The Anxiolytic-Like Effects of the Novel, Orally Active Nociceptin Opioid Receptor Agonist 8-[bis(2-Methylphenyl)methyl]-3-phenyl-8-azabicyclo[3.2.1]octan-3-ol (SCH 221510)

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Received January 21, 2008; accepted May 20, 2008

ABSTRACT

Orphanin FQ/nociceptin (OFQ/N) is the endogenously occurring peptide ligand for the nociceptin opioid receptor (NOP) that produces anxiolytic-like effects in mice and rats. The present study assessed the anxiolytic-like activity of 8-[bis(2-methylphenyl)methyl]-3-phenyl-8-azabicyclo[3.2.1]octan-3-ol (SCH 221510), a novel potent piperidine NOP agonist (EC50/H11005 12 nM) that binds with high affinity (Ki/H11005 0.3 nM) and functional selectivity (Ki/H11022 50-fold over the H11022-, H9260-, and H9254- opioid receptors). The anxiolytic-like activity and side-effect profile of SCH 221510 were assessed in a variety of models and the benzodiazepine, chlordiazepoxide (CDP), was included for comparison. The effects of chronic dosing of SCH 221510 were also assessed. Furthermore, the specificity of the anxiolytic-like effect of SCH 221510 was investigated with the NOP receptor antagonist 1-[(3R,4R)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2H-benimidazol-2-one (J-113397) and the opioid receptor antagonist naltrexone. Like CDP (1–30 mg/kg i.p.), SCH 221510 (1–30 mg/kg p.o.) produced anxiolytic-like effects in the elevated plus-maze (rat and gerbil), Vogel conflict (rat), conditioned lick suppression (rat), fear-potentiated startle (rat), and pup separation-induced vocalization (guinea pig) assays. In the Vogel conflict, the anxiolytic-like effect of SCH 221510 (10 mg/kg) was attenuated by J-113397 (3–10 mg/kg p.o.), but not naltrexone (3–30 mg/kg i.p.). Additionally, the anxiolytic-like effects of SCH 221510 did not change appreciably following 14-day b.i.d. dosing in rats (10 mg/kg). Furthermore, unlike CDP, SCH 221510 (3–30 mg/kg) produced anxiolytic-like activity at doses that did not disrupt overt behavior. Collectively, these data suggest that NOP agonists such as SCH 221510 may have an anxiolytic-like profile similar to benzodiazepines, with a reduced side-effect liability.
inhibitory actions on neurons within the dorsal raphe nucleus, locus coeruleus, periaqueductal gray, and amygdala (Connor et al., 1996; Vaughan and Christie, 1996; Vaughan et al., 1997). The distribution pattern of OFQ/N and the NOP receptor within limbic brain regions, together with the demonstrated inhibitory actions of OFQ/N on neurons within these areas, suggests that the OFQ/N-NOP system may have a modulatory role in the control of several behaviors, including attention, arousal, fear, and anxiety. Indeed, mice with a targeted deletion of the OFQ/N precursor showed an increased susceptibility to a variety of stressors (Koster et al., 1999), and intracerebroventricular injection of OFQ/N in both mice and rats produced behavioral changes consistent with an anxiolytic-like profile (Jenck et al., 1997; Griebel et al., 1999).

Furthermore, Jenck et al. (2000) demonstrated that Ro64-6198, a potent, nonpeptide agonist of the NOP receptor, produced a broad spectrum of anxiolytic-like activity in rats. Ro64-6198 increased exploration in the open arms of a rat elevated plus-maze (EPM), decreased the enhanced startle response in a fear-potentiated startle (FPS) paradigm, and increased the number of punished food deliveries in a Geller-Seifter procedure. These effects were similar to those produced by the benzodiazepine alprazolam. Unlike alprazolam, however, the anxiolytic-like effects of Ro64-6198 occurred at doses that did not affect locomotor activity or muscle tone. More recent studies with Ro64-6198 have extended the anxiolytic-like effects to other models, including rat conditioned lick suppression (CLS), separation-induced vocalization assays in both rat and guinea pig pups, mouse marble burying, and the mouse Geller-Seifter assay (Varty et al., 2005; Nicolas et al., 2006). Again, these studies suggested that, although there were mechanism-based side effects with NOP receptor agonists, such as sedation, motor impairments, and hypothermia (Higgins et al., 2001; Varty et al., 2005), there seemed to be a significant therapeutic window between efficacious and adverse effects. Therefore, collectively, these data suggest that nonpeptide NOP receptor agonists have the potential to serve as a novel class of anxiolytic agents with a reduced propensity to produce unwanted side effects. The present study has expanded this work further by testing an orally active compound unrelated to nopicetin or Ro64-6198. Specifically, we investigated the anxiolytic-like activity of a novel piperidine NOP receptor agonist, SCH 221510 (Fig. 1). The anxiolytic-like effects of SCH 221510 were assessed primarily in rats using EPM, Vogel conflict, CLS, and FPS assays; however, SCH 221510 was also tested in a gerbil EPM and a separation-induced vocalization assay in guinea pig pups to assess cross-species effects. Furthermore, to confirm the mechanism of action by which SCH 221510 produced its anxiolytic-like actions, antagonism studies were conducted using both the NOP receptor antagonist J-113397 (Ozaki et al., 2000) and the opioid antagonist naltrixone. Furthermore, to test for any tolerance to the anxiolytic-like effects, SCH 221510 was dosed for 14 days and tested in the rat Vogel conflict assay. Finally, SCH 221510 was assessed in spontaneous locomotor activity (LMA) and rotarod assays to assess any effects of motor function and coordination. It is noteworthy that the effects of SCH 221510 were compared with those of the benzodiazepine anxiolytic chlordiazepoxide (CDP) to provide a better understanding of the potential advantage an NOP receptor agonist may offer over benzodiazepine anxiolytics.

