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Pharmacology, Biochemistry and Behavior 88 (2008) 497–510
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

MPZP: A novel small molecule corticotropin-releasing factor type 1 receptor (CRF_1) antagonist

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Received 25 August 2007; received in revised form 13 October 2007; accepted 17 October 2007
Available online 23 October 2007

Abstract

The extrahypothalamic stress peptide corticotropin-releasing factor (CRF) system is an important regulator of behavioral responses to stress. Dysregulation of CRF and the CRF type 1 receptor (CRF_1) system is hypothesized to underlie many stress-related disorders. Modulation of the CRF_1 system by non-peptide antagonists currently is being explored as a therapeutic approach for anxiety disorders and alcohol dependence. Here, we describe a new, less hydrophilic (*cLogP* ~2.95), small molecule, non-peptide CRF_1 antagonist with high affinity (K_i=4.9 nM) and specificity for CRF_1 receptors: *N*,*N*-bis(2-methoxyethyl)-3-(4-methoxy-2-methylphenyl)-2,5-dimethyl-pyrazolo[1,5-*a*] pyrimidin-7-amine (MPZP). The compound was systemically administered to adult male rats in two behavioral models dependent on the CRF_1 system: defensive burying (0, 5, 20 mg/kg, n=6–11 for each dose) and alcohol dependence (0, 5, 10, 20 mg/kg, n=8 for each self-administration group). Acute administration of MPZP reduced burying behavior in the defensive burying model of active anxiety-like behavior. MPZP also attenuated withdrawal-induced excessive drinking in the self-administration model of alcohol dependence without affecting nondependent alcohol drinking or water consumption. The present findings support the proposed significance of the CRF_1 system in anxiety and alcohol dependence and introduce a promising new compound for further development in the treatment of alcohol dependence and stress-related disorders.
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Keywords: Corticotropin-releasing factor; Corticotropin-releasing hormone; Receptor; CRF_1; Antagonist; MPZP; Alcohol; Defensive burying; Anxiety; Dependence; Binding; Autoradiography

1. Introduction

Corticotropin-releasing factor (CRF) is a 41-amino acid residue peptide that mediates neuroendocrine (Vale et al., 1981) and behavioral responses to stress (Sutton et al., 1982; Britton et al., 1986a,b). CRF and its putative receptors are now recognized to have numerous endogenous functions and are currently being explored as therapeutic targets for intervention in stress-related disorders such as anxiety and alcohol dependence (Koob, 2003; Cowen and Lawrence, 2006; Gehlert et al., 2007; Heilig and Egli, 2006; Valdez, 2006).

CRF exerts its actions via two known receptors: Type 1 (CRF_1) (Chang et al., 1993; Chen et al., 1993; Perrin et al., 1993) and Type 2 (CRF_2) (Lovenberg et al., 1995). Both receptors belong to the B1 subgroup of G protein-coupled receptors linked to a number of intracellular signaling pathways,

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doi:10.1016/j.pbb.2007.10.008

including ligand-dependent increase of intracellular cyclic adenosine monophosphate (cAMP) (Chen et al., 1986; Giguere et al., 1982). CRF cell bodies, terminals, or CRF receptors are located in neuroendocrine structures, such as the paraventricular nucleus of the hypothalamus, median eminence, and anterior pituitary, as well as in extrahypothalamic brain regions of the "extended amygdala" that are important for behavioral responses to stress and addictive disorders (Bloom et al., 1982; Swanson et al., 1983).

Genetic and pharmacological evidence implicates CRF_1 in mediating anxiety-related behaviors in animals (Timpl et al., 1998; Smith et al., 1998; Heinrichs et al., 1997; Liebsch et al., 1995; McElroy et al., 2002; Zorrilla et al., 2002, 2003). CRF_1 knockout mice display less anxiety-like behavior (Timpl et al., 1998; Smith et al., 1998). Central administration of CRF mimics the behavioral responses to stress in rodents (Britton et al., 1986a,b; Sutton et al., 1982; Dunn and Berridge, 1990), and CRF_1 antagonists have opposing effects (Zorrilla and Koob, 2004).

Alcoholism is a chronically relapsing disorder characterized by cycles of repeated high alcohol intake and negative emotional consequences during withdrawal (Breese et al., 2005; Koob, 2003; Heilig and Egli, 2006). Alcoholics are thought to drink alcohol initially for its euphorigenic effects, and subsequently to avoid or reduce the negative emotional state experienced in the absence of the drug or to self-medicate preexisting negative emotional states (Koob, 2003; Cappell and LeBlanc, 1979; Lowman et al., 1996). CRF activation of CRF_1 receptors is hypothesized to play a significant role in the negative emotional state and alcohol-seeking behavior associated with withdrawal from chronic alcohol exposure in rats (Koob, 2003; Menzaghi et al., 1994; Valdez and Koob, 2004). Indeed, CRF_1 antagonists attenuate the elevated anxiety-like behavior (Overstreet et al., 2004) and increased drinking (Chu et al., 2007; Funk et al., 2007; Gehlert et al., 2007; Sabino et al., 2006) associated with withdrawal in dependent animals as well as the excessive drinking of genetically selected Marchigian Sardinian alcohol-preferring rats (Hansson et al., 2006).

Compounds that modulate the CRF_1 system are being developed for the treatment of alcohol dependence. Although peptide CRF_1 antagonists are available, they are not able to penetrate the blood–brain barrier, thereby limiting their clinical effectiveness for treating central nervous system (CNS) disorders. Alternatively, small molecule, non-peptide CRF_1 selective antagonists with appropriate physiochemical properties can readily reach the brain CRF system, and considerable effort is being made to develop and characterize such compounds (Zorrilla and Koob, 2004; Kehne and De Lombaert, 2002).

Most of the presently available non-peptide CRF_1 antagonists are more lipophilic than prototypical CNS therapeutics (Zorrilla and Koob, 2004). The purpose of the present study was to explore the pharmacological and behavioral properties of a non-peptide small molecule CRF_1 specific antagonist with hydrophilicity approaching that of typical CNS therapeutics. *N*,*N*-bis(2-methoxyethyl)-3-(4-methoxy-2-methylphenyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-7-amine (MPZP) was synthesized and characterized *in vitro* and *in vivo*. The defensive burying model of active anxiety-like behavior is highly dependent on brain CRF systems (Basso et al., 1999; Diamant et al., 1992; Korte et al., 1994; Zorrilla et al., 2003) and was used to test the anxiolytic-like properties of MPZP. MPZP then was tested on a well-established model of alcohol dependence in which rats allowed to self-administer alcohol exhibit enhanced intake following chronic exposure to alcohol vapor ("dependent") compared to rats not chronically exposed to alcohol vapor ("nondependent") (Roberts et al., 1996; Overstreet et al., 2002; Rimondini et al., 2002; Valdez et al., 2002). Our data demonstrate that MPZP has high specificity and affinity for CRF_1 receptors, has anxiolytic-like properties, and significantly reduces excessive alcohol self-administration in dependent rats without altering nondependent operant responding. The results suggest experimental and therapeutic potential for MPZP and other drug-like CRF_1 small molecules in stress-related disorders such as anxiety and alcohol dependence.

2. Materials and methods

2.1. Animals

Adult male Wistar rats were obtained from Charles River Laboratory (Kingston, NY). Rats were housed 2–3 per cage with food and water available *ad libitum*. Lights were on a 12 h light/dark cycle, with lights on at 0600. For the behavioral studies, animals were allowed 4–7 days of acclimation to the laboratory and were frequently handled prior to the start of both experiments. Brain tissue for receptor binding and autoradiography assays was obtained from alcohol-naive rats that were anesthetized with isofluorane and immediately decapitated. For autoradiography, brains were rapidly removed, snap-frozen in isopentane (2-methylbutane, Sigma, St. Louis, MO), and stored at −80°C until sectioning, as described below. For receptor binding assays, brains were rapidly removed and placed immediately on an ice-cold stage, and whole cerebellum was dissected out and immediately placed in cold homogenizing buffer for homogenization, as described below. All procedures met the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at The Scripps Research Institute.

2.2. Synthesis and in vitro characterization of N,N-bis(2-methoxyethyl)-3-(4-methoxy-2-methylphenyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-7-amine (MPZP)

MPZP was synthesized as described in Gilligan et al. (2000) and Arvanitis and Chorvat (1998). Binding activity of MPZP was determined in a competition assay using $[^{125}I]Tyr^0$-sauvagine (2200 Ci/mmol; Perkin Elmer, Waltham, MA) as the radioligand. Cerebellum was homogenized in homogenizing buffer (Dulbecco's phosphate-buffered saline [PBS]: 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 2.7 mM KCl, 138 mM NaCl, pH 7.2, supplemented with 10 mM $MgCl_2$, 2 mM EGTA) using a Polytron (Dispersing and Mixing Technology, Kinematica,

Littau-Lucerne, Switzerland) at setting 6 for 2×15 s on ice. The homogenate was centrifuged at 45,000 ×*g* for 20 min at 4°C. The pellet was resuspended and spun at 45,000 ×*g* for 20 min at 4°C. The final pellet was resuspended in assay buffer (homogenizing buffer supplemented with protease inhibitor; 1 tablet/10 ml; Sigma CAT#S8829-20TAB, St. Louis, MO, pH 7.4) using a Polytron. The reaction was initiated by adding 0.05 ml of [^{125}I]Tyr^0-sauvagine to 1.5 ml polypropylene tubes containing 0.1 ml of membrane preparation (~2 mg protein/ml) and 0.05 ml of a CRF_1 antagonist at logarithmic interval concentrations from 10^{-6} to 10^{-11} M. MPZP binding affinity was compared to that of DMP904, a structurally related reference compound that exhibits high, selective affinity for CRF_1 receptors (Gilligan et al., 2000) and which dose-dependently occupies brain CRF_1 receptors, reduces anxiety-like behavior, and prevents stress-induced increases in circulating corticosterone levels following oral dosing (Lelas et al., 2004). Total binding was determined using assay buffer in lieu of a CRF_1 antagonist, and nonspecific binding was determined in the presence of 1 μM of the unlabeled homologous ligand (Hoare et al., 2004, 2005, 2006; Gross et al., 2005). The final radioligand concentration was 0.2 nM, and the reaction was incubated at room temperature for 2 h. The reaction tubes were centrifuged at 12,000 rpm for 5 min to terminate the reaction. The supernatant was removed and the pellets washed twice with ice-cold washing buffer (DPBS with 0.01% Triton-X100). Tubes then were centrifuged at 12,000 rpm, and the supernatant was removed. The pellet-containing tip was cut off and counted in an automated 10-detector gamma counter (MicroMedic Apex, ICN Biomedical, Costa Mesa, CA) at 80% efficiency. Six independent radioligand displacement assays, each involving a freshly prepared membrane preparation from a unique brain and freshly prepared solutions, were performed on different days using duplicate replicates for each data point. In each assay, the total radioligand bound was less than 10% of the total amount of radioligand added to the tube.

