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CP-154,526: A potent and selective nonpeptide antagonist of corticotropin releasing factor receptors

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ABSTRACT **Here we describe the properties of CP-154,526, a potent and selective nonpeptide antagonist of corticotropin (ACTH) releasing factor (CRF) receptors. CP-154,526 binds with high affinity to CRF receptors (K_i < 10 nM) and blocks CRF-stimulated adenylate cyclase activity in membranes prepared from rat cortex and pituitary. Systemically administered CP-154,526 antagonizes the stimulatory effects of exogenous CRF on plasma ACTH, locus coeruleus neuronal firing and startle response amplitude. Potential anxiolytic activity of CP-154,526 was revealed in a fear-potentiated startle paradigm. These data are presented in the context of clinical findings, which suggest that CRF is hypersecreted in certain pathological states. We propose that a CRF antagonist such as CP-154,526 could affirm the role of CRF in certain psychiatric diseases and may be of significant value in the treatment of these disorders.**

Corticotropin releasing factor (CRF) is a 41-amino acid peptide initially identified as a hypothalamic factor responsible for stimulating corticotropin (ACTH) secretion from the anterior pituitary (1, 2). CRF causes a rapid increase in plasma ACTH and glucocorticoid levels when given intravenously (3). Activation of the hypothalamic–pituitary–adrenal (HPA) axis can also result from release of CRF from the paraventricular nucleus of the hypothalamus in response to various stressors (1, 4). In the central nervous system, both CRF-like immunoreactivity and high affinity CRF receptors are heterogeneously distributed in the brain (5, 6). Characterizations of these extrahypothalamic CRF systems demonstrate that, in parallel with its actions on the HPA axis, CRF also acts as a neurotransmitter or neuromodulator to coordinate stress-induced neural responses in the brain (7, 8).

Intracerebroventricular administration of CRF to rats leads to a constellation of neurochemical, neurophysiological, and behavioral sequelae that include activation of central noradrenergic systems and enhancement of behavioral responses to external stimuli (9–13). In this regard, increases in norepinephrine turnover (10) and in the firing rate of locus coeruleus neurons (13) have been observed following CRF injection. Physiological stressors such as nitroprusside infusions also increase locus coeruleus neuronal firing, an effect blocked by a CRF antagonist (α-helical CRF_{9-41}) and consequently thought to be mediated by endogenous CRF (14, 15). The response to hemodynamic stress in this case can be desensitized by chronic treatment with tricyclic antidepressants, suggesting that one possible mode of action of antidepressants might be to alter central CRF neurotransmission (16). In behavioral paradigms, CRF injection i.c.v. produces anxiogenic-like effects in several rodent models (e.g. 17–20). These effects are antagonized by central infusion of peptide antagonists (α-helical CRF_{9-41} and D-Phe CRF_{12-41}), suggesting the involvement of CRF in anxiety and the utility of CRF antagonists as anxiolytics. The persistence of behavioral activation in hypophysectomized and dexamethasone-treated rats reinforces the notion that it occurs independent of the HPA axis (21).

Various clinical findings suggest that CRF is hypersecreted in certain pathological states. For example, cerebrospinal fluid levels of CRF are elevated in chronically depressed patients (22, 23) but return toward normal 24 h after a course of treatment with electroconvulsive therapy (24). Dexamethasone nonsuppression, the failure of an exogenously administered glucocorticoid to lower cortisol levels, is observed in 40% of depressed patients and is consistent with hyperactivity of the HPA axis in this population (25). Elevations in neuronal CRF activity result in the down-regulation of CRF receptors, which could underlie the blunted ACTH response to i.v. CRF observed in depressed patients, bulimics, and victims of childhood sexual abuse (26–28). CRF receptor densities postmortem are in fact diminished in the brains of suicide victims, supporting the hypothesis that brain CRF systems may be hyperactive in individuals suffering from severe melancholia (29). Although changes in HPA function may result from hypersecretion of CRF, studies in primates have shown that a major component of CRF release in brain appears to be extrahypothalamic in origin in that cerebrospinal fluid levels of CRF do not correlate with HPA axis activity (30).

