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Antidepressant-Like Effects of the Corticotropin-Releasing Factor 1 Receptor Antagonist, SSR125543, and the Vasopressin 1b Receptor Antagonist, SSR149415, in a DRL-72 s Schedule in the Rat

Caroline Louis*[,1], Caroline Cohen[1], Ronan Depoortère[1] and Guy Griebel[1]

[1]*CNS Research Department, Sanofi-Aventis, Bagneux, France*

The vasopressin 1b receptor antagonist, SSR149415, and the corticotropin-releasing factor 1 receptor antagonist, SSR125543, are orally active non-peptidic compounds with anxiolytic- and antidepressant-like activities in animal models. Presently, SSR149415 and SSR125543 were evaluated in a differential reinforcement of low-rate 72 s (DRL-72 s) schedule, a procedure known to respond differentially to antidepressants and anxiolytics. Male Wistar rats were trained to lever-press for food reinforcement, but only lever-presses occurring after a 72 s delay were reinforced; otherwise, presses were not rewarded, and the timer was reset to 0 s. The selective serotonin reuptake inhibitor, fluoxetine, and the benzodiazepine anxiolytic, diazepam, were tested in parallel. SSR149415 (10–30 mg/kg, i.p.) and SSR125543 (30 mg/kg, i.p.) increased the percentage of responses emitted in the inter-response time (IRT) bin (49–96 s), which resulted in a greater number of reinforced presses. Both compounds shifted the frequency distribution of responses toward longer IRT durations, with a preservation of the bell shape of the IRT distribution curve. Fluoxetine (10 mg/kg, i.p.) had an effect on DRL-72 s similar to that of SSR149415 and SSR125543. By contrast, diazepam increased the number of responses in IRT bin (0–12 s), and the IRT distribution curve was shifted toward shorter IRT durations and flattened. In summary, these results show that SSR149415 and SSR125543 displayed antidepressant-like activity in a DRL-72 s schedule in rat, confirming their therapeutic potential for the treatment of pathological states induced by chronic frustration such as depression.
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INTRODUCTION

Arginine vasopressin (AVP), a cyclic nonapeptide synthesized in the hypothalamus, participates in the hypothalamic–pituitary–adrenal (HPA) axis function by regulating the secretion of pituitary adrenocorticotropin hormone (ACTH) and by potentiating the stimulatory effects of corticotropin-releasing factor (CRF)—also denominated corticotropin-releasing hormone (CRH) (Antoni, 1993). AVP and CRF play a central role in the coordination of neuroendocrine, autonomic, and behavioral responses to stress (Carrasco and Van de Kar, 2003). Besides their participation in HPA axis regulation, AVP and CRF have extra-hypothalamic functions, as AVP and CRF receptors have been found outside the hypothalamus. Vasopressin (V) V1a and V1b receptors have been detected in the septum, cortex, and hippocampus (Lolait *et al*, 1995; Stemmelin *et al*, 2005), and CRF binding sites in the olfactory bulb, hippocampus, hypothalamus, striatum, cerebellar cortex, medulla, midbrain, pons and spinal cord (De Souza, 1987), locus coeruleus (Merchenthaler *et al*, 1982; Vigh *et al*, 1982; Wynn *et al*, 1984), and raphe nuclei (Lowry *et al*, 2000; Roche *et al*, 2003).

There has been increased interest in the antidepressant potential of drugs that block receptors for CRF, leading to the pharmaceutical development of several compounds that are antagonists at the CRF1 receptor (Griebel *et al*, 2002c; Keck and Holsboer, 2001; Okuyama *et al*, 1999; Schulz *et al*, 1996; Millan *et al*, 2001; Lelas *et al*, 2004; Seymour *et al*, 2003). The CRF1 receptor antagonist SSR122543 has been reported to induce anxiolytic- and antidepressant-like activities in several animal models of psychiatric diseases (Gully *et al*, 2002; Griebel *et al*, 2002b, c; Alonso *et al*, 2004). Likewise, antagonists at central vasopressin receptors are of

*Correspondence: Dr C Louis, CNS Research Department, Sanofi-Aventis, 31 Ave P Vaillant-Couturier, Bagneux 92220, France, Tel: +33 1 45 36 24 91, Fax: +33 1 45 36 20 70,
E-mail: caroline.louis@sanofi-aventis.com
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potential interest for the treatment of depression and stress-related disorders. SSR149415 is the first orally active non-peptidic V1b receptor antagonist. It produced a powerful inhibition of vasopressin- and CRH-induced release of ACTH, blocked the stress-induced elevation of plasma ACTH, and had anxiolytic- and antidepressant-like effects in various animal models (Griebel *et al*, 2002b; Serradeil-Le Gal *et al*, 2002; Alonso *et al*, 2004).

