ELSEVIER

Pharmacology, Biochemistry and Behavior 69 (2001) 445–449

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Involvement of the hypothalamus–pituitary–adrenal axis in antidepressant activity of corticotropin-releasing factor subtype 1 receptor antagonists in the rat learned helplessness test

Kazuaki Takamori*, Naoya Kawashima, Shigeyuki Chaki, Atsuro Nakazato, Kazuya Kameo

CNS Diseases Research, Laboratory-1, Medicinal Research Laboratories, Taisho Pharmaceutical Co., Ltd., 1-403 Yoshino-cho, Saitama, Saitama 330-8530, Japan

Received 31 October 2000; received in revised form 27 February 2001; accepted 7 March 2001

Abstract

Effects of corticotropin-releasing factor (CRF) subtype 1 receptor antagonists on learned helplessness (LH) were examined in rats. Repeated administration of CRF_1 receptor antagonists, CRA1000 (3 mg/kg, po) and CP-154,526 (10 mg/kg, po), and tricyclic antidepressant, imipramine (10 mg/kg, po), for 8 days significantly decreased the number of escape failures in LH. On the other hand, acute treatment of adrenocorticotropin (ACTH) abolished the decreased number of escape failures seen with imipramine. Likewise, in this ACTH model, the CRA1000- and CP-154,526-induced decrease in the number of escape failures was no longer observed. The CRF_1 receptor is apparently involved in the produced escape failures in LH, and the attenuated LH seen with CRF_1 receptor antagonists was abolished by ACTH. It would thus appear that the attenuated LH seen with CRF_1 receptor antagonists depends on the hypothalamus–pituitary–adrenal (HPA) axis.

Keywords: ACTH; CP-154,526; CRA1000; CRF; HPA; Learned helplessness

1. Introduction

Corticotropin-releasing factor (CRF) is a 41-amino acid peptide that regulates the release of adrenocorticotropin (ACTH) from the anterior pituitary (Chen et al., 1993). In depressive subjects, there are elevated CRF concentrations in the cerebrospinal fluid, thus, indicating that hypersecretion of CRF may be related to stress (Darnell et al., 1994; Nemeroff et al., 1984). The CRF receptor has been divided into two major subtypes, termed CRF_1 and CRF_2, as based on molecular cloning techniques (Chen et al., 1993; Perrin et al., 1995) and CRF_2 has the isoforms, $CRF_{2\alpha}$ and $CRF_{2\beta}$ (Chalmers et al., 1995; Lovenberg et al., 1995).

Expression of CRF_1 mRNA in the hypothalamic PVN was found to be increased under various kinds of stress (Luo et al., 1994; Makino et al., 1995). Stress-induced increase in CRF_1 mRNA levels in the PVN corresponded to the increase in CRF binding (Luo et al., 1994), but not $CRF_{2\alpha}$ mRNA levels in the PVN (Makino et al., 1997). Thus, the CRF_1 receptor may be involved in situations involving stress.

Acute treatment with specific nonpeptide antagonists for the CRF_1 receptor can reverse many behavioral effects of CRF or stress, such as CRF-induced increased anxiety, as noted in an elevated plus-maze (Okuyama et al., 1999), swim stress-induced reduction of the time spent in the light area in the light/dark exploration task (Okuyama et al., 1999), emotional stress-induced inhibition of feeding behavior and increase in locomotor activity (Hotta et al., 1999) and inescapable shock-induced increase learned helplessness (LH) (Mansbach et al., 1997). Therefore, we asked if the CRF_1 receptor might mediate, at least in part, the stress model of anxiety and depression-like behavior.

Nakagawa et al. (1996, 1999) reported that LH is widely used as a depression model since it is sensitive to agents with a variety of antidepressant properties. In addition, other authors reported that acute treatment with a CRF_1 receptor antagonist attenuated LH (Darnell et al., 1994). Therefore, the CRF system may be involved in the LH induced by inescapable shocks. In rats given stress in LH,

* Corresponding author. Tel.: +81-48-663-1111; fax: +81-48-652-7254.

E-mail address: s17525@ccm.taisho.co.jp (K. Takamori).

