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Antidepressant-like effects of CP-154,526, a selective CRF_1 receptor antagonist

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Abstract

The effects of CP-154,526 (butyl-ethyl-[2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]-amine), a selective corticotropin releasing factor (CRF_1) receptor antagonist, were examined in the learned helplessness procedure, a putative model of depression with documented sensitivity to diverse classes of antidepressant drugs. Rats were exposed to a series of inescapable foot shocks on three consecutive days and tested in a shock-escape procedure on the fourth day. Animals exposed to 'helplessness' training performed poorly in the shock-escape test compared with control animals not receiving inescapable shocks. CP-154,526 (10–32 mg/kg, intraperitoneally) dose-dependently reversed the escape deficit when administered 60 min prior to the test session, but had no effect on the performance of control rats not receiving prior exposure to inescapable stress. In comparison, the tricyclic antidepressant imipramine (17.8 mg/kg) reversed the escape deficit after repeated, but not acute, administration. These data support evidence implicating stress systems in the pathophysiology of depression, and suggest potential efficacy of small-molecule CRF receptor antagonists in the treatment of affective disorders.

Keywords: CRF (Corticotropin-releasing factor); Depression; Learned helplessness; CP-154,526; (Rat)

1. Introduction

Stress has long been recognized as an etiological factor in depressive disorders (Anisman and Zacharko, 1982). Corticotropin releasing factor (CRF), a 41-amino-acid neuropeptide synthesized in the hypothalamus, is regarded as the primary mediator of behavioral as well as physiological responses to chronic stress (Owens and Nemeroff, 1991). Intracerebroventricular administration of CRF to laboratory animals produces behavioral effects similar to those observed in depression, such as diminished food intake, decreased sexual activity, and disturbed sleep patterns (Dunn and Berridge, 1990; Owens and Nemeroff, 1991). In depressed patients, several studies have reported elevated levels of CRF in cerebrospinal fluid, which then return to normal after successful therapy with antidepressive drugs or electroconvulsive shock (Nemeroff et al., 1984, 1991; Arato et al., 1986). In addition, some depressed patients display a blunted adrenocorticotropic hormone (ACTH) response to intravenously administered CRF, possibly due to downregulated CRF receptor populations at the level of the pituitary (e.g., Holsboer et al., 1984). Taken together, these data suggest that hypersecretion of CRF in response to chronic stress may be a key contributing factor in depression, and that blockade of CRF receptors in the central nervous system (CNS) may be useful in treating this disorder.

Specific peptide antagonists of CRF have been available for some time, and these molecules have been shown to reverse many of the behavioral effects of CRF (see review by Koob et al., 1993). However, peptide-based CRF receptor antagonists would be of limited clinical value because they do not penetrate the blood-brain barrier in significant quantities. Recently, we reported on the characterization of CP-154,526 (butyl-[2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]-ethyl-amine), a small-molecule CRF receptor antagonist with selectivity for the CRF_1 subtype (Schulz et al., 1996). This antagonist is readily absorbed by oral or parenteral routes, and displaces CRF from sites in both the CNS and pituitary. CP-154,526 reverses CRF-elicited increases in ACTH, prevents CRF-induced elevations in locus coeruleus cell firing, and blocks both CRF-enhanced and fear-potentiated

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startle responses in rats (Schulz et al., 1996). The latter result may indicate potential anxiolytic activity for CRF receptor antagonists, as the potentiated startle model has been shown to selectively identify compounds with anxiolytic effects in humans (Davis, 1992). However, the effects of CRF receptor antagonists remain largely unexplored in animal models recognized for their ability to detect efficacious antidepressants.

The 'learned helplessness' procedure (Maier and Seligman, 1976) has been described as a model of depression, in that it satisfies some criteria for face, predictive and construct validity (Wilner, 1984). In this procedure, animals are exposed to uncontrollable stressors and subsequently tested for acquisition of a learned response, typically avoidance or escape behavior from a noxious stimulus. Animals pre-exposed to uncontrollable stress display acquisition deficits that are prevented by a number of antidepressant medications and by electroconvulsive treatment (Sherman et al., 1982). 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 (Petty and Sherman, 1979). Because one common effect of antidepressants is a delayed downreguation of the hypothalamic-pituitary-adrenal axis (Brady, 1994), administration of a CRF receptor antagonist may represent a means to shorten the onset of therapeutic activity. Thus, the present study examined the effects of acutely administered CP-154,526 in comparison with the tricyclic antidepressant, imipramine. Positive results with CP-154,526 would indicate potential antidepressant activity of small-molecule CRF receptor antagonists, and would support the hypothalamic-pituitary-adrenal axis and/or sympathetic output systems influenced by CRF as a final common pathway in mediating the beneficial effects of several classes of antidepressants.

