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Evidence that the Lateral Septum is Involved in the Antidepressant-Like Effects of the Vasopressin V_{1b} Receptor Antagonist, SSR149415

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Previous experiments with the first selective nonpeptide vasopressin V_{1b} receptor antagonist SSR149415 ((2*S*, 4*R*)-1-[5-chloro-1-[(2,4-dimethoxyphenyl)sulfonyl]-3-(2-methoxyphenyl)-2-oxo-2,3-dihydro-1*H*-indol-3-yl]-4-hydroxy-*N*,*N*-dimethyl-2-pyrrolidinecarboxamide) have shown that the drug elicits anxiolytic- and antidepressant-like effects following systemic administration. Extrahypothalamic V_{1b} receptors have been suggested to be involved in these effects as evidenced by the findings that hypophysectomized rats were still sensitive to the antistress action of SSR149415. The first objective of the present work aimed at locating V_{1b} receptors in the rat limbic brain using anti-V_{1b} receptor immunohistochemistry. The immunolabeling revealed high densities of V_{1b} receptors in the lateral septum, the amygdala, the bed nucleus of the stria terminalis, the hippocampal formation, and in several cortical areas. Since the lateral septum is well known to participate in the modulation of emotional processes, the second objective of this study went on to evaluate the behavioral effects of an infusion of SSR149415 into the lateral septum and to determine whether its behavioral effects are mediated by this structure. Animals were tested in models classically used for the screening of anxiolytics (ie the punished drinking and elevated plus-maze tests) and antidepressants (ie the forced-swimming test). Bilateral intraseptal infusion of SSR149415 (10 and 100 ng) produced a decrease in immobility time in the forced-swimming test, indicating antidepressant-like effects. In contrast, the behavior of rats in the punished drinking procedure or in the elevated plus-maze test was not modified by intraseptal infusion of SSR149415. These findings suggest that V_{1b} receptors located in the lateral septum participate in the antidepressant- but not the anxiolytic-like action of SSR149415 in rats.

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INTRODUCTION

Vasopressin is a nonapeptide that is synthesized in hypothalamic nuclei. This peptide is critical for the regulation of the activity of the hypothalamo-pituitary-adrenocortical axis representing a major component of the stress response. Vasopressin promotes pituitary adrenocorticotropin release through its ability to potentiate the stimulatory effects of corticotropin-releasing factor (Aguilera and Rabadan-Diehl, 2000). Among the two $G_{q/11}$-coupled vasopressin receptors (V_{1a} and V_{1b}) found in the brain, the V_{1b} subtype mediates the pituitary actions of vasopressin. Although there is experimental evidence that the expression of V_{1b} receptor mRNA increases after chronic stress exposure (Rabadan-Diehl *et al*, 1997), the role of V_{1b} receptors in the regulation of the stress response is poorly understood. Recently, the first selective nonpeptide antagonist of the V_{1b} receptor, SSR149415 ((2*S*, 4*R*)-1-[5-chloro-1-[(2,4-dimethoxyphenyl)sulfonyl]-3-(2-methoxyphenyl)-2-oxo-2,3-dihydro-1*H*-indol-3-yl]-4-hydroxy-*N*,*N*-dimethyl-2-pyrrolidinecarboxamide), was described (Serradeil-Le Gal *et al*, 2002). Behavioral studies showed that SSR149415 is endowed with anxiolytic- and antidepressant-like properties (Griebel *et al*, 2002, 2003). For example, the drug displayed anxiolytic-like effects in the punished drinking and elevated plus-maze tests in rats. Furthermore, SSR149415 elicited antidepressant-like effects in the forced-swimming test in rats. In this latter test, hypophysectomy did not affect the effect of SSR149415, suggesting that extrahypothalamic V_{1b} receptors may be involved in the antidepressant-like action of SSR149415. Vasopressin-containing neurons have been described in the limbic brain areas such as the medial amygdala and the bed

