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The Pharmacology of CP-154,526, a Non-Peptide Antagonist of the CRH1 Receptor: A Review

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ABSTRACT

Since CRH has been shown to mediate stress-induced physiological and behavioral changes, it has been hypothesized that CRH receptor antagonists may have therapeutic potential in disorders that involve excessive CRH activity. CP-154,526 and its close analog antalarmin are potent, brain-penetrable, selective nonpeptide CRH1 receptor antagonists that were discovered in an effort to develop compounds with efficacy in CNS disorders precipitated by stress. Since its discovery many investigators have used CP-154,526 as a tool to study the pharmacology of CRH and its receptors and to evaluate its therapeutic potential in a variety of CNS and peripheral disorders. Systemically-administered CP-154,526 has been demonstrated to antagonize CRH- and stress-induced neuroendocrine, neurochemical, electrophysiological, and behavioral effects.

These findings support the hypothesis that CRH1 receptor antagonists may have therapeutic utility in a number of neuropsychiatric disorders. CP-154,526, as well as other CRH1 receptor antagonists that have since been discovered, have also shown activity in several preclinical models of anxiety, depression, and substance abuse, while having little effect on locomotor activity and motor function. Although these effects are on occasion inconsistent among different laboratories, clinical evaluation of CRH1 antagonists appears justified on the basis of these and clinical data implicating the involvement of CRH in several CNS disorders. The effects of CRH1 antagonists on cognition, neurodegeneration, inflammation, and the gastrointestinal system have not been as extensively characterized and additional studies will be necessary to evaluate their therapeutic potential in these areas.

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INTRODUCTION

Corticotropin-releasing hormone (CRH), a 41-amino-acid polypeptide was first identified and characterized by Vale et al. (189). It is a major physiological regulator of the hypothalamic-pituitary-adrenal (HPA) axis and the primary coordinator of endocrine, autonomic, immunologic, and behavioral responses to stress. In response to various stressors, CRH stimulates the biosynthesis and secretion of ACTH from the anterior pituitary, which releases glucocorticoids from the adrenal glands. In addition, CRH has been shown to mediate stress-induced physiological and behavioral changes, many of which occur independently of HPA axis activation since they are not prevented by dexamethasone or hypophysectomy (15). After central administration to rodents CRH has effects similar to those produced by stress, including autonomic and behavioral effects, such as increased heart rate and blood pressure (45) and stimulation/inhibition of gastrointestinal functions (182). It also reduces exploratory activity in novel surroundings (8,179), enhances fear responses (19), decreases sleep (170) and diminishes food intake (135). Shortly after the characterization of CRH, a truncated analog of CRH, α-helical $CRH_{(9–41)}$ (ah-CRH) was discovered (155) and shown to block the effects of CRH (16). Additional peptidic antagonists were subsequently identified (e.g., d-Phe $CRH_{(12–41)}$) (d-Phe CRH) (73). The extensive utilization of these tools was critical to progress in understanding the functional roles of CRH at multiple levels, including the HPA axis; the immune, autonomic, and gastrointestinal systems; and, especially, the brain. The finding that many of the behavioral effects listed above could be induced by either i.c.v. administered CRH or exogenous stressors, combined with the observation that these responses were blocked by peptidic CRH receptor antagonists, led to the hypothesis that CRH antagonists have psychotherapeutic potential in disorders resulting from the hypersecretion of CRH. At the same time clinical studies also suggested that dysregulation of CRH systems may underlie the pathologies of several neuropsychiatric disorders, including major depressive disorder (137) and a number of anxiety disorders (158).

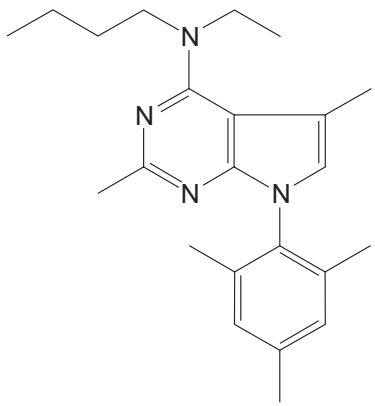
These compelling findings have driven the search for a small molecule CRH receptor antagonist that, by peripheral administration, mimics the pharmacological profile of the peptidic antagonists. Such a compound would require a high affinity for the CRH receptor, and would readily cross the blood-brain barrier. Its unbound fraction in plasma should be in equilibrium with the free fraction in brain sufficient to yield functionally relevant levels of receptor occupancy. Some additional properties desirable in a clinically useful agent include a high degree of receptor selectivity to minimize side effects; high metabolic stability to allow once-a-day dosing, and safety in at least two preclinical species. Ease of synthesis and chemical stability are also desirable to support large-scale manufacture.

CP-154,526 was the first potent and selective nonpeptide CRH1 antagonist to be described in the peer-reviewed literature (165). Initial studies with this compound indicated that it binds with high affinity to cortical and pituitary CRH receptors in multiple species, and that it behaves as a full antagonist. It was also found that systemically-administered CP-154,526 antagonizes the stimulatory effects of exogenous CRH on plasma ACTH, locus coeruleus cell firing, and acoustic startle responses. Additionally, potential anxiolytic activity was suggested by its ability to block, by systemic administration, fear-potentiated startle in rats. Unfortunately, CP-154,526 was found to have low oral bioavailability and, on the basis of *in vitro* findings in human liver microsomes, was predicted to have high hepatic clearance. These findings led to the conclusion that CP-154,526 would not

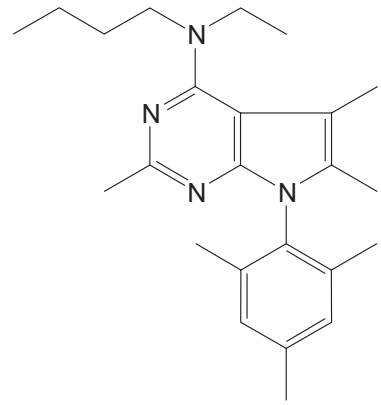
provide the best choice for proof of concept studies in the clinic and its development was halted preclinically. Since this initial report, however, many investigators have conducted studies with CP-154,526 to evaluate the potential therapeutic utility of CRH1 antagonists in a variety of CNS and peripheral disorders. Thus, although deemed not suitable for clinical development, this compound, as well as others that have since been discovered, has enjoyed widespread use as a tool to study the physiology of CRH and its receptors. This review summarizes the currently available literature on the pharmacology of CP-154,526, with particular emphasis on its effects in preclinical models of anxiety and depression.

CHEMISTRY

The synthesis of the pyrrolopyrimidine, CP-154,526 {butyl[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-*d*]pyrimidin-4-yl]ethylamine}, has been described in detail (28). Its close analog, CP-156,181, also known as antalarmin (196), is structurally very similar, containing an extra methyl group in position 6 of the pyrrolopyrimidine ring system, and is considerably easier to synthesize. Consequently antalarmin has also been used as a tool by many investigators and its pharmacology will also be described. While rigorous side-by-side comparisons of the two compounds have not been made, it is likely that they are very similar in their pharmacological, pharmacokinetic, and physicochemical properties.



CP-154,526



Antalarmin (CP-156,181)

IN VITRO PHARMACOLOGY

CRH belongs to a class of peptides (recently reviewed in 32) related by sequence homology, including fish urotensin I, amphibian sauvagine, mammalian urocortin (192) and the newly described peptides: urocortin II (UCN II), also known as stresscopin-related peptide (79,153) and UCN III, also known as stresscopin (79,105). The actions of CRH

and the UCNs are mediated by two major subtypes of CRH receptors belonging to the Class B sub-family of the super-family of seven transmembrane domain receptors, designated CRH1 and CRH2, and by the CRH binding protein (CRH-BP). Both CRH1 and CRH2 receptors are G_s-protein-coupled receptors and stimulate adenylate cyclase activity upon activation, thereby increasing intracellular cAMP levels. CRH has 30-fold higher affinity for CRH1 receptors (A. Schmidt, unpublished data), UCN has high affinity for both CRH1 and CRH2 receptors, and UCN II and UCN III have high affinity for CRH2 receptors (79,105,153).

Both CRH1 and CRH2 receptors are heterogeneously distributed throughout rat brain with the highest density of CRH1 receptors found in cortex, hypothalamus and pituitary; and the highest density of CRH2 receptors found in subcortical regions such as amygdala, lateral septum and hypothalamus. In contrast to the distribution of CRH1 and CRH2 receptors in rat brain, both CRH1 and CRH2 receptors are found in neocortex and pituitary of the rhesus monkey, suggesting that CRH2 receptors play a role in cognitive, behavioral and pituitary-adrenal function in primates (161). The CRH1 and CRH2 receptors have been cloned from various tissues including brain, pituitary, heart and skeletal muscle, in a number of different species including mouse (99,148,175,193), rat (24,109,149), and human (27,107,193). In rat brain, CRH1 receptor mRNA is found in neocortical, cerebellar and sensory relay structures, whereas CRH2 mRNA is found in lateral septal nucleus, choroid plexus, olfactory bulb, amygdaloid nuclei and various hypothalamic nuclei (22). In the periphery, CRF1 receptor mRNA is localized in the testis and intestine in mice (175), whereas CRH2 receptor mRNA is localized in heart and lung in rats (22). Three isoforms of the CRH2 receptor have been identified and designated CRH2α, CRH2β, and CRH2γ. Based on *in situ* hybridization studies, these isoforms are differentially distributed in rat CNS and periphery (110): $CRH2_\alpha$ receptor mRNA was found primarily in sub-cortical brain regions (hypothalamus, lateral septum and olfactory bulb), whereas the CRH2β receptor mRNA was found primarily in heart and skeletal muscle. In humans, the CRH2α receptor isoform predominates and is found in both central and peripheral locations (136). CRH2γ was derived from human amygdala (101). The CRH-BP is found in the brain (rat and human) as well as in plasma (human) where it regulates circulating levels of CRH.

Although CRH1 and CRH2 receptor genes encode proteins that share 70% identity at the amino acid level, they have distinguishable pharmacological properties. Rat/human CRH (r/hCRH), ovineCRH (oCRH) and bovine CRH have lower affinity for the CRH2 receptor than for the CRH1 receptor, whereas UCN, sauvagine, and urotensin I have similar affinities for the two receptor subtypes. UCN II and UCN III are reported to have higher affinity for CRH2 than for CRH1 receptors (79,105,153). The peptide CRH receptor antagonists, ah-CRH and d-Phe-CRH, have a slightly greater (four-fold) affinity for the CRH2 receptor, but are not sufficiently selective to serve as tools in probing the functional attributes of the receptor subtypes. More recently, two additional peptide antagonists, astressin (61) and the sauvagine analog, antisauvagine-30 (AS-30) (159) have become available. Astressin reportedly displays improved solubility and increased potency at both CRH1 and CRH2 receptors. AS-30 has been shown to display >300-fold selectivity for CRH2 over CRH1 receptors (159) and has, therefore, become an important tool for the characterization of CRH2 receptor function. Although some evidence suggests that the CRH1 receptor plays a role in regulating brain and pituitary function (i.e., behavioral and endocrinological responses to stress), while the CRH2 receptor may be more im-

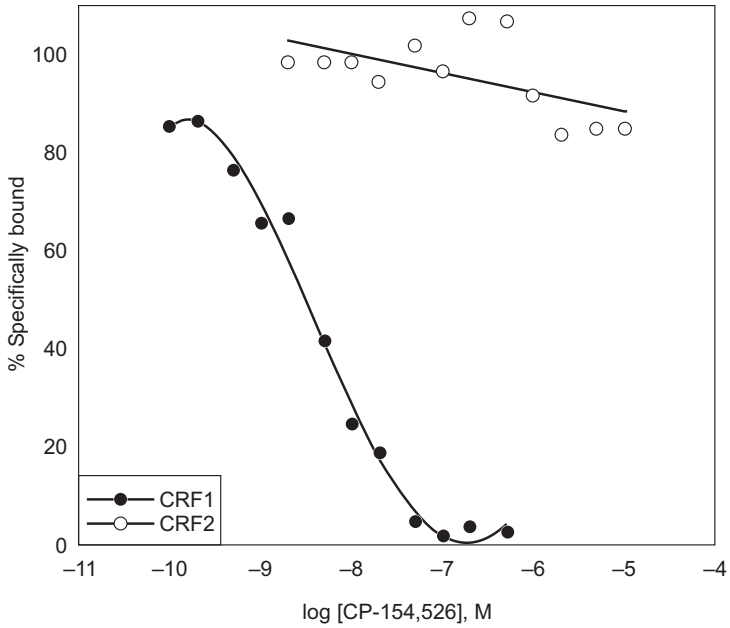


Fig. 1. Selectivity of CP-154,526 for CRH1 receptors. CP-154,526 potently inhibited [^{125}I]oCRH binding to CRH1 receptors in rat cortical membranes. In contrast, CP-154526 only weakly inhibited [^{125}I]sauvagine binding to CRH2 receptors in pig choroid plexus.

portant in the periphery (32). The precise roles of these receptor subtypes remain to be determined.

