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Vascular effects of [Arg^8]vasopressin in the isolated perfused rat kidney

Mariette Barthelmebs [a,*], Jean-Paul Krieger [a], Michèle Grima [a], Dino Nisato [b], Jean-Louis Imbs [a,c]

[a] *Institut de Pharmacologie (ERS 109 CNRS), Faculté de Médecine, 11 Rue Humann, Strasbourg, France*
[b] *Sanofi Recherche, Montpellier, France*
[c] *Service des Maladies vasculaires et Hypertension, Hôpital civil, Strasbourg, France*

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Abstract

Thee renal vascular effects of [Arg^8]vasopressin (vasopressin) were investigated in the isolated perfused rat kidney. Vasopressin (0.01–3 nM) elicited a dose-dependent vasoconstriction in kidneys from Sprague Dawley rats, with a EC_{50} value of 0.206 ± 0.044 nM. Inhibition of nitric oxide synthase by N^{ω}-nitro-L-arginine (100 μM) shifted the vasopressin-induced vasoconstrictor response curve to the left. Inhibition of cyclooxygenase by indomethacin (10 or 30 μM) blunted the constriction induced by low concentrations of the peptide. Vasopressin, like angiotensin II but not noradrenaline, induced tachyphylaxis. SR 49059 ((2*S*)1-[(2*R*,3*S*)-5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzene-sulfonyl)-3-hydroxy-2,3-dihydro-1*H*-indole-2-carbonyl]-pyrrolidine-2-carboxamide) (1–30 nM), a new potent and selective non-peptide vasopressin V_{1A} receptor antagonist, shifted the concentration-response curve for vasopressin to the right without decreasing the maximum contraction. Antagonism became competitive with a pA_2 value (±S.D.) of 9.72 ± 0.20 during inhibition of nitric oxide release. [Mpa^1,D-Arg^8]Vasopressin (desmopressin; 0.1–100 nM), or vasopressin (0.01–1 nM) after blockade of the vasopressin V_{1A} receptor by SR 49059, induced no vasopressin V_2 receptor-related renal relaxation in kidneys with vascular tone previously restored by noradrenaline or prostaglandin $F_{2\alpha}$. These findings indicate that in the isolated perfused rat kidney vasopressin is a potent renal vasoconstrictor. The constriction depends on activation of smooth muscle vasopressin V_{1A} receptors and is modulated by endothelial nitric oxide but not by prostacyclin or vasopressin V_2 receptor-related vasodilation.

Keywords: [Arg^8]Vasopressin; [Mpa^1,D-Arg^8]Vasopressin; SR 49059; Kidney, isolated, perfused, rat; Vasopressin V_{1A} receptor

1. Introduction

[Arg^8]Vasopressin (vasopressin) is a neurohypophyseal hormone involved in the regulation of body fluid volumes and blood pressure. Based on pharmacological and functional studies, three types of vasopressin receptors (V_2, V_{1A}, V_{1B}) have been distinguished (Jard, 1988). The vasopressin V_2 receptor is mainly involved in the aquaretic action of vasopressin, the vasopressin V_{1B} receptor has so far only been found in the adenohypophysis while the vasopressin V_{1A} receptor occurs in many tissues (blood vessels, liver, nervous system, mesangial cells).

The cardiovascular effects of vasopressin are complex (for review see Share, 1988; Laszlo et al., 1991). However, beside its effects on heart, baroreflex and modulation of other hormonal systems, vasopressin acts as a potent pressor hormone. It induces vasoconstriction of several different arteries (Martinez et al., 1994a,b; Katusic et al., 1986) while venules are less responsive (Altura, 1975). Since vasoconstriction is inhibited by a selective vasopressin V_1 receptor antagonist, this response has been linked to activation of vasopressin V_{1A} receptor. The vasopressin-induced vasoconstrictor response has also been reported to be modulated by a vasopressin-induced relaxation (Takayasu et al., 1993; Martinez et al., 1994a,b) and, in several dog arteries, vasodilation was the main action of the hormone (Katusic et al., 1986; Sai et al., 1995). The mechanisms involved in vasopressin-induced vasorelaxation were mainly of two types: (i) activation of endothelial vasopressin V_1 receptor with subsequent nitric oxide or prostacyclin release or (ii) stimulation of vasopressin V_2 receptor on endothelial and/or smooth muscle cells.

The study of the effects of vasopressin on renal circulation has led to divergent observations. In conscious or

* Corresponding author. Tel.: (33) 8835-8759; Fax: (33) 8836-0715.

