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An Overview of SSR149415, a Selective Nonpeptide Vasopressin V_{1b} Receptor Antagonist for the Treatment of Stress-Related Disorders

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ABSTRACT

Vasopressin (AVP) and corticotropin-releasing factor (CRF) are key mediators in the organism's neuro-adaptive response to stress. Through pituitary and central vasopressin V_{1b} receptors, AVP participates in the control of the hypothalamic-pituitary-adrenal axis (HPA) and is involved in various emotional processes. SSR149415 is the first selective, orally active vasopressin V_{1b} receptor antagonist yet described. It is a competitive antagonist with nanomolar affinity for animal and human V_{1b} receptors and displays a highly selective profile with regard to a large number of receptors or enzymes. *In vitro*, SSR149415 potently antagonizes functional cellular events associated with V_{1b} receptor activation by AVP, such as intracellular Ca^{2+} increase or proliferation in various cell systems. Pharmacological studies, performed by measuring ACTH secretion induced by various stimulants such as hormones (AVP or AVP + CRF) or physical stress (restraint or forced swimming stress and dehydration) in conscious rats or mice, confirm the antagonist profile of SSR149415 and its efficacy in normalizing ACTH secretion *in vivo*. SSR149415

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is active by the oral route, at doses from 3 mg/kg, it potentiates CRF effect and displays a long-lasting oral effect in the different models. At 10 mg/kg p.o. its duration of action is longer than 4 h. This molecule also decreases anxiety and exerts marked antidepressant-like activity in several predictive animal models. The anxiolytic effects of SSR149415 have been demonstrated in various Generalized Anxiety Disorders (GAD) models (four-plate, punished drinking, elevated plus-maze, light dark, mouse defense test battery, fear-potentiated startle and social interaction tests). It is as effective as the benzodiazepine diazepam in the acute stress exposure test. SSR149415 has similar efficacy to the reference antidepressant drug, fluoxetine, in acute (forced-swimming) and chronic (chronic mild stress and subordination stress) situations in rodents. SSR149415 also reduces offensive aggression in the resident-intruder model in mice and hamsters. Depending on the model, the minimal effective doses are in the range of 1–10 mg/kg i.p. or 3–10 mg/kg p.o. SSR149415 is devoid of adverse effects on motor activity, sedation, memory or cognitive functions and produces no tachyphylaxis when administered repeatedly. It is well-tolerated in animals and humans and exhibits an adequate ADME profile. Thus, SSR149415 is a new dual anxiolytic/antidepressant compound, which appears to be free of the known side effects of classical anxiolytic/antidepressant drugs. Clinical trials are in progress, they will hopefully demonstrate its therapeutical potential for treating stress-related disorders.

INTRODUCTION

Vasopressin (AVP) exerts a variety of biological effects in mammals. This hormone is actively involved in the regulation of water and solute excretion by the kidney and in a number of other physiological functions including blood pressure control, platelet aggregation, clotting factor release, liver metabolism, uterus contraction and cell proliferation. By acting centrally, AVP regulates interneuron communication, feeding, thermoregulation and numerous behavioral functions such as memory, social and sexual processes (3,9,24). AVP also participates in the control of the HPA axis by stimulating ACTH secretion at the pituitary level and steroid secretion such as aldosterone and corticosterone at the adrenal level. So far, three AVP receptors [vascular — V_{1a}, pituitary — V_{1b} (or V_3) and renal — V_2] have been cloned. They can be clearly identified by their primary structure, gene localization, mRNA distribution, pharmacology and functions (37). Through pituitary and central V_{1b} receptors, found in limbic structures such as the lateral septum and the ventral hippocampus, AVP participates in the control of the hypothalamic-pituitary-adrenal axis (HPA). It may be involved in various emotional processes such as stress-related disorders, anxiety and depression (8,10). With regard to pituitary V_{1b} receptors, AVP exerts a dual effect. It acts as a direct ACTH secretagogue and also potentiates the stimulatory effect of corticotropin-releasing factor (CRF) in animals and humans (16). Regulation of ACTH secretion is a critical component in the adaptive organism response to stress and AVP, together with CRF — it plays a major role during the adaptation to stress. The expression of AVP in the parvocellular neurons of the paraventricular nucleus and its secretion into the portal circulation increases under chronic stress conditions (1). In depressed patients, the number of hypothalamic neurons co-expressing AVP and CRF is substantially increased. These patients exhibit a high level of circulating AVP and hyperactivity of the corticotrope

axis (11,27). Very recent data from Wersinger et al. on the first V_{1b}-R knockout mice demonstrated the important role of the V_{1b} receptor in aggression and to a lesser extent in social recognition (39). Even if binding experiments have failed to identify V_{1b} receptors in the brain, *in situ* hybridization and immunohistochemistry studies in the rat show a wide distribution of central V_{1b} receptors in target areas such as the prefrontal cortex, hippocampus, hypothalamus, amygdala and lateral septum, supporting a functional role of AVP and the V_{1b} receptors in emotional situations (19,25,38).

SSR149415 is the first selective, orally active, non-peptide antagonist of the V_{1b} receptor described (33). It behaves as a potent, specific antagonist *in vitro* and *in vivo*. It inhibitis various AVP V_{1b} receptor-mediated pharmacological effects and controls ACTH secretion. Interestingly, SSR149415 exhibits a mixed anxiolytic/antidepressant profile in various relevant animal models without the known side effects of classical anxiolytic/antidepressant drugs (17). This chapter provides an up-to-date overview of the *in vitro* and *in vivo* properties of SSR149415 in several experimental models. The protocols used have been widely described in previous publications. For more details on the experimental conditions the readers are referred to other publications (17,18,33). All protocols performed have been approved by the Animal Care and Use Committee of Sanofi-Synthélabo Recherche.