While a CRF antagonist may be useful in treating some forms of psychiatric illness, it is likely that a peptide such as α-helical CRF_{9-41} would have limited utility due to poor bioavailability and difficulty in penetrating the blood–brain barrier. Therefore, we sought to identify a nonpeptide CRF receptor antagonist for potential use as a therapeutic agent. Here we describe the properties of CP-154,526, the first potent, selective nonpeptide CRF antagonist. In addition, data are presented indicating that this unique compound exhibits anxiolytic potential in animals.

MATERIALS AND METHODS

Receptor Binding. Assays were modified from those described previously (31). P2 membranes (1 mg wet weight/ml) from human neuroblastoma IMR32 cells were prepared in buffer [20 mM 1,4-piperazinediethanesulfonic acid (Pipes, pH 7.0), 10 mM $MgCl_2$, 2 mM EGTA, 0.04% BSA, 0.015% bacitracin, 100 units/ml aprotinin]. Aliquots of 100 μl were added to assay samples containing ^{125}I-labeled ovine CRF (^{125}I-oCRF; 40 pM) and antagonists or buffer in a final volume of 200 μl. Nonspecific binding was determined using 1 μM rat/human CRF. After a 2-h incubation at room temperature, assay samples were centrifuged for 10 min at 1300 × *g*. The supernatant was discarded. Samples were rinsed with 100 μl of ice-cold assay buffer and recentrifuged. Pellets were filtered onto Betaplate filtermats using a Skatron cell harvester (set-

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Abbreviations: CRF, corticotropin releasing factor; HPA, hypothalamic–pituitary–adrenal.

ting 222). Radioactivity was quantified using a Betaplate scintillation counter (LKB).

Adenylate Cyclase Measurements. Activity was determined by measuring the conversion of [α-^{32}P]ATP to [^{32}P]cAMP as described previously (32). The assay medium consisted of 100 mM Hepes (pH 7.4), 2 mM EGTA, 5 mM $MgCl_2$, 1 mM cAMP, 0.5 mM ATP, 0.5 mM isobutylmethylxanthine, 10 mM phosphocreatine, 120 units/ml creatine phosphokinase, 100 μM GTP, 1–2 μCi of [α-^{32}P]ATP, 0.25% BSA, 0–10 μM oCRF, and antagonists or buffer in a final volume of 100 μl. Incubations were initiated by the addition of washed rat cortical membranes (40 μg of protein). After a 15-min incubation at 30°C, reactions were terminated by the addition of 100 μl of 2% SDS. [^{32}P]cAMP was separated from [^{32}P]ATP by sequential elution over Dowex and alumina columns (33). [^{3}H]cAMP (40,000 dpm) was added to each column to monitor the recovery of cAMP. Radioactivity was quantified by liquid scintillation counting.

Plasma ACTH Measurements. Male Sprague–Dawley rats were injected s.c. with 3 ml/kg vehicle [5% Me_2SO, 5% Emuphlor, 90% saline (0.9%)] or CP-154,526. After 30 min, animals received i.v. injections (1 ml/kg, via lateral tail vein) containing vehicle (40 mM NaH_2PO_4, pH 7.4/0.1% BSA/0.01% ascorbic acid), 4 μg/kg CRF, or both CRF and 3 mg/kg α-helical $oCRF_{9-41}$. Rats were decapitated 30 min after i.v. injection, and trunk blood was collected for analysis of ACTH content by radioimmunoassay (ICN).

Locus Coeruleus Neuronal Recording. Extracellular single unit recordings of locus coeruleus neurons were made as described previously (34). Male Sprague–Dawley rats (275–375 g) were anesthetized with chloral hydrate (400 mg/kg i.p.) and supplemented as required; body temperature was maintained at 37 ± 1°C. Nominal coordinates for recording in the locus coeruleus were: anterior posterior = −1.0 mm from the interaural line, lateral = 1.1 mm from the midline, ventral = −5.0 to −7.0 mm from the brain surface; incisor bar was set −10.0 mm from the interaural line. Rat/human CRF (3.0 μg in 6.0 μl) was dissolved in HCl with a final acid concentration of 0.5 M. The i.c.v. cannula for CRF infusion was placed at lateral = 1.5 mm on the ipsilateral side, anterior posterior = −1.0 mm from bregma. CP-154,526 in an acid vehicle (HCl, final concentration 0.1 M) was administered via lateral tail vein 5–10 min prior to CRF. Aside from a transient increase in neuronal firing following injection, no vehicle effects on unit activity were observed.