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anesthetized animals, acute vasopressin infusion induces only a marginal increase in renal vascular resistance (Hofbauer et al., 1984), no effect (Gellai et al., 1984) or even a slight decrease (Oliver et al., 1982). In vitro however, vasopressin induces vasoconstriction in the isolated perfused rat kidney (Berecek et al., 1980), of rat renal artery rings (Katusic and Krstic, 1987) and of rabbit microperfused efferent (Edwards et al., 1989) or afferent glomerular arterioles (Weihprecht et al., 1991; Tamaki et al., 1996). Involvement of vasopressin V_1 receptor is suggested since vasopressin-induced vasoconstriction is antagonized by vasopressin V_1 receptor antagonists, $d(CH_2)_5[Tyr(Me)^2,Arg^8]$vasopressin or OPC21268 (Edwards et al., 1989; Tamaki et al., 1996). The possible modulation of the renal vasoconstrictor response by a vasodilator component has however received less attention.

The aim of our study was to analyse the renal vascular response to vasopressin and to evaluate the participation of a vasodilatory component. Our investigations were performed in vitro on isolated perfused rat kidneys. The direct vascular effects can thus be quantified without the intervention of sympathetic and humoral regulation of renal vasomotricity, particularly of high circulating levels of vasopressin caused by surgical stress and anaesthesia (Walker et al., 1983). We took advantage from the recent synthesis of SR 49059 ((2*S*)1-[(2*R*,3*S*)-5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzene-sulfonyl)-3-hydroxy-2,3-dihydro-1*H*-indole-2-carbonyl]-pyrrolidine-2-carboxamide), a new potent non-peptide antagonist of vasopressin V_{1A} receptor to further characterize renal vascular response to vasopressin. The biochemical and pharmacological properties of this antagonist have already been published (Serradeil-Le Gal et al., 1993). We also assessed the modulation of vasopressin-induced vasoconstriction by nitric oxide, prostaglandins or vascular vasopressin V_2 receptor.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (7–8 weeks old, 200–240 g, Iffa-Credo, L'Arbresle, France) were used. Animals were housed in a room at 20°C with a 12 h light/dark cycle (lights on at 6:00 am) and allowed free access to tap water and standard food (AO4 pellets, UAR, Villemoisson/Orge, France). The rats stayed in our animal facility for at least 1 week before the experiments.

2.2. The isolated perfused rat kidney preparation

After anaesthesia with sodium pentobarbital (45 mg/kg, i.p.), the right kidney of the rats was prepared without ischemia and perfused via the mesenteric artery, in an open circuit, as described previously (Schmidt and Imbs, 1980). The perfusion medium was a prewarmed (37°C), oxygenated (95% O_2/5% CO_2), colloid free Tyrode's solution of the following composition (mM): NaCl 137; KCl 2.7; $CaCl_2$ 1.8; $MgCl_2$ 1.1; NaH_2PO_4 0.42; $NaHCO_3$ 12; glucose 5; Ca-EDTA 0.026; pH was adjusted at 7.4. In some experiments (vasopressin-induced control response), kidneys were perfused with a Krebs solution (composition in mM: NaCl 118; KCl 4.75; $CaCl_2$ 1.9; $MgSO_4$ 1.19; KH_2PO_4 1.19; $NaHCO_3$ 25; glucose 11; pH 7.4). Following an equilibration period of 90 min, the perfusion flow rate was adjusted to 8 ml/min and kept constant (Gilson Minipuls 3, Bioblock, Illkirch, France). The perfusion pressure was continuously monitored (Statham P23 Db transducer, Statham Instruments, Hato Rey, Porto Rico) and recorded (Philips PM 8222, S.A. Philips, Bobigny, France).

2.3. Evaluation of vasoconstrictor responses

Increasing concentrations of vasopressin (0.03–3 nM) or of the vasopressin V_2 receptor agonist, [Mpa^1,D-Arg^8]vasopressin (desmopressin; 0.01–300 nM), were infused into the perfusate near the kidney. Final concentrations in the perfusate are given. Responses were compared to that induced in the same kidney by a supramaximal concentration of noradrenaline (10 μM). Concentration-response curves to angiotensin II (0.01–10 nM) and noradrenaline (0.05–0.75 μM) were also constructed. With vasopressor responses greater than 100 mm Hg, we observed some decrease in perfusion flow (less than 20%, volumetric measurement) despite a fixed flow rate on the perfusion pump. Vasoconstriction was therefore expressed as an increase in renal vascular resistance (calculated as the ratio of perfusion pressure/perfusion flow rate). Since desensitization may occur after a maximum response to vasopressin, only one dose-response curve was generated on a kidney. For each curve, the maximum effect (E_{max}) and the concentration inducing half maximum effect (EC_{50}, according to Ariëns and Van Rossum, 1957) were assessed.

