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Antidepressant-like effects of the vasopressin V1b receptor antagonist SSR149415 in the Flinders Sensitive Line rat

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Abstract

There is an increased interest in the potential of vasopressin receptor antagonists as antidepressants because of the involvement of vasopressin in stress-related behavioral changes. The present study sought to provide confirmatory evidence for the antidepressant-like effects of the selective vasopressin V1b receptor antagonist SSR149415, which had been previously demonstrated in a variety of animal models. The Flinders Sensitive Line (FSL) rat, a selectively bred animal model of depression, was chronically treated for 14 days with SSR149415 (1, 10, and 30 mg/kg), vehicle, or desipramine (5 mg/kg) as a positive control. Approximately 22–24 h after the last treatment, the rats were exposed to a single 5-min session in a cylinder containing 25 °C water and immobility was recorded. A control group of Flinders Resistant Line (FRL) rats was included as a reference group as well as one treated with 10 mg/kg SSR149415. Vehicle-treated FSL rats exhibited much more immobility than the FRL rats, and desipramine-treated FSL rats had much lower scores, as expected. Treatment with SSR149415 reduced immobility in the FSL rats at all doses, but only the higher doses reduced it such that they were no longer different from the FRL rats. In contrast, SSR149415 did not alter the lower immobility of the FRL rats. The social interaction test of anxiety was also examined in the FSL rats, at 20–22 h after the last of the 14 injections. Results showed that the 10 and 30 mg/kg doses of SSR149415 increased the time spent in social interaction in the FSL rats, suggesting anxiolytic effects. These findings confirm the antidepressant-like potential of SSR149415 and suggest that it may also have anxiolytic effects. It is likely that the strategy of testing selective vasopressin V1b receptor antagonists will be fruitful.

Keywords: Vasopressin V1b receptor; SSR149415; FSL rats; Animal model of depression; Forced swim test; Desipramine

1. Introduction

There has been increased interest in neuropeptide systems as novel targets for depression and anxiety (Holmes et al., 2003). Arginine vasopressin (AVP) is a nonapeptide that has been implicated in a variety of behavioral processes, including anxiety and depression (Bohus, 1977; de Wied et al., 1974; Dantzer and Bluthe, 1992; Engelmann et al., 1996). Evidence indicates that AVP may exist not only in the hypothalamus, where it participates in the hypothalamic–pituitary–adrenal axis (Engelmann et al., 2004; Volpi et al., 2004), but also in other key brain regions such as the medial amygdala (DeVries and Buijs, 1983). The projections from the amygdala include the lateral septum and ventral hippocampus and AVP's effects are mediated through G protein coupled V1a receptors (Lolait et al., 1995; Morel et al., 1992).

Studies using peptide V1 receptor antagonists have provided supporting evidence for the involvement of AVP in emotional behavior. The mixed V1a/b receptor antagonist d $(CH_2)_5$Tyr(-Et)VAVP had anxiolytic-like effects in the elevated plus maze test in rats after intra-septal administration (Liebsch et al., 1996). Anxiolytic-like effects in the elevated plus maze were also observed after the infusion of an antisense oligodeoxynucleotide to the V1a subtype mRNA into the septum of rats (Landgraf et al., 1995). The fact that V1b mRNA levels in the rat brain were increased following chronic immobilization stress (Aguilera and Rabadan-Diehl, 2000) further strengthens the relationship between AVP and emotional behavior.

Recently, the first non-peptide antagonist of the V1b receptor, SSR149415, was developed; this compound was highly selective for the V1b versus the V1a receptor (60- to

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800-fold) and was inactive at more than 90 other targets (Serradeil-Le Gal et al., 2002). This compound was found to be effective in a modified version of the Porsolt swim test, reducing immobility in a manner comparable to imipramine and fluoxetine after two injections between a 15-min pre-swim and a 6-min test 24 h later (Griebel et al., 2002). The drug was also effective in a chronic mild stress paradigm in mice where the drug was injected chronically (Alonso et al., 2004; Griebel et al., 2002). Thus, two different protocols for detecting antidepressant-like effects provided support for the antidepressant potential of SSR149415.

The present investigation sought to provide even stronger support for the antidepressant potential of SSR149415 by testing its effects in the Flinders Sensitive Line (FSL) rat, an animal model of depression with good predictive validity (Overstreet, 1993, 2002). The FSL rat is innately more immobile in the forced swim test than its control counterpart, the Flinders Resistant Line (FRL) rat, and exhibits a decrease in immobility following chronic, but not acute, treatment with desipramine and sertraline (Pucilowski and Overstreet, 1993). A key aspect of this model is that the putative antidepressants are given chronically for 14 days and the behavioral tests are carried out 24 h after the last treatment. Consequently, the FSL rat responds to other antidepressants but not the psychomotor stimulants amphetamine and scopolamine (see Overstreet, 2002; Overstreet et al., 1995, 2005).

