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Behavioral and neurobiological effects of the enkephalinase inhibitor RB101 relative to its antidepressant effects

Emily M. Jutkiewicz^{a,*}, Mary M. Torregrossa^e, Katarzyna Sobczyk-Kojiro^b, Henry I. Mosberg^b, John E. Folk^d, Kenner C. Rice^d, Stanley J. Watson^c, and James H. Woods^a

^a 1301 Medical Science Research Building III, Department of Pharmacology, University of Michigan Medical School, Ann Arbor, MI 48109-0632, United States

^b Department of Medicinal Chemistry, University of Michigan, Ann Arbor, MI 48109, United States

^c Mental Health Research Institute, Ann Arbor, MI 48109, United States

^d NIDDK, NIH, Bethesda, MD 20892, United States

^e Department of Neurosciences, Medical University of South Carolina, Charleston, SC 29425, United States

Abstract

Nonpeptidic delta-opioid receptor agonists produce antidepressant-like effects in rodents, and compounds that inhibit the breakdown of endogenous opioid peptides have antidepressant-like effects in animal models. In this study, the behavioral effects of the enkephalinase inhibitor, RB101 (*N*-[(*R*, *S*)-2-benzyl-3-[(*S*)(2-amino-4-methyl-thio)-butyldithio]-1-oxopropyl]-l-phenylalanine benzyl ester), were examined. Specifically, the effects of RB101 on convulsive activity, locomotor activity, and antidepressant-like effects in the forced swim test were studied in Sprague–Dawley rats, and the opioid receptor types mediating these effects were examined by antagonist studies. In addition, the effects of RB101 on brain-derived neurotrophic factor (BDNF) mRNA expression were evaluated in relation to its antidepressant effects. RB101 produced delta-opioid receptor-mediated antidepressant effects (32 mg/kg i.v. and 100 mg/kg i.p.) and increased locomotor activity (32 mg/kg i.v.) in rats. RB101 did not produce convulsions or seizures and did not alter BDNF mRNA expression. In conclusion, RB101 has the potential to produce antidepressant effects without convulsions.

Keywords

Behavior; Forced swim test; RB101; Brain-derived neurotrophic factor; (Rat)

1. Introduction

Nonpeptidic delta-opioid receptor agonists demonstrate antidepressant-like activity in animal models of depression (Broom et al., 2002; Jutkiewicz et al., 2004; Saitoh et al., 2004). In addition, delta-opioid receptor agonists stimulate neurobiological correlates of antidepressant activity in rat brains. For example, a single administration of the delta receptor agonist (+) BW373U86 has been shown to increase brain-derived neurotrophic factor (BDNF) mRNA expression in rat frontal cortex, hippocampus, and basolateral amygdala (Torregrossa et al., 2004). Increases in BDNF have been suggested to be responsible for the clinical efficacy of antidepressant treatment (Duman, 2002; Vaidya and Duman, 2001). The rapid increase in BDNF mRNA expression produced by delta receptor agonists suggests that compounds acting

*Corresponding author. Tel.: +1 734 764 4560; fax: +1 734 764 7118. E-mail address: ejutkiew@umich.edu (E.M. Jutkiewicz).

on the delta-opioid receptor system may be faster acting than traditional antidepressants, and a promising system for the development of a new class of antidepressants. However, delta-opioid receptor agonists also induce convulsions in a number of species potentially limiting their therapeutic utility (Comer et al., 1993; Dykstra et al., 1993; Negus et al., 1994; Pakarinen et al., 1995; Hong et al., 1998; Broom et al., 2002; Jutkiewicz et al., 2004). In order to develop delta-opioid receptor agonists as potential treatments for depression, compounds without seizure activity would be ideal. This study examines an alternative mechanism of activating the delta-opioid receptor in order to achieve antidepressant-like effects without convulsions. These results are discussed as compared to the effects observed with nonpeptidic delta-opioid receptor agonists.