To investigate the modulation of the vasopressin-induced vascular response by prostaglandins or nitric oxide, a specific inhibitor of cyclooxygenase, indomethacin (10 or 30 μM), or of nitric oxide synthase, N^{ω}-nitro-L-arginine (100 μM) (Ishii et al., 1990), was added to the perfusate, 90 and 30 min before studying the response to vasopressin, respectively.

In order to analyse the stability of vasoconstrictor responses to vasopressin, angiotensin II and noradrenaline, successive challenges with these subtances were given at 30 min intervals. In a first serie of experiments, each period comprised successively a challenge with vasopressin (0.3 or 1 nM), angiotensin II (3 nM) and noradrenaline (10 μM). In a second serie, each period comprised a challenge with vasopressin (100 nM) or angiotensin II (3 nM) followed by 10 μM noradrenaline.

2.4. Evaluation of vasodilator responses

The renal vascular tone of the isolated kidney was restored by a continuous perfusion of noradrenaline or prostaglandin $F_{2\alpha}$, at concentrations sufficient to increase perfusion pressure by about 40 and 25 mm Hg, respectively. We previously found that such moderate increases in perfusion pressure were stable (Schmidt and Imbs, 1980; Stephan et al., 1995). Cocaine (10 μM) and 17β-oestradiol (8.5 μM) were added to the perfusate when the tone was restored by noradrenaline, to block neuronal and extraneuronal uptake of the catecholamine. Responses to increasing concentrations of desmopressin (0.1–100 nM) or of vasopressin (0.01–1 nM) in the presence of the vasopressin V_{1A} receptor antagonist (30 or 100 nM SR 49059), were compared to the relaxation induced by a supramaximal concentration of acetylcholine (30 nM) and sodium nitroprusside (30 μM), respective markers for endothelium-dependent and -independent vasodilator responses. Relaxation was expressed as a percentage of the renal vascular tone established by noradrenaline or prostaglandin $F_{2\alpha}$.

2.5. Evaluation of the effects of SR 49059, a vasopressin V_{1A} receptor antagonist

Concentration-response curves of vasopressin were made in the presence of fixed concentrations of SR 49059 (1, 3, 10 and 30 nM) added to the perfusate 30 min before the agonist. Results were plotted according to Arunlakshana and Schild (1959) for pA_2 determination. Concentration ratio was calculated as the ratio of vasopressin concentrations giving half maximum vasoconstriction in kidneys in the presence or absence of the antagonist. The specificity of SR 49059 (30 nM) was tested using angiotensin II and noradrenaline-induced responses.

2.6. Drugs

The following drugs were used: vasopressin ([Arg^8]vasopressin; UCB Pharma, Nanterre, France); desmopressin ([Mpa^1,D-Arg^8]vasopressin; Bachem, Voisins le Bretonneux, France); acetylcholine hydrochloride, angiotensin II acetate, N^{ω}-nitro-L-arginine, noradrenaline hydrochloride, sodium nitroprusside (Sigma, St. Quentin Fallavier, France); indomethacin (Merck, Sharp and Dohme-Chibret, Paris, France), prostaglandin $F_{2\alpha}$ thromethamine salt (Prostine $F_{2\alpha}$, Upjohn laboratories, Paris, France), sodium pentobarbital (Sanofi Santé, Libourne, France). SR 49059 ((2*S*) 1-[(2*R*,3*S*)-(5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzene-sulfonyl)-3-hydroxy-2,3-dihydro-1*H*-indole-2-carbonyl]-pyrrolidine-2-carboxamide) was synthesized by Sanofi Recherche (lot MY-11-107, Montpellier, France). All other chemicals were of pro analysis quality from Merck (Darmstadt, Germany).

SR 49059 was dissolved in dimethylsulfoxide at a concentration of 2 mM and then diluted in perfusate to appropriate concentration. Peptides were prepared as stock solutions (1 mg/ml in distilled water), stored in fractions at −20°C and diluted to desired concentration with 0.28 M glucose just prior to use. To avoid adsorption of peptides, materials were silicone-treated (Aquasil, Interchim, Montluçon, France; 10 min contact with a 1% silicone solution). Indomethacin was dissolved in absolute ethanol (0.5 ml) and N^{ω}-nitro-L-arginine as hydrochloride before addition to the perfusate. Other drugs solutions were made with 0.28 M glucose.

2.7. Statistical analysis

Results are expressed as means ± S.E.M. unless otherwise stated. Differences were tested for statistical significance by the Student's *t*-test or a modified *t*-test according to Bonferroni for multiple comparisons. Variance or regression analysis were also used when appropriate. A *P* value less than 0.05 was considered significant. Concentration response curves were analysed by linear regression for the determination of the 50% effective concentration (EC_{50}). All statistics were run with BMDP Statistical Software (Statistical Software, Cork, Ireland) on an IBM PC compatible computer.

