Biochemical and Pharmacological Properties of SR 49059, a New, Potent, Nonpeptide Antagonist of Rat and Human Vasopressin V_{1a} Receptors

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Abstract

SR 49059, a new potent and selective orally active, nonpeptide vasopressin (AVP) antagonist has been characterized in several in vitro and in vivo models. SR 49059 showed high affinity for V_{1a} receptors from rat liver (K_i = 1.6±0.2) and human platelets, adrenals, and myometrium (K_i ranging from 1.1 to 6.3 nM). The previously described nonpeptide V_1 antagonist, OPC-21268, was almost inactive in human tissues at concentrations up to 100 μM. SR 49059 exhibited much lower affinity (two orders of magnitude or more) for AVP V_2 (bovine and human), V_{1b} (human), and oxytocin (rat and human) receptors and had no measurable affinity for a great number of other receptors. In vitro, AVP-induced contraction of rat caudal artery was competitively antagonized by SR 49059 (pA_2 = 9.42). Furthermore, SR 49059 inhibited AVP-induced human platelet aggregation with an IC_{50} value of 3.7±0.4 nM, while OPC-21268 was inactive up to 20 μM. In vivo, SR 49059 inhibited the pressor response to exogenous AVP in pithed rats (intravenous) and in conscious normotensive rats (intravenous and per os) with a long duration of action (> 8 h at 10 mg/kg p.o). In all the biological assays used, SR 49059 was devoid of any intrinsic agonistic activity. Thus, SR 49059 is the most potent and selective nonpeptide AVP V_{1a} antagonist described so far, with marked affinity, selectivity, and efficacy toward both animal and human receptors. With this original profile, SR 49059 constitutes a powerful tool for exploring the therapeutical usefulness of a selective V_{1a} antagonist. (*J. Clin. Invest.* 1993. 92:224–231.) Key words: SR 49059 • vasopressin • nonpeptide antagonist • V_{1a} receptor • human

Introduction

Vasopressin (AVP) exerts a variety of biological effects in mammals. It plays a major role in regulating water and solute excretion by the kidney and participates in the multifactorial regulation of a number of other physiological functions such as blood pressure control, platelet aggregation, ACTH secretion by the adenohypophysis, aldosterone secretion by the adrenals, Factor VIII secretion, liver glycogenolysis, and uterine motility (for review see reference 1). AVP is also involved in interneuronal communication in the central nervous system (2).

So far, three AVP receptor subtypes (V_2, V_{1a}, and V_{1b}) have been identified on pharmacological and functional bases. V_2 receptors are positively coupled to adenylylcyclase and play a predominant role in the antidiuretic response to AVP. V_{1a} and V_{1b} receptors mediate phospholipase C activation and intracellular calcium mobilization. V_{1b} receptors are involved in the stimulating effect of AVP on ACTH secretion. It is generally assumed that V_{1a} receptors mediate all other known AVP actions (3). Determination of the amino acid sequence of human (4) and rat (5) V_2 receptors, rat V_{1a} AVP receptors (6), and human oxytocin (OT)[1] receptors (7) clearly confirms that the receptors for neurohypophysial peptides constitute a subfamily of G protein–coupled membrane receptors.

AVP may be involved in several diseases and disorders such as diabetes insipidus (8), heart failure, hypertension, coronary renal vasospasm, hyponatremia, and dysmenorrhea (9, 10). The development of selective AVP antagonists appears essential for investigating the pathophysiological roles of AVP and could lead to new therapeutic agents. A number of potent and selective peptide antagonists have been produced (11, 12); however, the evaluation of their therapeutic utility has been severely hampered by their lack of oral activity (13). Recently, Yamamura et al. reported an orally effective nonpeptide AVP antagonist, OPC-21268, active in vivo on AVP V_{1a} receptors (14, 15).

In this study, we report the biochemical and pharmacological properties of SR 49059 ((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), the first member of an original chemical series of potent, selective, nonpeptide AVP V_{1a} antagonists resulting from the chemical optimization of a lead molecule found from random screening.

Special attention was paid to the evaluation of the pharmacological properties of SR 49059 with regard to the three different AVP receptor subtypes. Similar experiments were also performed with OT receptors, since it is well established that AVP and AVP analogues exhibit measurable affinity for those receptors (16). We also investigated the properties of SR 49059 on receptors from various animal species and on receptors from human origin, since marked species differences exist in the binding and functional potencies of certain AVP analogues (17–19). Finally, for the sake of comparison, we measured the activity of OPC-21268 in all the biological tests used.

We would like to dedicate this work to the memory of Dr. B. Cantau.

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1. *Abbreviations used in this paper:* DBP, diastolic blood pressure; OT, oxytocin; PAF, platelet-activating factor; SBP, systolic blood pressure.