Receptor Binding

Receptor binding studies demonstrate that CP-154,526 and antalarmin have high affinity for CRH1 receptors. In membranes prepared from rat, guinea pig, and monkey cortex and IMR32 neuroblastoma cells, CP-154,526 potently displaced [^{125}I]oCRH-labeled CRH1 receptors with K_i values <10 nM (e.g., rat cortical K_i = 5.7 nM, Fig. 1) (165). In contrast, CP-154,526 did not inhibit [^{125}I]sauvagine binding to CRH2 receptors in pig choroid plexus membranes (Fig. 1) or [^{125}I]r/hCRH labeling of the CRH-binding protein (CRH-BP) at concentrations <1 μM. CP-154,526 was found to have minimal affinity (IC_{50} values > 1 μM) for 40 additional CNS receptors, which confirms its high degree of selectivity for CRH1 receptors. Antalarmin displayed a profile very similar to that of CP-154,526, with high affinity for CRH1 receptors (K_i value = 3.2 nM) and reduced affinity for both CRH2 and CRH-BP (K_i values > 1 μM). The binding affinities of CP-154,526 and antalarmin at CRH1 receptors and their lack of affinity for CRH2 receptors have been confirmed by other laboratories (Table 1). Using [^{3}H]urocortin to label the CRH-BP, Ardati et al. (4) also confirmed the lack of affinity of CP-154,526 for CRH-BP (K_i > 1 μM). Several other potent non-peptide CRH1 antagonists have been discovered, such as R-121919, SSR125543A, NBI-27914, CRA-1000, CRA-1001, DMP-695, DMP-696, and DMP-904, all of which have K_i values less than 5 nM, except for CRA-1000 and CRA-1001, which have K_i values of approximately 30 nM (Table 1).

Effect on Adenylate Cyclase

Potent antagonist activity of CP-154,526 was demonstrated in adenylate cyclase studies utilizing membranes prepared from rat cortical tissue. Adenylate cyclase activity activated by 100 nM oCRH was potently and completely blocked by CP-154,526 in a concentration-dependent manner with an average K_i value of 3.7 nM (165). CP-154,526 had no effect on basal or forskolin-stimulated adenylate cyclase activity. CP-154,526 blocked CRH-stimulated adenylate cyclase activity in a number of different tissues (e.g., cortex and pituitary) as well as in various species (e.g., rat, guinea pig, human) with similar potencies as described for rat cortex. In membranes from an immortalized cell line derived from mouse locus coeruleus (Cath. a) that endogenously expresses both CRH1 and VIP receptors that are coupled to adenylate cyclase, CP-154,526 blocked CRH-stimulated, but not VIP-stimulated adenylate cyclase activity, demonstrating selectivity for CRH1 receptors (A. Schmidt, unpublished data). Several other laboratories have confirmed the *in vitro* antagonist activity of CP-154,526 and antalarmin at CRH1 receptors (summarized in Table 2) and the inactivity of CP-154,526 at CRH2 receptors (144,173).

Ex Vivo Binding

Ex vivo binding techniques were utilized to assess the ability of CP-154,526 to penetrate the blood-brain barrier. Animals were dosed orally with 3.2 mg/kg of CP-154,526 and euthanized one hour later. Frontal cortex and pituitary were collected and frozen and

TABLE 1. CRH1 receptor binding affinity for CP-154,526, antalarmin, and related compounds

Tissue	Compound	K_i, nM	IC_{50}, nM	Reference
Human IMR32 cells	CP-154,526	2.7		(165)
Rat pituitary	CP-154,526	1.4		(165)
Rat cortex	CP-154,526	5.7		(165)
Rat hippocampus	CP-154,526		0.5	(114)
Rat pituitary	CP-154,526		0.04	(114)
Human clone	CP-154,526		5	(21)
Rat cerebellum	CP-154,526	1		(52)
Human clone	CP-154,526	10		(52)
Rat pituitary	Antalarmin	1.9		(196)
Rat frontal cortex	Antalarmin	1.4		(196)
Human clone	Antalarmin	6		(60)
Human clone	R-121919	3.5		(70)
Human clone	SSR125543A	2		(60)
Human clone	NBI-27914	2		(108)
Human clone	CRA-1000		30	(21)
Human clone	CRA-1001		38	(21)
Human clone	DMP-695	3.3		(50)
Human clone	DMP-696	1.7		(67)
Human clone	DMP-904	1.0		(50)

[^{125}I]oCRH receptor binding was examined in unwashed homogenates subsequently prepared from these tissues. Animals that received CP-154,526 showed substantially lower specific [^{125}I]oCRH binding in both cortical (69% decrease) and pituitary (54% decrease) samples compared to control animals, indicating that CP-154,526 penetrated the blood-brain barrier very well at this dose (A. Schmidt, unpublished data). Time course studies were also performed with CP-154,526 (10 mg/kg) administered subcutaneously (s.c.). These studies demonstrated that CP-154,526 rapidly penetrated the blood-brain barrier (within 10 minutes) and remained in the brain for at least 7 h after administration (86% decrease in specific binding in cortical samples at 7 h; 19% decrease in specific binding in cortical samples at 18 h).

PHARMACOKINETICS

The pharmacokinetics of CP-154,526 has been studied in male Sprague–Dawley rats following an i.v. dose of 5 mg/kg and an oral dose of 10 mg/kg (28). Following i.v. administration, drug concentrations declined over time in a biphasic manner. High plasma clearance (82 mL/min/kg) and a large volume of distribution (6.7 L/kg) were observed, resulting in an elimination half-life of 1.5 h. Following p.o. administration in DMSO/Emulphor/ water (5:5:90; v/v/v), the mean maximal plasma concentration (C_{max}) of drug was 367 ng/mL and occurred at 0.5 to 1 h after administration. Oral bioavailability was estimated to be 37%. Assuming blood concentrations of CP-154,526 are similar to those in plasma, clearance exceeded hepatic blood flow. An oral bioavailability of 37% suggests that hepatic extraction of CP-154,526 was ≤63%. Recently Keller et al. (96) reported the pharmacokinetic profile of CP-154,526 in male Wistar rats following a dose of 13.7 μmol/kg (5 mg/kg, p.o.) and a dose of 2.7 μmol/kg (1 mg/kg, p.o.). CP-154,526 displayed a large volume of distribution (Vss = 105 L/kg), a systemic clearance of 36 mL/min/kg and oral bioavailability of 27%. Brain levels (cortex) after p.o. administration (840 nmol/g tissue) and a brain to plasma ratio of 2.5 (8 h timepoint) confirmed that good brain penetration was achieved. Habib et al. (63) characterized the pharmacokinetics of antalarmin in macaques. After p.o. administration (20 mg/kg), average plasma and CSF levels were 76 and 9.8 ng/mL, respectively, at 3 h after administration. Total clearance of antalarmin was 4.5 L/hr/kg, the elimination half-life was 7.8 h and oral bioavailability was 19%.

TABLE 2. Functional data for CP-154,526 and antalarmin

Tissue	Type of Assay	Compound		Reference
Rat cortex	cyclase	CP-154,526	K_i = 3.7 nM	(165)
Human clone	microphysiometry	CP-154,526	pK_b = 8.17	(173)
Human SH-SY5Y	cAMP	CP-154,526	pK_b = 7.76	(164)
Human clone	luciferase assay	CP-154,526	pA2 = 7.38	(144)
Human SH-SY5Y	cAMP	Antalarmin	pK_b = 9.19	(164)
Human Y79 cells	cAMP	Antalarmin	IC_{50} = 0.8 nM	(60)

IN VIVO PHARMACOLOGY

Neuroendocrine Effects

Studies examining receptor subtype localization and mRNA expression (32), as well as studies in mice lacking a functional CRH1 receptor (e.g., 188) have provided convincing evidence that activation of the CRH1 receptor mediates the secretion of ACTH from the anterior pituitary. The majority of experiments profiling the effects of nonpeptide CRH1 antagonists on HPA axis function are supportive of this view. *In vitro* CP-154,526 and antalarmin, as well as another antagonist, NBI 27914 (25), inhibited CRH-stimulated ACTH release in cultured rat anterior pituitary cells (127), and antalarmin blocked the production and release of cortisol in cultured human adrenal cells (198). Acute *in vivo* studies have also yielded the expected finding that pretreatment of rats with CP-154,526 and antalarmin prevents the increase in plasma ACTH caused by i.v. injection of CRH (165,196). In both of these studies, no effects of the antagonists on baseline ACTH levels were detected. However, 11 days (11) and 8 weeks of antalarmin (i.p., b.i.d.) administration (200), but not 1 week, significantly lowered basal ACTH and corticosterone concentrations in rats suggesting decreased adrenocortical responsiveness to ACTH, but no evidence of adrenal insufficiency. The ACTH and corticosterone responses to immobilization stress were unaffected suggesting that the response to acute stress remained intact.

This paradigm of reversing the endocrine effects of i.v. CRH has been proposed as a potential pharmacodynamic approach to validate mechanism of action and explore dose-response relationships of novel CRH receptor antagonists entering clinical development, since many groups have extensive experience studying the effects of i.v. CRH in humans. However, Zobel et al. (204) reported that administration of R121919 (also known as NBI 30775) to depressed subjects failed to attenuate the ACTH response to CRH (100 μg, i.v.). Further work will be required to determine whether this is a unique property of this compound relative to the other nonpeptide agents, or that rats and humans respond differently to these compounds. Another possibility is that even the highest dose used in the study (80 mg) failed to provide the levels of antagonist needed to sufficiently block CRH1 receptors. No exposure data are provided in the report, nor is the information available on plasma protein binding of this particular agent, but general animal studies using other antagonists employed doses well in excess of 1 mg/kg when demonstrating suppression of CRH-induced ACTH secretion (165,196).

Compared to animal studies utilizing exogenous CRH, laboratories using exposure to stressors as a means of activating the HPA axis have observed less consistency among the nonpeptide antagonists with regard to their ability to suppress elevations in ACTH. For example Deak et al. (35) found that antalarmin (i.p.) blocked the ACTH response to conditioned fear in rats, but had no effect on the ACTH or corticosterone responses to inescapable shock. Our group has found that CP-154,526 reliably blocks restraint stress-induced increases in plasma ACTH in rats, but was less likely to block the ACTH response to other stressors (e.g., cold stress; D. Schulz, unpublished observations). Others have reported that CP-154,526 attenuated the increases in plasma ACTH and c-fos mRNA expression in the paraventricular nucleus of the rat hypothalamus following 30 min of restraint stress (83). In the most complete study to date, Habib et al. (63) examined the effect of antalarmin in primates, showing that it prevented the plasma ACTH response, as well as the fear/anxiety behaviors produced by a social stressor, the presentation of another male in a non-familiar environment. The failure of CRH1 antagonists to affect stress-in-

duced activation of the HPA axis is often explained as being supportive of the hypothesis that other ACTH secretagogues besides CRH, particularly arginine-vasopressin, may be elevated in response to certain stressors (154).

Neurochemical Effects

Direct infusion of CP-154,526 into the locus coeruleus (LC) had no effect on microdialysate norepinephrine (NE) levels in rat prefrontal cortex, but significantly blocked the increase in NE caused by handling stress (94). However, treatment of rats with CP-154,526 (i.p.) did not alter extracellular levels of NE, dopamine (DA), or 5-HT in the prefrontal cortex even at doses shown to be active in the Vogel conflict anxiolytic model (131). In another study, small but statistically significant decreases in NE and 5-HT levels were detected in the hippocampus (but not in the prefrontal cortex) following treatment with CP-154,526 (32 mg/kg, i.p.) (88). Both antalarmin and SSR125543A have been shown to block the increases in extracellular NE levels in the cortex evoked by tail pinch (57). These data suggest a role for CRH1 receptors in the central release of NE in response to stress.