3. Results

3.1. Renal vasoconstrictor effects induced by vasopressin, angiotensin II and noradrenaline

Vasopressin caused renal vasoconstriction at a concentration as low as 0.03 nM. The response was concentration-dependent. For each concentration, the maximum vasoconstrictor response was reached within 3–5 min, declined thereafter partially despite continuous perfusion of the agonist. Prompt recovery to preinjection value was observed at the end of infusion of the agonist. Vasopressin induced a maximum increase in renal vascular resistance (E_{max}) of 34.0 ± 1.1 mmHg/ml per min at 0.3 or 1 nM (Fig. 1; Table 1). Vasoconstrictor response to a supramaximal concentration of vasopressin thereafter was depressed. Half maximum effect was obtained at 0.206 ± 0.044 nM (EC_{50}). Noradrenaline, which was tested at 10 μM in the same kidneys, induced a maximum vasoconstrictor response (32.5 ± 0.9 mmHg/ml per min increase in renal vascular resistance) similar to that of vasopressin. For vasopressin, data from kidneys perfused with Tyrode's or Krebs solutions ($n = 9$ and 5, respectively) were pooled since no difference in E_{max} or EC_{50} values were observed (E_{max} = 32.3 ± 1.0 and 36.9 ± 2.1 mmHg/ml per min; EC_{50} = 0.206 ± 0.049 and 0.192 ± 0.093 nM).

Angiotensin II also induced a concentration-dependent increase in renal vascular resistance resembling the vasopressin-induced response with the same threshold concen-

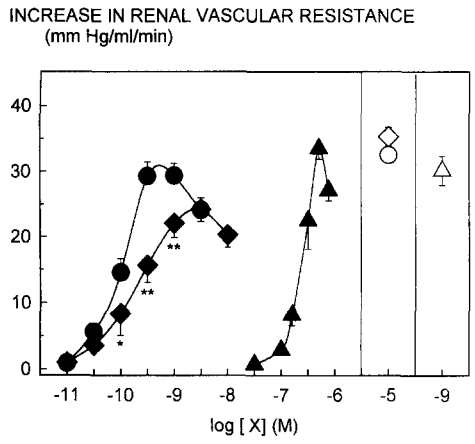

Fig. 1. Concentration-response curves for vasoconstriction induced by vasopressin (●, $n = 14$), angiotensin II (◆, $n = 6$) and noradrenaline (▲, $n = 5$) in the isolated perfused rat kidney. Response to noradrenaline (○, ◇, 10 μM) or vasopressin (△, 1 nM) was tested at the end of the experiments (open symbols). Data (means ± S.E.M.) are expressed as increase in renal vascular resistance. Statistical analysis was performed by Student's t-test comparing angiotensin II to vasopressin; * $P < 0.05$ and ** $P < 0.01$.

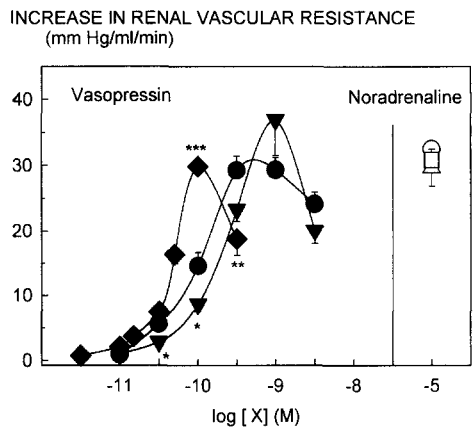

Fig. 2. Effects of indomethacin (10 μM, $n = 5$, ▼) or N^{ω}-nitro-L-arginine (100 μM, $n = 11$, ◆) on vasopressin-induced vasoconstriction in the isolated perfused rat kidney (control, $n = 14$, ●). Response to noradrenaline (10 μM) was tested at the end of each experiment (open symbols). Data (means ± S.E.M.) are expressed as increase in renal vascular resistance. Statistical analysis was performed by Student's t-test modified according to Bonferroni for multiple comparisons; * $P < 0.05$ and ** $P < 0.01$ versus control vasopressin.

tration (0.03 nM), an EC_{50} in the nanomolar range and also a diminished response during continuous perfusion or to a supramaximal concentration (Fig. 1, Table 1). However, the maximum response was only reached at 3 nM and was 25% lower than that of vasopressin ($P < 0.001$).

Noradrenaline induced a dose-dependent vasoconstriction which also diminished during continuous perfusion or after a maximum effective concentration (Fig. 1). EC_{50} values differed by more than a factor of thousand when compared to vasopressin or angiotensin II (Table 1). The maximum increase in renal vascular resistance was reached at 0.5 μM noradrenaline. E_{max} was similar to the E_{max} for vasopressin (Table 1). It was also comparable to the response elicited by 10 μM noradrenaline tested at the end of the experiments.