Methods

Materials

The nonpeptide molecules SR 49059 ((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); $\alpha_D 25 = -210°$ (concentration of SR 49059 of 2 mg/ml in $CHCl_3$) and OPC-21268 (1-[1-[4(3-acetyl-aminopropoxy) benzoyl]-4-piperidyl]-3,4-dihydro-2-(1H)-quinolinone) (Fig. 1) were synthesized in Sanofi Recherche, Montpellier, France. Both compounds were initially dissolved in DMSO at a concentration of 10^{-2} M and then diluted in the appropriate test solvent. AVP, d(CH_2)$_5$Tyr(Me)AVP (SKF-100273), ADP, arachidonic acid, collagen, thrombin, polybrene, PMSF, OT, and bacitracin were from Sigma Chemical Co. (L'isle d'Abeau, France). BSA type V was obtained from IBF (Villeneuve La Garenne, Paris, France). Platelet-activating factor (PAF) was purchased from Bachem Feinchemikalien (Bubendorf, Switzerland). DME and PBS were from Boehringer Mannheim (Meylan, France). EDTA, Tris, and DMSO were purchased from Merck-Clevenot (Nogent sur Marne, France). All other chemicals were from Prolabo (Paris, France). The radioligands [^{3}H]AVP, [^{3}H]OT (60 Ci/mmol), and ^{125}I-OT antagonist (d(CH_2)$_5$Tyr(Me)^2, Thr^4, Orn^8 [^{125}I]Tyr^9-NH_2) (2,000 Ci/mmol) were obtained from New England Nuclear (Paris, France). ^{125}I linear AVP antagonist (Phaa-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Arg-Tyr-NH_2) was synthesized as described in reference 20.

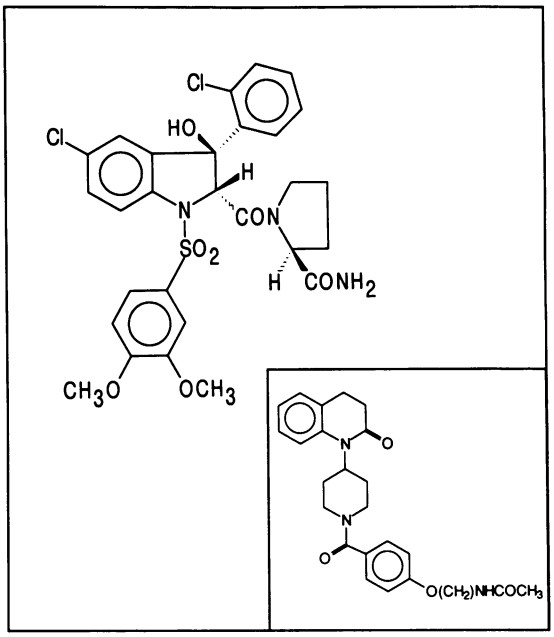

Figure 1. Structure of SR 49059. (*Inset*) Structure of OPC-21268.

Biological material

Human tissue samples from uterus, adrenals, kidneys, and hypophysis were collected in conformity with our national ethical rules. Uterus, adrenal, and kidney samples were immediately chilled in cold saline. Membranes were prepared within 3 h after collection. Hypophyses were collected within 6 h after death and immediately frozen in liquid nitrogen. Bovine kidneys were obtained from a local slaughterhouse. Mammary tissue was taken from 19-d Sprague-Dawley pregnant rats and stored in liquid nitrogen until used. Male Sprague-Dawley rats (Iffa-Credo, Lyon, France) were used for in vitro binding studies and isolated tissue preparations and for in vivo blood pressure measurements. Male Wistar Janssen rats (bred in-house) were used in pithed rat studies. Male New Zealand rabbits were used for in vitro isolated preparations.

Membrane preparations

Membranes from human tissues. For human adrenal membrane preparations, pieces of adrenal glomerulosa zonae were suspended in a cold buffer (10 mM Tris-HCl, pH 7.4, 3 mM $MgCl_2$, 1 mM EDTA, 250 mM sucrose, and 0.1 mM PMSF) and fragmented using a glass/glass Dounce homogenizer. The homogenate was filtered through glass wool and centrifuged for 15 min at 1,500 *g* at 4°C. The pellet was incubated for 20 min in ice-cold hypotonic buffer (10 mM Tris-HCl, pH 7.4, 3 mM $MgCl_2$, 1 mM EDTA, and 0.1 mM PMSF) and kept on ice to allow cell lysis. Lysed cells were recovered by centrifugation at 1,500 *g* for 15 min at 4°C, homogenized using a loose-fitting Dounce homogenizer in hypotonic buffer supplemented with 40% (vol/vol) glycerol, and stored at −20°C. Before experiments, glycerol was eliminated by washing the membranes in glycerol-free hypotonic buffer.

For the preparation of hypophyseal membranes, frozen entire pituitary glands were rapidly thawed at 37°C in isotonic buffer supplemented with 0.1 mM PMSF, and the adenohypophyses were separated. Adenohyphyseal membranes were prepared as described above for adrenal membranes.

All other membrane preparations from human platelets, myometrium, and kidneys were prepared as previously described by Vittet et al. (21) and Guillon et al. (16, 19), respectively.

Membranes from animal origins. Bovine kidney medullary and rat liver membranes were prepared by the method of Stassen et al. (22) and Prpic et al. (23), respectively.

For rat mammary gland membrane preparations, tissues were minced and homogenized in 50 mM 10% (wt/vol) buffer A Tris-HCl, pH 7.4, 320 mM sucrose, and 0.5 mM dithiothreitol and centrifuged at 900 *g* for 15 min; the pellet was resuspended in the buffer A and centrifuged as above. The two 900-*g* supernatants were filtered through cheesecloth and centrifuged at 70,000 *g* for 20 min. The pellet was washed with buffer B containing 50 mM Tris-HCl, pH 7.4, and 10 mM $MgCl_2$. Finally, the 70,000-*g* washed pellet was suspended in buffer B at a final concentration of ~ 8 mg protein/ml and stored as aliquots in liquid nitrogen.

