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Anxiolytic- and antidepressant-like profile of a new CRF_1 receptor antagonist, R278995/CRA0450

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Abstract

1-[8-(2,4-dichlorophenyl)-2-methylquinolin-4-yl]-1,2,3,6-tetrahydropyridine-4-carboxamide benzenesulfonate (R278995/CRA0450) is a newly synthesized corticotropin-releasing factor subtype 1 (CRF_1) receptor antagonist. In the present study, in vitro and in vivo pharmacological profiles of R278995/CRA0450 were investigated. R278995/CRA0450 showed high affinity for recombinant and native CRF_1 receptors without having affinity for the CRF_2 receptor. R278995/CRA0450 attenuated CRF-induced cyclic AMP formation in AtT-20 cells and CRF-induced forepaw treading in gerbils, indicating that R278995/CRA0450 is an antagonist of the CRF_1 receptor. In addition to CRF_1 receptor antagonism, R278995/CRA0450 showed high affinity for the σ_1 receptor, and attenuated (+)-SKF10,047-induced head-weaving behavior, suggesting σ_1 receptor antagonism. R278995/CRA0450 showed dose-dependent in vivo occupancy when assessed by ex vivo receptor binding, indicating good brain penetration. R278995/CRA0450 did not alter spontaneous anxiety when tested in the rat elevated plus maze (up to 3 mg/kg, p.o.) or lick suppression test (up to 10 mg/kg, i.p.). However, potent anxiolytic-like properties were observed in rats subjected to swim stress prior to testing on the elevated plus-maze, indicating activity primarily in tests taxing stress-induced anxiety. R278995/CRA0450 was inactive in mouse tail suspension, rat forced swim and rat differential-reinforcement-of-low-rate 72-s (DRL72), while it showed dose-dependent antidepressant-like effects in the rat learned helplessness paradigm and the olfactory bulbectomy model, demonstrating activity in a subset of animal models of depression associated with subchronic stress exposure. No or only mild effects were seen in tests of locomotor activity, motor coordination and sedation. These results indicate that R278995/CRA0450 is an orally active CRF_1 and σ_1 receptor antagonist with potent anxiolytic-like and antidepressant-like activities.

Keywords: CRF (corticotropin-releasing factor); CRF_1 receptor; Anxiety; Depression; Stress-related behavior; R278995/CRA0450

1. Introduction

Corticotropin-releasing factor (CRF), a 41-amino acid peptide originally isolated from ovine hypothalamus, is the primary hypothalamic factor driving stress-induced adrenocorticotropin secretion from the anterior pituitary (Vale et al., 1981; Rivier and Plotsky, 1986) and plays an important role in the adaptive responses to stress. CRF has also been suggested to play an important role as a neurotransmitter in the mediation of anxiety- and depression-related behaviour (Dunn and Berridge, 1990; Owen and Nemeroff, 1991).

CRF acts via two receptor subtypes, CRF_1 and CRF_2. Both belong to the G-protein-coupled receptor superfamily and are positively coupled to adenylate cyclase (Steckler and Holsboer, 1999). CRF_2 exists in three isoforms, $CRF_{2(a)}$, $CRF_{2(b)}$ and $CRF_{2(c)}$ (Hauger et al., 2003; Kostich et al., 1998; Lovenberg et al., 1995; Perrin et al., 1995). Nonselective peptidergic CRF receptor antagonists have been reported to prevent stress-induced behavior (Heinrichs et al., 1992; Menzaghi et al., 1994). In particular, the CRF_1 receptor has been emphasized to play a role in depression- and anxiety-related behavior: Knockout mice lacking the CRF_1 receptor have reduced levels of anxiety-related behavior when compared to performance of wild type controls (Timpl et al., 1998). Inactivation of the CRF_1 receptor with an antisense oligonucleotide reduced the anxiogenic-like effect of CRF (Skutella et al., 1998). Moreover, CRF_1, but

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not CRF_2, knockdown produced significant anxiolytic-like effects in defensive withdrawal relative to vehicle-treated and two missense oligonucleotide negative control groups (Heinrichs et al., 1997). Although more recent evidence suggests that the CRF_2 receptor is also involved in the modulation of anxiety- and depression-related behavior, the exact role remains to be elucidated (Bakshi et al., 2002; Takahashi et al., 2001).

Recently, a number of synthetic CRF_1 receptor antagonists have been reported (Gutman et al., 2000). These antagonists have been demonstrated to show anxiolytic-like and antidepressant-like activities in rodent models (Deak et al., 1999; Griebel et al., 2002; Gutman et al., 2003; Heinrichs et al., 2002; Lundkvist et al., 1996; Millan et al., 2001; Okuyama et al., 1999). This suggests that CRF_1 receptor antagonism could be beneficial in stress-related psychiatric disorders associated with an overactive CRF system. Indeed, patients with major depression or post-traumatic stress disorder have increased cerebrospinal fluid CRF levels (Nemeroff et al., 1984). Moreover, a blunted adrenocorticotropin response to i.v. injection of CRF has been shown in patients with depression, anorexia nervosa and post-traumatic stress disorders (Holsboer et al., 1984; Taylor and Fishman, 1988). Indeed, the results of the first open-label study with R121919, a CRF_1 receptor antagonist, in 20 patients with major depression, showed that the compound induced significant reductions in depression and anxiety scores after a 30-day treatment (Zobel et al., 2000).

Recently, we have synthesized a CRF_1 receptor antagonist, 1-[8-(2,4-dichlorophenyl)-2-methylquinolin-4-yl]-1,2,3,6-tetrahydropyridine-4-carboxamide benzenesulfonate (R278995/CRA0450) with excellent oral bioavailability. We report here the biochemical and psychopharmacological profiles of R278995/CRA0450 in various rodent models of anxiety and depression. Part of the present work was presented in abstract form at the annual meeting of Society for Neuroscience 2002 (Chaki et al., 2002; Steckler et al., 2002).

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (220–240 g, Charles River, Yokohama, Japan) were used to assess stress-induced anxiety-related behavior in the elevated plus-maze task and antidepressant-like effects in the forced swimming test. For lick suppression and differential-reinforcement-of-low-rate 72-s (DRL72), male Wistar rats (209–214 g, Charles River, Sulzfeld, Germany) were used. Male Wistar rats (Charles River, Yokohama, Japan), weighing 220–240 g, were also used for learned helplessness, olfactory bulbectomy, locomotor activity, rotarod and hexobarbital-induced sleeping, and weighing 75–160 g were used for (+)-SKF10,047-induced head weaving behavior. Male ICR mice (20–30 g, Charles River, Yokohama, Japan) were used for locomotor activity, rotarod test and hexobarbital-induced sleeping. Male NMRI mice (28.6–31.1 g, Charles River, Sulzfeld, Germany) were used for tail suspension and staircase. Male Mongolian gerbils (70–80 g, Charles River, Sulzfeld, Germany) were used for CRF-induced forepaw treading behavior. Brains/tissue from cynomolgus monkeys (2–3 kg, HAMRI, Sanwa, Japan) were used for receptor binding assay. Unless specified otherwise, animals were kept under 12:12 h light/dark cycle with lights on at 7:00 a.m. in a temperature- and humidity-controlled holding room. Food and water were available ad libitum. All studies were reviewed by the Taisho Pharmaceutical, Animal Care Committee and met the Japanese Experimental Animal Research Association standards, as defined in the Guidelines for Animal Experiments (1987). These were also in accordance with the European Communities Council Directive Nov. 1986 (86/609/EEC) and approved by the animal care and use committee of Johnson and Johnson Pharmaceutical Research and Development.

