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Functional and Pharmacological Characterization of the First Specific Agonist and Antagonist for the V1b Receptor in Mammals

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By activating three distinct vasopressin receptor isoforms called V1a-R, V1b-R (V3-R) and V2-R, vasopressin (VP) mediates a wide number of biological effects in mammals and may be involved in several pathological states. Up to now only specific V1a and V2 receptor agonists and antagonists have been successfully designed. The role of the V1b-R still remains partially unknown, due to the lack of selective V1b-R ligands and orally-active molecules, which are crucial tools for investigating the central and peripheral functions or pathological disorders associated with this receptor. In this review, we report the biological and pharmacological properties of the first two specific V1b-R ligands: d[Cha^4] AVP, a high affinity V1b-R agonist and SSR149415, a potent orally-active V1b-R antagonist with good selectivity with respect to other VP/OT receptor isoforms and able to control ACTH secretion *in vitro* and *in vivo*. Indeed, these molecules constitute invaluable tools for exploring the central and peripheral roles of VP mediated via V1b receptors. Interestingly, SSR149415 displays potent anxiolytic and antidepressant-like activities, indicating that this new class of drugs has a promising therapeutical potential in the treatment of stress-related disorders, anxiety and depression.

Keywords: ACTH; Anxiety; Depression; Vasopressin; V1b (V3) receptor; Stress

INTRODUCTION

In mammals, vasopressin (VP) is a nonapeptide synthesized centrally by the hypothalamus and released into the general circulation and into the hypophysial portal system. VP exerts several central and peripheral actions like water reabsorption by the kidney, platelet aggregation, liver glycogenolysis, vascular smooth muscle contraction and secretion of aldosterone, insulin, atrial natriuretic factor and corticotropin (ACTH) by the adrenal gland, the pancreas, the heart and pituitary, respectively (Jard, 1998). With oxytocin (OT), VP is also involved in interneuronal communications in the central nervous system and modulates behavioral functions such as memory, feeding, thermoregulation social and sexual processes (Dreifuss *et al.*, 1991; Barberis *et al.*, 1999).

VP participates in the control of the hypothalamic-pituitary-adrenal axis (HPA) at the pituitary level and controls ACTH secretion. VP exerts a dual effect as a direct ACTH secretagogue and also potentiates the stimulatory effect of corticotropin releasing factor (CRF) (Gillies *et al.*, 1982). Importantly, VP also plays a major role during adaptation to stress (Aguilera and Rabadan-Diehl, 2000). The expression of VP in the parvocellular neurons of the paraventricular nucleus and its secretion into the portal circulation increases under chronic stress conditions (Rabadan-Diehl *et al.*, 1995). In depressed human patients the number of hypothalamic neurons coexpressing VP and CRF is strongly increased. Moreover, high levels of circulating ACTH and hyperactivity of the corticotrope axis have also been observed in these patients (Raadsheer *et al.*, 1994). Very recently, data concerning the first V1b-receptor knockout mice have demonstrated the important role of VP receptors in aggression and, to a lesser extent, in social recognition (Wersinger *et al.*, 2002).

The different physiological actions of VP are triggered by three VP receptor isoforms, pharmacologically

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characterized and recently cloned in numerous mammalian species including man (Serradeil-Le Gal *et al.*, 1993; Jard, 1998; Barberis *et al.*, 1999). The V2 isoform is principally found in the kidney and is responsible for the antidiuretic effects of VP. It is positively coupled to adenylyl-cyclase via an αs heterotrimeric G protein. The more ubiquitous V1a isoform mediates the various actions of VP in platelets, blood vessels, liver, adrenal, uterus and brain. It induces activation of phospholipase Cβ. Finally, the V1b VP receptor is principally found in the pituitary and in the adrenal. Like the V1a receptor, it stimulates PLC β activity via an αq/α11 heterotrimeric G protein. Yet, its pharmacological binding profile is very different. Thus, vasopressin cyclopentamethylene antagonists like the Manning compound [d$(CH_2)_5$ Tyr(Me^2)AVP] exhibit a nanomolar affinity for rat and human V1a receptors and only a micromolar Ki for the V1b receptor subtype (Kruszynski *et al.*, 1980; Jard *et al.*, 1986).

Following years of intensive investigations in many laboratories, peptides and nonpeptides exhibiting a high affinity and good selectivity for some of the VP receptor isoforms were discovered (Barberis *et al.*, 1999). They are very useful tools for physiological studies and also represent drugs with high potential therapeutic interest. SR49059 (Serradeil-Le Gal *et al.*, 1993) and d[(CH_2)Tyr$(Me)^2$] AVP (Kruszynski *et al.*, 1980) are both potent and selective V1a receptor antagonists with nanomolar affinities in various species (see for review, Barberis *et al.*, 1999). F-180, a structural analogue of [Phe^2, Orn^8] vasotocin, is a potent V1a receptor agonist in human and bovine species (Andres *et al.*, 2002). Similarly, SR121463 is one of the most selective V2 receptor antagonists described so far with a potent affinity and an excellent isoform selectivity for VP receptors from various species (Serradeil-Le Gal *et al.*, 1996). Finally, d[Thi^3] VDAVP is an excellent selective V2 receptor agonist in the rat with nanomolar affinity (Ben Mimoun *et al.*, 2001). Until very recently attempts to design specific VP receptor agonists or antagonists exhibiting high selective affinity for the V1b receptor isoform were unsuccessful. On the basis of *in vivo* bioassays, previous studies indicated that d[D-3-Pal^2] AVP could be a V1b relatively specific agonist (Schwartz *et al.*, 1991). However, binding experiments performed both on rat and human membranes expressing VP receptors indicate that this peptide is totally non-selective with respect to the V1a receptor isoform (Barberis *et al.*, 1999).