0091-3057/01/$ – see front matter
PII: S0091-3057(01)00515-9

Table 1
Effects of ACTH on imipramine-induced decreased number of escape failures in the rat LH test

Drug	IS	Number of escape failures
Vehicle		
+ Vehicle	−	5.2 ± 1.8
+ Vehicle	+	24.3 ± 2.9[a]
+ Imipramine	+	5.1 ± 1.6[b]
ACTH		
+ Vehicle	+	22.8 ± 3.5
+ Imipramine	+	19.2 ± 4.4[c]

Imipramine (10 mg/kg, po) was administered once daily for 8 days. ACTH (100 μg/rat, ip) was injected after the last administration of imipramine on Day 8. Data are expressed as means with S.E. ($n = 15$). IS: inescapable shocks.

[a] $P < .01$ vs. ACTH 0 μg/rat + imipramine 0 mg/kg + nonshock group (Mann–Whitney U test).

[b] $P < .01$ vs. ACTH 0 μg/rat + imipramine 0 mg/kg + shock group (Mann–Whitney U test).

[c] $P < .01$ vs. ACTH 0 μg/rat + imipramine 10 mg/kg + shock group (Mann–Whitney U test).

these were increases in concentrations of ACTH and corticosterone in the serum, thus, indicating that LH was closely related to a hyperactivated hypothalamus–pituitary–adrenal (HPA) axis.

We examined the effects of repeated treatment of CRF_1 receptor antagonists, CRA1000 and CP-154,526 on the number of escape failures in LH; and effects of exogenous injection of ACTH on the decreased number of escape failures seen with CRF_1 receptor antagonists were observed. We report here the involvement of the HPA axis on the antidepressant activity of CRF_1 receptor antagonists in LH.

2. Method

Male Wistar rats (Charles River, Japan), weighing 180–220 g at the start of the experiments, were housed in groups of five per cage, under standard conditions; room temperature 23 ± 3°C; light–dark cycle (light phase 07:15–19:15 hours). The rats had free access to food and water except during the experiment.

This study reported here have been reviewed by Taisho Pharmaceutical Animal Care Committee and have met the Japanese Experimental Animal Research Association standards as defined Guidelines for Animal Experiments (1987).

LH in the shuttle box test was carried out according to Nakagawa et al. (1996, 1999) as a model of behavioral despair. Briefly, the two-way shuttle box (56 × 21 × 25 cm; Muromachi-Kikai, Japan) was divided into equal-sized chambers with the use of a steel divider. Floors of the chambers in the shuttle box consisted of stainless-steel rods. Scrambled shocks were delivered through a shock generator (SGS-001; Muromachi-Kikai).

Rats were given imipramine, CRA1000 or CP-154,526 orally once daily for 8 days. ACTH was immediately injected (ip) after the last imipramine, CRA1000 or CP-154,526 administration on Day 8. On Day 8, the rats were individually placed in the chamber and given 90 inescapable shocks (1.8 mA) of 10-s durations at 2-s intervals. Control rats were not given shocks. Immediately after the shock pretreatment session, the rats were given imipramine, CRA1000 or CP-154,526 orally. On Day 9 (24 h after the final imipramine, CRA1000 or CP-154,526 treatment), the rats underwent the 40-trial escape test. The animals were individually placed in the shuttle box and given a 5-min adaptation period. A tone signal was given during the first 5 s of each trial. If there was no avoidance response within this period, the tone signal remained on and a 1.8-mA shock (10-s duration) was delivered through the grid floor. In case of a no escape response within this period, both the tone