2.2. Materials

1-[8-(2,4-dichlorophenyl)-2-methylquinolin-4-yl]-1, 2, 3, 6-tetrahydropyridine-4-carboxamide benzenesulfonate (R278995/CRA0450) (Fig. 1) and *N*,*N*-dipropyl-2-[4-methyl-3-(2-phenylethoxy)phenyl]-ethylamine monohydrochloride (NE-100) were synthesized at Taisho Pharmaceutical Laboratories. [^{125}I]ovine CRF (specific radioactivity: 81.4 TBq/mmol), [^{125}I]sauvagine (specific radioactivity: 81.4 TBq/mmol) and [^{3}H](+)pentazocine (specific radioactivity: 1036 GBq/mmol) were purchased from Perkin-Elmer Life Sciences (Boston, MA, USA). The cyclic AMP assay system was purchased from Amersham International (Buckinghamshire, England). COS-7 cells (CV-1 cell line transformed with an origin defective mutant of SV40 which codes for wild type T antigen) and AtT-20 cells were purchased from American Type Culture Collection (Rocksville, MD, USA). Ovine CRF and sauvagine used for in vitro studies were purchased from Peninsula Laboratories

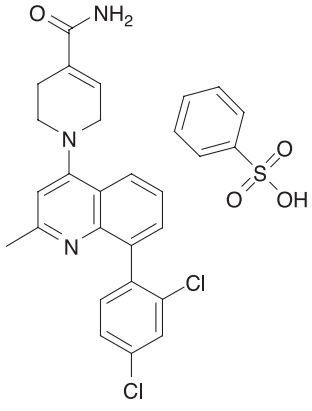

Fig. 1. Chemical structure of R278995/CRA0450.

(Belmont, CA, USA). Rat/human CRF was purchased from Calbiochem (San Diego, USA) and used for CRF-induced forepaw treading. All other chemicals used in this study were obtained commercially, and were of the highest purity available. For all in vitro studies, R278995/CRA0450 was dissolved in 0.1% dimethylsulfoxide. Prior to any experiment, it was shown that the concentration of dimethylsulfoxide did not affect the binding assays and cyclic AMP levels. For behavioral studies, R278995/CRA0450 was dissolved in 0.3% Tween 80/saline solution for oral (p.o.) administration, and dissolved in 10% hydroxypropyl-β-cyclodextrin solution for subcutaneous (s.c.) and intraperitoneal (i.p.) administration, unless stated otherwise. NE-100 was dissolved in distilled water and (+)-SKF10,047 in saline.

2.3. Cell cultures and transfection

COS-7 cells and AtT-20 cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum, 100 units/ml penicillin and 100 μg/ml streptomycin in a 5% CO_2 incubator at 37 °C. The monkey CRF_1 receptor cDNA inserted into pcDLΔPE was transfected into COS-7 cells using lipofectin (GIBCO BRL, Gaithersburg, MD, USA) according to the protocol provided by the manufacturer (Felgner et al., 1987). Seventy-two hours after transfection, COS-7 cells expressing the CRF_1 receptor were used for pharmacological experiments.

2.4. Binding for CRF_1 and CRF_2

2.4.1. Membrane preparations

COS-7 cells expressing the CRF_1 receptor were washed with phosphate buffered saline, scraped and pelleted by centrifugation. The animals (male Wistar rats, 220–240 g, Charles River, Yokohama, Japan; Cynomolgus monkeys, 2–3 kg, HAMRI) were sacrificed by decapitation and phlebotomy under anesthesia, respectively, and amygdala, pituitary and heart were rapidly dissected. Tissues and cell pellets were homogenized with 50 mM Tris–HCl buffer (pH 7.0) containing 10 mM $MgCl_2$ and 2 mM EDTA. In case of tissues, the nucleus and debris were removed by centrifugation for 5 min at 1500 rpm at 4 °C. Supernatant was centrifuged at 48,000 × *g* for 20 min at 4 °C. The pellet was washed twice with the buffer, and the final pellet was suspended in the assay buffer (50 mM Tris–HCl buffer, pH 7.0, containing 10 mM $MgCl_2$, 2 mM EDTA, 0.1% bovine serum albumin and 100 KU/ml aprotinin), and used as crude membrane preparations for binding studies. Protein concentration was determined according to Bradford (1976).

2.4.2. CRF binding

Binding assays for [^{125}I]ovine CRF (for CRF_1) and [^{125}I]sauvagine (for CRF_2) were carried out according to methods previously reported (De Souza, 1987; Grigoriadis et al., 1996), but with slight modifications. The reaction was initiated by incubating 0.5 ml of membrane preparations with 0.2 nM [^{125}I]ovine CRF or 0.2 nM [^{125}I]sauvagine. The reaction mixture was incubated for 2 h at 25 °C (for [^{125}I]ovine CRF binding) or at 23 °C (for [^{125}I]sauvagine binding), and terminated by rapid filtration through Whatman GF/C glass fiber filters presoaked with 0.3% polyethyleneimine, after which the filters were washed three times with 3 ml of phosphate buffered saline containing 0.01% Triton X-100. The radioactivity was quantified in a γ-counter. Nonspecific binding was determined either in the presence of unlabeled 1 μM ovine CRF (for [^{125}I]ovine CRF binding) or 1 μM sauvagine (for [^{125}I]sauvagine binding). Specific binding was determined by subtracting nonspecific binding from total binding. In the competition binding assay, the concentration of the test compound that caused 50% inhibition of specific radiolabeled ligand binding (IC_{50} values) was determined from each concentration–response curve. IC_{50} values were determined by the Marquardt–Levenberg nonlinear least-squares curve-fitting procedure of the MicroCal ORIGIN program (MicroCal, Northampton, MA, USA), and K_i value was calculated using K_d value computed from Scatchard plot analysis.

2.5. σ1 binding

Hartley male guinea pigs (250–300 g, Charles River, Yokohama, Japan) were sacrificed by decapitation and whole brain was rapidly dissected. The brain was homogenized with 50 mM Tris–HCl buffer (pH 7.4) and then centrifuged at 48,000 × *g* for 20 min at 4 °C. The pellets, washed once with the buffer, were suspended in Tris–HCl buffer (pH 7.4) and used as crude membrane preparation. Binding of [^{3}H](+)pentazocine was used to assess binding to σ_1 binding sites. Membranes (1 ml) were incubated with 2 nM [^{3}H](+)pentazocine at 25 °C for 120 min. Membrane-bound radioligand was separated from free radioligand by filtration over GF/B filter presoaked with 0.3% polyethyleneimine using a cell harvester and washed with 3 ml of the Tris–HCl buffer (pH 7.4) three times. The filter disks were counted in a liquid scintillation counter spectrometer. Nonspecific binding was determined in the presence of 10 μM haloperidol. In the competitive binding assay, IC_{50} and K_i value of the compound was determined as described under "CRF binding".

2.6. Determination of cyclic AMP in AtT-20 cells

AtT-20 cells grown in six-well plates were used. The culture medium was removed, the cells were washed with phosphate buffered saline and 1 ml of DMEM containing 1 mM isobutylmethylxanthine, a phosphodiesterase inhibitor, was added. The cells were incubated with R278995/CRA0450 for 20 min at 37 °C, and then 100 nM CRF was added to the medium and incubated for 5 min at 37 °C. The culture medium was then aspirated and the cells were washed twice with 3 ml of phosphate buffered saline. Two

ml of ice-cold 65% ethanol was added, and the cells were scraped from the wells. The supernatant was collected by centrifugation at 15,000 rpm for 15 min at 4 °C. Cyclic AMP formed in the cells was determined using a commercially available cyclic AMP enzyme immunoassay system.

2.7. Ex vivo receptor occupancy

Male Wistar Wiga rats weighing 180–200 g (Charles River, Sulzfeld, Germany) were used. R278995/CRA0450 was administered p.o. or s.c. at four doses ranging from 0.63 to 40 mg/kg. The animals were killed by decapitation 1 (s.c.) or 2 (p.o.) h after administration. Brains were immediately removed, and rapidly frozen in dry ice-cooled 2-methylbutane (−40 °C). Coronal sections (20 μm thick) were cut using a Reichert Jung 2800R cryostat (Cambridge Instruments, Cambridge, UK) and thaw-mounted on silanized microscope slides (Star Frost, Knittel Glaser, Germany). The sections were stored at −20 °C until use. After thawing, sections were dried under a cold stream air. Three adjacent brain slices from the same animal were collected per slide. Two brain slices were used to measure the total binding, and the third one was used for nonspecific binding. Total binding was measured by incubating sections with [^{125}I]sauvagine (0.3 nM) in 200 μl of 50 mM Tris–HCl buffer (pH 7.4) containing 2 mM EGTA, 10 mM $MgCl_2$, 0.1% bovine serum albumin, 2 μg/ml chymostatin, 4 μg/ml leupeptine, 40 μg/ml bacitracine for 45 min at room temperature. Nonspecific binding was measured by the addition of 1 μM r/hCRF to the incubation buffer. The reaction was stopped by washing the slides (4 × 1 min) with Tris–HCl buffer (pH 7.4) at 4 °C, followed by a rapid dip in cold distilled water and drying under a stream of cold air. Sections were exposed to Ektascan GRL films (Kodak). After development of films, autoradiograms were analyzed and quantified using a MicroComputer Imaging Device M1 image analysis system (Imaging Research, St. Catharines, Ontario, Canada). Optical densities in the anatomical region of interest were transformed into levels of bound radioactivity after calibration of the image analyzer with gray values generated by the co-exposed [^{125}I] Microscales standards (Amersham Pharmacia Biotech). Ex vivo receptor labeling by [^{125}I]sauvagine in the frontal cortex and pituitary was expressed as the percentage of receptor labeling in corresponding brain areas of vehicle-treated animals. The percentage of receptor occupancy was plotted against dosage, and the sigmoidal log dose–effect curve of best fit was calculated by nonlinear regression analysis, using the Graph Pad Prism program (San Diego, CA, USA). From these dose–response curves, ED_{50} values were calculated.