Therefore, the FSL rat model of exaggerated immobility was used in this study to evaluate the antidepressant potential of the V1b receptor antagonist SSR149415 and the tricyclic desipramine.

2. Materials and methods

2.1. Animals

The FSL and FRL rats were selected from breeding colonies maintained at the University of North Carolina Bowles Center for Alcohol Studies. They were housed in groups of three in temperature- and humidity-controlled rooms under a 12:12 light/dark cycle (lights on 0700–1900). Rats were randomly divided into five (FSL) or two (FRL) groups and then given the treatments described below. Fewer FRL groups were used because previous evidence indicated that the FRL rats, which exhibit a relatively low degree of immobility, do not exhibit decreases in immobility following many antidepressant treatments (see Overstreet, 2002; Overstreet et al., 2004a). These experiments were carried out according to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the UNC Institutional Animal Care and Use Committee.

2.2. Treatments

The following five treatment groups were established for the FSL rats: VEH — isotonic saline; SSR — 3, 10, and 30 mg/kg SSR149415; DMI — 5 mg/kg desipramine. The FRL rat groups were treated with VEH or 10 mg/kg SSR. The reason for this asymmetric design is related to the observations that the FRL rat, which is more active in the swim test, does not respond to classical and novel antidepressants with a decrease in immobility (Overstreet et al., 2004a,b; Pucilowski and Overstreet, 1993). The interest here was to determine whether the V1b receptor antagonist would be active in the FRL rats at a dose expected to be effective in the FSL rats. The DMI was dissolved in isotonic saline and the SSR was suspended in carboxymethylcellulose (CMC, 0.5%). The rats were injected i.p. once daily for 14 consecutive days between 0900 and 1100. Previous work indicated that there were no effects of the saline or CMC vehicles on swim test immobility (Overstreet et al., 2004a,b).

2.3. Behavioral tests

Approximately 20 h after the last treatment, FSL rats with the same treatment and similar body weights were placed in a square test arena (60×60 cm, marked with 16 15×15 cm squares on the floor; light level: 30 lux) for the testing of social interaction. This test of anxiety-like behavior (File and Seth, 2003) was included because there is evidence FSL rats spend less time in social interaction than FRL rats and that other antidepressants may increase the time spent in social interaction exhibited by the FSL rats (Overstreet et al., 2004a). The amount of time spent in social interaction (grooming, licking, sniffing, crawling over or under) was recorded during a 5-min session by an experienced observer who was blind to the treatment condition. This measure provides an index of anxiety-like behavior, with more “anxious” rats spending less time in social interaction (File and Seth, 2003; Overstreet et al., 2004a). In addition, a motor activity measure was collected: the total number of lines crossed during the session.

The swim tank was 18 cm in diameter and 40 cm tall. The tank was filled with enough 25 °C water so the rat could not touch the bottom with its hindlegs. The rat was placed in the swim tank for a single 5-min session 22–24 h after the last treatment and the seconds of immobility were scored by an observer blind to the treatment condition and rat strain being tested (Overstreet, 1993; Overstreet et al., 2004a,b; Zangen et al., 1997).

2.4. Statistical analyses

The data for the three measures were summarized into means ± S.E.M. for each of the 7 treatment groups. Graphical representations of the findings were compiled using Prism software. Initially, the data for each measure were subjected to one-way ANOVAs. If the ANOVA revealed significant group differences, follow-up Tukey’s tests were carried out to elucidate the pattern of group differences. The GBstat software package was used for the statistical analyses.

