ELSEVIER

Anxiolytic Properties of the Selective, Non-peptidergic CRF_1 Antagonists, CP154,526 and DMP695: A Comparison to Other Classes of Anxiolytic Agent

Mark J. Millan, Ph.D., Mauricette Brocco, Ph.D., Alain Gobert, Ph.D., Gilbert Dorey, Ph.D., Patrick Casara, Ph.D., and Anne Dekeyne, Ph.D.

The selective, non-peptidergic corticotropin-releasing factor $(CRF)_1$ receptor antagonists, CP154,526 and DMP695, dose-dependently increased punished responses of rats in a Vogel conflict test and enhanced social interaction (SI) of rats in an unfamiliar environment. They were, however, inactive in a plus-maze procedure and failed to reduce ultrasonic vocalizations (USV) associated with an aversive environment. In contrast, the benzodiazepine, chlordiazepoxide, was effective in all these procedures. Further, the serotonin $(5\text{-}HT)_{1A}$ agonist, flesinoxan, was active in each paradigm (except the plus-maze) while the $5\text{-}HT_{2C}$ antagonist, SB242,084, was effective in the SI and Vogel but not the plus-maze and USV procedures. In contrast to chlordiazepoxide, flesinoxan and SB242,084, CP154,526 did not modify dialysate levels of 5-HT, norepinephrine (NE) and dopamine (DA) in the frontal cortex (FCX) of freely moving rats. In conclusion, CP154,526 and DMP695 possess a common and distinctive profile of anxiolytic action expressed in the absence of an intrinsic influence upon monoamine release.

[Neuropsychopharmacology 25:585–600, 2001]

KEY WORDS: *CRF_1 Receptors; $5\text{-}HT_{1A}$ Receptors; $5\text{-}HT_{2C}$ Receptors; Benzodiazepines; Anxiety*

Corticotropin-releasing factor (CRF) plays a crucial role in modulation of the release of adrenocorticotrophic hormone from the anterior pituitary (De Souza 1995). In addition, CRF is broadly distributed throughout the mammalian central nervous system (CNS), being concentrated in corticolimbic regions such as amygdala, hippocampus, and periaqueductal gray (PAG) and frontal cortex (FCX), as well as the locus coeruleus (LC) and dorsal raphe nucleus (DRN), the origin of ascending adrenergic and serotonergic projections, respectively (Sawchenko et al. 1993; Van Bockstaele et al. 1996; Price et al. 1998; Steckler and Holsboer 1999). In line with this organization, independently of the hypothalamocorticotrophic axis, cerebral CRF-containing neurones fulfill an important role in the control of emotional behavior (Adamec and McKay 1993; De Souza 1995; Mitchell 1998; Steckler and Holsboer 1999; Smagin and Dunn 2000), actions expressed via two principal subtypes of CRF receptor, both of which positively couple to adenyl cyclase (Grigoriadis et al. 1996). CRF_1 receptors bind CRF and a related neuropeptide, urocortin, with high affinity whereas CRF_2 receptors display a

From the Psychopharmacology Department (MJM, MB, AD, AG), and Chemistry A Department (GD, PC), Institut de Recherches Servier, Centre de Recherches de Croissy, Paris, France.

Address correspondence to: M. J. Millan, Ph.D., Institut de Recherches Servier, Psychopharmacology Department, 125 Chemin de Ronde, 78290-Croissy-sur-Seine, France. Tel.: +33.1.55.72.24.25; Fax: +33.1.55.72.24.70; E-mail: mark.millan@fr.netgrs.com

Received November 30, 2000; revised February 19, 2001; accepted February 27, 2001.

Online publication: 3/2/00 at www.acnp.org/citations/Npp 03020186.

Published by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/01/$–see front matter
PII S0893-133X(01)00244-5

distinct preference for the latter (Donaldson et al. 1996; Grigoriadis et al. 1996). Of several splice variants of the latter, the $CRF_{2\alpha}$ subtype is found predominantly in the rodent CNS, notably in several corticolimbic regions enriched in CRF itself, such as the hippocampus, medial amygdala, septum, olfactory bulb, and raphe nuclei, although species differences in this regard should be pointed out (Chalmers et al. 1995; Sánchez et al. 1999). Studies of mRNA encoding CRF_1 receptors and of the corresponding peptide have established a contrasting pattern of distribution in rats with a predominance of CRF_1 over $CRF_{2\alpha}$ sites in anterior pituitary, FCX and basolateral amygdala, as well as high levels in the hippocampus and PAG (Potter et al. 1994; Chalmers et al. 1995; Sánchez et al. 1999; Chen et al. 2000).

These observations provide a neuroanatomical substrate for a potential role of CRF_1 and/or CRF_2 receptors in the control of mood (Coplan et al. 1996; Steckler and Holsboer 1999), and, in this regard, there is a diversity of evidence implicating CRF_1 sites in the modulation of anxious states. First, several studies have reported anxiogenic actions of CRF (and urocortin) upon i.c.v. administration (see Steckler and Holsboer, 1999) and similar effects have been seen upon direct introduction into the dorsal PAG (Martins et al. 1997), basolateral amygdala (Sajdyk et al. 1999), and hippocampus (Radulovic et al. 1999), structures possessing a high density of CRF_1 receptors. Second, direct evidence for participation of CRF_1 receptors in anxiogenic actions is derived from studies of antisense probes for their neutralization, i.c.v. administration of which attenuates induction of anxiety by CRF (Skutella et al. 1998). Under certain conditions, antisense probes against CRF_1, but not CRF_2, receptors also display intrinsic anxiolytic activity, although such actions are variable (Skutella et al. 1994; Liebsch et al. 1995; Heinrichs et al. 1997). Third, underpinning these observations, CRF_1 receptor-deficient mice display reduced anxiety in a variety of experimental paradigms (Timpl et al. 1998; Contarino et al. 1999; see also Steckler and Holsboer 1999). Fourth, contrariwise, mice over-expressing CRF_1 receptors or lacking CRF-binding hormone show an increase in anxious behavior (Skutella et al. 1994; Stenzel-Poore et al. 1994; Karoloyi et al. 1999).

The fifth line of evidence concerns actions of CRF_1 receptor antagonists. Peptidergic antagonists, such as 6-hel-$CRF_{9\text{-}41}$ and astressin, attenuate anxiogenic actions of CRF and stress (e.g., Menzaghi et al. 1994; Spina et al. 2000). In analogy, the novel, non-peptidergic CRF_1 antagonists, NBI27914, CRA1000, CRF1001 (all anilinopyrimidines), and CP154,526 (a pyrrollopyrimidine), inhibited the anxiogenic actions of CRF (Guanowsky et al. 1997; Smagin et al. 1998; Okuyama et al. 1999). However, like several studies of peptidergic antagonists in rats under non-stressed conditions (Heinrichs et al. 1992; Menzaghi et al. 1994; Spina et al. 2000), with the exception of NBI27914, they all failed to elicit anxiolytic activity alone in a plus-maze procedure (Lundkvist et al. 1996; Griebel et al. 1998; Okuyama et al. 1999). Further, whereas Griebel et al. (1998) reported modest anxiolytic actions of CP154,526 in a light-dark box paradigm in mice, in other studies, CP154,526, CRA1000, and CRA1001 were ineffective in this model unless mice were pre-exposed to stress (Guanowsky et al. 1997; Okuyama et al. 1999). The latter authors also documented their inactivity in a passive avoidance paradigm. As concerns other procedures, CP154,526 was active in "defensive withdrawal" procedures, decreased fear-potentiated startle model in rats, and suppressed separation-induced ultrasonic vocalizations (USV), whereas it was inactive in a conflict procedure in the rat and in a model of conditioned defeat in hamsters (Schulz et al. 1996; Griebel et al. 1998; Jasnow et al. 1999; Arborelius et al. 2000; Kehne et al. 2000). The novel phenylpyrimidine, R121919, is similarly active in a "defensive withdrawal" model (Heinrichs et al. 2000), and anxiolytic actions have also been claimed for a further pyrollopyrimidine derivative, antalarmin (Deak et al. 1999; Fiorino et al. 2000). On the other hand, while the pyrazolopyrimidine, DMP904, and the pyrazolotriazine, DMP696, were active in a rat model of "situational anxiety", CP154,526 was not effective in this paradigm (Gilligan et al. 1998; 2000; He et al. 2000).

Clearly, the above data are rather disparate, and several reports remain preliminary. To explain contrasting patterns of data regarding the potential anxiolytic actions of non-peptidergic CRF_1 antagonists, inter-species (and inter-strain) differences, as well as procedural variables and the level of stress, have been evoked (Griebel et al. 1998; Steckler and Holsboer 1999). Irrespective of underlying factors, the actions of non-peptidergic antagonists in therapeutically-pertinent models of potential anxiolytic activity are of critical importance as concerns the potential utility of CRF_1 receptor blockade in the clinical treatment of anxious states (Owens and Nemeroff 1991). To clarify such issues, one instructive approach may be to simultaneously examine the actions of chemically distinct CRF_1 antagonists in a number of contrasting procedures. In addition, it would be informative to directly compare their functional profiles to those of other classes of anxiolytic agent.

In light of the above comments, we compared the potential anxiolytic actions of CP154,526 in rats to those of a chemically distinct and novel CRF_1 antagonist, the triazolopyridine, DMP695 (Bakthavatchalam et al. 1998). Like CP154,526, DMP695 shows high affinity for both cloned, human (h)CRF_1 (K_i, 3.3 nM) and native, rat CRF_1 (K_i, 4.6 nM) receptors, at which it potently expresses antagonist properties in suppressing CRF-induced increases in cAMP levels (Bakthavatchalam et al. 1998; Gilligan et al. 2000; Gilligan PJ, personal communication). In contrast, it shows low affinity for $CRF_{2\alpha}$

and diverse other classes of receptor (Bakthavatchalam et al. 1998; Millan MJ et al., unpublished observations). Further, in vivo, DMP695 shows high bioavailability and is active in a model of situational anxiety in the rat—which is, interestingly, irresponsive to CP154,526 (Bakthavatchalam et al. 1998; Gilligan et al. 2000; He et al. 2000). In the present study, the actions of CP154,526 and DMP695 in plus-maze, Vogel conflict, SI, and conditioned USV procedures, were compared to those of the benzodiazepine (BZD), chlordiazepoxide; the serotonin $(5\text{-HT})_{1A}$ agonist, flesinoxan; and the 5-HT_{2C} antagonist, SB242,084 (Barrett and Gleeson 1991; Schreiber and De Vry 1993; Coplan et al. 1995; Griebel 1995; Miczek et al. 1995; Dekeyne et al. 2000; Millan et al. 2000a). Moreover, we determined their potential influence upon extracellular level of 5-HT, norepinephrine (NE) and dopamine (DA) in the FCX of freely moving rats.

METHODS

Animals

Unless otherwise specified, these studies employed male Wistar rats of 200–250 g and NMRI mice of 22–25 g (Iffa Credo, l'Arbresles, France) housed in sawdust-lined cages with unrestricted access to standard chow and water. There was a 12:12 hr light:dark cycle with lights on at 0730. Laboratory temperature and humidity were 21 ± 0.5°C and 60 ± 5%, respectively. Animals were adapted to laboratory conditions for at least a week prior to testing. They were used once only. All animal use procedures conformed to international European ethical standards (86/609-EEC) and the French National Committee (décret 87/848) for the care and use of laboratory animals.