**Materials and Methods**

**Opioid Receptor Binding Assays.** NOP receptor binding assays were performed as described by Fawzi et al. (1997) and Corboz et al. (2000). In brief, CHO cell membranes expressing human NOP receptors were incubated with [125I]-[Tyr14]-N/OFQ (PerkinElmer Life and Analytical Sciences, Waltham, MA) and increasing concentrations of compound in binding assay buffer containing 50 mM HEPES, pH 7.4, 2.5 mM CaCl2, 1 mM MgCl2, 10 mM NaCl, 0.025% bacitracin, and 0.1% bovine serum albumin. Assays were performed at room temperature for 60 min and were terminated by rapid filtration over GF/B membranes (PerkinElmer Life and Analytical Sciences). Radioactivity retained on filters was determined in a TopCount microplate scintillation counter (PerkinElmer Life and Analytical Sciences). All assays were performed in duplicates. Total and nonspecific binding was determined in quadruplicates. Opioid receptor binding assays (μ-, κ-, and δ-opioid receptors) were performed on CHO cell membranes expressing the human opioid receptors (Receptor Biology, Beltsville, MD) as described by Corboz et al. (2000). In brief, CHO cell membranes were incubated with [3H]diprenorphine (PerkinElmer Life and Analytical Sciences) and increasing concentrations of compounds in binding assay buffer for 60 min at room temperature. Assays were terminated by rapid filtration over GF/B membranes, and radioactivity retained on filters was counted in a TopCount microplate scintillation counter. Kᵣ values (n = 4–6/receptor) were determined using GraphPad Prism curve-fitting and data analysis software (GraphPad Software Inc., San Diego, CA).

**Opioid Receptor Functional Assays.** [35S]GTPᵢS binding to recombinant CHO cell membranes was performed according to the method of Fawzi et al. (1997), with some minor modifications. CHO cell membranes expressing human NOP receptors (4 μg) or human μ-, κ-, and δ-opioid receptors (5 μg) (Receptor Biology, Beltsville, MD) were incubated for 30 min at room temperature with 100–300 pM [35S]GTPᵢS (PerkinElmer Life and Analytical Sciences) in an assay mixture (200 μl) containing 50 mM HEPES, pH 7.4, 10 mM MgCl₂, 1 mg/ml bovine serum albumin, 120 mM NaCl, 0.2 mM EGTA, and 1 μM GDP. Assays were terminated by rapid filtration over GF/B filter plates (PerkinElmer Life and Analytical Sciences) (pre-soaked for 30 min in 10 mM K₂HPO₄) and washed seven times with 2 ml of cold (4–10°C) buffer containing 20 mM Tris-HCl, pH 8.0, 20 mM MgCl₂, and 100 mM NaCl. Filter-bound radioactivity was quantified on a TopCount (PerkinElmer Life and Analytical Sciences). Nonspecific binding was determined by performing the assay in the presence of 10 μM GTPᵢS. For the determination of the agonist-stimulated increase in the binding of [35S]GTPᵢS, membranes were preincubated with SCH 221510 for 60 min before the initiation of the assay. EC₅₀ values were determined using GraphPad Prism software.

**Subjects.** Male CD and Wistar (only for FPS studies) rats (200–500 g) and female Mongolian gerbils (30–50 g) were obtained from Charles River Laboratories (Kingston, NY). Male and female Dunkin Hartley guinea pig pups (100–250 g) were used for the vocalization studies (Charles River Laboratories). Pups were 2 to 3 days of age upon arrival and were used up to 18 days of age. Pups were housed three per cage with the dam, and rats and gerbils were housed three
per cage, under a 12-h light/dark cycle (lights on 6:00 AM) with constant temperature and humidity. Animals were given free access to food and water (except during Vogel and CLS studies; see below) for at least 1 week before experiments. Except in the CLS assay, animals were used once only, i.e., for a single administration of drug or vehicle. All studies took place during the light cycle between 9:00 AM and 5:00 PM. All studies were conducted at an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility in accordance with the National Institutes of Health Guide to the Care and Use of Laboratory Animals and the Animal Welfare Act, and guidelines established by the Institutional Animal Care and Use Committee.