CHEMISTRY

Structure and Chemical Properties

SSR149415 (Fig. 1) ((2S,4R)-1-[5-chloro-1-[(2,4-dimethoxyphenyl)sulfonyl]-3-(2-methoxyphenyl)-2-oxo-2,3-dihydro-1H-indol-3-yl]-4-hydroxy-N,N-dimethyl-2-pyrrolidine carboxamide, (–) isomer) originated from a new chemical series of potent and selective, non-peptide V_{1b} receptor antagonists (29). This molecule (3 asymmetric carbon atoms) belongs to the [1H]2,3-dihydroindol-2 family and results from chemical optimization in the field of the indoline/oxoindole chemical series which has previously yielded orally-active molecules highly selective for either the V_{1a} (SR49059) or for the V_2 (SR121463B) receptors (31,32). The compound is a chemically stable, white powder with a molecular weight of 630.1 (molecular formula: $C_{30}H_{32}ClN_3O_8S$). The recrystallized compound in an ethanol/H_2O mixture (6/4; v/v) forms a nonstoechiometric hydrate (melting point: 125–131°C). It is sparingly soluble in water at natural pH, slightly soluble in ethanol and soluble in methanol, ethylene glycol and dimethyl sulfoxide.

Recently based upon the crystallized structure of the bovine rhodopsine, a three-dimensional structural model of interaction between the V_{1b} receptor and SSR149415 has been developed. This proposed model was validated in binding and functional studies by combining three-dimensional modelling and site-directed mutagenesis approaches using SSR149415. This work emphasizes the key role of at least two amino acids, threonine 203 and methionine 324, located in different transmembrane helices (5^{th} and 7^{th}, respectively), responsible for the V_{1b} selectivity of SSR149415 compared to other AVP receptor subtypes (12). Obviously, such a model needs to be further validated and could serve as an interesting starting point for virtual screening.

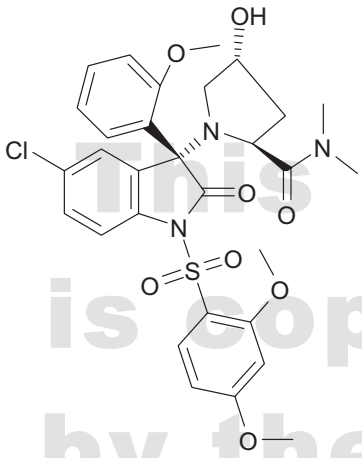

Fig. 1. Chemical structure of SSR149415, a selective V_{1b} receptor antagonist, (–) isomer of (2S,4R)-1-[5-chloro-1-[(2,4-dimethoxyphenyl)sulfonyl]-3-(2-methoxyphenyl)-2-oxo-2,3-dihydro-1H-indol-3-yl]-4-hydroxy-N,N-dimethyl-2-pyrrolidine carboxamide.

IN VITRO PHARMACOLOGY

Binding Studies and Selectivity Profile

SSR149415 exhibits an affinity for animal (mice, rat, hamster, and bovine) and human pituitary V_{1b} receptors in the nanomolar range, similar to that of the natural hormone, AVP, in these systems. As shown in Fig. 2A, SSR149415 interacts with human recombinant V_{1b} receptors in a competitive manner. The compound displays high affinities for both native and recombinant (CHO cells) human and rat V_{1b} receptors (human: K_i = 4.2 and 1.5 nM, respectively; rat: K_i = 3.7 and 1.3 nM respectively), 60- and 800-fold selectivity for human and rat V_{1b} as compared to V_{1a} receptors and displays weak affinity for V_2 and oxytocin (OT) receptors (Table 1). Moreover, SSR149415 (10 µM) has no measurable affinity for a variety (n = 100 assays) of receptors for neurotransmitters, neuropeptides and hormones, ion channels or enzymes.

Interestingly, SSR149415 has been recently tritiated and used in autoradiography experiments for labelling native V_{1b} receptors in human pituitary sections (Fig. 2B). An in-

TABLE 1. Affinity and selectivity profile of SSR149415 for rat and human vasopressin and oxytocin receptors

	K_i (nM)				
Species	V_{1b}		V_{1a}	V_2	OT
Humans	hypophysis	CHO cells	CHO cells	CHO cells	Ltk cells
	4.2 ± 1.1	1.5 ± 0.8	91 ± 23	1412 ± 314	174 ± 35
Rats	hypophysis	CHO cells	liver	kidney	mammary glands
	3.7 ± 1.3	1.3 ± 0.9	1050 ± 112	2897 ± 509	270 ± 39

Binding assays were performed using either CHO/Ltk cells transfected with the corresponding AVP/OT receptor, or native tissues constitutively expressing this receptor. Inhibition constants (K_i) were determined from competition experiments and calculated according to the Cheng and Prussoff equation. Values are the mean ± S.E.M. of at least 3 determinations.