Binding assays

V_{1a} binding assays using ^{125}I linear AVP antagonist. Binding assays on human adrenal membranes, myometrial membranes from nonpregnant uterus, or platelet membranes were performed in an incubation medium (200 μl) containing 10 mM Tris-HCl, pH 7.4, 3 mM $MgCl_2$, 1 mg/ml BSA, 0.05 mg/ml soy bean trypsin inhibitor, 0.5 mg/ml bacitracin, 0.1 mM PMSF, 0.5 mM EDTA, 10–60 pM ^{125}I-AVP linear antagonist, and increasing amounts of a test compound. The reaction was started by the addition of membranes (10–40 μg/assay) that were incubated at 30°C for 45 min. The reaction was stopped by adding 3 ml of ice-cold filtration buffer (10 mM Tris-HCl, pH 7.4, and 3 mM $MgCl_2$) followed by filtration through GF/C Whatman glass microfiber filters that had been soaked for at least 5 h in a solution containing 10 mg/ml BSA. Filters were washed five times with 3 ml of filtration buffer and counted for radioactivity by gamma spectrometry. All determinations were performed in duplicate. Nonspecific binding was determined in the presence of 0.3 μM unlabeled iodinated AVP linear antagonist or 1 μM AVP.

V_{1b}, V_2, and OT binding assays using tritiated ligands. Binding of [^{3}H]AVP to human pituitary membranes and binding of [^{3}H]OT to myometrial membranes from the pregnant uterus were conducted as indicated above except that the membrane concentration was 80–100 μg/assay and bound radioactivity was separated from free by filtration on RAWP 1.2-μM (Millipore Corp., Milford, MA) filter. Binding assays of [^{3}H]AVP to human or bovine kidney membranes were performed according to the method of Guillon et al. (19) and Crause and Fahrenholz (24), respectively. Binding of [^{3}H]AVP to rat liver membranes was performed as previously described (25).

OT binding assays using ^{125}I-OT antagonist. Rat mammary membranes (30 μg/assay) were incubated for 20 min at 30°C in an incubating medium containing 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 1 mM EGTA, 0.1 mg/ml BSA, 0.1 mg/ml bacitracin, 60 pM ^{125}I-OT

antagonist, and increasing concentrations of the compound to be tested. The content of the assay tubes was filtered through GF/C filters (Whatman Inc., Clifton, NJ) presoaked in 0.5% polyethyleneimine, then rinsed twice with 4 ml of ice-cold wash buffer. The radioactivity was counted in an LKB multiwell gamma counter (LKB Instruments S.A., Paris, France). Nonspecific binding was determined in the presing was determined in the presence of 10 μM unlabeled OT.

Binding data analysis. The IC_{50} value was defined as the concentration of inhibitor required to obtain 50% inhibition of the specific binding. Inhibition constant (K_i) values were calculated from the IC_{50} values using the Cheng and Prusoff equation (26). Data for equilibrium binding (K_d, B_{max}), competition experiments (IC_{50}, nHill), and kinetic constants (k_{obs}, k_{-1}) were analyzed using an iterative nonlinear regression program (27).

In vitro biological assays

Human platelet aggregation. Human blood was collected in a 10-mM trisodium citrate solution by venipuncture from nonsmoking healthy volunteers who were drug free for at least 1 wk. Platelet-rich plasma (PRP) was obtained by centrifuging the blood sample at 150 *g* for 15 min at 20°C. The resulting pellet was further centrifuged (2,000 *g* for 15 min at 20°C) to produce platelet-poor plasma (PPP). Platelet aggregation was measured turbidimetrically with a dual channel aggregometer (Chrono-Log Corp., Havertown, PA) according to the method of Born and Cross (28). The reaction was followed by monitoring changes in light transmission on 400-μl PRP aliquots, under continuous stirring (900 rpm) at 37°C, against 400 μl of PPP control. Various concentrations of SR 49059 or OPC-21268 were added to PRP 1 min before the addition of aggregating agents 50–100 nM AVP, 5 μM ADP, 10 μg/ml collagen, 0.5 μM PAF, 400 μM arachidonic acid, or 0.2 U/ml thrombin. In every case, a dose–response curve was first performed with AVP to assess the good reactivity of platelets to AVP, and inhibition experiments were conducted with an AVP concentration inducing ~ 80% of the maximal aggregating response. The inhibitory effects of the compounds were determined by measuring the height of the aggregation wave from the bottom of the shape change compared with the control response. IC_{50} (concentration required to inhibit 50% of control aggregating response) was estimated from the dose–response inhibition curve by an iterative nonlinear regression program (27).

Rat caudal artery and rabbit aorta contraction. A ring (2–3 mm wide) cut from the rat tail (caudal) artery or rabbit aorta was suspended in a temperature-controlled (37°C) organ bath (20 ml) and submitted to an initial tension of 0.5 g (tail artery) or 2 g (rabbit aorta).

The bathing solution contained (mM): 137 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.2 NaH_2PO_4, 15.5 $NaHCO_3$, and 11.5 glucose and was maintained at pH 7.4 by bubbling a 95% O_2-CO_2 gas mixture. Potassium-rich solution (40 mM) was obtained by substituting equimolar KCl for NaCl. The tension of the preparation was measured with an isometric force transducer and the experiments were performed with Robotized Isolated Organ System (IOS3). Various concentrations of SR 49059 and OPC-21268 were assessed on AVP (3 nM)-induced contraction of the rat caudal artery. Cumulative dose–response curves to AVP were constructed by the method of stepwise addition of the agonist according to Van Rossum (29) in the absence and presence of a fixed concentration of antagonist added 30 min before the agonist. The specificity of action of SR 49059 was studied on angiotensin II (10^{-8} M)-, norepinephrine (10^{-7} M)-, or KCl (40 mM)-induced rabbit aorta contraction. Control experiments, conducted in the absence of the antagonist, showed that no significant changes in sensitivity occurred between several stimulations by the agonist. Contractile responses were expressed as a percentage of the maximal control response to the agonist. PA_2 values were calculated by the method of Arunlakshana and Schild (30).