Electrophysiological Effects

Early studies established that by i.c.v. administration CRH enhances the firing rate of noradrenergic neurons in the rat LC (190). Studies in our laboratories confirmed this finding and showed that i.v. infusion of CP-154,526 blocked the CRH-induced increase in LC cell firing (165). Administration of CP-154,526 alone had no effect on baseline cell firing. These data support a role for CRH1 receptors in mediating the excitation of LC neurons. To our knowledge similar studies have not been performed with antalarmin or any of the other nonpeptide CRH1 antagonists. However, antalarmin was shown to partially reverse the inhibition of dorsal raphe cell firing produced by i.c.v. CRH (98).

Behavioral Pharmacology

Anxiety Models. The findings that panic disorder patients exhibit blunted ACTH responses to CRH administration (158) and that post-traumatic stress disorder patients exhibit elevated CSF levels of CRH (93) support a role for CRH in anxiety disorders. There is an abundance of preclinical behavioral evidence supporting a role for CRH in anxiety, as well (39,55,143). For example, by i.c.v. administration to rats, CRH suppressed punished responding in a conflict test (14), decreased social interaction (38), potentiated acoustic startle response (181), and decreased time spent on the open arm of the elevated plus maze (EPM) (5). Clinically effective anxiolytics produce the exact opposite effects. These findings led to the hypotheses that CRH plays a major role in anxiogenesis and that CRH receptor antagonists would have anxiolytic properties in humans. Indeed, studies with peptidic CRH receptor antagonists supported this hypothesis, since they were shown by many to antagonize the anxiogenic effects of CRH (16,55). However, in traditional models only a few studies have successfully demonstrated anxiolytic activity with the peptidic CRH receptor antagonists alone (e.g., 16). The interpretation of these findings is complicated by the fact that these compounds must be administered centrally. Over the past few years, the non-peptide CRH1 antagonists, CP-154,526 and antalarmin, have been tested by many different investigators in numerous anxiety models (previously reviewed in 55). Table 3 contains a summary of the effects obtained with these two compounds.

TABLE 3. The effects of CP-154,526 and antalarmin in anxiety/stress models

Model/Species	Drug/Dosing[a]	Activity	Positive Control/Activity[b]	Reference
Conflict Tests				
Punished drinking Male SD rats	1.0–32 mg/kg 30 min, s.c.	Not active	Diazepam/Y	(Unpublished data)
	0.56–17.8 mg/kg 30 min, i.p.	Not active	Diazepam/Y	
Punished drinking Male SD rats	0.62–20 mg/kg 30 min, i.p.	Not active	Diazepam/Y Buspirone/Y	(54)
Punished drinking Male Wistar rats	5–80 mg/kg 30 min, i.p.	Active at 80	Chlordiazepoxide/Y Flesinoxan/Y	(131)
Punished drinking Male rats	Antalarmin 3–30 mg/kg 30 min, i.p.	Active at 10 and 30	Diazepam/Y	(57)
Punished lever press (food) Male Wistar rats	2.5–10 mg/kg 30 min, i.p.	Not active	Diazepam/Y Buspirone/N	(54)
Elevated Plus Maze				
Traditional measures Male SD rats	1–10 mg/kg 30 min, s.c.	Not active	Diazepam/Y	(Unpublished data)
	10–32 μg 15 min, i.c.v.	Not active	None	
Traditional measures Male SD rats	1–10 mg/kg 30 min, i.p.	Active at 1 only	None	(114)
Traditional measures Male Wistar rats	0.63–80 mg/kg 30 min, i.p.	Not active	Chlordiazepoxide/Y Flesinoxan/N	(131)
Traditional + ethological Male SD rats	0.62–20 mg/kg 30 min, i.p.	Not active	Diazepam/Y	(54)
Traditional + ethological Male SD rats	Antalarmin 3–30 mg/kg 60 min, p.o.	Active at 10 and 30	Diazepam/Y	(131)
CRF-Induced Anxiety Male SD rats	0.3–10 mg/kg 90 min, p.o.	Active at 3–10	Diazepam/Y	(142)
Traditional measures Male CD-1 mice	1–10 mg/kg 30 min, i.p.	Not active	Diazepam/Y	(Unpublished data)
Traditional measures after social defeat stress Male CD-1 mice	Antalarmin 30 mg/kg i.p., 15 min pre-defeat	Active	Diazepam/Y	(57)
Light/Dark Exploration				
Male Balb/c mice	1.78–56 mg/kg 60 min, s.c.	Not active	Diazepam/Y	(Unpublished data)
Male Balb/c mice	5–40 mg/kg 30 min, i.p.	Active at 10, 20, 40	Diazepam/Y Buspirone/N	(54)
Male ICR mice	10–30 mg/kg 30 min, p.o.	Not active	Diazepam/Y	(142)
Male Balb/c mice	Antalarmin 1–30 mg/kg 30 min, p.o.	Not active	Diazepam/Y	(57)

TABLE 3 (continued)

Model/Species	Drug/Dosing[a]	Activity	Positive Control/Activity[b]	Reference
CRF-Induced Anxiety Male C57Bl/6 mice	0.32–3.2 μg 60 min, i.c.v.	Active at 3.2	α-helical CRF(9–41)/Y	(59)
CRF-Induced Anxiety Male C57Bl/6 mice	3.2–56 mg/kg 60 min, s.c.	Not active	None	(Unpublished data)
Swim-Induced Anxiety Male ICR mice	3–30 mg/kg 50 min, p.o.	Active at 10 only	Diazepam/Y	(142)
Acoustic Startle				
CRF-Enhanced Male SD rats	5.6 and 17.8 mg/kg 60–70 min, i.p.	Active at 17.8	D-Phe CRF(12–41)/Y	(165)
Fear-Potentiated Male SD rats	3.2–17.8 mg/kg 60 min, i.p.	Active at 10 and 17.8	None	(165)
Fear-Potentiated (rats)	17.8 mg/kg 60 min, p.o.	Active	None	(28)
Defensive Withdrawal				
Acute dosing Male Wistar rats	0.1–3.2 mg/kg 60 min, i.p.	Active at 1 and 3.2	None	(Unpublished data)
Acute dosing SD rats??	30 mg/kg 60 min, p.o.	Not active	Chlordiazepoxide/Y	(67)
Acute dosing SD rats	30 mg/kg ?? min, p.o.	Not active	Chlordiazepoxide/Y	(50)
Acute dosing Male SD rats	3.2 and 32 mg/kg ?? min, s.c.	Not active	None	(3)
Chronic dosing (rats) Male SD rats	3.2 mg/kg/day ×14 days ?? min, s.c.	Active	None	(3)
Conditioned Fear				
Freezing behavior Male SD rats	Antalarmin[c] 20 mg/kg 120 min, i.p.	Active	None	(35)
Freezing Behavior Male Wistar rats	1–32 mg/kg 30 min, i.p.	Active at 10–32	None	(74)
Ultrasonic Vocalizations Male Wistar rats	30 mg/kg 60 min, i.p.	Active	None	(97)
Ultrasonic Vocalizations Male Wistar rats	2.5–80 mg/kg 30 min, i.p.	Not active	Chlordiazepoxide/Y Flesinoxan/Y	(131)
Maternal Separation-Induced Vocalizations				
SD rat pups	5–40 mg/kg 30 min, i.p.	Active at 10–40	Diazepam/Y Buspirone/Y	(95)
Guinea pig pups	Antalarmin 3–30 mg/kg 180 min, i.p.	Active at 3 and 30 not 10	None	(57)
Miscellaneous Models				
Social Interaction Male SD rats	0.16–10 mg/kg 30 min, i.p.	Active at 2.5 only	Chlordiazepoxide/Y Flesinoxan/Y	(131)

TABLE 3 (continued)

Model/Species	Drug/Dosing[a]	Activity	Positive Control/Activity[b]	Reference
Male SD rats	2.5–40 mg/kg 30 min, s.c.	Active at 40	Chlordiazepoxide/Y Flesinoxan/Y	(131)
Stress-Induced Hyperthermia Male rats	Antalarmin 30 mg/kg 60 min, i.p.	Active	Diazepam/Y	(57)
Staircase Test After Cat Exposure Male rats	Antalarmin 0.3–10 mg/kg	Active at 1 only	Diazepam/N	(56)
Four-Plate Test Male NMRI mice	Antalarmin 30 mg/kg 60 min, p.o.	Active	Diazepam/Y	(57)
Defense Test Battery Swiss mice	5–20 mg/kg 30 min, i.p.	Active at 5–20	Diazepam/Y Buspirone/Y	(54)
Male OF1 mice	Antalarmin 1–30 mg/kg 60 min, p.o.	Active at 1,3, 10, and 30	Diazepam/Y	(57)
Free-Exploration Test Balb/c mice	5–20 mg/kg 30 min, i.p.	Not active	Diazepam/Y Buspirone/N	(54)
Conditioned Defeat Male Syrian hamsters	15 and 30 mg/kg 60 min, i.p.	Not active	D-Phe CRF(12–41)/Y	(90)
Acute Psychosocial Stress Male macaques	Antalarmin 20 mg/kg 90 min, p.o.	Active	None	(63)

[a] Drug administered was CP-154,526 unless otherwise noted as antalarmin.
[b] Indicates positive control used in the same study; Y = active and N = not active.
[c] Actual drug used is unclear since paper claims CP-154,526 = antalarmin.

Conflict Tests. Several findings suggest a role for CRH systems in conflict models of anxiety. For example, Britton et al. (14) reported that i.c.v. CRH decreased both punished and unpunished responding in a Geller–Seifter conflict test in rats, and that these responses were blocked by ah-CRH (16) and the anxiolytic benzodiazepine, chlordiazepoxide (14). Zhang and Barrett (203) demonstrated that CRH decreased punished responding in pigeons, as well. However, administration of ah-CRH (alone) was reported to be ineffective in the Geller–Seifter conflict procedure in rats (16). These results suggest that CRH has an anxiogenic effect and reinforce the notion that CRH receptors play a role in conflict-induced response suppression and that CRH antagonists may be anxiolytic. These factors led to an initial focus in our laboratory on the effects of CP-154,526 in a Vogel conflict test (194) in rats. Our paradigm, which uses water deprivation, a 3-min free-drinking period in the test chamber prior to drug administration, and a 10-min test period in which every 20th lick results in a shock (0.5 mA), reliably produces anticonflict effects with benzodiazepine anxiolytics. Extensive testing with CP-154,526 failed to produce an anticonflict effect (Table 3). Griebel et al. (54) reported similar negative results with CP-154,526 using two different conflict models. More recently, however, this same group reported significant anticonflict activity with antalarmin and SSR125543A,

under similar conditions (57). In addition, Millan et al. (131) showed that CP-154,526 produced an anticonflict effect at a dose considerably higher than those used in the other studies (80 mg/kg, i.p.). Another CRH1 antagonist, DMP695 was also active. In summary, some investigators have found significant anticonflict activity with CRH1 antagonists, including CP-154,526 and antalarmin, while others have not.

Elevated Plus Maze (EPM). Since CRH has also been shown to produce an anxiogenic-like response in the EPM test (e.g., 5), early studies with CP-154,526 in our laboratory focused on this test as described by Pellow and File (147). In these tests CP-154,526, by either systemic or i.c.v. administration to rats, failed to produce an anxiolytic-like response (Table 3). Two additional reports cited negative results with CP-154,526 in rats, including one in which doses up to 80 mg/kg were used (131) and one in which ethological measures were added (54). A study by Lundkvist et al. (114) reported, however, a significant effect with one dose of CP-154,526 (1 mg/kg, i.p.), but not at lower and higher doses. Two additional CRH1 antagonists, DMP695 (131) and CRA1000 (65), were also reported to be inactive in rats. In contrast, Griebel et al. (57) reported antalarmin and SSR125543A to be active after p.o. administration, although the latter compound appeared weak at best, with efficacy only vs. the "attempts" measure.