SI Test

As previously described (Dekeyne et al. 2000), male Sprague-Dawley rats of 240–260 g (Charles River, Saint-Aubin-les-Elbeuf, France) were individually housed for 5 days before testing. On the test day, they were placed in weight-matched pairs (±5 g) in opposite corners of a highly illuminated (300 lux), open-topped arena (57 × 36 × 30 cm) for a 10 min session. A camera was mounted 2 m above the arena and was connected to a monitor and a videotape recorder in an adjacent room. The observer recorded from the screen the duration of active social interaction: that is, the time spent in grooming, following, sniffing, biting, jumping, or crawling over or under the other animal. If animals remained adjacent to each other without any movement for more than 10 s, scoring was discontinued until active SI resumed. Animals were administered with drug or vehicle 30 min before testing, with each rat of the same pair receiving the same treatment.

Vogel Test

As previously described (Dekeyne et al. 2000), the test was conducted in polycarbonate cages (32 × 25 × 30 cm) possessing a grid floor with the spout of a water bottle located 6 cm above the floor. Both the grid and the spout were connected to an Anxiometer (Columbus Instruments, Ohio, USA) used to record licks and deliver electrical shocks. During the 3 days preceding testing, rats were housed by four and were restricted to 1 hr-per-day access to tap water (from 0900 to 1000). On day 4, just after water delivery, they were isolated in cages with a grid-floor. Testing took place on day 5. Each rat was placed in the test cage and the session was initiated after the animal had made 20 licks and received a first, mild shock (a single, 0.5 s constant current pulse of 0.3 mA intensity) through the spout. Thereafter, a shock was delivered to the animal every twentieth lick during a period of 3 min. Only animals that initiated the session within 5 min were studied further. Data were the number of licks emitted by the animal during the 3 min session. Certain control (vehicle) animals did not receive shocks during the session and were used to evaluate free drinking behavior. Drugs were given 30 min before testing. The percentage of drug effect was computed as [(drug − vehicle)/(vehicle non-shocked − vehicle)].

Plus-maze Test

As previously described (Millan et al. 1997), the experiments were performed in a white-mat-painted plus-maze constructed of wood and elevated to a height of 50 cm. The apparatus comprised two open arms (50 × 10 cm) and two enclosed arms of the same dimensions, with walls 40 cm high. The two open arms were opposite to each other. On the test day, each rat was administered with drug or vehicle and was placed, 30 min later, in the central square of the maze facing one of the enclosed arms. The number of entries and time spent in open, and enclosed arms were recorded by an observer situated 2 m from the maze. An entry was counted only when the rat had its four limbs in an individual arm. Data were the total number of entries, the percentage entries and the percentage time spent in open arms. Drugs were given 30 min before testing.

USV Test

As previously (Millan et al. 1997), there were 3 different experimental phases performed at intervals of 24 hr. On day 1 (training), rats were placed in a chamber equipped with a grid-floor and were exposed to 6 randomly-distributed electric shocks (800 μA and 8 s) over a 7 min period. On day 2 (selection), they were placed in the chamber for 2 min and received a single shock. They were returned to the chamber 30 min later and ul-

trasonic vocalizations recorded for 10 min. Only rats emitting ultrasonic vocalizations for a total duration of at least 90 s were examined further. On day 3, the procedure was identical to day 2, but rats were treated with drug or vehicle immediately after the 2 min session. Data were the total duration of ultrasonic vocalizations recorded over the 10 min session.

Rotarod Procedure in Mice

As described previously (Dekeyne et al. 2000), 30 min after drug or vehicle injection, mice were placed on the rotating bar of a Rotarod apparatus (Ugo Basile, Varese, Italy) that gradually accelerated from 4 to 40 rpm over a period of 300 s. The latency of mice to fall was determined with a cut-off of 360 s.

Spontaneous Locomotion in Rats

As previously described (Dekeyne et al. 2000), rats were individually placed for 12 min in transparent polycarbonate cages (45 × 30 × 20 cm) equipped with two rows of photocells 4 cm above the floor and 24 cm apart. A locomotion count corresponded to the consecutive interruption within 2 s of 2 infrared beams. Drugs or vehicle were given 30 min prior to testing.

Determination of Dialysate Levels of Monoamines

The protocol employed is described in detail elsewhere (Gobert et al. 2000). Briefly, the influence of drugs upon levels of DA, NE, and 5-HT in single dialysate samples of the FCX was determined employing HPLC plus coulometric detection in freely moving rats implanted one week before testing with a guide cannula in this region. Samples were taken every 20 min. Following 2 hr "equilibration," basal monoamine levels were monitored for 1 hr, then drugs were injected, and samples were taken for a further 3 hr. Changes were expressed relative to basal values (defined as 100%).

Drugs

For all drugs in all procedures, extensive dose-response relationships were examined. Incremental doses were tested until (1) statistical significance was attained, (2) the dose-response curve inflected, and/or (3) (for s.c. administration) the solubility limit was reached. All drug doses are in terms of the base. CP154,526 was administered i.p. as a suspension in carboxymethylcellulose (0.1%). Flesinoxan and DMP695 were administered s.c. in solution (sterile water). A few drops of lactic acid were added, and pH adjusted as close to normality (>5.0) as possible. SB242,084, chlordiazepoxide, and, for the Vogel test, DMP695, were administered i.p. as a suspension in water with a few drops of Tween 80. Drugs were injected in a volume of 1 ml/kg (rats) or 10 ml/kg (mice). Drug sources, salts, and structures were as follows: CP154,526 (butyl-ethyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo [2.3-d] pyrimidin-4-yl]amine) HCl, DMP695 (N-(2-chloro-4,6-dimethylphenyl)-1-[1-methoxymethyl-(2-methoxyethyl]-6-methyl-1H-1,2,3,triazolo[4,5-c]pyridin-4-amine) mesylate, SB242,084 (6-chloro-5-methyl-1-[6-(2-methylpyridin-3-yloxy) pyridin-3-yl carbamoyl] indoline) HCl and racemic (±) flesinoxan HCl were synthesized by Servier chemists (P. Casara and G. Lavielle). Chlordiazepoxide HCl was supplied from Produits Roche (Neuilly-sur-Seine, France).

Statistics

In all behavioral studies, dose-effects were analyzed employing one-way analysis of variance (ANOVA) followed by Dunnett's test. Where computable (USV and motor procedures), Inhibitory $Dose_{50}$s (ID_{50}s) plus 95% confidence limits (CL) were calculated. In the dialysis study, data were analyzed by ANOVA with sampling time as the repeated within-subject factor.

RESULTS

Vogel Conflict Test

Over a dose-range of 5.0–80.0 mg/kg, CP154,526 significantly, dose-dependently, and markedly increased punished responses in the Vogel procedure (Figure 1 and Table 1). DMP695 mimicked this effect of CP154,526 in monotonically enhancing punished responses over a dose range of 10.0–40.0 mg/kg, with the latter dose achieving statistical significance. Chlordiazepoxide similarly showed robust activity in the Vogel procedure over a dose-range of 5.0–20.0 mg/kg. Flesinoxan was also active, with statistical significance obtained at doses of 2.5 and 10.0 mg/kg, although the dose-response curve inflected at a dose of 40.0 mg/kg. SB242,084 displayed only modest activity in this procedure, attaining statistical significance at a dose of 15.0 mg/kg.

SI Test

In pairs of unfamiliar rats exposed to an unfamiliar environment, CP154,526 elicited a pronounced, dose-dependent, and significant increase in active SI at doses of 0.16–2.5 mg/kg, with a further increase in the dose to 10.0 achieving no additional effect (Figure 2 and Table 1). DMP695 also elicited a dose-dependent and significant facilitation of active SI. Although chlordiazepoxide evoked a robust increase in SI, its dose-response curve was clearly biphasic, inflecting at the highest dose (10.0 mg/kg). Flesinoxan similarly manifested a biphasic dose-response curve in this paradigm. Finally, SB242,084

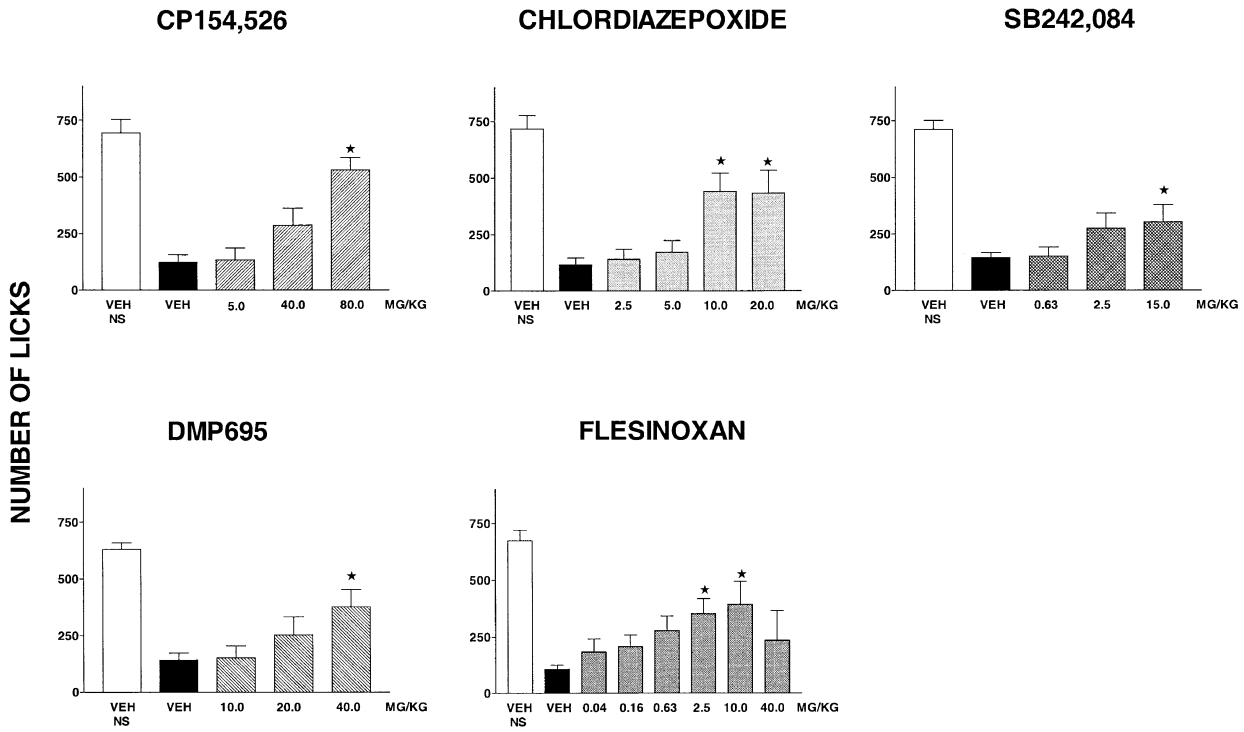

Figure 1. Actions in the Vogel test. VEH = vehicle; NS = non-stressed controls. Data are means ± SEMs. N = 5 per value. ANOVA as follows: CP154,526, $F(3,37) = 13.5$, $p < .01$; DMP695, $F(3,22) = 3.2$, $p < .05$; chlordiazepoxide, $F(4,41) = 5.5$, $p < .01$; flesinoxan, $F(6,63) = 3.0$, $p < .05$; and SB242,084, $F(3,58) = 2.9$, $p < .05$. Asterisks indicate significance of differences to corresponding vehicle values in Dunnett's test. *$p < .05$.

was potently active in enhancing SI. Simultaneous monitoring of other behaviours (rearing, locomotion and sleeping) revealed no significant effects of CP154,526 or DMP695 (not shown).