In vivo biological assays

Arterial blood pressure in the pithed rat. Rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.). After tracheal intubation, rats were artificially ventilated with room air using a rodent ventilator (Hugo Sachs Elektronik, March-Hugstetten, Germany) at a volume of 4.8 ml and at a rate of 60 strokes/min. Animals were kept warm at 37°C. The left carotid artery and the dorsal vein of the penis were cannulated for blood pressure measurements and intravenous injection of drugs, respectively. Diastolic blood pressure (DBP) was measured using a pressure transducer coupled to a polygraph (Gould Inc., Glen Burnie, MD). Rats were pithed (31) through the orbit with a steel rod. After stabilization of arterial blood pressure, SR 49059 (dissolved in 20% ethanol) or the vehicle was injected (0.5 ml/kg i.v.). 15 min later, a dose–pressor response curve was obtained for AVP (0.5 ml/kg i.v.). Full recovery was achieved with the lower doses of AVP, which elicited a rise in DBP of < 20 mmHg. At higher doses, AVP was injected cumulatively immediately after the maximal effect of the preceding dose (10 s). Only one full dose–response curve was obtained in each rat. The increase in DBP was expressed in millimeters of Hg. Maximal effect and ED_{50} were determined by an iterative computed program (32, 33). Results were analyzed by the Arunlakshana and Schild method applied to in vivo data (30, 34). In vivo pA_2 values were also calculated according to the method of Van Rossum adapted to in vivo conditions (29, 30).

Arterial blood pressure in conscious normotensive rats. Rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.). Both the jugular vein and the carotid artery were cannulated for the intravenous injection of drugs (0.1 ml/kg) and blood pressure measurements, respectively. The catheters, filled with an aqueous solution of 40% polyvinylpyrrolidone and 1,000 UI/ml heparin, were tunneled under the skin and exteriorized on the dorsal side of the neck. 24 h later, rats were maintained in individual boxes and the systolic blood pressure (SBP) was measured using a Statham P23I a pressure transducer (Gould-Statham, Oxnard, CA) coupled to a TA 2000 polygraph. Before administration of the test compound, exogenous AVP inducing a rise in SBP of ~ 40 mmHg (40 ng/kg) was injected intravenously (50 μl/100 g in saline) two or three times at 15-min intervals to establish a reproducible control pressor response and subsequently at 15, 30, 60, 120, 180, and 240 min after the dose. SR 49059 was solubilized in an aqueous 10% DMSO solution for intravenous injections and in 5% arabic gum per os; in each case, it was tested against the corresponding controls. Statistical analysis of the data was performed by ANOVA followed by a Dunnett's *t* test for multiple comparisons. The level of significance was taken as $P < 0.05$.

Analysis of data

All data are expressed as means±SE.

Results

Interaction of SR 49059 with rat and human AVP V_{1a} receptors and selectivity profile

A preliminary series of experiments (not shown) indicated that the recently designed radioiodinated ligand (^{125}I linear AVP antagonist), which was shown to be highly selective for rat V_{1a} receptors (20), also binds with high affinity (K_d values ranging from 10 to 150 pM) to membranes of human platelets, adrenals, and myometrium from nonpregnant uterus, three tissues in which the presence of AVP receptors of the V_{1a} subtype has been previously demonstrated (16, 21). Competition experiments with selective peptide agonists and antagonists indicated that the ^{125}I linear AVP antagonist labeled a major population of sites that had the expected pharmacological profile of V_{1a} receptors. This ligand was used for all further experiments on human V_{1a} receptors.

The nonpeptide antagonist SR 49059 dose dependently inhibited specific binding to V_{1a} receptors from rat liver and human platelets, adrenals, and myometrium. Measured K_i values (Table I) were in the nanomolar range and Hill coefficient values were close to unity, compatible with a single-site competitive model. Under the same experimental conditions OPC-

Table I. Affinity of SR 49059 for Rat and Human AVP V_{1a} Receptors in Comparison with OPC-21268

	K_i (nM)			
	Rat*	Human tissues‡		
	Liver	Platelets	Adrenals	Nonpregnant uterus
SR 49059	2.2±0.4	6.3±0.6	1.1±0.2	1.5±0.4
OPC-21268	350±30	>100,000	>10,000	15,000±2,000

Binding assays were performed as described in Methods. Inhibition constants (K_i) were determined from competition experiments calculated according to the equation of Cheng and Prusoff (26). Values are the mean±SE of at least three independent determinations.
* Competition experiments with [^{3}H]AVP.
‡ Competition experiments with ^{125}linear AVP antagonist.

21268 exhibited a low affinity for rat liver V_{1a} receptors and an even lower affinity for human V_{1a} receptors (K_i values > 10 μM).

Saturation binding experiments were performed on rat liver plasma membranes in the absence or presence of SR 49059 (0.5, 1, 2, and 4 nM). Scatchard analysis of data indicated that SR 49059 inhibited [^{3}H]AVP binding on rat liver membranes in a competitive manner, since in the presence of this molecule the apparent dissociation constant (K_d) was significantly changed, whereas the maximal binding capacity (B_{max}) was not modified (Fig. 2). The K_i value calculated from Scatchard plots (1.65±0.22 nM) was consistent with the K_i value obtained according to the Cheng and Prusoff equation (26) from competition experiments (2.20±0.40 nM).

