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Effects of the CRF1 antagonist SSR125543A on aggressive behaviors in hamsters

Catherine Farrokhi[a], D. Caroline Blanchard[b], Guy Griebel[c], Mu Yang[a], Coty Gonzales[a], Chris Markham[a], Robert J. Blanchard[a,*]

[a]*Department of Psychology, University of Hawaii, 2430 Campus Road, Honolulu, HI 96822, USA*
[b]*Pacific Biomedical Research Center, University of Hawaii, 1993 East West Road, Honolulu, HI 96822, USA*
[c]*Sanofi-Synthelabo Recherche, Bagneux, France*

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Abstract

Corticotropin-releasing factor (CRF) and its receptor subtypes have been implicated in endocrine and behavioral responsivity to stress and emotion, including fear, anxiety, and aggression. SSR125543A is a new nonpeptide selective antagonist at the CRF1 receptor that has been shown to produce an anxiolytic-like effect in a number of animal models of anxiety. The present study investigated effects of an oral dose of 10, or 30 mg/kg of SSR125543A on aggressive behaviors of resident male Syrian hamsters toward male intruders. The high dose (30 mg/kg) of the CRF1 receptor antagonist produced a higher latency to bite and lower lateral attack frequencies and chase durations, indicating a reduction in aggression toward intruders in resident hamsters. The same dose of SSR125543A also enhanced frequency and duration of olfactory investigation, indicating that neither avoidance of the opponent nor deficiency in social activity is responsible for the reduction in aggression seen in these animals.

Keywords: Corticotropin-releasing factor; Anxiolytic-like effect; Agression

1. Introduction

Corticotropin-releasing factor (CRF) is a 41-amino acid peptide that serves as the main hypothalamic factor stimulating corticotropin (ACTH) release from the anterior pituitary and, in turn, release of glucocorticoids from the adrenal cortex (Vale et al., 1981). CRF and the CRF-like peptide, urocortin, are also widely distributed in extrahypothalamic portions of the mammalian brain, as are a number of CRF-responsive receptor subtypes (Gray and Bingaman, 1996; Olschowka et al., 1982; Swanson et al., 1983). CRF receptor subtypes include CRF1 and CRF2, each with a number of splice variants and each uniquely distributed within the brain. In the rat brain, CRF1 appears to be the predominant CRF receptor in the pituitary, brain stem, cerebellum, amygdala, and cortex, while CRF2 is more prevalent in subcortical regions, including the lateral septum and the ventromedial nucleus of the hypothalamus, with moderate levels also in other hypothalamic nuclei, some nuclei of the amygdala, and in the midbrain raphe (Chalmers et al., 1996; Primus et al., 1997).

CRF appears to play a crucial role in both responsivity to stress and in emotional behavior (Arborelius et al., 1999; Owens and Nemeroff, 1991). Specifically, intracerebroventricular application of CRF produces many behavioral effects similar to those seen in stressful situations, increasing anxiety-related behaviors and aversion in place preference tests (for a review, see Cador et al., 1992; Griebel, 1999). Of the two receptor subtypes, there is much more evidence for a role of CRF1 receptors in emotion-linked behaviors, such as fear and anxiety (for a review, see Takahashi, 2001). Evidence for the involvement of the CRF1 receptor subtype in emotion includes data from studies of CRF1 receptor knockout mice, indicating that these animals show anxiolytic-like behaviors in tests such as the open-field, light–dark box, defensive-withdrawal, and elevated plus maze (Contarino et al., 1999; Smith et al., 1998; Timpl et al., 1998); and from work using CRF1 receptor antagonists, again tending to show anxiolytic-like effects in the elevated plus maze (Griebel et al., 1998; Lundkvist et al., 1996); defensive-

* Corresponding author. Tel.: +1–808–956–8004; fax: +1–808–956–9612.
E-mail address: Blanchar@hawaii.edu (R.J. Blanchard).

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withdrawal (for chronic but not acute administration; Arborelius et al., 2000); and potentiated startle (Schulz et al., 1996; Mansbach et al., 1997) paradigms.