When, however, CRH was administered i.c.v. to produce anxiogenic-like effects on the EPM, both CP-154,526 and CRA1000 were active (142). Similarly, the CRH1 antagonist, DMP696, exhibited an anxiolytic effect in rats that had undergone maternal separation as infants, but not in handled rats, while chlordiazepoxide had an anxiolytic effect in both groups (116). In fact, several older studies showed that peptide CRH receptor antagonists produced anxiolytic effects in the EPM test only after animals had been stressed (69,128). Similar effects have been observed in mice. Although CP-154,526 failed to show activity in a traditional model in our laboratory (Table 3), antalarmin and SSR125543A exhibited significant activity in mice that had been subjected to social defeat stress (57). SSR125543A was also active in the EPM test after mice had been subjected to a continuous mild stress procedure for 30 days (57). In summary, some investigators have found significant EPM activity with CRH1 antagonists, while others have not. However, when anxiety-like behaviors are increased by the administration of CRH or by stress treatments the anxiolytic-like activity of CRH1 antagonists appears to be enhanced.

Light/Dark Exploration Test. It was demonstrated in our laboratory that i.c.v. administration of CRH to C57BI/6 mice, a strain that normally exhibits high basal levels of exploration, reliably produced a dose-related anxiogenic-like effect in the light/dark (L/D) test (31), as measured by decreased L/D transitions, without affecting general locomotor activity (58). These effects were mimicked by restraint stress and systemic administration of FG-7142, an anxiogenic benzodiazepine inverse agonist, and were blocked by i.c.v. co-administration of ah-CRH. Subsequent studies (59) using i.c.v. co-administration of CP-154,526 and CRH revealed that CP-154,526 dose-dependently antagonized the anxiogenic-like effect of CRH (Fig. 2). However, systemically administered CP-154,526 failed to block the CRH-induced effect and failed to produce an anxiolytic-like effect in Balb/c mice (Table 3), a strain that spontaneously exhibits anxiogenic-like behavior and that has been described as an "emotional" strain (9). CP-154,526, antalarmin, SSR125543A, and CRA1000 were also found to be inactive by other investigators (57,142). In contrast, Griebel et al. (54) found that CP-154,526 produced significant anxiolytic-like effects on all three indices in the L/D test. In a similar "free-exploration"

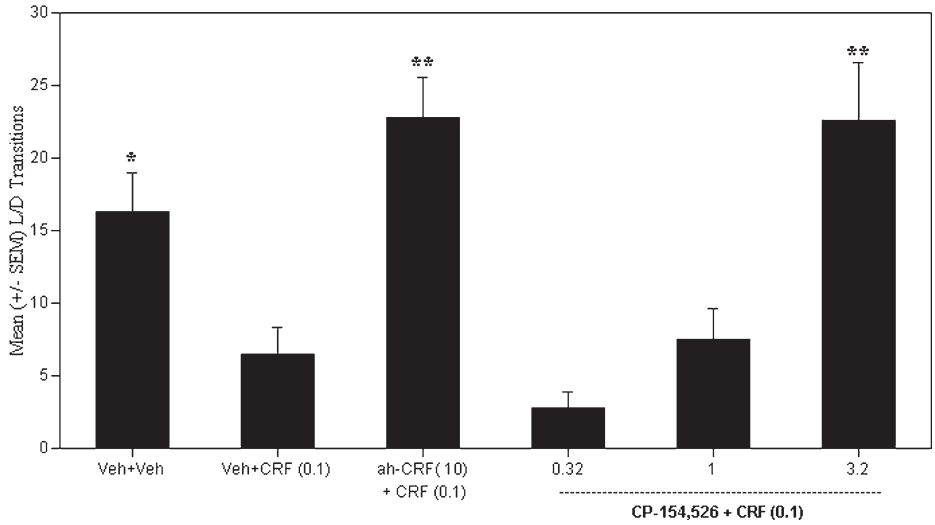


Fig. 2. Anxiolytic-like effect of CP-154,526 in the light/dark exploration test. Like ah-CRH, i.c.v. administration of CP-154,526 fully reversed the anxiogenic-like effect of i.c.v. CRH in C57Bl/6 mice. Numbers represent doses of the drugs (μg/mouse).

test, it was found that diazepam, but not CP-154,526 or buspirone, significantly increased novel unit changes and time spent in novel units. It was suggested that this procedure is less stressful than the L/D test, leading to weaker effects of CRH receptor antagonists (54). These same investigators failed, however, to show significant activity with antalarmin in the L/D test (57).

Similar to the EPM findings, when anxiety levels were increased by stress treatments the activity of these compounds appears to be enhanced. Thus, CP-154,526 and CRA1000 were both active in mice that received swim stress prior to L/D testing (142) and SSR125543A was active in mice that had been subjected to continuous mild stress for 30 days (57). In summary, only one study demonstrated significant activity with a CRH1 antagonist (CP-154,526) (alone) in the L/D test, while several have shown activity in stress-enhanced versions. However, although CP-154,526 was active in a CRH-enhanced test by i.c.v. administration, it was inactive by systemic administration. Unfortunately, other compounds have not been tested in this latter paradigm.

Acoustic Startle Models. Swerdlow et al. (181) were the first to report that by i.c.v. administration CRH increased acoustic startle responses, and that this effect was antagonized by ah-CRH. It has been hypothesized that this effect reflects increased fear or anxiety since an anxiolytic, chlordiazepoxide, also blocks this effect, without blocking a similar effect produced by d-amphetamine or strychnine (180). Several classes of anxiolytic drugs (33) and lesions of the amygdala (160), a key limbic structure involved in the mediation of fear and anxiety, have been shown to block fear-potentiated startle. Studies in our laboratories confirmed that i.c.v. CRH produced a significant enhancement of acoustic startle responses in rats (165). This response was completely blocked by coadministration of the peptide CRH receptor antagonist, d-Phe CRH. Moreover, unlike in the L/D test in mice, it has been found that systemic administration of CP-154,526 completely blocked

the CRH-induced effect. Neither compound affected startle when administered alone, suggesting little contribution of endogenous CRH to baseline startle responses. To investigate the role of CRH in the response produced by stress, CP-154,526 was tested in a fear-potentiated startle model (165). In this model rats exhibit potentiated startle responses when the acoustic stimulus is accompanied by a light stimulus that was previously paired with shock. CP-154,526 significantly blocked the fear-potentiated startle response without affecting baseline responses. In a subsequent study (28) CP-154,526 was active by p.o. administration as well. Thus, CP-154,526 appears to produce an anxiolytic-like effect in both the CRH-enhanced and fear-potentiated startle models, although to our knowledge, these effects have not yet been replicated by other laboratories.

Defensive Withdrawal. In this model anxiolytics have been shown to decrease the latency of rats to emerge from a small darkened chamber to explore a brightly lit open field (67). Several studies have suggested that CRH plays a role in these behaviors (172, 183). In addition, by i.c.v. administration ah-CRH had an anxiolytic-like effect in this test (183). Two investigators have reported that CP-154,526 is inactive in this model (50, 67). DMP696 (67) and DMP904 (50), however, were both reported to be active. Arborelius et al. (3) tested CP-154,526 using both acute and chronic s.c. dosing paradigms. Acute dosing with CP-154,526 had no effect, but the authors speculated, however, that the lack of significance was due to the small numbers of animals used ($N = 4–6$/group). When CP-154,526 was administered via minipumps for 14 days it significantly decreased the total withdrawal time. A recent experiment in our laboratories found that CP-154,526 significantly decreased the time spent in the chamber (MED = 1 mg/kg), when tested at 60 min after i.p. administration of the drug (J. Freeman, personal communication), suggesting that acute administration may be effective when the compound is administered i.p. Additional experiments to evaluate the reliability of this effect and to fully determine its dose-response are currently underway. In summary, CP-154,526 and two other antagonists have shown activity in the defensive withdrawal model, but several investigators have failed to obtain activity with acute administration of CP-154,526.

Conditioned Fear. It is well-known that cues present during exposure to a stressor, will later elicit the same physiological and behavioral responses as the stressor itself, a phenomenon known as "fear conditioning" (44). Brain CRH systems have been shown to play a role in the mediation of these responses, which include freezing and other fear-related behaviors, decreased appetite, potentiated startle responses, and increased autonomic nervous system activity. Serotonergic 5-HT_{1A} agonists and SSRIs have been reported to reduce freezing behavior in this model (84). Using different approaches, several investigators have studied the effects of CP-154,526 and antalarmin on fear-conditioned behaviors. In one study (35) antalarmin was administered prior to fear conditioning on day 1, prior to testing on day 2, or at both times. All three regimens reduced conditioned freezing behavior, suggesting that antalarmin blocked the development of fear conditioning as well as the expression of conditioned fear, and that CRH1 receptors are important in both processes. In other studies similar effects were obtained with CP-154,526 (74) and DPC904 (75). Using ultrasonic vocalizations (USVs) as a measure, Millan et al. (131) found no effect with CP-154,526 or DMP695 at doses shown to be active in conflict and social interaction tests in the same laboratory. In contrast, chlordiazepoxide and flesinoxan showed significant activity. In a study by Kikusui et al. (97) rats were exposed to conditioning sessions which consisted of a 10-min pre-shock period, a 10-min shock period, and a 5-min post-shock period, daily for 8 days. CP-154,526 (i.p.) and ah-CRH (i.c.v.) significantly de-

creased USVs in the preshock session, but not in the shock or post-shock sessions, suggesting a possible anxiolytic effect. Thus, CRH and CRH1 receptors appear to play a role in conditioned fear. However, CRH KO mice have been shown to exhibit fear-conditioned freezing behavior which was blocked by i.c.v. administration of either ah-CRH or CP-154,526 (197). In addition, Ho et al. (75) showed that intraseptal administration of an antisense oligonucleotide directed against the CRH2 receptor significantly reduced conditioned fear-induced freezing in rats. Combination of the antisense with a CRH1 antagonist, DPC904 (10 mg/kg, p.o.), resulted in a greater reduction in freezing behavior than with either agent alone. These data support the involvement of both CRH1 and CRH2 receptors, as well as other agonists in addition to CRH, in fear conditioning.

Maternal Separation-Induced Vocalization Model. The separation-induced vocalization (SIV) test in rats involves measurement of the ultrasonic vocalizations that are emitted by pre-weanling rat pups upon removal from their litter (85). Several studies suggest that CRH is involved in this behavior, but its precise role appears to be a complex one. Insel and Harbaugh (85) showed that CRH (0.01 and 0.1 μg, i.c.v.) decreased, and ah-CRH increased, SIVs in isolated rat pups. Harvey and Hennessy (66) found that higher doses of CRH (0.1 and 1 μg, i.c.v.) and ah-CRH (20 μg, i.c.v.), both decreased SIVs. One study using CP-154,526 has been reported (95) in which it dose-dependently reduced the number of SIVs without producing motor side effects, a profile similar to that of buspirone but not diazepam, which showed efficacy only at doses that produced motor side effects. The SIV test in guinea pig pups involves measurement of audible vocalizations upon removal from their litter (133). Hennessy et al. reported that peripherally (71) and centrally (72) administered CRH suppressed SIVs in isolated guinea pig pups, while i.c.v. administration of ah-CRH enhanced SIVs. Griebel et al. (57) found, however, that antalarmin and SSR125543A, administered i.p. 3 h prior to testing, significantly reduced SIVs. Thus, conflicting reports exist with respect to the activity of CRH antagonists in this test and it appears that more work needs to be done in this area to define the precise role of CRH in these behaviors.

Other Anxiety Models. CP-154,526 and antalarmin have been tested in several additional paradigms, summarized in Table 3 (Miscellaneous Models). Three rat models and three mouse models have been used. They included the social interaction test, the stress-induced hyperthermia test, and the staircase test in rats; as well as the defense test battery, the free exploration test, and the four-plate test in mice. In addition, CP-154,526 and antalarmin have been tested in a hamster conditioned defeat model, and an acute psychosocial stress model in macaques, respectively.

In the social interaction (SI) test Millan et al. (131) found that CP-154,526 and DMP695 produced pronounced, significant increases in active SI. CRA1000 was also found to significantly increase SI (65). In the staircase test (187) after exposure to a cat, rats were placed at the bottom of a staircase apparatus containing a cat odor-saturated brush on the top stair (56). Antalarmin, at 1 mg/kg, but not lower or higher doses, significantly increased time spent in contact with the brush. Griebel et al. (56) examined the effect of antalarmin in a stress-induced hyperthermia model, in which handling and isolation of rats in small novel cages significantly increases body temperature. In this model, antalarmin, SSR125543A, and diazepam significantly reduced the stress-induced hyperthermia response.