Drugs. SCH 221510 and J-113397 were synthesized by the Department of Chemical Research (Schering-Plough Research Institute, Kenilworth, NJ). CDP and naltrexone were purchased from Sigma-Aldrich (St. Louis, MO). SCH 221510 was administered via oral gavage. CDP, J-113397, and naltrexone were administered intraperitoneally. Drugs were administered in isotonic saline (CDP, J-113397, and naltrexone) or 0.4% methylcellulose (SCH 211510) at dosing volumes of 1 to 5 ml/kg (rat), 5 ml/kg (gerbil), or 1 to 2 ml/kg (guinea pig). SCH 221510 and J-113397 were administered 120 min before testing (except for the LMA study when SCH 221510 was administered 60 min before testing), and CDP and naltrexone were administered 30 and 135 min before testing, respectively. All drug doses are expressed as free base equivalents.

The route of administration and pretreatment time for SCH 221510 were determined from a pharmacokinetic study in which rats were dosed orally with SCH 221510, and samples were collected at various time points up to 8 h after administration. This study demonstrated good levels of SCH 221510 in the plasma (area under the curve 0–8 h = 352 ng·h/ml; Cmax = 50 ng/ml and brain (area under the curve 0–8 h = 473 ng·h/g; Cmax = 73 ng/g), with peak exposure from 120 to 240 min. Based on these data, a 120-min pretreatment time was chosen.

**Rat EP.** Testing was carried out in a Plexiglas plus-maze elevated to a height of 50 cm. The apparatus consisted of two open arms (50 × 10 cm) surrounded with a 0.5-cm lip, and two closed arms of the same dimensions with Plexiglas walls 50 cm in height arranged such that both open and closed arms faced each other. The floors of the EPM were matte black, and the walls of the closed arms were transparent to ensure consistent illumination in all parts of the EPM (approximately 500 lux). On the day of testing, rats were individually housed, brought to the testing room, and allowed to acclimate for at least 60 min before testing. Vehicle or test drugs were administered, and after an appropriate pretreatment time, rats were placed onto the central square of the apparatus facing an open arm. Two behavior types were scored by an observer, who was blinded to treatment, situated approximately 2 m from the EPM. Both traditional (number of entries and time spent) and ethological (number of head dips) (Cruz et al., 1994) behavioral measures were recorded during a 5-min test period. The EPM was thoroughly washed with dilute soap solution between testing each rat.

**Rat Vogel Conflict Procedure.** Testing took place in four standard operant boxes (32 × 25 × 25 cm) (MED Associates, Camden, VT). Each chamber contained a stainless steel drinking spout that protruded through the front wall. The spout was centered and elevated 3 cm above the floor of the chamber. The drinking spout was connected to an external 200-ml bottle that contained a 0.2% saccharin solution. Each operant box was located within a sound attenuating chamber identical to those described for the Vogel conflict procedure. Twenty rats were water-deprived for 20 h and trained under a schedule of CLS. Under this schedule, rats were presented with 20 trials that consisted of 23 s of unpunished drinking, followed by 7 s during which a tone was delivered to the apparatus. The first 2 s of tone presentation was unpunished, whereas every lick during the final 5 s was punished with scrambled shock delivery (0.7 mA; 500-ms duration). Rats were trained under this schedule, 5 days per week, until they made fewer than 5% of total licks during the period in which tone was presented. When rats had reached this criterion, they were tested during a session in which tone was presented but shock delivery was turned off. If rats made less than 5% of total licks in the tone periods under these conditions they were deemed to be conditioned and suitable for drug testing. On the day of testing, rats were brought to the test room and administered vehicle or a dose of drug. After an appropriate pretreatment time, rats were placed into the test apparatus, and the number of licks in both the unpunished (no tone) and punished (tone, no shock) components of the schedule were recorded for 20 trial presentations. Drug was administered using a within-subjects crossover design such that the effect of vehicle administration and up to four doses of drug was assessed over several different test sessions. Drug testing occurred no more than twice a week and only following sessions in which rats made less than 5% of total licks during the tone periods with the shock turned off. On intervening days, rats continued to be trained in sessions with both tone and concomitant shock.