The recently developed nonpeptide CRF1 antagonist 4-(2-chloro-4-methoxy-5-methylphenyl)-*N*-[(1*S*)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]5-methyl-*N*-(2-propynyl)-1,3-thiazol-2-amine hydrochloride (SSR125543A) is a 2-aminothiazole derivative. It shows nanomolar affinity for human-cloned or native CRF1 receptors and a 1000-fold selectivity for CRF1 versus CRF2a receptors or CRF-binding protein (Gully et al., 2002). It has been shown to have anxiolytic-like effects in a number of animal models of anxiety, including conflict procedures, social defeat-induced anxiety, and a defense test battery, as well as antagonizing stress-induced hyperthermia, distress vocalization, and cortical norepinephrine release (Griebel et al., 2002). In two models of depression, the forced swim test and the chronic mild stress test, SSR125543A produced antidepressant-like effects. These results (Griebel et al., 2002) indicate that SSR125543A shows activity in numerous tests of unavoidable stress exposure. Moreover, these effects were obtained after oral administration, as were (Gully et al., 2002) reductions in plasma ACTH levels elicited by a 15-min restraint stress or intracerebroventricular CRF administration in rats.

Aggression involves additional stressor-elicited behavior patterns. One group of these stressors, involving dominance or resource challenge to the animal, elicits offensive aggression; whereas threats from predators or attacking conspecifics to the body or life of the animal elicit defensive aggression (Blanchard and Blanchard, 1997). These two behavior patterns occur in different contexts and respond differentially to a number of drugs, as well as to motivational variables involving stress or emotion. Thus, a class of "serenic" drugs reduce offensive (Olivier et al., 1991) but not defensive (Blanchard et al., 1985) aggression, while fear-inducing situations also reduce offensive (Blanchard et al., 1988; Blanchard and Blanchard, 1989) but not defensive attack (Blanchard et al., 1980).

In isolated DBA/2 mice confronting a group-housed intruder, a paradigm likely to elicit largely offensive aggression, both CRF and the CRF agonist sauvagine, with intracerebroventricular infusion, reduced aggressive behavior and sociability, while increasing defensive behavior (Mele et al., 1987). When CRF was infused bilaterally into the amygdala and aggression measured in a paradigm that might have produced both offensive and defensive forms of aggression, lower doses increased the agonistic behaviors of rats, whereas higher doses decreased it (Elkabir et al., 1990). However, Habib et al. (2000) reported that the CRF1 antagonist antalarmin, given orally, tended to reduce aggression in monkeys. While these studies suggest that SSR125543A might impact aggression, their differences provide no consistent indication of the likely direction of effect. The present study evaluated this relationship in a hamster resident–intruder paradigm.

2. Method

2.1. Animals

Subjects were 72 male Syrian hamsters obtained from Charles River Laboratories (St. Louis, MO). At the time of testing, 48 of these were 75- to 80-day old animals that served as residents, while 24 were 55-day old stimulus animals placed as intruders into the resident's home cages. After arrival in the University of Hawaii Animal Facility, residents were singly housed for 2 weeks, while intruders were housed in groups of three over the same time period. All subjects were maintained under reversed light–dark cycle (lights off at 12:00 p.m.) in a temperature- and humidity-controlled room (68 °F) with food and water available ad libitum.

2.2. Drug

SSR125543A was prepared as a suspension in distilled water containing 5% dimethyl sulfoxide and 5% Cremophor EL, which alone served as vehicle. Compounds were administered per os at a constant volume of 5 ml/kg.

2.3. Procedure

Each subject was evaluated during a single test. During the 2 weeks prior to behavioral testing, all subjects were handled daily for 3 min. On the day of testing, residents were randomly assigned to the drug condition, and the drug was administered orally at doses of 0, 10, or 30 mg/kg ($n = 16$/group). Testing began 20 min later. Each resident was transported in its homecage to a small testing room located adjacent to the holding room and left undisturbed for a 5-min familiarization period; after which, an intruder was brought into the room and placed immediately into the homecage of the resident. Testing lasted 10 min or until five wounds were incurred by the intruder (at which point, testing was termi-

Table 1
Ethological measures (means)

	Dose (mg/kg)		
	0	10	30
Attack latency (s)	183.75	146.50	276.65
S.E.M.	54.23	46.64	60.17
Flank marking (frequency)	1.75	5.34	2.81
S.E.M.	0.56	1.60	1.08
Flank marking (s)	5.43	10.71	5.81
S.E.M.	2.72	3.01	2.38
On top (frequency)	3.56	3.68	2.12
S.E.M.	0.81	1.16	0.89
On top (s)	13.30	8.80	8.43
S.E.M.	3.75	3.38	4.30
Upright (frequency)	18.06	21.31	16.62
S.E.M.	2.51	3.27	1.70
Upright (s)	50.83	56.36	58.37
S.E.M.	8.23	10.26	8.20

nated). To ensure a relatively high level of conspecific interaction, residents and intruders were paired such that a minimum weight difference of 10 g separated the two. Subjects were videotaped via a video camera placed 1 m from the test cage. The test was also scored live by trained observers standing 1.5 m from the test cage. Behaviors scored live included

(a) latency to attack—time until residents exhibited lateral attack, biting, or on top of the intruder;
(b) latency to bite—bites were recorded when an attack resulted in sharp, high-pitched vocalization by the intruder;
(c) flank marking by the resident.