Opioid peptides have been demonstrated to have antidepressant actions in animal models of depression without producing overt convulsions. For example, enkephalins and endorphins decreased immobility in the forced swim test and in the learned helplessness paradigm in rats, demonstrating antidepressant-like effects (Kastin et al., 1978; Tejedor-Real et al., 1995, respectively); however, analogs of endogenous enkephalins and other opioid peptides have been shown to produce epileptiform activity and seizures (Haffmans and Dzoljic, 1983; Stutzmann et al., 1986; Walker and Yaksh, 1986; Snead, 1986; De Sarro et al., 1992). It has also been demonstrated that preventing the breakdown of endogenous opioid peptides with enkephalinase inhibitors produces antidepressant-like effects in animal models, but these compounds have not been evaluated in terms of convulsions or electroencephalographic changes. RB38A, a mixed inhibitor of enkephalinase, and RB38B, a selective inhibitor of endopeptidase EC 3.4.24.11, produced antidepressant-like effects in the learned helplessness paradigm following intracerebroventricular administration (Tejedor-Real et al., 1993, 1995). These effects were blocked by the nonselective opioid receptor antagonist naloxone, demonstrating an opioid-mediated effect.

Oral administration of the enkephalinase inhibitor BL-2401 demonstrated antidepressant-like effects in the forced swim test in mice, and these effects were blocked by the nonselective opioid receptor antagonist naloxone (Kita et al., 1997). Enkephalinase inhibitors elevate levels of endogenous opioids that act at multiple opioid receptors; therefore, the receptor mediating this antidepressant activity was unknown. However, Baamonde et al. (1992) and Tejedor-Real et al. (1998) demonstrated that the selective delta-opioid receptor antagonist, naltrindole, blocked the antidepressant-like effects produced by the enkephalinase inhibitor RB101 in the learned helplessness model of depression in mice. To further support this finding, intravenous administration of the selective delta-opioid peptide BUBU (Tyr-D.Ser-(*O*-tert-butyl)-Gly-Phe-Leu-Thr(*O*-Tert-butyl-OH) demonstrated antidepressant-like effects in the learned helplessness paradigm (Tejedor-Real et al., 1998). Although the enkephalinase inhibitor RB101 demonstrated antidepressant-like effects through the delta-opioid receptor, its antinociceptive effects were mediated through the mu-opioid receptor (Noble et al., 1992).

The enkephalinase inhibitor RB101 appears to produce antidepressant-like effects similar to those observed with nonpeptidic delta-opioid receptor agonists. However, enkephalins are promiscuous ligands in that they bind to multiple opioid receptor subtypes, and the contribution of elevated enkephalin levels to the different behavioral effects produced by RB101 has not been fully elucidated. In addition, the effects of RB101 on convulsions and seizure-like electroencephalographic (EEG) changes have not been evaluated. The present study characterized the behavioral and physiological effects of RB101 in Sprague-Dawley rats and the neurobiological correlates of the antidepressant-like effects of RB101. In particular, RB101 was evaluated based on behavioral effects observed with delta-opioid agonists: convulsions, EEG, locomotor stimulation, and antidepressant-like effects; and the involvement of opioid receptor subtypes was examined. In addition, the antidepressant-like effects produced by RB101 may be induced by increases in BDNF. However, the effect of endogenous opioids on

BDNF mRNA expression is unknown. Thus, changes in BDNF mRNA were measured following administration of RB101.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats (250–300 g), obtained from Harlan Sprague Dawley (Indianapolis, IN), were housed in groups of three or four animals per cage. All animals were fed on a standard laboratory diet and maintained on a 12 h light/dark cycle with lights on at 6:30 am at an average temperature of 21 °C. Studies were performed in accordance with the Declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. The experimental protocols were approved by the University of Michigan University Committee on the Use and Care of Animals.

2.2. Procedures

2.2.1. Intravenous (i.v.) catheter implantation—Catheters were constructed from approximately 15 cm of Micro-Renathane tubing (MRE-040, Braintree Scientific, Inc, Braintree, MA). Implantation of i.v. catheters was described previously (Baird et al., 2000). Briefly, rats were anesthetized with ketamine hydrochloride (100 mg/kg, i.m.) and xylazine hydrochloride (10 mg/kg, i.m.). The right jugular vein was isolated through a ventral incision in the neck. Approximately 3 cm of the catheter was inserted into the right jugular vein, and the tubing was sutured to the vein and to the surrounding tissues at 3–4 points in order to secure the catheter placement. The remaining tubing was threaded subcutaneously to a dorsal incision and held in place by sutures to the musculature directly below the incision. Three to four centimeters of tubing remained exposed outside the rat's body. The catheter was plugged with a stainless steel pin (McMaster-Carr, Cleveland, OH). Immediately after and every day following the surgery, the catheters were flushed with approximately 0.5 ml of heparinized saline (50 U/ml) to maintain catheter patency. Following surgery, rats were singly housed and allowed approximately 4–5 days of recovery from surgery before being used in an experiment.