3. Results

The data for the forced swim test are illustrated in Fig. 1. As expected, the FSL rats treated with vehicle were much more immobile than the FRL rats treated with vehicle. Consequently,

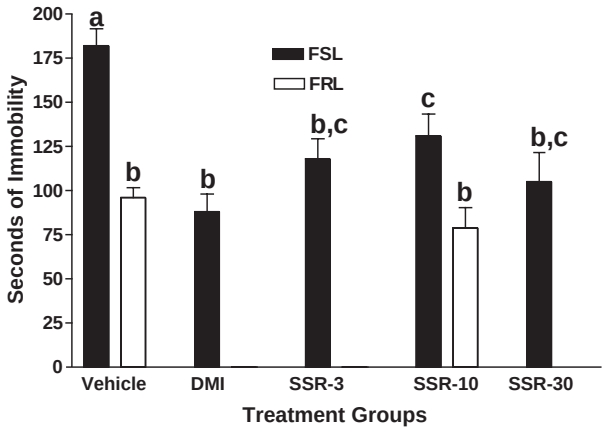

Fig. 1. Swim test immobility in FSL and FRL rats treated chronically (14 days) with vasopressin V1b receptor antagonist SSR149415, desipramine (DMI) or vehicle. Rats were tested for 5 min 24 h after the last of the 14 treatments. Data represent the mean sec±S.E.M. immobility for 8 rats per group. Groups with different letters are significantly different, according to ANOVA and Tukey's protected *t* tests.

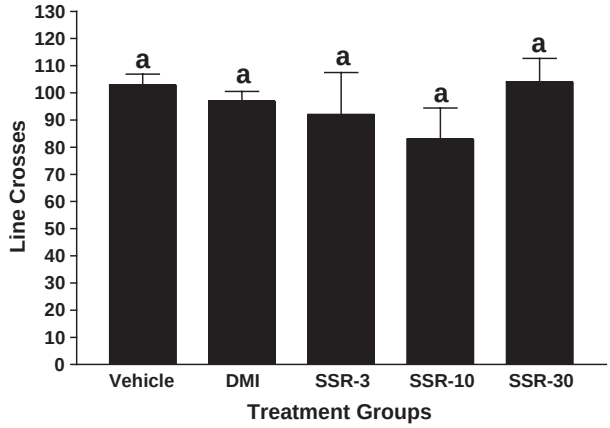

Fig. 3. Line crosses in FSL rats treated chronically (14 days) with vasopressin V1b receptor antagonist SSR149415, desipramine (DMI) or vehicle. Rats were tested as like-treated pairs in the social interaction arena for 5 min 22 h after the last of the 14 treatments. Groups were not significantly different from each other.

the overall one-way ANOVA was highly significant ($F[6,51]=11.12$, $p<0.001$). As expected, DMI significantly reduced immobility in the FSL rats (Fig. 1). Subsequent Tukey's protected *t* tests established that SSR149415 treatment led to a significant decrease in immobility in the FSL rats only (Fig. 1). Thus, this vasopressin V1b receptor antagonist has antidepressant-like effects in the FSL rat model of depression but does not affect the swim test behavior of the FRL rats.

Fig. 2, which illustrates the results for social interaction, indicates that SSR149415 has anxiolytic-like properties as well, as the time spent in social interaction is higher for the 10 and 30 mg/kg doses than it is for the vehicle-treated rats. The overall ANOVA revealed a significant difference among the groups ($F[4,35]=3.47$, $p<0.01$) and Tukey's tests confirmed the pattern of group differences as described in Fig. 2. Note that DMI also increased social interaction, but to a lesser extent than SSR149415 (Fig. 2). The slightly elevated time spent in social interaction was not significantly different from that of the group treated with vehicle, nor those of the groups treated with 10 or 30 mg/kg SSR149415.

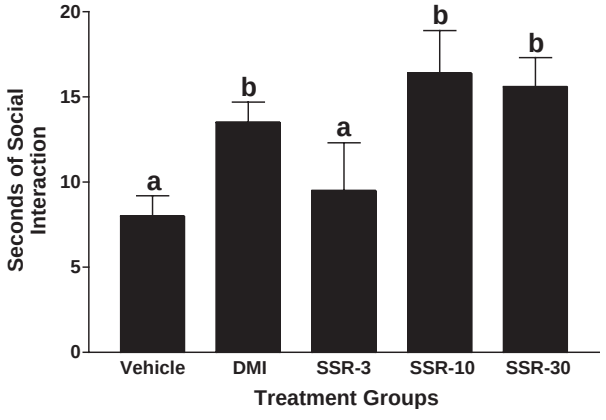

Fig. 2. Social interaction behavior in FSL rats treated chronically (14 days) with vasopressin V1b receptor antagonist SSR149415, desipramine (DMI) or vehicle. Rats were tested as like-treated pairs in the social interaction arena for 5 min 22 h after the last of the 14 treatments. Groups with different letters are significantly different, according to ANOVA and Tukey's protected *t* tests.

