPalAVP, at concentrations 1000 fold higher than those used for AVP, did neither increase baseline perfusion pressure nor enhance methoxamine responses. Since the potency of dDPalAVP on V_{1B} receptors has been reported to be only 36 times lower than that of AVP (Schwartz *et al*., 1991), these results clearly indicate that V_{1B} receptors are not involved in the potentiation of adrenoceptor-mediated vasoconstriction. The V_2 receptor-selective agonist dDAVP likewise did not potentiate methoxamine-induced vasoconstriction. With regard to its more than 2 fold increased potency on V_2 receptors, as compared to AVP (Manning *et al*., 1976), the lack of effect of dDAVP on methoxamine-related vasoconstriction rules out an involvement of V_2 receptors. It is worth noting here that vasopressin is a potent agonist at oxytocin receptors, which might also be present in vascular tissue (Yazawa *et al*., 1996; Wu *et al*., 1996). Therefore, activation of oxytocin receptors has to be taken into account as a potential pharmacological basis of vasopressin-induced augmentation of methoxamine responses. This possibility, however, appears unlikely because dPTyrMeAVP, which is a potent antagonist at oxytocin receptors (Bankowski *et al*., 1978), was unable to inhibit the potentiating effect of AVP.

The synthetic vasopressin analogue TGLVP has been designed as a pro-drug, which is slowly metabolized *in vivo* to [Lys^8]vasopressin (Forsling *et al*., 1980), to produce sustained vasoconstriction. Consistently, TGLVP did not elicit vasoconstriction *in vitro*, not even at concentrations 10,000 times higher than those used for AVP (Heinemann & Stauber, 1996). Conversely, TGLVP shared the ability of AVP to potentiate adrenoceptor-mediated vasoconstriction. Although the relative potency of TGLVP in this respect appears to be 1/100 of AVP, this compound might be a useful tool in exploring the mechanism behind vasopressin potentiation of adrenoceptor-mediated vasoconstriction.

The final set of experiments in this study was designed to investigate which vasopressin effect, direct vasoconstriction sensitive to SR 49,059, or enhancement of adrenoceptor-induced vasoconstriction insensitive to SR 49,059, operates under physiological conditions. In order to mimic *in vivo* vascular tone the *in situ* perfused mesenteric bed was precontracted by continuous methoxamine infusion and AVP was administered on top of adrenoceptor activation. In these experiments a concentration of SR 49,059, which nearly abolished the pressor response to AVP in the absence of methoxamine, was unable to inhibit the pressor effect of AVP in vessels preconstricted with methoxamine. This observation suggests that potentiation of adrenoceptor-induced vasoconstriction is an important component of the vasoconstrictor action of vasopressin under physiological conditions.

In summary, AVP elicits direct vasoconstriction *via* V_{1A} receptors in the rat mesenteric arterial bed, while its potentiating effect on pressor responses to adrenoceptor stimulation is insensitive to V_{1A}, V_{1B} and oxytocin receptor antagonists, and is not mimicked by selective V_{1B} or V_2 agonists. The lack of effect of SR 49,059 on potentiation of adrenoceptor-mediated pressor responses may be due to an unusual interaction of AVP with V_{1A} receptors. Alternatively, these findings might point to the existence of a yet unidentified vasopressin receptor subtype.

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