The selectivity of SR 49059 for AVP V_{1a} receptors was evaluated by measuring the ability of SR 49059 to inhibit the binding to other AVP receptor subtypes (V_{1b}, V_2) and to the related OT receptor, from both human and animal origins. Control experiments (not shown) using selective V_2, V_{1a}, or OT peptide ligands indicated that the major population of sites labeled with [^{3}H]AVP on renal membranes and with [^{3}H]OT on myometrial membranes from pregnant uterus were of the V_2 and OT subtypes, respectively. [^{3}H]AVP-labeled binding sites on human adenohypophysial membranes exhibited weak affinity for a selective V_{1a} antagonist ($d(CH_2)_5Tyr(Me)AVP$) and a selective V_2 agonist (dDAP), suggesting that these sites were of the V_{1b} subtype. As shown in Table II, SR 49059 displayed weak affinities for human and nonhuman V_2, V_{1b}, and OT receptors. Moreover, in a variety of binding assays SR 49059 did not interact with receptors of nonpeptide (histamine, adrenergic, dopamine, serotonine, adenosine, benzodiazepine, L-type calcium channel) or peptide ligands (neuropeptide Y, endothelin, CCK A, CCK B, neurotensin, etc.) (data not shown). Thus, SR 49059 appears to be the most potent nonpeptide molecule interacting selectively with both rat and human AVP V_{1a} receptors.

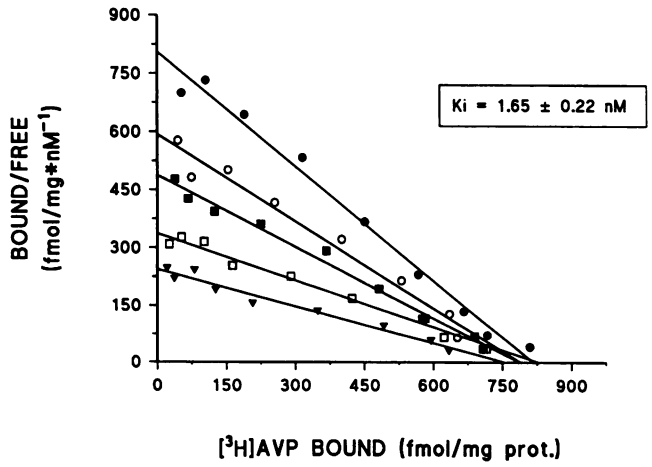

Figure 2. Scatchard plots of [^{3}H]AVP binding to rat liver plasma membranes without (●) or with 4 (▼), 2 (□), 1 (■), or 0.5 (○) nM SR 49059. Results represent data from a typical experiment performed in duplicate which was repeated three times.

Effect of SR 49059 on AVP-induced human platelet aggregation in vitro

As shown in Fig. 3, SR 49059 inhibited AVP-induced human platelet (PRP) aggregation in a concentration-dependent manner with an IC_{50} value of 3.7±0.4 nM, similar to that of the specific peptide V_1 antagonist, $d(CH_2)_5Tyr(Me)AVP$ (IC_{50} = 3.6±0.6 nM; data not shown). Under the same experimental conditions, OPC-21268 was inactive in concentrations up to 20 μM. This result was consistent with its low affinity for human platelet V_{1a} receptors (Table I). It is important to note that neither SR 49059 nor OPC-21268 had any agonist properties on platelet shape change or aggregation when tested alone. Moreover, the inhibitory effect of SR 49059 is specific since at 1 μM it had no action on human platelet (PRP) aggregation induced by PAF, collagen, thrombin, ADP, or arachidonic acid: responses observed in the presence of SR 49059 were 99±3, 103±1, 88±9, 103±1, and 91±3% of the control response (n = 3), respectively.

Effect of SR 49059 on AVP-induced contraction in isolated arteries in vitro

SR 49059 and OPC-21268 inhibited the contractile responses induced by AVP (3 nM) in isolated rat caudal artery rings with IC_{50} values of 1.6±0.2 and 156±22 nM, respectively (Fig. 4).

Table II. Binding Profile of AVP V_{1a} Antagonists, SR 49059 and OPC-21268: Affinity for AVP and OT Receptor Subtypes in Several Species

	K_i				
	V_{1b}* hypophysis	V_2* renal medulla		OT	
	Human	Bovine	Human	Rat‡ mammary glands	Human§ pregnant uterus
	nM				
SR 49059	220±30	280±20	275±50	1,080±115	130
OPC-21268	>10,000	>10,000	>10,000	>10,000	—

Binding assays were performed as described in Methods. Inhibition constants (K_i) were determined from competition experiments calculated according to the equation of Cheng and Prusoff (26). Values are the mean±SE of at least three independent determinations.
* Competition experiments with [^{3}H]AVP.
‡ Competition experiments with ^{125}I-OT antagonist.
§ Competition experiments with [^{3}H]OT. The value indicated was derived from a single myometrium sample.

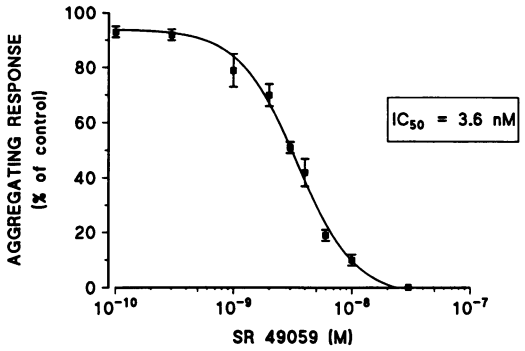

Figure 3. Dose-related inhibition of AVP-induced human platelet aggregation by SR 49059 (■). Results are expressed as percentage of the maximal aggregating responses mediated by 50–100 nM AVP on a human PRP fraction. Each data point represents the mean±SE of three independent experiments.