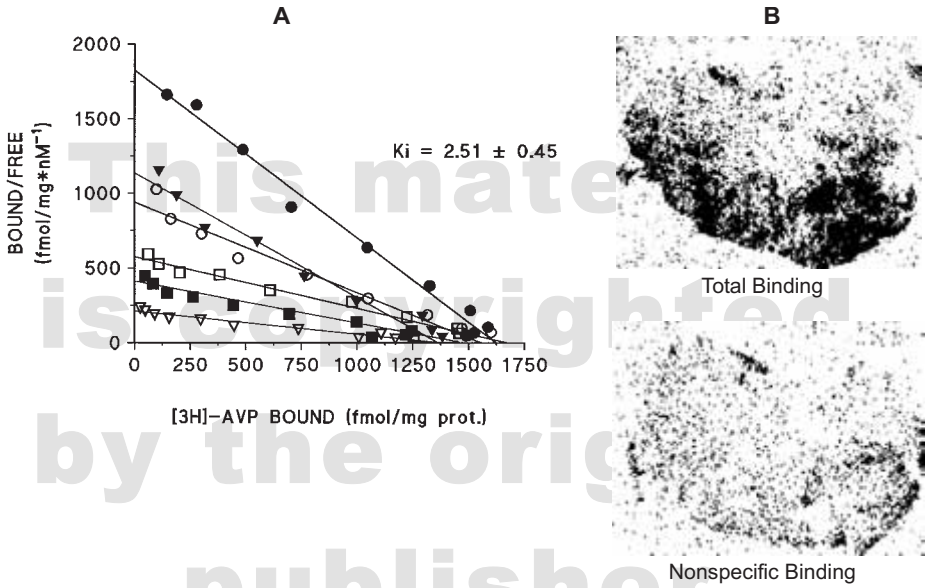

Fig. 2. Binding studies to human recombinant and native V_{1b} receptors using SSR149415 and [^{3}H]SSR149415. (**A**) Scatchard plots of [^{3}H]AVP binding to CHO cells transfected with the human V_{1b} receptors. Studies were conducted without (●) or with 0.9 (▼), 1.8 (○), 3.7 (□), 7.5 (■), and 15 (▽) nM SSR149415. Binding assays were performed for 45 min at 20°C in the presence of 30 µg/assay of CHO membranes as previously described (33). (**B**) Autoradiography using [^{3}H]SSR149415 in human pituitary sections. Autoradiograms were obtained by incubating adjacent sections with 1.5 nM [^{3}H]SSR149415 in the presence (non specific binding) or absence (total binding) of cold SSR149415 (1 µM).

tense labelling signal, totally displaced by cold SSR149415 or AVP (10 µM), is observed in corticotrophic cells showing the suitability of this molecule as a selective probe for the mapping and characterization of V_{1b} receptors. Binding studies with this antagonist as a radioligand are in progress, they will explore the central and peripheral localization of V_{1b} receptors. Up to now preliminary autoradiographic results using [^{3}H]SSR149415 on rat brain sections failed to produce evidence for clearly specific labelling. The main difficulties come from sticky properties of the radioligand, limited specific activity due to tritium in general and probably low level of the V_{1b} receptors in the brain. Some expected labelled areas are still under investigation, but a radioiodinated ligand would be more appropriate for establishing the cartography of the V_{1b} receptors in the brain.

Functional *in vitro* Studies

Earlier cellular events, provoked by the occupancy of V_{1b} receptors by AVP, include the activation of phospholipase C, protein kinase C and the mobilization of intracellular free Ca^{2+} mainly via $G_{q/11}$ G-protein recruitment (6,35). SSR149415 is a potent antagonist at the V_{1b} receptor as shown by its ability to inhibit vasopressin-induced Ca^{2+} increase in Chinese hamster ovary (CHO) cells expressing the human or rat V_{1b} receptor (K_i = 1.26 and 0.73 nM, respectively) and in In-R1-G9 cells, a hamster glucagon-secreting cell line

(K_i = 0.66 nM). SSR149415 is devoid of any agonist effect up to 10 μM when tested alone in these preparations (15,33).

Beside the phosphatidylinositol/Ca^{2+} signalling pathway, other intracellular events have also been associated with V_{1b} receptor activation (e.g., cAMP production, stimulation of DNA synthesis and cell proliferation), clearly depending on the level of V_{1b} receptor expression (37). The presence of V_{1b} receptors was reported in various tumors such as small cell lung cancer cells, and the V_{1b} receptor gene is overexpressed in corticotropin-secreting tumors (7,26). In CHO cells expressing the human or the rat V_{1b} receptors and in pancreatic In-R1-G9 cells, SSR149415 dose-dependently antagonizes the stimulation of cell proliferation by AVP (3 nM) with Ki values of 0.43, 0.41, and 0.71 nM, respectively, consistent with the V_{1b} receptor binding affinity of SSR149415 in these cells. Additionally, SSR149415 potently inhibits AVP-induced glucagon release in In-R1-G9 α-pancreatic cells (K_i = 1.2 nM) (15). Obviously, the relevance of this latter property of AVP at V_{1b} receptors needs to be further explored in *in vivo* pharmacological models using normal and diabetic animals. All these data point to the potent and full V_{1b} receptor antagonist properties of SSR149415 in various functional cellular models *in vitro*.

IN VIVO PHARMACOLOGY

Antagonist Properties of SSR149415 on ACTH Secretion *in vivo*

AVP, via pituitary V_{1b} receptor activation, exerts a dual effect as a direct ACTH secretagogue and also potentiates the stimulatory effect of CRF in many species including humans (13,16). Regulation of ACTH secretion is a critical component in the adaptive organism response to stress and elevated activity of the HPA system has been observed in depression and anxiety (22). The *in vivo* activity of SSR149415 has been studied in conscious mice and rats by measuring ACTH secretion induced by various stimulants such as hormones (AVP, AVP+CRF) and physical stress, such as restraint/swimming-stress or dehydration (33). In all these situations, SSR149415 at doses of 3 or 10 mg/kg p.o. or 10 mg/kg i.p. (depending on the model) exhibits an antagonist profile. As shown in Fig. 3A, in conscious rats SSR149415 has a long-lasting effect (longer than 4 h at 10 mg/kg p.o.) on potentiation of CRF by AVP, a typical V_{1b}-mediated effect. Conversely, the selective, orally active V_{1a} receptor antagonist, SR49059, was unable to inhibit AVP + CRF-induced ACTH secretion (Fig. 3B).