Inhibition of cyclooxygenase by indomethacin (10 or 30 μM) had no effect on basal renal vascular resistance (10.3 ± 0.5 and 9.6 ± 0.5 mmHg/ml per min in indomethacin-treated and control kidneys, respectively). Indomethacin (10 μM) blunted vasopressin-induced vasoconstriction at the lowest concentrations of the peptide (Fig. 2). E_{max} (36.9 ± 5.4 mmHg/ml per min) and EC_{50} value (0.219 ± 0.053 nM) were however not modified. Also vasoconstriction induced by 10 μM noradrenaline was not affected. Similar results were obtained in two experiments with 30 μM indomethacin (data not shown).

Inhibition of nitric oxide synthase by N^{ω}-nitro-L-arginine (100 μM) produced a small increase in basal renal vascular resistance (10.6 ± 0.3 and 12.0 ± 0.4 mmHg/ml per min before and after treatment by N^{ω}-nitro-L-arginine, respectively; paired Student's t-test, $P < 0.05$). The vasoconstrictor concentration-response curve to vasopressin was shifted to the left and the maximum response was now reached at a three or ten times lower concentration (Fig. 2). A lower EC_{50} value was calculated (0.062 ± 0.010 versus 0.206 ± 0.044 nM in control kidneys, $P < 0.05$). N^{ω}-Nitro-L-arginine however did not affect vasopressin-induced maximum response or the response to 10 μM noradrenaline.

Table 1
Vasoconstrictor effects induced by vasopressin, angiotensin II and noradrenaline in the isolated perfused rat kidney

	n	E_{max} (mm Hg/ml/min)	EC_{50} (nM)
Vasopressin	14	34.0 ± 1.1	0.206 ± 0.044
Angiotensin II	6	24.9 ± 1.7 [a]	0.221 ± 0.044
Noradrenaline	5	34.7 ± 0.6	309 ± 77 [a]
Variance analysis		$P < 0.001$	$P < 0.001$

Kidneys were perfused with Tyrode's or Krebs solutions, in an open circuit, with a constant flow rate of 8 ml/min. Maximum increases in renal vascular resistance (E_{max}) and half maximum effective concentrations (EC_{50}) are given as means ± S.E.M. Statistical analysis was performed by variance analysis and modified t-test according to Bonferroni for multiple comparisons; [a] $P < 0.001$ relative to vasopressin; n, number of experiments.

3.2. Stability of vasoconstrictor responses induced by vasopressin, angiotensin II or noradrenaline

The direct perfusion of a maximum effective concentration of vasopressin (0.3 or 1 nM) induced similar increases in renal vascular resistance than that observed after sequential additions of increasing concentrations (Fig. 1 and Fig. 3). The same was true for angiotensin II (3 nM) and noradrenaline (10 μM). The direct perfusion of a supramaximal concentration of vasopressin (100 nM) also induced a maximum response.

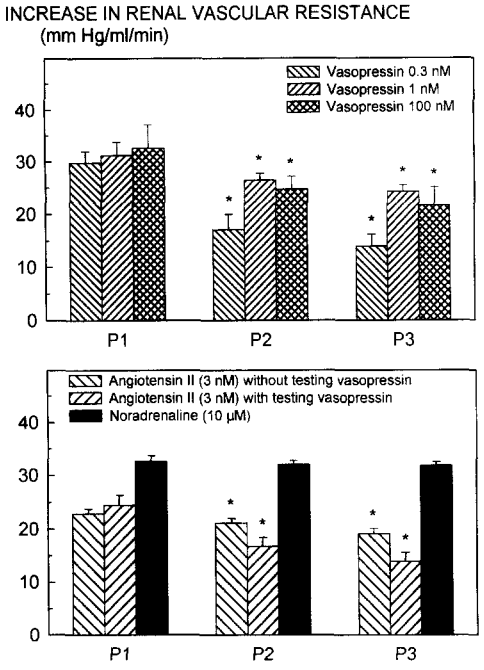

Fig. 3. Tachyphylaxis to vasopressin and angiotensin II in the isolated perfused rat kidney. Three successive challenges were given at 30 min intervals (P1, P2, P3). Each period comprised a challenge with vasopressin and/or angiotensin II followed by a challenge with noradrenaline. Vasoconstrictor responses to vasopressin (0.3 or 1 nM, $n = 5$ and 3, respectively, with angiotensin II tested on the same kidney, or 100 nM, $n = 4$, without testing angiotensin II) are shown in the upper panel. Vasoconstrictor responses to angiotensin II (3 nM, $n = 6$ with vasopressin tested on the same kidney and $n = 3$ without testing vasopressin) and to noradrenaline (10 μM, $n = 15$) are shown in the lower panel. Data (means ± S.E.M.) are expressed as increase in renal vascular resistance. Statistical analysis was performed by variance analysis with repeated measures; * time factor $P < 0.001$.