Recently, we described two new selective V1b compounds: SSR149415, a specific V1b receptor antagonist (Serradeil-Le-Gal *et al.*, 2002), and d[Cha^4] AVP, an agonist with high affinity and good selectivity with respect to other VP/OT receptors isoforms (Derick *et al.*, 2002).

In the present article, we will review the biochemical profiles and the pharmacological properties of these two new V1b ligands.

MATERIALS AND METHODS

d[Cha^4]AVP is an analogue of dAVP obtained by replacing the glutamine in position 4 by a cyclohexyl alanine residue (Fig. 1). It was synthesized by the manual solid phase method starting from BOC-Gly resin (terButyl Oxy Carbonyl-Glycine) as previously described (Derick *et al.*, 2002). Its homogeneity was confirmed by HPLC and its structure confirmed by electron spray mass spectrophotometry. The non-peptide V1b receptor antagonist, SSR149415, a *N*-arylsulfonyl-oxindole derivative, was synthesized at Sanofi-Synthélabo Recherche and its chemical structure determined by mass spectrometry, ^1H and ^{13}C NMR and infrared spectroscopy (Serradeil-Le-Gal *et al.*, 2002). Both compounds were dissolved in DMSO at a concentration of 10^{-2} or 10^{-3} M and diluted in the appropriate test solvent. All the pharmacological tests performed in various *in vitro* (CHO cell lines transfected with human VP/OT receptors, primary cultures of rat adenohypophysis, plasma membranes from rat tissues) or *in vivo* models in anaesthesized or conscious rats were

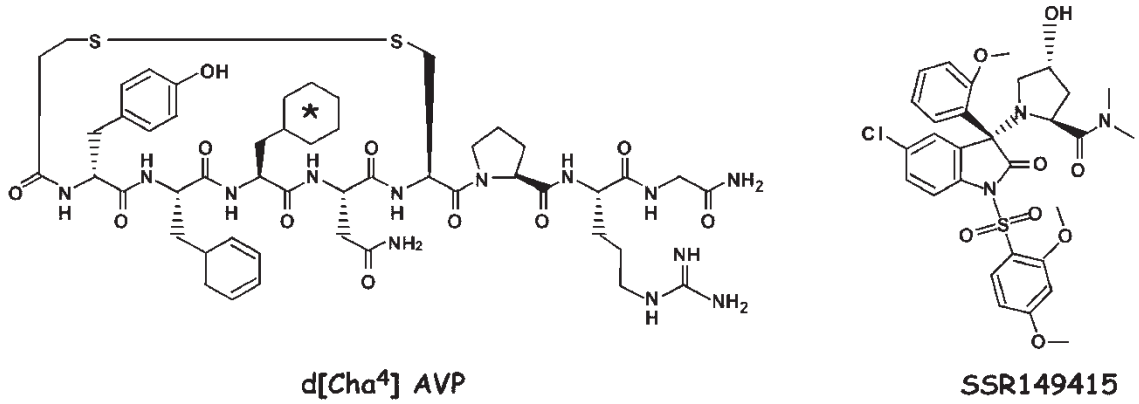

FIGURE 1 Chemical structures of d[Cha^4]AVP and SSR149415. d[Cha^4] AVP = [1-deamino, 4 cyclohexyl alanine] arginine vasopressin. *Indicates the cyclohexyl residue in position 4. SSR149415 = (2S,4R)-1-[5-chloro-1-[2,4-dimethoxyphenyl)sulfonyl]-3-(2-methoxy-phenyl)-2-oxo2,3-dihydro-1H-indol-3-yl]-4-hydroxy-*N*, *N*-dimethyl-2-pyrrolidine carboxamide.

conducted as previously described (Derick *et al.*, 2002; Griebel *et al.*, 2002; Serradeil-Le-Gal *et al.*, 2002).

Animal manipulations were performed according to the recommendations of the French Ethical Committee and under the supervision of authorized investigator.

