

## AVP V<sub>1b</sub> selective antagonist SSR149415 blocks aggressive behaviors in hamsters

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### Abstract

Arginine vasopressin (AVP) has been implicated in a variety of physiological and behavioral responses to stress. Synthesis of receptor-selective AVP agonist and antagonist compounds allows differential analysis of the specific roles of particular receptor subtypes with respect to these responses. Here, effects of the recently synthesized AVP V<sub>1b</sub> selective antagonist, SSR149415, were examined for offensive aggression in male Syrian hamsters, using a resident-intruder paradigm. Oral administration of vehicle or 1, 10, or 30 mg/kg of SSR149415 to resident hamsters was followed by evaluation of a range of aggression-related measures of residents confronted by intruders. The 10 and 30 mg/kg doses significantly reduced the duration of offensive sideways and chase behaviors, and the 30 mg/kg dose also reduced chase frequency. The 10 and 30 mg/kg dose also significantly reduced frequency and duration of olfactory investigation and duration of flank marking. These findings suggest a link between activity of the V<sub>1b</sub> receptor and the modulation of offensive aggression. These findings agree with previous research on V<sub>1b</sub> receptor effects in suggesting that antagonism of this receptor may be useful in modulating a range of emotional responses to highly stressful or threatening conditions.

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### 1. Introduction

Vasopressin (AVP), a nine-amino acid neurohypophyseal neuropeptide, acts as a neurotransmitter and neurohormone in a wide variety of autonomic and behavioral responses to stress (Aguilera and Rabadan-Diehl, 2000). AVP has been consistently implicated in the aggressive behaviors of a variety of species, including humans (Coccaro et al., 1998). In rodents, AVP infusions into the hypothalamus or amygdala enhance aggression in hamsters and rats (Bamshad and Albers, 1996; Delville et al., 1996a,b; Ferris et al., 1984), while AVP receptor antagonist infusions reduce

aggression (Ferris and Potegal, 1988). Similarly, central administration of AVP agonists and antagonists enhances or reduces, respectively, the scent marking (flank marking) that is particularly (Ferris et al., 1986), although not exclusively, associated with dominance in male–male interactions in many rodent species (Albers and Ferris, 1986; Bamshad and Albers, 1996; Ferris et al., 1985, 1988, 1993).

The action of AVP involves several receptor subtypes. Of these, AVP V<sub>1a</sub> has been more widely used in animal models, in part due to the availability of a range of orally active antagonists for this receptor (Serradeil-Le Gal et al., 2002a). However, a recent study (Wersinger et al., 2002) of vasopressin V<sub>1b</sub> receptor knockout mice has reported robust reductions in aggressive behavior for these animals. The V<sub>1b</sub> knockout mice also showed a less robust reduction in social investigation, significant only in the context of a stringent

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test involving familiar versus unfamiliar conspecific females. Wild-type and knockout mice were not different on a range of tests of sensory (olfaction, vision) and motor function, anxiety (open field, elevated plus maze), and spatial memory (Morris water maze), suggesting that AVP  $V_{1b}$  antagonists may be capable of modulating aggressive behavior with minimal impact on other behaviors.

SSR149415 is the first selective, nonpeptide vasopressin  $V_{1b}$  receptor antagonist (Serradeil-Le Gal et al., 2002b). It is orally active and shows competitive nanomolar affinity for animal and human  $V_{1b}$  receptors, with much lower affinity for rat and human  $V_{1a}$ ,  $V_2$ , and oxytocin receptors. Both in vitro and in vivo SSR149415 potently inhibited AVP-induced activity and reduced emotion-linked behavior in a variety of mouse models after both acute and repeated administration (Griebel et al., 2002). In particular, SSR149415 produced clearcut anxiolytic-like activity in models involving traumatic stress exposure, such as the social defeat paradigm and the mouse defense test battery, and reduced responsiveness to stress in the forced swimming test and the chronic mild stress model. Such findings emphasize a potential role for the  $V_{1b}$  receptor in the modulation of emotion-linked responding to stressful stimuli and suggest the value of analysis of the effects of SSR149415 on aggression.