Specificity of MPZP for other receptor, transporter, ion channel, or enzyme targets was determined in duplicate at a 1 μM concentration via the NovaScreen commercial screening service (GEN SEP I panel, Hanover, MD).

For CRF receptor autoradiography, brain tissue was sectioned coronally (20 μm) using a cryostat (−17°C). Sections were mounted on Superfrost Plus+charged glass slides (Fisher Scientific, Pittsburgh, PA), allowed to dry completely, and stored in airtight boxes at −80°C until the day of autoradiography. Autoradiography was performed using standard procedures based on the previous characterization of [^{125}I]Tyr^0-sauvagine (Grigoriadis et al., 1996). Slides containing triplicate adjacent brain sections were thawed to room temperature and allowed to dry completely. Each section then was outlined using a PAP pen (Calbiochem, San Diego, CA). Sections were incubated in assay buffer (DPBS with 10 mM $MgCl_2$, 2 mM EGTA, 1 tablet/100 ml protease inhibitor, 0.15% bovine serum albumin) for 15 min to remove endogenous ligand. Slides then were incubated under one of four conditions: (1) 0.2 nM [^{125}I]Tyr^0-sauvagine to determine total binding; (2) 0.2 nM radiolabeled sauvagine+1 μM R121919 (3-[6-(dimethylamino)-4-methyl-pyrid-3-yl]-2,5-dimethyl-*N*,*N*-dipropyl-pyrazolo[2,3-*a*]pyrimidin-7-amine, also referred to as NBI-30775) to determine non-CRF_1 (e.g., CRF_2) receptor binding; (3) 0.2 nM radiolabeled sauvagine+3 μM MPZP to determine binding using the experimental compound under study; (4) 0.2 nM radiolabeled sauvagine+0.3 μM unlabeled D-Phe-$CRF_{12–41}$, a subtype-nonspecific CRF receptor antagonist, to determine non-CRF_1/CRF_2 (e.g., nonspecific binding). After 2 h incubation at room temperature, unbound radioligand was removed via a brief dip in ice-cold assay buffer, followed by two 5 min rinses in ice-cold washing buffer (DPBS with 0.01% Triton-X100) and one brief dip in ice-cold distilled, deionized H_2O. Slides then were dried at room temperature and apposed to Kodak Biomax MR film for 2 days. Unlabeled peptides (sauvagine, D-Phe-$CRF_{12–41}$) were generously provided by Dr. Jean Rivier (The Salk Institute, La Jolla, CA). Images were captured using a light box and digital camera computer workstation using a MTI CCDC72 digital camera equipped with a 90 mm Tamron macro lens. The frame-grabber software was Scion FGC Capture, and image analysis was performed with ImageJ 1.39 (National Institutes of Health, Washington, DC).

2.3. MPZP preparation

MPZP was prepared for systemic administration by first solubilizing it in 1 M HCl (10% final volume). It then was diluted using 25% w/v hydroxypropyl β-cyclodextrin (HBC, Cargill, Cedar Rapids, IA) (80% final volume) and backtitrated under constant mixing, with descending concentrations of NaOH (2, 1, 0.1 M) (10% final volume) resulting in a final suspension of 10 mg/ml MPZP in 20% HBC (pH 4.5). Lower concentrations then were prepared by serial dilution with vehicle (20% HBC, pH 4.5). Animals were administered the appropriate dose via a 2 ml/kg injection (0–20 mg MPZP/2 ml 20% HBC vehicle/kg body weight). For the 0 mg/kg dose of MPZP, animals were given 2 ml 20% HBC vehicle/kg body weight.

2.4. Experiment 1 — effect of MPZP on anxiety-like behavior

The defensive burying test was used to assess the effects of MPZP on anxiety-like behavior (Treit et al., 1981; De Boer and Koolhaas, 2003; Zorilla et al., 2003). This model has been validated by anxiolytic and anxiogenic compounds, which decrease and increase defensive burying behavior, respectively (Korte et al., 1994; De Boer and Koolhaas, 2003). For two consecutive days before defensive burying testing, animals were acclimated to the testing apparatus by placing them for 45 min in the testing cage (a polycarbonate rat housing cage with 2 cm of bedding covering the floor and a small hole centered on a long dimension of the cage 1 in. above the bedding to accommodate the shock probe on the subsequent test day). On the day of testing, animals were brought into the anteroom at least 2 h before testing began. Subjects were subcutaneously pretreated with MPZP (0, 5, 20 mg/kg) in a between-subjects design 1 h before their test session. For testing, animals were placed individually in the test cage, and a

shock probe connected to a Coulbourn precision shocker (model E13-01, Coulbourn Instruments, Allentown, PA) delivered one 1.5 mA shock (lasting <1 s) upon contact. As soon as the animal was shocked (verified by a startle response), the probe current was deactivated, and the 10 min test began. Contact with the shock probe under these conditions results in the rat displacing bedding material with treading-like movements of the forepaws and shoveling movements of the head, often directed toward the shock probe. Latency to the first display of burying behavior and time spent burying (in four 2.5 min bins throughout the 10 min test) were assessed (Korte et al., 1994). Defensive burying testing occurred 2–6 h into the dark cycle. Tests were Videotaped, and two reliable raters naive to the treatment conditions of the animals independently scored burying behavior of each subject ($r=0.97$, total duration; $r=0.87$, latency to bury). Rater averages were used in statistical analysis. A total of 24 rats (MPZP doses: 0 mg/kg, $n=11$; 5 mg/kg, $n=6$; 20 mg/kg, $n=7$) were used for this experiment. The unequal sample sizes reflect that, due to the limited availability of synthesized MPZP, we could only include $n=6$ for the 5 mg/kg group and $n=7$ for the 20 mg/kg group.

2.5. Experiment 2 — effect of MPZP on excessive drinking in an animal model of alcohol dependence

The effect of MPZP on drinking behavior was studied in an established animal model of alcohol dependence. In this model, rats previously trained to self-administer alcohol exhibit increased anxiety-like behavior and enhanced alcohol intake during withdrawal from chronic, intermittent alcohol exposure (dependent) compared to rats not chronically exposed to alcohol vapor (nondependent) (O'Dell et al., 2004; Funk et al., 2006; see also Roberts et al., 1996; Overstreet et al., 2002; Rimondini et al., 2002; Valdez et al., 2002 for related models).

2.5.1. Acquisition of operant alcohol self-administration

Animals were allowed to self-administer alcohol or water orally in a concurrent, two-lever, free-choice contingency. A continuous reinforcement (fixed ratio-1) schedule was used in which each lever press was reinforced. Animals acquired alcohol self-administration using a variation of the previously described saccharin fading free-choice operant conditioning protocol (Samson, 1986). The present procedure culminates in pharmacologically relevant levels of alcohol self-administration, as defined by blood alcohol levels (BALs), in nondependent animals with limited access to alcohol over a 6-week period (Roberts et al., 1999). The modified procedure in the present study utilized a sweetened solution containing 3% glucose and 0.125% saccharin (Sigma, St. Louis, MO) instead of water restriction and 0.2% saccharin to initiate and maintain operant responding (Funk et al., 2006). Animals respond for the sweetened solution within 1–2 training sessions, making water restriction unnecessary. Operant sessions during training were conducted 5 days per week between 0900 and 1500 (lights on at 0600). Operant sessions were 30 min in duration, except during the initial days of training in which sessions lasted up to 2 h to permit acquisition of responding for the sweetened solution. Alcohol (10% w/v) then was added to the sweetened solution, and once mean responding stabilized (around one week) the glucose was removed from the solution, leaving only 0.125% saccharin and 10% w/v ethanol. Animals were kept at this stage until mean responding again stabilized (around 1 week), and saccharin concentrations were gradually reduced in ~50% successive steps over 2–10 days, ultimately leaving an unadulterated 10% w/v ethanol solution. Animals then were maintained on 10% w/v ethanol for at least 3 weeks, and stable responders (±25% across three consecutive sessions) were evenly divided into two groups matched for baseline responding and exposed to intermittent ethanol vapors (dependent) or air (nondependent) as described below. A total of 16 rats (dependent, $n=8$; nondependent, $n=8$) were used for this experiment.

2.5.2. Operant self-administration apparatus

The self-administration system consisted of test chambers (Coulbourn Instruments, Allentown, PA) contained within wooden sound-attenuated ventilated cubicles. The test chambers were equipped with two retractable levers located 4 cm above the grid floor and 4.5 cm to either side of a small stainless steel receptacle containing two drinking cups. Two infusion pumps (Razel Scientific Instruments, Stamford, CT) were connected to the system so that a lever press resulted in the delivery of 0.1 ml of solution. Tap water was delivered to one dish, and the experimental solution (e.g., sweetened solution or alcohol) was delivered to the other dish. Fluid delivery and recording of operant self-administration were controlled by a computer. Lever presses were not recorded during the 0.5 s interresponse time-out interval when solution was being delivered.

2.5.3. Solutions for oral self-administration

Alcohol (10% w/v) was prepared with 95% ethyl alcohol and tap water. Glucose (3%) and/or saccharin (0–0.125%; Sigma, St. Louis, MO) was added to the water or alcohol solutions to achieve the appropriate concentration.

2.5.4. Dependence induction by alcohol vapor chambers

A recent modification of the alcohol dependence model was made to reflect the natural progression of alcohol dependence in which alcohol exposure occurs in a series of extended exposures followed by periods of withdrawal (O'Dell et al., 2004). Chronic exposure to intermittent alcohol vapor exposure elicits even higher alcohol self-administration than continuous vapor (O'Dell et al., 2004), and the intermittent procedure therefore was used to induce dependence in trained animals in the present study. Vapors were delivered on a 14 h on/10 h off schedule for 4 weeks before post-vapor testing began. This schedule of exposure has been shown to induce physical dependence (O'Dell et al., 2004). In the chambers, 95% alcohol flows from a large reservoir to a peristaltic pump (model QG-6, FMI Laboratory, Fluid Metering Inc., Syosett, NY). Ethanol is delivered from the pump to a sidearm flask at a flow rate that can be regulated. The flask is placed on a heater so that the drops of alcohol hitting the bottom of the flask are vaporized.