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nucleus of the stria terminalis (Caffe *et al*, 1987; De Vries and Buijs, 1983), which both project to the lateral septum. This latter structure has been shown to be involved in the expression of anxiety-related behaviors such as those elicited in conflict procedures or exploration tests (Treit and Menard, 2000; Yadin *et al*, 1993). For example, lesion studies have demonstrated that the removal of the entire septum produces antianxiety effects in the elevated plus-maze test (Treit and Pesold, 1990). As a consequence, it has been suggested that the inhibition of the activity of the septum could reduce fear. Microinjection studies have also shown that the infusion of the benzodiazepine midazolam into the lateral septum (but not the medial septum) increased open arm activity in the elevated plus-maze and decreased burying behavior in the shock-probe test (Pesold and Treit, 1994), suggesting that the reduction in septal activity through the facilitation of the inhibitory activity of GABA produced an anxiolytic-like effect. Similarly, the participation of the lateral septum in the modulation of behavior in models sensitive to antidepressants has also been documented (Contreras *et al*, 1995). These authors showed that early lesions of the lateral septum altered both the duration of immobility in the forced-swim test and the response to antidepressants when rats were tested several weeks later. Furthermore, intraseptal infusions of the reference antidepressant imipramine produced antidepressant-like effects in the forced-swimming test in rats (Estrada-Camarena *et al*, 2002), as is the case with 5-HT_{1A} receptor ligands (Schreiber and De Vry, 1993), confirming that the lateral septum mediates the behavioral effects of antidepressants. Concerning vasopressinergic tone, it has also been reported that the release of the peptide in the septum modulates social memory (Dantzer *et al*, 1988), aggressive (Everts *et al*, 1997), and anxiety-related behaviors in rats (Liebsch *et al*, 1996). Furthermore, these authors and others (Landgraf *et al*, 1995) have shown that the intraseptal application of nonselective V_1 receptor antagonists or V_{1a} receptor antisense produces anxiolytic-like effects in the elevated plus-maze in rats. It was also demonstrated that swim stress enhances the septal release of vasopressin (Ebner *et al*, 1999), suggesting a close relationship between vasopressin released in the septum and stress-coping strategies. Using anti-V_{1b} receptor immunohistochemistry, the presence of V_{1b} receptors was demonstrated in various regions of the rat brain (Hernando *et al*, 2001), including the olfactory system, the basal forebrain and basal ganglia, the hippocampal formation, some amygdaloid, thalamic, and hypothalamic nuclei, some cortical areas, and the cerebellum. Although V_{1b} mRNAs were previously located in the rat septal area using RT-PCR (Lolait *et al*, 1995), data concerning the localization of the protein in the lateral septum using selective anti-V_{1b}R antibody are still lacking. In this context, the first objective of the present work was to study the immunohistochemical localization of V_{1b} receptors in the rat brain using a new and specific anti-V_{1b}R antibody. In order to confirm that extrahypothalamic V_{1b} receptors may be involved in the antistress action of SSR149415, the second objective of this study was to examine the behavioral effects of intraseptal infusion of the V_{1b} receptor antagonist using the punished drinking and elevated plus-maze tests, two tests classically used to monitor anxiolytic-like drug effects, and the forced-swimming test, a test generally used for the screening of antidepressants.

MATERIALS AND METHODS

Male Sprague–Dawley rats (200–250 g, Iffa Credo, Les Oncins, France) housed in groups of five upon arrival were used. After surgery, they were housed in single cages ($20 \times 30 \times 18$ cm) and maintained under a 12:12 LD cycle (lights on at 0700). Food and water was available *ad libitum*, except the days preceding the punished drinking test for which a 48-h water deprivation period was required. The behavioral experiments were performed between 0900 and 1500. All experimental procedures described herein were approved by the Animal care and Use Committee of Sanofi-Synthelabo Recherche and fully comply with French legislation on research involving laboratory animals.

Anti V_{1b}-R Immunohistochemistry

Biological material. Five rats were anesthetized with pentobarbital (Ceva Santé Animal, Libourne, France, 60 mg/kg, i.p.), then perfused intracardiacally with heparinized-phosphate-buffered saline (PBS, 0.01 M; pH 7.4), and paraformaldehyde 4% (Sigma, St Louis, MO, USA). Brains were removed from the skull and postfixed overnight in paraformaldehyde 4% at 4°C. The dehydration of the brains was performed with gradual concentrations of alcohol. Brains were subsequently embedded in paraffin-wax (Paraplast Plus, SPI, West Chester, PA, USA) blocks.

Immunohistochemical labeling. Tissue sections (5 μm) were mounted on electrostatically treated slides (SuperFrost Plus, Menzel-Glaser, Freiburg, Germany) and were processed for antigen retrieval, which includes deparaffinization and rehydration. Briefly, the slides were immersed in the Trilogy reagent (diluted 1:20 with distilled water) and heated to 90°C in a PC-controlled microwave oven (MicroMED BASIC, Milestone, Italy) for 20 min and left for the next 10 min in the microwave without heating. Slides were subsequently reloaded into the second container with hot Trilogy and kept in for 10 min at bench. The slides were then washed three times for 5 min with PBS (pH 7.4). For immunohistochemical detection of the V_{1b} receptor, sections were incubated with 3% H_2O_2 diluted in PBS for 15 min. After washing in PBS containing 0.05% Tween 20 to neutralize nonspecific binding sites, the sections were covered with PBS containing 5% normal goat serum (NGS) and 0.3% Triton X-100 for 20 min at room temperature (RT), then drained and incubated for 120 min at RT with rabbit anti-rat V_{1b} receptor antibody diluted (1:250) in PBS containing 1.5% NGS and 0.1% Triton X-100 (antibody diluting buffer). Subsequently, the slides were covered with the PicTure™-Plus KIT HRP (horseradish peroxidase-conjugated polymer coupled to goat anti-rabbit IgG antibodies) for 30 min at RT. Staining was completed by incubation with the peroxidase substrate diaminobenzidine (DAB) (Liquid DAB-Plus Substrate Kit, Zymed Laboratories Inc., San Francisco, CA, USA) for 8 min at RT. Sections were counter-stained with hematoxylin (Zymed Laboratories

Inc., San Francisco, CA, USA), a nuclear marker, then mounted with Entellan (Merck, Darmstadt, Germany) and coverslipped.