2. Materials and methods

2.1. Subjects

Adult male Sprague-Dawley rats (Charles River), weighing 300–350 g and aged 10–12 weeks at the time of testing, were used as subjects. Rats were housed in individual stainless-steel cages with continuous access to water and lab chow in an environmentally controlled animal facility (lights on 7:00 a.m.–7:00 p.m.). Behavioral experiments were begun after one week of acclimation to the animal facility.

2.2. Apparatus

All experimental manipulations were conducted in standard operant test chambers (BRS/LVE, Laurel, MD, USA) equipped with a grid floor, house light and two response levers. The chamber measured 30 cm in length by 25 cm in width, and each lever was mounted 5 cm above the floor, on opposite sides of the chamber's front panel. Levers were present during both conditioning and testing sessions. With the exception of time-out periods, all sessions were accompanied by illumination with a single 28 V, 0.1 A lamp anchored near the chamber's ceiling. Chambers were housed within ventilated, sound-attenuating cubicles (MED Associates, Georgia, VT, USA). Scheduling of stimuli and recording of responses was accomplished by a computer and associated interfacing hardware (MED Associates). Scrambled shock was delivered by programmable a.c. shockers (model E13-14, Coulbourn Instruments, Allentown PA, USA).

2.3. Procedure

We used a modified version of the learned helplessness procedure reported by Henn et al. (1985). The protocol was approved by the Institutional Animal Care and Use Committee at Pfizer Central Research, and is consistent with the Guide for the Care and Use of Laboratory Animals as promulgated by the National Institutes of Health. The first stage of testing consisted of exposure to fifty 1 s, 0.8 mA shocks, administered at irregular intervals over approximately 25 min. Subjects had no opportunity to terminate or avoid the shocks. These sessions took place on each of three consecutive days. Control animals were placed in the test cages for the same amount of time each day, but no shocks were delivered. 24 h following the final session of inescapable shocks, all animals were exposed to shock-escape testing, in which shocks could be terminated by a single response on either of the two response levers. Shocks were presented as 20 discrete trials; each trial began with the simultaneous onset of 0.8 mA scrambled shock, which cycled on and off every 0.35 s, and the illumination of the house light. If a response was not made within 45 s, the shock was terminated and the houselight extinguished. Recorded were 'successes', trials in which shock was terminated with a latency of less than 20 s; 'no-responses', those which were automatically terminated by the program, and mean response latency. A latency of 45 s was assigned to 'no-response' trials. A 24 s time-out, during which chamber illumination was extinguished, separated each trial from the next. For the various drug treatment conditions, order of dosing and test chambers were counterbalanced.

2.4. Drugs

For experiments examining the effects of acutely administered CP-154,526 HCl or imipramine HCl, single drug injections were administered prior to shock escape sessions only. For studies of repeated imipramine administration, drug injections were administered immediately following each session of inescapable shock, and 60 min prior to shock escape testing, for a total of four daily

injections. CP-154,526 was synthesized at Pfizer Central Research. It was suspended in 0.1% methylcellulose (pH 5) and injected intraperitoneally 60 min prior to shock escape sessions. Imipramine HCl was obtained from Sigma (St. Louis, MO, USA). It was dissolved in water and injected subcutaneously. All injections were administered in a volume of 1 ml/kg. Doses of CP-154,526 were calculated as the free base; imipramine doses refer to the salt.

3. Results

3.1. Effects of CP-154,526

Fig. 1 depicts the effects of acutely administered CP-154,526 on shock-escape performance. Because of capacity limitations, the drug was evaluated over successive experiments, each of which examined one dose. After determining that the control performances did not differ significantly across experiments, the results for all three studies were pooled for analysis. At 10 mg/kg, there was no effect on escape successes, but at 17.8 mg/kg a partial restoration of escape behavior was observed, and at 32 mg/kg CP-154,526 fully blocked the effects of prior exposure to inescapable shock. A two-way analysis of variance (ANOVA) with drug treatment and shock pre-exposure as factors revealed a significant effect of drug, $F(3,208) = 4.2$, $P = 0.006$, a significant effect of inescapable shock, $F(1,208) = 31.5$, $P = 0.0001$, and a significant drug-by-shock interaction, $F(3,208) = 3.7$, $P = 0.01$. The significant interaction, which indicated reversal of escape deficits by CP-154,526, was followed by one-way analyses of pre-shocked and non-pre-shocked treatment groups. In the pre-shocked group, there was a signficant effect of dose, $F(3,104) = 7.4$, $P = 0.0002$. Post-hoc Dunnett's tests using the vehicle group as the control revealed a significant effect of the 32 mg/kg dose. A one-way analysis of non-shocked groups confirmed that there was no effect of CP-154,526 on escape successes in the absence of inescapable shock pre-exposure, $F(3,104) = 0.2$. Similar analyses conducted with the other dependent variables resulted in nearly identical findings in this and subsequent experiments (data not shown).

