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Comparison of the V1b antagonist, SSR149415, and the CRF1 antagonist, CP-154,526, in rodent models of anxiety and depression

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Abstract

Vasopressin and corticotropin releasing factor (CRF) are both critical regulators of an animal's stress response and have been linked to anxiety and depression. As such, antagonists of the CRF1 and V1b receptor subtypes are being developed as potential treatments for affective disorders. The two most characterized V1b and CRF1 antagonists are SSR149415 and CP-154,526, respectively, and the present studies were designed to compare these two compounds in acute animal models of affective disorders. We employed five anxiety models: Separation-induced pup vocalizations (guinea pig and rat), elevated plus-maze (EPM), conditioned lick suppression (CLS), and marble burying (mouse); as well as three depression models: forced swim test (FST; mouse and rat) and tail suspension test (TST; mouse). SSR149415 (1–30 mg/kg) was active in the vocalization, EPM and CLS models, but inactive in marble burying. CP-154,526 (1–30 mg/kg) was active in vocalization models, but inactive in EPM, CLS, and marble burying. SSR149415 was inactive in all depression models; CP-154,526 was active in rat FST but inactive in mouse models. This work demonstrates the different profiles of V1b and CRF1 receptor antagonists and supports both approaches in the treatment of affective disorders.

Keywords: Vasopressin; V1b receptor; Corticotropin-releasing factor; CRF-1 receptor; HPA axis; Anxiety; Depression; Behavior; Rat; Mouse; Guinea pig

1. Introduction

The hypothalamic–pituitary–adrenal (HPA) axis regulates an animal's response to stress. Two key regulators of the HPA axis, vasopressin and corticotrophin-releasing factor (CRF) are released during stressful events and bind to receptors in the pituitary. There they synergistically trigger the release of adrenocorticotropin hormone (ACTH), which is circulated in the bloodstream to the adrenal glands. The stress hormone corticosterone (cortisol in primates) is released by the adrenal glands and binds to receptors in the pituitary, limbic structures and the hypothalamus. Sustained elevation of HPA activity is considered a causal factor in human affective disorders (Dinan, 1994; Gold et al., 1988). Dampening HPA axis activity has been hypothesized to be a potential avenue for the treatment of affective disorders (Holsboer, 1999).

There is strong evidence from human studies for a link between affective disorders and vasopressin and CRF. For example, high numbers of both vasopressin- (Purba et al., 1996) and CRF-expressing (Raadsheer et al., 1994) neurons have been reported in post mortem analyses of depressed patients as compared to controls. Also, relative to healthy controls, depressed patients have elevated concentrations of both vasopressin in plasma (Van Londen et al., 1997) and CRF in cerebrospinal fluid (CSF) (Nemeroff et al., 1984). Bremmer et al. (1997) reported that patients suffering from post-traumatic stress disorder have elevated levels of CRF in their CSF. Finally, antidepressant treatment has been shown to lower CSF concentrations of CRF in depressed patients (Nemeroff et al., 1991).

More recently, there has been increasing evidence to support central, non-HPA effects of both vasopressin and CRF.

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Neuroanatomically, vasopressin (Hernando et al., 1998) and CRF receptors (Primus et al., 1997) are localized in brain regions associated with anxiety and depression. Using in vivo studies, Appenrodt et al. (1998) demonstrated that central administration of vasopressin induced anxiety-like behavior, and this effect is intact in hypophysectomized animals (Appenrodt and Schwarzberg, 2000). Similarly, there is clear evidence of a central role for CRF in anxiety (see Zorrilla and Koob, 2004). For example, rodents that receive central administration of CRF (Dunn and Berridge, 1990) have been reported to have a high anxiety-like profile. Collectively, these studies suggest an important role of central receptors in mediating the effects of vasopressin and CRF.

In terms of endogenous receptors, four G-protein-coupled receptors are known to bind vasopressin: V1a, V1b, V2, and oxytocin; while two distinct subtypes of the CRF receptor have been identified: CRF1 and CRF2. Of these, the CRF1 and V1b receptor subtypes have been most thoroughly investigated and data from both pharmacological and non-pharmacological studies support the involvement of both receptors in anxiety-like and depression-like behaviors in rodents. For example, chronic stress is known to increase depression and stunt hippocampal neurogenesis, but this effect is blocked with chronic administration of either a V1b or a CRF1 antagonist (Alonso et al., 2004). As well, CRF1 knockouts have reduced anxiety relative to controls (Heinrichs et al., 1997; Smith et al., 1998). Pharmacologically, antagonism of either CRF1 or V1b receptors has been investigated pre-clinically and clinically for possible utility in treating affective disorders.

Preclinical studies also support the hypothesis that centrally located receptors mediate the anxiolytic-like and antidepressant-like effects of V1b and CRF1 receptor antagonists. Salomé et al. (2006) have demonstrated that central administration of a V1b antagonist can reduce anxiety-like and depression-like behavior. Additionally, Okuyama et al. (1999) have demonstrated that the anxiolytic-like effects of a variety of CRF1 antagonists are seen at doses lower than those required to blunt stress-induced elevations in ACTH. Jointly, these data point to a clear contribution of central receptors in the efficacy of both V1b and CRF1 receptor antagonists.