Slides were analyzed by transmission microscopy using a Leica microscope (Leitz DMREB, Bannockburn, IL, USA) equipped with a video CCD camera (Sony DCX-930, New York, NY, USA). Sections taken at different levels of the rat brain (according to the rat brain atlas of Paxinos and Watson, 1998) were used to index the immunoreactive signal of $V_{1b}R$; $+++$, $++$, or $+$ was used to denote strong, moderate, or low but positive signals, respectively. Micrographs were made using the MetaMorph 4.6r6 image analysis system (Universal Imaging Corporation™, Downingtown, PA, USA).

Control experiment. The specificity of the polyclonal rabbit anti-rat V_{1b} receptor antibody previously used in the literature (Hurbin *et al*, 2002; Folny *et al*, 2003) was confirmed in the present study: (1) by staining in the absence of the primary antibody, replaced by buffer dilution (negative control), and (2) by preabsorption of the rabbit anti-rat V_{1b} antibody with the rat immunogenic peptide sequence (10, 30, and 100 μg/ml). Rabbit anti-rat V_{1b} receptor antibody diluted 1:250 was incubated for 30 min at 37°C with rat peptide antigens (10, 30, and 100 μg/ml). The primary antibody not mixed with any of the peptides was subjected to the same procedure as the antibody preabsorbed with peptide antigen.

Chemicals and antibody. The Trilogy reagent for tissue deparaffinization and antigen retrieval was from Cell Marque (Hot Springs, AR, USA). The polyclonal anti-V_{1b} receptor antibody and the corresponding blocking peptide were purchased from Alpha Diagnostic (San Antonio, TX, USA). NGS, Triton X-100, Tween 20 and H_2O_2 were from Sigma (St Louis, MO, USA). Antigen–antibody complex were detected by using PicTure™-KIT HRP (horseradish peroxidase conjugated, a stable amino-acid polymer, which carries goat anti-rabbit IgG antibodies) and Liquid DAB-Plus Substrate Kit (Zymed Laboratories Inc., San Francisco, CA, USA).

Behavioral Experiments

Surgical procedures. Rats were anesthetized with Zoletil (Virbac, Carros, France, 60 mg/kg, i.p.) and placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). Two guide cannulae consisting of stainless-steel tubings (26 G, 12 mm, Cooper, London, UK) were implanted in the lateral septum so as to position the tips at 1 mm above the medial part (coordinates from bregma: AP: 0.0, ML: ±0.6, V: −4.7, according to the rat brain atlas of Paxinos and Watson, 1998). The guide cannulae were anchored to the skull with two jeweler screws (Plastic One, Ronaoke, VA, USA) and Paladur dental cement (Heraeus Kulzer, Hanau, Germany). After surgery, rats were allowed a 5-day recovering period. During this period, rats were handled twice before testing in order to verify that the cannulae obturators (A-M Systems, Carlsborg WA, USA) were kept in place. As a control experiment, guide cannulae were also implanted in the medial striatal region (AP: +0.3, ML: ±4.0, V: −4.8 from bregma).

Behavioral Testing

The punished drinking test. The procedure was a modification of that described by Vogel *et al* (1971). Rats, water deprived for 48 h before testing, were placed in cages with a stainless-steel grid floor. Each cage contained a drinking tube connected to a 50-ml burette filled with tap water. Trials were started when the rat's tongue had entered in contact with the drinking tube for the first time. An electric shock (0.6 mA, 600 ms) was delivered to the tongue every 20 licks. The number of shocks delivered was recorded during a 5-min period.

The elevated plus-maze test. The procedure is based on that described by Pellow *et al* (1985). The apparatus was made of polyvinylchloride. It was elevated to a height of 70 cm with two open (50 × 10) and two enclosed arms (50 × 10 × 50) arranged so that the arms of the same type were opposite to each other. The apparatus was equipped with infrared beams and sensors capable of measuring activity in the different arms. The illumination intensity measured in the open arms was 30 lux. Rats were placed in the center of the maze for a free exploration period of 4 min. Results were expressed as the mean ratio of time spent (or entries) in open arms to total time spent (or entries) in both open and closed arms and the mean number of entries in closed arms.