Similarly in mice, SSR149415, 30 mg/kg p.o., at 2 h after treatment almost totally suppressed the increase in ACTH after a 5 min forced-swimming stress (Fig. 4A) and significantly ($p < 0.05$) decreased the dramatic elevation in corticosterone (Fig. 4B). In other models, SSR149415 selectively antagonized stress-induced ACTH secretion without affecting basal ACTH levels (Fig. 4B). Importantly, SR149415, at doses up to 30 mg/kg p.o., is devoid of any significant effect on ACTH and corticosterone secretion following CRF stimulation in conscious rats showing normal response of the HPA axis to stressful stimulus under specific blockade of the V_{1b} receptors (not shown). These results demonstrate that SSR149415 is an orally-active V_{1b} receptor antagonist in rodents since it controls or normalizes elevated ACTH secretion induced by exogenous (AVP or AVP + CRF) or endogenous (AVP) hormonal stimulations as well as by stressful stimuli.

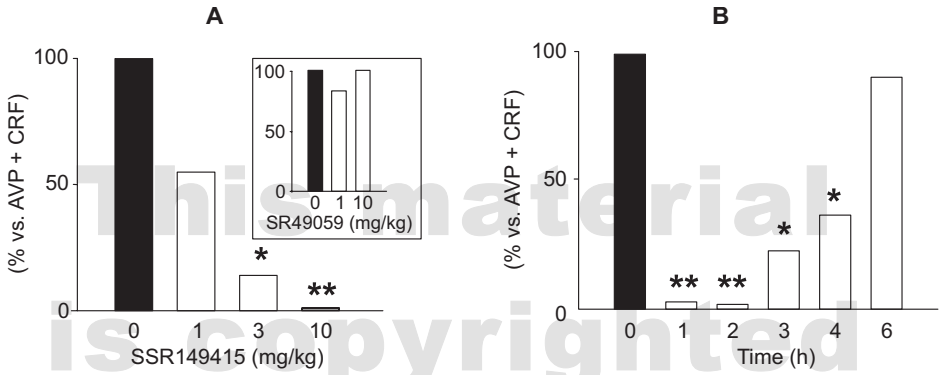

Fig. 3. Effect of oral SSR149415 on the potentiation of exogenous CRF by AVP on ACTH secretion in conscious rats. Dose-effect (A) and time-course (B) studies. (**A**) SSR149415 (1 to 10 mg/kg) was administered by gavage 2 h prior to CRF (0.1 μg/kg i.v.) and AVP (0.03 μg/kg i.v.) injections. **Inset:** Effect of a selective orally active V_{1a} receptor antagonist, SR49059 (1 to 10 mg/kg) in this model. (**B**) The time-course effect of SSR149415, 10 mg/kg p.o., on plasma ACTH secretion (n = 5 to 10). Values are expressed as the percentage of ACTH secretion vs. the CRF + AVP control. Statistical analysis was performed using a one-way ANOVA followed by a Dunnett's test or using the nonparametric Kruskal–Wallis test. The level of significance was taken as $P < 0.05$ for comparison with the control (*$P < 0.05$).

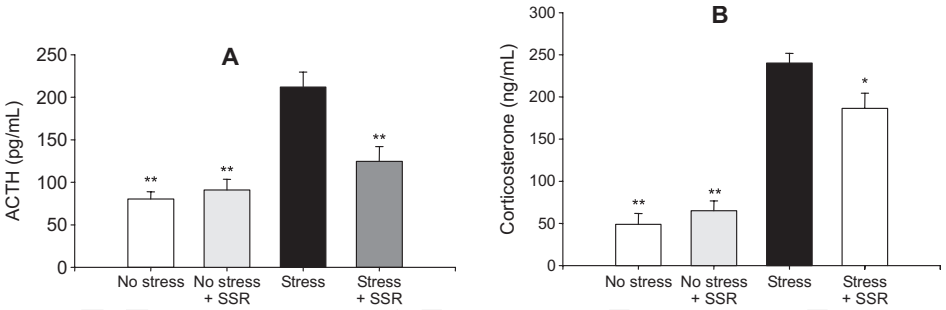

Fig. 4. Effect of SSR149415 p.o. on plasma ACTH (A) and corticosterone (B) concentrations after a 5-min swimming stress test in conscious mice. Mice (10 per group) received by oral route either vehicle (2 mL/kg 5% DMSO, 5% cremophor, 90% saline) or SSR149415 (30 mg/kg). At 2 h after treatment, mice were placed for 5 min into individual glass cylinders containing water. At the end of the immobilization period, they were immediately sacrificed by decapitation. Plasma ACTH (A) and corticosterone (B) levels were measured by RIA. Data are means ± S.E.M. Statistical significance was assessed by an Anova on transformed logarithmic data followed by Dunett's test (**$p < 0.01$ vs. the stress control group).

Anxiolytic-Like Profile of SSR149415

The behavioral effects of SSR149415 were investigated in various traditional tests for anxiolytics involving models of Generalized Anxiety Disorder (GAD), panic attack and acute stress disorders (Fig. 5 and Table 2). In conflict (punished drinking and four-plate) or exploration (elevated plus-maze and light/dark) models of GAD, in the fear-potentiated startle in rats and in social interaction test in gerbils, SSR149415 is active at doses ranging from 1 to 30 mg/kg (i.p. or p.o.), but the magnitude of anxiolytic-like action of