## 2. Method

### 2.1. Animals

Seventy-three adult male Syrian hamsters (*Mesocricetus auratus*, Charles River Laboratories) were used as subjects. Hamsters to be used as residents were housed individually for at least 2 weeks prior to the beginning of the experiment. Thirty-three smaller males were used as intruders. Intruders were group-housed (three/cage) in order to minimize aggression levels. There was a minimum 10-g weight difference between resident and intruder pairs. The weight range for residents was between 105 and 150 g, and intruder weights ranged from 95 to 140 g.

Animals were housed in Plexiglas cages (46.0×24.0×21.0 cm) with corn cob bedding in a temperature (69 °F) and humidity-controlled room with food and water available ad libitum. The colony room was maintained on a 14:10 light–dark cycle with lights off at 12:00 noon. Tests were run under red light illumination during the first 3 h of the dark phase of the light–dark cycle. All animals were handled daily for 10 days prior to the start of the study.

### 2.2. Drug

The vasopressin antagonist (SSR149415) was suspended in a solution of DMSO (5%) and cremophor (5%) and administered orally by gavage at doses of 1 ( $n=10$ ), 10 ( $n=10$ ), and 30 mg/kg ( $n=10$ ). The vehicle control group contained nine animals.

### 2.3. Procedure

A single nondrug screening test (resident–intruder) was run with each individually housed hamster to determine the baseline levels of aggression of the animal. Residents were transported to the testing room in their home cage and left undisturbed for a 5-min habituation period. An intruder was then placed into the home cage of the resident for a 10-min test period. Only resident males that showed a minimum of one bite during the test session were used in the drug test.

Tests with SSR149415 were run 48 h after the screening test. Twenty-five minutes after drug administration, residents were moved to the testing room. Intruders were introduced into the resident home cage 5 min later, for a 10-min test. Each resident was confronted with an intruder that was novel to that resident.

The protocols used in this experiment were in compliance with the regulations of the Institutional Animal Care and Use Committee at the University of Hawaii.

### 2.4. Behavioral measures

Behaviors that were scored live included attack latency, latency to bite, and number of bites.

- I. Offensive sideways (lateral attack): sideways movement toward the intruder. In the hamster this may include movement from a lateral posture with the flanks touching the floor.
- II. Bite: bites were counted if an offensive sideways, chase, or on-top-of behavior of the resident, involving proximity of the resident's snout to the body of the intruder, was accompanied by a loud, sharp vocalization by the latter (pinch vocalization).
- III. On-top-of: the subject stands over a supine or prone intruder.
- IV. Latency to attack: time until residents exhibited offensive sideways, biting, or on top of the intruder.
- V. Flank marking by the resident: rubbing the flanks against objects in the environment (e.g., Ferris et al., 1993).

Behaviors were also videotaped and scored by experienced behavior raters using a computerized scoring system ("Hind Sight"; developed by Dr. Scott Weiss). These behaviors included the frequency and duration of olfactory investigation, chasing, offensive sideways, and flank marking by the resident. Pinch vocalization could not be scored on the videotapes because there was no audio component to these tapes, precluding an indication of vocalization.

### 2.5. Statistical analysis

Data were analyzed by one-way ANOVA, followed by Newman–Keuls post hoc tests.

### 3. Results

#### 3.1. Screening tests

Thirty-four of the resident hamsters failed to bite during the no-drug screening test and were omitted from subsequent testing.

#### 3.2. Drug tests

##### 3.2.1. Chase

Drug differences were significant for both the frequency and duration of chase  $F(3,35)=5.02$ ,  $p<0.005$  and  $F(3,35)=2.90$ ,  $p<0.05$ , respectively. Newman–Keuls post hoc analyses indicated that the 10 and 30 mg/kg doses produced significant reductions in frequency and duration of chasing, compared to either the vehicle control or the 1 mg/kg dose ( $p<0.05$  or less for each difference; Fig. 1A,B).

##### 3.2.2. Offensive sideways (lateral attack; Fig. 1C,D)

ANOVA indicated significant main effects of drug for frequency and duration of offensive sideways behavior  $F(3,35)=2.96$ ,  $p<0.05$  and  $F(3,35)=7.62$ ,  $p<0.0005$ , respectively. Post hoc analyses indicated that the 10 and 30 mg/kg

dose groups showed reductions in both measures, compared to the vehicle control and 1 mg/kg animals ( $p<0.05$  or less in each case).