RESULTS AND DISCUSSION

Pharmacological Properties of d[Cha^4]AVP

As illustrated in Fig. 2A, VP analogues completely displaced, in a dose-dependent manner, [^{3}H]-AVP specific binding to membranes from CHO cells expressing the human V1b receptor (hV1b-R). Similar experiments were performed using membranes from CHO cell lines stably transfected with the human V1a, V2 and OT receptors (hV1a-R, hV2-R and hOT-R, respectively) (data not shown). These curves were fitted using an iterative nonlinear regression program and Ki's were calculated from IC_{50} values by using the Cheng and Prusoff equation. From these data, competitive interactions between [^{3}H]-arginine vasopressin (AVP) and the unlabeled analogues tested were observed. Dissociation constant values (Ki) deduced from these experiments are summarized in Table I. As shown in this table, deamination of AVP at position 1 to give dAVP led to a small increase of Ki for hV1b-R and also to some V1b selectivity as compared with other VP receptor isoforms. Similarly, modification of the glutamine residue in position 4 of dAVP led to d[Val^4] AVP which exhibited a better V1b/V1a receptor selectivity. This finding prompted further investigation of position 4 in dAVP with a variety of aliphatic amino acids (unpublished results) and led to the discovery of d[Cha^4] AVP when substituting the glutamine in position 4 by a cyclohexylalanine residue (Derick *et al.*, 2002).

Like the natural peptide AVP, d[Cha^4] AVP exhibited high affinity for hV1b-R together with excellent selectivity for other human VP/OT receptors, in contrast with AVP (Fig. 2, panel B). The relative affinity was at least 100-fold lower for hV1a, hV2 and hOT-R as compared to hV1b-R. This compound was also an excellent V1b ligand for the rat V1b VP receptor (rV1b-R). Its affinity was in the nanomolar range and its selectivity versus rat V1a and OT receptors was excellent (Fig. 2, panel B). d[Cha^4] AVP fulfils all the pharmacological criteria for a potent vasopressin analogue selective for the human V1b receptor versus other VP receptors and the OT receptor, and was found to be the first analogue selective for rat V1b versus V1a and OT receptors. However, d[Cha^4] AVP also exhibited relatively good affinity for the rat V2-R (Ki = 12.7 ± 2.8 nM) leading to a weak V1b/V2 selectivity ratio (about 4).

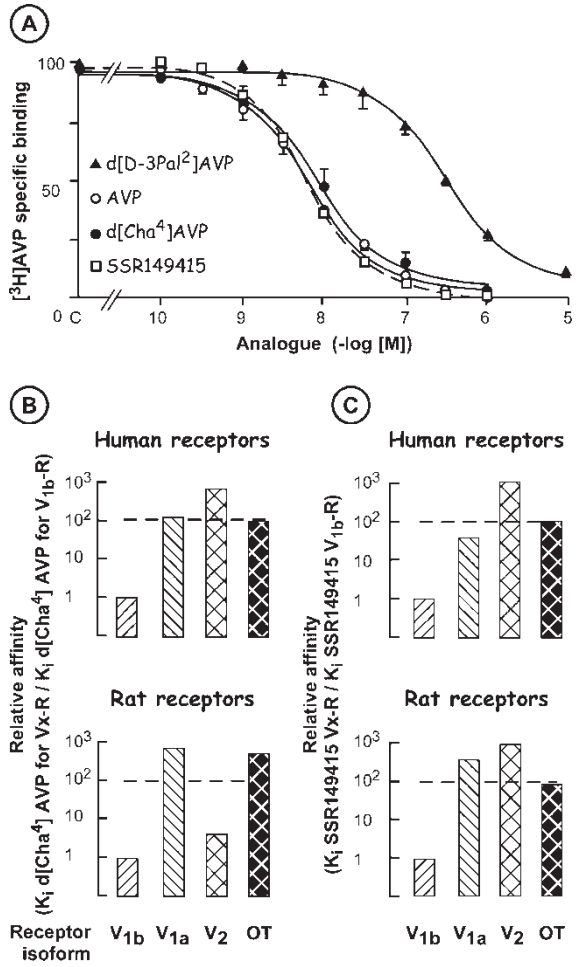

FIGURE 2 Affinity and selectivity of d[Cha^4] AVP and SSR149415 for rat and human VP/OT receptors. Panel A illustrates competition experiments using [^{3}H]-AVP as a radioligand and various unlabeled VP analogues on membranes from CHO cell lines stably transfected with the human V1b receptor isoform. Results are expressed as percent of specific binding measured in the absence of unlabelled VP analogues (Control, C). Panel B: Affinities (Ki) of d[Cha^4] AVP for the different VP/OT receptor isoforms from human and rat species are deduced from competition binding experiments as described in panel A. These values are normalized for each species to the affinity of d[Cha^4] AVP for the V1b receptor isoform. Note the species differences for d[Cha^4] AVP between rat and human V2-R. The dotted line corresponds to a receptor V1b selectivity of 100 fold. Panel C: Relative affinities (Ki) of SSR149415 for VP/OT receptor isoforms from human and rat species (see panel B for legend). Results are the mean ± SE mean of 3–4 separate experiments.