The forced-swimming test. The procedure was a modification of that described by Porsolt *et al* (1977). Animals were placed in an individual glass cylinder (diameter 17 cm, height 40 cm) containing water (height 24 cm, 22°C). Two swimming sessions were conducted (an initial 6-min pretest followed the same day by a 6-min test). The duration of immobility (in s) was measured manually during the 6-min test by an experimenter who was unaware of the drug treatments.

Drug Administration

Every rat was tested consecutively in the three behavioral tests which were run in the following order: (1) the punished drinking test, (2) the elevated plus-maze test, and (3) the forced-swimming test. Each rat received the same treatment during the 3 consecutive days. At 10 min prior to testing, rats received a bilateral injection of the drug using stainless-steel microinjection cannulae (30 G Cooper, London, UK). The cannulae were connected by polyethylene tubings (Plastic One, Ronaoke, VA, USA) to microsyringes (Exmire, Ito Corporation, Fuji, Japan) mounted on a motorized pump (CMA, Solna, Sweden).

SSR149415, isomer(−), was synthesized by the Medicinal Chemistry Department of Sanofi-Synthelabo. It was prepared as a solution in physiological saline 0.9% containing DMSO 5% (Sigma, Lyon, France) and Cremophor EL 5% (Sigma, Lyon, France). Aliquots containing 1 μg/0.6 μl SSR149415 were frozen and stored at −20°C. The following groups were constituted: control solution (saline 0.9% alone and a mixture of saline/DMSO/Cremophor) and SSR149415 (1, 10, and 100 ng dissolved in saline/DMSO/Cremophor). All drugs were injected in the lateral septum at a volume of 0.3 μl/side and at a rate of 0.2 μl/min. Cannulae were left in

place for an additional minute to avoid reflux of the drugs inside the guide cannulae. During the 2-min infusion, rats were slightly restrained by the experimenter. After injection, obturators were inserted and rats placed in their home cages. The same injection procedure was used for intrastriatal administration of SSR149415.

Histology and Controls

On the day after the completion of the last test, rats were killed with an overdose of pentobarbital (Virbac, Carros, France, 100 mg/kg, i.p.), and brains were removed and frozen. Brain slices (30 μm) were subsequently made using a cryostat and stained with cresyl violet. The placement of the microinjection cannulae was determined for each rat by an experimenter blind to the behavioral results. Cases where the tip of one or both cannulae was located outside the lateral septum were excluded for statistical analysis. Histological control for brain cannulae implanted in the medial striatum was carried out using the same procedure.

Statistics

All data were analyzed with one-factor analysis of variance (ANOVA). Significance was set at 0.05. In cases of a significant main effect, *post hoc* comparisons were performed with a Dunnett test.

RESULTS

Anti-V1$_b$R Immunohistochemistry

An overview of the rat brain regions where V_{1b} receptors were located using immunohistochemical detection is given in Table 1. There was no major difference in staining intensity between the different slices of the five rat brains. Moderate to intense cellular V_{1b}R immunoreactivity was observed in several limbic structures including the septum, the amygdala, the hippocampus, and the nucleus accumbens (see Figure 1). In the lateral septum, there was a moderate labeling of neuronal perikarya (dorsal, medial, and ventral parts, see Figure 1 for illustration). A similar positive immunoreactivity was observed in the posterolateral part of the bed nucleus of the stria terminalis (dorsal and ventral parts). The vertical and horizontal limb of the diagonal band of Broca as well as the medial septum presented low V_{1b}R immunoreactivity. V_{1b} receptors were also found in the different amygdaloid nuclei. A moderate labeling was observed in the rostral part of the medial, basolateral, and central nuclei, but a more intense staining was observed in the caudal region. The nucleus accumbens (core) and the caudate putamen (medial region) showed moderate V_{1b}R immunoreactivity. In the nucleus accumbens (shell), the staining intensity was low. In cortical areas, V_{1b}R immunoreactivity was observed in the frontal and in the cingulate cortices. A weaker staining was observed in the parietal cortex. All fields of the Ammon's horn displayed V_{1b}R immunoreactivity at the rostrocaudal level. Moderate staining was observed in the stratum radiatum from CA1 and CA2 and the labeling was found to be particularly intense in the CA3 and CA4 fields. V_{1b}R immunoreactivity was also found in the dentate gyrus, subiculum and entorhinal cortex. In the nucleus basalis magnocellularis, the intensity of the labeling was very low. The septofimbrial nucleus, the subfornical organ and the triangular septal nucleus showed intense V_{1b}R immunostaining. For all structures, perikarya and dendrites stainings were observed. However, in a few regions such as, for example, the frontal cortex, a dense V_{1b}R immunoreactivity was seen in the fibers.