##### 3.2.3. Olfactory investigation

Drug effects were significant for both number and duration of olfactory investigations by the resident  $F(3,35)=19.39$ ,  $p<0.00001$ ;  $F(3,35)=24.07$ ,  $p<0.00001$ , respectively. Post hoc analyses indicated that both the 10 and the 30 mg/kg groups showed reductions in this measure compared to the vehicle control and the 1 mg/kg dose groups ( $p<0.001$  or less in each case; Fig. 2A,B).

##### 3.2.4. Flank marking (Fig. 2C,D)

ANOVA indicated a significant effect of drug on the duration of flank marking  $F(3,35)=5.73$ ,  $p<0.005$ . Post hoc analysis showed that both the 10 and 30 mg/kg groups spent significantly less time flank marking than the vehicle control or 1 mg/kg animals ( $p<0.01$  or less in all cases). The drug effect on frequency of flank marking was not significant.

Of the remaining measures (Table 1), attack latency and on-top frequency and duration all showed substantial changes with the higher doses, in each case, suggestive of decreased aggression for animals given SSR149415. How-

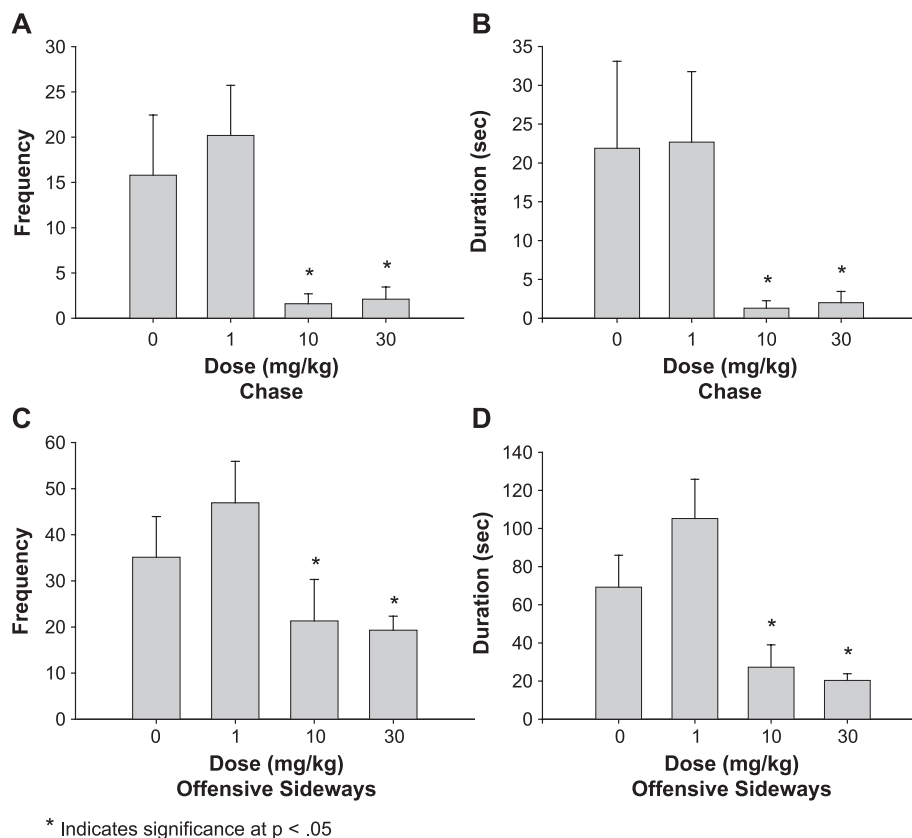


Fig. 1. Frequency and duration of chase and offensive sideways (lateral attack) behaviors for resident hamsters given vehicle or 1, 10, or 30 mg/kg SSR149415 and confronted by an intruder into their home cages. SSR149415 (10 and 30 mg/kg) significantly decreased frequency and duration of chase and frequency and duration of offensive sideways.