2.2.2. Electrode implantation surgery—To measure electroencephalographic changes in rats using a telemetry system, rats were implanted with 3-channel radiotransmitters (model F50-EET, Data Sciences International, St. Paul, MN) under ketamine (100 mg/kg, i.m.) and xylazine (10 mg/kg, i.m.) anesthesia. The transmitter was 4.5 cm long, 1.5 cm wide, 1 cm deep, and weighed approximately 13 g. Prior to implantation, the transmitter was cleaned in Exspor (base and activator concentrate; Alcide, Redmond, WA) for approximately 5 min and then soaked twice for 10 min in sterile saline. An incision in the skin and musculature of the peritoneal cavity was made, and the transmitter was oriented inside the peritoneal cavity. The transmitter was attached to the muscle wall of the peritoneum using non-absorbable nylon suture, and the skin over the muscle was closed. The biopotential leads (5) were passed through the muscle wall of the peritoneum using a 16 gauge needle and threaded subcutaneously emerging at an incision over the skull. The rat's head was placed in a stereotaxic instrument for stability during screw and biopotential lead attachment. After exposing the skull, the bone was scraped clean and dried. Five holes were drilled using a micro-drill with 0.7 mm steel burr (Fine Science Tools, Inc., Foster City, CA) for bilateral placement of epidural recording electrodes, which consisted of biopotential leads from the transmitter wrapped around stainless steel slotted, fillister screws (0.8 mm diameter, 0.12 in. long, Small Parts, Inc., Miami Lakes, FL). Screws were implanted at the following location bilaterally: approximately 1 mm posterior to bregma, 1.5 mm lateral to the midline and 1 mm anterior to lambda, 1.5 mm lateral to the midline. A reference wire was also placed 1–2 mm posterior to lambda. The biopotential leads were prepared by removing approximately 0.5 cm of silicone rubber tubing from the end of each wire. The exposed wires were stretched to allow easier and more secure wrap around the

skull screws. All skull screws and wires were anchored to the skull with dental acrylic cement. Following biopotential lead attachment, the incision on the head was closed with nylon suture. Following surgery, rats were singly housed and monitored daily for signs of recovery (normal eating, drinking, and defecation) prior to experimentation. Approximately 7 days after the EEG surgery, rats were implanted with i.v. catheters as described above, and allowed to recover for at least 5 days before testing.

2.2.3. Forced swim test—To measure antidepressant-like activity, six rats per test condition were subjected to a modified forced swim test as previously described (Broom et al., 2002). Briefly, rats were placed in a clear cylindrical acrylic container (46 cm tall×20 cm in diameter) filled with 30 cm of 25 °C (±1 °C) water. Each rat experienced one 15-min swim session. Test compounds were administered as a single 20-s intravenous (i.v.) injection 30 min prior to the forced swim test or as a single intraperitoneal (i.p.) injection 60 min prior to the forced swim test. Cylinder water was changed after every rat. Following each swim period, rats that received i.p. injections were removed from the water, towel-dried, and placed in a heated cage for 15 min. Following swimming, rats with i.v. catheters were sacrificed with i.v. pentobarbital to confirm catheter patency.

Videotaped 15-min test swims were scored for immobility, swimming, and climbing behaviors (Detke et al., 1995). These behaviors were defined as: *immobility* — floating in the water without struggling and using only small movements to keep the head above water; *swimming* — moving limbs in an active manner (more than required to keep head above water); *climbing* — making active movements with the forepaws in and out of the water, often directed at the wall of the swim tank. The individuals scoring the videotapes were blind to the drug treatments received by each rat. Every 5 s, the scorer rated the subject's behavior as one of the three behaviors, immobility, swimming, or climbing. The total counts of each behavior during the 15-min test swim were averaged within treatment groups.

Various doses of RB101 were injected i.v. 30 min before the swim or i.p. 60 min before swimming. In antagonism studies, rats were injected s.c. with a single dose of naltrindole or naltrexone 30 min prior to RB101 administration or nor-BNI (s.c.) 24 h prior to RB101 injection. For co-administration experiments with RB101 and SNC80, RB101 was administered i.v. 10 min prior to SNC80 (s.c.), and rats swam 30 min after SNC80 administration.