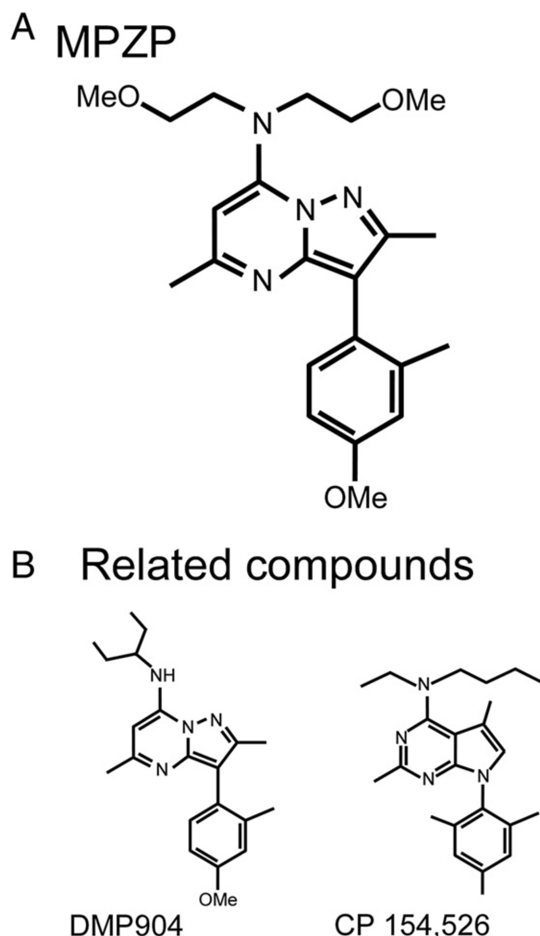

Fig. 1. Chemical structure of (A) *N*,*N*-bis(2-methoxyethyl)-3-(4-methoxy-2-methylphenyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-7-amine (MPZP) and (B) two related compounds: *N*-(1-ethylpropyl)-3-(4-methoxy-2-methylphenyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-7-amine (DMP904) and *N*-butyl-*N*-ethyl-2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (CP-154,526).

Air flow controlled by a pressure gauge is delivered to the flask and carries the alcohol vapors to the vapor chamber that contains the animal cages. The flow rate was set to deliver vapors that result in BALs between 0.125 and 0.250 g%.

Beginning 4 weeks after the onset of vapor exposure, post-vapor alcohol self-administration testing was conducted twice per week during acute withdrawal (6–8 h after cessation of daily vapor exposure). For testing the effects of MPZP on self-administration behavior, subjects were subcutaneously pre-treated with MPZP (0, 5, 10, 20 mg/kg) 1 h before their 30 min test session in a Latin square design with 3–4 days between tests. No carryover, order, or conditioning effects were detected.

2.5.5. Blood collection and measurement of blood alcohol levels

Throughout the time in vapors, blood samples were obtained 1–2 times per week to confirm that vapor-exposed animals had BALs between 0.125 and 0.250 g%. Vapor chambers were adjusted when BALs fell outside the 0.125–0.250 g% range, although this occurred rarely (<5% of the time spent in vapors). Blood samples were collected by the tail-snip method (0.1–0.2 ml) from all animals (both ethanol vapor-exposed dependent and control air-exposed nondependent groups) just after the vapors turned off (0800 h). Plasma (5 μl) was used for measuring BALs using an Analox AM 1 analyzer (Analox Instruments, Lunenburg, MA). The reaction is based on the oxidation of alcohol by alcohol oxidase in the presence of molecular oxygen (alcohol + O_2 → acetaldehyde + H_2O_2). The rate of oxygen consumption is directly proportional to the alcohol concentration. Single-point calibrations were done for each set of samples with reagents provided by Analox Instruments (0.025–0.400 g%). When dependent animals had BALs outside the 0.125–0.250 g% range, the evaporated ethanol values (ml/h) were adjusted to reestablish the correct range. As expected, BALs were always undetectable in nondependent animals, but tail bleeding was performed to control for any stress experienced during this procedure.

2.6. Statistical analyses

For analysis of competition binding assays, four-parameter logistic equations were fit to the mean % specific (total-nonspecific binding) [^{125}I]Tyr^0-sauvagine binding observed across concentrations of MPZP or the reference CRF_1 antagonist DMP904 in six independent experiments. The effect of MPZP on defensive burying behavior (latency to first bury and burying time) was analyzed by analysis of variance (ANOVA). One-way ANOVAs were used to analyze burying latency and total burying duration, with *Dose* a between-subjects factor. A two-way ANOVA was used to analyze burying duration across time, with *Dose* a between-subjects factor and *Time bin* (four 2.5 min bins) a repeated measure for duration of burying. Pre- *vs.* post-vapor operant responding (number of presses for alcohol or water, g/kg alcohol intake) was analyzed by two-way ANOVAs with *Test number* a within-subjects factor and *Vapor treatment* a between-subjects factor. The effect of MPZP on operant responding (number of presses

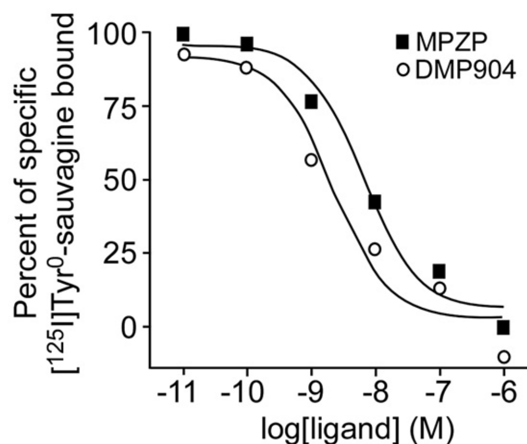

Fig. 2. Subtype nonselective binding affinity of MPZP and DMP904 for CRF receptors in rat cerebellar homogenates. The figure shows displacement of specific [^{125}I]-Tyr^0-sauvagine binding from rat cerebellar membrane homogenates by unlabeled MPZP or the reference CRF_1 antagonist DMP904. Data points represent mean inhibition observed across six independent experiments. Curves were fit using a four-parameter, single-site logistic regression equation.

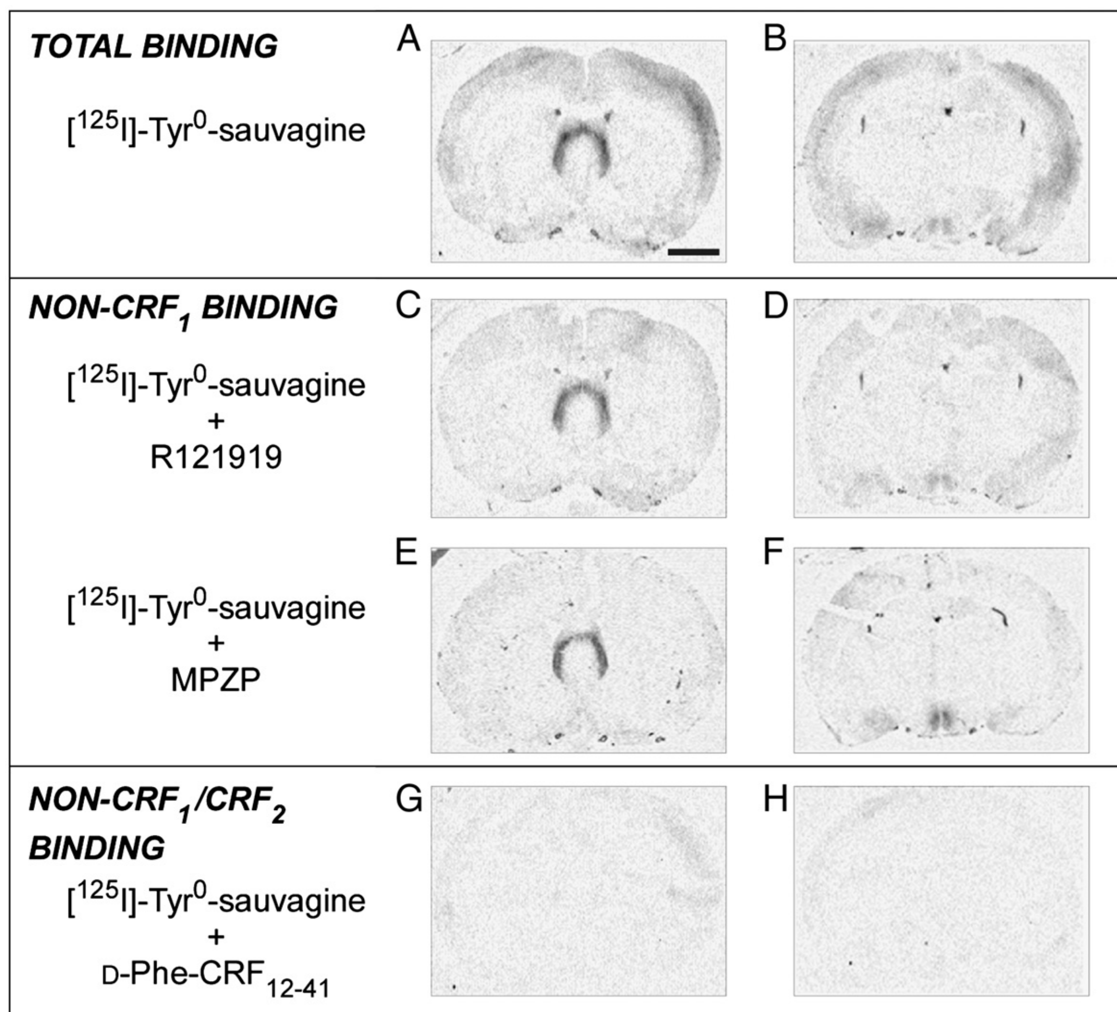

Fig. 3. Autoradiography of CRF receptors in rat brain. Slide-mounted coronal rat brain sections (20 μm) were incubated with $[^{125}I]Tyr^0$-sauvagine (0.2 nM) at the level of the lateral septum (*left panels*, A, C, E, G, bregma 0.20) or ventromedial nucleus of the hypothalamus (*right panels*, B, D, F, H, bregma −2.30). Representative autoradiographic images are shown from sections that were co-incubated with (A, B) assay buffer only ("total binding"), (C, D) the high-affinity selective CRF_1 antagonist R121919 (1 μM) to displace specific radioligand binding from CRF_1 receptors ("non-CRF_1 binding"), (E, F) MPZP, the putative, selective CRF_1 antagonist under study (3 μM), or (G, H) the subtype nonselective CRF_1/CRF_2 antagonist D-Phe-$CRF_{12–41}$ (300 nM) to displace specific radiolabel binding from CRF_1 and CRF_2 receptors ("non-CRF_1/CRF_2 binding"). Backgrounds were subtracted from all images using ImageJ (National Institutes of Health, Bethesda, MD). Scale bar = 2000 μm.

for alcohol or water, g/kg alcohol intake) was analyzed by a two-way ANOVA with *Dose* a within-subjects factor and *Vapor treatment* a between-subjects factor. Linear trend and sigmoidal regression analyses were used to characterize the dose–response curve of MPZP on operant responding. Unless stated otherwise, significant interactions were followed by Bonferroni/Dunn *post hoc* tests and $P \leq 0.05$ was considered statistically significant.