Table 1 Distribution of the V_{1b} Receptor Protein in Various Structures of the Rat Brain

Cortex	
Frontal cortex	++
Cingulate cortex	+
Entorhinal cortex	++
Parietal cortex	+
Basal ganglia	
Nucleus accumbens shell	+
Nucleus accumbens core	++
Caudate/putamen	+
Hippocampus	
Dentate gyrus	++
CA1	++
CA2	++
CA3	+++
CA4	+++
Subiculum	++
Basal forebrain	
Lateral septum	++
Medial septum	+
Triangular septal nucleus	+++
Septofimbrial nucleus	+++
Vertical diagonal band of Broca	+
Horizontal diagonal band of Broca	+
Nucleus basalis magnocellularis	+
Amygdala	
Central amygdala	++
Basolateral amygdala	++
Medial amygdala	++
Bed nucleus of the stria terminalis	++

+++, ++, or + denote strong, moderate, or low but positive signal, respectively.

Behavioral Experiments

Histological control. Slides stained with cresyl violet showed that the tip of the injection cannulae was located mostly in the medial part of the lateral septum (Figure 2). Rats were discarded from analysis when the lateral ventricle was damaged or when the implantation was outside the lateral septum. The final number of animals for the different groups were: Controls $N=10$, SSR149415 (1 ng) $N=8$,

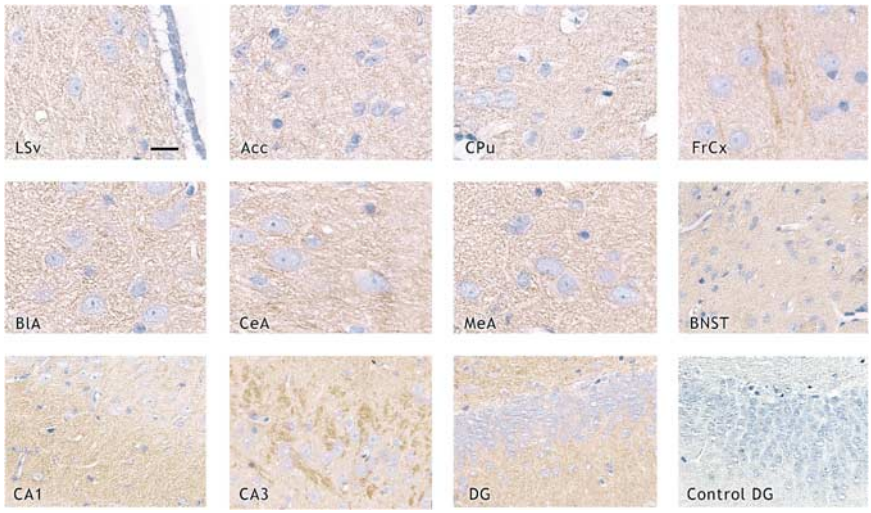

Figure 1 Localization of V_{1b} receptors in the rat brain using anti V_{1b}R immunohistochemistry. V_{1b}-R signal is light brown and hematoxylin-stained nuclei are blue. LSv: ventral part of the lateral septum; Acc: accumbens shell nucleus; CPu: caudate putamen; FrCx: frontal cortex; BLA: basolateral amygdala; CeA: central amygdala; MeA: medial amygdala; BNST: bed nucleus of the stria terminalis; CA1-CA3: Ammon's horn fields; DG: dentate gyrus. Bar: 10 μm except for BNST, CA1, CA3, DG = 15 μm.

Table 2 Effects of Intraseptal SSR149415 in the Punished Drinking and in the Elevated Plus-Maze Tests, and Intrastriatal SSR149415 in the Forced-Swimming Test, in Rats

	Septal infusion				Striatal infusion
	Punished drinking	Elevated plus maze			Forced swimming
SSR149415 (ng)	Punished responses	% Time open arms	% Entries open arms	Closed arms entries	Immobility time (s)
0	11.4 ± 2.20	15.4 ± 2.71	24.3 ± 4.48	14.0 ± 1.42	80.00 ± 7.29
1	13.6 ± 3.81	18.5 ± 4.31	25.5 ± 5.87	14.0 ± 1.61	
10	13.4 ± 3.27	21.7 ± 4.63	30.5 ± 4.13	13.2 ± 1.03	
100	9.80 ± 1.78	13.9 ± 3.53	18.5 ± 3.96	13.2 ± 0.88	101.1 ± 19.5

Data represent mean ± SEM.

SSR149415 (10 ng) $N = 10$, and SSR149415 (100 ng) $N = 15$. For the striatal infusion experiment, all cannulae tips were located in the medial part of the caudate nucleus (not shown).