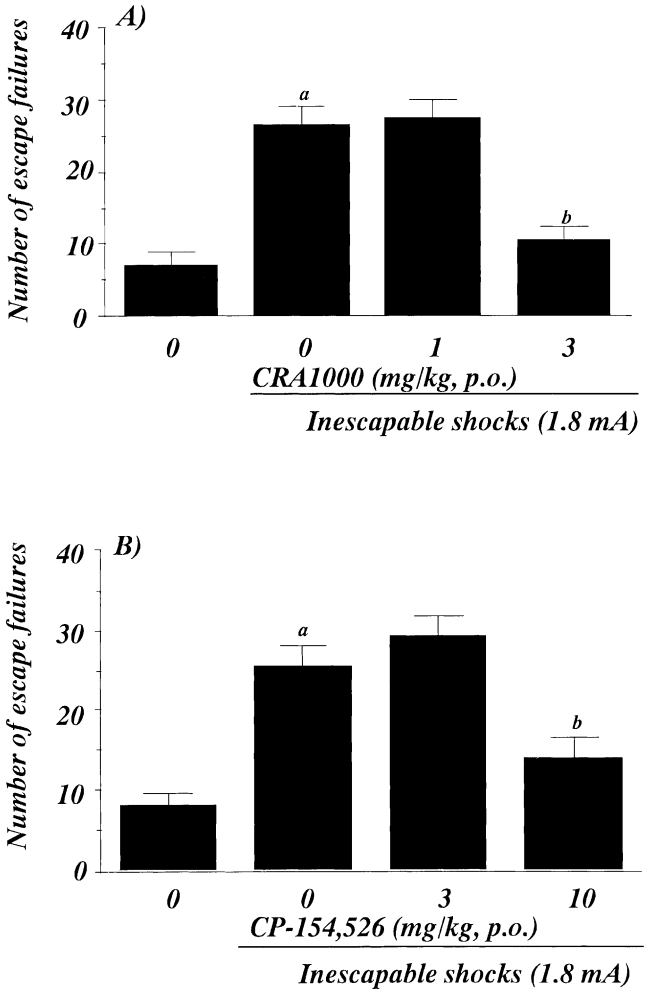

Fig. 1. Effects CRA1000 (A) and CP-154,526 (B) on escape failures in the LH test in rats. CRA1000 and CP-154,526 were administered orally once daily for 8 days. On Day 8, rats were given 90 inescapable shocks. On Day 9, rats were subjected to the 40-trial escape test. Data are expressed as means with S.E. ($n = 15$). (a) $P < .01$ vs. CRA1000 0 mg/kg or CP-154,526 0 mg/kg + nonshock group (Mann–Whitney U test). (b) $P < .01$, CRA1000 0 mg/kg or CP-154,526 0 mg/kg + shock group vs. CRA1000 1, 3 mg/kg or CP-154,526 3, 10 mg/kg + shock groups (Steel test).

signal and shock were automatically terminated. The inter-trial interval was 5s. We recorded the number of escape failures, which refers to a noncrossing response during the shock delivery.

CRA1000 hydrochloride and CP-154,526 hydrochloride were synthesized in the laboratories of Taisho Pharmaceutical (Saitama, Japan). Imipramine (Wako, Japan), CRA1000 and CP-154,526 were suspended in 0.3% Tween-80 solution. These drugs were administered orally in a volume of 2 ml/kg body weight. ACTH-(1–24)-Zinc (ACTH; Cortrosyn-Z; Daiichi Seiyaku, Japan) was given intraperitoneally in a volume of 0.2 ml/rat.

Data were expressed as the mean number of escape failures (±S.E.) recorded over 40 trials during each shuttle box session. A comparison between two groups was made using the Mann–Whitney *U* test. Between-groups comparisons were assessed using the Steel test. The level of statistical significance in each analysis was $P < .05$.