2.2.4. Convulsion observations—Immediately after an i.v. or an i.p. injection of RB101, rats were placed in a clean observation chamber filled with bedding for 20 min to observe for convulsions and cataleptic behaviors. In co-administration studies with RB101 and SNC80, rats were placed in the observation chambers for a total of 30 min. Following the 20-min observation period, rats were returned to the home cage prior to swimming.

2.2.5. EEG measurements—Rats were tested on an individual basis in their home cages, and behavioral observations for different rats never overlapped. Individual rats and their digital EEG traces were continuously monitored during the 1 h test session to identify behaviors, movement artifacts, convulsions, nonconvulsive seizures, or other abnormal behaviors that coincided with EEG changes. Prior to the start of an experiment, baseline data was collected for approximately 60 min. All EEG experiments were conducted between 8:00 am and 12:00 pm. During observations, food and water were removed to allow for easier observation. Occasionally, gentle tapping on the cage or removal of the cage lid was required to maintain the rat in an alert, awake state.

Signals detected by the biopotential leads were transmitted to a receiver (RPC-1, Data Sciences International, St. Paul, MN) located beneath a cage. The receiver sent the signal via a cable

connector to the Dataquest ART Exchange Matrix (Data Sciences International, St. Paul, MN) converting the analog signal into digital output, and the digital signal was stored on a computer. The signal was filtered for 60 Hz signal, the low pass filter was set to 0.3 Hz, and the high pass filter was fixed 70 Hz. Data analysis was conducted with Somnologica Software and DSI import modules (Medcare Flaga, Reykjavik, Iceland) and waveforms were evaluated at a 30 mm/s recording speed.

For these experiments, 6 rats were injected with 32 mg/kg RB101 i.v. and continuously monitored for 60 min following the injection. Behavioral changes and EEG changes were recorded for observed changes and the time of occurrence. After the 60-min observation period, rats were sacrificed with i.v. pentobarbital to confirm catheter patency.

2.2.6. Locomotor activity measurements—To measure changes in locomotor activity, singly housed rats were implanted with i.v. catheters and radiotransmitters (model ER-4000 E-Mitter, Mini Mitter Co., Bend, OR). Under ketamine (100 mg/kg, i.m.) and xylazine (10 mg/kg, i.m.) anesthesia, a transmitter was implanted inside the peritoneum of a rat, and an i.v. catheter was implanted as described above, at least 6 days before conducting an experiment. The transmitter produced locomotor activity signals that were sent to a receiver (model ER-4000 Receiver, Mini Mitter Co.) placed under the home cage of each rat. Data were collected and processed simultaneously by the Vital View data acquisition system (Mini Mitter Co.). Locomotor activity data were summed over 5 min intervals for 40 min of baseline activity and continuing at least 3 h after injections of test compounds. Mean activity counts across rats for each treatment group were averaged as time after injection ± 2 min.

Following collection of baseline activity levels, rats were injected i.v. with a single dose of RB101 (either 3.2, 10, or 32 mg/kg), vehicle RB101, or 3.2 mg/kg SNC80. For antagonism studies, rats were injected s.c. with sterile water, 1.0 mg/kg naltrindole, or 0.032 mg/kg naltrexone 30 min prior to 32 mg/kg RB101 (i.v.). Following the experiment, rats were injected with i.v. pentobarbital to confirm catheter patency.

2.2.7. ACTH and BDNF measurements—Blood plasma samples were assayed using radioimmunoassay kits for adrenocorticotropin (ACTH) (Nichols Institute Diagnostics, San Juan Capistrano, CA). The ACTH kit measures the amount of intact ACTH molecules that contain both N-terminal and C-terminal regions. Thus, ACTH fragments, its large precursor molecule proopiomelanocortin (POMC), and internal amino acid sequences are not measured in this radioimmunoassay kit.

BDNF mRNA levels were determined by a double-label in situ hybridization with a [^{35}S]-labeled BDNF RNA probe, as described previously (Torregrossa et al., 2004). The rat BDNF cDNA (described by Isackson et al., 1991) was donated by Drs. Gall and Lauterborn (University of California, Irvine). BDNF mRNA levels were quantified using NIH Image (Scion Image Corp., Frederick, MD) software. BDNF mRNA expression was examined in frontal cortex, hippocampus (CA1, the CA3, and the dentate gyrus regions), the basolateral amygdaloid complex, the endopiriform nucleus, and primary olfactory cortex. Each brain region was analyzed by creating an outline around the region and measuring both the left and right sides of the brain and from rostral–caudal sections approximately 100–200 μm apart. At least six sections per region per rat were quantified. The signal measurements were corrected for background and were determined as the mean radioactive intensity per pixel for that region. These signal values for each section were then averaged to obtain the mean signal for each region in each rat. These data points were then averaged per group and compared using a two-way ANOVA and Tukey's post hoc test.