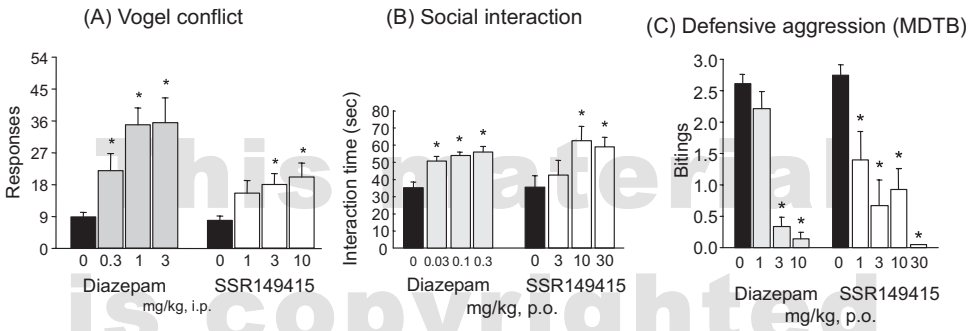

Fig. 5. Comparison of the effects of SSR149415 and diazepam in models of Generalized Anxiety Disorders (GAD): (A) the Vogel conflict test in rats; (B) social interaction test in gerbils; and (C) the mouse defense test battery (MDTB). Data represent means ± S.E.M.; *$P < 0.05$ (Kruskal–Wallis or Dunnett); $N = 13–20$.

SSR149415 is generally less than that of diazepam, used as a positive control in these experiments (Fig. 5A). Of note, flumazenil, a benzodiazepine receptor antagonist, does not block the anxiolytic-like activity of SSR149415, indicating that these effects are not mediated by $GABA_A$/benzodiazepine receptors (17). SSR149415 exhibits also some efficacy in the treatment of panic attacks since flight responses were weakly affected by SSR149415, at doses up to 30 mg/kg p.o., in the mouse defense test battery (MDTB).

TABLE 2. Efficacy of the V_{1b} receptor antagonist, SR149415, in acute and chronic stress or emotional situations in rodents. Comparison with diazepam and fluoxetine

Tests	SSR149415	Diazepam	Fluoxetine
Drinking conflict test in rats	(3)	(1)	(>20)
Elevated plus-maze in rats	10	3	(>10)
Fear-potentiated startle in rats	3	1	NA
Light/dark test in mice	(1)	(1)	(>20)
Four-plate test in mice	3	1	NA
Social interaction in gerbils	10	(0.1)	10
Mouse defense test battery	1	1	(5)[a]
Social defeat stress in mice	3	4	(20)
Conditioned fear stress in mice	10	(2)	NA
Distress vocalizations in rat pups	§10[a]	§1[a]	§3[a]
Distress vocalizations in guinea pig pups	(20)	NA	(3)
Forced-swimming in rats	10	NA	10
Chronic mild stress in mice	(10)[a]	NA	(10)[a]
Chronic subordination stress in rats	10[a]	NA	10[a]
Isolation-induced aggression in mice	1	NA	NA
Restraint-induced physiological changes in rats	30	(2)	NA
Restraint stress-induced ACTH release in rats	10	NA	NA
Tail pinch stress-induced NE release in rats	(10)	NA	NA

Data are expressed as MED by either oral, i.p. (), or s.c. § administration.
MED, minimal effective dose; NA, not available; [a]repeated-dose.

SSR149415 had marked anxiolytic-like activity in models involving acute and traumatic stress exposure, such as the social defeat paradigm, conditioned fear in mice, the defensive aggression in the MDTB (at 1 to 30 mg/kg, p.o.) (Fig. 5C) and maternal separation-induced vocalization in rat and guinea pig pups (at 10 and 20 mg/kg, i.p., respectively). Acute stress exposure has been associated with neurochemical modifications in specific brain regions. During stressful conditions, an increase in firing of the locus coeruleus neurons induces enhanced release of norepinephrine in several brain areas with noradrenergic innervation. The V_{1b} receptor antagonist, SSR149415, at 10 mg/kg, i.p., significantly antagonized the tail pinch stress-induced release of norepinephrine in the prefrontal cortex, an effect which could be prevented by other anti-stress compounds, such as CRF1 receptor antagonists (18).

Antidepressant Effects of SSR149415

SSR149415 exhibits marked antidepressant-like properties similar to those obtained with classical antidepressant drugs such as fluoxetine or imipramine in acute (forced-swimming) and chronic (chronic mild stress in mice and chronic subordination stress in rats) rodent models (Fig. 6, Table 2). In the forced swimming test, SSR149415, at 10 to 30 mg/kg, p.o., produces antidepressant-like effects (Fig. 6A). These effects can still be observed at higher doses of SSR149415 in hypophysectomized rats (17). These findings suggest that, in addition to pituitary-adrenal axis blockade, a central component is involved in the mechanism of action of this compound. Experiments with local injections in specific brain areas support this hypothesis. SSR149415 infusions into the lateral septum or the central nucleus of the amygdala (0.1 to 100 ng i.c.v.) induce dose-dependent antidepressant-like effects in the forced swimming test in rats whereas no effects are observed when the drug is administered to brain areas not related to emotionality (34). Indeed, these brain structures exhibit a high level of V_{1b} receptor protein or mRNA as demonstrated by immunohistochemistry or *in situ* hybridization (19,25,37). In the chronic mild stress model, repeated administration of SSR149415, 10 and 30 mg/kg, i.p., for 39 days, reduced degradation of the physical state, anxiety, despair and the loss of coping behavior produced by repetitive unpredictable stress applied 3 weeks before and maintained during SSR149415 treatment (Fig. 6B). Interestingly, a link between hippocampal neurogenesis and depression has been reported in animals and humans. The prefrontal cortex and hippocampus of both depressed and anxious patients revealed an atrophy or loss of neurons. Similarly, in the chronic mild stress model, depression is also associated with a dramatic decrease in hippocampal neurogenesis, a phenomenon reversed by chronic antidepressants, such as fluoxetine or imipramine (14,30). In this latter model, SSR149415, 30 mg/kg i.p., restored impaired neurogenesis in the dentate gyrus of the hippocampus as evidenced by an increased number of progenitor cells that incorporate the DNA synthesis marker, 5-bromo-2′-deoxyuridine (BrdU) and differentiate into mature neurons, as does the reference antidepressant drug, fluoxetine (10 mg/kg i.p.) tested under similar operating conditions (Fig. 6B) (2). Very recently, using x-ray irradiation specific to the hippocampus area, it has been shown that in the chronic mild stress (CMS) model neurogenesis is necessary for the demonstration of the antidepressant-like effect of fluoxetine while it is only partially required for demonstration of the same effect of SSR149415. This finding suggested that the antidepressant-like mechanisms of action of SSR149415 and fluoxetine are different and that this neurological process may be involved in the curative treatment