The two most extensively investigated V1b and CRF1 receptor antagonists are SSR149415 and CP-154,526, respectively. SSR149415 is potent at both the human and rat V1b receptors (Griffante et al., 2005) and in the rat, SSR149415 is highly selective over the V1a, V2 and oxytocin receptor subtypes (Griebel et al., 2002b). In terms of efficacy, SSR149415 has been shown to increase licks in a punished lick assay and time spent in the open arms of an elevated plus-maze (EPM) in rats (Griebel et al., 2002a). In the forced swim model (FST) of depression, SSR149415 significantly decreases the time rats spend immobile (Griebel et al., 2002a; Overstreet and Griebel, 2005). For a review of the effects of SSR149415 in animal models of anxiety-like behavior see Griebel et al. (2005). CP-154,526 is selective at the CRF1 receptor subtype with an affinity of 5.7 nM (rat) and has good brain penetration (Schulz et al., 1996). CP-154,526 has also been widely characterized across numerous animal models of anxiety. For example, it increases the amount of time that rats spend in the open arms of the EPM (Griebel et al., 1998; Lundkvist et al., 1996) and reduces separation-induced vocalizations in rat pups (Kehne et al., 2000). Antalarmin, a structurally similar CRF1 antagonist, lowers separation-induced calls in guinea pig pups (Griebel et al., 2002b). Results have varied in punished licking conflict tests. Griebel et al. (1998) tested CP-154,526 up to 20 mg/kg and reported no effect; Millan et al. (2001) on the other hand reported a significant increase in punished licking (at 80 mg/kg). In depression models, Overstreet et al. (2004) reported that CP-154,526 decreased immobility in the FST using Flinders Sensitive Line rats and Griebel et al. (1998) reported a similar effect using CD rats. For a review of the effects of CP-154,526 in animal models of anxiety and depression see Seymour et al. (2003).

The data collected to date for SSR149415 and CP-154,526 are from a variety of labs using different conditions and methods to assess compounds. Here, we directly compare these two compounds in animal models of anxiety and depression. Rat and guinea pig pup separation-induced vocalizations, rat EPM, rat conditioned lick suppression (CLS) and mouse marble burying were used as models of anxiety. Rat and mouse FST and a mouse tail suspension test were used as models of depression. The aim of these studies was to compare the profiles of these two novel approaches for the treatment of anxiety and depression.

2. Methods

2.1. Animals

Male CD rats were used in the EPM, FST (weighing 180–280 g) and the CLS assay (500–800 g). For the rat pup USV assay, male and female CD rats (7–10 days old weighing 25–30 g) were used. Male and female Hartley Guinea pig pups aged 5–21 days old were used for the guinea pig pup vocalization studies. Male CD1 mice (25 g) were used in the marble burying, forced swim and tail suspension tests. All animals were obtained from Charles River Laboratories and were naïve at the time of the study, except the CLS animals, which were pre-conditioned prior to testing. The animals were group housed for all studies except the CLS for which the animals were single housed. All studies were conducted during the light phase of a 12 h light cycle (lights on 7 a.m., lights off 7 p.m.). Food and water were available *ad libitum*; CLS rats were restricted to 1 h of water each day to ensure motivation to lick for a 0.2% saccharin solution. Animal care and testing procedures were conducted in conformity with the Schering-Plough Institutional Animal Care and Use Committee, and in compliance with the NIH "Guide to the Care and Use of Laboratory Animals" and the Animal Welfare Act.

2.2. Drugs

SSR149415, synthesized by the Chemical Research at the Schering-Plough Research Institute, was suspended in 0.4% Tween 80. CP-154,526 (Synchem Inc.) was dissolved in 1% 2-

hydroxypropyl-beta-cyclodextrin. Chlordiazepoxide hydrochloride (CDP; Sigma Chemical Co., St. Louis, MO) and imipramine hydrochloride (MP Biomedicals, Inc., Aurora, OH) were administered in 0.9% saline. All drugs were delivered intraperitoneally at 2 ml/kg for the rat and guinea pig, and 10 ml/kg for the mouse. SSR149415 was administered 15 min prior to testing; CP-154,526, CDP, and imipramine were administered 30 min prior to testing. The optimal route of administration and pre-treatment time for SSR149415 were determined by pharmacokinetics (PK) studies in rats conducted at the Schering-Plough Research Institute. These PK studies demonstrated that administering SSR149415 via the IP route in a 1% Tween 80 vehicle gave the highest plasma and brain levels 15 min post-administration (plasma: 996 ng/ml; brain: 19 ng/g; see Fig. 1). Route, vehicle, and pre-treatment time for CP-154,526, CDP, and imipramine were chosen based on previously published work. All doses are expressed as free base.

2.3. Vasopressin binding assays

Competition binding assays were performed using 5–10 μg of membrane protein from CHO-K1 cells over-expressing the guinea pig, human, mouse or rat V1a, V1b, V2 or oxytocin receptor, 2 nM [^{3}H]Vasopressin, 8-arginine (for the V1a, V1b and V2 receptors) or [^{3}H]Oxytocin (for the oxytocin receptor) and SSR149415 (10 pM–10 μM) in 50 mM BisTrisPropane, pH 7.4, 5 mM $MgCl_2$ and 0.1% BSA (200 μl total volume). Non-specific binding was determined in the presence of 1 μM AVP or oxytocin. The reaction mixtures were incubated for 60 min at room temperature, and then filtered through Millipore MAFC glass fiber filter plates presoaked in 0.5% (v/v) polyethyleneimine. The filters were washed three times with 150 μl of ice cold 50 mM BisTrisPropane, pH 7.4, and filter-associated radioactivity was measured in a Packard TopCount scintillation counter.