TABLE I Binding properties of the selective V1b receptor agonist, d[Cha^4]AVP, the selective V1b receptor antagonist, SSR149415, and AVP and related VP analogues for human VP/OT receptors

	Ki (nM)			
Analogues	hV1b-R	hV1a-R	hV2-R	hOT-R
AVP	0.68 ± 0.01	1.1 ± 0.1	1.2 ± 0.2	1.7 ± 0.2
dAVP	0.37 ± 0.06	3.8 ± 0.1	5.0 ± 0.4	nd
d[Val^4] AVP	0.29 ± 0.1	11.4 ± 1.6	1.2 ± 0.1	nd
d[Cha^4] AVP	1.2 ± 0.1	151 ± 11	750 ± 120	240 ± 50
SSR149415	1.5 ± 0.8	91 ± 23	1412 ± 314	174 ± 35

Binding assays were performed on plasma membranes from CHO cells stably transfected with human VP or OT receptor isoforms and the Ki's deduced from dose-binding inhibition experiments as illustrated in Fig. 2. Results are the mean ± SE mean obtained from 4 to 6 independent experiments. nd: not done.

Functional Properties of d[Cha^4] AVP in Various *In Vitro* and *In Vivo* V1b Biological Models

In CHO cell lines stably transfected with hV1b-R, both AVP and d[Cha^4] AVP stimulated total inositol phosphate accumulation in a dose-dependent manner with a nanomolar Kact in good correlation with the Ki determined for these peptides. Both peptides maximally stimulated inositol phosphate accumulation to the same extent (Fig. 3, panel A).

To validate the agonistic properties of d[Cha^4] AVP in a physiological model *in vitro*, we tested its ability to induce ACTH secretion by rat pituitary cells in primary culture. As shown in Fig. 3, panel B, AVP and d[Cha^4] AVP both stimulated ACTH release. As in CHO cells expressing the V1b-R, these effects were dose-dependent and half maximal effects were observed at nanomolar concentration (Kact = 2.0 ± 0.6 and 1.0 ± 0.2 nM, respectively). In contrast, compared to AVP, d[Cha^4] AVP was found to be 27% less efficient in maximally stimulating ACTH suggesting some partial agonist properties.

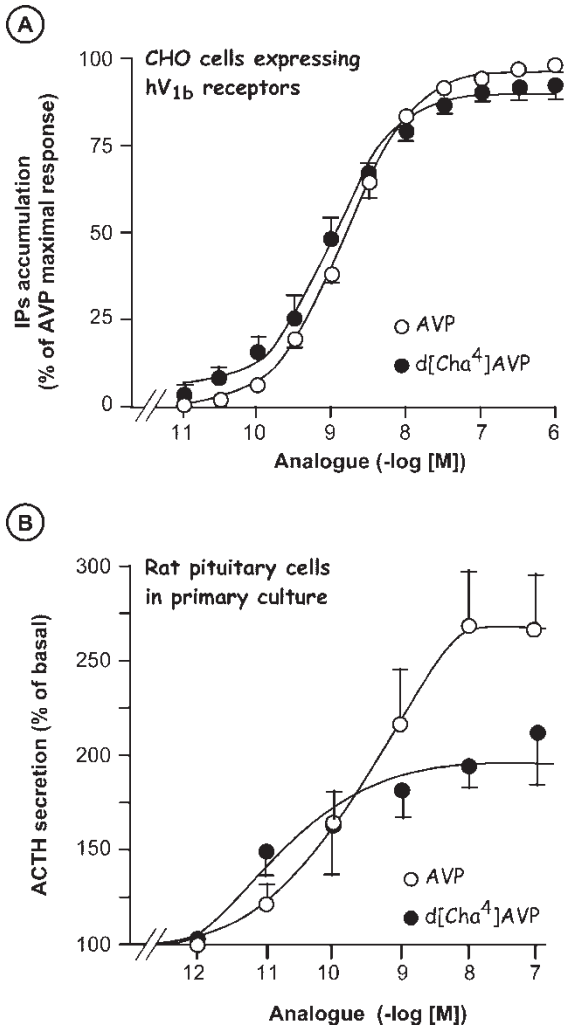

FIGURE 3 *In vitro* properties of d[Cha^4] AVP on rat pituitary primary cultures and on CHO cell lines expressing human V1b receptors. Panel A: CHO cells stably transfected with human V1b receptors and prelabeled with [^{3}H]-myo-inositol were incubated 15 min at 37°C with or without increasing amounts of AVP or d[Cha^4]AVP (Derick *et al.*, 2002). Total inositol phosphates (IPs) that accumulated were measured and expressed as percent of the maximal AVP response. Panel B: rat pituitary cells in primary culture were incubated for 2 h at 37°C with or without (basal) increasing amounts of AVP or d[Cha^4] AVP. ACTH released in the incubation medium was measured by RIA and plotted as percent of basal secretion. Results are the mean ± SE mean of 3–4 separate experiments each performed in triplicate.