In this preparation, SR 49059 (3–30 nM) produced parallel rightward shifts in the AVP concentration–response curve without significantly affecting the maximal contractile response (Fig. 5). Schild analysis of these data yielded a pA_2 value of 9.42 with a slope of 1.08, indicating competitive antagonism (Fig. 5, *inset*).

In isolated rabbit aorta SR 49059 did not modify the contractile responses induced by high potassium concentration (40 mM), angiotensin II (10 nM), or norepinephrine (100 nM). Mean variations with respect to control values were: 13±1, 1±1, and 2±2% ($n = 4$), respectively. This indicates the absence of interaction of SR 49059 with voltage-dependent calcium channels, angiotensin II, and alpha 1 adrenergic receptors. SR 49059 alone did not elicit any contractile response in either rat tail artery or rabbit aorta.

Effect of SR 49059 in the pithed rat in vivo

In the pithed rat AVP dose-dependently increased DBP with an in vivo pD_2 value of 9.94±0.12 ($n = 6$) (not shown). Increasing intravenous doses of SR 49059 shifted the dose–pressor response curve for AVP dose-dependently to the right in a parallel manner without significantly modifying the maximal AVP hypertensive effect, suggesting competitive antagonism (Fig. 6). Schild analysis of data yielded an in vivo pA_2 value of 7.68 with a slope not significantly different from unity. This in vivo

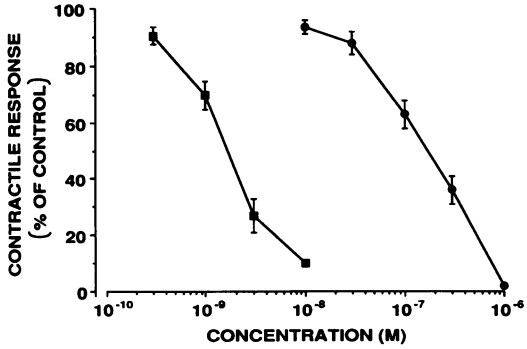

Figure 4. Inhibition curves of AVP-induced contraction by SR 49059 (■) and OPC-21268 (●) in isolated rat caudal artery. Results are expressed as a percentage of the maximal control responses to AVP (3 nM) and are the mean±SE of four experiments.

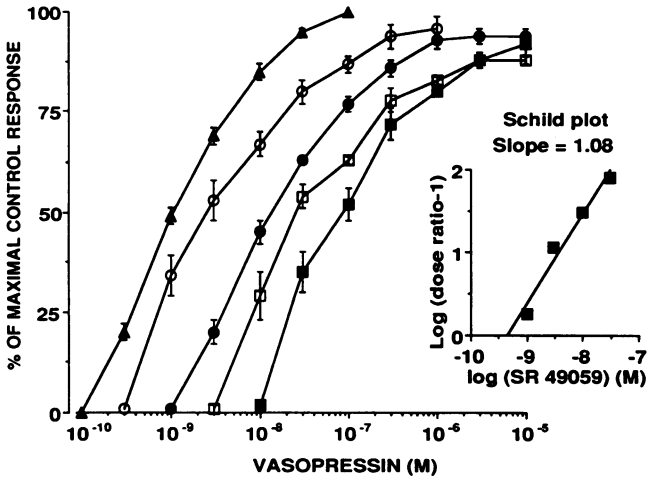

Figure 5. Concentration–response curves of AVP-induced contraction in isolated rat caudal artery without (▲) and with 1 (○), 3 (●), 10 (□), 30 (■) nM SR 49059. Results, expressed as a percentage of the maximal control response mediated by AVP, are the mean±SE of five experiments. (*Inset*) Schild analysis of data (pA_2 9.42; slope 1.08).

pA_2 value was in good agreement with that determined by the Van Rossum method (29) (7.55±0.12) ($n = 16$), indicating that SR 49059 exerted a competitive inhibition on the hypertensive effects of AVP. Under the same experimental conditions OPC-21268 also demonstrated a competitive antagonism as already reported (14), with an in vivo pA_2 value of 6.02±0.12 ($n = 17$) (not shown). For both SR 49059 and OPC-21268 no agonistic properties were observed in this in vivo model. The pressor response to angiotensin II was not significantly attenuated by SR 49059 at doses of 0.1 and 0.3 mg/kg i.v. (data not shown).

Effect of SR 49059 in conscious normotensive rats in vivo

In conscious rats, 40 ng/kg AVP induced a raise in SBP from 138±2 to 184±2 mmHg ($n = 59$). After several AVP injections no tachyphylaxis occurred in this model (Fig. 7). Bolus intravenous injection of SR 49059 (0.01–1 mg/kg) produced dose-dependent inhibition of the pressor response to exogenous AVP (40 ng/kg i.v.) (Fig. 7 *A*). The maximal inhibitory effect appeared 15 min after injection with a magnitude of 58, 81, and 87% inhibition at 0.01, 0.1, 0.3, and 1 mg/kg, respectively (n = 5–8 per group). The action of SR 49059 lasted ~ 30 min at 0.01 mg/kg and 4 h at 1 mg/kg.