2.4. Anxiety studies

2.4.1. Rat pup ultrasonic vocalizations (USVs)

To induce calls, pups were placed individually into a glass beaker inside a sound-attenuating chamber for 10 min while calls were being recorded. Atop the beaker a Noldus Mini-3 bat detector recorded their calls. Calls were digitized with a Noldus audiofilter and the number and duration of calls were recorded and quantified with Ultravox software (Noldus Information Technology, Leesburg, VA). Call number was measured as a count of USVs an animal made during the 10 min test; call duration was defined as the cumulative time that the animals spent calling during the 10 min test. Because of inter-pup variability, before each test session, an experimenter listened to the calls manually and set the range of the detector to optimize call detection for the animal.

Twenty-four hours prior to testing, each pup was pre-screened for 10 min. Animals with fewer than 100 vocalizations were excluded from the study. The animals were then assigned to groups based on the number of calls to ensure that high and low callers were evenly distributed across the groups. On test days, animals were dosed and returned to the litters for the appropriate pre-treatment time. Following USV recording, the extent of drug-induced sedation was measured by assessing righting reflex and negative geotaxis latencies. Righting reflex latencies were generated by placing the animals on their backs and manually timing the latency to return all four paws to the ground. Negative geotaxis latencies were generated by placing the animal on an inclined plane (25°) such that their heads were facing directly down and manually recording the latency to turn 90° such that all four paws were perpendicular to the edge of the plane.

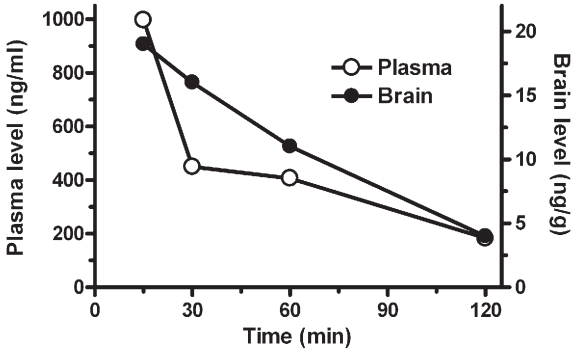

Fig. 1. Pharmacokinetic profile of SSR149415 following IP administration in the rat in a 1% Tween 80 vehicle. Animals were dosed at 10 mg/kg. Left axis represents plasma level (open circle) and right axis represents brain levels (filled circle). $n=6$ rats per time point.

2.4.2. Guinea pig pup vocalizations

Guinea pig pups were separated from their mothers and littermates for weighing and dosing then returned. Following the appropriate pre-treatment time, the pups were removed from their mothers and taken to a separate room. Their calls were counted manually for 5 min by an observer blind to the drug treatment of the animals.

2.4.3. Elevated plus-maze (EPM)

Following administration of the test compound, rats were placed at the center of a black plastic EPM constructed in house. The arms of the maze measured 50 cm in length, 8 cm in width, and were elevated 70 cm above the floor. The closed arms were enclosed with plastic on three sides measuring 40 cm in height. Activity on the maze was monitored for 5 min by a camera mounted directly above the maze. Ethovision software was used to analyze activity (Noldus Information Technology). Between animals the maze was cleaned. The room in which the studies were conducted was illuminated in red light, which measured 40 lx in the open arms and 13.5 lx in the closed arms.

2.4.4. Conditioned lick suppression (CLS)

Water-restricted rats were initially trained daily (20 trials per day) to lick a spout for a 0.2% saccharin solution. During each 30 s trial, a tone was played for 7 s. During the final 5 s of the tone, each lick produced a mild foot shock (intensity: 0.7 mA; duration: 0.5 s). This procedure was followed until all rats learned to reliably suppress licking during the 7 s punished phase.

Table 1
Binding affinities of SSR149415 at the vasopressin receptor subtypes

Receptor	Species	Ki (nM)
V1a	Human	22±4
	Mouse	1446±303
	Rat	613±114
V1b	Guinea pig	0.8±0.1
	Human	0.6±0.02
	Mouse	0.7±0.1
	Rat	1±0.3
V2	Human	325±61
	Rat	160±26
Oxytocin	Human	19±2
	Mouse	398±15
	Rat	40±4

Each value is the average of three determinations, each performed in duplicate. Data are mean±SEM.

On test days, the 20 trials were identical to training trials with the exception that the shock was turned off during the 7 s punished phase. The number of "punished" and unpunished licks was recorded automatically with a lickometer. All studies were conducted using a within-groups design. The order of treatment was counter-balanced across the rats. Between test days, the animals were given a minimum two-day washout period.

2.4.5. Marble burying

Mice were placed in a Plexiglas container (46 cm long by 24 cm wide) in which fine sawdust was placed to a depth of 5 cm. Fifteen marbles (1 cm diameter) were placed so that they rested on top of the sawdust and were evenly spaced. Mice were placed individually into the containers and left undisturbed for 60 min, following which they were removed and an observer blind to the drug condition of the animals counted the unburied marbles. Marbles were considered buried if two-thirds, or more, of the surface area was covered in sawdust.