Fig. 3 demonstrates that none of the treatments significantly altered locomotor activity in the social interaction test. The ANOVA was not significant ($F[4,35]=0.82$, NS) and all of the groups are remarkably similar. This lack of difference suggests that the differences in swim test immobility or time spent in social interaction cannot be accounted for by differences in general activity. Thus, SSR149415 had relatively selective antidepressant- and anxiolytic-like effects in the FSL rat.

4. Discussion

The findings for the swim test confirm previous conclusions that SSR149415 had antidepressant-like effects (Griebel et al., 2002; Alonso et al., 2004). However, the design used in the present study, which included an animal model of depression, a relatively long-term treatment protocol and the forced swim test 22–24 h after the last treatment, provides convincing data suggesting that adaptive changes produced by SSR149415 underlie the anti-immobility effects. The fact that there were no effects on locomotor activity suggests that a general increase in activity does not occur under the treatment conditions used. A selective antidepressant-like effect appears to have occurred.

The antidepressant-like effects of this V1b receptor antagonist imply that there could be an overactive or up-regulated vasopressinergic system in the FSL rats. Since there is evidence that the cholinergic system positively modulates the vasopressinergic system (e.g., Cavun et al., 2004), an up-regulated system might indeed exist in the FSL rats. Several lines of evidence suggest that the cholinergic hypersensitivity itself cannot account for the exaggerated immobility of the FSL rats (see Overstreet, 2002; Overstreet et al., 2005, for reviews). Studies with other animal models suggest that the amygdala (Ebner et al., 2002), lateral septum (Stemmelin et al., 2005), and/or paraventricular nucleus of the hypothalamus (Wotjak et al., 2001) could be key brain sites to examine for vasopressin differences in the FSL and FRL rats. To date, however, testing

of vasopressin levels and/or vasopressin receptor concentrations in select brain regions of the FSL rat has not been carried out. The FSL rats do show decreases in neuropeptide Y, another peptide that has been implicated in depression (Jimenez-Vazquez et al., 2000; Overstreet, 2002).

Recent studies in humans confirm the need for further study of the vasopressinergic system in FSL rats. For example, one genetic study provided evidence for a specific haplotype of the V1b gene that offered protection against depressive disorders (van West et al., 2004). A biochemical study that focused on the suprachiasmatic nucleus (SCN) reported increased vasopressin neurons but reduced mRNA (Zhou et al., 2001). This finding provided mixed support for the hypothesis of the authors that there should be reduced vasopressin in the SCN because of circadian abnormalities in depressed individuals. The work with rodents (Aguilera and Rabadan-Diehl, 2000; Landgraf et al., 1995; Griebel et al., 2002) suggests that the central vasopressin system may be overactive in depressed individuals. However, although there have been reports of elevated plasma vasopressin in depressed individuals (van Londen et al., 2001), there do not appear to be any other brain site studies than the report on the SCN (Zhou et al., 2001), so we know little about central vasopressinergic systems in depressed individuals. Thus, a comprehensive examination of regional brain vasopressin systems in the FSL rat, an animal model of depression, could provide valuable new information.

These findings are also consistent with a range of other reports of antidepressant action in the FSL and FRL rats (see Overstreet, 2002, Overstreet et al., 2005, for reviews). For example, the psychomotor stimulants amphetamine and scopolamine, which produce false positives in the standard swim test (Borsini and Meli, 1988), do not alter swim test immobility in the FSL rats using the treatment conditions used here (Overstreet et al., 1995). It is also important to emphasize that the swim test immobility of the FRL rats was not altered by treatment with 10 mg/kg SSR149415 (Fig. 1), nor with citalopram or CP-154,526, a corticotrophin releasing factor (CRF) receptor antagonist (Overstreet et al., 2004a), nor with desipramine (Overstreet and Griebel, 2004).

The increase in social interaction observed in the SSR149415-treated groups is consistent with an anxiolytic action. This finding supports a previous report indicating an anxiolytic profile for SSR149415 (Griebel et al., 2002). However, the previous studies employed predominantly acute protocols with the behaviors being assessed shortly after the drug was injected. In the current protocol the behavior was assessed about 20 h after the last treatment. Thus, an adaptive change has occurred that permits the FSL rats to engage in more social behavior. Note that SSR149415 was more effective than DMI in its anxiolytic profile, suggesting that it might have therapeutic potential in a wider variety of patients. It was previously shown that DMI did not significantly alter entries into the center of an open field arena, another index of anxiety-like behavior (Overstreet et al., 2004a). This finding may account for why DMI has only shown efficacy as an antidepressant drug (McLeod et al., 2000; Sasson et al., 1999). Because many depressed individuals may also experience anxiety, SSR149415 may be more widely used.

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