Other behaviors were scored from the videotapes, including olfactory investigation, chase, lateral attack, upright, on-top-of, and hanging from the cage tops. All of these behaviors, with the exception of hanging, were exhibited by the resident animal (for ethological measures, see Table 1).

2.4. Statistical analysis

Data were analyzed using one-way ANOVA, with subsequent comparisons between groups analyzed using Newman–Keuls post hoc tests.

3. Results

(a) Olfactory investigation—Dose effects were significant for both the frequency [$F(2,45) = 3.61$; $P < .05$] and duration [$F(2,45) = 5.36$; $P < .01$] of olfactory investiga-

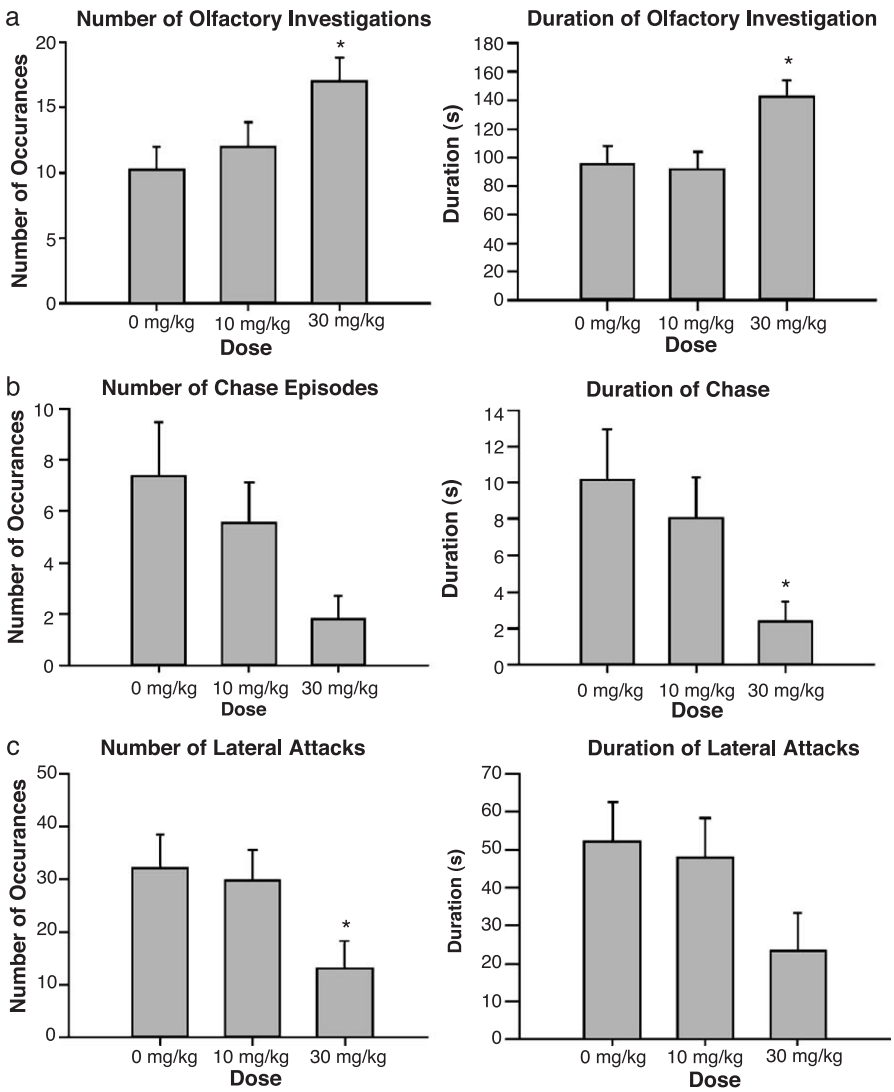

Fig. 1. Effects of CRF1 Antagonist SSR125543A on the frequency and duration of (a) olfactory investigation, (b) chase, and (c) lateral attack of resident hamster intruder. Vertical bars represent S.E.M.; * $P < .05$, in comparison to controls.

tion (Fig. 1a). Post hoc analysis revealed that both measures were significantly higher in high-dose (30 mg/kg) animals compared to either the vehicle controls or the low dose (10 mg/kg) groups ($P < .05$).