Acoustic Startle. Male Sprague–Dawley rats were used as subjects. Startle experiments were conducted using previously described equipment (35). In the CRF-enhanced startle experiments, rats were implanted with intracerebroventricular cannulae 1 week after arrival. Four days later, subjects were exposed to 20 120-dB[A] acoustic startle stimuli, interspersed by 15 sec of background noise. Results from this baseline matching test were used to assign treament groups. Two days later, rats were exposed to two startle sessions, one before administration of drugs and one afterward. The first session consisted of 60 trials as described above and served as a reference for the startle amplitudes measured after drug administration. Because the results were not significantly different after normalization than when absolute startle amplitudes were analyzed, only the absolute startle scores after drug administration are reported here. After the first startle session, rats were administered rat/human CRF (1 μg, i.c.v. in 2 μl) or vehicle 60–70 min prior to the second startle session, which consisted of 169 trials as described above, separated by 20-sec periods of background noise. D-Phe CRF_{12-41} (3.2 μg, i.c.v. in 5 μl) was administered 10 min before CRF; CP-154,526 (5.6 or 17.8 mg/kg, s.c.) was administered immediately following CRF. Each animal was tested on two occasions in this manner, with CRF administered once and vehicle once. Antagonist treatment served as a between-subjects factor, with subjects receiving either antagonist or vehicle on both days of testing.

In the potentiated startle experiments, rats were baseline-matched as described above. One day later, subjects were exposed to a conditioning session in which illumination of an incandescent light (25 watts AC) was paired with presentation of a scrambled foot shock (1.2 mA), delivered through a metal grid inserted into the startle cylinder. Twenty such pairings were presented, 2 min apart, in darkened startle chambers. One group of animals, "No Shock," was exposed to the light presentations without the accompanying shocks. Three days later, rats were exposed to a startle session in which some of the 108 dB startle stimuli were preceded by a light presentation and others were not.

RESULTS AND DISCUSSION

Binding of CP-154,526 to CRF Receptors. High speed screening of compound libraries with a radioligand binding assay had previously led to the discovery of potent nonpeptide NK_1 antagonists (36). Accordingly, a similar approach was taken to assay compounds for their ability to inhibit binding of radiolabeled oCRF (^{125}I-oCRF) to the human CRF receptor in membranes prepared from IMR32 cells, a human neuroblastoma cell line. This effort yielded a low affinity lead (800 nM) that served as a starting point for subsequent chemical modifications (37). Directed synthesis then resulted in a series of novel pyrrolo[2,3-*d*]pyrimidines (38) exemplified by CP-154,526 (Fig. 1; butyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]-ethylamine). CP-154,526 bound to CRF receptors in IMR32 cells with a K_i of 2.7 nM and showed similar high affinity for cerebral cortical and pituitary sites labeled by ^{125}I-oCRF in multiple species (Table 1). Compared with α-helical $oCRF_{9-41}$, binding affinity for CP-154,526 was greater regardless of the tissue source. Competition curves for all tissue preparations were monophasic, with Hill slopes approximating 1.0. CP-154,526 also was examined in radioligand binding assays for more than 40 other receptors, and in no case did it compete for binding with an IC_{50} of less than 1 μM, which demonstrates its high degree of selectivity.

In a series of preliminary studies, CRF_1 and CRF_2 receptors were expressed in Chinese hamster ovary cells, and the affinity of CP-154,526 for each receptor subtype was determined (39). CP-154,526 competed for ^{125}I-oCRF binding to the CRF_1 receptor subtype with a K_i of 2.7 nM. In contrast, the K_i for inhibition of binding by ^{125}I-sauvigine to CRF_2 receptors was >10 μM.

Blockade of CRF-Induced Activation of Adenylate Cyclase. The functional consequences of CP-154,526 binding to CRF receptors were determined by studying its effects on the *in vitro* activation of adenylate cyclase by CRF reported by others in brain tissue (40, 41) and pituitary (42, 43). In the present

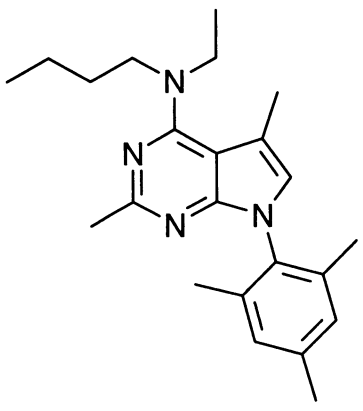

FIG. 1. Structure of CP-154,526.