The differential reinforcement of low-rate 72 s (DRL-72 s) schedule has been proposed as a behavioral screening model for antidepressant agents (O'Donnell and Seiden, 1983). It is an operant procedure in which a rat is required to withhold a lever-press response for at least 72 s in order to obtain a reward. If a response is emitted before the 72 s elapsed, the time counter is reset to 0. This schedule of reinforcement generates low rates of lever-pressing, typically in the range of 75–120 presses per hour (Seiden *et al*, 1985), and the frequency of reinforced responses is very low (generally less than 7–8%). The typical effect of tricyclic antidepressants (McGuire and Seiden, 1980a, b), monoamine oxidase inhibitors (O'Donnell and Seiden, 1982; Marek and Seiden, 1988), selective serotonin (5-HT) reuptake inhibitors (Cousins and Seiden, 2000; Sokolowski and Seiden, 1999; Dekeyne *et al*, 2002), and electroconvulsive shocks (Seiden *et al*, 1985) is to increase time elapsed between responses, leading to an increase in the frequency of reinforced responses.

In the present study, the effects of i.p. treatment with SSR149415 and SSR125543 were evaluated on a DRL-72 s schedule, and compared to those of the selective 5-HT reuptake inhibitor antidepressant fluoxetine and the benzodiazepine anxiolytic diazepam, for internal validation.

MATERIALS AND METHODS

Animals

Male Wistar rats (Iffa Credo, Les Oncins, France) were singly housed under a 12 : 12 light–dark cycle (lights on at 0700) in a room at 22°C, 50% humidity, with *ad libitum* access to water except during operant sessions. Their weight was kept at 450 ± 50 g by feeding with 20 g of food chow given at the end of the day and over the week-end. All experiments were approved by the in-house Ethics Committee and performed in accordance with the 'European Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (European Union Directive #86/606/EEC) and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

Apparatus

The experiments were carried out in eight identical rat operant chambers (Med Associates, East Fairfield, VT, USA), each fitted with a 2.8 W overhead house light and a stainless steel rods floor. A 4.8×1.9 cm lever was positioned on the right side of a food tray, which was connected to a food pellets (45 mg, Formula P, Noyes, Research Diets, New Jersey, USA) dispenser. Each operant chamber was enclosed in a ventilated and sound-attenuating cubicle; all events were recorded and controlled by the 'Med-PC' software.

Acquisition of the Operant Behavior

Rats were first trained (5 days a week) in daily 30 min sessions to press a lever to obtain a food pellet under a continuous reinforcement—fixed time 60 s concurrent schedule (ie if the rat did not press the lever within 60 s, a reinforcement was automatically delivered). When rats obtained at least 100 pellets per training session, they were subjected to a differential reinforcement of low-rate (DRL) 15 s schedule. More explicitly, a lever-press occurring before a delay of 15 s had elapsed was not rewarded and the timer was reset to 0 s for a further 15 s cycle. Session duration of these DRL sessions was set to 60 min. Across successive DRL sessions, the timing of the DRL schedule was progressively increased from 15 to 30 s to the final timing of 72 s. In order to acquaint them to the i.p. injection procedure, rats were injected with saline 30 min pre-session once they attained the DRL-72 s stage. Once performance had stabilized (ie less than 10% variation of total number of responses during six consecutive DRL-72 s vehicle sessions, and less than seven reinforcers per session), rats were subjected to pharmacological challenge sessions.

Pharmacological Studies

Each rat received four drug treatments, with doses administered in a mixed order. For a given drug treatment, control values were calculated by averaging the performance of all vehicle sessions immediately preceding all drug sessions. Furthermore, a stability criterion (less than 10% variation of total number of responses between the vehicle session immediately before the drug session and the six vehicle sessions preceding the start of the pharmacological study: *vide supra*) was in effect in-between each drug session.