The mouse defense test battery is a model of antipredator defensive behavior that was designed to assess the defensive reactions of mice to a natural predator, the rat, and is

claimed to provide behavioral measures capable of differentiating between various classes of anxiolytic drugs (53). In this model mice are placed into an oval runway and behaviors are recorded before, during, and after presentation of an anesthetized rat. Griebel et al. (54) found that CP-154,526 significantly decreased several behaviors in this test, as did buspirone and diazepam. In a later study (57) antalarmin and SSR125543A also showed activity in this test. In the four-plate test, mice are placed into a cage with a floor composed of four rectangular metal plates (10) and a shock is presented every time the subject crosses over to a different plate. Antalarmin significantly increased the number of plate crossings (57), although the magnitude of this effect was considerably weaker than that produced by diazepam.

The conditioned defeat model exploits the fact that Syrian hamsters will attack conspecific intruders that are introduced into their home cage, a phenomenon believed to reflect behaviors that occur in the wild. Repeated brief defeats by a larger, aggressive resident produce defensive and submissive behaviors, even in response to a smaller, non-aggressive male, a phenomenon known as conditioned defeat (CD). Animals that have been defeated display an activated HPA axis, as indicated by increased ACTH, β-endorphin, cortisol and corticosterone release, while dominant animals do not (80–82). In addition, data suggest that CD results in a wide range of physiological, behavioral and autonomic changes in the defeated animal, including increased anxiety, and a host of other changes that are reminiscent of CRH- and stress-induced effects (90). Jasnow et al. (90) found that i.c.v. d-Phe-CRH significantly reduced the expression of CD, as measured by a significant decrease in the duration of submissive/aggressive behaviors. Systemic administration of CP-154,526 was without effect on these behaviors, but significantly reduced plasma ACTH levels, suggesting that central CRH systems mediate the expression of CD and behavioral responses to social stress in hamsters, but that CRH1 receptors may not be involved.

When male rhesus macaques that are unfamiliar to each other are placed in unfamiliar adjacent cages a reproducible, intense anxiety/aggression response is elicited, as interpreted by the presence of stress-induced behaviors (63). In a "psychosocial stress" study, antalarmin was given prior to the stress session, and behaviors were recorded by an observer that was blind to treatment. The stress session significantly increased the occurrence of anxiety/aggressive behaviors, as well as plasma ACTH, cortisol, norepinephrine and epinephrine levels. Significant increases in CSF CRH, but not arginine vasopressin concentrations, were also observed. Antalarmin administration produced significant reductions in all measures, reducing the behavioral, as well as the physiological responses to the psychosocial stress.

Depression Models. There is an abundance of clinical evidence supporting a role for CRH in depression (77). In depressed patients, several studies have reported elevated levels of CRH in cerebrospinal fluid (CSF), which then return to normal after successful therapy with antidepressant drugs or electroconvulsive shock (ECS) (2,137,139). In addition, some depressed patients display a blunted ACTH response to i.v. CRH, which has been hypothesized to be due to CRH receptor populations at the level of the pituitary that have been downregulated as a consequence of chronic CRH hypersecretion (e.g., 76). Post-mortem studies in depressed or suicide patients reveal a decrease in the number of CRH1 receptors (138) consistent with the hypothesis that CRH is hypersecreted in depressed patients. In addition, i.c.v. administration of CRH to animals produces behavioral effects similar to those observed in depression, such as diminished food intake, decreased

sexual activity, and disturbed sleep patterns (39,143). Taken together, these findings suggest that hypersecretion of CRH in response to chronic stress may be a key contributing factor in depression, and that blockade of CRH receptors in the CNS may be useful in treating this disorder. Because one common effect of ADs is a delayed downregulation of the HPA axis (12), administration of a CRH receptor antagonist may represent a means to shorten the onset of antidepressant activity. Positive results with CRH1 antagonists in preclinical depression models would strengthen the rationale for antidepressant activity of CRH1 antagonists. Table 4 contains a summary of the effects of CP-154,526 and antalarmin in depression models.

TABLE 4. The effects of CP-154,526 and antalarmin in depression models

Model/Species	Drug/Dosing	Activity	Positive Control/Activity[a]	Reference
Learned Helplessness				
Male SD rats	CP-154,526 60 min, 10–32 mg/kg i.p. (pre-test)	Active at 32 mg/kg	Imipramine/Y (chronic)	(122)
Male SD rats	Antalarmin[b] 20 mg/kg 120 min, i.p. (pre-shock and pre-test)	Not active	None	(35)
Male Wistar rats	CP-154,526 10–30 mg/kg 60 min, p.o. (pre-shock)	Active at 30 mg/kg	Imipramine/N (acute)	(184)
Male Wistar rats	CP-154,526 10–30 mg/kg 0 min, p.o. (post-shock) or 60 min, p.o. (pre-test)	Not active	Imipramine/N (acute)	(184)
Male Wistar rats	CP-154,526 3–10 mg/kg p.o. for 8 days	Active at 3 mg/kg	Imipramine/Y (chronic)	(185)
Behavioral Despair (Swim)				
Male SD rats	Antalarmin 3–30 mg/kg, p.o. 15 min post-swim (Day 1) 60 min pre-swim (Day 2)	Active at 3–30 mg/kg	Fluoxetine/Y	(57)
Tail-Suspension Test				
Male ddY mice	CP-154,526 0.3–3 mg/kg 45 min, s.c.	Not active	Imipramine/Y	(201)
YM643-induced immobility Male ddY mice	CP-154,526 0.3–3 mg/kg 45 min, s.c.	Active at 3 mg/kg	Imipramine/Y	(201)
Olfactory Bulbectomy				
Hyperemotionality score Male Wistar rats (acute and chronic — 7 days)	CP-154,526 0.3–10 mg/kg 120 min, p.o.	Active at 10 mg/kg (acute and chronic)	None	(142)

[a] Indicates positive control used in the same study; Y = active and N = not active.
[b] Actual drug used is unclear since paper claims antalarmin = CP-154,526.

Learned Helplessness. The antidepressant efficacy of CP-154,526 has been evaluated by several investigators using the learned helplessness (LH) paradigm (166), which has been described as a model of depression in that it satisfies some of the criteria for face, predictive and construct validities (199). In this procedure, animals are exposed to an uncontrollable stressor and subsequently tested for acquisition of a learned response, typically avoidance or escape behavior from a noxious stimulus. Animals pre-exposed to uncontrollable stress typically display acquisition deficits that are prevented by a number of ADs and by electroconvulsive therapy (169). Importantly, the time course for the protective effect of antidepressants in this model mirrors their clinical time course, in that repeated but not acute drug administration is effective (150).

In a study in our laboratories (122) rats exposed to inescapable shocks (IS) performed poorly in a shock-escape test compared with control animals that did not receive inescapable shocks. A single administration of CP-154,526 (10–32 mg/kg, 60 min, i.p. prior to testing) dose-dependently reversed the escape deficit, but had no effect on the performance of control rats that were not exposed to inescapable stress. The antidepressant, imipramine, reversed the escape deficit after repeated, but not acute, administration. It is likely that these effects were not due to a general anti-stress action, because the performance of control animals exposed to the same shock-escape session on the final test day was not affected by the drug. In addition, efficacy was observed in the same dose range previously reported to be effective against the startle-enhancing and ACTH-enhancing effects of CRH in the rat (165), suggesting that the effects on LH are mediated by CRH1 receptors in the CNS.

In a study performed by Deak et al. (35) LH was produced using a different procedure. Rats were restrained in tubes with their tails exposed and shocks were applied to the tail. Twenty-four hours later they were placed into shuttleboxes for fear conditioning and escape learning. Antalarmin (20 mg/kg) was administered 120 min prior to the IS session and again 120 min prior to testing on day 2. Animals that received IS treatment on day 1 showed enhanced freezing behavior on day 2. Antalarmin totally blocked this effect and reduced the overall level of fear conditioning. Antalarmin had no effect on the escape deficits produced by IS or on escape behavior in control subjects, and had no effect on the cortisol or ACTH responses produced by IS, but blocked the ACTH response produced by exposure to two footshocks. It was concluded that these data contradict those of Mansbach et al. (122), possibly due to methodological differences.

In another LH study (184) CP-154,526 (10 and 30 mg/kg, p.o.) was administered 60 min prior to the IS session (acquisition phase), immediately after the shock session (consolidation phase), or 60 min before escape testing (retention phase). When administered prior to the shock session, CP-154,526 significantly decreased the number of escape failures on day 2, while administration at either of the other two times had no effect on escape failures. These results were in contrast to those of Mansbach et al. (122) who reported that CP-154,526 (at the same i.p. dose) was efficacious when administered prior to testing, albeit different procedures were used in the two studies. Another CRH1 antagonist, CRA1000, produced similar effects to those of CP-154,526. It was concluded that blockade of CRH1 receptors prior to stress can alleviate stress-induced changes in behavior, and that CRH1 antagonists may be rapidly acting antidepressants.

In a LH study utilizing chronic drug treatment (185) rats were given imipramine (10 mg/kg, p.o.), CP-154,526 or CRA1000 (p.o.) daily for 8 days. On day 9 animals were subjected to active avoidance and the number of escape failures was recorded. Imipramine

reduced the number of escape failures and ACTH administration (100 mg/rat, i.p.) blocked this effect. CP-154,526 (10 mg/kg) and CRA1000 (3 mg/kg) significantly decreased the number of escape failures, and the effects of both compounds were blocked by ACTH. It was concluded that CRH acting at CRH1 receptors plays a role in the development of LH, and that the antidepressant-like activity of CRH1 antagonists in LH may be mediated via the HPA axis. Thus, the effects of CRH1 antagonists in the LH paradigm appear to be somewhat controversial and conclusions regarding the role of CRH1 receptors await further studies using additional compounds that are now available.

Behavioral Despair. Griebel et al. (57) tested the effect of antalarmin (3–30 mg/kg, p.o.) in the behavioral despair test in rats (151) when administered 15 min after the pre-test swim on day 1 and 60 min prior to testing on day 2. Like fluoxetine and SSR125543A, antalarmin significantly decreased immobility in this test, an indication of AD-like activity. Similarly, Harro et al. (65) showed that CRA1000 significantly decreased immobility by subchronic i.p. administration. Therefore, several CRH1 antagonists have shown activity in this test in rats.

Tail-Suspension Test After YM643. CP-154,526 has also been tested in an interesting new depression model. YM643, a consensus interferon-a (IFN-a), is a synthetic IFN that is effective in treating chronic hepatitis C (201), but also produces a variety of psychiatric symptoms, including depression, which is successfully treated with antidepressants (104). Using the mouse tail suspension test, Yamano et al. (201) demonstrated that i.v. and i.c.v. administration of YM643 dose-dependently increased immobility time. CP-154,526 (0.3–3 mg/kg, s.c.) dose-dependently and completely antagonized the immobility produced by YM643, while having no effect on immobility exhibited by vehicle-treated controls. This was in contrast to imipramine, which decreased the immobility in both groups. These results were interpreted to suggest that IFN-α induces depression-like behavior in mice via a central mechanism and involves activation of CRH1 receptors via IFN-α-induced CRH release. It was also suggested that endogenous CRH may not be involved in depression-like behavior induced by tail-suspension stress, since CP-154,526 was not active in vehicle-treated animals.

Olfactory Bulbectomy. CP-154,526 has also been tested in olfactory bulbectomized rats, another model that is hypothesized to produce depressive-like symptoms in rats (191). Using a "hyperemotionality" behavioral scale as a measure, Okuyama et al. (142) found that CP-154,526 and CRA1000 significantly reduced the behaviors observed in bulbectomized rats.

Drug Addiction Models. Much evidence supports a role for CRH in the dependence and abstinence syndromes associated with drugs of abuse. For example, increased CRH release has been demonstrated during withdrawal from ethanol and cannabinoids (130,156) and intra-amygdaloid infusion of ah-CRH reversed the anxiogenic-like effects of ethanol withdrawal in an EPM test (152). CRH also appears to play a role in relapse, the prevention of which is a significant problem in individuals with a history of substance abuse. Studies suggest that several factors induce craving and precipitate drug-seeking behavior and relapse, including stress (42,100), reexposure to the drug itself (reviewed in 34) and exposure to cues associated with the drug (e.g., 40). The effect of stress is partially mimicked by i.c.v. injections of CRH (168). Several investigators have used CP-154,526 as a tool to explore the role of CRH1 receptors in these phenomena.