Table 1. Competition by CP-154,526 and alpha helical oCRF$_{9-41}$ for ^{125}I-oCRF (40 pM) binding to cerebral cortical and pituitary membranes

Tissue	CP-154,526		α-helical oCRF$_{9-41}$	
	pK_i	K_i (nM)	pK_i	K_i (nM)
IMR32	−8.56 ± 0.18	2.7	−7.51 ± 0.15	31
Rat pituitary	−8.84 ± 0.15	1.4	−7.52 ± 0.09	30
Guinea pig pituitary	−8.54 ± 0.08	2.9	−7.58 ± 0.13	26
Bovine pituitary	−8.46 ± 0.01	3.5	−8.03 ± 0.13	9.3
Rat cortex	−8.25 ± 0.02	5.7	−7.48 ± 0.06	33
Guinea pig cortex	−8.12 ± 0.16	7.5	−7.29 ± 0.13	51
Dog cortex	−8.22 ± 0.38	6.0	−7.93 ± 0.11	12
Marmoset cortex	−8.03 ± 0.10	9.3	−7.07 ± 0.22	85

Values are from at least three experiments performed in triplicate, with four to fourteen concentrations of competitor used to determine individual K_i values. Data are shown as both geometric mean pK_i ± SEM and derived K_i.

experiments, CRF activated adenylate cyclase in rat cortical membranes in a concentration-dependent manner (Fig. 2 *inset*); a challenge concentration of 100 nM oCRF was then selected to establish antagonist potency. Like α-helical oCRF$_{9-41}$, CP-154,526 completely blocked the activation of adenylate cyclase caused by 100 nM oCRF (Fig. 2) with an apparent K_i of 3.7 nM (geometric mean of 3 experiments = −8.43 ± 0.1). CP-154,526 alone did not alter basal or forskolin-stimulated adenylate cyclase. Moreover, it did not affect activation of adenylate cyclase mediated by histamine H_2 or noradrenergic β receptors in rat brain (data not shown), indicating that its ability to block CRF-stimulated adenylate cyclase activity is due to a specific antagonism of CRF receptors.

Blockade of CRF-Induced ACTH Secretion. To examine the effect of CP-154,526 on CRF receptors *in vivo*, changes in plasma ACTH were determined in rats administered a challenge dose of 4 μg/kg oCRF i.v. Previous studies have shown that this dose elicits a half-maximal increase in plasma ACTH levels measured 30 min later (3). As shown in Fig. 3, the CRF-induced increase in plasma ACTH was blocked by both

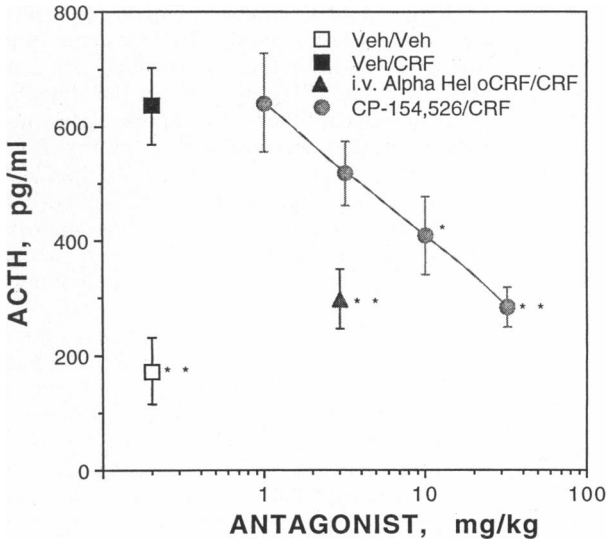

FIG. 3. Antagonism of CRF-stimulated ACTH elevations in rat plasma by α-helical oCRF$_{9-41}$ and CP-154,526. Rats received s.c. pretreatment of CP-154,526 30 min before i.v. injection of 4 μg/kg CRF. α-helical oCRF$_{9-41}$ (3 mg/kg) was administered i.v. concurrently with CRF. Data above are from one of three identical experiments, with 6 rats per treatment group for each experiment. Statistical significance: *,**, $P < 0.05, 0.01$ *versus* s.c. vehicle/CRF group. Overall ID$_{50}$ for CP-154,526 from three experiments was 10 mg/kg.