Data

The following parameters were automatically recorded by the Med-PC software: the total number of lever-presses emitted during the session, the number of food pellets obtained (ie the number of reinforced responses), and the inter-response time (IRT, the time elapsed between two lever-presses; Richards *et al*, 1993). IRTs were subsequently split into nine bins (IRT bin (0–12 s), IRT bin (13–24 s),…, IRT bin (85–96 s), and IRT bin (>96 s)). From these raw data, the percentage of lever-presses emitted in each of the nine 12 s bins and the percentage of lever-presses reinforced were calculated using a Macro procedure with the Excel® software (percentages were calculated as a function of the total number of lever-presses emitted during the session). The percentages of lever-presses during two IRT bins in particular were analyzed: (1) the IRT bin (0–12 s), corresponding to the 'burst responses' and shown to be sensitive to benzodiazepine-like compounds (Richards *et al*, 1993); (2) the IRT bin (49–96 s), as it has been shown to be particularly sensitive to antidepressant drugs (Cohen *et al*, 1997).

Statistical Analysis

The percentage of lever-presses emitted in bins (0–12 s) and (49–96 s), the percentage of lever-presses reinforced, and

the total number of lever-presses were analyzed with a Friedman's test, followed by *post hoc* tests with a Dunn's correction factor for a comparison between drug and control (vehicle-treated) groups. A Cochran–Mantel–Haenzsel test was applied to analyze the effects of drug treatments on the distribution of responses across the IRT bins, followed by *post hoc* tests with a Bonferroni–Holm correction factor for a comparison between drug- and control (vehicle)-treated groups. Statistical analyses were performed with the SAS system 8.2 (SAS Institute Inc., Cary, NC, USA).

Drugs

Fluoxetine (Sigma Aldrich, Saint Quentin Fallavier, France) and diazepam (Roche, Basel, Switzerland) were dissolved in saline with Tween80® (circa 2% v/v). SSR149415 and SSR125543, synthesized by the Chemistry Department of sanofi-aventis, were suspended in saline with methylcellulose (0.6% w/v). Drugs or the appropriate vehicles were administered i.p. (1 ml/kg body weight) 30 min pre-session. Doses are expressed as the free base of the compounds.

RESULTS

Fluoxetine

Fluoxetine (10 mg/kg, i.p.) significantly (Friedman's $H(3) = 8.55$, $p < 0.05$) increased the percentage of lever-presses occurring in the IRT bin (49–96 s), which translated into a significant (Friedman's $H(3) = 20.25$, $p < 0.001$) augmentation of the percentage of reinforced responses. Fluoxetine did not affect the percentage of 'burst responses' (ie percentage of responses in the IRT bin (0–12 s)). All these effects are illustrated in Figure 1, where it can be seen that fluoxetine dose-dependently shifted the IRT distribution curves toward longer durations, starting with the dose of 2.5 mg/kg, in a significant manner (Cochran's QSMH(8) = 85.50, $p < 0.0001$). Overall, fluoxetine significantly ($H(3) = 15.00$, $p < 0.01$) reduced the total number of responses (Table 1).

SSR149415

Similarly to fluoxetine, the V1b receptor antagonist SSR149415 (10 and 30 mg/kg, i.p.) significantly (Friedman's $H(3) = 13.95$, $p < 0.01$) increased the percentage of lever-presses emitted in the IRT bin (49–96 s). This effect is coherent with a significant (Friedman's $H(3) = 14.55$, $p < 0.01$) increase in the percentage of reinforced responses and with the fact that SSR149415 significantly (Cochran's QSMH(8) = 141.48, $p < 0.0001$) and dose-dependently (3–30 mg/kg) shifted the IRT distribution curves toward longer durations, starting from the IRT bin (49–60 s) (Figure 2). The compound also decreased the total number of responses in a significant way (Friedman's $H(3) = 13.50$, $p < 0.01$). However, it did not modify the percentage of 'burst responses'.

SSR125543

The CRF1 receptor antagonist SSR125543 (30 mg/kg, i.p.) had an overall effect on DRL-72 s performance similar

Table 1 Effects of Fluoxetine, the V1b Receptor Antagonist SSR149415, the CRF1 Receptor Antagonist SSR125543, and Diazepam, on the Percentage of Responses Emitted in the IRT Bins (49–96 s) and (0–12 s), on the Percentage of Reinforced Lever Presses, and on the Total Number of Lever Presses