However, when kidneys were challenged a second and a third time with vasopressin or angiotensin II at 30 min intervals, vasoconstrictor responses progressively decreased (variance analysis with repeated measures, time factor, $P < 0.001$) (Fig. 3). This was true whatever vasopressin (100 nM) or angiotensin II (3 nM) were tested alone or in combination (0.3 or 1 nM vasopressin and 3 nM angiotensin II). In contrast, the vasoconstrictor response to noradrenaline, included as reference compound in all kidneys, was remarkably stable (Fig. 3).

Taking into account tachyphylaxis which seems to occur with the peptides after maximum receptor stimulation, we established only one concentration-response curve to vasopressin (or angiotensin II) on each kidney preparation.

3.3. Effects of SR 49059 on vasopressin-induced renal vasoconstriction

When administered alone, SR 49059, up to the highest concentration tested (300 nM), failed to modify renal vascular resistance although the kidneys were responsive to 10 μM noradrenaline as usual.

SR 49059 (1–30 nM) inhibited the vasoconstrictor responses induced by vasopressin in a concentration-dependent manner (Fig. 4). The antagonist produced a rightward shift of concentration-response curve, without affecting maximum constriction. On the Arunlakshana and Schild plot (Arunlakshana and Schild, 1959), a linear relationship was observed between the logarithm of (concentration-ratio for vasopressin − 1) and the negative logarithm of the molar concentration of SR 49059 ($r = 0.940$, $P < 0.001$). The slope of the regression line (-1.33 ± 0.13; slope ± S.D.) differed however significantly from -1 ($P < 0.05$). An apparent pA_2 (± S.D.) was calculated as 9.14 ± 0.20. When the EC_{50} value for vasopressin in the presence of N^{ω}-nitro-L-arginine was taken as the EC_{50} value in the absence of antagonist, SR 49059 acted as a competitive antagonist. Schild analysis yielded a pA_2 (± S.D.) of 9.72 ± 0.20 with a slope of 1.20 ± 0.12 (statistically not different from -1).

SR 49059 (30 nM) did not affect the angiotensin II-induced dose-response curve, whatever the E_{max} (24.5 ± 0.9 mmHg/ml per min) or the EC_{50} value (0.21 ± 0.03 nM) are considered. SR 49059 also had no antagonistic effect on the vasoconstriction induced by 10 μM noradrenaline tested at the end of the experiments (Fig. 4).

3.4. Search for a renal vascular vasopressin V_2 receptor-related response

When infused at increasing concentrations (0.01–100 nM), desmopressin did not markedly modify renal vascular resistance. Only a small increase (about 5 mmHg/ml per min) was observed at 100 nM, although the kidney preparations responded to noradrenaline (10 μM) as usual.

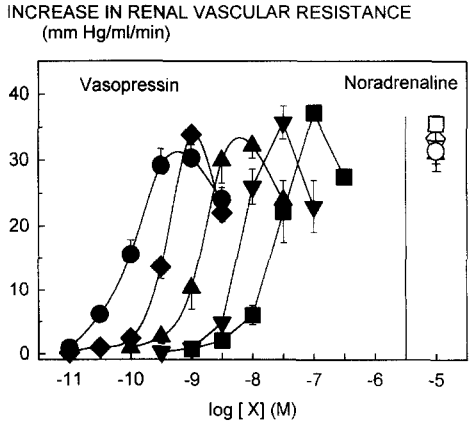

Fig. 4. Inhibition of vasopressin-induced vasoconstriction in the isolated perfused rat kidney by the vasopressin V_{1A} selective antagonist, SR 49059. The antagonist was added to the perfusate 30 min before the initiation of the vasopressin concentration-response curve. Symbols refer to control response induced by vasopressin ($n = 14$, ●), or responses in the presence of SR 49059 at 1 nM (◆), 3 nM (▲), 10 nM (▼) or 30 nM (■) concentrations ($n = 4$ for each). Response to noradrenaline (10 μM) was tested at the end of each experiment (open symbols). Data (means ± S.E.M.) are expressed as increase in renal vascular resistance.

To further investigate a potential vasodilatory effect of desmopressin, renal perfusion pressure was restored by noradrenaline (37 ± 9 nM, mean increase in perfusion pressure 41.0 ± 2.1 mmHg, $n = 3$) or prostaglandin $F_{2\alpha}$ (0.82 ± 0.18 μM, mean increase in perfusion pressure 24.7 ± 1.4 mmHg, $n = 4$). Whatever the constrictor used, desmopressin (0.03–100 nM) failed to induce any relaxation. A small vasoconstriction occurred again at 100 nM. In the same kidneys however, acetylcholine (30 nM) and sodium nitroprusside (30 μM) induced the usual observed relaxations, 99 ± 4 and 109 ± 3% reversal of the increase in renal vascular tone, respectively ($n = 7$).