Self-Administration. To examine the role of CRH in the maintenance of ongoing cocaine self-administration, Goeders and Guerin (51) investigated the effect of CP-154,526

on i.v. cocaine self-administration in rats. CP-154,526 (i.p.) significantly reduced the number of responses on the cocaine lever, to a level similar to that observed during cocaine extinction. Responding was decreased across several doses of cocaine, with the dose-response curve for self-administration shifted downward and flattened, suggesting that CP-154,526 was decreasing cocaine-induced reinforcement. The effects of CP-154,526 were deemed not to be due to nonspecific effects since food-maintained responding during the same session was unaffected by drug treatment. These results suggested that CRH1 receptors are involved in the maintenance of ongoing cocaine self-administration. Furthermore, responding on the cocaine lever following CP-154,526 treatment was significantly suppressed during the first 15 min of the session, a time period during which rats typically sample the cocaine lever during extinction, suggesting that CRH receptors may also be involved in some of the conditioned effects of cocaine. This latter effect was addressed in a subsequent study (62), in which rats were trained to respond on a multiple schedule of cocaine and food reinforcement, and responding during extinction was used as a model of cue-induced craving. CP-154,526 (20 mg/kg, i.p.) significantly decreased responding on the cocaine-associated lever during extinction, suggesting an involvement of CRH1 receptors in cue-induced craving. In addition, since CP-154,526 did not reduce plasma corticosterone, it was concluded that the HPA axis was not involved in these effects, and that extrahypothalamic CRH1 receptors were probably involved. It was concluded that CRH1 receptors are involved in cocaine-seeking behavior and, therefore, may be a good target for cocaine addiction therapy.

Drug Withdrawal. CRH has also been reported to be involved in the anxiogenic and aversive symptoms of withdrawal from several drugs of abuse, including alcohol (129), opiates (68), cannabinoids (156), and cocaine (162). Iredale et al. (87) investigated the effects of CP-154,526 on the physical symptoms associated with opiate withdrawal. Rats were implanted with morphine pellets and subjected to naltrexone-precipitated withdrawal. Consistent with a report that i.c.v. administration of ah-CRH reduces morphine withdrawal (17), CP-154,526 (20 mg/kg, i.p.) significantly attenuated the withdrawal response, as measured by decreases in writhing, chewing, weight loss, lacrimation, salivation, irritability, and diarrhea. In addition, CRH1 mRNA was significantly downregulated in the basolateral nucleus of the amygdala and the parietal cortex at 6 h after precipitation of withdrawal, consistent with other reports that agonist treatment and repeated stress decreases expression of the CRH1 receptor (86). Another experiment using a more severe withdrawal paradigm demonstrated that CRH1 mRNA was also significantly down-regulated in the frontal cortex, striatum, and nucleus accumbens, but not in the periaqueductal gray or hypothalamus. These findings were taken as evidence that the CRH1 receptor subtype plays an important role in withdrawal-induced symptoms and is acutely activated during opiate withdrawal, leading to decreased receptor expression in brain regions implicated in withdrawal. In addition, these data imply that a CRH1 antagonist could be useful for the treatment of opiate withdrawal, as well as stress-induced opiate-seeking behavior. Using a different withdrawal-induction procedure Lu et al. (112) found that CP-154,526 (i.p.) significantly attenuated the withdrawal response, confirming most of the effects observed by Iredale et al. (87), and adding efficacy vs. several additional signs, including jumping, teeth chatter, shakes, and piloerection. In addition, i.c.v. ah-CRH showed a similar profile, while AS-30 had no effect on any sign except diarrhea. It was concluded that the anti-withdrawal effects of ah-CRH and CP-154,526 clearly implicate the CRH1 receptor in the opiate abstinence syndrome.

Relapse Models. Studies suggest that CRH plays a role in stress-induced relapse to drug-seeking. Shaham et al. (168) examined the effects of CP-154,526 on reinstatement of heroin- and cocaine-seeking induced by footshock. In this study, rats were trained to self-administer heroin or cocaine for 9–12 days. Extinction sessions were introduced for 14 days, followed by reinstatement tests after exposure to footshock. The footshock stressor reliably reinstated the extinguished cocaine and heroin self-administration and CP-154,526 (15 and 30 mg/kg, s.c.) significantly attenuated this reinstatement. CP-154,526 had no effect on lever pressing for sucrose, suggesting that the suppression in the stress-induced reinstatement procedure was not due to a drug-induced performance deficit. Thus, the results of this study are in agreement with those utilizing peptide CRH receptor antagonists. Shaham et al. (167) showed that in animals trained to self-administer heroin (167) and cocaine (43), footshock stress-induced, but not drug-induced, reinstatement was attenuated by i.c.v. injections of ah-CRH and d-Phe CRH, respectively. In heroin-trained rats, both ah-CRH and CP-154,526 produced only a 50% reduction in footshock-induced responses, suggesting the possible involvement of additional transmitters in addition to CRH (168). In cocaine-trained animals, however, d-Phe CRH and CP-154,526 attenuated footshock-induced responding to no-shock levels. Lê et al. (103) found that administration of CP-154,526 (i.p.) and d-Phe-CRH (i.c.v.) dose-dependently attenuated footshock stress-induced reinstatement of alcohol-taking behavior. This effect was also reinstated in adrenalectomized (ADX) rats and in ADX rats that received corticosterone replacement, suggesting that extra-hypothalamic CRH systems are involved in stress-induced reinstatement of alcohol-seeking.

Lu et al. (111) investigated the effect of different CRH receptor antagonists on the maintenance and reactivation of morphine-induced conditioned place preference (CPP) induced by morphine or footshock stress, respectively. Pretreatment with ah-CRH (i.c.v.), CP-154,526 (i.p.), or AS-30 (i.c.v.), had no effect on morphine-CPP, maintained by morphine itself. However, ah-CRH and CP-154,526 significantly inhibited footshock-induced maintenance of morphine-CPP, while AS-30 was without effect. In saline-maintained animals, a single injection of morphine reactivated the morphine-CPP, an effect that was attenuated by ah-CRH, but not by CP-154,526 or AS-30. Footshock stress was also shown to reactivate the morphine-CPP, and this effect was blocked by ah-CRH and CP-154,526, but not by AS-30. It was concluded that CRH1 receptors mediate the stress-induced maintenance and reactivation of morphine CPP and that CRH1 antagonists may be of value in the treatment and prevention of relapse in opiate addiction. A follow-up study (112) showed that the reactivation of morphine-CPP after a 28 day extinction (saline) period by a single injection of morphine was also blocked by CP-154,526 (i.p.). Based on these findings a similar experiment was conducted using reactivation of cocaine-CPP by cocaine and stress (113). A single injection of cocaine reactivated cocaine-CPP after a 28 day extinction period. Pretreatment with CP-154,526 (i.p.) or AS-30 (i.c.v.) were without effect, while pretreatment with ah-CRH (i.c.v.) significantly attenuated this reactivation. Both ah-CRH and CP-154,526, however, attenuated the reactivation of cocaine-CPP induced by footshock stress. It was concluded that the CRH1 receptor mediates the stress-induced reactivation of cocaine-CPP. The lack of effect of CP-154,526 vs. cocaine-induced reactivation was interpreted to suggest that CRH1 and CRH2 receptors are not the only receptors involved in the relapse to cocaine dependence, and that the CRH system plays different roles in modulating stress- and cocaine-induced relapse to drug dependence. These data point to a role for CRH in the relapse to drug-taking behavior in-

duced by stressors, and suggest that CRH1 antagonists may be useful for the treatment of relapse to several drugs of abuse.

Locomotor Activity and Motor Effects. In-house locomotor activity studies and behavioral observations showed that CP-154,526 at doses up to 32 mg/kg s.c. failed to produce decreases in locomotor activity in non-habituated rats and mice (P. Seymour, unpublished data). Additionally, no stimulant-like effects were observed in habituated rats and mice. Many additional investigators have confirmed these effects and have extended these findings to other tests of motor function as well. Table 5 contains a summary of the effects

TABLE 5. The effects of CP-154,526 on motor functions in rodents

Test/Species	Drug/Dosing	Effects	Comparator/Effect[a]	Reference
Locomotor Activity (Automated Chambers)				
Male SD rats Unhabituated 12 h data collection	CP-154,526 0.32–32 mg/kg 0 min, s.c.	No Effect	None	(Unpublished data)
Male SD rats Overnight habituation 5 h data collection	CP-154,526 0.32–32 mg/kg 0 min, s.c.	No Effect	None	(Unpublished data)
Male Wistar rats 60 min habituation 3 h data collection	CP-154,526 32 mg/kg 0 min, i.p.	No Effect	None	(74)
Male Wistar rats Unhabituated	CP-154,526 2.5–80 mg/kg 30 min, i.p.	No Effect Trend at 80 mg/kg	Chlordiazepoxide/decrease	(131)
Male C57Bl/6 mice Unhabituated 60 min data collection	CP-154,526 3.2–32 mg/kg 0 min, s.c.	No Effect	None	(Unpublished data)
Male C57Bl/6 mice 30 min habituation 60 min data collection	CP-154,526 3.2–32 mg/kg 0 min, s.c.	No Effect	None	(Unpublished data)
Male ICR mice Unhabituated 30 min data collection	CP-154,526 1–100 mg/kg 30 min, p.o.	No Effect	Diazepam/decrease	(142)
Rotarod (Motor Coordination)				
Male ICR mice	CP-154,526 10–100 mg/kg 30 min, p.o.	No Effect	Diazepam/decreased time spent	(142)
Male NMRI mice	CP-154,526 2.5–80 mg/kg, i.p.	No Effect	Chlordiazepoxide/decreased latency to fall	(131)
Miscellaneous Models				
Time on Inclined Plane SD rat pups	CP-154,526 5–40 mg/kg 35 min, i.p.	No Effect	Diazepam/decreased time Buspirone/no effect	(95)
Negative Geotaxis SD rat pups	CP-154,526 5–40 mg/kg 30 min, i.p.	No Effect	Diazepam/increased latency Buspirone/increased latency	(95)

[a] Indicates comparator drug used in the same study and effect observed with the comparator drug.

of CP-154,526 and antalarmin in various tests of motor function in rodents. Without exception, significant effects on motor function have not been reported with these two compounds. Similarly, no effects have been reported with SSR125543A, CRA1000, or DMP904 (50,57,142). Millan et al. (131), however, reported that DMP695 significantly decreased locomotor activity in rats (MED = 10 mg/kg) and decreased the latency to fall in the rotarod test in mice. It has been suggested, therefore, that in comparison to the benzodiazepine anxiolytics this drug class represents an improvement with respect to effects on motor function in the dose range at which desirable behavioral effects are observed. With regard to effects on CRH-induced locomotor activity, studies in our laboratories revealed that i.c.v. administration of CRH dose-dependently increased locomotor activity in habituated rats, with a minimal effective dose of 3.2 μg (P. Seymour, unpublished data). At 56 μg i.c.v., co-administered ah-CRH partially blocked this effect, while CP-154,526 failed to reliably do so after either s.c. or i.c.v. administration, suggesting that the locomotor stimulant effect of CRH may not be CRH1-mediated. Nijsen et al. (140) found that in rats 10 and 25 μg of CP -154,526 (i.c.v.) had no effect on gross locomotor activity (either baseline or CRH-induced changes). A trend toward attenuation (50%) was evident at each dose, but it appeared that high variability precluded statistical significance, very similar to our in-house results. To our knowledge, other CRH1 antagonists have not been tested against this endpoint.

Learning and Memory. The precise role of CRH in cognitive processes remains unclear, but evidence suggests that it plays a modulatory role. Chen et al. (26) found that intra-LC CRH significantly improved retention in a passive avoidance task. CRH, by i.c.v. administration, improved performance in a T-maze task, an effect that was blocked by ah-CRH (102). Similarly, Behan et al. (6, 7) showed that CRH binding protein (CRH-BP) inhibitors elevated brain levels of "free CRH" and improved cognition as well. Zorrilla et al. (205) found that potency in a spatial navigation task was correlated to the ligands' affinities for the CRH-BP. Moreover, improved performance occurred in the absence of anxiogenic-like effects, which occurred at higher doses of the inhibitor and were accompanied by a loss of performance enhancement. Thus, it appears that moderate levels of CRH stimulation positively affect cognitive processes, while high levels may have negative effects.