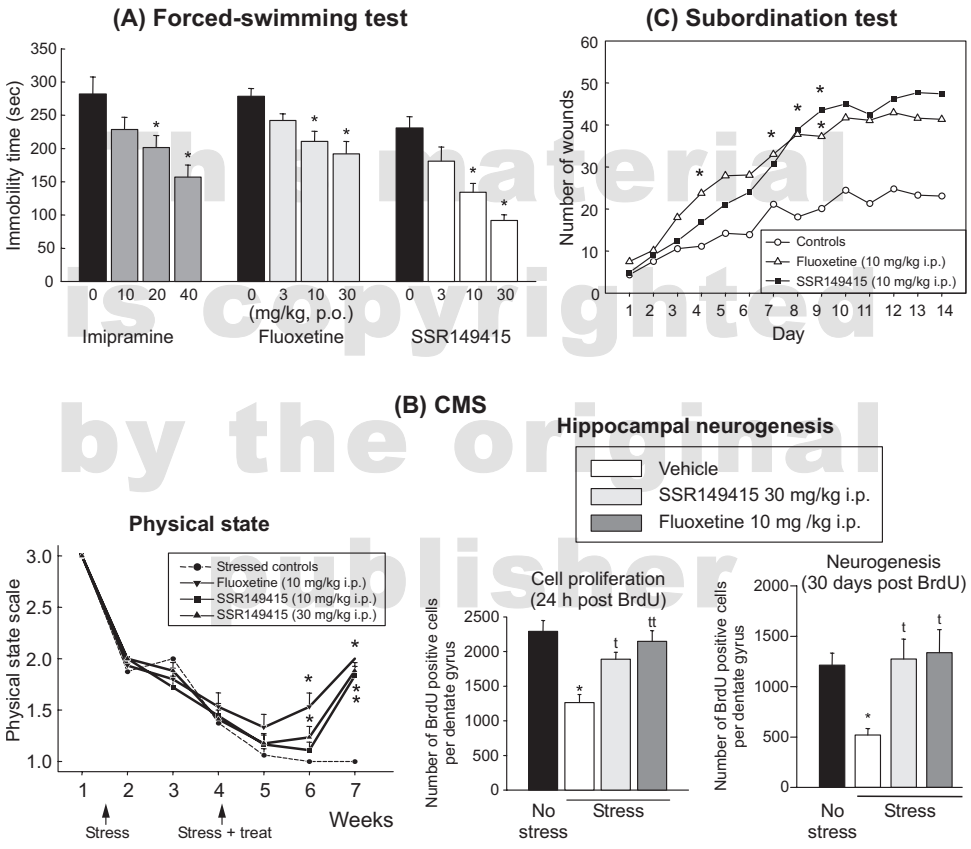

Fig. 6. Antidepressant-like effects of SSR149415 in comparison with the reference antidepressant drug, fluoxetine, in: (A) the forced swimming test in rats; (B) the chronic mild stress in mice; and (C) the subordination stress in rats after repeated administration. (**A**) The drugs were administered p.o. twice (15 min after the first swimming session on day 1 and 60 min before swimming session 2 on the second day); the duration of immobility was measured for a 6-min period. Data represent means ± S.E.M.; $*P < 0.05$ (Dunnett); $N = 7$ to 13. (**B**) Effect of repeated administration of SSR149415 in the CMS. The CMS consists of the sequential application of a variety of mild stressors in a schedule that lasts for 3 weeks and continued for 4 week treatment with vehicle or drug (SSR149415, 10 and 30 mg/kg i.p. or fluoxetine, 10 mg/kg i.p.); The physical state of the coat was an indicator of the depressive state of the stressed mice. Hippocampal neurogenesis was used as another indicator, it was evaluated at the end of antidepressant treatment. Cell proliferation (24 h post BrdU) and mature neurons (30 days post BrdU) were measured in the granular cell layer of the dentate gyrus. Data represent means ± S.E.M.; $*P < 0.05$ (Dunnett); $N = 15$–18. (**C**) Effect of repeated administration of SSR149415 in the subordination test in rats. Treatment with drugs or vehicle lasted for 2 weeks (SSR149415 30 mg/kg i.p. or fluoxetine, 10 mg/kg i.p.). The increased number of wounds observed in the drug groups reflects a higher number of attacks of the subordinate against the dominant rat. Data represent mean ± S.E.M.; $*P < 0.05$ (Dunnett); $N = 15$ to 18.

of depression (36). In the chronic subordination stress model, SSR149415, administered repeatedly to subordinate male rats, reduced defensiveness and behavioral inhibition such as mounting of female in the presence of dominant and normalized HPA axis parameters (i.e. ACTH secretion) (Fig. 6C). In all these models, the antidepressant-like profile of SSR149415 is comparable to that of the classical antidepressant, fluoxetine (10 mg/kg, i.p.), confirming the potential of V_{1b} receptor antagonists in depressive states (4,18).