**Rat Fear-Potentiated Startle.** Testing took place in an eight-chamber startle system (SR-LAB; San Diego Instruments, San Diego, CA). Rats were conditioned to associate 20 presentations of a light cue (3500-ms duration) with the presentation of a mild foot shock (0.4 mA; 500-ms duration) at an average intertrial interval of 90 s. The shock was presented during the last 500 ms of light presentation such that the light and shock terminated at the same time. Rats were exposed to two of these conditioning sessions, 24 h apart. Twenty-four hours after the second conditioning session, rats were tested in a baseline FPS session consisting of 20 startle trials (105 dB; average intertrial interval, 25 s; range, 20–30 s). Ten of these startle trials occurred in the presence of a light cue (light + startle trials), and the remaining 10 trials were startle-alone trials. In addition, five startle-alone trials were presented immediately before and immediately after presentation of the 20 test trials. The following day, rats were divided into equal, balanced groups (n = 12–14) and retested in the FPS startle session after vehicle or drug treatment. Treatments were balanced across the startle chambers to allow for any interchamber differences in stimulus presentation or startle response sensitivity.

**Gerbil EPM.** The gerbil EPM consisted of two open arms (30 × 8 cm) and two closed arms of the same dimensions with clear Plexiglas walls 20 cm in height, arranged such that both open and closed arms
faced each other. The walls of the closed arms were clear to allow for constant illumination in all parts of the EPM. The floor of the EPM was constructed of black Plexiglas with 6.5-mm-diameter holes incorporated into the floor, approximately 10 mm apart. These holes allowed the gerbils to grip the surface, as their movement seemed to be impaired on the slick Plexiglas surfaces traditionally used to construct rat and mouse EPMs. The EPM was elevated to a height of 35 cm (for details, see Varty et al., 2002).

Drug or vehicle were administered to gerbils, and after an appropriate pretreatment time, gerbils were placed into the center area of the EPM facing an open arm. An observer, situated approximately 2 m from the EPM, recorded the amount of time each gerbil spent exploring the open and closed arms, and the number of entries into the open and closed arms, for the duration of a 5-min test session. An entry into a specific arm was scored when the gerbil placed all four paws into the arm. A risk assessment behavior, i.e., the number of open arm head dips, was also measured. The EPM was thoroughly cleaned with dilute soap solution before testing of each gerbil.

Guinea Pig Pup Vocalization. To induce and record vocalizations, pups were separated from the dam and littermates by being placed into a Plexiglas box and then transported to a separate room. Pups typically vocalize within 1 min of being separated. The number of vocalizations produced during a 5-min test period was recorded by an observer in the room. At least 24 h before the first drug study, baseline vocalizations were recorded for 5 min, and only pups that produced at least 200 vocalizations were used in subsequent studies. During drug studies, pups were administered the drug and placed back with the dam and littermates for the appropriate pretreatment time. After the pretreatment time, the pups were separated and tested for 5 min. Pups were reused in up to three studies or up to 18 days of age with at least 3 days between tests.

Rat LMA and Rotarod. Spontaneous LMA was measured using eight digital activity monitors (Accuscan Instruments, Inc., Columbus, OH). Each apparatus consisted of a clear Plexiglas box (40 x 40 x 30 cm) placed within the activity monitor. LMA was detected via photocell interruptions, and data were recorded and analyzed by a computer connected to the apparatus. During testing, rats were allowed to acclimate to the test room for at least 60 min. Rats were dosed with vehicle or test drug, and then after a suitable pretreatment time, they were placed into the apparatus and allowed to freely explore for 60 min. Activity was recorded in 5-min bins, and as total activity for the duration of the test session. The activity chambers were cleaned with a dilute soap solution after each test run. Rotarod testing took place on a standard rat rotarod apparatus (Ugo Basile, Comerio, Italy) that consisted of a circular drum (10 cm in diameter), separated into four sections by plastic flanges (30 cm in diameter). This setup allowed four rats to be tested simultaneously. The rotarod was programmed to rotate at 8 revolutions per minute. On the day before testing, rats were given two training sessions during which they were placed onto the rotating rod and allowed to maintain themselves for up to 120 s. Rats that could not maintain themselves on the rotarod for 120 s during the second training session were excluded from further study. On the day of testing, rats were administered test drug and placed on the rotarod. The time that rats maintained themselves on the rotarod was measured for up to 120 s.

Statistical Analysis. In vivo data were analyzed using one-way analyses of variance (GraphPad Instat, version 3.06; GraphPad Software Inc.). Where appropriate, post-hoc comparisons were made using Dunnett’s t test, with a 5% significance level.