2.8. CRF-induced forepaw treading in Mongolian gerbils

Mongolian gerbils were pretreated with R278995/CRA0450 (2.5–40 mg/kg) or NE-100 (40 mg/kg) p.o. and then CRF (1 μg) was injected i.c.v. 30 or 60 min later. Forepaw treading was measured by an observer unaware of the drug treatment for 5 min.

2.9. Anxiolytic-like activity

2.9.1. Elevated plus-maze task in rats, basal and stress-induced anxiety-related behavior

The swim stress consists of placing rats in a 40-cm-tall, 20-cm-wide cylindrical plastic container containing 25 cm of water maintained at 25 ± 1 °C. Duration of the swim stress was 2 min, and the elevated plus-maze test was done 5 min after the swim stress. The elevated plus-maze test was based on that validated for the rat by Guimaraes et al. (1991). The apparatus consisted of a plus-shaped maze, elevated 50 cm from the floor, with two opposite open arms, 50 × 10 cm, crossed at right angles by two arms of the same dimensions, but enclosed by 40-cm-high walls with an open roof. In addition, a 1-cm-high edge made of Plexiglas surrounded the open arms to avoid falls. Illumination measured at the center of the maze was 40 lx. Each rat was placed in the center of the plus-maze facing one enclosed arm. The amount of time spent in open arms of the maze was recorded with a video tracking system (video tracking system CompACT VAS for windows plus-maze ver.3.05, Muromachi Kikai Tokyo, Japan). Rats were naïve to the apparatus. R278995/CRA0450 (0.1–3 mg/kg) or NE-100 (1–10 mg/kg) was administered p.o. 30 min prior to swim stress. We observed that diazepam (3 mg/kg, p.o.) significantly reversed stress-induced decrease in time spent in open arms (data not shown). When the effect of R278995/CRA0450 in non-stress conditions was investigated, the elevated plus-maze test was run 30 min after the oral administration with either the test compound or vehicle.

2.9.2. Lick suppression

Four operant chambers (MedAssociates, Georgia, VT, USA), each fitted with a water spout, a shock grid and a house light, were used. The spout and the grid were connected to a lickometer and a shock generator, which were controlled by a computer equipped with the MedPC software version 4.0. The boxes were housed in dark, ventilated and sound-attenuating compartments. First, rats were water deprived for 24 h. Thirty min prior to test, they received an i.p. injection with R278995/CRA0450 (0.1–10 mg/kg). A test session lasted 3 min and started once the rat had made 20 licks on the spout to ensure knowledge about the position of the spout. Every 20th lick, the rat received a mild shock (0.5 mA, 500 ms). Rats failing to make the 20 licks to start the session within 15 min were excluded. The number of licks made was registered by the computer.

2.10. Antidepressant-like activity

2.10.1. Forced swimming test in rats

The effect of the compound was evaluated according to the method described by Detke et al. (1995). Swimming

sessions were conducted by placing rats in cylinders containing 25 °C water, 30 cm deep, so that rats could not support themselves by touching the bottom with their feet. Two swimming sessions were conducted. An initial 15-min pretest was followed 24 h later by a 5-min test. R278995/CRA0450 (0.3–3 mg/kg) was administered p.o. during the period between these two sessions (24 and 1 h prior to the test). Following the initial swimming session, the rats were removed from the cylinders, placed in a heated cage for 15 min, and then returned to their home cages. Test sessions were videotaped from the front of the cylinders for later scoring. The water in the cylinders was changed after every trial. The total duration of immobility was measured for a 5-min period. Both 3 mg/kg p.o. of imipramine and fluvoxamine significantly reduced immobility time in this paradigm (data not shown).

2.10.2. Tail suspension in mice

NMRI mice were taped by their tail on a metal hook in 6 test chambers (15 width × 19 cm high) made of white plastic walls and black plastic floor (Biosep, France). Each hook was connected to a computerized strain gauge that was adjusted to detect all movements of the animals (Tail suspension software, Biosep). Total duration of immobility was measured over a period of 6 min. R278995/CRA0450 (0.1–10 mg/kg) was administered s.c. 30 min prior to the test.

2.10.3. Differential-reinforcement-of-low-rate (DRL) 72-s schedule in rats

Eight rat operant chambers (MedAssociates), each fitted with two levers, a food pellet dispenser centred between the two levers, an attachable water bottle and a house light, were connected to a computer equipped with the Med PC software version 1.15. The boxes were housed in dark, ventilated and sound-attenuating compartments. Food-deprived Wistar rats (food access was restricted to 1 h at the end of the day) were trained overnight to press the right lever for food pellets (45 mg, Bioserv). Subsequently, the schedule was changed: rats received another set of overnight sessions, but now they had to withhold responding for at least 72 s before obtaining a reinforcer (DRL 72 s). Pressing the lever at shorter time intervals resulted in resetting of the timer and the animal had to wait for at least another 72 s before a lever press would result in the delivery of a food pellet. Once animals responded reliably, training commenced during the day, 5 days per week. Each session now lasted for 60 min and the animals were adapted to receive i.p. injections of saline, 60 min prior to the session.

Upon stable performance over at least five sessions, treatment with R278995/CRA0450 (0, 3.0, 10 mg/kg, i.p., 60 before test, $n = 10$/group) commenced. During each session, the number of responses made and the pellets earned were recorded. The ratio of these measures (number of responses/number of pellets earned) was taken as a measure of efficacy. Control values were obtained from sessions on the days immediately preceding the drug test days to ensure appropriate baseline responding. Both desipramine and paroxetine were active in this paradigm (data not shown).

2.10.4. Learned helplessness test in rats

Learned helplessness test in the shuttle box test was carried out according to the method reported by Takamori et al. (2001). The two-way shuttle box (56 × 21 × 25 cm; Muromachi-Kikai, Japan) was divided into equal-sized chambers with 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, Japan). For the study of acute drug effects, rats were given R278995/CRA0450 (0.1–3 mg/kg) or NE-100 (0.1–1 mg/kg) p.o. 30 and 10 min, respectively, before first exposure to the inescapable shock session on day 1. Rats were individually placed in the chamber and given 90 inescapable shocks (1.8 mA) of 10-s duration at 2-s intervals. Control rats were treated identically, except that they were not exposed to foot shocks. On day 2, the rats were subjected to the 40-trial escape test. The animals were individually placed in the shuttle box. Following 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 no escape response within this period, both the tone signal and shock were automatically terminated. The inter-trial interval was 5 s. The number of escape failures, which refers to a non-crossing response during the shock delivery, was recorded. In case of chronic administration, R278995/CRA0450 (0.1–1 mg/kg) was administered p.o. once daily for 8 days, following which learned helplessness test was carried out as described above. Imipramine (10 mg/kg, p.o.), when administered sub-chronically, but not acutely, showed antidepressant-like effect in this test (data not shown).