2.5. Depression assays

2.5.1. Forced swim test (FST) — rat

The forced swim procedure took place over a two-day period. On day one, the animals were placed in a swim chamber (height: 54 cm; diameter: 24 cm) for 15 min. An additional 15 min after this initial exposure, animals were assigned to a

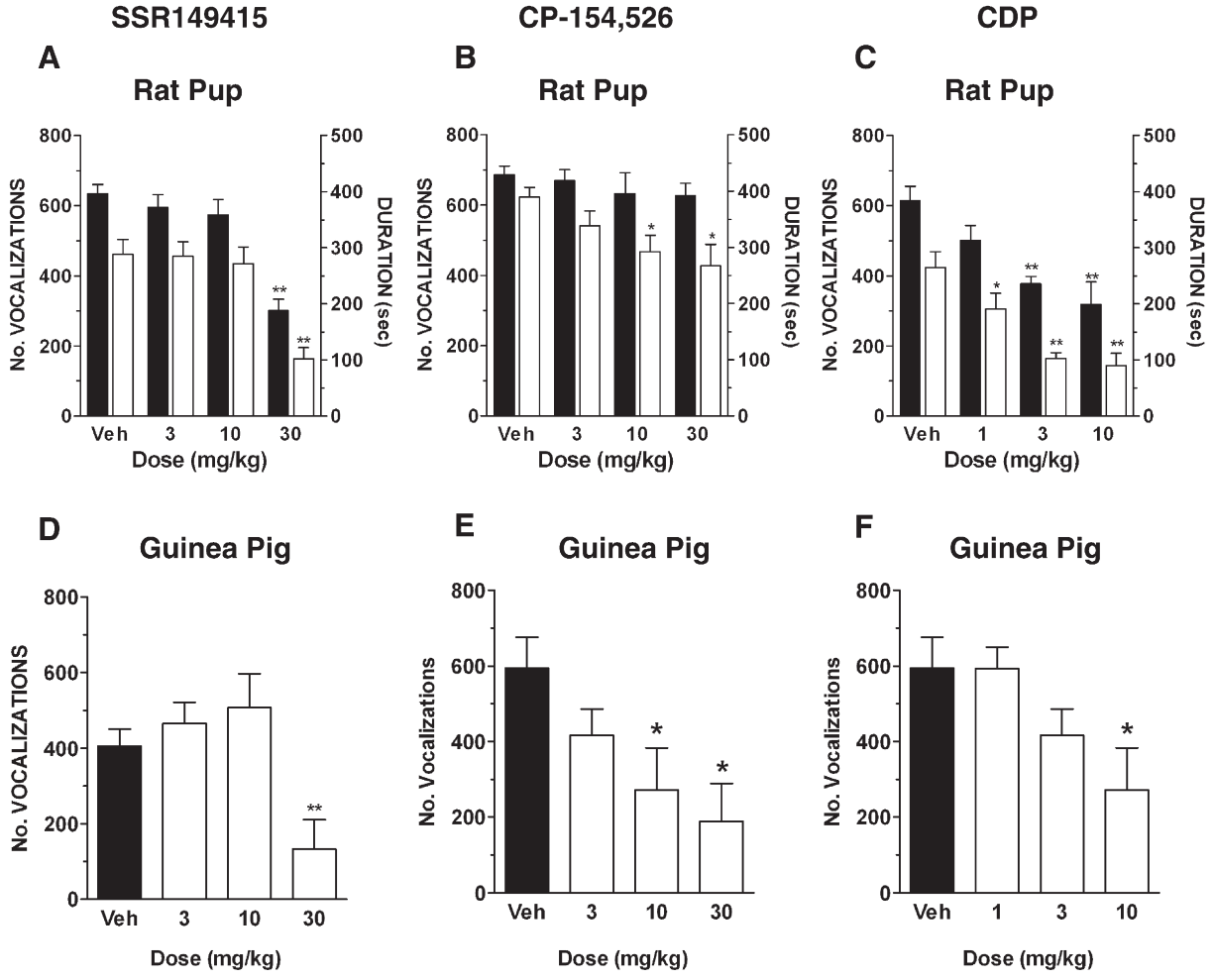

Fig. 2. The effects of SSR149415, CP-154,526, and chlordiazepoxide (CDP) in the rat pup ultrasonic vocalization (USV) and guinea pig pup vocalization (GPPV) assays. For rat pup USV studies ■ = number of vocalizations, □ = total duration of vocalizations in seconds. For the GPPV studies, ■ represents vehicle animals, □ represents drug-treated animals. The data represent mean±SEM; *$p<0.05$; **$p<0.01$ vs. vehicle (Veh).

Table 2
Effect of SSR149415, CP-154,526 and CDP on negative geotaxis and righting reflex latencies in the rat pup

Compound	Dose (mg/kg)	Negative geotaxis (s)	Righting reflex (s)
SSR149415	0	18.5±3.0	1.1±1.8
	3	19.8±3.1	1.8±0.6
	10	18.9±3.2	2.2±0.8
	30	18.3±2.8	1.4±0.2
CP-154,526	0	15.7±3.4	1.1±0.2
	3	16.8±3.1	1.2±0.1
	10	13.8±2.7	1.0±0.1
	30	8.6±2.3	1.3±0.1
CDP	0	13.3±2.8	1.2±0.5
	3	11.6±2.7	1.4±0.2
	6	18.9±2.7	1.5±0.3
	10	25.9±2.1**	3.7±0.6**

Data represent group means±SEM.
**$p<0.01$ vs. 0 mg/kg.

drug/dose and the appropriate treatment was delivered. On the test day (day two) the animals were again dosed in accordance with their group assignment. Following the appropriate pre-treatment time, rats were placed in the swim chamber for 6 min. During this time, an expert observer blind to the group assignment of the animals measured the duration each rat spent immobile. Immobility was defined as the minimum movements necessary to keep the head above water.

2.5.2. Forced swim test (FST) — mouse

Mice were placed for 6 min in a glass cylinder (height: 50 cm; diameter: 8 cm) filled ¾ full of room temperature water (~22 °C). The mice were in the cylinders for 6 min. During the final 4 min, an observer blind to the condition of the animals timed the total duration that the animals spent immobile. Immobility was defined as the minimum movements necessary to keep the animal's head above water.