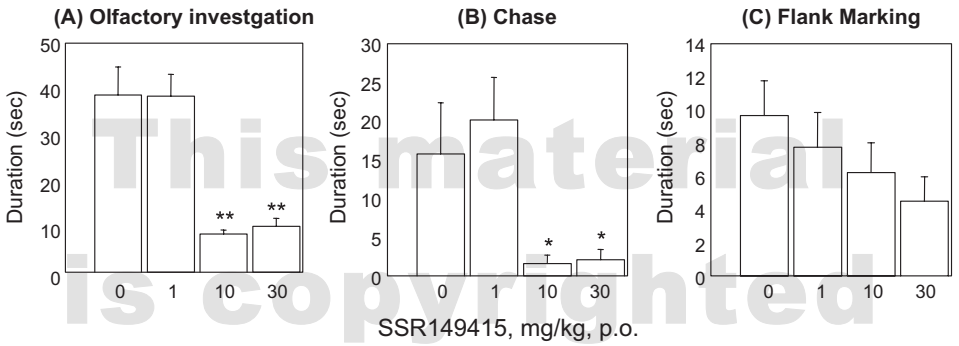

Fig. 7. Effect of SSR149415 on offensive aggression in the resident-intruder model in hamsters. (**A**) Olfactory investigation; (**B**) Chase; and (**C**) Flank marking behavior were recorded between isolated and intruder hamsters. Data represent means ± S.E.M.; **P* < 0.05 (Dunnett); *N* = 10 to 13.

SSR149415 Reduces Offensive Aggression

In the resident-intruder aggression model in mice, SSR149415 significantly reduces the duration of fighting between isolated and intruder mice. Similarly in hamsters, this compound reduces chase, lateral attack behavior and flank marking, major components of dominance behavior (Fig. 7) (5). These results show that SSR149415 reduces offensive behavior and are in accordance with findings from Wersinger et al. showing that V_{1b}-R knockout mice demonstrate less aggressivity (39).

SAFETY PHARMACOLOGY

The highly selective V_{1b} profile of SSR149415 is an important characteristic of this molecule. First, *in vitro*, this compound has low affinity for the 3 other AVP/OT related rat and human receptors (Table 1). Second, at the highest doses used to block the V_{1b} receptors *in vivo* (10 mg/kg i.p. and 30 mg/kg p.o.), SSR149415 did not modify the V_{1a}-vascular response to AVP in conscious rats; it also did not affect urine flow rate controlled by renal V_2 receptors, in either normally hydrated or vasopressin-deficient Brattleboro rats. Third, SSR149415, at 10 µM did not interact with a large number of receptors, ion channels or enzymes. Finally, SSR149415, at 1 µM, had only antagonist properties in functional assays *in vitro* (Ca^{2+} or cAMP) related to V_{1a}, OT and V_2 receptor activation. No V_{1b}, V_{1a}, V_2 or OT receptor agonist properties of SSR149415 have been detected even at high concentrations of this drug (33). Because of the highly selective pattern of activity, this drug can be expected to exhibit a particularly safe profile. Indeed, *in vivo* pharmacological studies with SSR149415, by acute or repeated administration to mice and rats, have confirmed the good tolerability of this drug. It should be noted that anxiolytic/antidepressant-like properties of the drug were still apparent during repeated administration, indicating the absence of tachyphylaxis with SSR149415.

When administered at doses up to 100 mg/kg the drug is well tolerated. Unlike classical anxiolytics (e.g., diazepam) SSR149415 does not significantly modify the performance of mice in the rotarod and traction tests. Unlike reference antidepressant drugs

TABLE 3. Summary of the side effect profile of the V_{1b} receptor antagonist, SSR149415, in animal models. Comparison with diazepam

	MED or $^{a}ED_{50}$, mg/kg, p.o. or (i.p.)	
Tests	SSR149415	Diazepam
EEG in rats	>30	(1)
Locomotor activity	>100	10
Traction test in mice	>100	6[a]
Rotarod in mice	>100	9[a]
Morris water maze in mice	>30	1
Morris water maze in rats	>30	10

MED, minimal effective dose; EEG, Electroencephalography; $^{a}ED_{50}$, Effective dose yielding 50% of the maximal effect.

SSR149415, at doses up to 30 mg/kg, does not modify sleep patterns or impair learning of mice or rats in the Morris water maze. The lack of activity in these models indicates that SSR149415 is devoid of central effects not related to emotionality (Table 3). In rats and dogs the compound was well absorbed and its plasma concentrations increased dose-dependently. Safety pharmacological studies demonstrated good tolerability of SSR149415 in mice and rats. No toxic effects and no deaths were observed after acute, repeated or chronic treatments. Additionally, SSR149415 exhibits an adequate ADME profile.

CLINICAL STUDIES

SSR149415 has been administered to humans. It was well absorbed with excellent tolerability and bioavailability at all doses tested. No serious adverse effects have been recorded.

CONCLUSIONS

The discovery of the first selective and orally active V_{1b} receptor antagonist, SSR149415, offers the opportunity to extensively characterize the AVP/V_{1b} receptor system in various *in vitro* and *in vivo* models. The results demonstrate that, via AVP V_{1b} receptor activation, AVP controls emotional processes or stress-related disorders suggesting that V_{1b} receptor blockade may represent an innovative treatment for some forms of anxiety and depression. Hyperactivity of the HPA axis is commonly observed in clinical depression and anxiety, and it has been shown that antidepressant drugs normalize HPA activity and cortisol levels in depressed patients (22). The AVP/V_{1b} receptor system appears to control stress response by a dual peripheral and central action through pituitary V_{1b} receptors. AVP, produced by hypothalamic neurons, activates ACTH release and consequently cortisol secretion. Moreover, AVP, released in several CNS areas binds to central V_{1b} receptors in limbic structures (i.e. lateral septum, hippocampus or amygdala) and produces stress-adaptive responses. Our findings with SSR149415 in various