Behavioral controls. In order to verify that the DMSO and Cremophor combination was devoid of behavioral effects and, therefore, could be considered as a valid control, performances of saline-infused animals were compared to those of rats that received saline/DMSO/Cremophor. Results showed that there was no significant difference between the two control groups for each of the variables measured in the punished drinking test (number of shocks), elevated plus maze (% time spent and % entries in the open arms and number of entries in closed arms), and forced-swimming test (immobility time) (data not shown). As a result, the saline/DMSO/Cremophor combination was used as the control solution.

Behavioral Testing

Effects of intraseptal infusion of SSR149415 in the punished drinking test. The bilateral infusion of SSR149415 (1–100 ng) into the lateral septum did not modify significantly the number of punished responses ($F3,39 = 0.51$, $p = 0.68$, see Table 2).

Effects of intraseptal infusion of SSR149415 in the elevated plus-maze test. The bilateral infusion of SSR149415 into the lateral septum failed to modify the behaviors of animals in the elevated plus maze. ANOVA revealed that neither the indices of anxiety (ie the % time spent or % entries in the open arms) nor the presumed measure of activity was changed significantly after drug challenge (respectively, $F3,39 = 0.84$, $p = 0.48$; $F3,39 = 1.35$, $p = 0.27$, and $F3,39 = 0.13$, $p = 0.94$, see Table 2).

Effects of intraseptal infusion of SSR149415 in the forced-swimming test. The bilateral infusion of SSR149415 into the lateral septum modified significantly immobility time ($F3,39 = 6.85$, $p < 0.001$). *Post hoc* comparisons showed that the doses of 10 and 100 ng SSR149415 induced a significant reduction of this measure when compared to the saline/DMSO/Cremophor control group ($p < 0.05$ and < 0.01, respectively). The lowest dose of SSR149415 (1 ng) tested was devoid of effects in this test (Figure 3).

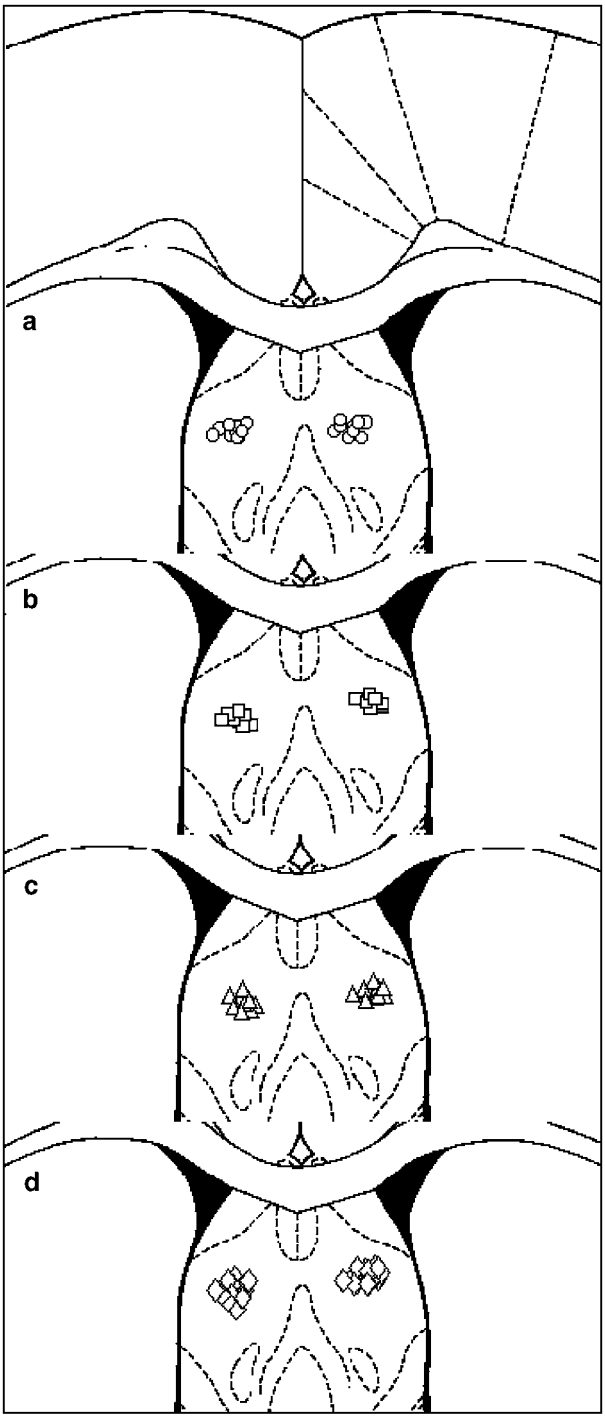

Figure 2 Schematic drawings of the localization of bilateral cannulae tips in the lateral septum of the rats treated with (a) control solution, (b) SSR149415 (1 ng), (c) SSR149415 (10 ng), or (d) SSR149415 (100 ng), according to the rat brain atlas of Paxinos and Watson (1998). AP: +0.2 mm from bregma.