2.5.3. Tail suspension test (TST) — mouse

Mice were suspended from their tails for 6 min such that their noses were approximately 5 cm from the surface. The behavior of each mouse in the final 4 min was scored as either immobile or struggling by an observer blind to treatment. The total time spent immobile was recorded.

2.6. Statistical analysis

One-way ANOVAs were used to analyze all studies. Dunnett's post hoc tests were employed to assess the statistical significance of individual doses versus vehicle. Significance was defined as $p<0.05$.

3. Results

3.1. Vasopressin receptor binding affinities

SSR149415 had affinities at the rat (Ki: 1.0±0.3 nM), mouse (Ki: 0.7±0.1 nM), guinea pig (Ki: 0.7±0.7 nM), and human (0.6±0.02 nM) V1b receptor. For full affinity characterization including selectivity over V1a, V2 and oxytocin receptor subtypes see Table 1.

3.2. Anxiety studies

3.2.1. Rat pup USV

SSR149415 significantly lowered both the number [$F(3,48)=17.87$, $p<0.01$] and duration [$F(3,48)=11.17$, $p<0.05$] of separation-induced USVs in the rat pup (Fig. 2A). For both measures, only the 30 mg/kg dose was significantly lower than vehicle. SSR149415 had no effect on righting reflex latency [$F(3,48)=0.87$, $p>0.05$] or negative geotaxic latency [$F(3,48)=0.04$, $p>0.05$] (Table 2).

CP-154,526 had no significant effect on the number of USVs the rat pups emitted but did significantly lower the overall duration of USVs [$F(3,44)=4.46$, $p<0.01$] at doses of 10 mg/kg and 30 mg/kg (Fig. 2B). CP-154,526 had no effect on negative geotaxic latency [$F(3,44)=1.72$, $p>0.05$] or righting reflex latency [$F(3,44)=0.89$, $p>0.05$] (Table 2).

CDP significantly lowered both the number [$F(3,56)=9.22$, $p<0.01$] and duration [$F(3,56)=11.95$, $p<0.01$] of separation-induced USVs (Fig. 2C). All doses tested (1, 3, and 10 mg/kg) lowered call duration, whereas only the 3 and 10 mg/kg doses lowered call number. Additionally, CDP significantly reduced both righting reflex latency [$F(3,56)=6.22$, $p<0.01$] and negative geotaxic latency [$F(3,56)=7.29$, $p<0.01$] at a dose of 10 mg/kg, indicating that the drug produced other behavioral effects in the animals (Table 2).

3.3. Guinea pig pup vocalizations

SSR149415 significantly lowered the number of separation-induced vocalizations in guinea pig pups [$F(3,38)=5.95$, $p<0.01$] at a dose of 30 mg/kg (Fig. 2D). Similarly, CP-154,526 significantly lowered vocalizations [$F(3,36)=3.54$, $p<0.05$] at doses of 10 mg/kg and 30 mg/kg (Fig. 2E). CDP also dose-dependently reduced the number of guinea pig pup calls [$F(3,28)=3.90$, $p<0.05$] at 10 mg/kg (Fig. 2F).

Table 3
Effect of SSR149415, CP-154,526 and CDP in the rat elevated plus-maze

Compound	Dose (mg/kg)	% Open arm entries	% Open arm duration	% Open arm distance
SSR149415	0	12.6±6.4	17.8±8.8	15.6±8.0
	3	20.4±9.5	18.7±4.6	10.2±2.0
	10	12.9±4.2	20.1±4.5	12.0±2.7
	30	27.0±10.4#	36.3±11.6#	10.93±2.9
CP-154,526	0	24.3±4.1	15.2±4.1	7.5±2.0
	3	11.8±4.9	19.1±5.9	12.3±2.3
	10	27.9±8.0	22.8±9.7	8.2±3.8
	30	21.4±4.8	10.1±2.3	6.8±1.3
CDP	0	22.1±4.9	24.0±3.3	11.5±2.2
	3	57.4±5.8**	53.5±3.7**	8.9±1.9
	6	54.1±7.4**	51.6±3.9**	18.7±2.9
	10	45.9±6.9*	51.1±5.5**	26.0±4.0**

Data represent group means±SEM.
**$p<0.01$. *$p<0.05$. #$p<0.1$ vs. 0 mg/kg.

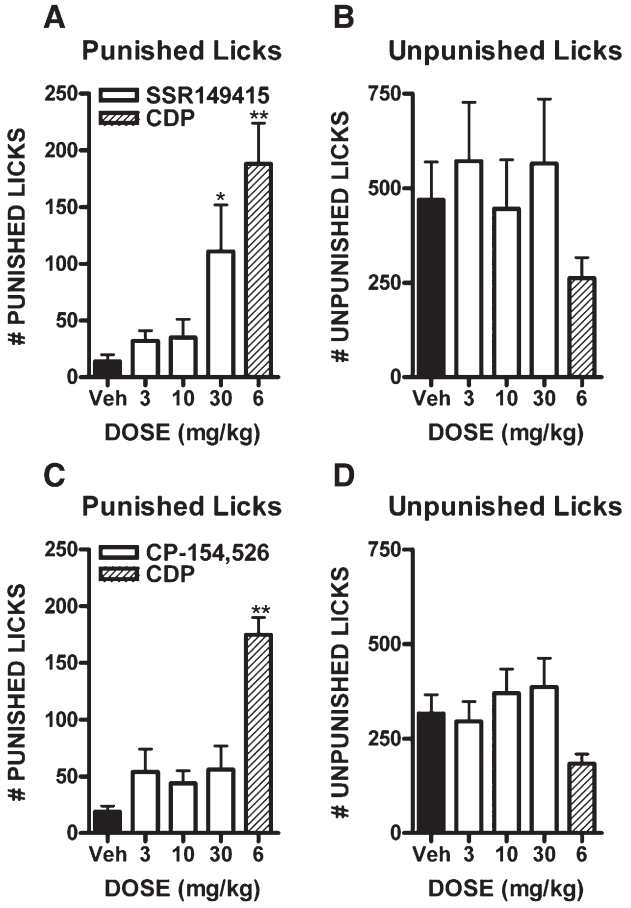

Fig. 3. The effects of SSR149415, CP-154,526 and CDP in the rat conditioned lick suppression (CLS) assay. The data represent mean±SEM; **$p<0.01$ vs. vehicle (V).