animal models support this dual hypothesis. Firstly, SSR149415 decreases elevated ACTH/cortisol secretion induced by various stimulants such as hormones (AVP, AVP + CRF) and physical stress (restraint or forced swimming test and dehydration) without modifying normal HPA axis response to stressful stimuli as observed in a CRF stimulation test in rats (33). Secondly, in the forced swimming test antidepressant-like effects are observed in both normal and hypophysectomized rats (even if less intense) suggesting that not only pituitary-adrenal axis blockade is involved in the mechanism of action of this compound (17). Moreover, SSR149415, by i.c.v. route into the lateral septum or the amygdala in rats, induced marked dose-related antidepressant-like effects showing that central V_{1b}-R participate in these effects. SSR149415 treatment is also associated with neurochemical modifications in specific brain regions, such as the control of norepinephrine release under stressful conditions in the prefrontal cortex or the restoration of hippocampal neurogenesis impaired by chronic stress (18). Very recently, an interesting neurochemical study using microdialysis associated with behavioral investigations provided new insight into the central mechanism of action of SSR149415 supporting both anxiolytic and antidepressant-like properties of the molecule. Peripheral administration of SSR149415 (3 to 30 mg/kg i.p.) produced significant and specific increases in extracellular concentrations of norepinephrine and gamma butyric acid (GABA) in the rat frontal cortex without affecting other neurotransmitter levels such as serotonin, dopamine, and glutamate. The authors reported increases in extracellular norepinephrine similar in magnitude to those observed with reference antidepressant compounds such as venlafaxine or desipramine. Additionally, the anxiolytic-like activity of SS149415 may be mediated by increase in prefrontal GABA levels. Thus, selective inhibition of V_{1b} receptors by SSR149415 is associated with central neurochemical modifications able to explain the dual anxiolytic/antidepressant profile of the molecule (28). The control of corticosteroid receptor expression could be also an important target in the mechanism of action of SSR149415. A disturbance of the corticosteroid feed-back has been associated with the pathology of stress and depression explaining increased levels of CRF and AVP and the elevated activity of the HPA axis (21). It has been shown that AVP and CRF regulate corticosteroid receptor levels in the hippocampus and anterior pituitary. During intermittent restraint stress the expression a non-selective $V_{1b/1a}$ receptor antagonist increased in both tissues (23).

Whether V_{1b} receptor blockade will provide an efficacious alternative to existing drug treatments for depression and anxiety disorders remains a crucial question. Clinical trials will be the next step in the evaluation of SSR149415. As reviewed in this chapter, SSR149415 decreases anxiety in rodents and is as effective as diazepam in acute or traumatic stress exposure. Its profile is similar to that of fluoxetine in all the acute and chronic depression models tested. Indeed, depression and anxiety are heterogenous disorders with various etiologies and their heterogeneity explains the difficulties in treating these diseases with a specific drug. This also explains the large number of treatment-resistant patients. SSR149415 constitutes a new approach for the treatment of anxiety and/or depression through a novel mechanism of action. Presently, it is too early to speculate on the spectrum of activities and efficacy of this molecule in patients with anxiety and depression alone or in addition to standard treatments. A more rapid onset of action is also expected for future classes of antidepressants, but animal studies performed with SSR149415 in appropriate models (i.e., CMS) do not support this expectation. Due to the highly selective

profile of this molecule, targeted only toward V_{1b} receptors, one could expect fewer side effects with SSR149415 than with currently used anxiolytic/antidepressant drugs. *In vivo* pharmacological studies with SSR149415, by acute and repeated adminstration and even at high doses, confirmed the excellent tolerability of this drug, absence of tachyphylaxis and of central effects not related to emotionality. This drug had also no effects on motor activity or sleep pattern. Although SSR149415 had no effect on spatial memory in the Morris water maze test in either mice or rats, it is premature to conclude that the drug has no effect on learning and memory. Additional studies in other memory models are necessary to get a clearer picture of the possible action or lack of effect of SSR149415 on cognition. Also, it remains to be determined whether long-term treatment with SSR149415 may influence memory processes.

In conclusion, SSR149415 is the first selective V_{1b} receptor antagonist described. It represents a unique tool for further exploration of the poorly understood role of pituitary and extrapituitary V_{1b}-R. With the help of this molecule the major role of the AVP/V_{1b} receptor system in controlling affective disorders has been revealed. Clinical evaluation with SSR149415 is eagerly awaited to confirm the therapeutic potential of V_{1b} receptor blockade in the treatment of stress-related disorders. Morerover, other functions for the AVP V_{1b}-R and other therapeutic indications for antagonists should be further explored using selective V_{1b} ligands. Recent data in V_{1b}-R knockout mice suggest a role for V_{1b}-R in schizophrenia (20).

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Abbreviations

ACTH, adrenocorticotropic hormone;
AVP, arginine vasopressin;
CMS, chronic mild stress;
CRF, corticotropin-releasing factor;
EEG, electroencephalography
GAD, Generalized Anxiety Disorder;
MED, minimal effective dose;
MDTB, mouse defense test battery;
OT, oxytocin;
SR49059, ((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);
SR121463B, (1-[4-(N-tert-butylcarbamoyl)-2-methoxybenzene sulfonyl]-5-ethoxy-3-spiro-[4-(2-morpholino-ethoxy)cyclohexane]indol-2-one, fumarate, equatorial isomer);
SSR149415, ((2S,4R)-1-[5-chloro-1-[(2,4-dimethoxyphenyl)sulfonyl]-3-(2-methoxyphenyl)-2-oxo-2,3-dihydro-1H-indol-3-yl]-4-hydroxy-N,N-dimethyl-2-pyrrolidine carboxamide.

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