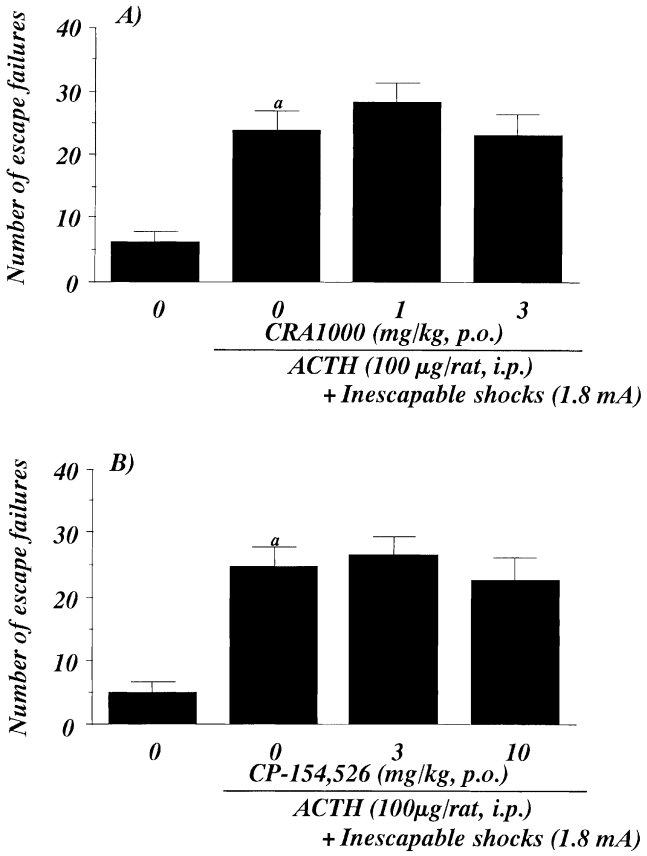

Fig. 2. Effects of ACTH on CRA1000- or CP-154,526-induced the decreased number of escape failures in the rat LH test. CRA1000 or CP-154,526 was administered once daily for 8 days. ACTH was injected after the last administration of CRA1000 or CP-154,526 on Day 8. On Day 8, rats given 90 inescapable shocks. On Day 9, rats were subjected to the 40-trial escape test. Data are expressed as means with S.E. ($n = 15$). (a) $P < .01$ vs. ACTH 0 μg/rat + CRA1000 0 mg/kg or CP-154,526 0 mg/kg + non-shock group (Mann–Whitney *U* test). There was no significant difference between ACTH 0 μg/rat + CRA1000 0 mg/kg or CP-154,526 0 mg/kg + shock group and ACTH 100 μg/kg + CRA1000, 3 mg/kg or CP-154,5263, 10 mg/kg + shock group (Steel test).

3. Results

Repeated administration of imipramine 10 mg/kg (po) significantly decreased the number of escape failures in the LH ($P < .01$; Table 1). On the other hand, acute treatment of ACTH (100 μg/rat, ip) abolished the decreased number of escape failures seen with imipramine (10 mg/kg; $P < .01$). ACTH given alone did not affect the number of escape failures in the control group in case of shock. In addition, ACTH given alone did not affect the number of escape failures in the c control group in case of nonshock. The number of escape failures of nonshock group in ACTH-treated was 5.7 ± 2.1 ($n = 15$).

Repeated administration of CRA1000 3 mg/kg (po) significantly decreased the number of escape failures in the LH ($P < .01$; Fig. 1A). On the other hand, ACTH (100 μg/rat, ip) abolished the decreased number of escape failures seen with CRA1000 (3 mg/kg; $P < .01$; Fig. 2A). ACTH given alone did not affect the number of escape failures in the control group in case of shock (Fig. 2A).

As shown in Fig. 1B, CP-154,526 10 mg/kg (po) significantly decreased the number of escape failures ($P < .01$). In contrast, ACTH (100 μg/rat, ip) abolished the decreased number of escape failures seen with CP-154,526 (10 mg/kg; Fig. 2B). ACTH given alone did not affect the number of escape failures in the control group in case of shock (Fig. 2B).

4. Discussion

In the present study, the rats exposed to the inescapable shocks showed a subsequent increase in escape failures in the escape test. Repeated treatment with imipramine decreased the increased number of escape failures induced by the inescapable shocks (Table. 1). Therefore, our present results were in agreement with reported data (Martin et al., 1987; Sherman and Petty, 1982; Sherman et al., 1982) and confirmed that antidepressants are effective in the LH paradigm.

We found here that repeated treatment of CRA1000 (Okuyama et al., 1999) and CP-154,526 (Schulz et al., 1996) decreased the number of escape failures (Fig. 1). Our present findings agreed with other reports that acute treatment of CP-154,526 attenuated escape failure by inescapable shocks (Mansbach et al., 1997). These results suggest that CRF_1 receptors are involved in the escape failures related to inescapable shocks.