2.10.5. Olfactory bulbectomy-induced hyperemotionality in rats

Animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p.), and placed in a stereotactic apparatus (Narishige, Tokyo, Japan). The olfactory bulbs were removed by suction. Postoperatively, animals were housed single caged, 14 days post-surgery, hyperemotionality was measured. Only rats that exhibited hyperemotionality (score>14) were selected for further studies. Hyperemotionality of olfactory bulbectomized rats was measured by scoring the emotional responses to (1) air blowing on the dorsum, (2) a rod presented in front of the snout, (3) resistance upon capturing and handling, and (4) reaction upon tail pinch. These responses were graded as follows: 0, no reaction; 1, slight; 2, moderate; 3, marked; or 4, extreme response (Shibata et al., 1984). In each emotional response, vocalization during the test was also scored and graded as follows, 0, no vocalization; 1, occasional vocalization; or 2, marked vocalization. In case of acute administration,

Table 1
Receptor profile of R278995/CRA0450

Receptor/ligand	Species	Receptor source	K_i (nM)
CRF_1/[^{125}I]ovine CRF	monkey	recombinant (COS-7)	54.0 ± 3.7
	monkey	amygdala	48.9 ± 5.5
	monkey	pituitary	72.7 ± 11.6*
	rat	pituitary	53.2 ± 1.4*
	mouse	AtT-20 cells	52.6 ± 8.6
CRF_2/[^{125}I]sauvagine	rat	heart	>10,000
σ_1/[^{3}H](+)pentazocine	guinea pig	whole brain	1.10 ± 0.2

* IC_{50} (nM).

R278995/CRA0450 (3 and 10 mg/kg) was administered p.o. 120 min, and NE-100 (0.1–1 mg/kg) was administered p.o. 10 or 120 min, prior to measurement of emotional responses. In case of chronic administration, R278995/CRA0450 (3 and 10 mg/kg) was administered p.o. once daily for 10 days, and emotional responses were measured 120 min after the final administration. Imipramine (10 mg/kg, p.o.) significantly reduced hyperemotionality in olfactory bulbectomized rats (data not shown).

2.11. (+)-SKF10,047-induced head-weaving behavior in rats

Immediately after administration of (+)-SKF10,047 (20 mg/kg, s.c.), the number of head weaving movements were counted for 15 min. NE-100 (0.01–0.1 mg/kg) or R278995/CRA0450 (0.003–0.03 mg/kg) were administered p.o., 10 min or 30 min prior to administration of (+)-SKF10,047, respectively. The ED_{50} values of NE-100 or CRA0450 were calculated as the percentage inhibition of head weaving behavior relative to the number of head weaving movements in animals receiving vehicle alone.

2.12. General behavior

2.12.1. Spontaneous locomotor activity in rats and mice

Spontaneous locomotor activity was determined as reported (Okuyama et al., 1999). Animals were housed individually in transparent acrylic cages (47 × 28.5 × 29.5 cm for rats; 30 cm in diameter × 30 cm high for mice), and spontaneous locomotor activity was recorded every 10 min for 60 min, using a SCANET apparatus (Neuroscience Japan) placed in a sound-proof box. R278995/CRA0450 (1–100 mg/kg) was administered p.o. 30 min before the start of measurements.

2.12.2. Rotarod performance in rats and mice

The rotarod performance were carried out according to Okuyama et al. (1999). The rotarod (Campdem Instruments, UK), consisted of a gritted plastic roller (3 cm in diameter, 9 cm long) flanked by two large round plates to prevent the animals from escaping and was run at 10 rpm. All animals were given control trials prior to the test. A rat or a mouse was placed on the roller, and the length of time it remained there was measured. A maximum of 2 min was allowed for each animal. R278995/CRA0450 (1–100 mg/kg) was administered p.o. 30 min prior to the test.

2.12.3. Staircase in mice

The staircase apparatus (Campden Inst., Loughborough, UK) consisted of a start compartment with hinged lid (80 × 55 × 52 mm). A narrow corridor with a central plinth (75 × 9 × 23 mm) extended from the start compartment. On each side of the plinth was a narrow removable trough in form of a staircase with eight steps, each step (7 × 2 mm) containing a 3-mm-deep food well which can hold a 20 mg food pellet. A mouse can climb onto the plinth and reach down left or right to grasp and retrieve the pellets. Scraping pellets up the side was prevented by a 3 mm lip on each side of the plinth. Food deprived mice were trained to retrieve the pellets on both side within a 10 min session. Once stable performance was reached (after 9 training days), animals were habituated to the injection procedure for 2 days, following which treatment with R278995/CRA0450, 0.63–40 mg/kg s.c., 60 min prior to the test, commenced.

2.12.4. Potentiation of hexobarbital-induced anesthesia in rats and mice

Hexobarbital-induced anesthesia was carried out as reported (Okuyama et al., 1999). Hexobarbital-induced anesthesia of rats or mice was estimated, based on the duration of righting reflex loss. Hexobarbital (70 mg/kg, i.p.) was administered 30 min after the oral administration of R278995/CRA0450 (1–100 mg/kg).

2.13. Statistical analysis

Data from the elevated plus-maze, forced swim, spontaneous locomotor activity, rotarod performance, hexobarbital-induced anesthesia and (+)-SKF10,047-induced head weaving behavior were analyzed by one-way analysis of variance

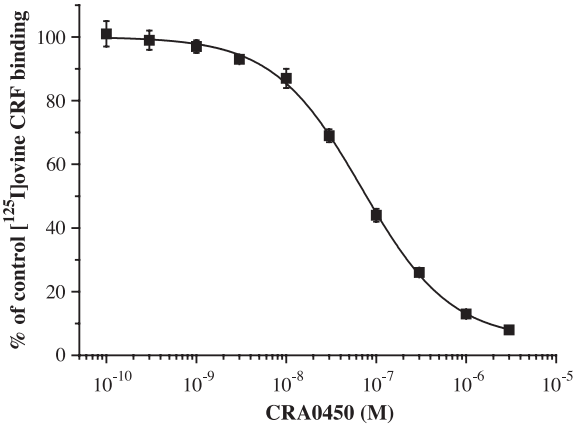

Fig. 2. Inhibition curve of [^{125}I]ovine CRF binding to membranes of COS-7 cells expressing recombinant monkey CRF_1 receptor by R278995/CRA0450. Similar inhibition curves by R278995/CRA0450 were obtained in other membrane preparations.

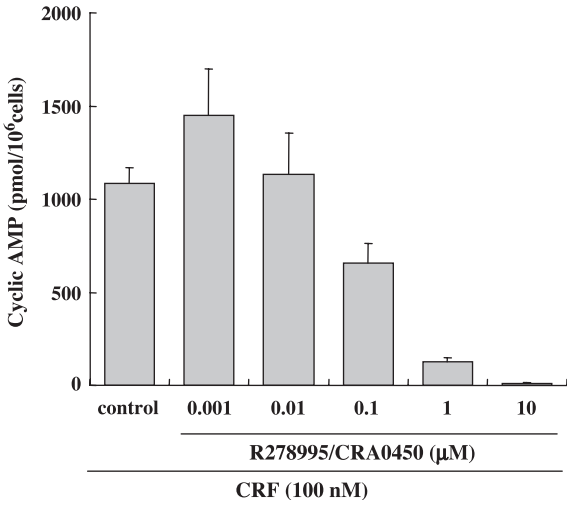

Fig. 3. Effects of R278995/CRA0450 on CRF-induced increase in cyclic AMP accumulation in AtT-20 cells. Results are means ± S.E.M.s obtained from three experiments.

and significant differences between groups were determined using Dunnett's test. In case of learned helplessness, the comparison between groups was made using the Mann–Whitney *U*-test. Between-group comparisons were done using the Steel test. In case of olfactory bulbectomy, performance of bulbectomized and sham-lesioned rats was compared using the Mann–Whiteney *U*-test; between-group comparisons were made using non-parametric Dunnett's test. For lick suppression, tail suspension, DRL72 and the mouse staircase test, data were analyzed using the Kruskal–Wallis test. For piano playing behavior, data were analyzed using Wilcoxon–Mann–Whitney rank sum test. The ED_{50} values were calculated from the dose–response of each compound, using nonlinear least-squares regression analysis.