USV Test

Administered over a dose-range corresponding to doses active in the Vogel and SI models, neither CP154,526 nor DMP695 significantly reduced USV in rats re-exposed to an environment in which they had previously received an aversive stimulus (Figure 3 and Table 1). In distinction, chlordiazepoxide showed dose-dependent activity in this model. Flesinoxan also displayed marked activity, whereas SB242,084 was ineffective.

Plus-maze Test

Administered over a broad dose-range, CP154,526 did not significantly modify the number or percentage of

Table 1. Summary of Drug Actions in Models of Potential Anxiolytic versus Motor Activity

	Vogel Conflict		Social Interaction		USV		Plus-Maze		Rotarod (mice)		Spont Loc	
Drug	MED	% MOE (Dose)	MED	% MOE (Dose)	ID_{50} (95% CL)	% MOI (Dose)	MED	% MOE (Dose)	ID_{50} (95% CL)	% MOI (Dose)	ID_{50} (95% CL)	% MOI (Dose)
CP154,526	80.0	71 (80.0)	2.5	23 (2.5)	> 80.0	19 (80.0)	> 80.0	6 (80.0)	> 80.0	24 (10.0)	NC	51 (80.0)
DMP695	40.0	48 (40.0)	40.0	15 (40.0)	> 40.0	38 (10.0)	> 40.0	41 (0.63)	11.1 (2.2–55.7)	68 (40.0)	12.2 (2.5–58.3)	62 (40.0)
Chlordiazepoxide	10.0	50 (10.0)	5.0	38 (5.0)	3.3 (1.3–7.0)	100 (40.0)	2.5	279 (2.5)	3.6 (1.0–13.8)	72 (10.0)	9.5 (3.4–26.6)	96 (40.0)
Flesinoxan	2.5	51 (10.0)	2.5	61 (10.0)	0.2 (0.1–0.4)	96 (0.63)	> 10.0	57 (0.01)	3.5 (1.3–9.8)	80 (10.0)	3.8 (1.8–8.1)	85 (10.0)
SB242,084	15.0	28 (15.0)	0.16	51 (0.63)	> 10.0	46 (0.63)	> 10.0	86 (0.63)	> 10.0	0 (10.0)	NC	60 (40.0)

USV = Ultrasonic vocalizations; Spont Loc = Spontaneous locomotion; MED = Minimal Effective Dose; % MOE = % Maximal Observed Effect; % MOI = % Maximal Observed Inhibition; ID = Inhibitory Dose and NC = not computable. Doses are in mg/kg. For plus-maze, MED and % MOE correspond to % entries in open arms.

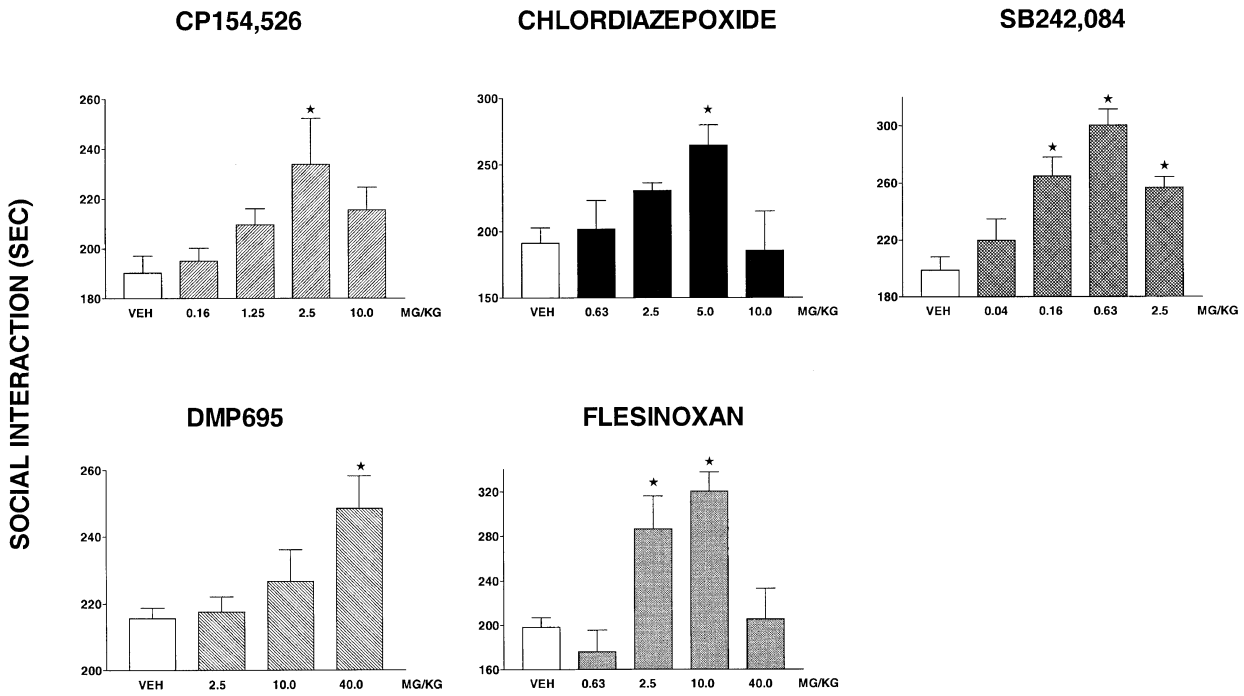

Figure 2. Actions in the Social Interaction test. VEH = vehicle. Data are means ± SEMs. N = 5 per value. ANOVA as follows: CP154,526, $F(4,31) = 3.2$, $p < .05$; DMP695, $F(3,18) = 4.8$, $p < .05$; chlordiazepoxide, $F(4,31) = 4.1$, $p < .01$; flesinoxan, $F(4,32) = 8.6$, $p < .01$; and SB242,084, $F(4,27) = 12.9$, $p < .01$. Asterisks indicate significance of differences to corresponding vehicle values in Dunnett's test. *$p < 0.05$.

open arm entries in the plus-maze procedure (Figure 4 and Table 1). It also did not significantly affect the total number of arm entries. DMP695 similarly failed to increase presence in the open arms and, at the highest dose tested, it tended to decrease entries and time in open arms. In distinction, chlordiazepoxide evoked a significant increase in entries and time in open arms, although its dose-response curve was biphasic. Both flesinoxan and SB242,084 were ineffective in increasing presence in open arms. They did not suppress total arm entries at any dose examined.

Motor Behavior

CP154,526 did not significantly affect behavior in the rotarod test in mice (Table 2). It also did not significantly affect spontaneous locomotor activity in rats, although it tended to decrease activity at the highest dose evaluated. DMP695 elicited a dose-dependent reduction in latency to fall in the rotarod test in mice, and a dose-dependent reduction in spontaneous locomotor activity in rats. Chlordiazepoxide elicited a pronounced and dose-dependent ataxia in the rotarod test in mice and also reduced spontaneous locomotor activity in rats. Flesinoxan also showed clear activity in both procedures. Finally, SB242,084 was ineffective in the rotarod procedure and reduced locomotion only at the highest dose tested.

Modulation of Dialysate Levels of Monoamines in FCX

CP154,526 failed to modify extracellular levels of 5-HT, NE or DA in the FCX of freely moving rats (Figure 5 and Table 3). In contrast, chlordiazepoxide evoked a marked and sustained diminution of levels of 5-HT, NE and DA. Flesinoxan markedly diminished levels of 5-HT, whereas those of NE and DA were simultaneously elevated. On the other hand, SB242,084 elevated levels of both NE and DA without modifying concentrations of 5-HT. (Because of insufficient quantities available, DMP695 was not examined in this procedure.)

DISCUSSION

Vogel Test

In line with a potential role in conflict paradigms, i.c.v. administration of CRF decreased punished responses in pigeons (Zhang and Barrett 1990). Further, employing a Vogel procedure, it was demonstrated that anxiogenic actions of social defeat are abolished in CRF_1 knock-out mice (Van Gaalen et al. 1999), although a concurrent reduction of non-punished responses complicated interpretation of these data. In fact, α-helical-CRF_{9-41} was ineffective in a Geller-Seifter conflict paradigm in rats (Britton et al. 1986) and a similar lack of activity was documented for CP154,526 at doses of 0.6–20.0 mg/kg,

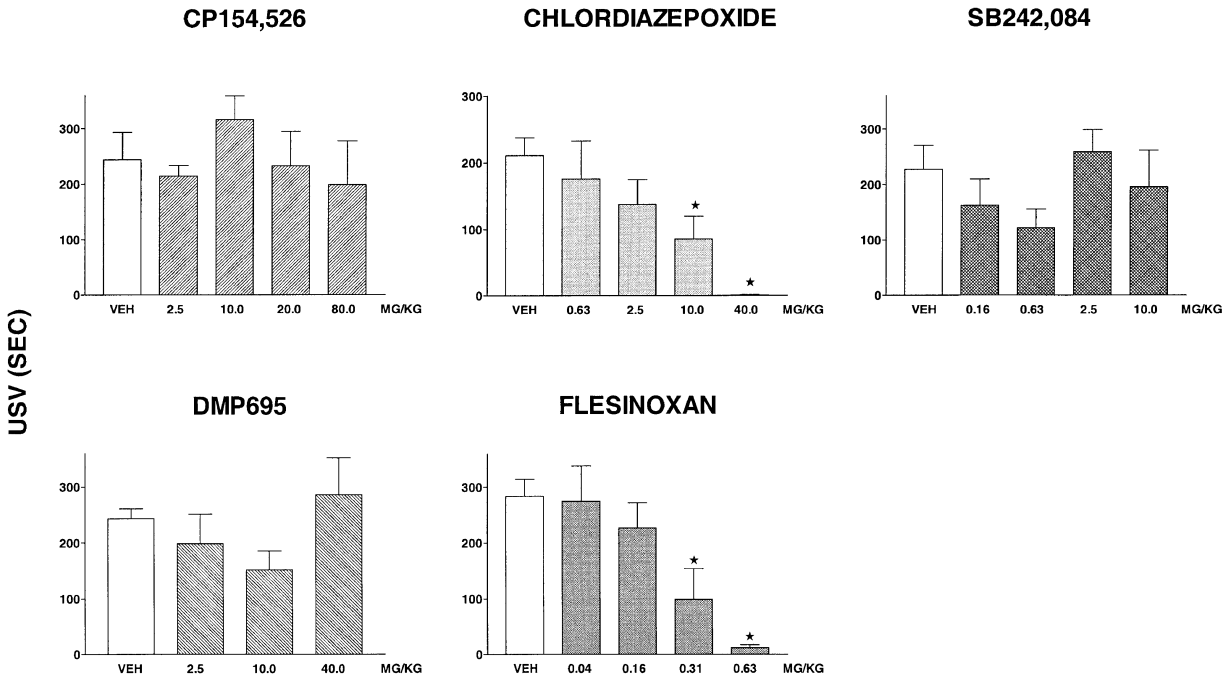

Figure 3. Actions in the USV test. VEH = vehicle. Data are means ± SEMs. N = 5 per value. ANOVA as follows: CP154,526, $F(4,32) = 0.6$, $p > .05$; DMP695, $F(3,18) = 1.9$, $p > .05$; chlordiazepoxide, $F(4,31) = 4.5$, $p < .01$; flesinoxan, $F(4,29) = 7.1$, $p < .01$; and SB242,084, $F(4,25) = 1.1$, $p > .05$. Asterisks indicate significance of differences to corresponding vehicle values in Dunnett's test. *$p < .05$.

i.p. (Griebel et al. 1998). Over this dose-range, CP154,526 similarly did not modify punished responses in the Vogel test, but, at a higher dose (80.0), a robust response was seen herein. Although CRF may enhance nociception (Millan 1999), CP154,526 is inactive in diverse algesiometric models (not shown), and any potential antinociceptive effect of CP154,526 is unlikely to be involved (Barrett and Gleeson 1991). In addition, although inactivation of CRF_1 receptors attenuates the suppressive influence of stress upon food intake, nonpeptidergic CRF_1 antagonists exert little influence upon food and water intake (see Steckler and Holsboer 1999), and, at anxiolytic doses, CP154,526 did not affect food or water consumption (not shown). The observation that DMP695 evoked a comparable increase in punished responses underpins findings with CP154,526 and, collectively, the above-discussed data support a role of CRF_1 receptors in modulation of emotionality in conflict procedures.