3.4. EPM

SSR149415 did not significantly increase the percent of open arm distance [$F(3,23)=1.50$, $p>0.05$]. There was a trend to increase the percent of entries into the open arms [$F(3,23)=2.48$, $p<0.1$] and percent time spent in the open arms [$F(3,23)=2.33$, $p=0.1$]. CP-154,526 had no effect on percent open arm entries [$F(3,36)=0.50$, $p>0.05$], duration [$F(3,36)=1.02$, $p>0.05$], or distance [$F(3,36)=0.76$, $p>0.05$]. CDP increased the percent of open arms entries [$F(3,44)=11.63$, $p<0.01$], time spent on the open arms [$F(3,44)=6.02$, $p<0.01$] and distance traveled on the open arms [$F(3,44)=7.33$, $p<0.01$] at a dose of 10 mg/kg (Table 3).

3.5. CLS

SSR149415 dose-dependently increased licking in the "punished" component of the test [$F(5,66)=7.94$, $p<0.01$] with the 30 mg/kg and CDP (6 mg/kg) dose groups significantly different from vehicle (Fig. 3A). There were no effects on unpunished licking [$F(5,66)=0.76$, $p>0.05$] (Fig. 3C).

There was a significant main effect of group in the CP-154,526 study [$F(5,90)=17.74$, $p>0.01$], however, only CDP (6 mg/kg) significantly increased "punished" licking relative to vehicle (Fig. 3B); none of the doses of CP-154,526 differed significantly from vehicle. Although there was a significant main effect for group on the unpunished licking measure [$F(5,90)=4.76$, $p>0.01$], no dose of CP-154,526 increased unpunished licking (Fig. 3D). CDP significantly lowered unpunished licking.

3.6. Marble burying

Although there was a significant main effect for the number of marbles buried in the SSR149415 study [$F(3,36)=3.37$, $p>0.05$], none of the dose groups significantly differed from vehicle in a post hoc analysis. The 30 mg/kg dosed group did bury significantly fewer marbles at a less stringent p-value of $p>0.1$. CP-154,526 had no effect on marble burying behavior [$F(3,36)=2.57$, $p>0.05$]. CDP dose-dependently reduced the number of marbles the animals buried [$F(3,34)=10.72$, $p<0.01$] at a dose of 10 mg/kg (Fig. 4).

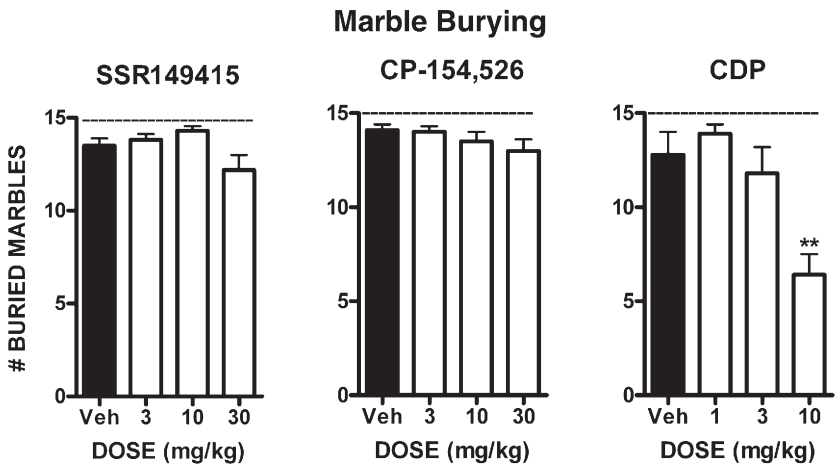

Fig. 4. The effects of SSR149415, CP-154,526 and CDP on marble burying behavior in mice. Dashed line indicates maximum number of unburied marbles. The data represent mean±SEM; **$p<0.01$ vs. vehicle (Veh).