Drugs	Doses (mg/kg)	Percentage bin (49–96 s)	Percentage reinf. presses	Percentage bin (0–12 s)	Total presses
Fluoxetine	0	24.3±4.5	3.2±0.7	10.3±1.4	100.3±4.5
	2.5	36.1±5.8	9.3±1.4*	10.9±2.7	86.8±4.0
	5	34.7±4.4	8.9±1.5	8.4±2.0	84.5±3.0*
	10	41.9±5.5*	12.9±2.1**	8.3±1.6	79.3±4.8**
SSR149415	0	22.6±4.7	4.1±0.9	9.5±1.1	99.7±5.0
	3	32.2±4.8	6.3±0.9	8.3±1.2	91.1±5.0
	10	38.5±4.3	13.1±1.9**	7.2±1.2	77.5±3.4**
	30	44.8±3.8**	14.4±4.1*	10.4±2.0	77.8±4.1**
SSR125543	0	26.9±4.7	5.1±1.0	13.6±1.8	98.3±5.5
	3	31.9±4.8	5.5±1.1	9.4±1.8	91.9±5.4
	10	37.8±5.6	11.0±2.9	8.9±2.0	82.9±6.5
	30	41.6±4.9*	16.2±3.3*	10.6±2.4	75.3±6.0*
Diazepam	0	30.4±6.0	5.2±1.1	10.2±2.0	94.6±7.0
	1	33.0±4.8	10.2±2.3	12.7±3.3	90.0±6.5
	3	29.1±4.4	16.3±1.9**	18.0±2.2	79.9±5.8
	10	19.9±3.8	12.1±2.5**	22.4±4.3*	60.1±13.7*

*$p < 0.05$, **$p < 0.01$ *vs* vehicle-treated group (0): *post hoc* tests with a Dunn's correction, following a significant Friedman analysis. $N = 8$ rats per drug treatment.

to that produced by SSR149415 and fluoxetine. It significantly increased the percentage of responses occurring in the IRT bin (49–96 s) and the percentage of reinforced responses (Friedman's $H(3)=8.85$, $p<0.05$, and 11.40, $p<0.01$, respectively). It did not modify the percentage of 'burst responses', but significantly (Friedman's $H(3)=12.15$, $p<0.01$) reduced the total number of lever-presses. The Cochran–Mantel–Haenzsel analysis revealed that SSR125543 significantly (QSMH(8) = 98.49, $p<0.0001$) affected the distribution of responses across the IRT bins, with a rightward shift of the curve. The similarity in the effects of fluoxetine, SSR149415, and SSR125543 on performance under a DRL-72 s schedule can be clearly captured by comparing Figures 1–3.

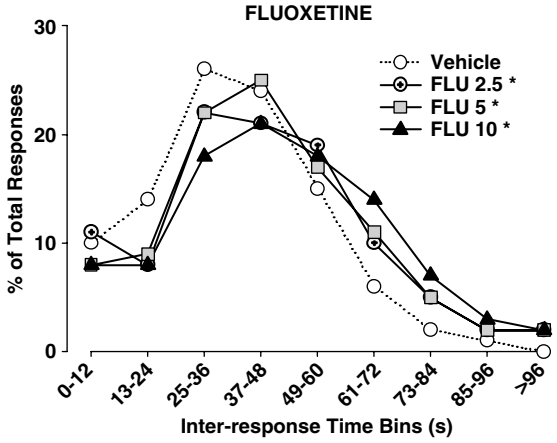

Figure 1 Effect of fluoxetine on the IRT distribution on a DRL-72 s operant schedule in rat. Data are expressed as the average percentage of lever-presses emitted during each IRT bin (with respect to the total number of lever-presses). Error bars (minimal value = 0.13; maximal value = 4.44) are omitted for clarity. Drug or vehicle was injected i.p., 30 min pre-session. *$p<0.05$ *post hoc* test (Bonferroni–Holm's correction factor) *vs* vehicle-treated group, following a significant Cochran–Mantel–Haenszel analysis. $N=8$ rats per group.

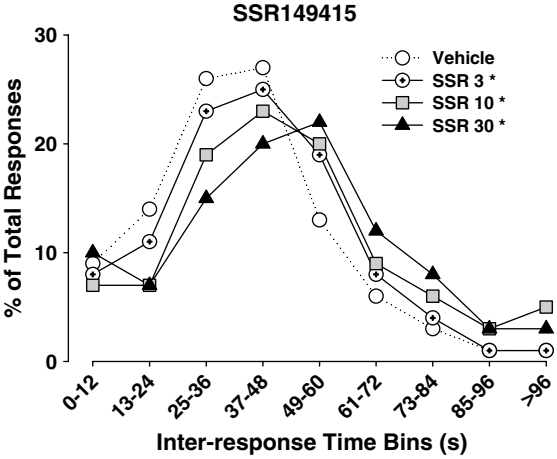

Figure 2 Effect of the V1b receptor antagonist, SSR149415, on the IRT distribution on a DRL-72 s operant schedule in rat. Refer to legend of Figure 1 for details. Error bars (min. value = 0.18; max. value = 3.75) are omitted for clarity. $N=8$ rats per group.