3. Results

3.1. Affinity and selectivity of R278995/CRA0450 for CRF1 receptors

R278995/CRA0450 inhibited [^{125}I]ovine CRF binding to membranes of COS-7 cells expressing the monkey CRF_1 receptor with an K_i value of 54.0 ± 3.7 nM (Table 1). R278995/CRA0450 also showed comparative affinity for native CRF_1 receptors endogenously expressed on monkey amygdala, monkey pituitary, rat pituitary and AtT-20 cells, mouse pituitary tumor cells (Table 1). Competition curve of R278995/CRA0450 was monophasic, and Hill slope was approximately 1.0 (Fig. 2). By contrast, R278995/CRA0450 did not show affinity for the CRF_2 receptor on rat heart membranes up to 10 μM (Table 1), and displayed negligible affinity for 52 other binding sites including receptors of nonpeptide (adrenergic, dopamine, histamine, muscarine,

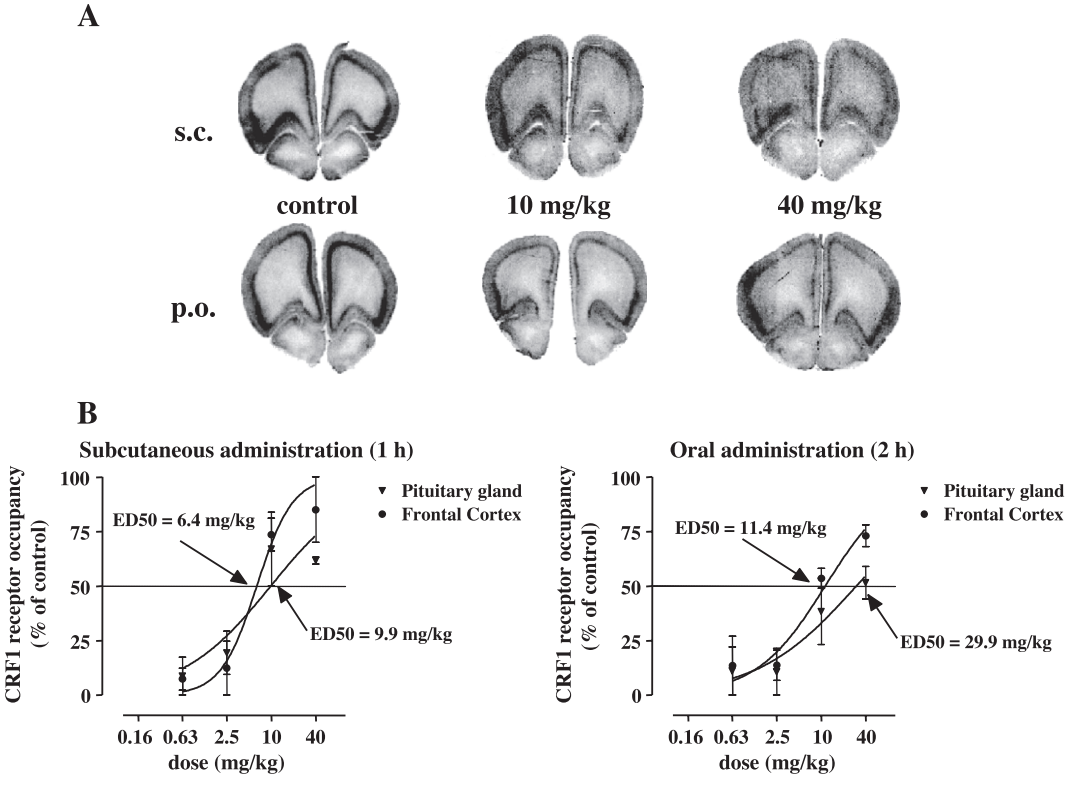

Fig. 4. Ex vivo receptor occupancy of R278995/CRA0450. Ex vivo receptor occupancy was assessed by [^{125}I]sauvagine binding to slices of rat brain. (A) Representative autoradiogram of [^{125}I]sauvagine binding to slices of rat brain. (B) Dose–response curve of the ability of R278995/CRA0450 in vivo administration to prevent [^{125}I]sauvagine binding to the rat frontal cortex and pituitary ex vivo.

serotonin, benzodiazepine, cannabinoid, γ-aminobutyric acid, glutamate, Ca^{2+}, Na^{+}) or peptide ligands (angiotensin, bradykinin, cholecystokinin, neurokinin) nor transporters (norepinephrine, serotonin, dopamine) at 1 μM, as determined at Cerep (data not shown). With regard to affinity for transporters of serotonin and norepinephrine, R278995/CRA0450 did not inhibit them even at 10 μM, as determined by [^{3}H]paroxetine (for serotonin) and [^{3}H]nisoxetine (for norepinephrine) bindings to membranes of rat brain. R278995/CRA0450, by contrast, potently interacted with σ_1 receptor with an K_i value of 1.10 ± 0.2 nM (Table 1), although it did not show affinity for σ_2 receptor at 1 μM, as determined by [^{3}H]1,3-di(2-tolyl)-guanidine binding to membranes of guinea pig brain in the presence of 100 nM (+)pentazocine.

3.2. Antagonism of CRF-induced increase in cyclic AMP formation in AtT-20 cells

Ovine CRF concentration-dependently increased cyclic AMP formation in AtT-20 cells, which selectively express the CRF_1 receptor, as shown by reverse transcription-polymerase chain reaction (data not shown). R278995/CRA0450 concentration-dependently attenuated the increase in cyclic AMP formation stimulated by 100 nM CRF in AtT-20 cells with an IC_{50} value of 105.0 ± 16.3 nM (Fig. 3).

3.3. In vivo receptor occupancy

In vivo receptor occupancy of R278995/CRA0450 was determined by using ex vivo receptor binding of [^{125}I]sauvagine on brain slices. Both s.c. and p.o. administration of R278995/CRA0450 dose-dependently inhibited [^{125}I]sauvagine binding to the slices of rat frontal cortex and pituitary (Fig. 4A,B). ED_{50} values were as follows: 6.4 mg/kg for the frontal cortex, 9.9 mg/kg for the pituitary (s.c.); 11.4 mg/kg for the frontal cortex, 29.8 mg/kg for the pituitary (p.o.).

3.4. Antagonism of CRF-induced forepaw treading in Mongolian gerbils

Intracerebroventricular injection of CRF (1 μg) in Mongolian gerbils induced vertical up-and-down movements of one or both forepaws. R278995/CRA0450 attenuated CRF-induced forepaw treading in a dose-dependent manner with a lowest active dose of 20 mg/kg p.o. (60 min pre-treatment) (Fig. 5A). Pretreatment with R278995/CRA0450, 30 min p.o. prior to CRF injection showed a comparable activity with 60 min pre-treatment, albeit it did not reach statistically significance (Fig. 5B).

3.5. Elevated plus-maze test in rats

Swim stress significantly reduced the time spent in open arms in elevated plus-maze task. R278995/CRA0450 dose-dependently reversed swim stress-induced reduction in time

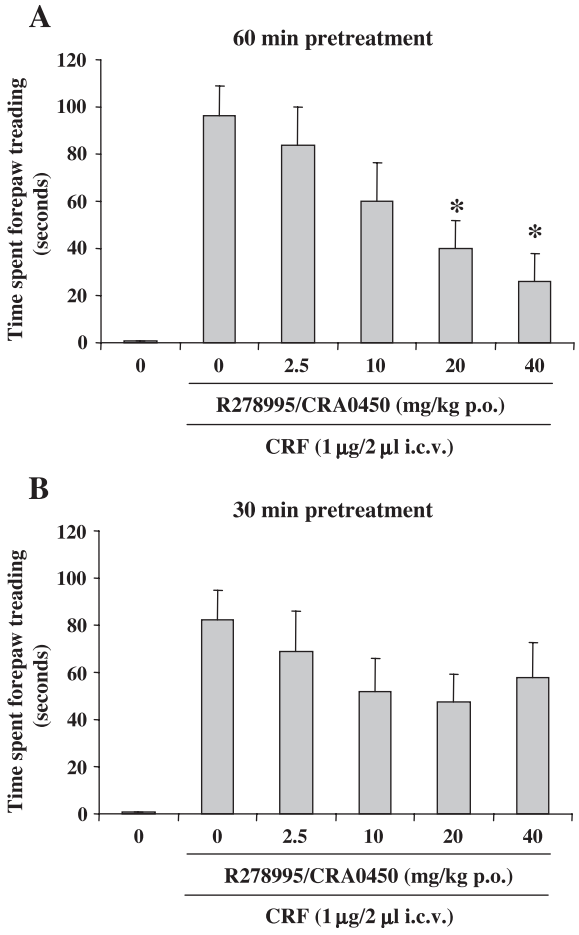

Fig. 5. Effects of R278995/CRA0450 on CRF-induced forepaw treading in Mongolian gerbils. R278995/CRA0450 was administered 60 (A) and 30 (B) min prior to i.c.v. injection of CRF (1 μg). Vertical up-and-down movements of one or both forepaws induced by i.c.v. injection of CRF was measured. *$P < 0.05$ versus CRF (i.c.v.) alone (Wilcoxon–Mann–Whitney rank sum test).

spent on the open arms (lowest active dose 1 mg/kg, p.o.) (Fig. 6A). In contrast, R278995/CRA0450 failed to show anxiolytic-like activity in the elevated plus-maze test in non-stressed rats up to 3 mg/kg p.o. (Fig. 6B).