In vivo pharmacological experiments performed in rats showed that, as compared to AVP, d[Cha^4] AVP exhibited a very low pressor activity (376 and 0.07 International units/mg, respectively). In contrast, its antidiuretic activity was only reduced by 60% compared with AVP (antidiuretic activity = 332 and 133 International unit/mg for AVP and d[Cha^4] AVP, respectively). We also measured the effects of peptide injection on ACTH and corticosterone plasma concentrations in order to evaluate the ability of d[Cha^4] AVP to stimulate the HPA axis. At up to 5 μg peptide injected/200 g body weight, d[Cha^4] AVP only partially (37%) stimulated ACTH secretion compared with a maximal dose of AVP (1 μg peptide injected/200 g body weight) (Derick *et al.*, 2002). In contrast, the corticosterone responses were similar whichever peptide was tested at 1 μg/200 g body weight (7-fold stimulation as compared to basal control values measured 15 min after injection).

Altogether, experiments performed on transfected cell lines, on rat pituitary cells in primary culture, and in rats *in vivo* clearly showed that d[Cha^4] AVP could be considered to be a potent V1b agonist. *In vitro*, it behaves as a full agonist on inositol phosphate accumulation and as a partial agonist on ACTH secretion in rat pituitary cells (Fig. 3). *In vivo*, as compared to AVP, this peptide showed partial agonist properties, namely in its ability to stimulate ACTH secretion (Derick *et al.*, 2002). These discrepancies between *in vivo* and *in vitro* data may be due to (1) a weaker bioavailability of d[Cha^4] AVP, (2) pharmacokinetic differences with the natural hormone AVP, or (3) different coupling properties of d[Cha^4] AVP/V1b receptor complexes in *in vivo* or *in vitro* models, namely by their abilities to interact with heterotrimeric G proteins and to stimulate phospholipase C. By contrast, d[Cha^4] AVP and AVP stimulate corticosterone release with the same maximal efficiency. These results indicate that as previously observed in rats (Ramachandran, 1984–1985), only a fraction of the ACTH released by the pituitary is necessary to induce a full adrenal response. Yet, we cannot also exclude a specific action of d[Cha^4] AVP at sites other than the corticotrope V1b receptor to influence corticosterone secretion. Additional experiments are now in progress to test this possibility.

Pharmacological Profile of SSR149415 at VP/OT Receptors

As shown in Table I and Fig. 2, SSR149415 displays high nanomolar affinity for rat and human V1b-R and, like d[Cha^4] AVP, totally antagonizes [^{3}H]-AVP specific

binding to V1b-R with an affinity close to that of the natural hormone, AVP. Binding competition and saturation experiments indicate a competitive interaction of SSR149415 at hV1b-R (Serradeil-Le-Gal *et al.*, 2002). Additionally, this compound exhibits selectivity for the V1b receptor isoform versus V1a, V2 and OT-R, both from rat and human origin. Its Ki values for these receptor subtypes were at least 60-fold weaker than for the V1b-R (Fig. 2, panel C). Thus, SSR149415 has a selective V1b profile similar to that previously described for d[Cha^4] AVP at human VP/OT receptors, but it displays much more selectivity than d[Cha^4] AVP at rat V1b-R since it does not interact significantly at rat V2-R (Fig. 2, panels B and C). The high degree of selectivity of SSR149415 for the V1b receptors was also demonstrated in several biological assays *in vitro*: up to 10 μM, SSR149415 does not interact with a number of other receptors or enzymes ($n = 100$).

To assess potential interaction with V1a and V2 receptors *in vivo*, the antidiuretic and pressor responses to SSR149415 were also studied in conscious rats. SSR149415 (30 mg/kg p.o.) neither modified the basal nor the hypertensive response to exogenous AVP and had no significant effects on urine excretion volume both in normal and genetically vasopressin-deficient Brattleboro rats, a sensitive strain that is used for detecting potential agonist antidiuretic activity (Serradeil-Le-Gal *et al.*, 2002).

Functional Properties of SSR149415 in Various *In Vitro* and *In Vivo* Models

To determine its agonist or antagonist profile *in vitro*, the effect of SSR149415 was studied on calcium transients and intracellular calcium mobilization ([Ca^{2+}]i) in CHO cells stably transfected with the human or rat V1b-R. When tested alone up to 1 μM, SSR149415 was devoid of any effect on [Ca^{2+}]i. On the contrary, it dose-dependently antagonized the AVP-induced [Ca^{2+}]i increase with a Ki (1.26 ± 0.6 nM) in close relationship with the nanomolar affinity reported at V1b-R (Serradeil-Le-Gal *et al.*, 2002).