Diazepam

Contrary to what was observed with fluoxetine, SSR149415, and SSR125543, diazepam, up to 10 mg/kg i.p., did not affect the percentage of responses emitted in the IRT bin (49–96 s), but significantly (Friedman's $H(3)=7.95$, $p<0.05$) increased the percentage of 'burst responses' cumulated in the IRT bin (0–12 s). The total percentage of reinforced responses was also significantly (Friedman's $H(3)=11.40$, $p<0.01$) augmented by diazepam. Although this effect might at first seem paradoxical (as the percentage of responses emitted in the IRT bin (49–96 s) tended to be diminished), it most likely resulted from an increase in the percentage of responses emitted in the IRT bin (>96 s) (see last quartet of symbols on the foremost right part of Figure 4). The distribution of the percentage of responses in each IRT bin was significantly (Cochran's QSMH(8) = 112.93, $p<0.0001$) affected by the treatment, with a loss of the bell shape of the curve at higher doses. Finally, the total number of responses was significantly (Friedman's $H(3)=7.95$, $p<0.05$) reduced by diazepam.

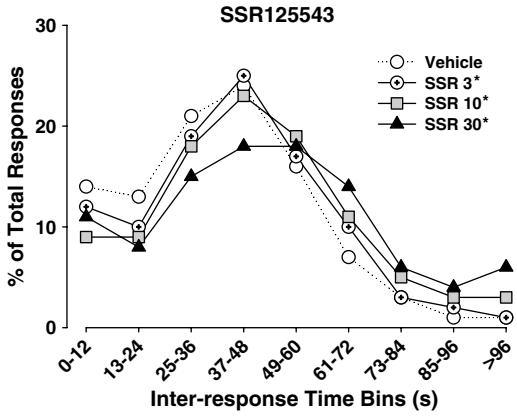

Figure 3 Effect of the CRF1 receptor antagonist, SSR125543, on the IRT distribution on a DRL-72 s operant schedule in rat. Refer to legend of Figure 1 for details. Error bars (min. value = 0.76; max. value = 3.27) are omitted for clarity. $N=8$ rats per group.

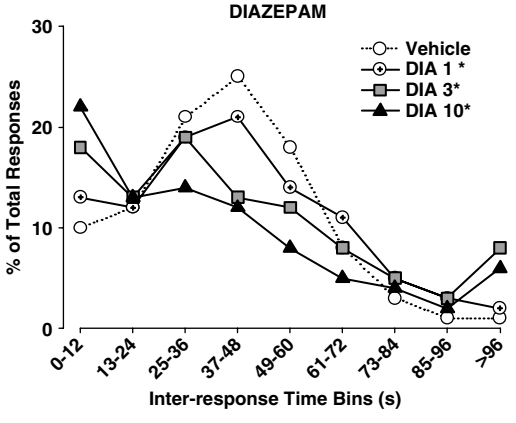

Figure 4 Effect of diazepam on the IRT distribution on a DRL-72 s operant schedule in rat. Refer to legend of Figure 1 for details. Error bars (min value = 0.30; max. value = 4.34) are omitted for clarity. $N=8$ rats per group.

DISCUSSION

This paper is the first to report an activity of two new potential antidepressants, SSR149415, an antagonist at the V1b receptor, and SSR125543, an antagonist at the CRF1 receptor, in a DRL-72 s schedule. These results are consonant with those previously obtained in other tests sensitive to antidepressants (Griebel *et al*, 2002b, c). It is worth noting that another CRF1 receptor antagonist, R278995/CRA0450, has been recently reported to be inactive in the DRL-72 s schedule (Chaki *et al*, 2004). This apparent discrepancy between our result and those of Chaki and collaborators may be explained by the fact that, in addition to the antagonistic activity at the CRF1 receptor, R278995/CRA0450 also showed a high affinity for the Sigma1 receptor. What impact this latter affinity might have on the CRF1 receptor antagonism-induced antidepressant-like effect on DRL-72 s performance remains to be further delineated.