Results

Opioid Receptor Binding. SCH 221510 binds to the human NOP receptor with 0.3 ± 0.05 nM affinity (n = 6; Fig. 2). In the same assay, the endogenous peptide OFQ/N has a binding affinity of 0.02 nM (data not shown). The binding affinities of SCH 221510 at the human μ-, κ-, and δ-opioid receptors were 65 ± 10 nM (n = 5), 131 ± 33 nM (n = 4), and 2854 ± 144 nM (n = 4), respectively. Based on these affinities, SCH 221510 exhibits 217-, 437-, and >9500-fold selectivity versus μ-, κ-, and δ-opioid receptors, respectively. The affinity of SCH 221510 at the NOP receptor was measured across multiple species, including mouse, rat, and guinea pig, and no marked differences in affinity were noted (Kᵢ value for mouse = 1.15 ± 0.2 nM; rat = 0.42 ± 0.01 nM; and guinea pig = 1.35 ± 0.07 nM). Furthermore, SCH 221510 was screened against a panel of 104 proteins, including G protein-coupled receptors, ion channels, and kinases, and SCH 221510 did not interact with any of these targets at relevant concentrations (IC₅₀ > 3 μM; data not shown).

Opioid Receptor Functional Potency. Using a GTPγS functional assay, SCH 221510 stimulated the human NOP receptor, with an EC₅₀ value of 12 ± 3 nM (n = 8; Fig. 2). In the same assay, OFQ/N has an EC₅₀ value of 7 ± 2 nM (Fig. 2). Additionally, SCH 221510 acts as a full agonist at the NOP receptor, as highlighted by the OFQ/N-like maximal effect in the assay.
The potencies of SCH 221510 at the human μ-, κ-, and δ-opioid receptors were 693 ± 179 nM (n = 6), 683 nM (n = 1), and 8071 nM (n = 1), respectively. Based on these functional potencies, SCH 221510 exhibits 58-, 57-, and >673-fold selectivity versus μ-, κ-, and δ-opioid receptors, respectively.

**Rat EPM.** SCH 221510 significantly increased the time that rats spent on the open arms of the EPM \([F(3,77) = 6.3; p < 0.01]\) (Fig. 3) and the number of open arm entries \([F(3,77) = 5.5; p < 0.01]\). Specifically, doses of 1 and 10 mg/kg increased open arm time, whereas doses of 3 and 10 mg/kg increased open arm entries. Additionally, SCH 221510 produced a significant increase \([F(3,77) = 9.5; p < 0.01]\) in the number of open arm head dips at doses of 3 and 10 mg/kg (VEH = 2.7 ± 0.8; 1 mg/kg = 5.4 ± 0.8; 3 mg/kg = 7.7 ± 0.8, p < 0.01; and 10 mg/kg = 9.3 ± 1.3, p < 0.01). Likewise, CDP (1–10 mg/kg) produced significant increases in both open arm time \([F(3,16) = 5.6; p < 0.01]\) and open arm entries \([F(3,16) = 6.5; p < 0.01]\) (Fig. 3), as well as open arm head dips \([F(3,16) = 13.7; p < 0.01]\) (VEH = 3.8 ± 0.5; 1 mg/kg = 4.8 ± 1; 3 mg/kg = 13.2 ± 0.7, p < 0.01; and 10 mg/kg = 9 ± 0.9, p < 0.01).

**Vogel Conflict.** SCH 221510 (1–30 mg/kg) dose-dependently increased the number of punished licks in a Vogel conflict assay (Fig. 4). The effects of SCH 221510 were significantly different from vehicle control \([F(4,97) = 6.8; p < 0.01]\) after doses of 3 to 30 mg/kg. CDP produced significant increases in punished licks at a dose of 6 mg/kg \([F(3,14) = 3.8; p < 0.05]\) (Fig. 4). At higher doses, CDP did not increase punished licking further, possibly due to nonspecific effects interfering with the anxiolytic-like actions. The increases in punished licks observed following administration of SCH 221510 seemed to be due to specific anxiolytic-like actions as SCH 221510 (3–30 mg/kg) did not change the number of unpunished licks measured in a Vogel test in which no shock was presented (VEH = 654 ± 103 licks; 3 mg/kg = 610 ± 62; 10 mg/kg = 679 ± 66; and 30 mg/kg = 704 ± 109).

**Rat CLS.** In an assay of conditioned anxiety, SCH 221510 (1–10 mg/kg) produced significant \([F(3,27) = 6.5; p < 0.01]\), dose-related increases in the number of licks that had been previously suppressed by tone paired with shock delivery (Fig. 4). SCH 221520 increased licking significantly at a dose of 10 mg/kg. CDP (1–10 mg/kg) also significantly increased

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Fig. 3. Effect of SCH 221510 (1–10 mg/kg) and CDP (1–10 mg/kg) in rat EPM. Top, time spent in open arms. Bottom, number of entries into open arms. Each bar indicates a mean ± S.E.M. of 19 to 21 rats at each dose. Significant differences from vehicle (0 mg/kg) are indicated by ***, p < 0.01 and *, p < 0.05.
licking that had been suppressed previously \(F(3,44) = 7.2; p < 0.01\), with licks increasing significantly following CDP doses of 6 and 10 mg/kg (Fig. 4). Additionally, neither SCH 221510 \(F(3,27) = 1.3; p = 0.29\) nor CDP \(F(3,44) = 0.7; p = 0.57\) had any effect on licking during the unpunished phase of the test.