Under a vascular tone restored by noradrenaline or prostaglandin $F_{2\alpha}$ and also in the presence of SR 49059 (30 or 100 nM) to block the vasopressin V_{1A} receptor, vasopressin (0.01–1 nM) did not induce any relaxation. Here also the usual degree of relaxation to acetylcholine and sodium nitroprusside was observed (90 ± 3 and 92 ± 2% relaxation, $n = 5$).

4. Discussion

Our results confirm previous in vitro findings showing that vasopressin is a potent renal vasoconstrictor (Berecek et al., 1980; Katusic and Krstic, 1987; Edwards et al., 1989). Present data obtained in the isolated rat kidney show that the EC_{50} value for vasopressin is in the nanomolar range as reported for renal artery rings (Katusic and Krstic, 1987), glomerular afferent or efferent arterioles (Edwards et al., 1989; Tamaki et al., 1996) and for other arterial segments (Martinez et al., 1994a,b).

To examine the possibility that the vasopressin-induced vasoconstriction is modulated by a concomitant vasopressin-induced relaxation, we considered several possibilities: vasopressin-induced release of nitric oxide, direct interaction with vasopressin V_2 receptor or release of prostanoids. Inhibition of nitric oxide synthase by N^{ω}-nitro-L-arginine shifted the vasoconstrictor dose-response curve of vasopressin in the isolated kidney to the left. Such an effect was not observed after inhibition of cyclooxygenase by indomethacin. Since vascular nitric oxide mainly originates from endothelial cells (Moncada et al., 1991), the present results suggest that endothelium-released nitric oxide can modulate vasopressin-induced vasoconstriction. Similar results or more pronounced potentiations have been reported for other resistance vessels (Takayasu et al., 1993; Touma et al., 1995) but not in all studies (Martinez et al., 1994a,b; Bax et al., 1995). Modulation by endothelial nitric oxide was also lacking in a large conduit vessel such as renal artery (Katusic and Krstic, 1987).

We did not find evidence for renal vascular effects related to vasopressin V_2 receptor activation. As expected, desmopressin only induced a moderate vasoconstriction at a high concentration compatible with some affinity for the vasopressin V_1 receptor. Under conditions allowing renal endothelium-dependent and -independent relaxations to be observed, desmopressin induced no vasodilation. Furthermore, when kidneys were pretreated by SR 49059 to block the vasopressin V_{1A} receptor, we also failed to observe relaxation with vasopressin infusion. Accordingly, a vasopressin V_2 receptor antagonist did not modify vasopressin-induced vasoconstriction of rabbit efferent arterioles (Edwards et al., 1989). These results are however in contrast with vasopressin V_2 receptor-induced renal vasodilation reported on rabbit microperfused afferent arterioles (Tamaki et al., 1996) and in anaesthetized or conscious dogs (Naitoh et al., 1993; Aki et al., 1994). Taking all together, these findings suggest that the distribution of vascular vasopressin V_2 receptors varies according to vessels and species.

In the isolated perfused rat kidney, the modulation of vasopressin-induced vasoconstriction is likely the result of vasopressin V_{1A} receptor stimulation on endothelial cells as reported on dog basilar and pulmonary arteries (Katusic et al., 1984; Sai et al., 1995). However, we cannot exclude the possible stimulation of nitric oxide release from endothelial cells, by elevated shear stress during vasopressin-induced vasoconstriction (Lamontagne et al., 1992).

Present data do not support a role for release of vasodilatory prostanoids to counteract vasopressin-induced renal vasoconstriction, consistent with previous in vitro data (Katusic and Krstic, 1987). Moreover, after inhibition of cyclooxygenase, we observed a paradoxical decrease in vasopressin-induced renal vasoconstriction at the lowest vasopressin concentrations. This observation is consistent with vasopressin-induced release of vasoconstrictor prostanoids, perhaps thromboxane-like as reported in the rat coronary circulation (Lee et al., 1991).