SSR149415 and SSR125543 have shown effects on DRL-72 s parameters very similar to those observed with fluoxetine, by maintaining the bell shape of the IRT distribution curve, and by displacing it toward longer durations (via an increase of the number of reinforced presses and a decrease of the total number of responses). Our results with fluoxetine in this paradigm are in line with those reported by Seiden *et al* (1985).

The few data available on the effects of vasopressin or CRF in DRL schedules indicate a global deleterious effect on operant behavior: in rats submitted to a DRL-60 s, CRF (0.5–1 μg, i.c.v.) decreased the total number of responses, without increasing the number of reinforced responses. The IRT distribution curve was flattened without being displaced toward longer or shorter durations, except that the number of responses in IRT bins >60 s was increased (Britton and Koob, 1989). The suppression of operant responding produced by CRF was confirmed in a fixed-interval/fixed ratio schedule (Ahlers *et al*, 1992). Vasopressin has been tested in a DRL-15 s schedule (van Haaren *et al*, 1986): its main effect was a decrease in the number of responses accompanied by a decrease of delivery of reinforcers (the IRT distribution of responses was not shown). Our results clearly show that a CRF1 receptor antagonist (SSR125543) and a V1b receptor antagonist (SSR149415) also decreased the total number of responses, but contrary to CRF and vasopressin, they maintained the bell shape of the IRT distribution curve and displaced it toward longer durations, as did fluoxetine. The consequence of such an alteration of the temporal timing of responses is an increase in the response frequency cumulated in the IRT bin (49–96 s) for fluoxetine, SSR149415, and SSR125543. The latter two compounds did not affect 'burst responding' (ie responses emitted in the IRT bin (0–12 s)), whereas another antidepressant, imipramine, decreased it (McGuire and Seiden, 1980a, b; Cohen *et al*, 1997). The ability to decrease 'burst responses' does not seem to be essential to support antidepressant activity as fluoxetine does not affect this parameter (our results; Cousins and Seiden, 2000).

The rightward shift in the IRT distribution curve may result from different processes, such as (1) a modification of timing behavior (ability to make temporal discrimination), (2) an enhanced capacity to wait before emitting a response, and/or (3) the ability to cope with the aversive nature of having to wait before obtaining a reward.

Firstly, an alteration of timing behavior induced by SSR149415 and SSR125543 could explain the shift of the distribution of responses toward longer durations. Using a temporal discrimination task, Bizot (1997) showed that clomipramine, a tricyclic antidepressant active in the DRL-72 s schedule (Bayley *et al*, 1998), increased the perceived length of the time during which the light stimulus was presented. This was interpreted by Bizot as resulting from a slowing-down of the speed of the 'internal clock' in antidepressant-treated rats. Whether or not SSR125543 and SSR149415 modify the speed of this 'internal clock' remains to be demonstrated.

Secondly, the antidepressant-induced shift in the IRT distribution toward longer durations, with preservation of the bell shape of the curve, may be due to an enhanced capacity to wait (Soubrié and Bizot, 1990; Bizot *et al*, 1999). Using a task that requires rats to choose between an immediate small reward and a larger but delayed reward in a T-maze, it was found that antidepressants led rats to choose more frequently the larger but delayed reward (Bizot *et al*, 1988). These authors suggested that the effects observed in the T-maze paradigm and in the DRL-72 s schedule may involve a common antidepressant-induced increase in waiting capacity. This latter suggestion can be rejected at least for a V1b antagonist, as in the T-maze paradigm, SSR149415 did not increase the number of choice for the large but 25 s delayed reward (Thiébot, unpublished data). It remains to be determined whether or not SSR125543 has such an ability to enhance capacity to wait in a T-maze task.

Thirdly, SSR149415 and SSR125543 have been shown to be particularly active under stressful conditions (Gully *et al*, 2002; Griebel *et al*, 2002b, c; Serradeil-Le Gal *et al*, 2002). Interestingly, a lack of reinforcement after a response can induce a frustration caused by the non-delivery of an expected reward ('frustrative non-reward'; Dantzer, 1977; Gray, 1977). Thus, it cannot be excluded that daily exposure to DRL-72 s procedure—and its intrinsic 'frustrative non-reward' component—could induce a chronic state of stress accompanied by an alteration of the HPA axis function. Indeed, it has been shown that frustrative non-reward leads to an increase in plasma levels of ACTH and cortisol (in squirrel monkeys) or corticosterone (in rats) (Lyons *et al*, 2000; Romero *et al*, 1995). SSR149415 and SSR125543 might thus normalize the HPA overactivity induced by the DRL-72 s schedule in the same way that they block restraint stress-induced release of ACTH (Gully *et al*, 2002; Serradeil-Le Gal *et al*, 2002), and the physical degradation observed in mice submitted to a chronic mild stress (Alonso *et al*, 2004).