Effect of bilateral infusion of SSR149415 into the medial striatum in the forced-swimming test. The infusion of a high dose of SSR149415 (100 ng) in the medial striatum did not modify significantly the behavior of animals in the forced-swimming test ($F1,14 = 0.83$, $p = 0.38$) (Table 2).

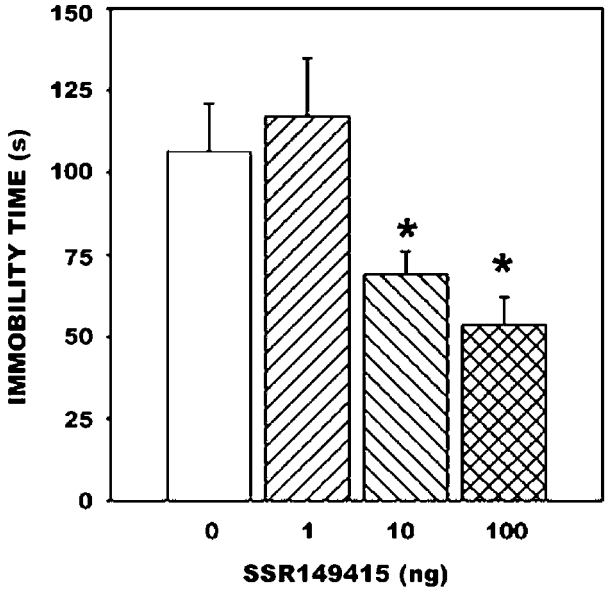

Figure 3 Effects of intraseptal SSR149415 in the forced-swimming test in rats. Data represent mean ± SEM; *$p < 0.05$ or less (Dunnett).

DISCUSSION

The first part of the present study described the localization of V_{1b} receptors in the rat limbic brain using anti-V_{1b} receptor immunohistochemistry. Results revealed that V_{1b} receptors are present in the rat brain with a large distribution throughout the limbic system. In particular, a substantial amount of V_{1b} receptors was detected in the lateral septum, the hippocampus, the amygdaloid region and the cortex. Second, we assessed the behavioral effects of local infusion of SSR149415 in the lateral septum, a region involved in stress-related behaviors. The behavioral experiments showed that local infusion of SSR149415 in the lateral septal area induced clearcut and dose-dependent antidepressant-like effects in the forced-swimming test, while similar treatment did not modify anxiety-related behaviors in the punished drinking and elevated plus-maze tests.

To date only a few studies have examined the expression of V_{1b} receptor mRNA or protein in the rat brain (Lolait *et al*, 1995; Vaccari *et al*, 1998; Hernando *et al*, 2001). Using RT-PCR, Lolait *et al* (1995) provided the first evidence of extrapituitary V_{1b} receptor mRNA expression in the rat brain tissues. Together with other authors (Vaccari *et al*, 1998; Hernando *et al*, 2001), they showed that V_{1b} receptors are present in the central nervous system, including the basal ganglia, septohippocampal and several cortical regions. In the present study, using a new anti-V_{1b} receptor antibody, we detected the presence of V_{1b} receptors in additional regions of the limbic system such as the diagonal band of Broca, all the regions of the hippocampal formation (fields of the Ammon's horn, dentate gyrus, subiculum and entorhinal cortex), the central, medial, and basolateral amygdaloid nuclei, and the bed nucleus of the stria terminalis. Our results clearly indicate that V_{1b} receptors are largely distributed outside the hypothalamic regions of the rat brain. This suggests that vasopressin, through V_{1b} receptors, may play an important modulatory role in limbic

functioning, reinforcing the idea that the peptide is involved in stress-related behaviors, and that V_{1b} receptors probably mediate its effects. Hopefully, the availability of selective V_{1b} receptor radioligands will certainly help to obtain a more complete picture of the distribution of V_{1b} receptors throughout the central nervous system. In addition, ultrastructural studies are required to determine whether V_{1b} receptors are distributed on pre- or postsynaptic membranes. This will help to better understand the regulatory role vasopressin exerts on synaptic transmission.