Rats were divided into four treatment groups using a 2×2 design, where they received a subcutaneous (s.c.) injection of naltrindole or vehicle 15 min before an i.v. injection of 32 mg/kg RB101 or vehicle. Five minutes prior to the first injection blood was collected from the tail vein by tail nick (as described by Houshyar et al., 2001), to establish baseline levels of ACTH. Blood samples were then taken 5 min after the first injection, and 5, 15, 30, 60, 90, 120, and 170 min after the second injection. Three hours after the injection of RB101 or vehicle, rats were sacrificed by rapid decapitation, and their brains were taken and frozen in isopentane at −40 °C, and were stored at −80 °C for later analysis of BDNF mRNA levels.

2.3. Drugs

RB101 (*N*-[(*R*, *S*)-2-benzyl-3-[(*S*)(2-amino-4-methyl-thio)-butyldithio]-1-oxopropyl]-l-phenylalanine benzyl ester) was synthesized in the laboratory of Henry I. Mosberg and was dissolved in a 1:1:8 ethanol, emulphor, and sterile water solution. RB101 was injected (i.v. or i.p.) in a volume of 1 ml/kg, except doses higher than 10 mg/kg due to solubility limitations. The largest volume administered was 5 ml/kg to obtain a dose of 100 mg/kg. Naltrindole and naltrexone (NIDA, Research Resource Drug Supply System) were administered s.c. 30 min before RB101 administration in a volume of 1 ml/kg. Norbinaltorphimine (nor-BNI) (NIDA, Research Resource Drug Supply System) was administered s.c. 24 h before RB101 in a volume of 1 ml/kg. [(+)-4-[(α R)- α -[(2*S*,5*R*)-2,5-dimethyl-4-(2-propenyl)-1-piperazinyl]-(3-methoxyphenyl)methyl]-*N,N*-diethylbenzamide (SNC80) was synthesized according to the published protocol (Calderon et al., 1994), dissolved in 8% 1 M HCl, and administered in a volume of 1 ml/kg.

3. Results

In the forced swim test, RB101 administered i.v. produced a dose-dependent decrease in immobility ($F(4, 29) = 11.89$; $P < 0.0001$), a significant increase in swimming behaviors ($F(4, 29) = 22.52$; $P < 0.0001$), but did not alter climbing ($F(4, 29) = 0.75$; $P = 0.57$) (Fig. 1) when administered 30 min prior to swimming. A dose of 32 mg/kg RB101 (i.v.) significantly decreased immobility ($P < 0.01$) and significantly increased swimming ($P < 0.01$). This decrease in immobility was no longer observed 60 min after RB101 injection (i.v.) (data not shown). Following i.p administration, RB101 produced a significant decrease in immobility ($F(3, 20) = 4.91$; $P = 0.01$) (Fig. 2), but did not alter swimming ($F(3, 20) = 1.28$; $P = 0.31$) or climbing ($F(3, 20) = 0.96$; $P = 0.44$). By post hoc comparison, 100 mg/kg RB101 (i.p.) significantly decreased immobility ($P < 0.01$). In addition, RB101 did not produce convulsions in any rats at any dose tested (data not shown).

In the antagonism studies, naltrindole dose-dependently blocked the decrease in immobility produced by 32 mg/kg RB101 i.v. ($F(5, 35) = 15.38$; $P < 0.0001$) (Fig. 3A). Doses of 1.0 and 10 mg/kg naltrindole significantly blocked the effects of RB101, generating immobility scores similar to control values ($P < 0.05$ and $P < 0.01$, respectively, as compared with vehicle naltrindole pretreatment to 32 mg/kg RB101; $P > 0.05$ for both doses of naltrindole with 32 mg/kg RB101 as compared to vehicle naltrindole pretreatment to vehicle RB101). However, the effects of RB101 were not blocked by mu-selective doses of naltrexone ($P > 0.05$) or the kappa-selective receptor antagonist nor-BNI ($P > 0.05$) (Fig. 3B and C, respectively).