The activity of chlordiazepoxide in the Vogel test coincides with numerous reports of anxiolytic actions of BZDs in conflict paradigms (Barrett and Gleeson 1991; Griebel et al. 1998; Dekeyne et al. 2000). Further, the enhancement of punished responses by flesinoxan extends observations with various 5-HT_{1A} agonists and with flesinoxan in other conflict models (Coplan et al. 1995; Griebel 1995; Dekeyne et al. 2000). Nevertheless, actions of 5-HT_{1A} agonists are less marked than those of BZDs (Barrett and Gleeson 1991; Sanger 1992; Coplan et al. 1995; King et al. 1997; Millan et al. 1997). 5-HT_{2C} receptor antagonists are also less efficacious than BZDs in conflict procedures (Cervo and Samanin, 1995; Kennett et al. 1996; Griebel et al. 1997). Indeed, SB242,084 displayed only modest activity in the Vogel paradigm herein, although it enhanced punished responses in a Geller-Seifter conflict procedure (Kennett et al. 1997).

Plus-maze Test

Robust anxiogenic actions of CRF, reversible by nonpeptidergic and peptidergic CRF_1 antagonists as well as antisense probes, have been observed in mice and rats employing the plus-maze procedure (Heinrichs et al. 1992; Menzaghi et al. 1994; Martins et al. 1997; Okuyama et al. 1999; Spina et al. 2000). In addition, a reduction in anxiety was detected in studies of CRF_1 knock-out mice (Smith et al. 1998; Contarino et al. 1999), implying that endogenous CRF can mediate anxiety under these conditions. However, in the above-cited studies, intrinsic, anxiolytic actions of CRF_1 antagonists were not reported. In fact, only a preliminary report has appeared of anxiolytic actions of the CRF_1 antagonists, NBI27914 and NBI30545, in this paradigm (Wilcoxon et al. 1999). These findings contrast with the lack of activity of CRA1000, CRA100 (Okuyama et al. 1999) and, as shown herein, DMP695 and CP154,526. The latter was ineffective over a broad dose-range (0.63-80.0), supporting observations of Griebel et al. (1998). Moreover, al-

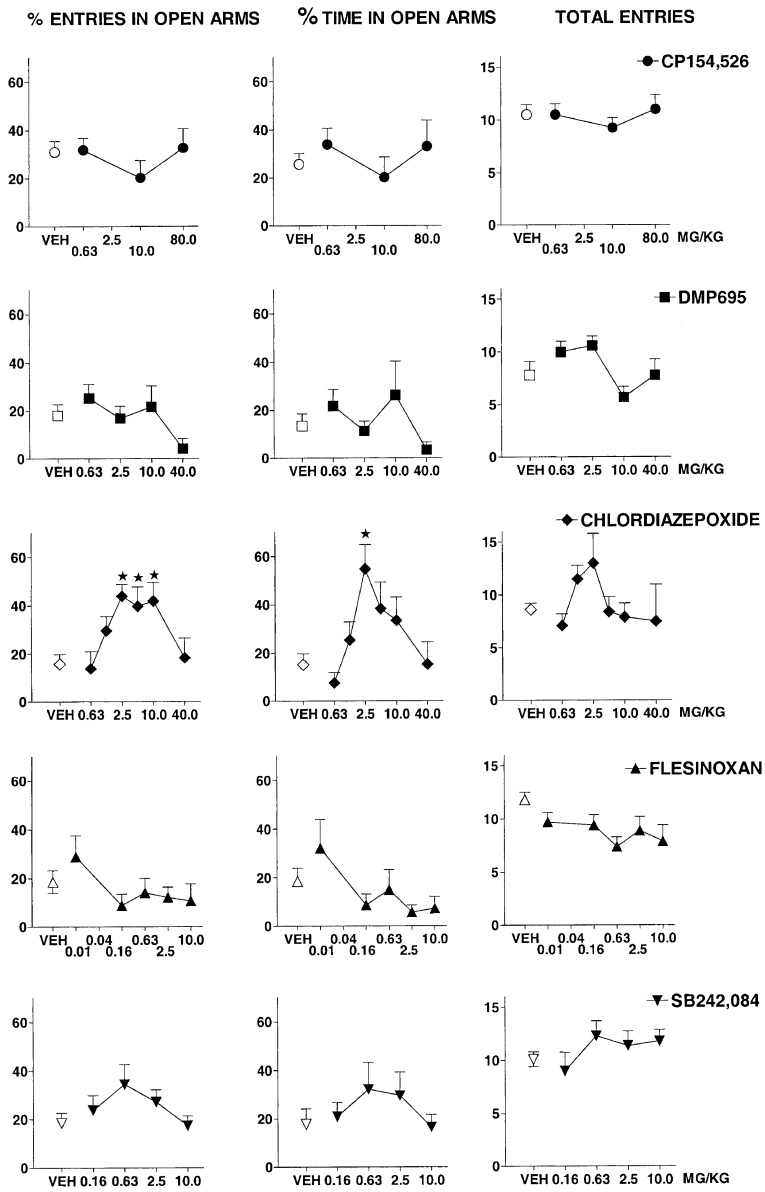

Figure 4. Actions in the plus-maze test. VEH = vehicle. Data are means ± SEMs. N = 6 per value. ANOVA as follows: For % entries in open arms (left panels): CP154,526, $F(3,28) = 0.8$, $p > .05$; DMP695, $F(4,31) = 1.3$, $p > .05$; chlordiazepoxide, $F(6,49) = 4.1$, $p < .01$; flesinoxan, $F(5,41) = 1.5$, $p > .05$; and SB242,084, $F(4,37) = 1.6$, $p > .05$. For % time in open arms (middle panels): CP154,526, $F(3,28) = 0.7$, $p > .05$; DMP695, $F(4,31) = 1.2$, $p > .05$; chlordiazepoxide, $F(6,49) = 4.0$, $p < .01$; flesinoxan, $F(5,41) = 2.1$, $p > .05$; and SB242,084, $F(4,37) = 0.8$, $p > .05$. For total entries (right panels): CP154,526, $F(3,28) = 0.5$, $p > .05$; DMP695, $F(4,31) = 3.1$, $p < .05$; chlordiazepoxide, $F(6,49) = 1.6$, $p > .05$; flesinoxan, $F(5,41) = 2.0$, $p > .05$; and SB242,084, $F(4,37) = 1.1$, $p > .05$. Asterisks indicate significance of differences to corresponding vehicle values in Dunnett's test. *$p < .05$.

though Lundkvist et al. (1996) reported "signs" of anxiolytic activity at 1.0 mg/kg, this action was absent at higher doses and expressed non-specifically inasmuch as closed arm entries also increased. There are several possible explanations for this lack of activity.

First, a preliminary report claimed that CP154,526 has weak partial agonist actions at CRF_1 receptors (Grosjean-Piot et al. 1997), but this contention has not been confirmed and does not apply to DMP695, which was also ineffective (Schulz et al. 1996; Steckler and Holsboer 1999; Gilligan et al. 2000). Further, this explanation would not account for a lack of anxiolytic activity of other "pure" antagonists mentioned above. Second, contrasting actions of non-peptidergic antagonists might reflect differences in their mode of interactions at CRF_1 receptors, which may possess multiple binding sites and/or various isoforms (Gilligan et al. 2000). Third, there may be species differences between mice and rats inasmuch as anxiolytic effects of CRF_1 receptor deletion were demonstrated in the former with the plus-maze procedure, while essentially negative findings with CRF_1 antagonists have been described in the latter (Steckler and Holsboer 1999). Fourth, the level of "stress" may be a critical variable, with the effect of CRF_1 receptor blockade being proportional to the degree of stress experienced (Griebel et al. 1998; Okuyama et al. 1999; Steckler and Holsboer 1999; Keck et al. 2000).

Irrespective of such considerations, potential anxiolytic actions of CRF_1 receptor antagonists in the plus-maze and other procedures reflecting exploratory activity, such as the light-dark box and "defensive withdrawal" models, would be of interest to document further (Griebel et al. 1998; Okuyama et al. 1999; Steckler and Holsboer 1999; Arborelius et al. 2000). These variable data may be con-

Table 2. Influence of Drugs on Motor Behavior

Drug	Dose	Rotarod (mice)	Spontaneous Locomotion (rats)
CP154,526	Vehicle	195 ± 26	54 ± 6
	2.5	209 ± 42	45 ± 2
	10.0	148 ± 23	47 ± 6
	40.0	—	48 ± 14
	80.0	246 ± 37	26 ± 5
DMP695	Vehicle	236 ± 36	58 ± 11
	0.63	222 ± 24	54 ± 6
	2.5	—	43 ± 8
	10.0	97 ± 33*	29 ± 4*
	40.0	75 ± 31*	24 ± 2*
Chlordiazepoxide	Vehicle	198 ± 41	50 ± 10
	0.63	161 ± 18	—
	2.5	123 ± 23	46 ± 9
	10.0	55 ± 6*	31 ± 9
	40.0	—	2 ± 1*
Flesinoxan	Vehicle	300 ± 18	39 ± 5
	0.63	249 ± 34	59 ± 10
	2.5	214 ± 53	17 ± 4*
	10.0	59 ± 7*	6 ± 2.5*
SB242,084	Vehicle	229 ± 30	61 ± 8
	0.63	267 ± 32	52 ± 5
	2.5	232 ± 32	60 ± 5
	10.0	244 ± 38	58 ± 5
	40.0	—	24 ± 4*

Doses are in mg/kg. Data are means latency to fall (s) ± SEMs (rotarod) or means locomotion counts ± SEMs (spontaneous locomotion). $n \geq 5$ per value. ANOVA as follows. Rotarod: CP154,526, $F(3,19) = 1.5$, $p > .05$; DMP695, $F(3,19) = 6.4$, $p < .01$; chlordiazepoxide, $F(3,17) = 5.0$, $p < .05$; flesinoxan, $F(3,17) = 10.4$, $p < .01$; and SB242,084, $F(3,18) = 0.3$, $p > .05$. Spontaneous locomotion: CP154,526, $F(4,29) = 1.6$, $p > .05$; DMP695, $F(4,20) = 3.7$, $p < .05$; chlordiazepoxide, $F(3,23) = 5.9$, $p < .01$; flesinoxan, $F(3,26) = 13.2$, $p < .01$; and SB242,084, $F(4,34) = 3.6$, $p < .05$. Asterisks indicate significance of differences to vehicle values in Dunnett's test following ANOVA. *$p < .05$.

trasted with the reproducible anxiolytic actions of BZDs, such as chlordiazepoxide (Griebel et al. 1997, 1998). On the other hand, data with 5-HT_{1A} agonists have proven highly variable, with both anxiolytic and anxiogenic actions, possibly mediated by pre- and postsynaptic 5-HT_{1A} receptors, respectively (Schreiber and De Vry 1993; Andrews et al. 1994; Coplan et al. 1995; Collinson and Dawson 1997; Millan et al. 1997). Indeed, although flesinoxan enhanced open arm entries in a mouse plus-maze, it concurrently reduced total arm entries (Rodgers et al. 1994) and specific anxiolytic actions of flesinoxan were not found herein. While the 5-$HT_{2B/2C}$ antagonist, SB206,553, showed anxiolytic actions in a plus-maze study (Griebel et al. 1997), these authors underlined its "weaker anxiety-reducing potential" than BZDs, and SB242,084 was inactive in the present model. Nevertheless, for both 5-HT_{1A} and 5-HT_{2C} receptor ligands, quantification of anxiety-related behaviors other than arm entries may enhance test sensitivity (Rodgers and Cole 1994), and the exploitation of such an approach might similarly improve detection of potential actions of CRF_1 antagonists.