3.6. Lick suppression test in rats

Intraperitoneal administration of R278995/CRA0450 did not affect the number of licks up to 10 mg/kg (Table 2).

3.7. Despair models: forced swimming test in rats and tail suspension test in mice

Antidepressant-like activity of R278995/CRA0450 was evaluated in two behavioral despair models. R278995/CRA0450 had no effect on immobility time in forced swimming in rats up to 3 mg/kg p.o. (Table 2). Likewise, immobility remained unaltered by subcutaneous administration of R278995/CRA0450 up to 10 mg/kg in mouse tail suspension (Table 2).

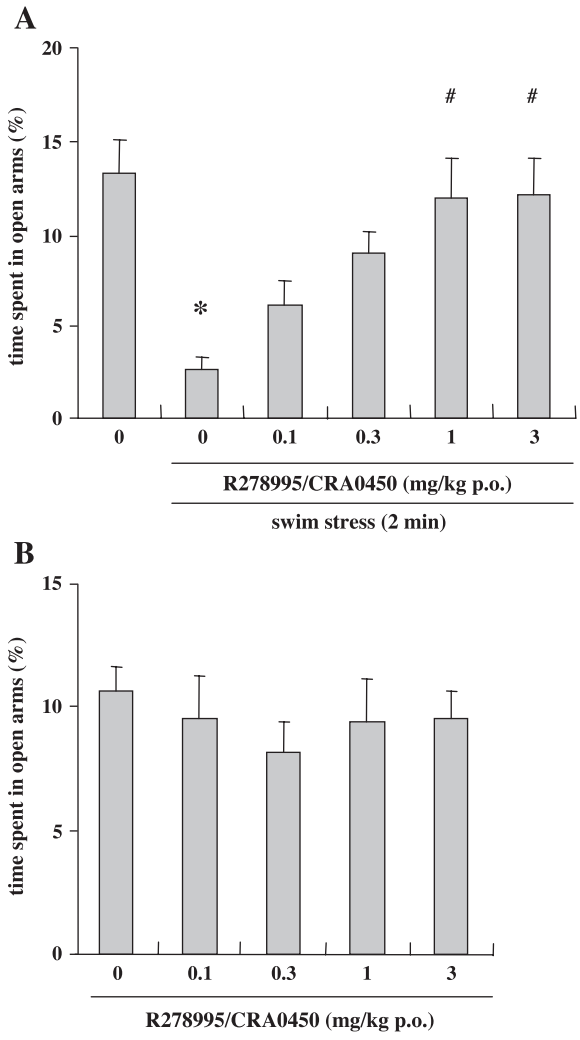

Fig. 6. Effects of R278995/CRA0450 in the elevated plus-maze task under stressed (A) and basal (B) conditions in rats. Results are shown as means ± S.E.M.s from 15 rats. *$P<0.05$ versus non-stress (*t*-test); $^{\#}P<0.05$ versus swim stress group (Dunnett's test).

3.8. DRL72 in rats

R278995/CRA0450 did not alter the number of responses made or the number of pellets earned when administered i.p. or p.o. up to 10 mg/kg (Table 2).

3.9. Learned helplessness test in rats

As compared with control animals, stressed animals exposed to inescapable shock (i.e., rendered 'helpless') exhibited an increased number of escape failures (Fig. 7A,B). We have previously shown that sub-chronic administration (8 days), but not acute administration, of imipramine reversed escape failures (Takamori et al., 2001). In contrast, 3 mg/kg R278995/CRA0450 p.o. significantly decreased the number of escapable failures already after acute administration when given prior to inescapable shock (acquisition phase; Fig. 7A). Comparable effects were seen following sub-chronic administration (8 days) of R278995/CRA0450 (Fig. 7B).

Table 2
Effects of R278995/CRA0450 in anxiety/depression models

Paradigm	Species	LAD
Lick suppression	rat	>10 mg/kg i.p.
Tail suspension	mouse	>10 mg/kg s.c.
Forced swimming	rat	>3 mg/kg p.o.
DRL72	rat	>10 mg/kg p.o./i.p.

LAD: lowest active dose.

3.10. Olfactory bulbectomy-induced hyperemotionality in rats

Emotional responses were significantly increased in olfactory bulbectomized rats when compared with sham-operated rats (Fig. 8A,B). These emotional responses of

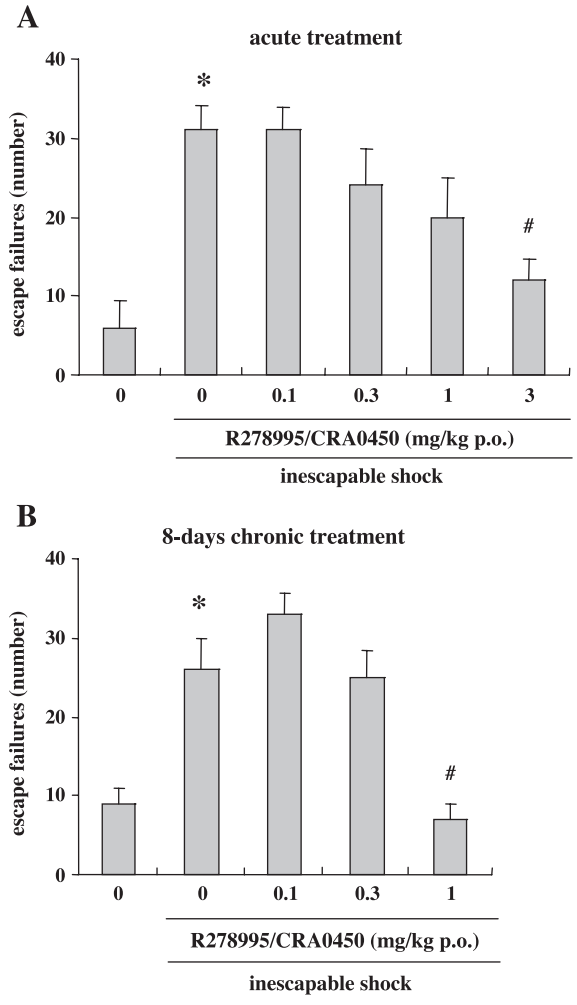

Fig. 7. Effects of R278995/CRA0450 on learned helplessness in rats. The compound was administered 30 min prior to inescapable shock (A) or chronically once daily for 8 days (B). Data represent means ± S.E.M.s from 10 rats. *$P<0.05$ versus non-inescapable shock group (Mann–Whitney *U*-test); $^{\#}P<0.05$, versus inescapable shock group (Steel test).