In locomotor activity studies, baseline levels of activity were low for all groups of rats (Fig. 4A). Following an i.v. injection of vehicle at time 0, locomotor activity counts increased and returned to baseline values within 30–45 min. The lower doses of RB101 tested, 3.2 and 10 mg/kg i.v., did not greatly alter activity levels as compared to vehicle control. Only the high dose of RB101, 32 mg/kg i.v., significantly increased locomotor activity counts above control values lasting for approximately 100–120 min. SNC80 at a dose 3.2 mg/kg i.v. produced an increase in locomotor activity above vehicle levels for approximately 60 min. The locomotor

stimulating effects of 32 mg/kg RB101 i.v. were decreased by 1.0 mg/kg naltrindole, but not by the mu-selective dose of naltrexone (Fig. 4B).

In EEG studies, 3.2 mg/kg SNC80 i.v. elicited overt convulsions with simultaneous epileptiform activity immediately after or during the injection in all rats tested ($N = 6$). A representative trace from one rat is shown in Fig. 5A. Following the convulsion, there was a period of catalepsy coinciding with postictal suppression and EEG desynchronization. During the cataleptic period, some rats demonstrated myoclonic twitches with concurrent single spikes or sharp waves. Approximately 4.5 min after the convulsion, the EEG returned to baseline or pre-injection activity. At this time, the rats were very active demonstrating high levels of locomotor activity. Unlike SNC80, all rats injected with 32 mg/kg RB101 i.v. failed to produce any epileptiform activity (Fig. 5B, representative trace), such that post-injection measurements appeared similar to pre-injection recordings. Following the injection of RB101, rats were active, but did not show convulsive or pro-convulsive behaviors.

As a 10 min pretreatment to the delta-opioid receptor agonist SNC80, RB101 slightly enhanced the convulsive properties of SNC80, as demonstrated by a small leftward shift in the convulsion dose effect curve (Fig. 6A). A dose of 1.0 mg/kg SNC80 with a pretreatment of vehicle RB101 produced convulsions in 83% of rats studied. With a pretreatment of 3.2 mg/kg RB101 i.v., a similar percentage of rats demonstrated convulsions induced by 1.0 mg/kg SNC80 (i.v.). However, 10 mg/kg RB101 administered as a 10 min pretreatment to 1.0 mg/kg SNC80 increased the number of rats convulsing to 100%.

In the forced swim test, 3.2 and 10 mg/kg RB101 enhanced the antidepressant-like properties of SNC80 as demonstrated by a small leftward shift in the dose effect curve (Fig. 6B). For example, with a vehicle RB101 pretreatment, 3.2 and 10 mg/kg SNC80 (i.v.) significantly decreased immobility as compared to control values; however, only the highest dose of SNC80 (10 mg/kg) decreased immobility to values below 100 counts of immobility. With pretreatments of 3.2 and 10 mg/kg RB101, 3.2 mg/kg SNC80 also decreased immobility below control values, and reduced the counts of immobility below 100. A pretreatment of 10 mg/kg RB101 alone also slightly decreased immobility as compared to control; however, 3.2 mg/kg RB101 alone did not alter immobility in the forced swim test.

BDNF mRNA levels were not significantly changed 3 h after i.v. injection of 32 mg/kg RB101 in any of the brain regions examined, and BDNF levels were not affected by pretreatment with naltrindole (Fig. 7). In addition, naltrindole had no effect on BDNF mRNA expression when given alone.

Finally, a dose of 32 mg/kg RB101 increased ACTH to 327 (± 113.3) pg/ml as compared to 125.5 (± 102.3) pg/ml in vehicle-treated rats at 15 min post-injection. Naltrindole failed to block the RB101-induced increase in ACTH (274 $\pm$ 89.6 pg/ml) (data not shown).