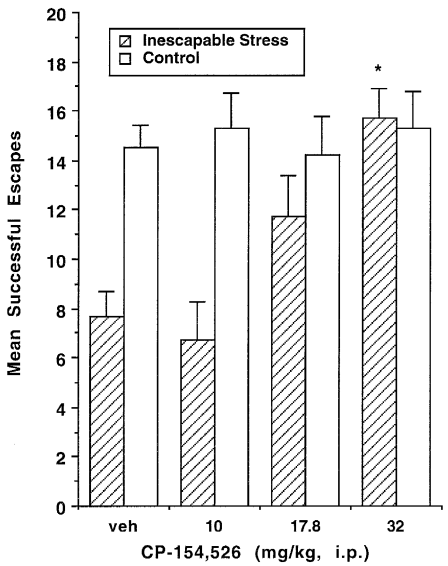

Fig. 1. Effects of CP-154,526 (±S.E.M.) on shock-escape performances in rats with or without previous exposure to inescapable shock. Shown are the mean number of successful escapes (latency of less than 20 s) out of a total of 20 trials. Asterisk indicates significant increase in escape success vs. vehicle controls following significant ANOVA ($n = 18–54$).

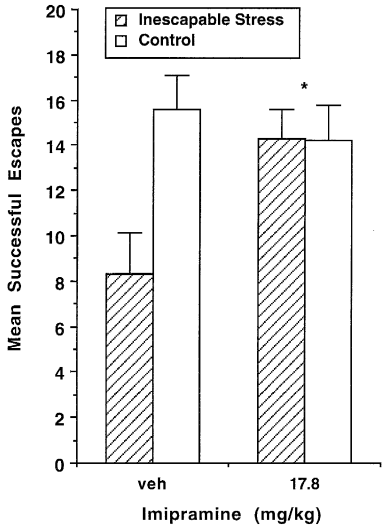

Fig. 2. Effects of repeated administration of imipramine (17.8 mg/kg) on shock-escape performance. Shown are the mean number of successful escapes (latency of less than 20 s) out of a total of 20 trials. Asterisk indicates significant reversal of the escape deficit as demonstrated by significant drug-by-shock interaction ($n = 18$).

3.2. Effects of imipramine

The effects of repeated injections of 17.8 mg/kg imipramine on escape successes are illustrated in Fig. 2. There was a significant main effect of shock pretreatment, $F(1,68) = 5.2$, $P = 0.02$, no main effect of drug, and a significant drug-by-shock-pretreatment interaction, $F(1,68) = 5.6$, $P = 0.02$, indicating a reversal of the escape deficit by imipramine.

The effects of acutely administered imipramine, at doses of 17.8 and 32 mg/kg, are shown in Fig. 3. As in experiments with CP-154,526, results from the control groups did not differ between the two single-dose experiments. At 17.8 mg/kg there was no effect of imipramine in either group; at 32 mg/kg there was a slight increase in successes in the group pre-exposed to inescapable shock, but this was accompanied by a decrease in success rate in the control group. These latter results may have been the result of marked sedation noted in animals treated with 32 mg/kg imipramine. Two-way ANOVA confirmed the

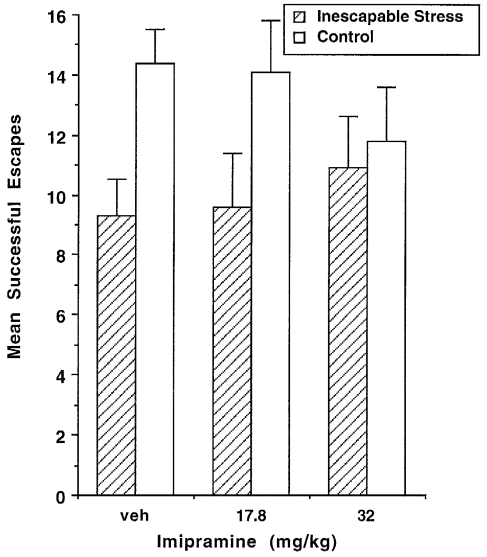

Fig. 3. Effects of acute administration of imipramine on shock-escape performance. Shown are the mean number of successful escapes (latency of less than 20 s) out of a total of 20 trials ($n = 18–36$).

presence of a significant main effect of inescapable shock, $F(1,138) = 8.9$, $P = 0.003$, but there was no main effect of drug or a drug-by-shock-pretreatment interaction.