SI Test

Extensive studies of social stress, such as aggressive encounters leading to "social defeat," suggest an important role of CRF_1 receptors in the modulation of emotionality in interaction with conspecifics (Heinrichs et al. 1992; Menzaghi et al. 1994; Liebsch et al. 1995; 1999; Jasnow et al. 1999; Spina et al. 2000). Such observations are pertinent to the SI model whereby unfamiliar pairs of rats are introduced to an unfamiliar, "stressful" environment. Under these conditions, i.c.v. administration of CRF (or urocortin) acts anxiogenically in suppressing active SI (Dunn and File, 1987; Moreau et al. 1997; Sajdyk et al. 1999). This action involves, at least partially, engagement of CRF_1 receptors in the amygdala and is blocked by non-peptidergic CRF_1 antagonists (Sajdyk et al. 1999; Steckler and Holsboer 1999). The present studies amplify, thus, such observations in demonstrating dose-dependent increases in SI with both CP154,526 and DMP695.

The enhancement of SI by chlordiazepoxide underpins studies with other BZDs, although dose-response curves inflect at high doses concomitant with onset of motor-suppressive properties (Griebel 1995; Dekeyne et al. 2000). 5-HT_{1A} agonists are likewise effective in the SI model (Schreiber and De Vry 1993; Griebel 1995; Dekeyne et al. 2000), observations supported by the present findings with flesinoxan. Finally, the present data confirm the robust anxiolytic actions of SB242,084, in analogy to other 5-HT_{2C} antagonists in this procedure (Kennett et al. 1996; 1997; Dekeyne et al. 2000).

USV Test

Central administration of CRF potentiates expression of conditioned fear in rats, an action to which CRF_1 receptors in the PAG and hippocampus contribute, although a role of other structures should not be excluded (Schulz et al. 1996; Tershner and Helmstetter, 1996; Martins et al. 1997; Deak et al. 1999; Radulovic et al. 1999). Evidence that endogenous pools of CRF facilitate psychological stress was provided by Schulz et al. (1996), who showed that CP154,526 blocks both CRF- and fear-induced potentiation of the acoustic startle response in rats. In contrast, in the present model of conditioned fear, CP154,526 and DMP695 failed to inhibit USV. This lack of activity corresponds to the report by Okuyama et al. (1999) that CRA1000 and CRA1001 do not influence conditioned fear in a passive avoidance paradigm. On the other hand, CP154,526 was reported to reduce USV following separation in young rats (Kehne et al. 2000), while Deak et al. (1999), employing the freezing response, observed significant anxiolytic effects of antalarmin. Thus, the precise measure of anxiety may be a decisive variable determining drug actions, although many other factors, such as procedural details and the level of stress, may also underlie contrasting patterns of data.

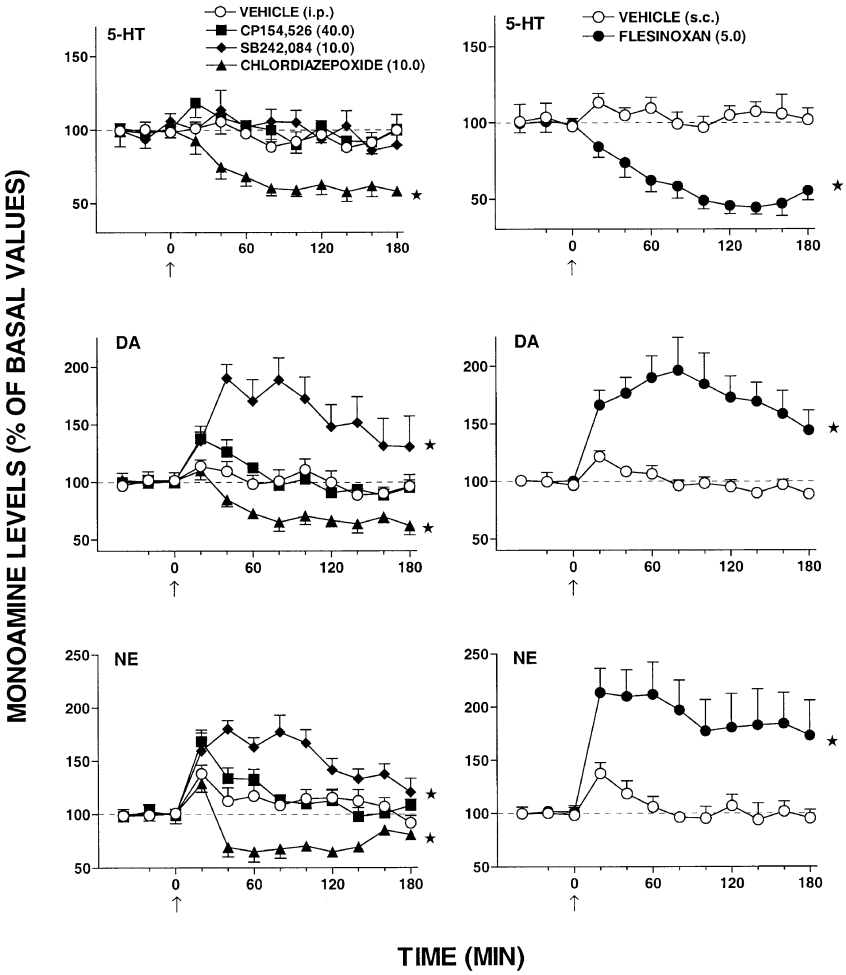

Figure 5. Influence upon extracellular levels of 5-HT, NE, and DA in dialysates of frontal cortex. Doses are indicated in mg/kg. Dialysate levels are expressed as a percentage of basal, pre-injection values, which were defined as 100%. These were 0.84 ± 0.09, 0.96 ± 0.06, and 1.79 ± .10 pg/20 μl dialysate for 5-HT, DA and NE, respectively. Data are means ± SEMs. $n = 5$ per value. ANOVA with dose as between factor and time as within factor was performed over 20–180 min. 5-HT: CP154,526, $F(1,10) = 1.0$, $p > .05$; chlordiazepoxide, $F(1,9) = 70.9$, $p < .01$; flesinoxan, $F(1,12) = 50.6$, $p < .01$; and SB242,084, $F(1,10) = 1.1$, $p > .05$. DA: CP154,526, $F(1,10) = 0.5$, $p > .05$; chlordiazepoxide, $F(1,9) = 14.5$, $p < .01$; flesinoxan, $F(1,12) = 24.1$, $p < .01$; and SB242,084, $F(1,10) = 9.9$, $p < .05$. NE: CP154,526, $F(1,10) = 1.1$, $p > .05$; chlordiazepoxide, $F(1,9) = 13.0$, $p < .01$; flesinoxan, $F(1,12) = 11.2$, $p < .01$; and SB242,084, $F(1,10) = 14.2$, $p < .05$. Asterisks indicate significant differences between the drug-treated group and the vehicle-treated group. *$p < .05$.

This inactivity of CP154,526 and DMP695 contrasts to dose-dependent actions of chlordiazepoxide and other BZDs in this paradigm (Molewijk et al, 1995; Millan et al. 1997). On the other hand, SB242,084 was ineffective, in analogy to other 5-HT_{2C} antagonists (Sànchez and Mørk 1999; Dekeyne A, unpub. obs.). This inactivity may be related to the contention that the USV model mimics a " panic-like" state (Molewijk et al. 1995; Jenck et al. 1998) since, in contrast to other forms of anxiety, activation of 5-HT_{2C} receptors (probably in the PAG) reduces emotionality under such conditions (Jenck et al. 1998). A relationship to panic states has also been claimed for potent actions of flesinoxan and other 5-HT_{1A} agonists in the USV procedure (Griebel 1995; Millan et al. 1997; Sànchez and Mørk 1999), which were corroborated herein. However, the exacerbation of panic states by flesinoxan in man (Van Vliet et al. 1996; Jenck et al. 1999) questions this interpretation.

Table 3. Area-under-the-Curve (AUC) Analysis of the Influence of Drugs upon Extracellular Levels of 5-HT, NE, and DA in Dialysates of Frontal Cortex

			% AUC		
Drug	**Dose**	**Route**	**5-HT**	**DA**	**NE**
Vehicle	—	i.p.	95.6 ± 2.3	100.9 ± 2.8	113.1 ± 3.2
Vehicle	—	s.c.	104.6 ± 2.4	100.2 ± 2.0	105.7 ± 3.7
CP154,526	40.0	i.p.	100.8 ± 3.0	105.1 ± 2.9	119.7 ± 3.7
Chlordiazepoxide	10.0	i.p.	66.2 ± 2.5*	74.0 ± 2.9*	77.9 ± 3.5*
Flesinoxan	5.0	s.c.	57.7 ± 2.8*	173.2 ± 6.4*	192.3 ± 9.3*
SB242,084	10.0	i.p.	99.8 ± 2.8	157.9 ± 6.7*	153.4 ± 4.7*

Values are means ± SEMs. See legend to Fig. 5 for details and statistical analyses. *$p < .05$ to corresponding vehicle.

Motor Behavior

The potential influence of CRF_1 receptors upon motor behavior critically depends upon novelty, suggesting that anxiety itself impacts upon this parameter (Steckler and Holsboer 1999). In fact, no major alteration of motor behavior is apparent in mice lacking the gene encoding CRF_1 receptors, their neutralization with antisense, or treatment with selective CRF_1 receptors antagonists (Griebel et al. 1998; Smith et al. 1998; Liebsch et al. 1999; Okuyama et al. 1999; Steckler and Holsboer 1999), and CP154,526 modified neither spontaneous locomotor behavior nor rotarod performance in mice. Indeed, anxiolytic actions of CP154,526 were exerted in the absence of marked motor perturbation. On the other hand, DMP695 interfered with motor behavior. The reason for this difference remains to be clarified since receptorial interactions of DMP695 at sites other than CRF_1 receptors have not been described (Bakthavatchalam et al. 1998; Newman-Tancredi A and Millan MJ, unpublished observations). In any case, the decrease in motor function elicited by DMP695 clearly cannot underlie an increase in responses in the Vogel procedure and an increase in active SI.