3. Results

3.1. Synthesis and in vitro characterization of MPZP

Fig. 1 compares the structure of MPZP with those of the pyrazolopyrimidine DMP904 and another widely studied CRF_1 antagonist, the pyrrolopyrimidine CP-154,526. Like the other ligands, MPZP has a heterocycle "core" unit and a confirmation-stabilizing ortho- and para-substituted "down" phenyl unit. Unlike DMP904 and CP-154,526, however, MPZP includes polar methoxy substituents in the "top" branched alkyl chains, intended to yield a compound with more "drug-like" lipophilicity (Zorrilla and Koob, 2004).

Fig. 2 shows data indicating that MPZP displaced specific $[^{125}I]$-Tyr^0-sauvagine binding from rat cerebellar homogenates on a similar order of potency as DMP904 ($pIC_{50} = 8.21 + 0.18$ *vs.* $8.67 + 0.27$, or $IC_{50} = 6.1$ *vs.* 2.1 nM, respectively), indicating that MPZP is a high-affinity CRF receptor ligand. Hill slopes approximated unity for both MPZP (0.91 ± 0.15) and DMP904 (0.85 ± 0.19), consistent with a one-binding site mode of competition, and estimated K_i values (95% confidence interval) were 4.9 (1.3–18.3) and 1.7 (0.3–15.1) nM, respectively. Specificity of MPZP for CRF_1 *vs.* CRF_2 receptors was determined via receptor autoradiography (Fig. 3) in which 3 μM MPZP did not displace $[^{125}I]$-Tyr^0-sauvagine binding from rat lateral septum or ventromedial hypothalamus, choroid

Table 1
Binding affinities for MPZP (1 μM): Pharmacological specificity for corticotropin-releasing factor receptors

Receptor tested	% Inhibition	Reference ligand	Reference ligand affinity (K_i, M)	Radioligand
Neurotransmitter related				
Adenosine, nonselective	0.7	Neca	5.99e−9	[^{3}H]Neca
Adrenergic, α_1, nonselective	−12.3	Phentolamine	6.62e−9	[^{3}H]7-meoxy-prazosin
Adrenergic, α_2, nonselective	15.8	Phentolamine	5.86e−9	[^{3}H]Rx 821002
Adrenergic, β, nonselective	−2.7	Alprenolol HCl	5.61e−9	[^{3}H]dihydro alprenolol
Dopamine transporter	−15.8	GBR12909	1.46e−8	[^{3}H]WIN 35,428
Dopamine, nonselective	5.0	Spiperone HCl	7.84e−10	[^{3}H]spiperone
γ-aminobutyric acid-A ($GABA_A$), agonist site	3.7	GABA	1.10e−8	[^{3}H]GABA
$GABA_A$, benzodiazepine, α_1 site	−3.0	Ro 15-1788 (flumazenil)	2.03e−9	[^{3}H]flunitrazepam
$GABA_B$	−17.6	(±) Baclofen	1.06e−6	[^{3}H]CGP 54626A
Glutamate, α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) site (ionotropic)	12.1	(±) AMPA HBr	3.66e−8	[^{3}H]AMPA acid
Glutamate, kainate site (ionotropic)	42.5	Kainic acid	8.79e−9	[^{3}H]kainic acid
Glutamate, *N*-methyl-D-aspartate (NMDA) agonist site (ionotropic)	−5.5	NMDA	1.23e−5	[^{3}H]CGP 39653
Glutamate, NMDA, glycine (strychnine-insensitive site) (ionotropic)	15.5	MDL 105,519	2.66e−8	[^{3}H]MDL 105,519
Glycine (strychnine-sensitive)	−12.9	Strychnine nitrate	4.42e−8	[^{3}H]strychnine
Histamine, H_1	5.1	Triprolidine	4.13e−9	[^{3}H]pyrilamine
Histamine, H_2	26.5	Tiotidine	2.66e−8	[^{125}I]aminopotentidine
Histamine, H_3	35.6	*N-a*-methylhistamine (NaMH)	5.75e−10	[^{3}H]*N-a*-mehistamine
Melatonin, nonselective	28.0	Melatonin	5.52e−10	[^{125}I]2-iodomelatonin
Muscarinic, M_1	−5.3	(−)Scopolamine, MeBr	7.32e−11	[^{3}H]scopolamine, *N*-methyl
Muscarinic, M_2	3.9	(−)Scopolamine, MeBr	3.02e−10	[^{3}H]scopolamine, *N*-methyl
Muscarinic, nonselective, central	7.4	Atropine sulfate	6.02e−10	[^{3}H]quinuclidinyl benzilate
Muscarinic, nonselective, peripheral	−8.3	Atropine sulfate	6.22e−10	[^{3}H]quinuclidinyl benzilate
Nicotinic, neuronal (α-bungarotoxin-insensitive)	−3.4	(±) Epibatidine	7.12e−11	[^{3}H]epibatidine
Norepinephrine transporter	11.9	Desipramine HCl	1.19e−9	[^{3}H]nisoxetine
Opioid, nonselective	5.8	Naloxone HCl	2.15e−9	[^{3}H]naloxone
Orphanin, Orl1	−3.7	Nociceptin	1.47e−9	[^{3}H]nociceptin
Serotonin transporter	11.9	Imipramine HCl	1.63e−8	[^{3}H]citalopram, *N*-methyl
Serotonin, nonselective	30.7	Methysergide maleate	5.41e−9	[^{3}H]lysergic acid diethylamide
Sigma, nonselective	0.7	Haloperidol	4.14e−9	[^{3}H]1,3-di-*o*-tolylguanidine
Steroids				
Estrogen	−29.1	17-β-estradiol	4.18e−10	[^{3}H]estradiol
Testosterone (cytosolic)	−8.2	Methyltrienolone	5.38e−10	[^{3}H]methyltrienolone
Ion channels				
Calcium channel, L-type (dihydropyridine site)	−8.4	Nifedipine	9.96e−10	[^{3}H]nitrendipine
Calcium channel, N-type	40.6	*W*-conotoxin GVIA	1.55e−11	[^{125}I]conotoxin GVIA
Potassium channel, atropine-sensitive	12.3	Glibenclamide	4.67e−10	[^{3}H]glibenclamide
Potassium channel, Ca^{2+} Act, VI	11.7	Apamin	6.86e−11	[^{125}I]apamin
Potassium channel, I_{Kr} (hERG)	6.3	Terfenadine	2.23e−6	[^{3}H]astemizole
Sodium, site 2	−19.0	Aconitine	1.42e−6	[^{3}H]batrachotoxin A 20-*a*-Benzo
Second messengers				
Nitric oxide, NOS (neuronal-binding)	18.6	Nitro-L-arginine	1.73e−8	[^{3}H]nitro-L-arginine
Prostaglandins				
Leukotriene, LTB4 (Blt)	7.1	LTB4	4.54e−10	[^{3}H]LTB4
Leukotriene, LTD4 (Cyslt1)	3.6	LTD4	5.32e−9	[^{3}H]LTD4
Thromboxane A2	8.6	Pinane-thromboxane A2	5.11e−8	[^{3}H]SQ 29,548
Growth factors/hormones				
Corticotropin-releasing factor (CRF), nonselective	**93.7**	**Tyr^0-oCRF**	**4.58e−9**	**[^{125}I]Tyr^0-oCRF**
Oxytocin	−4.1	Oxytocin	2.31e−9	[^{3}H]oxytocin
Platelet activating factor (PAF)	15.9	C_{16}-PAF	1.66e−9	hexadecyl-[^{3}H]-acetyl-PAF
Thyrotropin-releasing hormone (TRH)	−3.5	TRH	8.54e−8	[^{3}H]-(3-methyl-His^2)TRH
Brain/gut peptides				
Angiotensin II, AT1	2.0	Angiotensin II (human)	2.35e−8	[^{125}I]-(Sar^1-Ile^8) angiotensin
Angiotensin II, AT2	−3.0	Angiotensin II (human)	1.21e−9	[^{125}I]Tyr^4-angiotensin II

(*continued on next page*)

Table 1 (*continued*)

Receptor tested	% Inhibition	Reference ligand	Reference ligand affinity (K_i, M)	Radioligand
Brain/gut peptides				
Bradykinin, BK2	−5.8	Bradykinin trifluoroacetate salt	3.51e−10	[^{3}H]bradykinin
Cholecystokinin, CCK1	−3.7	CCK-8 (sulfated)	3.83e−11	[^{125}I]CCK-8
Cholecystokinin, CCK2	6.5	CCK-8 (sulfated)	8.63e−10	[^{125}I]CCK-8
Endothelin, ET-A	2.4	Endothelin-1	1.48e−10	[^{125}I]endothelin-1
Endothelin, ET-B	3.5	Endothelin-1	1.43e−10	[^{125}I]endothelin-1
Galanin, nonselective	2.5	Galanin (porcine)	5.87e−10	[^{125}I]galanin
Neurokinin, NK1	3.8	Substance P	3.51e−9	[^{3}H]substance P
Neurokinin, NK2	3.8	Neurokinin A	6.70e−10	[^{125}I]neurokinin A
Neurokinin, NK3	−13.6	Eledoisin	6.72e−9	[^{125}I]eledoisin
Vasoactive intestinal peptide (VIP), nonselective	16.6	VIP	2.40e−9	[^{125}I]VIP
Vasopressin 1	−0.02	Arg^8-vasopressin (AVP)	4.37e−9	[^{3}H]phenyl 3,4,5-8-AVP
Enzymes				
Decarboxylase, glutamic acid	−5.6	Aminooxy acetic acid	1.67e−10	[^{14}C]glutamic acid
Esterase, acetylcholine	4.6	Eserine (physostigmine)	3.61e−7	acetylthiocholine
Oxidase, monoamine oxidase A, peripheral	22.0	Ro 41-1049	1.64e−8	[^{14}C]-5-hydroxytryptamine (serotonin)
Oxidase, monoamine oxidase B, peripheral	−2.6	Ro 16-6491 HCl	4.40e−8	[^{14}C]phenylethylamine
Transferase, choline acetyl	−12.6	Bromoacetylcholine bromide	2.46e−9	[^{14}C]acetyl coenzyme

Data are expressed as % mean inhibition of specific binding from duplicate samples.

plexus, or cerebral arterioles, regions that are rich with CRF_2, but not CRF_1, receptors (Grigoriadis et al., 1996; Heinrichs et al., 2002). In contrast, MPZP displaced most [^{125}I]-Tyr^0-sauvagine binding from cortex and basolateral amygdala, regions which contain abundant levels of CRF_1 receptors. Binding also remained in amygdaloid nuclei that contain high CRF_2 receptor distribution, such as the medial amygdala. Thus, MPZP has high specificity for CRF_1 and no measurable specificity for CRF_2 receptors at up to 3 μM concentrations. The pattern of residual [^{125}I]-Tyr^0-sauvagine binding in the presence of MPZP resembled that observed in the presence of R121919, a recognized high-affinity, highly selective CRF_1 antagonist (Heinrichs et al., 2002; Chen et al., 2004) (Fig. 3).