4. Discussion

The present results demonstrate that the selective CRF_1 antagonist CP-154,526 is effective in an animal model shown to be predictive of antidepressant activity in humans. The effects of CP-154,526 in this procedure were not likely 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 were not affected by the drug. CP-154,526 reversed escape deficits in the same dose range (10–32 mg/kg) previously reported to be effective against the startle-enhancing and ACTH-enhancing effects of CRF in the rat (Schulz et al., 1996), suggesting that the effects on learned helplessness are mediated by CRF receptors in the CNS. Moreover, CP-154,526 does not bind appreciably to over 40 other receptors tested (Schulz et al., 1996).

One particularly noteworthy aspect of the antidepressant-like effect of CP-154,526 is its acute efficacy in the learned helplessness model. As reported previously, the tricyclic antidepressant imipramine was not effective upon acute treatment, but required multiple injections before a reversal of escape deficits was observed (Petty and Sherman, 1979). Activity in the model was observed only after 3–4 days of treatment with imipramine, a finding subequently confirmed with nortriptyline (Telner et al., 1981). Other studies have also demonstrated activity of diverse classes of antidepressants after 4–7 days of treatment (Leshner et al., 1979; Sherman et al., 1982; Henn et al., 1985). Although the present experiments do not represent true chronic treatment (i.e., > 2 weeks) with antidepressants, our observations are consistent with the delayed therapeutic action of antidepressive drugs in patients, and substantial evidence exists to suggest that these drugs are effective only after modifying the number or sensitivity of biogenic amine receptors (e.g., Blier et al., 1987; Riva and Creese, 1989).

With respect to stress systems, the locus coeruleus has been shown be be a key mediator of neurogenic responses to stress (Valentino et al., 1993). The activity of noradrenergic neurons in the locus coeruleus is greatly increased by stress, resulting in elevated sympathetic outflow and enhanced arousal. The locus coeruleus is also rich in CRF immunoreactivity (Swanson et al., 1983), and stress- or CRF-induced increases in locus coeruleus activity are blocked by CRF receptor antagonists (Valentino et al., 1983; Valentino and Wehby, 1988). Tricyclic antidepressants acutely elevate CNS levels of norepinephrine and decrease locus coeruleus cell firing, but after chronic administration have been reported to decrease locus coeruleus firing, norepinephrine levels and activity of the catacholamine biosynthetic enzyme, tyrosine hydroxylase (Brady, 1994; Roffler-Tarlov et al., 1973). However, a few reports have suggested increases in noradrenergic activity following chronic antidepressant treatment, as measured by extracellular norepinephrine and changes in α_2-adrenoceptor density (e.g., Tanda et al., 1996; Jimenez-Rivera et al., 1995). Decreases in noradrenergic activity may be the result of a compensatory response to antidepressant administration, and are opposite to the effects of chronic stress on the locus coeruleus-noradrenergic system (Brady, 1994). Animals displaying 'helpless' behavior following inescapable stress display exaggerated release of hippocampal norepinephrine when a mild stressor is subsequently applied (Petty et al., 1994), further implicating noradrenergic pathways in the effects of uncontrollable stress. Thus, behavioral deficits observed in animals following inescapable stressors could be due to a conditioned increase in activity of the locus coeruleus-noradrenergic system, which could in turn be acutely terminated by a CRF receptor antagonist.

Chronic stress also increases the activity of the hypothalamic-pituitary-adrenal system, leading to a decrease in hypothalamic CRF concentration, possibly as a result of hypersecretion (Chappell et al., 1986). Administration of imipramine, the monoamine oxidase inhibitor phenelzine or the selective serotonin reuptake inhibitor (SSRI) fluoxetine decreased CRF mRNA expression in the paraventricular nucleus, the site from which CRF is released into the hypophyseal portal circulation, but this effect was only observed after long-term (8 weeks) administration (Brady et al., 1991, 1992). Moreover, imipramine has been shown to prevent stress-induced increases in hypothalamic CRF mRNA (Lopez et al., 1994). Taken together with evidence from the locus coeruleus-noradrenergic system, these data

suggest a convergent role for CRF-influenced stress systems in depression and its treatment by drugs with varying mechanisms of action. Because norepinephrine can stimulate the release of hypothalamic CRF, it has been suggested that locus coeruleus-noradrenergic and CRF-hypothalamic-pituitary-adrenal pathways may mutually reinforce one another (Southwick et al., 1992). CRF, therefore, may represent a final common pathway for the therapeutic effects of antidepressants, many of which appear to produce a functional diminution of CRF activity in the brain. By accomplishing this acutely, a CRF receptor antagonist might produce antidepressant effects with more rapid onset. Through their actions on systems thought to be important to physiological and behavioral responses to chronic stress, CRF receptor antagonists may also be found to have therapeutic effects in a number of psychiatric and psychosomatic disorders, such as anorexia nervosa, post-traumatic stress disorder (Southwick et al., 1992) and irritable bowel syndrome (Friedman, 1991; Monnikes et al., 1993).

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