A decrease in the renal vasculature sensitivity to vasopressin was found in three different situations: (i) during continuous perfusion of the peptide, (ii) with a supramaximal concentration tested after exposure to a high concentration causing a maximal response and (iii) with repeated challenge at 30-min intervals with a maximal effective concentration. Since no evidence for vascular metabolism of neurohypophyseal hormones has been reported (Lauson, 1967) and since hyporeactivity to vasopressin also appeared after inhibition of the release of vasoactive autacoids, desensitization might have occurred. Desensitization during continuous perfusion of a drug is widespread (Furchgott, 1972; Ullian and Linas, 1990) and was found in our hands with all the three constrictors we used. A long duration desensitization was however only observed with vasoactive peptides. Depletion of intracellular messengers or receptor uncoupling might have occurred together with receptor endocytosis as reported previously to occur for vasopressin V_{1A} (Lutz et al., 1991) and angiotensin AT_1 receptors (Ullian and Linas, 1990). Tachyphylaxis to vasopressin was inconsistently found and was practically absent in human and rabbit vessels (Ohlstein and Berkowitz,

1986; Edwards et al., 1989). Although the reason for this difference is presently not known, a larger vasopressin V_{1A} receptor reserve could be involved.

SR 49059 is the most potent and selective non-peptide vasopressin V_{1A} receptor antagonist described so far (Serradeil-Le Gal et al., 1993). In the isolated rat kidney, SR 49059 inhibited vasopressin-induced vasoconstriction from a low concentration of 1 nM. Present results confirm the specificity of the antagonist previously documented in the rabbit aorta (Serradeil-Le Gal et al., 1993). At a concentration of 10 nM, no interaction was obvious with the angiotensin AT_1 or α-adrenoceptors involved in the vasoconstrictor responses to angiotensin II and noradrenaline, respectively. SR 49059 acted as a competitive antagonist on vasopressin-induced contraction in rat caudal and human coronary arteries, with pA_2 values close to 9.50 (Serradeil-Le Gal et al., 1993; Bax et al., 1995). The slope of the Schild plot we observed in the renal vasculature, although only marginaly different from -1, gives evidence of a non-competitive antagonism. Vasopressin-induced vasoconstriction is not a completely pure response since it was modulated by nitric oxide and prostanoids. Of interest, the concentration-response curves of vasopressin seemed steeper in the presence of SR 49059, as was the case after inhibition of nitric oxide synthesis. When the dose-response curve for vasopressin in the presence of N^{ω}-nitro-L-arginine was taken as the reference curve, SR 49059 acted as a competitive antagonist, with a pA_2 of 9.72, in the same range as observed in other vessels (Serradeil-Le Gal et al., 1993; Bax et al., 1995).

In contrast to in vitro studies, vasopressin induced only weak renal vasoconstriction in vivo (McVicar, 1988) but more often had no significant effect (Heyndricks et al., 1976; Hofbauer et al., 1983; Gellai et al., 1984; Serre et al., 1994). In the same studies, vasoconstriction to vasopressin was particularly marked in muscle, skin, mesenteric and iliac vascular beds. These results suggest that the potent direct renal vasoconstrictor effect of vasopressin is blunted in vivo by systemic effects or modulation of the renal sympathetic innervation which is lacking in vitro. Vasopressin is known to decrease heart rate and cardiac output (Hofbauer et al., 1984) and to enhance baroreceptor sensitivity (Cowley et al., 1984). Alternatively, vasopressin-induced renal vascular effects depend on the range of doses tested and the endogenous level of the hormone which varies with anesthesia and surgical stress (Walker et al., 1983). Finally, release of prostanoids (Oliver et al., 1982: Hofbauer et al., 1983) or nitric oxide could also blunt vasopressin-induced vasoconstriction.

Inhibition of vasopressin V_1 receptor had no effect on renal blood flow in normal hydrated rats (Gellai et al., 1984) but induced renal vasodilation in dehydrated animals (Aisenbrey et al., 1981). The threshold concentration for vasopressin to induce vasoconstriction in our study was at 0.03 nM. Such a concentration is found in the circulation after water deprivation, hemorrhagic or ischemic shock, congestive heart failure and even cigarette smoking (Laszlo et al., 1991). Present results suggest that hypersensitivity to vasopressin could occur during endothelium dysfunction which has been reported in essential hypertension, hyperlipidaemia, diabetes mellitus, ischemia and aging (Vanhoutte, 1991; Lüscher et al., 1993; Lefer and Lefer, 1993). Consistently, an increased constrictor response to vasopressin was observed in mesenteric arterioles from aging rats (Touma et al., 1995) and a vasopressin V_1 receptor antagonist partially blunted the hypertension observed after inhibition of nitric oxide synthesis (Manning et al., 1994).

In conclusion, our study shows that vasopressin is a potent renal vasoconstrictor, at least equipotent to angiotensin II in the isolated rat kidney. The constrictor response is mediated by vasopressin V_{1A} receptors and attenuated by a concomitant vasodilation mediated by endothelial release of nitric oxide. Prostaglandins do not markedly contribute to the vasopressin-induced vasoconstriction. The low threshold concentration for vasopressin (0.03 nM) suggests that renal vascular tone could be increased in pathophysiological situations with elevated plasma vasopressin levels and/or with hypersensitivity to vasopressin.

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