Pharmacological selectivity of MPZP was further assessed using the NovaScreen commercial binding assay screening service (GEN SEP I panel, Hanover, MD). As expected, MPZP (1 μM) inhibited 93.7% of specific [^{125}I]Tyr^0-oCRF binding to cortical membrane preparations. In contrast, MPZP did not exhibit high potency for any of 62 other receptors, transporters, ion channels, or enzymes studied (all <43% inhibition of specific binding/activity) further confirming high selectivity of this compound for CRF_1 receptors (Table 1).

Although the binding affinity of MPZP for CRF_1 receptors is slightly less potent than that of DMP904 and CP-154,526, MPZP has lipophilicity 2 to 3.5 orders lower than those of these reference compounds and in a range more typical of CNS-acting therapeutics (compare *cLogP* and *cLogD* across compounds, Table 2) (Zorrilla and Koob, 2004). The molecular volume and polar surface area of MPZP, like the other CRF_1 ligands, are consistent with an absorbable, blood–brain barrier-penetrating molecule (Kelder et al., 1999; Zhao et al., 2007; Fu et al., 2005; Liu et al., 2004).

3.2. Experiment 1 — effect of MPZP on anxiety-like behavior

A model of active anxiety-like behavior highly regulated by the CRF system (Treit et al., 1981; De Boer and Koolhaas, 2003; Korte et al., 1994) was used to assess the anxiolytic properties of MPZP (Fig. 4). MPZP significantly increased the latency to bury [$F(2,23)=4.64$, $P=0.04$], with *post hoc* analyses showing that both the 5 and 20 mg/kg doses of MPZP increased the latency to start burying compared to vehicle (0 mg/kg) pretreatment (Fig. 4A). Systemic pretreatment with MPZP also dose-dependently reduced the total duration of defensive burying behavior [$F(2,23)=3.63$, $P=0.04$]. As shown in Fig. 4B, *post hoc* analyses indicated that the 20 mg/kg dose of MPZP significantly reduced the duration of burying across the 10-min observation period compared to vehicle (0 mg/kg) pretreatment. Thus, MPZP, a CRF_1 ligand, potently decreased shock-elicited active anxiety-like behavior in the defensive burying test, supporting proposed anxiolytic properties of this compound.

Table 2
Selected physiochemical properties of MPZP and reference CRF_1 antagonists

	MPZP	DMP904	CP-154,526
CAS registry number	202579-76-8	303579-74-6	157286-86-7
cLogP	2.95±1.13	4.80±1.10	6.63±1.30
cLogD, pH 7	2.93	4.80	6.15
pK_a	5.32±0.30	4.46±0.40	7.20±0.30
Polar surface area (Å^2)	61.1	51.5	29.0
Molar volume (cm^3/mol)	346.2±7.0	311.2±7.0	342.0±7.0

Physiochemical properties were calculated using Advanced Chemistry Development (ACD/Labs) Software v.8.14 for Solaris (ACD/Labs). CAS, Chemical Abstracts Service.

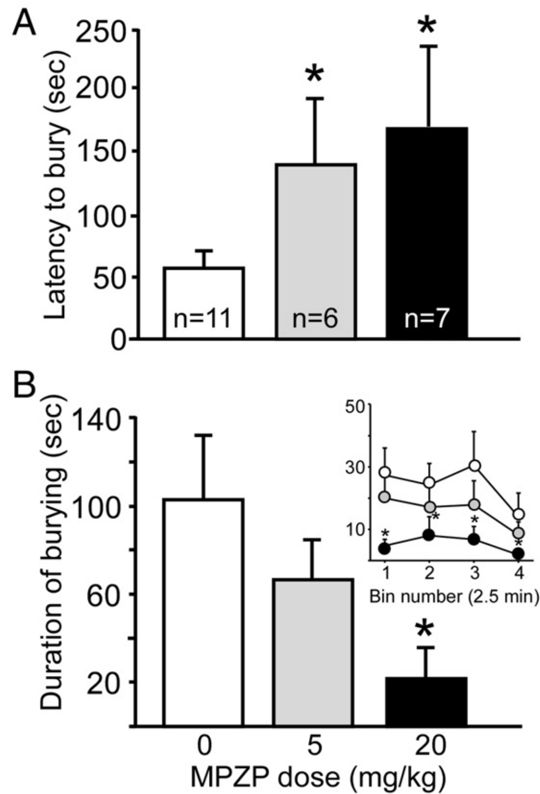

Fig. 4. The anxiolytic-like effect of MPZP in the defensive burying model of active anxiety-like behavior. (A) Latency to bury. MPZP increased the latency to first engage in burying behavior following contact with the shock probe. (B) Total burying duration (s) and burying duration (s) across time (2.5 min bins) (inset). MPZP reduced defensive burying time, but the attenuating effects of MPZP on burying duration did not significantly differ across 2.5 min bins. *$P<0.05$ compared to vehicle (0 mg/kg MPZP) treated controls. Data are shown as mean ± SEM (n=6–11 rats per dose).

3.3. Experiment 2 — effect of MPZP on excessive drinking in an animal model of alcohol dependence

Fig. 5 illustrates alcohol and water self-administration behavior before and after dependence induction via chronic intermittent alcohol vapor exposure. Post-vapor testing was conducted when dependent animals were in acute withdrawal (6–8 h after removal from vapors). The increased responding for alcohol observed at this time-point in dependent animals is consistent with previous studies of the dependence model during acute (2 h) (Roberts et al., 1996; O'Dell et al., 2004; Funk et al., 2006), 6–8 h (Sabino et al., 2006), or protracted 2-week (Roberts et al., 2000) withdrawal from alcohol vapors. There were main effects of *Vapor treatment* [g/kg intake: $F(1,98)=4.79$, $P=0.04$, Fig. 5A; trend toward main effect of *Vapor treatment* on alcohol responses: $F(1,98)=3.88$, $P=0.06$, n.s.] and *Test number* [g/kg intake: $F(7,98)=7.04$, $P<0.0001$, Fig. 5A; alcohol responses: $F(7,98)=7.51$, $P<0.0001$, Fig. 5B], and an interaction between the two factors [g/kg intake: $F(7,98)=5.76$, $P<0.0001$, Fig. 5A; alcohol responses: $F(7,98)=4.87$, $P<0.0001$, Fig. 5B] on alcohol self-administration. *Post hoc* analyses indicated that post-vapor g/kg intake and lever responses for alcohol were higher in dependent animals compared to both nondependent animals and pre-vapor responding (all Ps<0.05, Fig. 5A and B). Pre-vapor alcohol self-administration was not different between the two groups (Ps>0.05, Fig. 5A and B). Self-administration of water was

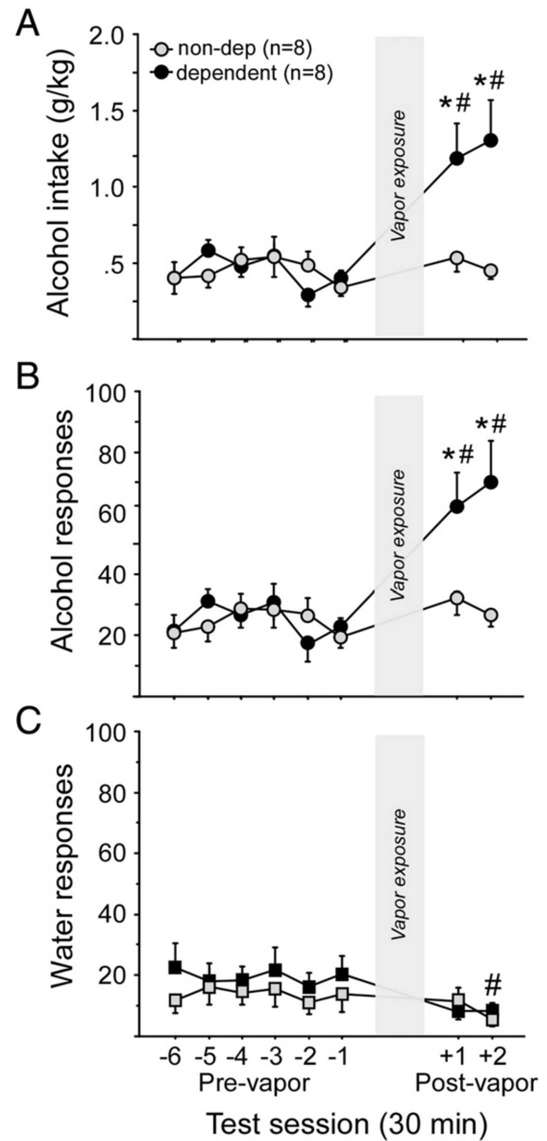

Fig. 5. Operant self-administration behavior (A, g/kg intake; B, lever presses/alcohol response; C, lever presses/water response) prior to and following dependence induction via chronic intermittent alcohol vapor exposure (gray shading). Post-vapor testing was conducted when dependent animals were in acute withdrawal (6–8 h after removal from vapors). There were main effects of *Vapor treatment* (dependent *vs.* nondependent animals) and *Test session* (pre- *vs.* post-vapor tests) and an interaction between these two factors on alcohol self-administration. *Post hoc* analyses indicated that post-vapor alcohol self-administration in dependent animals was higher than post-vapor alcohol self-administration in nondependent animals (* in A, B) and compared to pre-vapor alcohol self-administration (# in A, B). There was a main effect of *Test session* on water self-administration such that post-vapor water self-administration was slightly, but significantly, lower on the post-vapor test 2 compared to pre-vapor water self-administration tests (# in C). However, there were no differences in water self-administration either before or after vapors in dependent animals compared to nondependent controls. *Compared to nondependent controls. #Compared to pre-vapor test sessions ($P<0.05$). Data are shown as mean ± SEM (n=8 per vapor treatment group).

slightly, but significantly, lower post-vapor [main effect of test number, $F(2,28)=6.36$, $P=0.005$; all pre-vapor tests > post-vapor test 2, $P<0.05$, Fig. 5C]. Water responses did not differ between nondependent and dependent animals before or after vapor exposure (no main effect of *Vapor treatment* or *Vapor treatment* × *Test number* interaction, Ps > 0.05, Fig. 5C). The data demonstrate that chronic intermittent alcohol vapor exposure in dependent animals elicits increased alcohol drinking during acute withdrawal.