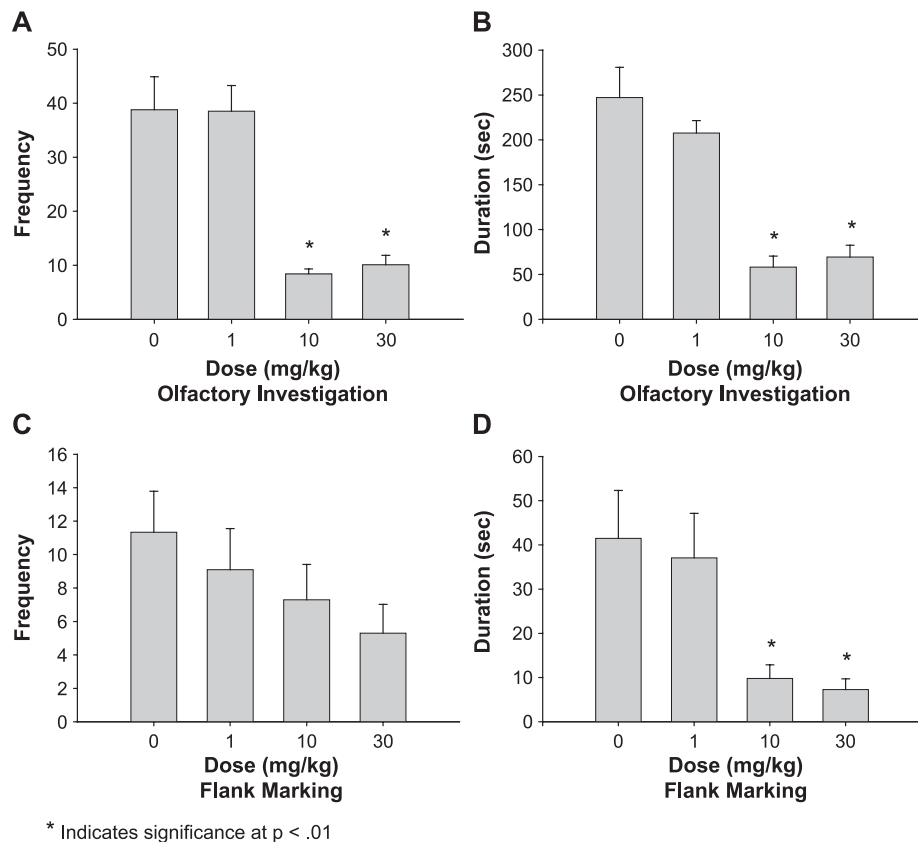


Fig. 2. Frequency and duration of olfactory investigation of the intruder and flank marking for resident hamsters given vehicle or 1, 10, or 30 mg/kg SSR149415. SSR149415 (10 and 30 mg/kg) significantly decreased frequency and duration of olfactory investigation and duration but not frequency of flank marking.

ever, none of these measures showed a statistically significant main effect of drug administration.

#### 4. Discussion

SSR149415 produced a consistent antiaggression effect at both of the higher doses (10 and 30 mg/kg), reducing frequency and duration of offensive sideways, and chase, as well as olfactory investigation and flank marking, behaviors that commonly precede and accompany offensive attack by resident hamsters. As some active behaviors did not decline with SSR149415, these effects do not appear to reflect activity reduction or sedative effects, nor have central sedative effects been reported in other studies using this compound (Griebel et al., 2002).

Ferris et al. (1986) reported that AVP antagonist-mediated reductions in flank marking for dominant hamsters are associated with enhanced flank marking for their untreated subordinate opponent, suggesting that flank marking during male encounters is an important component of dominance behavior rather than a simple marker of the animal's presence. Other investigators have described flank marking as a communicative behavior within the agonistic context (Hennessey et al., 1992), suggesting a role in the communication of dominance. Thus, the present reductions in flank marking are in agreement with chase and offensive sideways reductions, suggesting a decrement in the communicative as well as the affective components of aggressive/dominance behavior.

These findings are in agreement with a recent report that SSR149415 reduced the duration of fighting in an isolation-

Table 1

Mean scores of behaviors observed during resident–intruder pairing after administration of vehicle solution or 1, 10, or 30 mg/kg SSR149415

Behavior	0 mg/kg	1 mg/kg	10 mg/kg	30 mg/kg
On-top frequency	5.56±2.47	2.60±0.91	1.10±0.64	2.20±0.88
On-top duration	14.72±10.00	4.35±2.53	3.23±1.99	5.50±2.46
Attack latency	38.44±11.75	86.60±57.30	119.20±56.75	81.60±32.15
Pinch vocalization latency	106.22±63.02	110.30±58.24	182.50±51.99	108.20±30.30
Pinch vocalization number	6.67±3.11	9.20±2.28	14.20±6.38	9.90±1.57