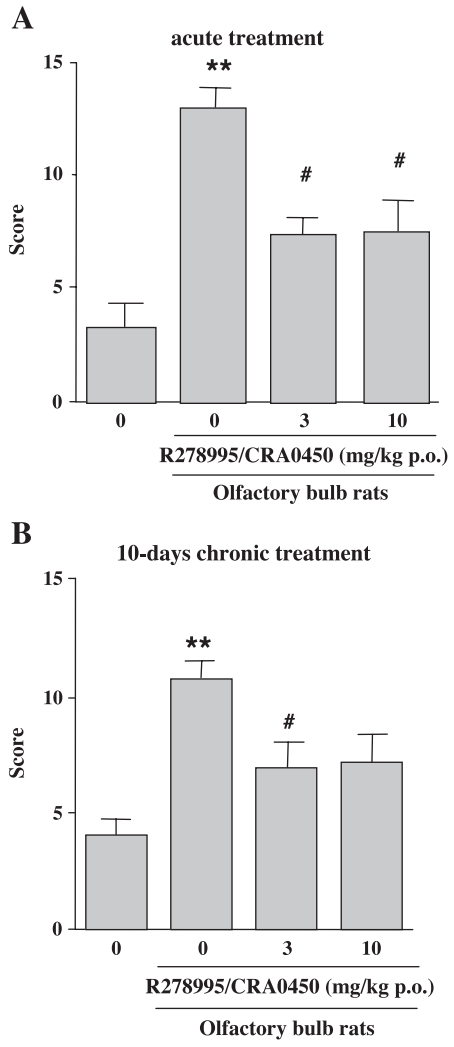

Fig. 8. Effects of R278995/CRA0450 on hyperemotionality of olfactory bulbectomized rats. The compound was administered 2 h prior to the test (A) or chronically once daily for 10 days (B). Data represent means ± S.E.M.s from 7 (A) or 6–8 (B) rats. *$P<0.05$, **$P<0.01$ versus sham-lesioned rats (Mann–Whitney U-test); $^{\#}P<0.05$, versus vehicle-treated bulbectomized rats (Dunnett's test).

olfactory bulbectomized rats were significantly reduced by both acute administration (Fig. 8A) and sub-chronic administration (10 days) of R278995/CRA0450 (Fig. 8B).

3.11. Side effect liabilities of R278995/CRA0450

R278995/CRA0450 significantly inhibited spontaneous locomotor activity in both rats (lowest active dose 10 mg/kg, p.o.) and mice (lowest active dose 100 mg/kg, p.o.) (Table 3). R278995/CRA0450 did not affect rotarod performance in either rats or mice up to 100 mg/kg p.o. (Table 3), but impaired the number of pellets earned in the mouse staircase test at a dose of 40 mg/kg s.c. (Table 3). R278995/CRA0450 did not show any effects on hexobarbital-induced sleeping time in either rats or mice up to 100 mg/kg p.o. (Table 3).

Table 3
Side effect liabilities of R278995/CRA0450

Type of behavior	Species	LAD
Locomotor activity	mouse	100 mg/kg p.o.
Locomotor activity	rat	10 mg/kg p.o.
Rotarod	mouse	>100 mg/kg p.o.
Rotarod	rat	>100 mg/kg p.o.
Staircase	mouse	40 mg/kg s.c.
Potentiation of hexobarbital-induced anesthesia	mouse	>100 mg/kg p.o.
Potentiation of hexobarbital-induced anesthesia	rat	>100 mg/kg p.o.

LAD: lowest active dose.

3.12. Antagonism of σ1-related behavior in rats

We have previously demonstrated that (+)-SKF10,047-induced head-weaving behavior in rats is a sensitive model to assess the antagonistic activity of compounds acting at the σ_1 receptor (Okuyama et al., 1993). Therefore, the in

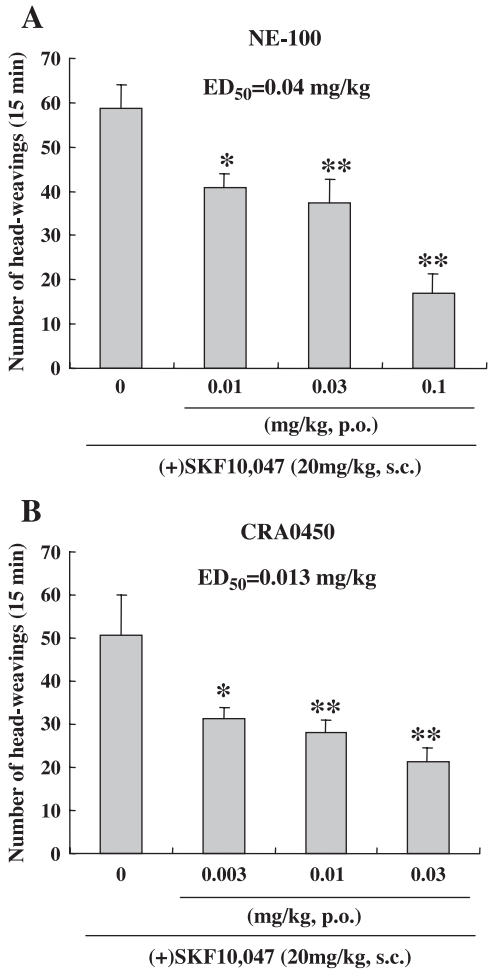

Fig. 9. Effects of NE-100 (A) and R278995/CRA0450 (B) on (+)-SKF10,047-induced head-weaving behavior in rats. Data represent means ± S.E.M.s from eight rats. *$P<0.05$, **$P<0.01$ versus (+)-SKF10,047 treated rats (Dunnett's test).

Table 4
Effects of NE-100 in CRF-induced behavior and depression models

Paradigm	Species	LAD
CRF-induced forepaw treading	mongolian gerbil	>40 mg/kg p.o.
Elevated plus-maze in stressed rats	rat	>10 mg/kg p.o.
Learned helplessness (acute)	rat	>1 mg/kg p.o.
Olfactory bulbectomy-induced hyperemotionality (acute)	rat	>1 mg/kg p.o.

LAD: lowest active dose.

vivo interaction of R278995/CRA0450 with the σ_1 receptor was evaluated in (+)-SKF10,047-induced head-weaving behavior in rats. R278995/CRA0450, as well as NE-100, a specific and potent σ_1 receptor antagonist, dose-dependently attenuated (+)-SKF10,047-induced head-weaving behavior with ED_{50} values of 0.013 and 0.04 mg/kg, respectively (Fig. 9A,B).

3.13. Effect of NE-100 on CRF-induced forepaw treading and depression models

NE-100 did not induce any effect on CRF-induced forepaw treading in gerbils up to 40 mg/kg p.o. (Table 4). Moreover, NE-100 had no effects in elevated plus-maze in stressed rats, learned helplessness or on hyperemotionality in olfactory bulbectomized rats up to 1 or 10 mg/kg p.o. (Table 4).

4. Discussion

In the present study, we demonstrated that R278995/CRA0450 is a CRF_1 receptor antagonist and that R278995/CRA0450 showed potent anxiolytic-like and antidepressant-like activities in various rodent experimental models.

R278995/CRA0450 exhibited affinity for the CRF_1 receptor, both for recombinant and native receptors expressed in brain tissues and cells, but had no affinity for the CRF_2 receptor or a variety of other receptors, transporters and ion channels, with the exception of the σ1 receptor, where affinity was high. There were no species differences in CRF_1 affinity, as R278995/CRA0450 showed comparable affinities for monkey, rat and mouse CRF_1 receptors.

CRF receptors have been reported to be coupled to GTP binding protein (Gs), and activate adenylate cyclase activity, which leads to accumulation of cyclic AMP in targeted cells (Bilezikjian and Vale, 1983). Thus, agonistic/antagonistic activity of R278995/CRA0450 was assessed by cyclic AMP formation in AtT-20 cells, an anterior pituitary cell line which has been demonstrated to express only the CRF_1 receptor among CRF receptor subtypes by reverse transcription-polymerase chain reaction, and to be a suitable in vitro model to investigate the functional consequences mediated through the CRF_1 receptor (Chaki et al., 1999). In AtT-20 cells, R278995/CRA0450 attenuated the CRF-induced increase in cyclic AMP formation, indicating that R278995/CRA0450 is an antagonist at the CRF_1 receptor.

To determine whether R278995/CRA0450 can penetrate the brain to occupy CRF_1 receptors in target regions, in vivo receptor occupancy was investigated by using ex vivo receptor binding of [^{125}I]sauvagine binding. R278995/CRA0450, when administered p.o. or s.c., dose-dependently inhibited [^{125}I]sauvagine binding in the frontal cortex and the pituitary, indicating that R278995/CRA0450 occupied CRF_1 receptors expressed in the brain and in the pituitary when administered peripherally. This result is supported by our pharmacokinetic results, showing high brain levels of R278995/CRA0450 after oral administration (1716 ng/g at 1 h and 2425 ng/g at 4 h after 10 mg/kg oral dosing; unpublished result).