α-helical oCRF$_{9-41}$ administered i.v. as a positive control and s.c. pretreatment with CP-154,526. The effect of CP-154,526 was dose-dependent, with an ID$_{50}$ from three separate assays of 13 ± 1.5 mg/kg. Using this paradigm, it was not possible to draw conclusions regarding the relative potencies of the two antagonists because different routes of administration were employed. Even though 10 mg/kg of CP-154,526 significantly blocked the effect of supranormal levels of CRF, this dose alone did not affect basal ACTH levels (data not shown). This dichotomy supports the notion that endogenous CRF is not the exclusive mediator of tonic ACTH secretion (2).

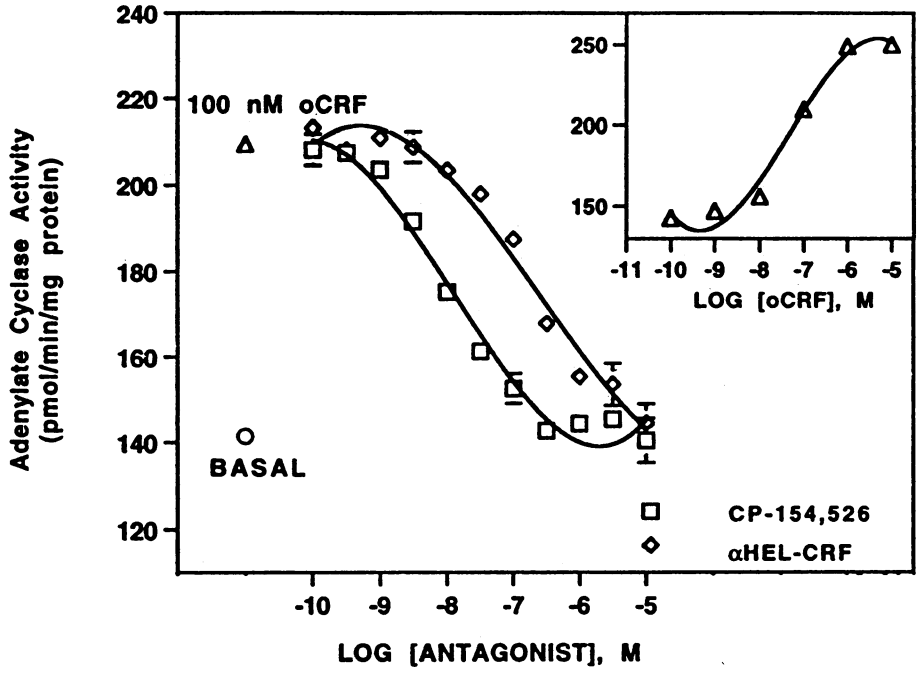

FIG. 2. Antagonism of 100 nM CRF-stimulated adenylate cyclase activity in rat cortical membranes by CP-154,526 and α-helical oCRF$_{9-41}$ in a representative single experiment with triplicate determinations. Mean K_i determined from three experiments were 3.7 nM for CP-154,526 and 30 nM for α-helical oCRF$_{9-41}$. (*Inset*) Concentration-dependent activation of adenylate cyclase by CRF in the same experiment.

Blockade of CRF-Induced Excitation of Locus Coeruleus Neuronal Firing. Administration of r/h CRF (3.0 μg in 6.0 μl) via a cannula implanted in the lateral ventricle yielded a net excitation of locus coeruleus cell firing of 102 ± 19% (mean ± SEM, $n = 15$), similar in magnitude to that reported previously (13). Onset of the excitation occurred within the first 2 min following injection; times to peak effects were variable, occasionally requiring 10–15 min (Fig. 4A). Injection i.c.v. with the CRF vehicle (0.5N HCl) did not alter baseline neuronal activity. Repeated administration of CRF to the same neuron gave generally smaller increases in excitation, a finding noted previously by others (44).