4. Discussion

The enkephalinase inhibitor RB101 (i.v.) demonstrated antidepressant-like effects in the forced swim test in rats, presumably by increasing endogenous levels of delta-opioid receptor-preferring neurotransmitter met-enkephalin (Daugé et al., 1996). This antidepressant activity was blocked with the selective delta-opioid receptor antagonist naltrindole, but not with mu-selective doses of naltrexone nor the kappa-selective receptor antagonist, nor-BNI. These findings confirm previous studies demonstrating that the antidepressant-like effects of RB101 are delta-opioid receptor-mediated (Baamonde et al., 1992; Tejedor-Real et al., 1998). These results further support previous findings that mu- and kappa-opioid receptor activation does not produce antidepressant-like effects in the forced swim test in rats (Broom et al., 2002). These data also demonstrated that i.p. injection of RB101 produces antidepressant-like effects

in the forced swim test; however, doses, at least 3-fold higher, were required. Overall, RB101 was not as efficacious as the nonpeptidic delta-opioid receptor agonists SNC86 and SNC80 in the forced swim test (Jutkiewicz et al., 2004).

RB101 (i.v.) increased locomotor activity in the home cage only at doses that demonstrated antidepressant-like properties (32 mg/kg). These effects were blocked by the delta receptor-selective antagonist naltrindole, but not by mu-selective doses of naltrexone, demonstrating that RB101-induced locomotor stimulation was mediated by delta-opioid receptors alone.

In contrast to the nonpeptidic delta-opioid receptor agonists that produce antidepressant-like effects in the forced swim test, RB101 did not produce convulsions following i.p. or i.v. administration. In addition, RB101 did not produce epileptiform activity, unlike the nonpeptidic delta-opioid receptor agonists SNC80. These studies demonstrated that activation of delta-opioid receptors might have potential therapeutic benefit for treating depression without convulsive effects.

In the current studies, RB101 is less potent in producing antidepressant-like effects as compared to previous studies that demonstrated antidepressant activity at 5 mg/kg in mice (Baamonde et al., 1992) and rats (Tejedor-Real et al., 1998). This discrepancy may be explained by the time of administration prior to testing. In previous studies, RB101 was administered either 10 min before the forced swim test in mice (Baamonde et al., 1992) or 15 min before testing in the learned helplessness model in rats (Tejedor-Real et al., 1998). Based on these procedural differences, a higher dose was required for longer pretreatment times in the current studies.

Interestingly, 32 mg/kg RB101 i.v. produced antidepressant-like effects as well as locomotor stimulation. In previous studies with mice, locomotor activity increases were also observed at similar doses as those that produced antidepressant-like effects (Baamonde et al., 1992). Locomotor stimulation occurring simultaneously with antidepressant-like effects is sometimes considered a false-positive result in the forced swim test as well as other behavioral models of depression. Previous studies have demonstrated that these two behavioral outputs can be separated (Broom et al., 2002). In the present experiments, the increases in locomotor activity that persisted longer than the antidepressant-like effects were observed, suggesting that locomotor activity alone cannot explain decreases in immobility in the forced swim test. However, these data also suggest that RB101 may be a false positive in the forced swim test.

In the studies investigating the combined administration of RB101 with SNC80, RB101 enhanced slightly both the convulsive and antidepressant-like effects of SNC80. These data do not support the theory that neuropeptides acting at opioid receptors may have anticonvulsant properties (Tortella, 1988). In addition, RB101 did not produce a larger SNC80-induced decrease in immobility, but simply shifted the SNC80 dose effect curve to the left. However, this shift is limited, such that only convulsive doses of SNC80 (i.v.) still produced antidepressant-like effects. These results demonstrate that elevating endogenous opioids enhanced the antidepressant-like effects of delta-opioid receptor agonists, but worsened the convulsive effects of delta-opioid receptor agonists.

RB101 did not alter BDNF mRNA expression when given acutely, unlike the delta-opioid receptor agonist (+)BW373U86 (Torregrossa et al., 2004), although it does produce similar behavioral antidepressant-like effects. It is unclear why RB101 did not increase BDNF mRNA expression, but one possibility is that the amount of delta receptor stimulation induced by endogenous enkephalins is not sufficient to affect BDNF mRNA expression. As previously stated, RB101 is less efficacious than (+)BW373U86. In addition, RB101 produced an increase in ACTH levels that was not blocked by naltrindole. A variety of stressors and exogenously administered corticosterone have been shown to decrease BDNF mRNA expression in several brain regions, particularly the hippocampus (Rasmussen et al., 2002; Smith et al., 1995;

Hansson et al., 2003). Therefore, the increase in stress hormones with RB101 might have the net effect of no change in BDNF.