Fig. 6 illustrates the effect of MPZP on alcohol (g/kg intake) and water (responses) self-administration in dependent and nondependent animals. Overall, dependent animals self-administered significantly more alcohol than nondependent animals [main effect of *Vapor treatment*: $F(1,42)=32.61$, $P<0.0001$, Fig. 6A]. In addition, there was a main effect of MPZP [$F(3,42)=3.07$, $P=0.03$] and a *Vapor treatment* × *Dose* interaction [$F(3,42)=3.30$, $P=0.03$; 0 mg/kg dose *vs.* 20 mg/kg dose, $P=0.005$, dependent group only] on alcohol self-administration (g/kg intake, Fig. 6A). Linear contrast analyses detected a *Vapor treatment* × *Dose* interaction [$F(1,14)=6.31$, $P=0.02$], such that MPZP dose-dependently reduced alcohol self-administration (g/kg intake) in dependent animals [$F(1,7)=6.87$, $P=0.03$] but not in nondependent animals [$F(1,7)=0.01$, $P=0.95$, Fig. 6A]. Sigmoidal regression showed a significant sigmoidal dose–response fit to the MPZP-induced reduction of alcohol self-administration in dependent animals ($r^2=0.907$, $P<0.05$; $ED_{50}=10.7$ mg/kg MPZP). MPZP had no effect on water self-administration in either dependent or nondependent animals.

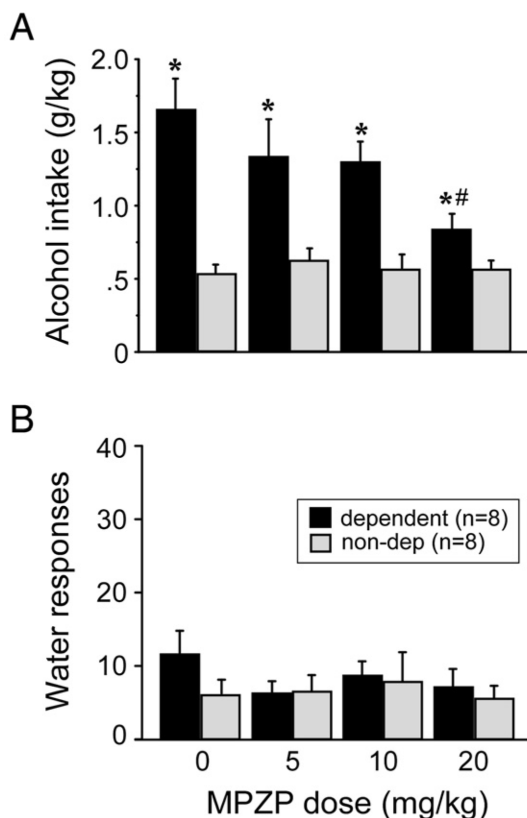

Fig. 6. The effect of MPZP on operant self-administration of (A) alcohol (g/kg) and (B) water (responses) in dependent and nondependent rats. Testing was conducted when dependent animals were in acute withdrawal (6–8 h after removal from vapors). There were main effects of *Vapor treatment* (dependent *vs.* nondependent animals) and MPZP dose (0, 5, 10, 20 mg/kg) on alcohol self-administration (g/kg intake) detected using ANOVA and dose–response fit analyses. Overall, dependent animals self-administered significantly more alcohol than nondependent animals (* in A). MPZP significantly reduced alcohol self-administration only in dependent animals, indicated by a significant downward sigmoidal trend ($r^2=0.907$, $P<0.05$; $ED_{50}=10.7$ mg/kg MPZP, no indicator) and a reduction with the 20 mg/kg dose compared to vehicle (0 mg/kg MPZP) (# in A). MPZP had no effect on alcohol self-administration in nondependent animals (A) or on water self-administration (responses) in either dependent or nondependent animals (B). *Note*: Alcohol self-administration data are expressed in g/kg intake, a more pharmacologically informative measure of alcohol consumption than lever responses, but the pattern of changes seen for alcohol responses was similar to that for g/kg (dependent animals: 0 mg/kg = 89 ± 11, 5 mg/kg = 72 ± 14, 10 mg/kg = 70 ± 7, 20 mg/kg = 47 ± 7; nondependent animals: 0 mg/kg = 32 ± 4, 5 mg/kg = 37 ± 5, 10 mg/kg = 32 ± 5, 20 mg/kg = 33 ± 4). *Compared to nondependent controls. #Compared to vehicle (0 mg/kg MPZP) ($P<0.05$). Data are shown as mean ± SEM ($n=8$ per vapor treatment group; MPZP doses were administered using a within-subjects Latin square design).

4. Discussion

The present report describes the initial pharmacological and behavioral characterization of a non-peptide small molecule, high-affinity CRF_1 specific antagonist, *N*,*N*-bis(2-methoxyethyl)-3-(4-methoxy-2-methylphenyl)-2,5-dimethyl-pyrazolo [1,5-*a*]pyrimidin-7-amine (MPZP), not previously reported in the peer-reviewed literature. MPZP exhibits lipophilicity more characteristic of existing CNS-acting drugs and substantially lower than that of many CRF_1 antagonist predecessors (Zorrilla and Koob, 2004). Our data demonstrate that MPZP has high specificity and affinity for CRF_1 receptors, has potent anxiolytic-like activity, and significantly reduces the increased levels of alcohol drinking seen during acute withdrawal in dependent animals without altering operant responding in nondependent subjects. The results suggest possible experimental and clinical indications for MPZP in further understanding and treating stress-related disorders such as anxiety and alcohol dependence.

The defensive burying model is a test of active anxiety-like behavior (Treit et al., 1981; De Boer and Koolhaas, 2003) and has been validated by several anxiolytic and anxiogenic compounds (Korte et al., 1994; De Boer and Koolhaas, 2003). Defensive burying is highly dependent on the extrahypothalamic CRF system (Basso et al., 1999; Korte et al., 1994). CRF administration increases defensive burying in rats (Diamant et al., 1992), and CRF antagonists block this response (Basso et al., 1999). Thus, the ability of MPZP to robustly attenuate burying behavior in the present study confirms a specific role of CRF_1 in mediating defensive burying behavior (Zorrilla et al., 2003) and suggests that MPZP may be a potent anxiolytic-like drug. MPZP also significantly reduced excessive drinking during withdrawal in alcohol-dependent animals similarly to other non-peptide CRF_1 antagonists (Chu et al., 2007; Funk et al., 2007; Gehlert et al., 2007; Sabino et al., 2006) without decreasing alcohol self-administration in nondependent animals. In addition, MPZP had no effect on nondependent binge drinking of sweetened alcohol in another study (Ji et al., in press). The fact that MPZP does *not* reduce binge-like self-

administration of sweetened alcohol (Ji et al., in press), self-administration of alcohol in nondependent animals (present report), or self-administration of water in dependent or nondependent animals (present report) not only argues against sedative effects of MPZP at the doses tested but also confirms specificity of this compound for the dependence model. Withdrawal-induced drinking in dependent animals in the present study is hypothesized to be motivated in part by an attempt to reduce the anxiety-like state associated with withdrawal (Valdez et al., 2002). Motivational signs of withdrawal (e.g., anxiety, dysphoria, malaise) are considered important in the maintenance and relapse of alcohol consumption in human alcoholics (Koob, 2003; Cappell and LeBlanc, 1979; Lowman et al., 1996), arguably more important than physical symptoms of withdrawal. The effects of MPZP on alcohol self-administration may be due, at least in part, to its anxiolytic-like properties.

MPZP may affect anxiety-like behavior and alcohol drinking via action on CRF_1 cells of the extrahypothalamic CRF system in the extended amygdala. The CRF peptidergic system is distributed throughout the brain, with high concentrations of cell bodies in the paraventricular nucleus of the hypothalamus and in extrahypothalamic areas of the extended amygdala. Extrahypothalamic CRF cell groups include the bed nucleus of the stria terminalis (BNST) and central (CeA) and basolateral subdivisions of the amygdala (Bloom et al., 1982), regions that are known to mediate anxiety-like behavior (Walker and Davis, 1997). Acute withdrawal from alcohol is accompanied by increased CRF release in the CeA (Merlo-Pich et al., 1995; Zorrilla et al., 2001) and lateral BNST (Olive et al., 2002) as well as increased anxiety-like behavior (Baldwin et al., 1991; Rassnick et al., 1993). Administration of nonspecific CRF receptor antagonists directly into the CeA reduces anxiety-like behavior (Rassnick et al., 1993) and decreases excessive alcohol intake (Funk et al., 2006) associated with acute withdrawal in dependent rats.

Many studies indicate that anxiolytic-like actions of CRF_1 antagonists are doubly dissociable from their actions to block pituitary CRF_1 receptors (and thereby corticosterone responses) (Zorrilla and Koob, 2004). Several CRF_1 antagonists exert anxiolytic-like behavior at doses that do not alter adrenocorticotropic hormone or corticosterone responses. Likewise, other CRF_1 antagonists can alter hypothalamic–pituitary–adrenal responses without affecting anxiety-like behavior. The same dissociation from an hypothalamic–pituitary–adrenal axis mechanism also may apply to the ability of CRF_1 antagonists to attenuate dependence-induced excessive drinking, given that intracerebral CRF_1 antagonist administration reduces ethanol self-administration (Funk et al., 2006).

MPZP has physiochemical properties that are consistent with a CNS-acting, blood–brain barrier-penetrating compound with adequate solubility. This set of characteristics includes a *cLogP* and physiological *cLogD* of between 0 and 3 (Lin and Lu, 1997; Zorrilla and Koob, 2004), a polar surface area of less than 106 Å^2 (Zhao et al., 2007), and, perhaps even more preferred, of ~60 Å^2 or less (Kelder et al., 1999; Ertl et al., 2000), a molar volume <350 cm^3/mol, and a relatively neutral (weak acid) acid-base ionization/dissociation constant (Lewi et al., 2004; Fischer et al., 1998). These physiochemical properties, especially the reduced lipophilicity of MPZP, compare favorably to those of many previously reviewed, first-generation CRF_1 receptor antagonists (Zorrilla and Koob, 2004). The physiochemical properties also are strong *in silico* predictors of human pharmacokinetic and toxicity measures, including drug transport processes, plasma protein binding, volume of distribution, and Ames genotoxicity (Osterberg and Norinder, 2001; Norinder and Osterberg, 2001; Votano et al., 2004; Lobell and Sivarajah, 2003; Lombardo et al., 2002), and suggest that MPZP may exhibit more drug-like properties than first-generation CRF_1 antagonists. Future studies of this promising compound may determine whether MPZP shares the desirable pharmacokinetic and pharmacodynamic properties possessed by newer CRF_1 antagonist series that are not yet widely available to the academic community (Gehlert et al., 2007; Ising et al., 2007; Gross et al., 2005).