In a separate series of experiments, intravenous administration of CP-154,526 5–10 min prior to the CRF challenge blocked the excitation in a dose-dependent manner (Fig. 4). The responses of neurons pretreated with doses of 3.0 and 5.6 mg/kg i.v. of CP-154,526 were significantly different from those given CRF alone (one-way analysis of variance, *post hoc t* test). The ID_{50}, calculated from regression analysis of the dose response curve, was 2 mg/kg i.v. Immediately following the injection of CP-154,526 or the drug vehicle (acidified saline, final concentration 0.1 M HCl), cells generally showed a transient increase in neuronal firing. Administration of the vehicle alone did not alter the CRF response.

Selectivity of the blockade by CP-154,526 was probed with substance P-induced excitation of locus coeruleus cell firing. Substance P given alone (1.0 μg in 6.0 μl of saline i.c.v.) increased firing rate by 102 ± 28% (mean ± SEM, $n = 4$). During the same recording session, CP-154,526 at 5.6 mg/kg i.v., the highest dose tested against the CRF challenge, was ineffective at blocking the response to substance P (net excitation = 101 ± 11%).

Blockade of CRF-Enhanced and Fear-Potentiated Acoustic Startle. Fig. 5 illustrates the effects of i.c.v. CRF on acoustic startle. As reported previously, (12, 19, 20), CRF (1 μg) produced a significant [$F(1, 21) = 9.6$, $P < 0.006$] and long lasting enhancement of startle (top panel). Co-administration of the peptide antagonist D-Phe CRF_{12-41} (3.2 μg i.c.v.) resulted in a complete blockade of the CRF response, as indicated by a significant interaction [$F(1, 21) = 5.1$, $P < 0.05$]. D-Phe CRF_{12-41} produced no effect on startle when given alone. In earlier reports (12, 20), α-helical $oCRF_{9-41}$ at doses of 25–50 μg antagonized the startle-enhancing effects of CRF. That we observed full blockade of the CRF effect with a D-Phe CRF_{12-41} dose of 3.2 μg agrees with its greater potency in other behavioral procedures (45).

The center and bottom panels of Fig. 5 depict the effects of CP-154,526 on CRF-enhanced startle. As before, CRF produced a large increase in startle when given alone [$F(1, 20) = 20.5$, $P < 0.001$]. At a dose of 5.6 mg/kg i.p., CP-154,526 blocked approximately 50% of the CRF response, an effect that fell just short of significance [$F(1, 20) = 3.7$, $P = 0.07$]. A larger dose of CP-154,526 completely antagonized the CRF effect [$F(1, 22) = 18.1$, $P < 0.001$]. Although the startle procedure itself has been shown to elevate HPA activity in some rat strains (46), neither CP-154,526 or D-Phe CRF_{12-41} significantly affected startle when administered alone, suggesting little contribution of endogenous CRF activity to baseline startle amplitude in the present experiments. Together with the locus coeruleus data, our finding that CP-154,526 blocked the actions of i.c.v. CRF on acoustic startle demonstrated that this antagonist was effective in central as well as peripheral measures of CRF activity.

The experiments with fear-potentiated startle were intended to determine whether endogenous activation of CRF systems in response to a psychological stressor would be similarly affected. Fig. 6 presents the results of potentiated startle experiments with CP-154,526. Pairing of electric shock with presentations of the light conditioned stimulus produced a significant potentiation of startle amplitude when the light alone was presented immediately prior to startle-inducing noises 2 days after conditioning. Compared with animals never receiving shock, rats having received the conditioning trials displayed significantly greater potentiated startle ($P < 0.05$, Dunnett's test following significant analysis of variance). CP-154,526 given at doses of 10.0 and 17.8 mg/kg i.p. produced a significant blockade of potentiated startle ($P < 0.05$), whereas doses of 3.2 and 5.6 mg/kg dose were ineffective. Baseline startle, as measured by absolute amplitudes following "no-light" trials, was slightly lower than vehicle controls in the presence of CP-154,526 at the two highest doses, but these effects did not reach significance.