Remarkably, when administrated orally to conscious rats, SR 49059 (0.3–10 mg/kg) inhibited AVP-induced hypertension in a concentration-dependent manner (Fig. 7 *B*). It is interesting to note that the onset of effect occurred rapidly after per os SR 49059 treatment. The inhibitory effect was maximal after 15 min at 0.3 mg/kg but was maintained for at least 4 h at higher doses (1, 3, and 10 mg/kg), demonstrating a long-lasting oral effect of SR 49059 to counteract the hypertensive response of AVP in vivo; at 10 mg/kg p.o. this antihypertensive action lasted significantly ($P < 0.05$) more than 8 h (data not shown).

It is important to note that no agonistic properties were observed with SR 49059 at these intravenous and per os doses. Moreover, SR 49059 (1 mg/kg i.v.) exhibited no inhibitory effect on the hypertension induced by angiotensin II (100 ng/kg i.v.) and norepinephrine (300 ng/kg i.v.).

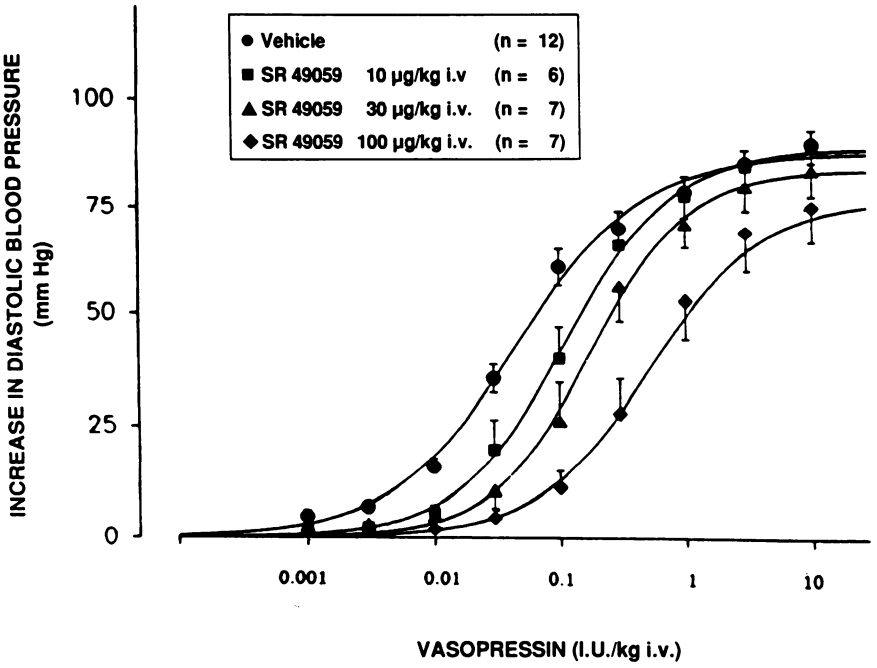

Figure 6. Inhibitory effects of SR 49059 on the cumulative dose–pressor response curves to AVP in the pithed rat. Data are expressed as an increase in DBP and represent the mean±SE of 6–12 determinations per group.

Discussion

For several years the literature has widely reported the participation of AVP in several regulations, especially in cardiovascular, central, and endocrine functions. Furthermore, many clues provide evidence that AVP might be intimately involved in several disorders and diseases including hypertension, heart failure, and vasospasm (for a review see reference 9). Conse-