Our present findings support the widely accepted data from behavioral studies. Specific nonpeptide CRF_1 receptor antagonists reversed many behavioral effects, CRF-enhanced and fear-potentiated acoustic startle (Schulz et

al., 1996). In addition, such antagonists showed anxiolytic-like activity in the elevated plus-maze test (Lundkvist et al., 1996; Okuyama et al., 1999) or antidepressive-like activity in the LH test (Mansbach et al., 1997) in olfactory bulbectomized-induced hyperemotion (Okuyama et al., 1999) in rats. These observations suggest that the brain CRF_1 receptor plays an important role in integrating stress responses, such as neuroendocrine-related ones and behavior.

CRF is involved in alteration of neuroendocrine systems responding to stress through activation of the HPA axis (River and Plotsky, 1986; Schulz et al., 1996). Secretion of ACTH from the pituitary gland has been thought to be the trigger eliciting physiological functions mediated by CRF (River and Plotsky, 1986; Schulz et al., 1996). In laboratory animals, helplessness behavior was seen to be sensitive to corticosterone (Papolos et al., 1993), thereby indicating that the HPA axis is functionally involved in the LH. These observations, in combination with our present findings, suggest that the antidepressant activity of CRF_1 receptor antagonists in LH may be mediated through the HPA axis. Our present data support this hypothesis.

We found here that repeated treatment with CRA1000 and CP-154,526-led to a decreased number of escape failures, which was abolished by exogenously injected ACTH. These results indicate that the antidepressant activity of CRF_1 receptor antagonists is mediated through the HPA axis in LH. Furthermore, exogenously injected ACTH attenuated the imipramine-induced reduced duration of immobility time in FS (Kitamura et al., 1997). Our present result is in agreement with the above report. We found that ACTH abolished the decreased number of escape failures of imipramine in LH (Table 1). It was clear that the antidepressant activity, not only of the CRF_1 receptor antagonists but also imipramine, depends on the HPA axis.

It is widely accepted that repeated treatment of imipramine decreased CRF concentration in rat brain (Fadda et al., 1995) and increased CRF binding in rat brain (Grigoriadis et al., 1989). Because norepinephrine can stimulate the release of CRF, the change in CRF contents may represent a final common pathway for antidepressants to expert therapeutic effects.

In summary, the CRF_1 receptor is apparently involved in the resultant escape failures in LH, and the attenuated LH seen with CRF_1 receptor antagonists was abolished by ACTH. In conclusion, the attenuated LH seen with CRF_1 receptor antagonists apparently depends on the HPA axis.

References

Chalmers DT, Lovenberg TW, De Souza EB. Localizaton of novel corticotropin-releasing factor (CRF_2) mRNA expression to specific subcortical nuclei in rat brain: comparison with CRF_1 mRNA expression. J Neurosci 1995;15:6340–50.

Chen R, Lewis KA, Perrin MH, Vale WW. Expression cloning of human corticotropin-releasing factor receptor. Proc Natl Acad Sci USA 1993; 90:8967–9871.

Darnell A, Bremner JD, Licinio J, Krystal J, Nemeroff CB, Owens M, Erdos J, Charnev DS. CSF levels of corticotropin-releasing factor in chronic post-traumatic stress disorder. Soc Neurosci Abstr 1994;20:17.

Fadda P, Pani L, Porcella A, Fratta W. Chronic imipramine, L-sulpiride and mianserin decreased corticotropin releasing factor revels in the rat brain. Neurosci Lett 1995;192:121–3.

Grigoriadis DE, Pearsall D, De Souza EB. Effects of chronic antideressamt and benzodiazepine treatment on corticotropin-releasing factor receptors in rat brain and pituitary. Neuropsychopharmacology 1989;2:53–60.

Hotta M, Shibasaki T, Arai K, Demura H. Corticotropin-releasing factor receptor type 1 mediates emotional stress-induced inhibition of food intake and behavioral changes in rats. Brain Res 1999;823:221–5.

Kitamura Y, Mochizuki D, Hashimoto S, Ikuhara T, Kogami Y, Sagai H, Yamamoto S. Pharmacological profile of AZ-16596, a novel 5-HT_{1A} receptor full agonist: 2. Pharmacological characterization. Jpn J Pharmacol 1997 (Suppl 73) 206 pp.