In summary, the present report introduces a new compound, MPZP, with high affinity and specificity for CRF_1 receptors. Systemic pretreatment with MPZP reduced anxiety-like behavior in the defensive burying model and reduced alcohol self-administration in alcohol-dependent rats. This compound also may have more general applications. CRF and its receptors are hypothesized to play a critical role in addiction to other drugs of abuse. Withdrawal from chronic nicotine, opiates, cannabinoids, and cocaine elicits increased release of CRF in the CeA and/or increased anxiety-like behavior (Contarino and Papaleo, 2005; George et al., in press; Heinrichs et al., 1995; Rodriguez de Fonseca et al., 1997; Zorrilla et al., 2001; Weiss et al 2001). Many drug withdrawal-induced changes can be reversed by CRF antagonists (Weiss et al., 2001). Altogether, the findings suggest that MPZP or related compounds may have therapeutic potential for treating pathological anxiety and drug addiction.

Acknowledgements

This is publication number 19119 from The Scripps Research Institute. The authors thank Maury Cole, Yanabel Grant, Elena Crawford, Maegan Mattock, Robert Lintz, and Molly Brennan for excellent technical assistance and Mike Arends for editorial assistance. The authors also thank Dr. Jean Rivier (The Salk Institute) for providing sauvagine and D-Phe-$CRF_{12–41}$. Supported by the Pearson Center for Alcoholism and Addiction Research, the Skaggs Institute for Chemical Biology, National Institutes of Health grants AA06420, AA08459, and AA12602 from the National Institute on Alcohol Abuse and Alcoholism, DK26741 from the National Institute of Diabetes and Digestive and Kidney Diseases, and a Hungarian State Eötvös Fellowship to Éva Fekete. GFK, EPZ, KDJ, and PW have filed a United States provisional patient application (no. 60/972, 409) relevant to the present work.

References

Arvanitis AG, Chorvat RJ. Azolotriazines and pyrimidines [patent number WO9803510]. 1998.

Baldwin HA, Rassnick S, Rivier J, Koob GF, Britton KT. CRF antagonist reverses the "anxiogenic" response to ethanol withdrawal in the rat. Psychopharmacology 1991;103:227–32.

Basso AM, Spina M, Rivier J, Vale W, Koob GF. Corticotropin-releasing factor antagonist attenuates the "anxiogenic-like" effect in the defensive burying paradigm but not in the elevated plus-maze following chronic cocaine in rats. Psychopharmacology 1999;145:21–30.

Bloom FE, Battenberg EL, Rivier J, Vale W. Corticotropin-releasing factor (CRF): immunoreactive neurones and fibers in rat hypothalamus. Regul Pept 1982;4:43–8.

Breese GR, Chu K, Dayas CV, Funk D, Knapp DJ, Koob GF, et al. Stress enhancement of craving during sobriety: a risk for relapse. Alcohol Clin Exp Res 2005;29:185–95.

Britton KT, Lee G, Dana R, Risch SC, Koob GF. Activating and 'anxiogenic' effects of corticotropin releasing factor are not inhibited by blockade of the pituitary–adrenal system with dexamethasone. Life Sci 1986a;39:1281–6.

Britton KT, Lee G, Vale W, Rivier J, Koob GF. Corticotropin releasing factor (CRF) receptor antagonist blocks activating and 'anxiogenic' actions of CRF in the rat. Brain Res 1986b;369:303–6.

Cappell H, LeBlanc AE. Tolerance to, and physical dependence on, ethanol: why do we study them? Drug Alcohol Depend 1979;4:15–31.

Chang CP, Pearse II RV, O'Connell S, Rosenfeld MG. Identification of a seven transmembrane helix receptor for corticotropin-releasing factor and sauvagine in mammalian brain. Neuron 1993;11:1187–95.

Chen FM, Bilezikjian LM, Perrin MH, Rivier J, Vale W. Corticotropin releasing factor receptor-mediated stimulation of adenylate cyclase activity in the rat brain. Brain Res 1986;381:49–57.

Chen R, Lewis KA, Perrin MH, Vale WW. Expression cloning of a human corticotropin-releasing-factor receptor. Proc Natl Acad Sci U S A 1993;90:8967–71.

Chen C, Wilcoxen KM, Huang CQ, Xie YF, McCarthy JR, Webb TR, et al. Design of 2,5-dimethyl-3-(6-dimethyl-4-methylpyridin-3-yl)-7-dipropylaminopyrazolo[1,5-*a*]pyrimidine (NBI 30775/R121919) and structure–activity relationships of a series of potent and orally active corticotropin-releasing factor receptor antagonists. J Med Chem 2004;47:4787–98.

Chu K, Koob GF, Cole M, Zorrilla EP, Roberts AJ. Dependence-induced increases in ethanol self-administration in mice are blocked by the CRF1 receptor antagonist antalarmin and by CRF1 receptor knockout. Pharmacol Biochem Behav 2007;86:813–21.

Contarino A, Papaleo F. The corticotropin-releasing factor receptor-1 pathway mediates the negative affective states of opiate withdrawal. Proc Natl Acad Sci U S A 2005;102:18649–54.

Cowen MS, Lawrence AJ. Alcoholism and neuropeptides: an update. CNS Neurol Disord Drug Targets 2006;5:233–9.

De Boer SF, Koolhaas JM. Defensive burying in rodents: ethology, neurobiology and psychopharmacology. Eur J Pharmacol 2003;463:145–61.

Diamant M, Croiset G, de Wied D. The effect of corticotropin-releasing factor (CRF) on autonomic and behavioral responses during shock-prod burying test in rats. Peptides 1992;13:1149–58.

Dunn AJ, Berridge CW. Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? Brain Res Rev 1990;15:71–100.

Ertl P, Rohde B, Selzer P. Fast calculation of molecular polar surface area as a sum of fragment based contributions and its application to the prediction of drug transport properties. J Med Chem 2000;43:3714–7.

Fischer H, Gottschlich R, Seelig AJ. Blood–brain barrier permeation: molecular parameters governing passive diffusion. Membr Biol 1998;165:201–11.

Fu XC, Song ZF, Fu CY, Liang WQ. A simple predictive model for blood–brain barrier penetration. Pharmazie 2005;60:354–8.

Funk CK, O'Dell LE, Crawford EF, Koob GF. Corticotropin-releasing factor within the central nucleus of the amygdala mediates enhanced ethanol self-administration in withdrawn, ethanol-dependent rats. J Neurosci 2006;26:11324–32.

Funk CK, Zorrilla EP, Lee MJ, Rice KC, Koob GF. Corticotropin-releasing factor 1 antagonists selectively reduce ethanol self-administration in ethanol-dependent rats. Biol Psychiatry 2007;61:78–86.

Gehlert DR, Cippitelli A, Thorsell A, Le AD, Hipskind PA, Hamdouchi C, et al. 3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-8-(1-ethylpropyl)-2,6-dimethyl-imidazo[1,2-*b*]pyridazine: a novel brain-penetrant, orally available corticotropin-releasing factor receptor 1 antagonist with efficacy in animal models of alcoholism. J Neurosci 2007;27:2718–26.

George O, Ghozland S, Azar MR, Zorrilla EP, Parsons LH, O'Dell LE, et al. A neurobiological mechanism for the "hook" in nicotine dependence. Proc Natl Acad Sci USA 2007;104:17198–203.

Giguere V, Labrie F, Cote J, Coy DH, Sueiras-Diaz J, Schally AV. Stimulation of cyclic AMP accumulation and corticotropin release by synthetic ovine corticotropin-releasing factor in rat anterior pituitary cells: site of glucocorticoid action. Proc Natl Acad Sci U S A 1982;79:3466–9.

Gilligan PJ, Baldauf C, Cocuzza A, Chidester D, Zaczek R, Fitzgerald LW, et al. The discovery of 4-(3-pentylamino)-2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)-pyrazolo-[1,5-*a*]-pyrimidine: a corticotropin-releasing factor (hCRF1) antagonist. Bioorg Med Chem 2000;8:181–9.

Grigoriadis DE, Liu XJ, Vaughn J, Palmer SF, True CD, Vale WW, et al. 125I-Tyro-sauvagine: a novel high affinity radioligand for the pharmacological and biochemical study of human corticotropin-releasing factor 2 alpha receptors. Mol Pharmacol 1996;50:679–86.

Gross RS, Guo Z, Dyck B, Coon T, Huang CQ, Lowe RF, et al. Design and synthesis of tricyclic corticotropin-releasing factor-1 antagonists. J Med Chem 2005;48:5780–93.

Hansson AC, Cippitelli A, Sommer WH, Fedeli A, Bjork K, Soverchia L, et al. Variation at the rat Crhr1 locus and sensitivity to relapse into alcohol seeking induced by environmental stress. Proc Natl Acad Sci U S A 2006;103:15236–41.

Heilig M, Egli M. Pharmacological treatment of alcohol dependence: target symptoms and target mechanisms. Pharmacol Ther 2006;111:855–76.

Heinrichs SC, Menzaghi F, Schulteis G, Koob GF, Stinus L. Suppression of corticotropin-releasing factor in the amygdala attenuates aversive consequences of morphine withdrawal. Behav Pharmacol 1995;6:74–80.

Heinrichs SC, Lapsansky J, Lovenberg TW, De Souza EB, Chalmers DT. Corticotropin-releasing factor CRF1, but not CRF2, receptors mediate anxiogenic-like behavior. Regul Pept 1997;71:15–21.

Heinrichs SC, De Souza EB, Schulteis G, Lapsansky JL, Grigoriadis DE. Brain penetrance, receptor occupancy and antistress in vivo efficacy of a small molecule corticotropin releasing factor type I receptor selective antagonist. Neuropsychopharmacology 2002;27:194–202.

Hoare SR, Sullivan SK, Schwarz DA, Ling N, Vale WW, Crowe PD, et al. Ligand affinity for amino-terminal and juxtamembrane domains of the corticotropin releasing factor type I receptor: regulation by G-protein and nonpeptide antagonists. Biochemistry 2004;43:3996–4011.

Hoare SR, Sullivan SK, Ling N, Crowe PD, Grigoriadis DE. Mechanism of corticotropin-releasing factor type I receptor regulation by nonpeptide antagonists. Mol Pharmacol 2005;68:260.

Hoare SR, Brown BT, Santos MA, Malany S, Betz SF, Grigoriadis DE. Single amino acid residue determinants of non-peptide antagonist binding to the corticotropin-releasing factor1 (CRF1) receptor. Biochem Pharmacol 2006;72:244–55.