While the influence of CRF_1 antagonists upon motor behavior requires further characterization, BZDs, such as chlordiazepoxide, profoundly disrupt motor function. Further, although 5-HT_{1A} receptor agonists, such as flesinoxan, are not sedative in humans; they perturb motor behavior in rodents (Coplan et al. 1995; Millan et al. 1997). Contrariwise, selective 5-HT_{2C} receptor antagonists like SB242,084 compromise motor function only at high doses in rodents (Kennett et al. 1996, 1997; Griebel et al. 1998; Dekeyne et al. 2000).

Monoaminergic Transmission

DRN-serotonergic neurones are susceptible to modulation by CRF, likely acting via CRF_1 receptors (Chalmers et al. 1995; Ruggiero et al. 1999). The predominant influence of CRF (at low concentrations) upon serotonergic neurones is inhibitory. This action is attenuated by antalarmin, which itself does not modify electrical activity (Price et al. 1998; Kirby et al. 2000). Correspondingly, CP154,526 did not affect dialysate levels of 5-HT in the FCX. Thus, in distinction to BZDs and 5-HT_{1A} agonists, such as chlordiazepoxide and flesinoxan, respectively, which reduce extracellular levels of 5-HT (Millan et al. 1997), suppression of serotonergic transmission is unlikely to fulfill a major role in the anxiolytic actions of CRF_1 antagonists. Nevertheless, very recently, CP154,526 (32 mg/kg, i.p.) was reported to transiently and slightly (15% relative to basal values) suppress extracellular levels of 5-HT in the hippocampus of conscious rats (Isogawa et al. 2000). Thus, the potential influence of various CRF_1 antagonists upon 5-HT release in the FCX and other regions under "resting" and "anxious" conditions would benefit from additional study.

In contrast, CRF potently enhances the activity of LC-derived adrenergic pathways (Valentino et al. 1993; Curtis et al. 1997), an action which may, in principal, enhance anxiety (Charney et al. 1995; see Millan et al. 2000b), and which is prevented by intracerebral application of non-peptidergic CRF_1 antagonists (Smagin et al. 1995; Curtis et al. 1997; Page and Abercrombie 1999) and CP154,526 (Braselton et al. 1996). These antagonists do not themselves suppress adrenergic activity, and CP154,526 failed to modify FCX levels of NE herein, in line with the lack of influence of local administration of CP154,526 into the LC upon adrenergic transmission (Kawahara et al. 2000). However, a mild ($\sim$15% relative to basal values) diminution in frontocortical release of NE upon i.p. administration of CP154,526 (32 mg/kg) was recently documented by Isogawa et al. (2000). Further, CP154,526 and peptidergic CRF_1 antagonists abrogate the induction of corticolimbic NE release provoked by stress (Shimizu et al. 1994; Smagin et al. 1997; Kawahara et al. 2000), an action which may contribute to their anxiolytic properties. Although this provides a parallel with BZDs, which also inhibit LC-adrenergic neurones, the significance of modulation of adrenergic transmission to anxious states remains under discussion (Swiergiel et al. 1992; Valentino et al. 1993; Weiss et al. 1994; Millan et al. 2000b). In any event, a lack of intrinsic influence of CRF_1 receptor antagonists upon LC-adrenergic projections contrasts strikingly to the pronounced excitation elicited by 5-HT_{1A} agonists and 5-HT_{2C} antagonists, such as flesinoxan and SB242,084, respectively (Figure 5; Gobert et al. 2000; Millan et al. 2000a). Finally, the lack of influence of CP154,526 upon frontocortical (Figure 5) and hippocampal (Isogawa et al. 2000) levels of DA, which have been implicated in anxious states (Morrow et al. 1999), may be differentiated from their suppression by chlordiazepoxide and other BZDs, and their facilitation by 5-HT_{1A} agonists, such as flesinoxan, and 5-HT_{2C} antagonists, such as SB242,084 (see Millan et al. 2000a).

GENERAL DISCUSSION

As summarized in Table 4, CP154,526 and DMP695 displayed similar profiles of anxiolytic activity, consistent with a common role of CRF_1 receptors in their actions. In line with this contention, in extensive binding studies performed both in our laboratory (Newman-Tancredi A and Millan MJ, unpublished observations) and elsewhere (Schulz et al. 1996; Bakthavatchalam et al. 1998; Gilligan PJ, personal communication), as compared to CRF_1 receptors, CP154,526 and DMP695 displayed affinities at least 100-fold lower for multiple serotonergic receptors and diverse other receptors, enzymes

Table 4. Summary of Overall Functional Profiles Characterized in the Present Studies

Drug	Class	Vogel	SI	USV	PM	RR	SLR	5-HT	NE	DA
CP154,526	CRF_1 ANT	yes	yes	no	no	—	—	—	—	—
DMP695	CRF_1 ANT	yes	yes	no	no	†	†	NT	NT	NT
Chlordiazepoxide	BZD	yes	yes	yes	yes	†	†	†	†	†
Diazepam	BZD	yes	yes	yes	yes	†	†	†	†	†
Flesinoxan	$5\text{-}HT_{1A}$ AGO	yes	yes	yes	no	†	†	†	‡	‡
8-OH-DPAT	$5\text{-}HT_{1A}$ AGO	yes	yes	yes	no	†	†	†	‡	‡
SB242,084	$5\text{-}HT_{2C}$ ANT	yes	yes	no	no	—	†	—	‡	‡
SB206,553	$5\text{-}HT_{2C}$ ANT	yes	yes	no	no	†	†	—	‡	‡

BZD = benzodiazepine; ANT = antagonist; AGO = agonist; SI = social interaction; USV = ultrasonic vocalizations; PM = plus maze; RR = rotarod; SLR = spontaneous locomotion in rats; 5-HT = serotonin; NE = norepinephrine; DA = dopamine and NT = not tested. – = no effect; † or ‡ = decrease or increase, respectively. In addition to drugs examined herein, to facilitate comparisons, data are summarized for several other agents previously studied in these procedures under identical conditions (Millan et al. 1997; Dekeyne et al. 2000; Gobert et al. 2000; Millan et al. 2000a).

and channels. Further, as judged by doses blocking the actions of exogenous CRF, doses active in the present anxiolytic procedures correspond well to those required to occupy CRF_1 receptor in vivo (Schulz et al. 1996; see Steckler and Holsboer 1999). Thus, it appears that other factors account for certain differences in the functional profiles of CP154,526 versus DMP695. Notably, DMP695 has a more pronounced influence than CP154,526 upon motor function and a relatively weak activity in the social interaction procedure. Interestingly, while the latter difference between DMP695 and CP154,524 concerns their active dose-ranges, previous studies have found qualitative differences between the anxiolytic profiles of various CRF_1 antagonists. For example, as compared to DMP695 and other CRF_1 antagonists, CP154,526 is inactive in a rat model of "situational anxiety" (Gilligan et al. 2000; Gilligan PJ, personal communcation). As mentioned above in relation to the plus-maze model, variables underlying such differences may include partial agonist activity, differential involvement of multiple CRF_1 receptor isoforms and/or binding sites, and contrasting interactions with "stress"—which is more pronounced for the Vogel test than for the Social Interaction procedure. The resolution of such questions will require further comparative studies of CP154,526, DMP695 and additional CRF_1 antagonists in the present and other functional models.

Finally, although chlordiazepoxide attenuates anxiogenic actions of CRF, and similarities between CRF_1 antagonists and BZDs have been pointed out, the anxiolytic profiles of CP154,526 and DMP695 contrast with those of chlordiazepoxide and other BZDs. Although the latter may more broadly display anxiolytic properties, certain anxious states might be specifically responsive to CRF_1 antagonists (Steckler and Holsboer 1999; Gilligan et al. 2000). The lack of activity of CP154,526 and DMP695 in the USV protocol differentiates them from $5\text{-}HT_{1A}$ agonists. In fact, notwithstanding the lack of intrinsic influence of CRF_1 antagonists upon monoaminergic transmission, the anxiolytic profile of CP154,526 and DMP695 most closely resembled that of $5\text{-}HT_{2C}$ antagonists (Table 4). Indeed, in the light of mutual sites of action in the hippocampus and amygdala, studies of a possible interrelationship between CRF_1 and $5\text{-}HT_{2C}$ antagonists in the modulation of anxious states would be of interest.

CONCLUSIONS

In conclusion, the selective, non-peptidergic CRF_1 antagonists, CP154,526 and DMP695, showed a similar profile of potential anxiolytic activity in rats. Their pattern of action could be distinguished from BZDs and $5\text{-}HT_{1A}$ agonists, and resembled $5\text{-}HT_{2C}$ antagonists. Nevertheless, in contrast to the other drug classes, CP154,526 did not influence extracellular levels of monoamines in FCX, and CRF_1 antagonists likely possess a distinctive mechanism of action requiring further elucidation. Indeed, additional work is also necessary to more precisely characterize the potential utility of CRF_1 receptor antagonists in the clinical treatment of anxious disorders.

ACKNOWLEDGMENTS

We thank H. Gressier, B. Denorme, C. Melon, L. Cistarelli, L. Iob, S. Girardon, and J. Mullot for technical assistance, and Marianne Soubeyran for preparation of the manuscript.

REFERENCES

Adamec RE, McKay D (1993): The effects of CRF and α-helical CRF on anxiety in normal and hypophysectomized rats. J Psychopharmacol 7:346–354

Andrews N, Hogg S, Gonzales LE, File SE (1994): $5\text{-}HT_{1A}$ receptors in the median raphe nucleus and dorsal hippocampus may mediate anxiolytic and anxiogenic behaviours respectively. Eur J Pharmacol 264:259–264

Arborelius L, Skelton KH, Thrivikraman KV, Plotsky PM, Schulz DW (2000): Chronic administration of the selective corticotropin-releasing factor 1 receptor antagonist

CP-154,526: Behavioral, endocrine and neurochemical effects in the rat. J Pharmacol Exp Ther 294:588–597

Bakthavatchalam R, Arvanitis AG, Gilligan PJ, Olson RE, Robertson DW, Trainor GL, Smith SC, Fitzgerald LW, Zaczek R, Shen H, Christ DW (1998): The discovery of DMP 695: An orally active corticotropin-releasing hormone (CRH_1) receptor antagonist. ACS National Meeting, Boston, MA, Aug 23–27

Barrett JE, Gleeson S (1991): Anxiolytic effects of 5-HT_{1A} agonists, 5-HT_2 antagonists and benzodiazepines: conflict and drug discrimination studies. In Rogers RJ, Cooper SJ (eds), 5-HT_{1A} agonists, 5-HT_3 Antagonists and Benzodiazepines: Their Comparative Behavioural Pharmacology. Chichester, Wiley & Sons, pp 59–105

Braselton J, Chen Y, Sprouse J (1996): Blockade of CRF-induced excitation of locus coeruleus cell firing by CP-154,526, a non-peptide antagonist of central CRF receptors. Soc Neurosci Abst 22:1554

Britton KT, Lee G, Vale W, Rivier J, Koob GF (1986): Corticotropin releasing factor (CRF) receptor antagonist blocks activating and "anxiogenic" actions of CRF in the rat. Brain Res 369:303–306

Cervo L, Samanin R (1995): 5-HT_{1A} receptor full and partial agonists and 5-HT_{1C} (but not 5-HT_3) receptor antagonists increase rates of punished responding in rats. Pharmacol Biochem Behav 52:671–676