In vivo pharmacology performed measuring ACTH secretion induced by various stimulants such as hormones (AVP, AVP + CRF) and physical-emotional stress, such as restraint, demonstrated a full antagonist profile for SSR149415. In all these situations, SSR149415 did not modify basal ACTH plasma levels up to 30 mg/kg p.o. (not shown) but reduced stimulated-ACTH secretion which constitutes a critical response of the organism to stress in emotional situations (Aguilera and Rabadan-Diehl, 2000). As shown in Fig. 4, panel A, AVP increased the concentration of plasma ACTH by 3.5-fold 10 min after injection. SSR149415 (1–30 mg/kg) antagonized AVP-induced ACTH secretion in a dose-dependent manner by the intraperitoneal route. It is important to note that, in these models, SSR149415 demonstrated high potency by the oral route on the potentiation of the CRF

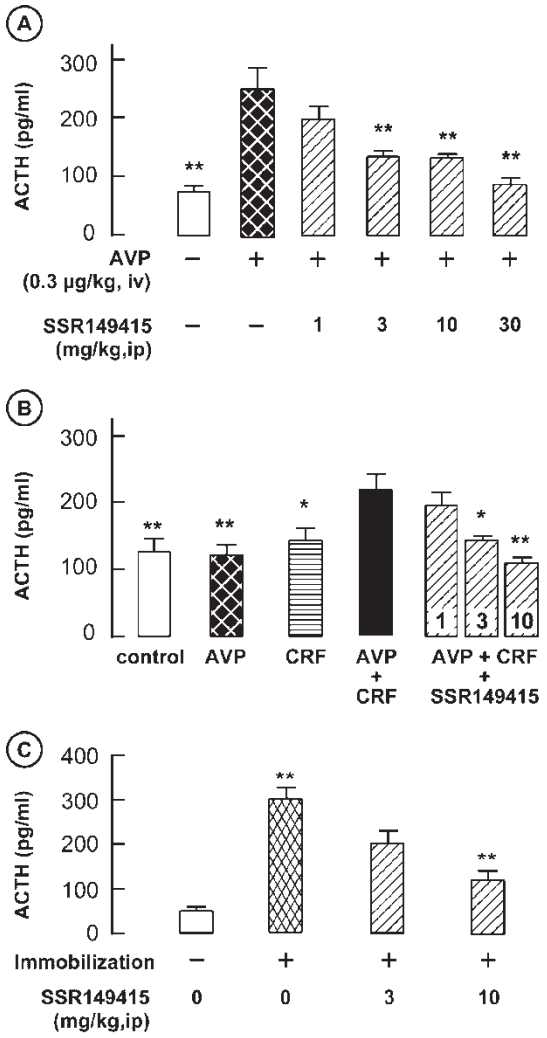

FIGURE 4 *In vivo* properties of SSR149415 on ACTH secretion in the rat. Panel A: Conscious rats were administered increasing amounts of SSR149415 (i.p.) or vehicle 15 min before stimulation with 0.3 μg/kg i.v. AVP. Plasma ACTH levels were measured by RIA 10 min after the AVP challenge. B. Effects of oral SSR149415 on the potentiation by AVP of exogenous CRF stimulation of ACTH secretion in conscious rats. 1–10 mg/kg SSR149415 was administered by gavage 2 h before 0.1 μg/kg i.v. CRF and 0.03 μg/kg/i.v. AVP injections. Plasma ACTH levels were measured by RIA 10 min after the AVP and CRF challenge. Panel C: Effect of SSR149415 (i.p.) on restraint-stress-induced ACTH secretion in conscious rats. Rats injected 30 min before the experiment with 3 or 10 mg/kg i.p. SSR149415 or vehicle (0) were submitted or not to a restraint test of 15 min of immobilization as previously described (Serradeil-Le-Gal *et al.*, 2002). Thereafter rats were killed and plasma ACTH levels measured by RIA. Results are the mean ± SE mean of 8–19 animals (*$P < 0.05$; **$P < 0.01$).

effect by AVP, a mechanism described as a typical V1b-R-mediated effect. Significant inhibition of ACTH secretion was observed with a dose of 3 mg/kg p.o., and total blockade occurred at 10 mg/kg p.o. (Fig. 4, panel B) and this effect lasted for more than 4 h (Serradeil-Le-Gal *et al.*, 2002). Conversely, a selective, orally-active V1a receptor antagonist, SR49059, was unable to inhibit AVP plus CRF-induced ACTH secretion demonstrating a specific V1b-mediated effect (Serradeil-Le Gal *et al.*, 1993). Various physical stresses induce ACTH secretion. The stress-induced release of ACTH is believed to involve

the activation of several humoral and neural pathways, including that mediated by AVP. As shown in Fig. 4, panel C, in rats submitted to an immobilization period of 15 min, there was a significant increase (more than 5-fold) in plasma ACTH levels. Pretreatment with SSR149415 (3–10 mg/kg i.p.) 30 min before the restraint stress period, caused a dose-dependent inhibition of the increase in plasma ACTH in comparison with stressed animals treated with the corresponding vehicle. Since the effects of SSR149415 occur rapidly in the *in vivo* models and as this nonpeptide molecule exhibits a high stability, we can speculate that it is the parent molecule and not a metabolite which supports its activity. Besides the well-known CRF system, several pieces of evidence have shown that the regulation of ACTH secretion and consequently of the HPA axis is also mediated by AVP and V1b receptors. Thus, SSR149415 offers a new tool for controlling emotional or physical stress. Indeed, several neuroendocrine studies strongly suggest that dysregulation of the HPA system plays a causal role in the development and the course of diseases such as post-traumatic stress disorders, depression and addiction (Holsboer, 1999).