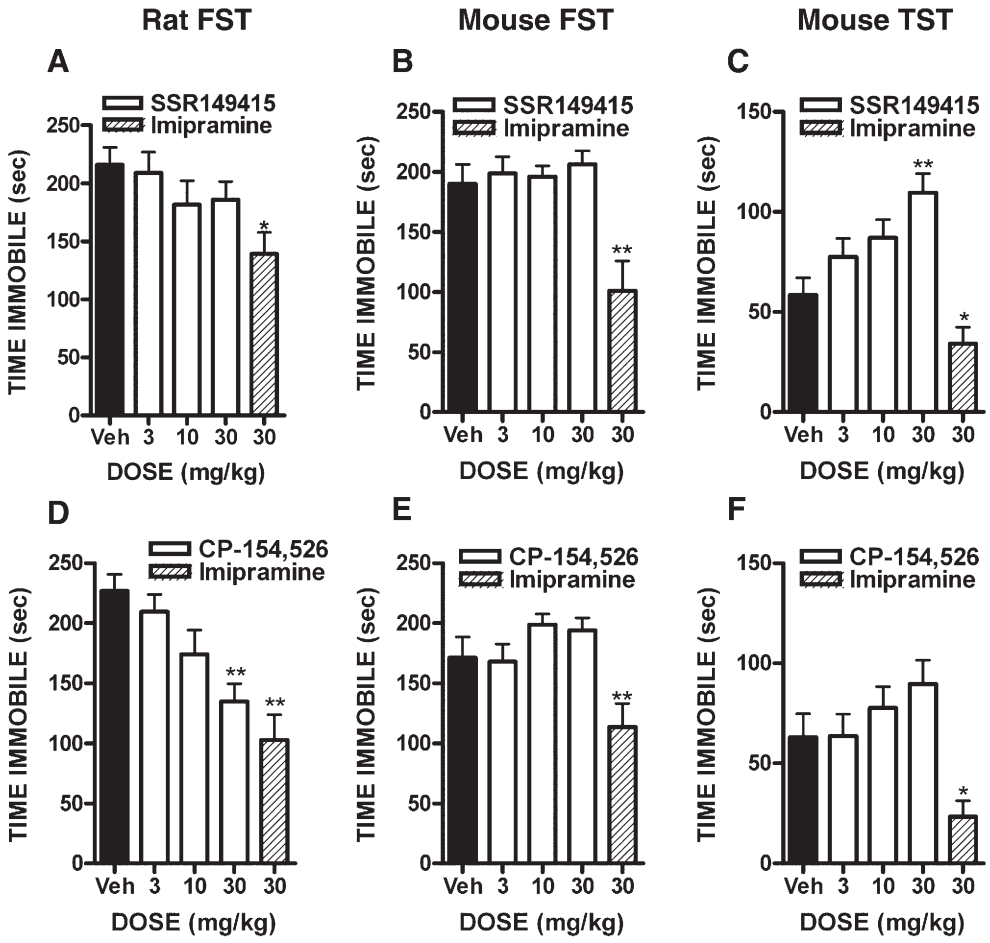

Fig. 5. The effects of SSR149415, CP-154,526 and imipramine in rodent forced swim and tail suspension assays. For all tests, closed bars represent vehicle, open bars represent drug-treated animals, and hatched bars represent imipramine-dosed animals. Data represent group means ± SEM; *$p<0.05$; **$p<0.01$ vs. vehicle (Veh).

3.7. Depression studies

3.7.1. FST — rat

SSR149415 was tested in the rat FST at doses of 3, 10, and 30 mg/kg with an imipramine-dosed (30 mg/kg) positive control group. There was a main effect of treatment [$F(4,55)=2.89$, $p<0.05$] but only imipramine significantly reduced immobility relative to vehicle (Fig. 5A). CP-154,526 was tested at doses of 1, 3, and 10 mg/kg and produced a significant reduction in immobility [$F(4,69)=8.71$, $p<0.01$] at a dose of 10 mg/kg (Fig. 5D), while imipramine was also effective at 30 mg/kg.

3.7.2. FST — mouse

There was a significant main effect of time immobile for both the SSR149415 [$F(4,41)=8.45$, $p<0.01$] and CP-154,526 [$F(4,55)=8.45$, $p<0.05$] mouse forced swim studies. However, in both studies, only the imipramine-dosed animals displayed significantly reduced immobility relative to vehicle-dosed animals (Fig. 5B and E).

3.7.3. TST — mouse

There was a main effect for treatment in the SSR149415 TST [$F(4,87)=9.62$, $p<0.01$]. However, no dose of SSR149415 produced a significant reduction in immobility relative to vehicle. The 30 mg/kg group was significantly more immobile than the vehicle group in a post hoc analysis. Imipramine produced a significant reduction in immobility (Fig. 5C). In the CP-154,526 TST, there was a main effect of treatment [$F(4,49)=3.26$, $p<0.05$], but only the imipramine-dosed group was significantly less immobile than vehicle (Fig. 5F).

4. Discussion

Vasopressin V1b and CRF1 receptor antagonists are currently being developed as putative novel treatments for affective disorders. Both receptors are located in the pituitary where they control the release of stress hormones, as well as centrally in brain regions associated with anxiety and depression. The present work was designed to directly compare these two pharmacological approaches. To that end, the V1b receptor antagonist, SSR149415, and the well-characterized CRF1 receptor antagonist, CP-154,526, were tested across a variety of established rodent models. Acute administration of both SSR149415 (Serradeil-Le Gal et al., 2002) and CP-154,526 (Schulz et al., 1996; Jutkoewicz et al., 2005) dose-dependently lowers stress-induced elevations of plasma ACTH,

which indicates that they are effective at lowering HPA axis activity. Both drugs have been reported to be highly selective for their target receptors in rat; however, recently Griffante et al. (2005) reported that SSR149415 is not selective for the V1b receptor over the oxytocin receptor in humans. Our human binding data confirm this lack of selectivity and we demonstrated that the compound is highly selective for the V1b receptor in the rat, which is consistent with Serradeil-Le Gal et al. (2002). We also found that CP-154,526 has high affinity for the CRF1 receptor (data not reported), which is consistent with work previously published by Schulz et al. (1996).