**Rat FPS.** SCH 221510 (3–10 mg/kg) dose-dependently reduced FPS, with a significant main effect of treatment \(F(3,44) = 2.8; p = 0.05\). Although post hoc analysis did not reveal any significance for a specific dose group compared with vehicle, clearly the amount of FPS is attenuated following a dose of 10 mg/kg (Fig. 5). It is noteworthy that SCH 221510 attenuated FPS without affecting the unconditioned startle response \(F(3,44) = 0.53; p = 0.66\). Likewise, CDP also dose-dependently reduced FPS, with a significant main effect of treatment \(F(3,52) = 2.7; p = 0.05\) and with a significant reduction following a dose of 10 mg/kg (Fig. 5). Although CDP did not significantly reduce the startle response \(F(3,52) = 2.4; p = 0.08\), there was a clear trend for a dose-related reduction, and this is probably due to the sedative-like effects seen at higher doses.

**Gerbil EPM.** SCH 221510 significantly increased the time that gerbils spent on the open arms of the EPM \(F(3,20) = 4.4; p < 0.05\) at doses of 3 and 10 mg/kg (Fig. 6). There was no effect on the number of open \(F(3,20) = 1.2; p = 0.35\) and closed \(F(3,20) = 0.19; p = 0.91\) arm entries. Furthermore, SCH 221510 produced a significant increase \(F(3,20) = 6.6; p < 0.01\) in the number of open arm head dips at 3 and 10 mg/kg (VEH = 0.8 ± 0.5; 1 mg/kg = 1.5 ± 1.5; 3 mg/kg = 13 ± 9.1, \(p < 0.05\); and 10 mg/kg = 14.2 ± 9.4, \(p < 0.01\)).

**Guinea Pig Pup Vocalization.** SCH 221510 (0.3–3 mg/kg) dose-dependently attenuated separation-induced vocalizations in guinea pig pups \(F(3,37) = 41; p < 0.01\), with significant reductions at all three doses (Fig. 6).

**Vogel Conflict: Antagonism Study.** In studies to determine specificity of the anxiolytic-like effects, the significant anxiolytic-like effect of SCH 221510 (10 mg/kg) was attenuated by pretreatment with the NOP receptor antagonist...
**Fig. 5.** Effect of SCH 221510 (3–10 mg/kg) and CDP (1–10 mg/kg) in the rat FPS procedure. Top, percentage of FPS. Bottom, mean startle response. Each bar indicates mean ± S.E.M. of 12 to 14 rats at each dose. Significant differences from vehicle (0 mg/kg) are indicated by *, p < 0.05.

**Fig. 6.** Effect of SCH 221510 in the gerbil EPM (1–10 mg/kg) and guinea pig pup vocalization assay (0.3–3 mg/kg). Each bar indicates mean ± S.E.M. of six gerbils or 10 to 11 pups at each dose. Significant differences from vehicle (0 mg/kg) are indicated by *, p < 0.05 and **, p < 0.01.
J-113397 (3–10 mg/kg) (Fig. 7). In contrast, pretreatment with the nonspecific, opioid receptor antagonist naltrexone (3–10 mg/kg) did not alter the anxiolytic-like effects of SCH 221510 (Fig. 7).

**Vogel Conflict: Chronic Dosing Study.** An acute injection of 10 mg/kg SCH 221510 increased the number of punished licks in rats treated chronically with vehicle (VEH/SCH), compared with the VEH/VEH group. There was no difference between VEH/VEH treated rats and those that received SCH 221510 chronically, but received vehicle on test day (SCH/VEH), suggesting no carryover effects of the chronic administration. In the SCH/SCH group, 10 mg/kg SCH 221510 increased the number of punished licks significantly \[F(3,16) = 9.3; p < 0.01\] (Fig. 8) following chronic administration, suggesting that there is no tolerance to the anxiolytic-like effects upon chronic exposure.

**Rat LMA and Rotarod.** SCH 221510 (3–30 mg/kg) did not markedly affect spontaneous LMA or motor coordination as measured by an open field activity monitor and rotarod, respectively (Fig. 9). In contrast, 3 to 30 mg/kg CDP produced dose-related decreases in both spontaneous LMA \[F(3,44) = 15.8; p < 0.01\] and rotarod performance \[F(3,44) = 39.9; p < 0.01\], with significant reductions at doses of 10 and 30 mg/kg (Fig. 9).