Taken together, these functional data indicate that SSR149415 is a full selective V1b antagonist both *in vitro* and *in vivo*. Interestingly, due to its nonpeptide nature and its low molecular weight, this antagonist may be used *in vivo* either via intraperitoneal injection or by oral administration and displays a long lasting effect (up to 4 h at 10 mg/kg p.o.) (Serradeil-Le-Gal *et al.*, 2002).

Anxiolytic- and Antidepressant-like Properties of SSR149415 in Rodents

As, firstly, VP was previously shown to be involved in various types of behavioral processes (De Wied, 1965), secondly, intraseptal application of a mixed V1a/V1b antagonist, d(CH2) Tyr $(Et)^2$ VAVP was found to produce anxiolytic-like effects in rats (Liebsch *et al.*, 1996) and thirdly, V1b receptor density increases in human depressed subjects (Raadsheer *et al.*, 1994) or in rats subjected to restraint-stress (Rabadan-Diehl *et al.*, 1995), the behavioral profile of SSR149415 was explored in a variety of traditional and atypical tests for anxiolytics and antidepressants in rodents (Griebel *et al.*, 2002; Serradeil-Le-Gal *et al.*, 2002).

As an illustration, results obtained with SSR149415 in the punished drinking test, a classical model of anxiety in rats (Vogel *et al.*, 1971), are reported here. SSR149415 significantly increased punished responding at 3 and 10 mg/kg i.p., suggesting anxiolytic-like properties as with diazepam, a well-known benzodiazepine (BZ) agonist used as a positive control in this model (Fig. 5, panel A). The magnitude of the anxiolytic-like action of SSR149415 was somewhat less than that obtained with diazepam. Flumazenil, a BZ antagonist, did not block the anxiolytic-like activity of SSR149415, providing strong evidence that SSR149415 effects are not mediated by an action at

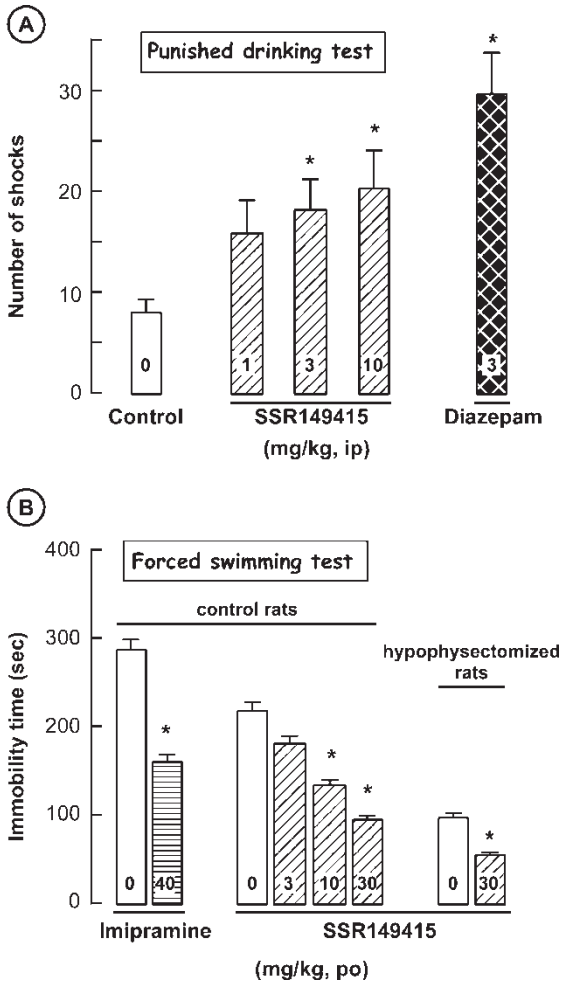

FIGURE 5 Anxiolytic- and antidepressant-like properties of SSR149415 in rats. Panel A: Effect of SSR149415 in the punished-drinking conflict test. Rats deprived of water for 48 h were administered increasing doses of SSR149415, diazepam or vehicle (control) and placed in a cage containing a drinking tube connected to a water buret. The trial started 30 min after drug injection, but only when the rat's tongue touched the drinking tube for the first time. An electric shock (0.6 mA, 500 ms) was then delivered to the tongue after every 20 licks and the number of shocks was recorded during a 5 min period. Results are the mean ± SE mean of 13–20 animals. Panel B: Effect of SSR149415 in the forced-swimming test. Control or hypophysectomized (3 weeks) rats were administered SSR149415, imipramine or vehicle (p.o.) 24 h and 60 min before the start of the trial. Rats were placed individually in a glass cylinder containing water and their immobility during the swimming session measured for a 6-min period. Results are the mean ± SE mean of 7–13 animals ($*P < 0.05$).

the γ-aminobutyric acid type A ($GABA_A$) BZ receptor, and more probably are V1b-Receptor mediated. The anxiolytic-effects of SSR149415 were largely confirmed in several other models, including conflict paradigms (e.g. four-plate test in mice), exploration-based tests (e.g. elevated plus-maze in rats) and more ethologically-orientated procedures (e.g. mouse defense test battery). Interestingly, these effects were still apparent following repeated treatment, indicating that no tolerance to the anxiolytic-like action or SSR149415 had developed. (Griebel *et al.*, 2002; Serradeil-Le-Gal *et al.*, 2002.).