Lovenberg TW, Chalmers DT, Liu C, De Souza EB. CRF2α and CRF2β receptor mRNAs are differentially distributed between the rat central nervous system and peripheral tissues. Endocrinology 1995;136:4139–42.

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

Luo Y, Kiss A, Makara G, Lolait SJ, Aguilera G. Stress-specific regulation of corticotropin-releasing hormone receptor expression in the paraventricular and supraoptic nuclei of the hypothalamus in the rat. J Neuroendocrinol 1994;6:689–96.

Makino S, Schulkin J, Smith MA, Pacak K, Palkovits M, Gold PW. Regulation of corticotropin-releasing hormone receptor messenger ribonucleic acid in the rat brain and pituitary by glucocorticoids and stress. Endocrinology 1995;136:4517–25.

Makino S, Takemura T, Asaba K, Nishiyama M, Takao T, Hashimoto K. Differential regulation of type-1 and type-2a corticotropin-releasing hormone receptor mRNA in the hypothalamic paraventricular nucleus of the rat. Mol Brain Res 1997;47:170–6.

Mansbach RS, Brooks EN, Chen YL. Antidepressant-like effects of CP-154,526, a selective CRF_1 receptor. Eur J Pharmacol 1997;323:21–6.

Martin P, Soubrie P, Simon P. The effect of monoamine oxidase inhibitors compared with classical tricyclic antidepressants on learned helplessness paradigm. Prog Neuro-Psychopharmacol Biol Psychiatry 1987;11:1–7.

Nakagawa Y, Ishima T, Ishibashi Y, Tsuji M, Takashima T. Involvement of GABAB receptor systems in action of antidepressants: II. Baclofen attenuates the effect of desipramine whereas muscimol has no effect in learned helplesssness paradigms in rats. Brain Res 1996;728:225–30.

Nakagawa Y, Sasaki A, Takashima T. The GABAB receptor antagonist CGP36742 improves learned helplessness in rats. Eur J Pharmacol 1999;381:1–7.

Nemeroff CB, Winderlov E, Bissette G, Wallens S, Karlsson I, Eklund K, Kilts CD, Loosen PT, Vale W. Elevated concentrations of CSF corticotropin releasing factor-like immunoreactivity in depressed patients. Science 1984;226:1342–4.

Okuyama S, Chaki S, Kawashima N, Suzuki Y, Ogawa S, Nakazato A, Kumagai T, Okubo T, Tomisawa K. Receptor binding, behavioral, and electrophysiological profiles of nonpeptide corticotropin-releasing factor subtype 1 receptor antagonists CRA1000 and CRA1001. J Pharmacol Exp Ther 1999;289:926–35.

Papolos DF, Edwards E, Marmur R, Lachman HM, Henn FA. Effects of the antiglucocorticoid RU 38486 on the induction of learned helpless behavior in Sprague–Dawley rats. Brain Res 1993;615:304–9.

Perrin M, Donaldson C, Chen R, Bount A, Berggren T, Bilezikjan L, Sawchenko P, Vale WW. Identification of a second corticotropin-releasing factor receptor gene and characterization of a cDNA expressed in heart. Proc Natl Acad Sci USA 1995;92:2969–73.

River C, Plotsky PM. Mediation by corticotropin-releasing factor (CRF) of adrenohypophysial hormone secretion. Annu Rev Physiol 1986;48: 475–94.

Schulz DW, Mansbach RS, Sprouse J, Braselton JP, Collins J, Dunaiskis A, Farach S, Schmidt AW, Seeger T, Seymour P, Tingley FD, Winsten EN, Chen YL, Heym J. CP-154,526: a potent and selective non-peptide antagonist of corticotropin-releasing factor receptors. Proc Natl Acad Sci USA 1996;93:10477–80.

Sherman AD, Petty F. Additivity of neurochemical changes in learned helplessness and imipramine. Behav Neural Biol 1982;35:344–53.

Sherman AD, Sacquitne JL, Petty F. Specificity of learned helplessness model of depression. Pharmacol, Biochem Behav 1982;16:449–54.