Chalmers DT, Lovenberg TW, De Sousa EB (1995): Localization of novel corticotropin-releasing factor receptor (CRF_2) mRNA expression to specific subcortical nuclei in rat brain; comparison with CRF_1 receptor mRNA expression. J Neurosci 15:6340–6350

Charney DS, Bremmer JD, Redmond DE Jr (1995): Noradrenergic neural substrates for anxiety and fear: Clinical associations based on preclinical research. In Bloom FE, Kupfer DJ (eds), Psychopharmacology: The Fourth Generation of Progress. New York, Raven Press, pp 387–395

Chen Y, Brunson KL, Müller MB, Cariaga W, Baram TZ (2000): Immunocytochemical distribution of corticotropin-releasing hormone receptor Type-1 (CRF_1)-like immunoreactivity in the mouse brain: Light microscopy analysis using an antibody directed against the C-terminus. J Comp Neurol 420:305–323

Collinson N, Dawson GR (1997): On the elevated plus-maze: The anxiolytic-like effects of the 5-HT_{1A} agonist, 8-OH-DPAT, but not the anxiogenic-like effects of the 5-HT_{1A} partial agonist, buspirone, are blocked by the 5-HT_{1A} antagonist, WAY 100635. Psychopharmacology 132:35–43

Contarino A, Dellu F, Koob GF, Smith GW, Lee K-F, Vale W, Gold LH (1999): Reduced anxiety-like and cognitive performance in mice lacking the corticotropin-releasing factor receptor 1. Brain Res 835:1–9

Coplan JD, Andrews MW, Rosenblum LA, Owens MJ, Friedman S, Gorman JM, Nemeroff CB (1996): Persistent elevations of cerebrospinal fluid concentrations of cortocotropin-releasing factor in adult nonhuman primates exposed to early-life stressors: implications for the pathophysiology of mood and anxiety disorders. Proc Natl Acad Sci USA 93:1619–1623

Coplan JD, Wolk SI, Klein DF (1995): Anxiety and the serotonin$_{1A}$ receptor. In Bloom FE, Kupfer DJ (eds), Psychopharmacology: The Fourth Generation in Progress. New York, Raven Press, pp 1301–1310

Curtis AL, Lechner SM, Pavcovich LA, Valentino RJ (1997): Activation of the locus coeruleus noradrenergic system by intracoerulear microinfusion of corticotropin-releasing factor: Effects on discharge rate, cortical norepinephrine levels and cortical electroencephalographic activity. J Pharmacol Exp Ther 281:163–172

Deak T, Nguyen KT, Ehrlich AL, Watkins LR, Spencer RL, Maier SF (1999): The impact of the nonpeptide corticotropin-releasing hormone antagonist antalarmin on behavioral and endocrine responses to stress. Endocrinology 140:79–86

Dekeyne A, Brocco M, Adhumeau A, Gobert A, Millan MJ (2000): The selective serotonin 5-HT_{1A} receptor ligand, S15535, displays anxiolytic-like effects in the social interaction and Vogel models and suppresses dialysate levels of 5-HT in the dorsal hippocampus of freely-moving rats: a comparison with other anxiolytic agents. Psychopharmacology 152:55–66

De Souza EB (1995): Corticotropin-releasing factor receptors: Physiology, pharmacology, biochemistry and role in central nervous system and immune disorders. Psychoneuroendocrinology 20:789–819

Donaldson CJ, Sutton SW, Perrin MH, Corrigan A, Lewis KA, Rivier JE, Vaughan JM, Vale WW (1996): Cloning and characterization of human urocortin. Endocrinology 137:2167–2170

Dunn AJ, File SE (1987): Corticotropin-releasing factor has an anxiogenic action in the social interaction test. Horm Behav 21:193–202

Fiorino DF, Kagaya T, Shibata H, Nishizawa Y (2000): The CRF receptor 1 antagonist, antalarmin, decreases anxiety as assessed by the shock-probe burying test in rats. Soc Neurosci Abst 26:2265

Gilligan PJ, Baldauf CA, Cocuzza AJ, Chidester DR, Fitzgerald LW, Zaczek R, Shen H-SL (1998): Pyrazole-(1,5-a)-pyrimidine CRF antagonists: Synthesis and structure-activity relationships. ACS National Meeting, Boston, MA, Aug 23–27

Gilligan PJ, Robertson DW, Zaczek R (2000): Corticotropin releasing factor (CRF) receptor modulators: Progress and opportunities for new therapeutic agents. J Med Chem 43:1641–1660

Gobert A, Rivet J-M, Lejeune F, Newman-Tancredi A, Adhumeau-Auclair A, Nicolas J-P, Cistarelli L, Melon C, Millan MJ (2000): Serotonin$_{2C}$ receptors tonically suppress the activity of mesocortical dopaminergic and adrenergic, but not serotonergic, pathways: A combined dialysis and electrophysiological analysis in the rat. Synapse 36:205–221

Griebel G (1995): 5-hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. Pharmacol Ther 65:319–395

Griebel G, Perrault G, Sanger DJ (1997): A comparative study of the effects of selective and non-selective 5-HT_2 receptor subtype antagonists in rat and mouse models of anxiety. Neuropharmacology 36:793–802

Griebel G, Perrault G, Sanger DJ (1998): Characterization of the behavioral profile of the non-peptide CRF receptor antagonist CP-154,526 in anxiety models in rodents. Psychopharmacology 138:55–66

Grigoriadis DE, Lovenberg TW, Chalmers DT, Liaw C, De

Souza EB (1996): Characterization of corticotropin-releasing factor receptor subtypes. Ann NY Acad Sci 780:60–80

Grosjean-Piot O, Burgevin MC, Achard D, Pauchet C, Betschart J, Imperato A, Dubroeucq MC (1997): In vitro and in vivo characterizaton of CP-154,526, a non-peptide CRF1 receptor antagonist with partial agonist properties. Eur Neuropsychopharmacol 7:S276–S277

Guanowsky V, Chen YL, Seymour PA (1997): Anxiolytic effect of the CRF antagonist, CP-154,526, in a light/dark anxiety test in mice. Soc Neurosci Abst 23:522

He L, Gilligan PJ, Zaczek R, Fitzgerald LW, McElroy JF, Shen H-SL, Saye JA, Kalin NH, Shelton S, Christ D, Trainor G, Hartig P (2000): 4-(1,3-Dimethoxyprop-2-ylamino)-2,7-dimethyl-8-(2,4-dichlorophenyl)-pyrazolo[1,5-α]-1,3,5-triazine: A potent, orally biovailable CRF_1 receptor antagonist. J Med Chem 43:449–456

Heinrichs SC, De Souza EB, Schulteis G, Lapsansky JL, Grigoriadis DE (2000): Brain penetrance, receptor occupancy and anti-stress in vivo efficacy of a small molecule corticotropin-releasing $factor_1$ receptor selective antagonist. Soc Neurosci Abst 26:2149

Heinrichs SC, Pich EM, Miczek KA, Britton KT, Koob GF (1992): Corticotropin-releasing factor antagonist reduces emotionality in socially defeated rats via direct neurotropic action. Brain Res 581:190–197

Heinrichs SC, Lapsansky J, Lovenberg TW, De Souza EB, Chalmers DT (1997): Corticotropin-releasing factor CRF_1, but not CRF_2, receptors mediate anxiogenic-like behavior. Regul Peptides 71:15–21

Isogawa K, Akiyoshi J, Hikichi T, Yamamoto Y, Tsutsumi T, Nagayama H (2000): Effect of corticotropin releasing factor receptor 1 antagonist on extracellular norepinephrine, dopamine and serotonin in the hippocampus and prefrontal cortex of rats in vivo. Neuropeptides 34:234–239

Jasnow AM, Banks MC, Owens EC, Huhman KL (1999): Differential effects of two corticotropin-releasing factor antagonists on conditioned defeat in male syrian hamsters (Mesocricetus auratus). Brain Res 846:122–128

Jenck F, Martin JR, Moreau JL (1999): The $5\text{-}HT_{1A}$ receptor agonist flesinoxan increases aversion in a model of panic-like anxiety in rats. J Psychopharmacol 13:166–170

Jenck F, Moreau JL, Berendsen HHG, Boes M, Broekkamp CLE, Martin JR, Wichmann J, Van Delft AML (1998): Antiaversive effects of $5\text{-}HT_{2C}$ receptor agonists and fluoxetine in a model of panic-like anxiety in rats. Eur Neuropsychopharmacol 8:161–168

Karoloyi IJ, Burrows HL, Ramesh TM, Nakajima M, Lesh JS, Seong E, Camper SA, Seasholtz AF (1999): Altered anxiety and weight gain in cortocotropin-releasing hormone-binding protein-deficient mice. Proc Natl Acad Sci USA 96:11595–11600

Kawahara H, Kawahara Y, Westerink BHC (2000): The role of afferents to the locus coeruleus in the handling stress-induced increase in the release of noradrenaline in the medial prefrontal cortex: a dual-probe microdialysis study in the rat brain. Eur J Pharmacol 387:279–286

Keck ME, Weh T, Wigger A, Renner U, Engelmann M, Holsboer F, Landgraf R (2000): Anxiolytic effects of the high-affinity CRH_1 receptor antagonist R121919 is dependent on the innate emotionality in rats. Soc Neurosci Abst 26: 2149

Kehne JH, Coverdale S, McCloskey TC, Hoffman DC, Cassella JV (2000): Effects of the CRF_1 receptor antagonist, CP 154,526, in the separation-induced vocalization anxiolytic test in the rat pups. Neuropharmacology 39:1357–1367

Kennett GA, Wood MD, Bright F, Cilia J, Piper DC, Gager T, Thomas D, Baxter GS, Forbes IT, Ham P, Blackburn TP (1996): In vitro and in vivo profile of SB 206553, a potent $5\text{-}HT_{2C}/5\text{-}HT_{2B}$ receptor antagonist with anxiolytic-like properties. Br J Pharmacol 117:427–434

Kennett GA, Wood MD, Bright F, Trail B, Riley G, Holland V, Avenell KY, Stean T, Upton N, Bromidge S, Forbes IT, Brown AM, Midlemiss DN, Blackburn TP (1997): SB 242084, a selective and brain penetrant $5\text{-}HT_{2C}$ receptor antagonist. Neuropharmacology 36:609–620

King CMF, Gommans J, Joordens RJE, Hijzen TH, Maes RAA, Olivier B (1997): Effects of $5\text{-}HT_{1A}$ receptor ligands in a modified Geller-Seifter conflict model in the rat. Eur J Pharmacol 325:121–128

Kirby LG, Rice KC, Valentino RJ (2000): Effects of corticotropin-releasing factor on neuronal activity in the serotonergic dorsal raphe nucleus. Neuropsychopharmacology 22:148–162

Liebsch G, Landgraf R, Engelmann M, Lörscher P, Holsboer F (1999): Differential behavioral effects of chronic infusion of CRH1 and CRH2 receptor antisense oligonucleotides into the rat brain. J Psychiatr Res 33:153–163

Liebsch G, Landgraf R, Gerstberger R, Probst JC, Wotjak CT, Engelmann M, Holsboer F, Montkowski A (1995): Chronic infusion of a CRH_1 receptor antisense oligodeoxynucleotide into the central nucleus of the amygdala reduced anxiety-related behavior in socially defeated rats. Regul Peptides 59:301–310