The behavioral experiments aimed at determining a possible site of action of the antistress-like effects of the selective nonpeptide V_{1b} receptor antagonist, SSR149415. A previous report (Griebel *et al*, 2002) has indicated that the antidepressant-like effects of SSR149415 do not only involve the blockade of V_{1b} receptors of the hypothalamus since these effects were still observed in hypophysectomized rats. Here, SSR149415 was directly infused into the lateral septum, a brain structure enriched in V_{1b} receptors as evidenced by the current immunohistochemistry data. Moreover, as indicated above, the lateral septum plays a key role in the modulation of emotional behaviors. Results showed that intraseptal application of SSR149415 elicited antidepressant- but not anxiolytic-like effects. The decrease in immobility in the forced-swimming test may be indicative of an antidepressant-like action of the drug. However, one cannot totally rule out the possibility that this effect may be the consequence of a nonspecific (ie motor) action of the drug. Data from the elevated plus-maze on closed arms entries may shed some light on this issue since this presumed index of locomotor activity was not changed over the entire dose-range tested (1–100 ng). Moreover, when administered systematically, SSR149415 did not modify several locomotor parameters assessed in different behavioral tests such as line crossings in the defense battery (Griebel *et al*, 2002) or swimming abilities in the Morris water maze task observed after repeated administrations of the drug as was the case here (Griebel *et al*, 2003), suggesting that the V_{1b} receptor antagonist was devoid of central effects not related to emotionality. Although a leakage of the infused solution to adjacent brain areas (bed nucleus of the stria terminalis for instance) cannot be excluded, it is unlikely that the antidepressant-like effects of SSR149415 are due to the blockade of V_{1b} receptors located in other structures since the volume injected in the lateral septum was rather small (0.3 μl). Moreover, a ventricular contamination or diffusion through the tissue is unlikely as the infusion of the compound into the striatum, a nearby structure, was devoid of any antidepressant-like effect. This latter result also suggests that V_{1b} receptors present in the striatum (see immmunohistochemistry data of the present study) are not involved the antidepressant-like effects of SSR149415. It is noteworthy that the clear reduction in immobility time in the forced-swimming test following intraseptal infusion of SSR149415 paralleled that observed after systemic administration of the compound (Griebel *et al*, 2002), suggesting that V_{1b} receptors located in the lateral septum play a key role in the mediation of the antidepressant-like effects of SSR149415 in rats. However, these effects do not fit well with those described in another study (Ebner *et al*, 1999), where increases in floating time were observed after intraseptal infusion of the mixed $V_{1a/1b}$ peptide antagonist $d(CH_2)_5Tyr(Me)AVP$ using a retrodialysis technique in rats. The discrepancies between our results and those of Ebner could be explained by differences in both the administration (microinjection *vs* retrodialysis) and the testing (single *vs* test-retest) procedures, which could have differentially affected vasopressinergic tone and the subsequent effects of the blockade of the vasopressinergic transmission. Another explanation could be that the behavioral effects induced by the mixed peptide antagonist resulted in the preferential blockade of the V_{1a} receptor subtype, which may exert an opposite action of that of the V_{1b} subtype. To illustrate this idea, a recent study using knockout mice for either V_{1b} or V_{1a} receptors on social aggression showed that aggression was decreased in $V_{1b}^{-/-}$ mice, while it was increased in $V_{1a}^{-/-}$ mice (Wersinger *et al*, 2003). Altogether, these results suggest opposite roles for V_{1b} and V_{1a} receptors in the mediation of behavioral responses, reinforcing the idea that the blockade of V_{1b} receptors may be relevant for the treatment of stress-related disorders. Although our results on the antidepressant-like effects of intraseptal SSR149415 reproduce those obtained in a previous experiment using systemic administration, they do not exclude that V_{1b} receptors located in other limbic regions, such as, for example, the amygdala, may also participate in the action of the V_{1b} antagonist. It has been demonstrated that the intra-amygdala delivery of the mixed $V_{1a/1b}$ peptide antagonist $d(CH_2)_5Tyr(Me)AVP$ by using a retrodialysis technique may modify stress-coping strategies in a swim test (Ebner *et al*, 2002). The present study revealed that intraseptal infusion of SSR149415 had no effect on anxiety-related behaviors as measured in the punished drinking and elevated plus-maze tests. In previous studies, anxiolytic effects of systemic administration of SSR149415 were described in the punished drinking and the elevated plus-maze tests, although the effects of the compound were weaker than those observed with the benzodiazepine anxiolytic diazepam (Griebel *et al*, 2002). Although it is recognized that the activity of the lateral septum mediates anxiety-related behaviors in tests based on exploration or conflict situations (Treit and Menard, 2000), septal V_{1b} receptors may not be involved in the mediation of anxiolytic-like effects of SSR149415. We demonstrated that other structures such as the different amygdaloid nuclei contain V_{1b} receptors. These brain areas are also known to mediate anxiety-related behaviors observed in the elevated plus-maze or in the punished drinking test (Graeff *et al*, 1993), suggesting that V_{1b} receptor located in the amygdala may mediate the anxiolytic effects of SSR149415.

In conclusion, this study demonstrated that the blockade of septal V_{1b} receptors with SSR149415 induced robust antidepressant-like effects resembling those observed after systemic administration of the compound. However, the brain region involved in the mediation of the anxiolytic-like action of SSR149415 remains to be determined.

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