SSR149415 and CP-154,526 were tested in five rodent models of anxiety which are sensitive to a wide range of established anxiolytics: the rat pup USV assay (Olivier et al., 1994; Iijima and Chaki, 2005); the guinea pig pup vocalization assay (Molewijk et al., 1996); the CLS assay (Kilts et al., 1981; Lu et al., 2002); EPM (Pellow et al., 1985); and the marble burying test (Njung'e and Handley, 1991). When the two compounds are compared across our anxiety models, SSR149415 was generally more efficacious than CP-154,526. In the EPM, SSR149415, but not CP-154,526, increased the percent of entries into and percent time spent in the open arms, which is consistent with findings from Griebel et al. (2002a,b). In the rat pup USV assay, SSR149415 lowered separation-induced vocalizations when recorded by two different dependent measures; number and duration of calls. CP-154,526 conversely was only effective in lowering total USV duration. Moreover, 30 mg/kg of SSR149415 inhibited rat pup USV duration by 65%, whereas the equivalent dose of CP-154,526 produced only a 32% inhibition of call duration. It is possible that higher doses of CP-154,526 would produce a greater effect; however, a 30 mg/kg dose of CP-154,526 should result in significant brain exposure and coverage of the CRF1 receptor based on pharmacokinetic data in the rat (see Chen et al., 1997). Neither SSR149415 nor CP-154,526 produced any overt signs of sedation in the rat pup. This is in contrast to CDP, which impaired both negative geotaxic latency and righting reflex latency making it difficult to dissociate the anxiolytic-like effect of CDP from sedation. Previously published data in separation-induced vocalization assays are conflicting for SSR149415. In the rat pup assay, Serradeil-Le Gal et al. (2005) reported that it significantly lowered rat pup calls at 10 mg/kg; whereas Iijima and Chaki reported that the drug did not lower calls up to 30 mg/kg. CP-154,526 has previously been shown to be efficacious in the rat model (Kehne et al., 2000; Iijima and Chaki, 2005).

The most compelling difference between the compounds was in the CLS assay. Specifically, SSR149415 but not CP-154,526 produced anxiolytic-like effects. Given that vasopressin is involved in water intake, it is possible that affects on thirst drive could account for the increase in punished drinking. However, a lack of effect of SSR149415 on unpunished drinking suggests that thirst is not responsible for the reduction in punished licking. Previously, both drugs have been tested in similar conflict models. In support of the present findings, Griebel et al. (2002a,b) reported that SSR149415 was active in a punished drinking assay. The data for CP-154,526 in conflict models of anxiety are inconsistent. Several labs have reported that CP-154,526 is inactive in a punished drinking assay (e.g., Griebel et al., 1998; Seymour et al., 2003), while, Millan et al. (2001) reported that CP-154,526 increased punished licking in a similar model.

Neither compound worked well in the acute models of depression in which they were tested. CP-154,526 was active in the rat FST test but not in any of the mouse models tested; SSR149415 was not active in any acute depression assay. Our data with CP-154,526 are consistent with published work, which in general suggests that CRF1 antagonists exhibit relatively weak and inconsistent activity in these models, compared to established antidepressants (Chaki et al., 2004; Jutkoewicz et al., 2005; Li et al., 2005). The results with SSR149415 are in contrast to those of Griebel and colleagues (Griebel et al., 2002a,b, 2005) who reported activity with SSR149415 in both the mouse TST and rat FST. In our studies, SSR149415 was administered following a shorter pre-treatment time compared to Griebel et al. However, pharmacokinetic studies demonstrated that testing at this pre-treatment time results in maximal brain exposure, suggesting that the pre-treatment time used was appropriate. The discrepancies with Griebel and colleagues might therefore result from differences in the behavioral assays, or possibly the formulation of the drug. Further studies are needed to clarify this.

Although V1b receptors are abundant in the pituitary, there is evidence that the efficacy of SSR149415 is mediated, in part, by central V1b receptors. This non-pituitary contribution to the antidepressant-like efficacy of the compound was demonstrated by Griebel et al. (2002a,b) who reported that hypophysectomized rats were sensitive to the effects of the drug in the forced swim assay. Moreover, Stemmelin et al. (2005) have shown that direct infusion of SSR149415 into the lateral septum, a region that lacks CRF1 receptors (Chalmers et al., 1995), reduced immobility in the forced swim test. More recently, Salomé et al. (2006), found that injections of SSR149415 into three separate amygdaloid nuclei (basolateral, central, and medial) produced antidepressant-like effects in the forced swim model.

In contrast to the depression studies, there is less evidence that a purely central mechanism mediates the anxiolytic-like effects of SSR149415. While Salomé et al. (2006) demonstrated that discrete injection of SSR149415 into the basolateral amygdala produced anxiolytic-like effects in the EPM, the contribution of pituitary V1b receptors can not be excluded at this point. Therefore, an alternative possibility for our findings is that, given the very poor brain penetration of SSR149415 in our hands (see Fig. 1), it is likely that we primarily antagonized pituitary V1b receptors over central V1b receptors, which could account for the greater efficacy in anxiety, compared to depression models.

Finally, given that activation of either the CRF or vasopressin system produces a stress response in animals, one possibility that was not tested in these studies is that dual antagonism of V1b and CRF1 activity may provide better activity in our models compared to antagonizing the receptors in isolation. Further work is required to assess this possibility.

In summary, our data are consistent with the hypothesis that both V1b and CRF1 antagonists have potential for the treatment of

anxiety and depression. Moreover, the compounds were, on the whole, more effective in tests with a high stress component (e.g., CLS, pup vocalization assays) and less effective in assays with low stress (e.g., marble burying, EPM). Furthermore, on balance, V1b antagonism was more effective than CRF1 antagonism in animal models of anxiety-like behavior, despite the fact that SSR149415 had poorer brain penetration than CP-154,526. Our studies, therefore, provide clear differentiation between the mechanisms of systemically administered V1b and CRF1 antagonists.

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