**Discussion**

SCH 221510 is a novel, high-efficacy NOP receptor agonist that binds to the NOP receptor with an affinity of 0.3 nM and has a functional in vitro potency (EC50) of 12 nM. SCH 221510 displays 217-fold binding selectivity and 57-fold functional selectivity for the NOP site, compared with the other opioid receptors.

In preclinical animal models used to identify potential anxiolytic-like activity, SCH 221510 produces robust effects that are similar to the effects produced by the benzodiazepine CDP. Additionally, unlike CDP, SCH 221510 produces anxiolytic-like activity at doses that do not seem to produce nonspecific disruption of overt behaviors such as locomotor activity.

The anxiolytic-like effects of SCH 221510 observed in the present studies mimic effects observed following central administration of OFQ/N directly into the CNS of both mice and rats. Jenck et al. (1997) showed that OFQ/N increased exploration of the brightly lit arena of a mouse light/dark apparatus and increased open arm exploration in a rat EPM. Furthermore, OFQ/N increased responding in a mouse Geller-Seifter test. Likewise, Griebel et al. (1999) demonstrated that centrally administered OFQ/N had a role in the adaptive responses to unavoidable stressful stimuli in the ethologically based mouse defense test battery. In addition, data generated from knockout mice further support a modulatory role for OFQ/N in the control of stress and anxiety. For example, Köster et al. (1999) showed that homozygous OFQ/N knockout mice displayed an enhanced anxiety-like phenotype when exposed to an open field or EPM. Additionally, these mice had elevated basal circulating levels of corticosterone, as well as an enhanced corticosterone response after a period of mild stress. Finally, the OFQ/N knockout mice did not adapt to repeated swim stress. However, the
group of Devine et al. have demonstrated that central administration of OFQ/N induces anxiogenic-like effects in mice (Fernandez et al., 2004) and rats (Green et al., 2007) in the elevated plus-maze, open field, and light-dark box models. The present experiments with SCH 221510 are consistent, however, with previous findings with both OFQ/N (Jenck et al., 1997; Griebel et al., 1999) and the nonpeptide agonist Ro64-6198 (Jenck et al., 2000; Varty et al., 2005) that demonstrated robust anxiolytic-like profiles in multiple species and across a larger number of behavioral models. Reasons for these discrepancies are not clear and warrant further study.

The anxiolytic-like effects of SCH 221510 were broad ranging, because effects were observed in assays that used acute, nonconditioned fear such as the EPM and separation-induced vocalization assays, classic punishment assays such as the Vogel conflict procedure, and conditioned fear assays such as CLS and FPS. It is noteworthy that the anxiolytic-like effects of SCH 221510 were evident in three species, similar to CDP. The magnitude of the effects observed was qualitatively similar to those observed following administration of the clinically used benzodiazepine CDP. In the rat EPM, SCH 221510 and CDP produced anxiolytic-like effects, with CDP having greater absolute effects on the traditional measures of open arm time and entries. In the gerbil EPM, SCH 221510 produced anxiolytic-like effects as measured by open arm time, and the magnitude of the effect was similar to CDP, based on a previous report from our group [Veh = 50 (9 s, 1 mg/kg CDP = 127 (13 s) (Varty et al., 2002). Furthermore, in both EPMs, SCH 221510 reduced open arm headdips, which is also consistent with an anxiolytic-like profile (Cruz et al., 1994). SCH 221510 also produced a clear attenuation of vocalizations in guinea pig pups separated from their dam, a model sensitive to the acute anxiolytic effects of both benzodiazepines and selective serotonin reuptake inhibitor antidepressants (Kramer et al., 1998). In a similar manner to the EPM studies, SCH 221510 and CDP increased the number of punished licks in a Vogel conflict procedure, with CDP having a somewhat greater effect. The effects of SCH 221510 in the Vogel procedure were reversed by the NOP antagonist J-113397, but not by the opioid antagonist naltraxone, indicating a selective action through the NOP receptor. Of interest, in this assay SCH 221510 produced dose-related increases in punished licking, whereas CDP increased punished licks at low to intermediate doses, before