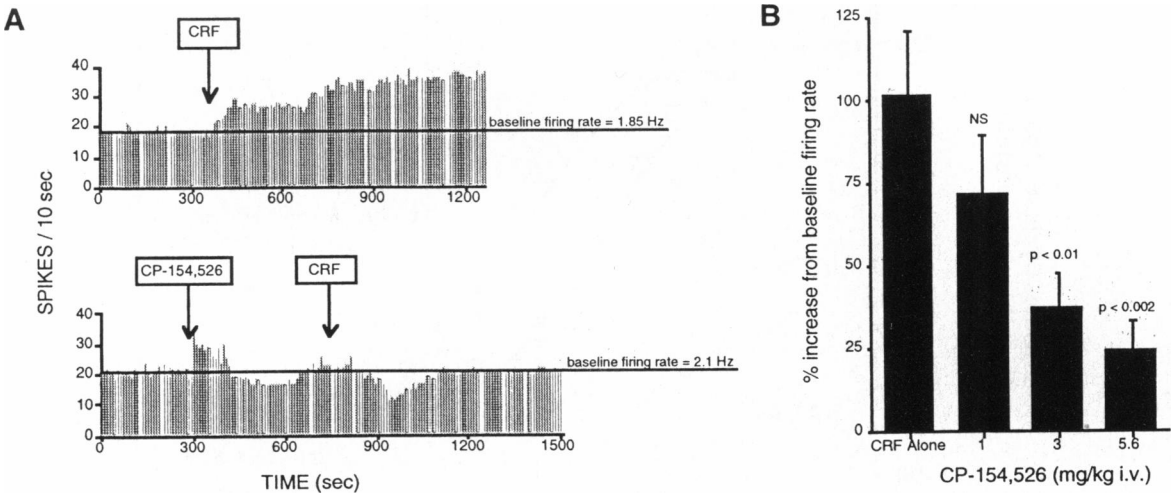

FIG. 4. Effect of CP-154,526 on CRF-induced excitation of locus coeruleus cell firing. (*A*) Representative firing rate histograms of two spontaneously active locus coeruleus neurons recorded in chloral hydrate anesthetized rats. Upper tracing shows the effects of CRF (3 μg) given by slow i.c.v. infusion at the arrow. In this neuron, unit activity gradually increased by 100% above baseline firing rate. Lower tracing shows in a second neuron blockade of the CRF-induced excitation by pretreatment with CP-154,526 (5.6 mg/kg i.v.). Firing rate immediately following CP-154,526 was transiently increased in most cells tested likely as a result of the acid vehicle (4 of 5 cells at 5.6 mg/kg i.v.); a more slowly developing transient decrease following CRF was also frequently observed in CP-154,526-treated animals. (*B*) Dose-dependent blockade of CRF-induced excitation of locus coeruleus neurons by CP-154,526. $n = 15$ cells (CRF given alone) or 5–6 cells (pretreatment with CP-154,526 given at 1, 3, or 5.6 mg/kg i.v. 5–10 min before CRF.