(b) Latency to bite—ANOVA indicated a statistically significant dose effect on the latency of the resident to bite the intruder [$F(2,45) = 4.35$; $P < .02$]. Post hoc analysis revealed that the high dose subjects showing higher latencies to bite the intruder, compared to either control or low-dose subjects ($P < .05$ in each case).

(c) Chase—Dose effects were significant for the duration of chase behavior [$F(2,45) = 3.29$; $P < .05$], with high-dose subjects exhibiting lower chase durations compared to either vehicle or low dose animals (Fig. 1b).

(d) Lateral attack—The main effect of drug was significant for the number of lateral attacks [$F(2,45) = 3.24$; $P < .05$]. Post hoc analysis indicated that high-dose animals made significantly fewer lateral attacks compared to either vehicle or low-dose animals ($P < .05$ for each; Fig. 1c).

Although the dose effect on flank markings approached an acceptable level of statistical significance [$F(2,45) = 2.81$; $P < .07$], no other measures were statistically significant.

4. Discussion

This pattern of results—higher latency to bite with lower lateral attack frequency and chase duration—indicates that high-dose (30 mg/kg) SSR125543A, given orally, reduced aggression toward intruders in resident hamsters. The finding of enhanced frequency and duration of olfactory investigation indicates that the same SSR125543A dose failed to reduce social activity. Thus, deficiencies in social (and, by inference, general) activity do not appear to be responsible for the reduction in aggression seen in these animals.

These CRF antagonist findings are different from reports (Mele et al., 1987) that intracerebroventricular infusions of CRF agonists reduce aggressive behavior in isolated DBA/2 mice, as do high-dose CRF infusions in the amygdala in male rats (Elkabir et al., 1990), but are consonant with reports (Habib et al., 2000) that another CRF1 receptor antagonist, antalarmin, tended to reduce aggression in male rhesus macaques. While Tazi et al. (1987) found that intracerebroventricular infusion of the CRF antagonist alpha-helical CRF-(9–41) blocked fighting in rats produced by higher levels of shock, it is notable that shock-elicited fighting is defensive rather than offensive, such that the relevance of this study to effects of centrally administered CRF receptor antagonists on aggression is questionable. Thus, these findings and the present results consistently suggest that the mode of drug delivery (central vs. oral) may have an important influence on the systems involved.

Oral administration of 30 mg/kg SSR125543A in rats significantly lowered basal ACTH levels and also reduced the enhancement of ACTH following CRH injection (Gully et al., 2002). Other systemically administered CRF1 antagonists (Rivier, 2002; Rivier et al., 2003) have also been shown to reduce CRF- or stress-induced activation of the hypothalamic pituitary adrenal (HPA) axis. Thus, the behavioral changes seen with oral administration of SSR125543A may reflect effects on peripheral aspects of HPA axis functioning. Aggression effects of HPA axis activity variations have been reported by Haller et al. (1998, 2000, 2002). These include higher rates of aggression during portions of the light–dark cycle when glucocorticoid levels are increasing (Haller et al., 1998); striking reductions in aggression after blocking the high-affinity mineralocorticoid receptor (MR) with spironolactone (Haller et al., 1998, 2002); and reduced aggression in males with decreased plasma corticosterone, following treatments with the corticosterone synthesis inhibitor metyrapone (Haller et al., 2000). Haller et al. (1998) suggest that acute increases in plasma glucocorticoids, such as those that occur in both resident and intruder males during a confrontation, facilitate the particular behavior that is predominant for the animal in that context. This suggests that both offensive and defensive forms of aggression might respond to reductions in the glucocorticoid release normally associated with the stress of either challenge or of conspecific attack, thus raising the possibility that treatments reducing the systemic activation of the HPA axis might prove to modulate nonoffensive aggression as well as the offensive aggression reported here, in addition to other stress-related behaviors, such as those linked to anxiety and, potentially, depression.

This view is consistent with additional effects of SSR125543A, given intraperitoneally or per os, including anxiolytic-like effects in stress-induced conflict procedures, a defeat-induced anxiety paradigm, and the mouse defense test battery (MDTB). It is notable that in the MDTB, the specific defensive behavior changes included reduced defensive threat and attack, suggesting that its mitigating effects on offensive aggression may be paralleled by similar reductions in defensive aggression. Finally, SSR125543A increased the number of punished crossings in a four-plate test, antagonized stress-induced hyperthermia, distress vocalization, and cortical norepinephrine release (Griebel et al., 2002). These findings provide considerable evidence for the view that systemic administration of SSR125543A may be capable of modulating a range of stress-induced behaviors—a view with which the present findings of reduced offensive aggression following per os administration of this compound are in clear agreement.

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