induced aggression paradigm in mice (Griebel et al., 2003). They are also concordant with reports (Wersinger et al., 2002) that mice deficient in the vasopressin  $V_{1b}$  receptor show reduced aggression. They also agree with a body of previous studies reporting that infusion of AVP into the anterior hypothalamus, lateral septum, bed nucleus of the stria terminalis, central gray, or amygdala increases the rate of aggressive behaviors or flank marking in hamsters (Bamshad et al., 1997; Ferris et al., 1984, 1985, 1990, 1994, 1997, 1999), and that AVP antagonists or lesions in these areas reduce aggression or flank marking in response to application of the agonist (Bamshad and Albers, 1996; Ferris and Potegal, 1988; Ferris et al., 1985, 1990, 1993). This association between vasopressin and aggression has also been confirmed in other species, including mice (Compaan et al., 1993) and rats (Everts et al., 1997). It is particularly interesting to note that a history of abuse/stress in young hamsters produces selective changes in attack; reduced toward same-sized conspecific opponents but enhanced toward younger males, and that these changes are associated with reduced density of vasopressin neurones and fibers in the hypothalamus (Ferris, 2000), suggesting that vasopressin's enhancement of aggression may be limited to offensive aggressive behavior.

While it has long been known that  $V_1$ -like receptors are involved in modulating the effects of AVP infusion on aggressive behaviors (Albers et al., 1986), studies using receptor subtype-selective AVP antagonists to investigate flank marking have concentrated on the  $V_{1a}$  receptor (e.g., Bamshad and Albers, 1996). The present data indicate that the  $V_{1b}$  receptor is also involved, and that antagonism of this receptor produces a sharp and consistent reduction in both aggressive behaviors and in the olfactory investigation and flank marking that accompany it.

While the Wersinger et al. (2002) study failed to find differences in initial durations of social/olfactory investigation of female conspecifics, the sexual behaviors of the  $V_{1b}$  receptor knockout mice in that study were also normal, suggesting that inactivity of  $V_{1b}$  receptors does not have a general impact on olfaction per se, but instead produces changes in particular behavior systems. This view is supported by Wersinger et al.'s additional (2002) suggestion, based on findings of relatively normal *c-fos* expression in chemosensory-responsive regions in their  $V_{1b}$  receptor knockout mice, that deficits in chemosensory detection and transmission are not responsible for the behavioral changes of these animals.

A number of previous studies have found vasopressin antagonists to disrupt olfactory memory (see Engelmann et al., 1996 for review). In particular, a social recognition memory paradigm, typically involving use of a prepubertal male as the social stimulus, has been developed to evaluate olfactory recognition and its memory trace (e.g., Dantzer, 1998; Le Moal et al., 1987). AVP agonists enhance, while antagonists disrupt this memory in male (Bluthe and Dantzer, 1993; Bluthe et al., 1990; Dantzer, 1998; Dantzer

et al., 1988; Le Moal et al., 1987), but not female (Bluthe and Dantzer, 1990) rats. However, the relevance of these studies to the present finding is unclear. The typical measure of social recognition memory is a decrease in the duration of olfactory investigation of the social (prepubertal male) stimulus during a second exposure to the same animal at a fixed time after the first exposure. Such ratio measures are hard to compare to the results of a single exposure, and a reduced olfactory investigation time during the latter might signify either a decrease or an increase in the investigating animal's ability to process such olfactory stimuli, or, indeed, in its motivation to do so. In addition, these studies involved manipulations that were not specific to the  $V_{1b}$  receptor. Thus, although vasopressinergic mechanisms do appear to be involved in relatively short-term ( $\approx 45$  min) social recognition memories, this phenomenon does not implicate olfactory deficits in the reduced aggression associated with  $V_{1b}$  receptor antagonism.

Finally, the effects of SSR149415 on aggressive behaviors in hamsters provide parallels to the effects of this compound in other tests of responsivity to high level stress, including reductions in anxiolytic-like activity in the Mouse Defense Test Battery and the Social Defeat paradigm; and in measures of depression-like behaviors in the Chronic Mild Stress model (Griebel et al., 2002). Notably, it did not display a clear benzodiazepine-like profile in some additional tests involving milder stressors, such as the Elevated Plus Maze (Griebel et al., 2002, 2003). These findings suggest that the  $V_{1b}$  receptor may be particularly involved in responsivity to high-level stressors, and that antagonism of these receptors may be useful in modulating a variety of such responses.

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