Ising M, Zimmermann US, Künzel HE, Uhr M, Foster AC, Learned-Coughlin SM, et al. High-affinity CRF1 receptor antagonist NBI-34041: preclinical and clinical data suggest safety and efficacy in attenuating elevated stress response. Neuropsychopharmacology 2007;32:1941–9.

Ji D, Gilpin NW, Richardson HN, Rivier CL, Koob GF. Effects of naltrexone, duloxetine, and a CRF_1 receptor antagonist on binge-like alcohol drinking in rats. in press.

Kehne J, De Lombaert S. Non-peptidic CRF_1 receptor antagonists for the treatment of anxiety, depression and stress disorders. Curr Drug Targets CNS Neurol Disord 2002;1:467–93.

Kelder J, Grootenhuis PD, Bayada DM, Delbressine LP, Ploemen JP. Polar molecular surface as a dominating determinant for oral absorption and brain penetration of drugs. Pharm Res 1999;16:1514–9.

Koob GF. Alcoholism: allostasis and beyond. Alcohol Clin Exp Res 2003;27:232–43.

Korte SM, Korte-Bouws GAH, Bohus B, Koob GF. Effect of corticotropin-releasing factor antagonist on behavioral and neuroendocrine responses during exposure to defensive burying paradigm in rats. Physiol Behav 1994;56:115–20.

Lelas S, Wong H, Li YW, Heman KL, Ward KA, Zeller KL, et al. Anxiolytic-like effects of the corticotropin-releasing factor1 (CRF1) antagonist DMP904 [4-(3-pentylamino)-2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)-pyrazolo-[1,5-*a*]-pyrimidine] administered acutely or chronically at doses occupying central CRF1 receptors in rats. J Pharmacol Exp Ther 2004;309:293–302.

Lewi P, Arnold E, Andries K, Bohets H, Borghys H, Clark A, et al. Correlations between factors determining the pharmacokinetics and antiviral activity of HIV-1 non-nucleoside reverse transcriptase inhibitors of the diaryltriazine and diarylpyrimidine classes of compounds. Drugs R D 2004;5:245–57.

Liebsch G, Landgraf R, Gerstberger R, Probst JC, Wotjak CT, Engelmann M, et al. Chronic infusion of a CRH1 receptor antisense oligodeoxynucleotide into the central nucleus of the amygdala reduced anxiety-related behavior in socially defeated rats. Regul Pept 1995;59:229–39.

Lin JH, Lu AY. Role of pharmacokinetics and metabolism in drug discovery and development. Pharmacol Rev 1997;49:403–49.

Liu X, Tu M, Kelly RS, Chen C, Smith BJ. Development of a computational approach to predict blood-brain barrier permeability. Drug Metab Dispos 2004;32:132–9.

Lobell M, Sivarajah V. In silico prediction of aqueous solubility, human plasma protein binding and volume of distribution of compounds from calculated pKa and AlogP98 values. Mol Divers 2003;7:69–87.

Lombardo F, Obach RS, Shalaeva MY, Gao F. Prediction of volume of distribution values in humans for neutral and basic drugs using physicochemical measurements and plasma protein binding data. J Med Chem 2002;45:2867–76.

Lovenberg TW, Liaw CW, Grigoriadis DE, Clevenger W, Chalmers DT, De Souza EB, et al. Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. Proc Natl Acad Sci U S A 1995;92:836–40.

Lowman C, Allen J, Stout RL. Replication and extension of Marlatt's taxonomy of relapse precipitants: overview of procedures and results. Addiction 1996;91:s51–71 (Suppl.).

McElroy JF, Ward KA, Zeller KL, Jones KW, Gilligan PJ, He L, et al. The CRF (1) receptor antagonist DMP696 produces anxiolytic effects and inhibits the stress-induced hypothalamic–pituitary–adrenal axis activation without sedation or ataxia in rats. Psychopharmacology 2002;165:86–92.

Menzaghi F, Howard RL, Heinrichs SC, Vale W, Rivier J, Koob GF. Characterization of a novel and potent corticotropin-releasing factor antagonist in rats. J Pharmacol Exp Ther 1994;269:564–72.

Merlo-Pich E, Lorang M, Yeganeh M, Rodriguez de Fonseca F, Raber J, Koob GF, et al. Increase of extracellular corticotropin-releasing factor-like immunoreactivity levels in the amygdala of awake rats during restraint stress and ethanol withdrawal as measured by microdialysis. J Neurosci 1995;15:5439–47.

Norinder U, Osterberg T. Theoretical calculation and prediction of drug transport processes using simple parameters and partial least squares projections to latent structures (PLS) statistics: the use of electrotopological state indices. J Pharm Sci 2001;90:1076–85.

O'Dell LE, Roberts AJ, Smith RT, Koob GF. Enhanced alcohol self-administration after intermittent versus continuous alcohol vapor exposure. Alcohol Clin Exp Res 2004;28:1676–82.

Olive MF, Koenig HN, Nannini MA, Hodge CW. Elevated extracellular CRF levels in the bed nucleus of the stria terminalis during ethanol withdrawal and reduction by subsequent ethanol intake. Pharmacol Biochem Behav 2002;72:213–20.

Osterberg T, Norinder U. Prediction of drug transport processes using simple parameters and PLS statistics. The use of ACD/logP and ACD/ChemSketch descriptors. Eur J Pharm Sci 2001;12:327–37.

Overstreet DH, Knapp DJ, Breese GR. Accentuated decrease in social interaction in rats subjected to repeated ethanol withdrawals. Alcohol Clin Exp Res 2002;26:1259–68.

Overstreet DH, Knapp DJ, Breese GR. Modulation of multiple ethanol withdrawal-induced anxiety-like behavior by CRF and CRF1 receptors. Pharmacol Biochem Behav 2004;77:405–13.

Perrin MH, Donaldson CJ, Chen R, Lewis KA, Vale WW. Cloning and functional expression of a rat brain corticotropin-releasing factor (CRF) receptor. Endocrinology 1993;133:3058–61.

Rassnick S, Heinrichs SC, Britton KT, Koob GF. Microinjection of a corticotropin-releasing factor antagonist into the central nucleus of the amygdala reverses anxiogenic-like effects of ethanol withdrawal. Brain Res 1993;605:25–32.

Rimondini R, Arlinde C, Sommer W, Heilig M. Long-lasting increase in voluntary ethanol consumption and transcriptional regulation in the rat brain after intermittent exposure to alcohol. FASEB J 2002;16:27–35.

Roberts AJ, Cole M, Koob GF. Intra-amygdala muscimol decreases operant ethanol self-administration in dependent rats. Alcohol Clin Exp Res 1996;20:1289–98.

Roberts AJ, Heyser CJ, Koob GF. Operant self-administration of sweetened versus unsweetened ethanol: effects on blood alcohol levels. Alcohol Clin Exp Res 1999;23:1151–7.

Roberts AJ, Heyser CJ, Cole M, Griffin P, Koob GF. Excessive ethanol drinking following a history of dependence: animal model of allostasis. Neuropsychopharmacology 2000;22:581–94.

Rodriguez de Fonseca F, Carrera MRA, Navarro M, Koob GF, Weiss F. Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. Science 1997;276:2050–4.

Sabino V, Cottone P, Koob GF, Steardo L, Lee MJ, Rice KC, et al. Dissociation between opioid and CRF1 antagonist sensitive drinking in Sardinian alcohol-preferring rats. Psychopharmacology 2006;189:175–86.

Samson HH. Initiation of ethanol reinforcement using a sucrose-substitution procedure in food- and water-sated rats. Alcohol Clin Exp Res 1986;10:436–42.

Smith GW, Aubry JM, Dellu F, Contarino A, Bilezikjian LM, Gold LH, et al. Corticotropin-releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. Neuron 1998;20:1093–102.

Sutton RE, Koob GF, Le Moal M, Rivier J, Vale W. Corticotropin-releasing factor produces behavioural activation in rats. Nature 1982;297:331–3.

Swanson LW, Sawchenko PE, Rivier J, Vale W. The organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. Neuroendocrinology 1983;36:165–86.

Timpl P, Spanagel R, Sillaber I, Kreese A, Reul JM, Stalla GK, et al. Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor 1. Nat Genet 1998;19:162–6.

Treit D, Pinel JP, Fibiger HC. Conditioned defensive burying: a new paradigm for the study of anxiolytic agents. Pharmacol Biochem Behav 1981;15:619–26.

Valdez GR. Development of CRF1 receptor antagonists as antidepressants and anxiolytics: progress to date. CNS Drugs 2006;20:887–96.

Valdez GR, Koob GF. Allostasis and dysregulation of corticotropin-releasing factor and neuropeptide Y systems: implications for the development of alcoholism. Pharmacol Biochem Behav 2004;79:671–89.

Valdez GR, Roberts AJ, Chan K, Davis H, Brennan M, Zorrilla EP, et al. Increased ethanol self-administration and anxiety-like behavior during acute withdrawal and protracted abstinence: regulation by corticotropin-releasing factor. Alcohol Clin Exp Res 2002;26:1494–501.

Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates the secretion of corticotropin and beta-endorphin. Science 1981;213:1394–7.

Votano JR, Parham M, Hall LH, Kier LB. New predictors for several ADME/Tox properties: aqueous solubility, human oral absorption, and Ames genotoxicity using topological descriptors. Mol Divers 2004;8:379–91.

Walker DL, Davis M. Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. J Neurosci 1997;17:9375–83.

Weiss F, Ciccocioppo R, Parsons LH, Katner S, Liu X, Zorrilla EP, et al. Compulsive drug-seeking behavior and relapse. Neuroadaptation, stress, and conditioning factors. Ann N Y Acad Sci 2001;937:1–26.

Zhao YH, Abraham MH, Ibrahim A, Fish PV, Cole S, Lewis ML, et al. Predicting penetration across the blood–brain barrier from simple descriptors and fragmentation schemes. J Chem Inf Model 2007;47:170–5.

Zorrilla EP, Koob GF. The therapeutic potential of CRF_1 antagonists for anxiety. Expert Opin Investig Drugs 2004;13:799–828.

Zorrilla EP, Valdez GR, Weiss F. Changes in levels of regional CRF-like-immunoreactivity and plasma corticosterone during protracted drug withdrawal in dependent rats. Psychopharmacology 2001;158:374–81.

Zorrilla EP, Schulteis G, Ormsby A, Klaasen A, Ling N, McCarthy JR, et al. Urocortin shares the memory modulating effects of corticotropin-releasing factor (CRF): mediation by CRF_1 receptors. Brain Res 2002;952:200–10.

Zorrilla E, Fekete E, Mason BJ, Wirsching P, Janda KD, Koob GF. CRF1 receptor antagonists for anxiety. Eur Neuropsychopharmacol 2003;13 (Suppl. 4):s130–1.