Intracerebroventricular injections of CRF induce a high frequency tremor of the forelimbs in gerbils called forepaw treading or piano playing, which has been considered to be mediated via the CRF_1 receptor (Gully et al., 2002). Oral administration of R278995/CRA0450 attenuated CRF-induced forepaw treading, although it did not reach 50% inhibition after 30 min pre-treatment. It is conceivable that there might be a difference in the pharmacokinetic profile between rats and gerbils, or that higher amounts of R278995/CRA0450 might be needed to antagonize the excess amount (1 μg) of exogenous CRF when compared to endogenous CRF levels, although these possibilities need further investigation. However, in vivo occupancy and CRF-induced forepaw treading clearly shows that R278995/CRA0450 occupies and antagonizes CRF_1 receptors in the brain when administered systemically.

Exposure to 2 min swim stress led to a marked reduction of exploration of the open arms of the elevated plus-maze, which is indicative of increased levels of anxiety-related behavior. R278995/CRA0450 ameliorated the swim stress-induced anxiety-like behavior, but failed to show any effect under non-stressed conditions in the elevated plus-maze. These results are in good agreement with previous findings with other selective CRF_1 receptor antagonists such as CRA1000 and CRA1001 (Okuyama et al., 1999), and with results obtained by others using other CRF1 receptor antagonists (Griebel et al., 1998,2002). The effect of R278995/CRA0450 was more potent than another CRF1 receptor antagonist, CP-154,526, and excellent oral bioavailability and brain penetration of R278995/CRA0450 may be attributed to the oral activity. Moreover, it has been reported that CRF_1 receptor antagonists show no or only weak effects on anxiety-related behavior under basal conditions, but reverse the increase in anxiety-related behavior in rats exposed to stress (Menzaghi et al., 1994; Griebel et al., 2002; Heinrichs et al., 2002). The present results are in line with these findings, which suggests that CRF_1 receptor antagonists may be more effective under conditions where

endogenous CRF activity is stimulated, i.e., under stressful conditions.

R278995/CRA0450 failed to show a significant effect in the lick suppression paradigm, consistent with previous findings with other CRF_1 receptor antagonists, such as CP-154,526 (Griebel et al., 1998), although more recent studies reported that SSR125543A and antalarmin were active in lick suppression (Griebel et al., 2002). Although the reason for this discrepancy is unclear, it can be suggested that CRF_1 receptor antagonists may have weak effects in those models in which "classical" anxiolytics such as benzodiazepines are effective, but have potent anxiolytic-like activity in models taxing stress-induced anxiety.

There are several lines of evidence suggesting that CRF_1 receptor antagonists may be effective in treating subjects with depression, not at least of which is the open-label study with R121919, showing significant reductions in depression and anxiety scores (Zobel et al., 2000). To elucidate antidepressant-like effects, we investigated the effect of R278995/CRA0450 in several animal models of depression. R278995/CRA0450 failed to show any effect in behavioral despair models such as rat forced swimming and mouse tail suspension. Likewise, R278995/CRA0450 did not show any effect in DRL72 in rats. However, various CRF_1 receptor antagonists have been reported to be effective in learned helplessness even when acutely treated, while clinically active antidepressants such as tricyclics and selective serotonin reuptake inhibitors are only effective after 3–4 days of treatment (Takamori et al., 2001). In this paradigm, R278995/CRA0450 exhibited antidepressant-like effects even after acute administration prior to the acquisition phase. Therefore, it may be speculated that R278995/CRA0450 may exert antidepressant-like activity with earlier onset. Further evidence supporting the important role of CRF in the modulation of this type of behavior comes from studies showing that i.c.v. injections of CRF induced escape deficits similar to those seen in learned helplessness (Ronan et al., 2000). Intra-caudal dorsal raphe nucleus injection of the CRF receptor antagonist, D-Phe CRF-(12–41), when injected before inescapable shock, inhibited learned helplessness behavior, while injection of CRF into the same site mimicked the effect of inescapable shock (Hammack et al., 2002). This suggests that an elevated CRF level might be involved in the mediation of the behavioral effects induced by inescapable shock and CRF_1 receptor antagonists may attenuate learned helplessness behavior by blocking this excess CRF activity.

Neuronal projections from the olfactory bulbs to the limbic system have a major influence on emotional behavior (Herbert, 1985). The removal of the olfactory bulbs leads to characteristic changes in behavior, referred to as the "bulbectomy syndrome", which includes increased activity in a novel environment (Mudunkotuwa and Horton, 1996), hyperemotionality (Okuyama et al., 1999), performance deficit in passive avoidance (Thorne and Rowles, 1988) and increased muricidal behavior (Shibata et al., 1984). These abnormalities can be reversed by chronic antidepressant treatment (Mudunkotuwa and Horton, 1996; Shibata et al., 1984). Likewise, CRF_1 receptor antagonists such as CRA1000, CRA1001 and CP-154,526 reduce hyperemotionality induced by bulbectomy. This effect was more potent after 14 days of chronic administration than after acute administration. In the present study, we also observed significant effects with R278995/CRA0450 following both acute and 10-day chronic administration. Of note, it has been reported that olfactory bulbectomy induces an increase in brain CRF levels (Bissette, 2001). Therefore, it is conceivable that activation of the CRF system may be, at least in part, responsible for the behavioral abnormalities observed in olfactory bulbectomized rats, which could explain why CRF_1 receptor antagonists are effective in this animal model of depression.

In addition to the antagonistic activity at the CRF_1 receptor, R278995/CRA0450 showed high affinity for the σ_1 receptor. The σ_1 receptor is also presumed to be involved in anxiety and depression. σ_1 receptor ligands showed antidepressant-like activity in tail suspension (Ukai et al., 1998), forced swimming (Urani et al., 2001) and induced anxiolytic-like activity in a conditioned fear paradigm (Kamei et al., 1996). Moreover, igmesine, which binds preferentially to σ receptors, has been demonstrated to show antidepressant-like effects in both preclinical (Kinsora et al., 1998) and clinical studies (Leadbetter et al., 1999). Therefore, the interaction of R278995/CRA0450 with the σ_1 receptor might be involved in its anxiolytic-like and antidepressant-like effects, although it is not clear whether R278995/CRA0450 acts as an antagonist or an agonist at σ_1 receptor on a molecular basis. In the present study, R278995/CRA0450 antagonized (+)-SKF10,047-induced head-weaving behavior comparable to NE-100, a putative σ_1 receptor antagonist with high affinity, indicating that R278995/CRA0450 may interact with the σ_1 receptor in the same way as NE-100 does. It has been reported that NE-100 reverses anxiolytic- and antidepressant-like effects of putative σ_1 receptor agonists, including (+)-SKF10,047 (Noda et al., 2000; Ukai et al., 1998). In contrast, NE-100 per se did not show any effect on anxiety- and depression-like behavior in these studies (Noda et al., 2000; Ukai et al., 1998). Moreover, NE-100 failed to show any effect in elevated plus-maze in stressed rats, in the learned helplessness task and in bulbectomized rats, even at a dose 100-times higher than the one which is effective in antagonizing (+)-SKF10,047-induced head-weaving. Taking into account that R278995/CRA0450 has σ_1 receptor antagonistic properties comparable to NE-100, at least in the (+)-SKF10,047-induced head weaving model, it is likely that R278995/CRA0450 exerts its antidepressant-like activity primarily by blocking the CRF_1 receptor.

Many drugs acting on the central nervous system cause unwanted side effects such as prolongation of sleep, sedation and impaired motor coordination. Unlike anxiolytics

such as benzodiazepines, R278995/CRA0450 did not show any significant effects on rotarod performance or hexobarbital-induced sleeping. R278995/CRA0450 inhibited spontaneous locomotor activity and staircase performance, but this effect occurred at much higher doses than the pharmacologically effective doses. Therefore, R278995/CRA0450 may show less unwanted central nervous system side effects sometimes seen in patients on antidepressants and/or anxiolytics.

In conclusion, this study demonstrates that R278995/CRA0450 could ameliorate anxiety-like and depression-like behavior in various animal models. Moreover, R278995/CRA0450 may be relatively devoid of the unwanted side effects caused by classical anxiolytics. R278995/CRA0450 should prove effective for the treatment of subjects suffering from anxiety or depression disorders.

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