The potential antidepressant-like properties of SSR 149415 were investigated in the forced-swimming test

initially described as a valid model of depression in rodents (Porsolt *et al.*, 1977). In normal rats, SSR149415 (10 and 30 mg/kg p.o.) significantly decreased immobility time in a dose-dependent manner, suggesting an antidepressant-like activity (Fig. 5, panel B). This effect was comparable to that of imipramine, fluoxetine and venlafaxine (data not shown), all clinically-effective antidepressant molecules. We also showed that the antidepressant-like properties of SSR149415 were still present, albeit at higher doses, in hypophysectomized rats. These data suggest that this action does not necessarily involve HPA blockade and that extrahypothalamic V1b receptors could be involved in the antidepressant-like effects of SSR149415. The antidepressant potential of SSR149415 was confirmed further in a chronic model of depression, namely the chronic mild stress (CMS) test in mice (Willner *et al.*, 1992). Repeated administration of SSR149415 (10 and 30 mg/kg i.p.) for 39 days in the CMS showed that the drug was well tolerated and significantly reduced the degradation of the physical state, anxiety, despair and the loss of coping behavior produced by stress. It is noteworthy that at the end of the 7-week stress period, mice treated with SSR149415 displayed a physical state comparable with non-stressed controls (Griebel *et al.*, 2002).

It is important to underline that SSR149415 was devoid of central effects unrelated to emotionality. When administered up to 100 mg/kg, the drug was well tolerated, and SSR149415 did not significantly modify performance of mice in the rotarod and traction tests. Neither did the drug modify sleep patterns following EEG analysis nor impair learning in the Morris water maze up to 30 mg/kg in mice or rats (Griebel *et al.*, 2002).

Clearly, these findings and the selective V1b profile of SSR149415 support direct V1b-R-mediated effects in the various stress models studied. Thus, SSR149415 controls AVP-stimulated ACTH secretion *in vivo* and exhibits a potent anxiolytic and antidepressant-like profile in rodents. Clinical studies will hopefully confirm its therapeutical potential for the treatment of affective disorders.

CONCLUSIONS

Molecular cloning of V1b-R and stable expression of this cloned isoform in mammalian cells has provided key information concerning: expression of its mRNA in rat and human tissues, its localization in the rat central nervous system and its pharmacological properties (Lolait *et al.*, 1995; Thibonnier *et al.*, 1997; Vaccari *et al.*, 1998). However, the precise functional roles of the V1b-R are still obscure due to the lack of selective V1b-R ligands and orally-active molecules. We reported here the biological and pharmacological properties of the first two specific V1b-R ligands: d[Cha^4] AVP, a high affinity V1b-R agonist and SSR149415, a potent orally-active V1b-R antagonist with good selectivity with respect to other VP receptor isoforms and the OT receptor. Indeed, these molecules provide invaluable tools for exploring the role of VP on central and peripheral V1b receptors and pathological disorders associated with this receptor.

This study also reinforces the strong pharmacological differences previously observed concerning VP receptors from various species (Barberis *et al.*, 1999; Ben Mimoun *et al.*, 2001; Andres *et al.*, 2002). Thus dCha^{4}AVP is a specific human and bovine V1b agonist and a mixed V1b/V2 agonist in the rat. Yet by combining a nanomolar concentration of dCha^{4}AVP with 100 nM of SR 121463, a specific non peptidic V2 antagonist (Serradeil-Le Gal *et al.*, 1996) it is thus possible to characterize unambiguously the V1b receptor subtype in rat tissues. Using d[Cha^4] AVP and SSR149415, we demonstrated that, by specific interaction with V1b-R in the anterior pituitary, AVP is an important physiological regulator of the HPA axis and thus controls ACTH release, which regulates the adaptative responses to stress in mammals. Besides the antagonistic properties of SSR149415 on stimulated ACTH secretion *in vivo*, we also showed that SSR149415 exhibits potent anxiolytic and antidepressant-like activities, indicating that this new class of drugs has a promising therapeutical potential in the treatment of stress-related disorders, such as anxiety and depression. In addition, recent gene expression studies (tissue localization of the V1b-R protein and its mRNA) and pharmacological experiments using non specific V1b-analogues suggest that other organs like the brain, pancreas, adrenals and kidneys may be targets for V1b-Receptor selective ligands (Lee *et al.*, 1995; Grazzini *et al.*, 1996; Hernando *et al.*, 2001; Saito *et al.*, 2002). Thus, there may be other potential uses of this new type of drug.

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