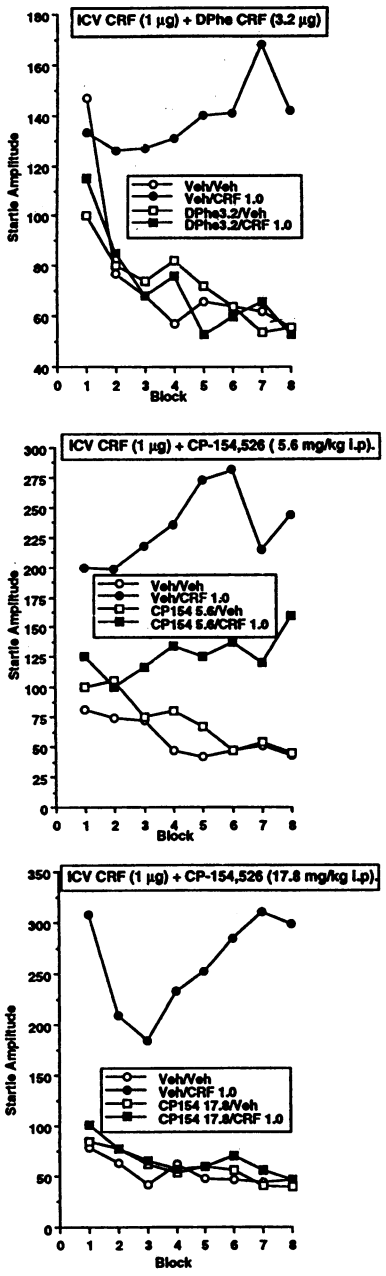

FIG. 5. Effects of CRF antagonists on CRF-enhanced increases in acoustic startle. A dose of 1 μg of CRF i.c.v. was administered alone and in combination with D-Phe CRF_{12-41} 3.2 μg i.c.v. (*top*), CP-154,526 5.6 mg/kg i.p. (*center*), and CP-154,526 17.8 mg/kg i.p. (*bottom*). Data are presented as mean startle amplitudes for each of eight blocks containing 21 trials each. (n = 9–12).

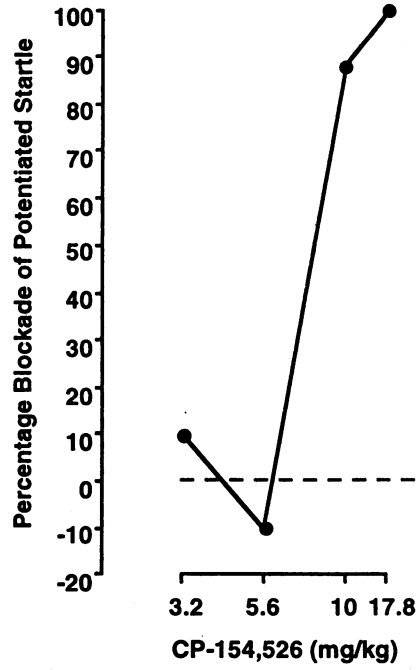

FIG. 6. Effect of CP-154,526 i.p. on potentiated startle in rats. Animals were exposed to 108 dB[A] acoustic startle stimuli, some of which were presented in darkness and others in the presence of a conditioned stimulus (electric light, 25 w) formerly paired with electric shock. Data are expressed as the percentage blockade of potentiated startle as compared with vehicle-treated controls. (n = 12).

CONCLUDING REMARKS

In earlier reports, the stimulation of locus coeruleus cell firing by CRF has served as evidence for a hypothesized role for this peptide in modulating noradrenergic tone during periods of stress (14, 15). A dampening of this system by a CRF antagonist would mimic the action of chronic treatment with tricyclic antidepressants in attenuating stress-induced locus coeruleus activation (16). To the extent that such findings predict clinical activity, CRF antagonists may represent a novel approach to reducing the severity or episodic frequency of affective disorders.

The increases in acoustic startle produced by i.c.v. administration of CRF have been suggested to be reflective of increased fear or anxiety, as administration of the anxiolytic chlordiazepoxide blocks the effect of CRF but not the startle-increasing effects of amphetamine or strychnine (19). Moreover, lesions of the amygdala, a key limbic structure in the mediation of fear and anxiety responses to stressors, abolish potentiated startle (47). Several classes of anxiolyic-like drugs also block potentiated startle (see review by Davis, ref. 48), and peptide CRF antagonists are known to be effective in other models of anxiety (e.g. ref. 45). Consequently, it is possible that a CRF antagonist would be a useful medication in the treatment of some disorders in which anxiety is a prominent feature.

In conclusion, CP-154,526 is the first nonpeptide antagonist of CRF receptors and possesses clear pharmaceutical advantages over peptide antagonists. CP-154,526 potently and selectively blocks CRF receptor-mediated activity *in vitro* as shown in adenylate cyclase assays and attenuates the activation of the HPA axis caused by exogenous CRF. CP-154,526 readily enters the CNS following peripheral administration, as demonstrated by its ability to antagonize the electrophysiological and behavioral effects of CRF infused directly into the brain. The finding that CP-154,526 is highly selective for the CRF_1 receptor subtype, coupled with the observation that CP-154,526 almost completely blocks the effects of CRF in all systems reported here, strongly suggests that these actions of CRF are mediated exclusively by the CRF_1 receptor subtype. Taken together, these data suggest that CP-154,526 will serve as a useful tool for further probing the functional importance of brain CRF systems. In the clinic, CP-154,526 may have important therapeutic utility in treating depression and anxiety as well as other diseases where excessive stimulation of CRF receptors contributes to pathology.

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