For the most part, the effects of CP-154,526 and antalarmin on cognitive processes have not been systematically studied. On the one hand, one might expect that CRH1 antagonists may disrupt cognitive processes, since CRH appears to play a facilitatory role. In fact, CRH1 knockout mice showed evidence of impaired spatial recognition memory (29). Several studies suggested, however, that CRH1 antagonists are not disruptive. For example, Okuyama et al. (142) tested the effects of CP-154,526 (10 and 100 mg/kg, p.o.) and CRA1000 in a passive avoidance test in rats. Compounds were administered 30 min prior to the acquisition trial. Neither compound had a significant effect on step-through latencies when tested 24 h later, while diazepam (10–30 mg/kg, p.o.) significantly decreased latencies, suggesting that antagonist activity at CRH1 receptors did not negatively affect retention. Additionally, animals treated with CP-154,526 prior to avoidance training in a learned helplessness (LH) study (see LH section above, 122) did not show the escape deficits observed in the vehicle-treated shocked group and had no effect on acquisition in the non-shocked group. These effects were not, however, replicated by Deak et al. (35), who found that antalarmin had no effect on the escape deficits in a LH study.

Takamori et al. (184) also found that both CP-154,526 and CRA1000 only reversed LH when administered prior to the inescapable shock session. Deak et al. (35) also found that antalarmin totally blocked the induction of contextual fear conditioning, a finding that could reflect either anxiolysis or an amnestic effect. Further studies using the currently available tools are needed to evaluate the effects of CRH1 antagonists on cognitive processes.

Effects on Neurodegeneration

Studies suggest that CRH plays a role in the neurodegeneration induced by ischemia, excitotoxicity and traumatic brain injury (TBI) (115,157,178). In addition, by i.c.v. administration to immature rats CRH has been shown to produce apoptosis in hippocampal CA3 neurons (18). The ischemia- and excitotoxicity-induced effects were blocked by ah-CRH (115,178) and the TBI-induced effects were blocked by d-Phe CRH. The peptidic antagonist, astressin, has also been demonstrated to have a neuroprotective effect on hippocampal cells following kainic-acid-induced seizures (118). Moreover, Mackay et al. (117) found that by i.v. administration the CRH1 antagonist, R121920, afforded significant neuroprotective effects in two rat models of permanent focal ischemia. These results suggest that CRH promotes neurodegeneration and that CRH1 antagonists may have neuroprotective effects.

To our knowledge, only one study documenting a neuroprotective effect of antalarmin has been published. In this study, rat PC12 cells were treated with CRH and apoptosis was measured at several time points (36). CRH (1–10 nM) increased the number of apoptotic cells produced by serum deprivation in a concentration- and time-dependent manner. This effect was completely blocked by antalarmin (10 nM) and ah-CRH (1 μM), suggesting that the proapoptotic effect of CRH was CRH1 receptor-mediated. It was also found that serum deprivation induced the expression of Fas ligand, an effect that was increased by treatment with CRH. The CRH-induced Fas effect was also blocked by antalarmin (10 nM), which had no effect on its own, suggesting that CRH acts via the CRH1 receptor to activate intracellular pathways that lead to Fas ligand induction and apoptosis. CRH also activated ERK1/2 and p38 MAPK and the p38 MAPK inhibitor, SB203580, blocked CRH-induced apoptosis, suggesting that p38 MAPK mediates the proapoptotic effect of CRH. It was concluded that CRH promotes apoptosis in PC12 cells via the CRH1 receptor, which induces Fas ligand production via activation of p38.

In some models, CRH itself has been found to be neuroprotective. Lezoualc'h et al. (106) reported that CRH protected against oxidative insults in primary neuronal cultures and in tumor cell lines. Pedersen et al. (145) found that CRH and UCN were neuroprotective in cultured rat hippocampal neurons (0.5–5 pM) subjected to both oxidative and excitotoxic insults. UCN was 10-fold more potent than CRH, while the CRH2 receptor-selective peptide, UCN II was ineffective. Antalarmin (10 nM) completely blocked the neuroprotective effects of both CRH and UCN, while AS-30 was without effect, suggesting that these effects are CRH1 receptor-mediated. CRH has also been reported to be neuroprotective *in vivo* following hypoxia in rats (47). Thus, the precise roles of CRH and UCN in neurodegeneration remain to be elucidated and await further research using the tools that are now available.

Effects on Autonomic Nervous System

When administered i.c.v. CRH has been shown to produce a marked hypertension response in rats, presumably by acting on central CRH receptors (134). By i.v. administration, however, CRH produces a hypotensive effect (30). This latter effect was blocked by ah-CRH. In our laboratories, CP-154,526 (10 mg/kg s.c. and 5 mg/kg, i.v.), as well as another aminopyrazole CRH1 antagonist, did not reduce either the magnitude or the duration of the CRH-induced hypotension in anesthetized male Sprague–Dawley rats (S. Gonsalves, unpublished data). In agreement with Corder et al. (30) ah-CRH (50 nmol, i.v.) completely blocked this response, suggesting that it may be CRH2-receptor-mediated.

Briscoe et al. (13) found that by i.c.v. administration CRH (1 μg/kg) produced a significant hypertensive response in rats, which lasted for the duration of the 45-min recording session. Antalarmin (i.p.) had no effect alone, but significantly blocked the CRH-induced response. By i.v. administration CRH (10 mg/kg) produced a significant decrease in mean arterial pressure (MAP). Similar to our in-house results with CP-154,526, antalarmin failed to reverse this effect, while i.v. administration of ah-CRH (120 μg/kg) produced a total blockade. It was speculated that the hypertensive effect is mediated via central CRH1 receptors, although the involvement of peripheral receptors could not be ruled out. It was also suggested that the hypotensive effect is mediated via peripheral CRH2 receptors, since ah-CRH has limited access to brain, and antalarmin failed to affect the response. Nijsen et al. (140) found that i.c.v. administration of CP-154,526 (10 and 25 μg) to male Wistar rats had no effect on baseline heart rate or plasma NE and epinephrine (E) levels, but partially blocked i.c.v. CRH (2 μg)-induced increases. It was suggested that endogenous central CRH does not play a role in the tonic regulation of autonomic nervous system activity under resting, low arousal conditions and that centrally administered CRH activates the sympathetic nervous system, at least in part via the CRH1 receptor. The lack of a complete blockade of CRH-induced effects by CP-154,526 suggests that another receptor, such as the CRH2 receptor, may also be involved. Yokotani et al. (202) found that i.c.v. administration of r/hCRH and UCN (0.5, 1.5, and 3 nmol/rat) significantly increased plasma levels of E and NE, with effects at some doses lasting out to the end of the experiment (120 min). By i.v. administration neither peptide had an effect on plasma catecholamine levels. By i.c.v. administration CP-154,526 (1.2 and 2.4 μmol) significantly attenuated the E and NE increases produced by CRH and UCN (1.5 nmol). It was concluded that CRH1 is primarily involved in the CRH- and UCN-induced activation of central sympathetic outflow, but that central CRH2 receptors may also be involved in the UCN-induced activation.

Effects on Immune System/Inflammation

It is well accepted that CRH and stress can suppress immune function. By subacute i.c.v. administration CRH has been shown to reduce lymphocyte proliferation and the phagocytic responses of neutrophils (174). Additionally, the immune system of CRH Tg mice is profoundly suppressed (176). Thus, CRH influences the immune system indirectly, through activation of the HPA axis and sympathetic nervous system. However, it also appears that CRH influences the immune system directly, through local modulatory actions of peripheral CRH, also known as “immune” CRH (41,92). CRH immunoreactivity and mRNA have been demonstrated in human leukocytes (177) and CRH is produced

locally by fibroblasts and endothelial cells in inflamed tissues (91), where it has been postulated to have a proinflammatory role. This is thought to occur via activation of mast cells, which are located perivascularly, close to nerve endings, and which degranulate in response to acute psychological stress (171). However, additional data suggest a possible anti-inflammatory role for CRH (46). Several investigators have used CP-154,526 and antalarmin to study the role of CRH in immunosuppression and inflammation. In a study evaluating the anti-inflammatory properties of CRH1 antagonists, Lundkvist et al. (114) found that CP-154,526 (i.p.) potently reduced the fever response to the endogenous pyrogen, interleukin-1β (i.p.), mimicking the effects of i.c.v. ah-CRH. Webster et al. (196) investigated the anti-inflammatory properties of antalarmin (i.p.), which was found to significantly inhibit the inflammation produced by s.c. carrageenin in rats, as measured by a 36% reduction in the leukocyte concentration of exudates. Suppression was also obtained by polyclonal anti-CRH (27%).

To investigate the proinflammatory properties of CRH, Theoharides et al. (186) tested the ability of CRH to activate skin mast cells and to increase vascular permeability. They found that by intradermal (i.d.) administration in rats CRH (0.1–10 μM) induced mast cell degranulation and increased capillary permeability, as measured by extravasation of Evans blue dye. Pretreatment with antalarmin (10 mg/kg, i.v.) significantly inhibited dye extravasation in response to 1 and 10 μM CRH. No effects were observed vs. substance P-induced plasma extravasation. It was hypothesized that during stress, CRH is released from postganglionic sympathetic nerves and/or peripheral sensory afferents and activates skin mast cells via a CRH1-dependent mechanism, leading to vasodilation and increased vascular permeability. Singh et al. (171) found that by intradermal administration UCN, at concentrations as low an 10 nM, also induced mast cell degranulation and increased vascular permeability. Antalarmin and astressin attenuated the vascular permeability response produced by UCN, but not that produced by Substance P or histamine.

To investigate the role of CRH in inflammatory processes and a possible link between CRH and iNOS, Cantarella et al. (20) studied the effects of CRH on cytokine-induced nitrite production and iNOS protein in murine endothelioma H5V and HUVEC cells, the latter of which solely express CRH2 receptors. It was found that CRH has an inhibitory effect on cytokine-stimulated iNOS protein and nitrite release by H5V cells, but has a stimulatory effect on these events in HUVEC cells. It was also found that H5V cells expressed both CRH1 and CRH2 receptor mRNAs, whereas HUVEC cells expressed only CRH2 mRNA. AS-30, but not CP-154,526 blocked the CRH-induced increase in nitrites and iNOS expression in H5V cells. AS-30 also inhibited these effects in HUVEC cells. It was concluded that CRH has an activating effect on endothelial cells and possibly, an inhibitory role in the late phase of the inflammatory response.

Effects on Gastrointestinal System and Feeding Behavior

There is substantial evidence supporting a role for CRH in stress-induced alterations in gastrointestinal (GI) functioning and feeding behavior. Both central and peripheral activation of CRH signaling pathways have been implicated. By central administration CRH has been shown to decrease feeding behavior (e.g., 146), inhibit gastric emptying (123), and accelerate colonic transit (124), mimicking the effects of stress. Recent studies have shown that by peripheral administration both CRH and UCN produce similar effects (119,141,195). In addition, by peripheral administration peptidic CRH receptor antago-

nists that do not readily gain access to the CNS attenuate stress-induced stimulation of colonic transit (119), supporting a role for peripheral CRH in colonic motility. The presence of functional CRH binding sites in the gut (89), the direct stimulatory action of CRH on intestinal myenteric neurons (64), and the finding that CRH is not transported into the brain from the blood (126) provide support for an initial peripheral site of action for CRH (163). Data showing that CRH applied to colonic tissue *in vitro* increases colonic motility and epithelial ion secretion through CRH receptors (119,121) also support this notion. However, the source of the systemic and/or local intestinal CRH release is presently unclear. CP-154,526, antalarmin, and other CRH1 antagonists have been used extensively as tools to explore the role of CRH and its receptors in stress-induced GI effects and appetite suppression.

With regard to stress-induced suppression of feeding behavior, Wang et al. (195) showed that by i.p. administration CRH and UCN decrease food intake in fasted mice. This effect was blocked by i.p. administration of astressin B and AS-30, but was not blocked by the CRH1 antagonists, CP-154,526 or DMP904 (10 mg/kg, i.p.). NBI -27914 (i.c.v. and p.o.) was also ineffective at blocking the inhibition of feeding behavior produced by i.c.v. CRH in mice (146). Similarly, antalarmin (i.p.) was ineffective at blocking the effects of i.p. CRH and UCN in marsupials (78). Thus, it appears that the suppression of feeding produced by CRH-related peptides and stress may be CRH2 receptor-mediated.