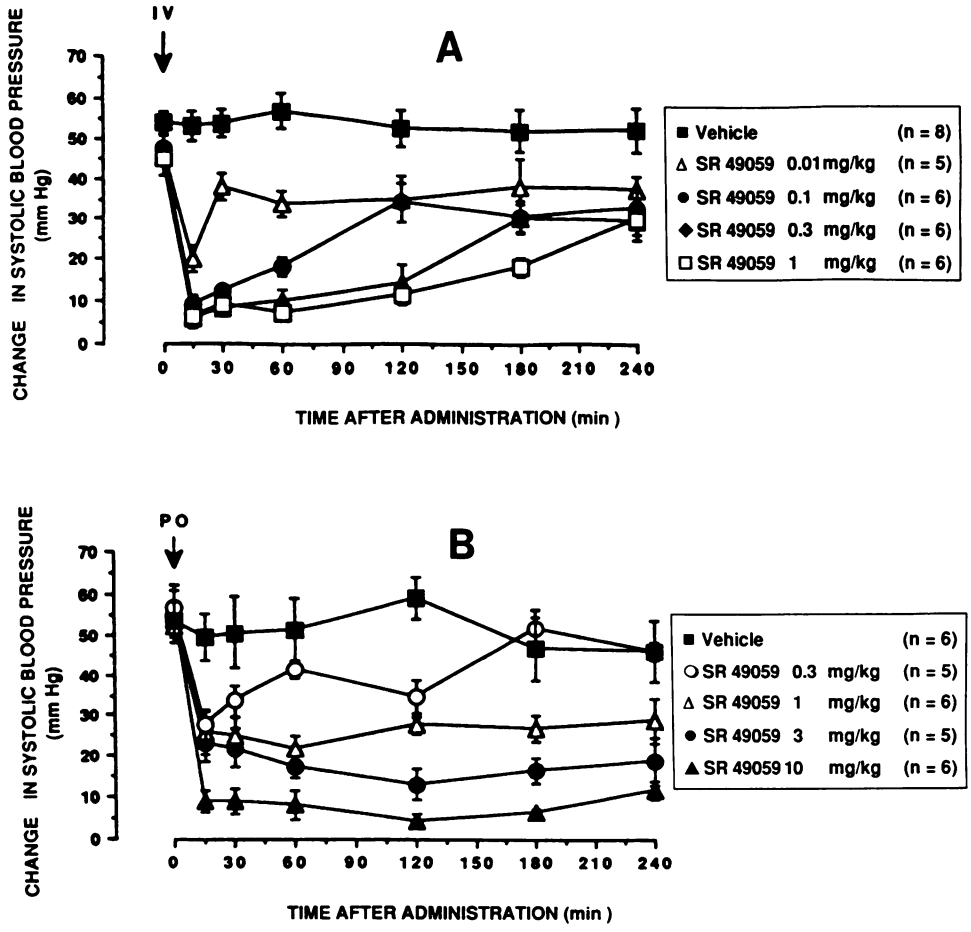

Figure 7. Time course of the inhibitory effect of SR 49059 intravenously (*A*) and per os (*B*) on exogenous AVP (40 ng/kg)-induced hypertension in conscious, normotensive rats. Data are expressed as a change in SBP, observed after each AVP injection, and represent the mean±SE of five to eight determinations per group.

quently, the development of potent and specific nonpeptide AVP antagonists with good oral bioavailability may offer novel therapeutic approaches, but also the possibility of elucidating the pathophysiological role of AVP.

In this study we describe and characterize a new potent and selective orally effective, nonpeptide antagonist for AVP V_{1a} receptors, SR 49059. Since it has been reported that AVP V_{1a} receptors are present not only in the liver (35), but also in adrenals (36), nonpregnant uterus (16), and platelets (21), we used these target tissues from rat and human origin to evaluate the affinity of the nonpeptide antagonist SR 49059 for AVP receptors. In our binding studies, SR 49059 displayed high affinity and selectivity for the AVP V_{1a} subtype as this compound competitively inhibits [^{3}H]AVP binding to rat liver V_{1a} receptors and ^{125}I linear AVP antagonist binding to AVP V_{1a} receptors on several human tissues; a remarkably good potency was observed in adrenals (K_i = 1.1±0.2 nM), uterus (K_i = 1.5±0.4 nM), and platelets (K_i = 6.3±0.6 nM). Under the same experimental conditions, OPC-21268 exhibited for rat liver V_{1a} receptors a K_i value of 350±30 nM, in agreement with the original published value (14); SR 49059 exhibited a 150-fold higher affinity than OPC-21268. It is of interest that even larger discrepancies appeared between SR 49059 and OPC-21268 when tested on human tissues. OPC-21268 exhibited very low affinity for several human V_{1a} receptors. Moreover, this low affinity for AVP platelet receptors is well correlated with the inactivity observed for OPC-21268 (20 μM) on AVP-induced human platelet aggregation, whereas SR 49059 displays high potency (IC_{50} = 3.6±0.4 nM).

The fact that SR 49059 and OPC-21268 interact differently in binding and functional studies depending on the mammal target cell tissue confirmed the existence of great species differences between rat and human V_{1a} receptors as already reported in the liver (18, 35) and in the brain for other neuropeptides such as neurotensin and substance P (37). The ability of nonpeptide molecules like SR 49059 to efficiently discriminate between closely structurally related receptor subtypes suggests that such chemical structures could be useful tools for identifying new receptor subtypes, as was shown for angiotensin II receptors (38).

It is also important to underline the selective profile of SR 49059 as evidenced in vitro in binding tests and functional studies. SR 49059 has a slight affinity for V_{1b}, V_2, and OT receptors from human origin and demonstrates no affinity for several peptide and nonpeptide receptors. Moreover, SR 49059 potently inhibited AVP-induced platelet aggregation without affecting aggregation induced by ADP, PAF, collagen, or thrombin. These observations, together with an absence of activity on KCl-, norepinephrine-, and angiotensin II–induced rabbit aorta contraction, indicate the high selectivity of SR 49059 for AVP V_{1a} receptors.

Because AVP is one of the most potent contractile agents described so far and could be involved in the control of blood pressure via vascular smooth muscle cell V_{1a} receptors (39, 40), the effect of SR 49059 was investigated in vascular tissue in vitro and in arterial blood pressure in vivo. In this study, specific dose-dependent inhibition of AVP-induced contraction could be shown in isolated rat caudal artery in which SR 49059 elicits a 100-fold higher competitive response than OPC-21268 with a pA_2 value of 9.2. SR 49059, investigated in both pithed and normotensive conscious rats, antagonizes the pressor response to exogenous AVP at intravenous doses ranging from 0.01 to 1 mg/kg. As shown in the pithed rat, this antagonism is also competitive. Furthermore, per os administration of SR 49059 in the conscious rat at doses of 0.3–10 mg/kg demonstrates the good bioavailability of SR 49059 since effective doses were ~ 10 times higher than those observed for intravenous administration. In addition, SR 49059 has a long-lasting effect in this vascular model as its protective effect at 10 mg/kg lasts for > 8 h.

All these observations allow us to conclude that SR 49059 can be considered as the most potent and selective synthetic V_{1a} antagonist yet described in vitro and in vivo in relevant models. Indeed, this molecule displays high affinity, selectivity, and efficacy at V_{1a} receptors in human tissues where OPC-21268 is almost ineffective; SR 49059, with a marked inhibitory effect on AVP-induced human platelet aggregation, has a potency similar to that of the V_{1a} peptide antagonist d(CH_2)$_5$Tyr(Me)AVP (41). But unlike peptide molecules, SR 49059 displays good oral bioavailability and a lack of agonistic properties in vitro and in vivo. Therefore, with this original potency SR 49059 may be a promising tool for further investigating the pathophysiological role of AVP and for exploring the therapeutic uses of a selective AVP V_{1a} antagonist.

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