The long-term effects of RB101 are not currently known, but it is possible that chronic administration of RB101 would increase the expression of BDNF or its receptor TrkB. Chronic administration of many known antidepressants increases BDNF and TrkB mRNA expression in the hippocampus, while a single administration has either no effect or reduces BDNF mRNA expression (Nibuya et al., 1995; Torregrossa et al., 2004). RB101 may require multiple days of administration before influencing BDNF levels, as with other antidepressants, however, we would have expected a single administration to be sufficient, as with the nonpeptidic delta-opioid receptor agonist (+)BW373U86 (Torregrossa et al., 2004). It is also possible that increases in BDNF expression are not required for the antidepressant-like effect of RB101.

In conclusion, these results confirmed previous work demonstrating that the enkephalinase inhibitor RB101 had antidepressant-like activity in the forced swim test in rats. The present study also demonstrated that the antidepressant-like effects of RB101 were mediated through the delta-opioid receptor. Interestingly, RB101 produced antidepressant-like effects through the delta-opioid receptor without producing convulsions or seizure-like activity. In addition, RB101 stimulated locomotor activity by activating delta-opioid receptors and not mu-opioid receptors. However, locomotor-stimulating and antidepressant-like effects were observed at similar doses, suggesting that these two behavioral outcomes may be related. Also, RB101-induced elevation of endogenous enkephalins did not protect against nonpeptidic agonist SNC80-induced convulsions, but did slightly enhance the SNC80-induced antidepressant-like effects. Overall, these data demonstrate that it may be possible to develop antidepressants that act at delta-opioid receptors without convulsant properties.

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Fig. 1.

The effects of RB101 (i.v.) in the forced swim test in Sprague–Dawley rats ($n = 6/\text{dose}$). A single dose of RB101 was injected 30 min before the forced swim test. The columns and vertical lines represent the mean and S.E.M. for immobility (open columns), swimming counts (single-hatched columns), and climbing counts (double-hatched columns). ** $P < 0.001$ compared with vehicle as determined by Dunnett's post hoc test.

Fig. 2.

The effects of RB101 (i.p.) in the forced swim test in Sprague–Dawley rats ($n = 5\text{--}6/\text{dose}$). A single dose of RB101 was injected 60 min before the forced swim test. The columns and vertical lines represent the mean and S.E.M. for immobility (open columns), swimming counts (single-hatched columns), and climbing counts (double-hatched columns).

Fig. 3.

The effects of naltrindole (A), mu-selective doses of naltrexone (B), or nor-BNI (C) administered (s.c.) prior to 32 mg/kg RB101 (i.v.) in the forced swim test in Sprague-Dawley rats ($n = 6$ /condition). The columns and vertical lines represent the mean and S.E.M. for immobility (open columns), swimming counts (single-hatched columns), and climbing counts (double-hatched columns).

Fig. 4.

(A) The effects of RB101 (i.v.) alone on locomotor activity in Sprague-Dawley rats ($n = 6/\text{dose}$). Locomotor activity counts before time 0 were baseline levels of activity before injection that occurred at time 0. (B) The effects of naltrindole or mu-selective doses of naltrexone administered (s.c.) prior to 32 mg/kg RB101 (i.v.) on locomotor activity.

Fig. 5. Representative traces showing the effects of i.v. administration of 3.2 mg/kg SNC80 (A) and 32 mg/kg RB101 (B) on electroencephalographic recordings. The asterisks indicate the time of injection, and the time lines indicate 2-min intervals. Baseline amplitude is 150–200 μ V.

Fig. 6.

The effects of RB101 (i.v.) administered 10 min prior to a single dose of SNC80 (i.v.) on the convulsive (A) and antidepressant-like effects (B) in Sprague-Dawley rats. For measure of antidepressant-like effects, data are represented as counts of immobility only.

Fig. 7.

The effect of 32 mg/kg RB101 (i.v.) (), 10 mg/kg naltrindole (s.c.) (), and 10 mg/kg naltrindole given 15 min prior to 32 mg/kg RB101 () on BDNF mRNA expression, in comparison to vehicle controls (). Quantification of BDNF mRNA signal was obtained from photomicrographs of X-ray films exposed for 14 days after in situ hybridization with the antisense cRNA probe to rat BDNF mRNA. BDNF mRNA levels were obtained from frontal cortex, and three hippocampal subfields, the CA1, CA3, and dentate gyrus regions (panel A), and from the basolateral amygdale (BLA), endopiriform nucleus (EN), and olfactory cortex (Olf. Ctx.) (panel B). Data are expressed as mean±S.E.M.