Lundkvist J, Chai Z, Teheranian R, Hasanvan H, Bartfai T, Jenck F, Widmer U, Moreau J-L (1996): A non peptide corticotropin releasing factor receptor antagonist attenuates fever and exhibits anxiolytic-like activity. Eur J Pharmacol 309:195–200

Martins AP, Marras RA, Guimaräes FS (1997): Anxiogenic effect of corticotropin-releasing hormone in the dorsal periaqueductal grey. Neuroreport 8:3601–3604

Menzaghi F, Howard RL, Heinrichs SC, Vale W, Rivier J, Koob GF (1994): Characterization of a novel and potent corticotropin-releasing factor antagonist in rats. J Pharmacol Exp Ther 269:564–572

Miczek KA, Weerts E, Vivian JA, Barros HM (1995): Aggression, anxiety and vocalizations in animals: $GABA_A$ and 5-HT anxiolytics. Psychopharmacology 121:38–56

Millan MJ (1999): The induction of pain: an integrative review. Prog Neurobiol 57:1–164

Millan MJ, Hjorth S, Samarin R, Schreiber R, Jaffard R, De Ladonchamps B, Veiga S, Goument B, Peglion J-L, Spedding M, Brocco M (1997): S15535, a novel benzodioxopiperazine ligand of serotonin $(5\text{-}HT)_{1A}$ receptors: II. Modulation of hippocampal serotonin release in relation to potential anxiolytic properties. J Pharmacol Exp Ther 282:148–161

Millan MJ, Lejeune F, Gobert A (2000a): Reciprocal autoreceptor and heteroreceptor control of serotonergic, dopaminergic and noradrenergic transmission in the frontal cortex: relevance to the actions of antidepressant agents. J Psychopharmacol 14:114–138

Millan MJ, Lejeune F, Gobert A, Brocco M, Auclair A, Bosc C, Rivet J-M, Lacoste JM, Cordi A, Dekeyne A (2000b): S18616, a highly-potent spiroimidazoline agonist at α_2-adrenoceptors: II. Influence on monoaminergic transmission, motor function, and anxiety in comparison with dexmedetomidine and clonidine. J Pharmacol Exp Ther 295:1206–1222

Mitchell AJ (1998): The role of corticotropin releasing factor in depressive illness: a critical review. Neurosci Biobehavioral Rev 22:635–651

Molewijk HE, van der Poel AM, Mos J, van der Heyden JAM, Olivier B (1995): Conditioned ultrasonic distress vocalizations in adult male rats as a behavioral paradigm for screening anti-panic drugs. Psychopharmacology 117:32–40

Moreau JL, Kilpatrick G, Jenck F (1997): Urocortin, a novel neuropeptide with anxiogenic-like properties. Neuroreport 8:1697–1701

Morrow BA, Elsworth JD, Rasmusson AM, Roth RH (1999): The role of mesoprefrontal dopamine neurons in the acquisition and expression of conditioned fear in the rat. Neuroscience 92:553–564

Okuyama S, Chaki S, Kawashima N, Suzuki Y, Ogawa S-I, Nakazato A, Kumagai T, Okubo T, Tomisawa K (1999): Receptor binding, behavioral, and electrophysiological profiles of nonpeptide corticotropin-releasing factor subtype 1 receptor antagonists CRA1000 and CRA1001. J Pharmacol Exp Ther 289:926–935

Owens MJ, Nemeroff CB (1991): Physiology and pharmacology of corticotropin-releasing factor. Pharmacol Rev 43:425–473

Page ME, Abercrombie ED (1999): Discrete local application of corticotropin-releasing factor increases locus coeruleus discharge and extracellular norepinephrine in rat hippocampus. Synapse 33:304–313

Potter E, Sutton S, Donaldson C, Chen R, Perrin M, Lewis K, Sawchenko PE, Vale W (1994): Distribution of corticotropin-releasing factor receptor mRNA expression in the rat brain and pituitary. Proc Natl Acad Sci USA 91:8777–8781

Price ML, Curtis AL, Kirby LG, Valentino RJ, Lucki I (1998): Effects of corticotropin-releasing factor on brain serotonin activity. Neuropsychopharmacology 18:492–502

Radulovic J, Rühmann A, Liepold T, Spiess J (1999): Modulation of learning and anxiety by corticotropin-releasing factor (CRF) and stress: Differential roles of CRF receptors 1 and 2. J Neurosci 19:5016–5025

Rodgers RJ, Cole JC (1994): The elevated plus-maze: pharmacology, methodology and ethology. In Cooper SJ, Hendric CA (eds), Ethology and Psychopharmacology. Chichester, John Wiley, pp 9–44

Rodgers RJ, Cole JC, Davies A (1994): Antianxiety and behavioral suppressant actions of the novel 5-HT_{1A} receptor agonist, flesinoxan. Pharmacol Biochem Behav 48:959–963

Ruggiero DA, Underwood MD, Rice PM, Mann JJ, Arango V (1999): Corticotropic-releasing hormone and serotonin interact in the human brainstem: behavioral implications. Neuroscience 91:1343–1354

Sajdyk TJ, Schober DA, Gehlert DR, Shekhar A (1999): Role of corticotropin-releasing factor and urocortin within the basolateral amygdala of rats in anxiety and panic responses. Behav Brain Research 100:207–215

Sànchez C, Mørk A (1999): N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline studies on the role of 5-HT_{1A} and 5-HT_2 receptors in mediating foot-shock-induced ultrasonic vocalisation in adults rats. Euro Neuropsychopharmacology 9:287–294

Sánchez MM, Young LJ, Plotsky PM, Insel TR (1999): Autoradiographic and in situ hybridization localization of corticotropin-releasing factor 1 and 2 receptors in nonhuman primate brain. J Comp Neurol 408:365–377

Sanger DJ (1992): Increased rates of punished responding produced by buspirone-like compounds in rats. J Pharmacol Exp Ther 261:513–517

Sawchenko PE, Imaki T, Potter E, Kovacs K, Imaki J, Wale W (1993): The functional neuroanatomy of corticotropin-releasing factor. Ciba Found Symp 172:5–21

Schreiber R, De Vry J (1993): Neuronal circuits involved in the anxiolytic effects of the 5-HT_{1A} receptor agonists 8-OH-DPAT, ipsapirone and buspirone in the rat. Eur J Pharmacol 249:341–351

Schulz DW, Mansbach RS, Sprouse J, Baselton JP, Collins J, Corman M, Dunaiskis A, Faraci S, Schmidt AW, Seeger T, Seymour P, Tingley III FD, Winston EN, Chen YL, Heym J (1996): CP-154,526: A potent and selective nonpeptide antagonist of corticotropin releasing factor receptors. Proc Natl Acad Sci USA 93:10477–10482

Shimizu N, Nakabe H, Hori T, Hayashi Y (1994): CRF receptor antagonist attenuates stress-induced noradrenaline release in the medial prefrontal cortex of rats. Brain Res 654:145–148

Skutella T, Montkowski A, Stöhr T, Probst JC, Landgraf R, Holsboer F, Jirikowski GF (1994): Corticotropin-releasing hormone (CRH) antisense oligodeoxynucleotide treatment attenuates social defeat-induced anxiety in rats. Cell Mol Neurobiol 14:579–588

Skutella T, Probst JC, Renner U, Holsboer F, Behl C (1998): Corticotropin-releasing hormone receptor (type I) antisense targeting reduces anxiety. Neuroscience 85:795–805

Smagin GN, Dunn AJ (2000): The role of CRF receptor subtypes in stress-induced behavioural responses. Eur J Pharmacol 405:199–206

Smagin GN, Howell LA, Ryan DH, De Souza EB, Harris RB (1998): The role of CRH2 receptors in corticotropin-releasing factor- and urocortin-induced anorexia. Neuroreport 9:1604–1606

Smagin GN, Swiergiel AH, Dunn AJ (1995): Corticotropin-releasing factor administered into the locus coeruleus, but not the parabrachial nucleus, stimulates norepinephrine release in the prefrontal cortex. Brain Res Bull 36:71–76

Smagin GN, Zhou J, Harris RBS, Ryan DH (1997): CRF receptor antagonist attenuates immobilization stress-induced norepinephrine release in the prefrontal cortex in rats; Brain Res Bull 42:431–434

Smith GW, Aubry J-M, Dellu F, Contarino A, Bilezikjian LM, Gold LH, Chen R, Marchuk Y, Hauser C, Bentley CA, Sawchenko PE, Koob GF, Vale W, Lee K-F (1998): Corticotropin releasing factor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. Neuron 20:1093–1102

Spina MG, Basso AM, Zorrilla EP, Heyser CJ, Rivier J, Vale W, Merlo-Pich E, Koob GF (2000): Behavioral effects of central administration of the novel CRF antagonist astressin in rats. Neuropsychopharmacology 22:231–239

Steckler T, Holsboer F (1999): Corticotopin-releasing hormone receptor subtypes and emotion. Biol Psychiatry 46:1480–1508

Stenzel-Poore MP, Heinrichs, SC, Rivest S, Koob GF, Vale WW (1994): Overproduction of corticotropin-releasing factor in transgenic mice: A genetic model of anxiogenic behavior. J Neurosci 14:2579–2584

Swiergiel AH, Takahashi LK, Rubin WW, Kalin NH (1992): Antagonism of corticotropin-releasing factor receptors in the locus coeruleus attenuates shock-induced freezing in rats. Brain Res 587:263–268

Tershner SA, Helmstetter FJ (1996): Injections of corticotropin-releasing factor into the periacqueductal grey enhance pavlovian fear conditioning. Psychobioloy 24: 49–56

Timpl P, Spanagel R, Sillaber J, Kresse A, Reul JMHM, Stalla GK, Blanquet V, Steckler T, Holsboer F, Wurst W (1998): Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor 1. Nat Genet 19:162–166

Valentino RJ, Foote SL, Page ME (1993): The locus coeruleus as a site for integrating corticotropin-releasing factor and noradrenergic mediation of stress responses. Ann NY Acad Sci 697:173–188

Van Bockstaele EJ, Colago EEO, Valentino RJ (1996): Corticotropin-releasing factor-containing axon terminals synapse onto catecholamine dendrites and may preferentially modulate other afferents in the rostral pole of the nucleus locus coeruleus in the rat brain. J Comp Neurol 364:523–534

Van Gaalen MM, Wurst W, Holsboer F, Steckler T (1999): Reduced anxiety-related behaviour in CRH1 knockout mice following social defeat. J Psychopharmacol 13:A33

Van Vliet IM, Westenberg GM, den Boer JA (1996): Effects of the 5-HT_{1A} receptor agonist flesinoxan in panic disorder. Psychopharmacology 127:174–180

Weiss JM, Stout JC, Aaron MF, Quan N, Owens MJ, Butlers PD, Nemeroff CB (1994): Depression and anxiety: Role of the locus coeruleus and corticotropin-releasing factor. Brain Res Bull 35:561–572

Wilcoxon K, Chen C, Huang C, Haddach M, Xie M, Wing L, Grigoriadis D, De Souza EB, McCarthy JR (1999): Design and syntheses of NBI 30545 and analogues as potent CRF receptor antagonists. ACS National Meeting, Anaheim, CA, Mar 21–25

Zhang L, Barrett JE (1990): Interactions of corticotropin-releasing factor with antidepressant and anxiolytic drugs: behavioral studies with pigeons. Biol Psychiatry 27:953–967