SSR149415- and SSR125543-treated rats did not display a behavioral profile of responses comparable to that of rats treated with the prototypical anxiolytic diazepam, used here as a comparator. This latter compound decreased the total number of responses and increased the proportion of reinforced presses, as did SSR149415 and SSR125543, but the IRT distribution curve clearly lost its bell shape. Responses were more frequent in low IRT bins, as described previously (Sanger and Blackman, 1989; Richards *et al*, 1994), and the frequency of responding progressively declined as the time intervals increased (except at the

sedative dose of 10 mg/kg, for the last IRT bin (>96 s)). Clearly, SSR149415 and SSR125543 are devoid of a benzodiazepine-like activity in the present DRL-72 s schedule, although both compounds have been shown to display anxiolytic-like activity in a variety of animal models (Griebel *et al*, 2002a, c).

The neurochemical mechanisms responsible for the antidepressant-like effects of SSR149415 and SSR125543 in the DRL-72 s schedule are not clear. The idea that the blockade of the HPA axis by SSR149415 and SSR125543 may account for these effects is challenged by findings indicating that extra-hypothalamic structures might also be involved in the antidepressant-like effects of CRF1 receptor antagonists and SSR149415 (Griebel *et al*, 2002b; Stemmelin *et al*, 2005). It is possible that the effects of SSR149415 and SSR125543 on DRL-72 s performance result from an interaction with monoaminergic systems, such as the serotonergic (5-HT) and norepinephrine (NE) neurotransmissions (Carrasco and Van de Kar, 2003; Lowry *et al*, 2000; Day *et al*, 2004; Price *et al*, 1998). It has been demonstrated that the activity of classical antidepressants in DRL-72 s schedules relies on their ability to modulate 5-HT and NE neurotransmission (Britton and Koob, 1989; Richards and Seiden, 1991; Richards *et al*, 1993; Balcells-Olivero *et al*, 1998; Dekeyne *et al*, 2002). Experiments with the CRF1 receptor antagonist CP154,526 have shown that this compound did not alter basal levels of cortical 5-HT, NE, and dopamine, in freely moving rats (Millan *et al*, 2001). Similarly, SSR125543 (10 mg/kg, i.p.) was found not to alter NE extracellular levels in the cortex (Steinberg, personal communication). At present, the exact extent of the implication of these catecholaminergic and indolaminergic neurotransmissions in the activity of SSR125543 is still not resolved.

Recent findings with SSR149415 indicate that it also does not affect tissue or extracellular levels of 5-HT in the hippocampus and the prefrontal cortex (PFC) and levels of dopamine in the PFC, but it increases extracellular levels of NE in the PFC from 10 mg/kg i.p. in the rat (Ring *et al*, 2004; Steinberg, personal communication). It can be speculated that this latter neurochemical effect may be responsible for the activity of SSR149415 in the DRL-72 s procedure, as the specific NE reuptake inhibitor desipramine, which also increases extracellular levels of NE in the PFC (Tanda *et al*, 1996; Mateo *et al*, 1998), is active in DRL schedules (O'Donnell and Seiden, 1983; Richards and Seiden, 1991; Dekeyne *et al*, 2002). This hypothesis needs to be reconciled with the report that SSR149415 blocked the stress-induced release of cortical NE (Griebel *et al*, 2002a, c). This apparent paradox underlines the fact that the mechanisms through which V1b receptor (and also CRF1 receptor) antagonists are active tightly depend on the experimental conditions to which the animal is submitted (ie acute *vs* chronic stress).

In conclusion, the non-peptidic compounds, SSR149415 and SSR125543, antagonists at V1b and CRF1 receptors, respectively, displaced the IRT distribution of response frequency toward longer durations. The mechanism(s) by which these compounds increased time length interval between responses remains to be understood. Nonetheless, these results suggest further that these compounds have a potential therapeutic interest for the treatment of depression and/or disorders related to chronic frustration-induced psychological disabilities.

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