**Fig. 9.** Effect of SCH 221510 (3–30 mg/kg) (left) and CDP (3–30 mg/kg) (right) on LMA (top) and rotarod performance (bottom). Each bar indicates mean ± S.E.M. of eight rats at each dose. Significant differences from vehicle (0 mg/kg) are indicated by ***, p < 0.01.
exhibiting a typical bell-shaped function as the dose was increased. The loss in efficacy observed with CDP is presumably due to nonspecific sedative effects interfering with licking behavior. Additionally, SCH 221510 did not alter levels of unpunished licking in the Vogel procedure or, in separate studies, alter nociceptive thresholds in analgesia assays (data not shown). These data indicate the effects of SCH 221510 in the Vogel assay were not due to nonspecific actions on thirst drive or changes in how the rats perceived the aversive nature of the electric shock punishment. In assays of conditioned fear, SCH 221510 released suppressed licking behavior to a similar degree to CDP, and it attenuated FPS to a similar degree to CDP, without affecting the startle response. Collectively, therefore, the differences in the profile of activities observed with SCH 221510 and CDP in the present study suggest that NOP agonists may have greater clinical impact in learned anxiety compared with acute anxiety states. Indeed, Griebel et al. (1999) showed that OFQ/N did not selectively alter the flight responses of mice in a defensive test battery assay, a marker thought to highlight antipanic actions. In addition, Jenck et al. (2000) showed that the NOP agonist Ro64-6198 was ineffective in a model of panic-like behavior, namely, electrical stimulation of the periaqueductal gray. The degree and type of anxiolytic-like action that NOP agonists produce clinically remains to be investigated.

SCH 221510 did not produce profound disruption of spontaneous or forced motor behavior at doses that produced anxiolytic-like activity. This is in contrast to the effects observed with CDP where dose-related decreases in spontaneous and forced motor behavior were observed. Although clinically effective, benzodiazepine use is associated with the potential to produce sedation, amnesia, and muscle relaxation, as well as tolerance and dependence (Busto and Sellers, 1991; Roth and Roehrs, 1992). The fact that SCH 221510 and Ro64-6198 (Jenck et al., 2000) did not produce these effects at active doses, or as with Ro64-6198 (Dautzenberg et al., 2001), the effects of SCH 221510 did not decrease after a chronic dosing regime, suggests that drugs of this class may be devoid of these side-effect liabilities. Presently, it is not clear how SCH 221510 and other NOP agonists produce their anxiolytic-like activity within the CNS. Histological studies have demonstrated OFQ/N and NOP receptors in several brain regions, including the dorsal raphe nucleus, associated with the control of anxiety/stress and emotion (Darland et al., 1998; Mollereau and Mouledous, 2000). Electrophysiological studies in brain slices have demonstrated that OFQ/N exerts a depressant-like effect on neuronal activity in these areas (Connor et al., 1996; Vaughan and Christie, 1996; Vaughan et al., 1997). In vitro, OFQ/N has been shown to inhibit serotonin release from cortical serotonergic nerve terminals (Siniscalchi et al., 1999). The dorsal raphe nucleus is the main cell body region of the serotonergic neuronal system in the CNS, and neuronal hyperactivity in this area has been associated with increased anxiety (Eison and Eison, 1994; Coplan et al., 1995). Furthermore, there is substantial preclinical evidence showing the anxiolytic actions of agents that either decrease presynaptic release of serotonin (Sánchez, 1993; Menard and Treit, 1999) or block postsynaptic serotonin receptors within this neuronal system (Griebel, 1995; Menard and Treit, 1999). Thus, normalization of serotonergic neuronal activity by NOP agonists in these areas may be responsible for the anxiolytic-like effects observed. Furthermore, OFQ/N has been shown to decrease the release of tachykinins from peripheral tissues (Fischer et al., 1998). Neurokinin NK1 and NK2 antagonists have also been shown to produce anxiolytic-like actions preclinically, and NK1 antagonists have been shown to do the same in clinical trials (Walsh et al., 1995; Teixeira et al., 1996; Kramer et al., 1998). Therefore, a mechanism whereby OFQ/N in some way decreases the release of central tachykinins such as substance P may also produce the observed effects of OFQ/N and the small molecule NOP agonists. Finally, Köster et al. (1999) showed that OFQ/N knockout mice had higher levels of circulating corticosterone, suggesting that OFQ/N has a regulatory role in the hypothalamic-pituitary-adrenal axis. Acute administration of agents that decrease activity of the hypothalamic-pituitary-adrenal axis have been shown to have anxiolytic-like actions (Schultz et al., 1996; Webster et al., 1996). Therefore, NOP agonists may mediate their actions by suppressing the increase in hypothalamic-pituitary-adrenal axis activity normally associated with stressful events (but see Devine et al., 2001). However, the exact mechanism by which SCH 221510 produces the effects observed in the present studies remains to be elucidated.

In summary, the present study highlights the anxiolytic-like actions of the selective NOP agonist SCH 221510. The anxiolytic-like effects observed were qualitatively similar to those of the benzodiazepine CDP. Unlike the benzodiazepines, however, the effects observed occurred at doses that did not disrupt ongoing behavior and did not tolerate after chronic dosing. This suggests that nonpeptide NOP agonists may represent a novel class of anxiolytic agents devoid of side effects associated with this major clinical drug class.

References


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