Several studies have implicated a role for CRH2 receptors in CRH-, UCN- and stress-induced inhibition of gastric emptying. Nozu et al. (141) demonstrated that stress and i.v. administration of CRH and UCN inhibited gastric emptying in rats and that these effects were antagonized by astressin (i.v.), but not by antalarmin or NBI-27914 (i.p. and i.v., respectively). Similarly, Million et al. (132) showed that the inhibitory effects of i.v. CRH and stress on gastric emptying were blocked by astressin-2B (s.c.), but not by CP-154,526. In mice, CRH and UCN, by i.p. administration, inhibited gastric emptying. This effect was blocked by astressin and AS-30 (i.p.), but not by CP-154,526 or NBI-27914 (i.p.) (125,195).

In contrast to the inhibitory effects of CRH on gastric emptying, several investigators have provided convincing evidence that CRH-related peptides and stress stimulate colonic motility and that this effect is modulated by CRH1 receptors. Maillot et al. (119) showed that i.p. administration of CRH, rUCN and amphibian sauvagine, and stress stimulate colonic transit in rats, as measured by a dose-dependent stimulation of fecal output. CRH was the most potent of the peptides tested. These effects were antagonized by astressin (i.p.), as well as CP-154,526 (s.c.). Similarly, Million et al. (132) found that the stimulatory effects of i.v. CRH and stress on colonic transit were blocked by CP-154,526 (s.c.), but not by the CRH2 receptor-selective antagonist astressin-2B. The induction of diarrhea by i.v. CRH in rats was also blocked by CP-154,526 (s.c.) (163). In mice, the CRH1 receptor-selective peptides, r/hCRH and oCRH, increased colonic transit, while the CRH2 receptor-selective peptides, mUCN II and mUCN III (i.p.) had no effect. In addition, the effect of CRH was blocked by astressin, CP-154,526 and NBI-27914 (i.p.), but not by AS-30 (i.p.). Thus, it appears that CRH acts peripherally to stimulate colonic motility and that CRH1 receptors play a role in the stimulation of colonic motor function induced by peripheral CRH or acute stress. These results may have clinical relevance since i.v. administration of CRH to humans has been shown to increase colonic contractions in humans and that the colonic motor response to CRH was exacerbated in patients with irritable

bowel syndrome (48). CRH1 antagonists, therefore, may have therapeutic potential in patients with colonic hypersensitivity to stress.

Effects on Reproductive System

CRH is produced in several organs of male and female reproductive systems (e.g., 37,49). Recent studies in which antalarmin has been used as a tool have begun to elucidate the role of CRH1 receptors in several reproductive processes. Data from Makrigiannakis et al. (120) suggest that locally-produced embryonic and endometrial CRH plays a role in both the inflammatory process of embryonic implantation and the anti-rejection process that protects the fetus from the maternal immune system. CRH stimulated the expression of FasL on trophoblast and maternal decidual cells at the maternal-fetal interface, which potentiated the apoptosis of activated T cells. Antalarmin (20 and 50 mg/kg, s.c.) administered b.i.d. to rats on days 1 through 6 of pregnancy, reduced the number of implantation sites and live embryos by 50 and 70%, respectively. This effect did not occur in mothers lacking T cells or after syngeneic matings. Immunohistochemistry showed that in antalarmin-treated animals, the implantation sites were characterized by a marked reduction in FasL expression. When antalarmin was administered from day 5 through the end of pregnancy, there was no difference in the number of animals delivered, body weight and length, or appearance. It was suggested that CRH1 receptor blockade prevents implantation in rats by reducing the inflammatory-like reaction of the endometrium to the invading blastocyst, and that CRH1 antagonists might represent a new class of nonsteroidal inhibitors of early stage pregnancy. It was also suggested that, given the promise of CRH1 antagonists as antidepressants and anxiolytics, their ability to cause hypofertility and early miscarriage should be thoroughly studied. Alternatively, the lack of abortifacient or fetotoxic effects when administered in mid- and late- gestation suggests that CRH1 antagonists could be useful in the protection of the fetus from maternal stress or to prevent premature labor.

In sheep, the fetal release of ACTH produces a cortisol surge, which leads to the onset of parturition. The role of CRH in this event is not clear since other factors such as AVP and estrogens are also known to play a critical role. Chan et al. (23) found that fetal vein infusion of antalarmin in timed-pregnant sheep delayed the onset of parturition. Fetal ACTH and cortisol did not change in the antalarmin-treated sheep after 3 days of infusion, while significant increases in both were observed in vehicle-treated sheep. These data suggest that CRH1 receptor antagonism in the fetus can delay the onset of parturition, and support the hypothesis that hypothalamic CRH is a primary factor in the onset of parturition.

Aggelidou et al. (1) hypothesized that placentally-derived CRH plays a central role in coordinating the transition of the uterus from a relaxed state to one of contraction during labor. Since *in vitro* studies have demonstrated that CRH activates myometrial CRH receptors that are coupled to the adenylate cyclase system only during pregnancy, and nitric oxide is thought to play a role in uterine contractility, the possible interaction between CRH and the nitric oxide/cGMP system was investigated. It was found that human pregnant myometrial cell cultures incubated with CRH, but not UCN II or Ucn III, showed increased expression of eNOS, nNOS, mRNA, and protein and increased guanylate cyclase activity. These effects were blocked by antalarmin and astressin, but not by AS-30, suggesting that they are CRH1 receptor-mediated. Inhibition of PKA activity and acti-

vation of PKC also blocked the effects, leading to the conclusion that during pregnancy, CRH activates intracellular signals that contribute to the maintenance of myometrial quiescence.

SUMMARY AND CONCLUSIONS

Studies with CP-154,526 have shown that it is a potent and selective CRH1 receptor antagonist that readily penetrates the CNS, confirming its utility for the characterization of the effects of CRH and urocortin at CRH1 receptors *in vivo*. Systemically-administered CP-154,526 antagonized stress-induced NE release in brain and the stimulatory effects of exogenous CRH on plasma ACTH levels. It also antagonized the stimulatory effects of exogenous CRH on cell firing in the LC and the dorsal raphe nucleus. Its effects on stress-induced ACTH release, however, appear to be less reliable, suggesting the involvement of other agonists or CRH2 receptors in the endocrine response to stress. As the nonpeptide CRH1 antagonists advance through development it will be important to determine which particular stressful events having deleterious clinical consequences are best addressed by this class of agents.

Testing of CP-154,526 and antalarmin, as well as other CRH1 antagonists, in anxiety assays has yielded mixed results, especially when traditional models such as conflict tests and the EPM test are used. Several factors may contribute to this variability, such as differences among laboratories with respect to behavioral methodologies, compound preparation and compound administration. Additionally, the compounds may be inherently weak as anxiolytics, in contrast to the benzodiazepines (BDZs) which are highly effective and reliably produce robust effects in most laboratories. Alternatively, the models that are traditionally used may actually be BDZ-selective models that are not suitable for the testing of non-BDZ anxiolytics. It has been speculated that basal levels of stress or anxiety may be higher in the studies in which CRH1 antagonists have shown activity, resulting in a greater production of CRH and, therefore, greater efficacy of CRH1 antagonists (e.g., 54). It seems intuitive that the greater the involvement of endogenous CRH the greater the efficacy a CRH receptor antagonist would have. However, the involvement of CRH in the traditional models has not been thoroughly studied and has been largely inferred as a result of efficacy with CRH receptor antagonists. Results appear more promising, however, when anxiogenic-like behaviors are enhanced by CRH administration or by stress treatments.

Results of tests that are more ethological in nature also appear promising, but much of this work has not yet been replicated by other laboratories. In addition, the reliability and predictive validity of some of these models have not been thoroughly investigated. Ultimately, the efficacy of CRH1 antagonists in human anxiety disorders will require clinical testing, which appears justified on the basis of the available preclinical data. It is proposed that only after a clinical determination of efficacy is made can the appropriate preclinical models for this mechanism be identified.

The preclinical antidepressant data available with CP-154,526 and antalarmin are limited and somewhat controversial (e.g., learned helplessness), but appear promising. However, the available clinical data implicating a role for CRH in depression warrant the clinical testing of CRH1 antagonists in depression. Of course, the ultimate proof that CRH hyperactivity contributes to the pathology of depressive disorders still awaits the full ad-

vancement of non-peptidic antagonists that, when dosed orally, can achieve significant blockade of CRH receptors in the brain. Recently a small open label clinical trial with R-121919, a potent and selective CRH1 antagonist, was conducted and significant improvements in both depressive and anxiety symptoms were reported (204). Although this was an open label study and needs to be replicated using a double-blind placebo-controlled design, the findings are encouraging.

Several lines of evidence suggest that CRH1 receptors play a major role in substance abuse and that CRH1 receptor antagonists have therapeutic potential in substance abuse disorders. CP-154,526 reduced cocaine self-administration and reduced cocaine-appropriate responding during cocaine extinction trials, suggesting that CRH1 receptors are involved in the maintenance of cocaine abuse, cocaine cue-induced craving, and cocaine-seeking behavior. CP-154,526 attenuated the signs observed during withdrawal from morphine, suggesting an important role for CRH1 receptors in the opiate abstinence syndrome. In addition, CP-154,526 blocked the stress-induced reinstatement of alcohol, heroin, and cocaine administration, as well as stress-induced reinstatement of morphine-CPP, strong evidence for the potential efficacy of CRH1 antagonists in relapse to drug-taking behavior induced by stress.

The effects of CP-154,526, antalarmin, and CRH1 antagonists in general, on cognitive, neurodegenerative, and inflammatory processes have not been extensively investigated. Although a facilitatory role in cognitive processes has been implicated with CRH, solid evidence for disruption of cognitive processes by CRH1 antagonists has not been demonstrated and it is possible that these agents may actually improve cognitive processes in highly stressful situations. In addition, although one CRH1 antagonist (R121920) has been shown to be neuroprotective *in vivo*, these effects have not yet been replicated, and similar effects have not been demonstrated with other members of the class. CP-154,526 and antalarmin have been reported to possess intriguing antiinflammatory-like properties by a few investigators. The data suggest that peripherally-active CRH1 antagonists may hold promise in inflammatory disorders and in skin disorders that are precipitated or exacerbated by stress, such as psoriasis, atopic dermatitis, urticaria and eczema (186). Research in the GI area has yielded data suggesting that CRH1 receptors are involved in stress-induced colonic motility and that CRH1 antagonists may be useful in irritable bowel syndrome (IBS) and in colonic hypersensitivity to stress. However, additional work is necessary to evaluate therapeutic potential in this area, as well. Finally, the effects of CRH1 antagonists on reproductive function remains largely unexplored, and their effects on these processes will have to be carefully monitored in toxicology studies prior to their widespread therapeutic use.

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Addendum

ah-CRH: α-helical corticotropin-releasing factor (9–41)
AD: antidepressant
AS-30: antisauvagine-30 = [D-Phe^{11},His^{12}]sauvagine (11–40)
CRA1000: 2-(N-(2-methylthio-4-isopropylphenyl)-N-ethylamino-4-(4-(3-fluorophenyl)-1,2,3,6-tetrahydropyridin-1-yl)-6-methylpyrimidine
CRA1001: 2-(N-(2-bromo-4-isopropylphenyl)-N-ethylamino-4-(4-(3-fluorophenyl)-1,2,3,6-tetrahydropyridin-1-yl)-6-methylpyrimidine

DMP 695: N-(2-chloro-4,6-cimethylphenyl)-1-[1-methoxymethyl-(2-methoxyethyl]-6-methyl-1H-1,2,3,triazolo[4,5-c]pyridin-4-amine mesylate
DMP 696: 8-(2,4-dichlorophenyl)-N-[2-methoxy-1-(methoxymethyl)ethyl]-2,7-dimethyl-pyrazolo[1,5-a]-1,3,5-triazin-4-amine
DMP 904: 4-(3-pentylamino)-2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)-pyrazolo-[1,5-a]-pyrimidine
FG-7142: N-methyl-9H-pyrido[3,4-b]indole-3-carboxamide
mUCN: mouse urocortin
mUCN III: mouse urocortin III
NBI 27914: 5-chloro-N-(cyclopropylmethyl)-2-methyl-N-propyl-N′-(2,4,6-trichlorophenyl)-4,6-pyrimidinediamine
R121919 (NBI 30775): 3-phenyl pyrazolo-pyrimidine
R121920: 7-(dipropylamino)-2,5-dimethyl-3-[2–(dimethylamino)-5-pyridyl]pyrazolo[1,5-a]pyrimidine
SB 203580: 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl) imidazole
SSR1: selective serotonin uptake inhibitor
SSR125543A: 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]5-methyl-N-(2-propynl)-1,3-thiazol-2-amine

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