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The Opioid Receptor Like-1 Receptor Agonist Ro 64-6198 (1S,3aS-8–2,3,3a,4,5,6-Hexahydro-1H-phenalen-1-yl-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one) Produces a Discriminative Stimulus in Rats Distinct from That of a μ, κ, and δ Opioid Receptor Agonist Cue

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ABSTRACT

Male Wistar rats were trained to discriminate either the opioid receptor like (ORL)-1 receptor agonist Ro 64-6198 (1S,3aS-8–2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one) or morphine from saline using a two-choice, food reinforced, operant procedure. Acquisition of Ro 64-6198 discrimination was relatively slow (mean trials to criterion 113 ± 6), and a final 4 mg/kg dose (initial training dose 2 mg/kg) was required to establish appropriate stimulus control. In comparison, a separate group of rats attained a morphine (2 mg/kg) discrimination in 44 ± 4 trials. In tests of substitution, Ro 64-6198 produced a dose-related generalization to its own cue (ED_{50} of 1.1 mg/kg i.p.), yet only weakly generalized to the morphine cue (19% at 10 mg/kg i.p.). In contrast, morphine generalized completely to the morphine cue (ED_{50} of 0.7 mg/kg s.c.), yet only partially generalized to the Ro 64-6198 cue (40% at 6 mg/kg s.c.). The κ opioid receptor agonist U50,488 [trans-3,4-dichloro-N-methyl-N(2-[1-pyrrolidinyl]cyclohexyl) benzeneacetamide methanesulfonate] (0.3–6 mg/kg s.c.) and the δ opioid receptor agonist SNC-80 [(+)-4-[(αR)-α-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-N,N-diethylbenzamide] (0.3–6 mg/kg i.p.) failed to evoke significant generalization to either cue. The μ opioid receptor agonists codeine (0.3–20 mg/kg) and buprenorphine (0.01–1 mg/kg) completely generalized to the morphine cue, but only buprenorphine partially generalized to the Ro 64-6198 cue. Naloxone pretreatment completely blocked the morphine cue (ED_{50} of 0.005 mg/kg s.c.), yet only weakly attenuated the Ro 64-6198 cue at 0.3 mg/kg. Finally, the selective ORL-1 antagonist J-113397 [1-[(3R,4R)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1, 3-dihydro-2H-benzimidazol-2-one] completely blocked the Ro 64-6198 cue at a dose (30 mg/kg i.p.) that had no effect against the morphine cue. The present studies demonstrate that rats may be trained to discriminate Ro 64-6198 from saline, and the pharmacological characteristics of this cue are most consistent with ORL-1 receptor activation.

Opioid receptors are currently classified into four subclasses, the δ opioid peptide receptor, κ opioid peptide receptor, μ opioid peptide receptor, and the nociceptin/orphaninFQ peptide receptor (also termed ORL-1) subclass (Calo et al., 2000; Snyder and Pasternak, 2003). The ORL-1 receptor represents the most recent addition to this family, identified by various laboratories during efforts to clone subtypes to the μ, κ, or δ receptor subclasses (for recent reviews, see Calo et al., 2000; Mogil and Pasternak, 2001).

The ORL-1 receptor has a pharmacology that is clearly distinct from the other opioid subclasses. For example, naloxone, a relatively nonselective antagonist at μ, κ, and δ receptors, has low affinity for the ORL-1 subtype (Calo et al., 2000; Mogil and Pasternak, 2001). Furthermore, nonpeptide ligands apparently selective for the ORL-1 receptor have been identified, notably the agonist Ro 64-6198 (Jenck et al., 2000), and the antagonist J-113397 (Ozaki et al., 2000). Ro 64-6198 seems to function as a full agonist, equivalent to the likely endogenous agonist orphaninFQ/nociceptin (OFQ/N; Calo et al., 2000) in cell-based systems expressing the human ORL-1 receptor, and with at least 100-fold selectivity versus

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ABBREVIATIONS: ORL-1, opioid receptor like; Ro 64-6198, 1S,3aS-8–2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one; OFQ/N, orphaninFQ/nociceptin; U50,488, trans-3,4-dichloro-N-methyl-N(2-[1-pyrrolidinyl]cyclohexyl) benzeneacetamide methanesulfonate; SNC-80, (+)-4-[(αR)-α-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-N,N-diethylbenzamide; J-113397, 1-[(3R,4R)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1, 3-dihydro-2H-benzimidazol-2-one; CPP, conditioned place preference; MOR, morphine group; RO, Ro 64-6198 group.

other opioid receptors (Jenck et al., 2000; Hashiba et al., 2002). One of the most compelling demonstrations of the apparent in vivo selectivity of Ro 64-6198, at least in the mouse, is the demonstration that many neurological effects produced by this drug, e.g., hypolocomotion, ataxia, and hypothermia, are abolished in ORL-1 receptor-deficient mice (Higgins et al., 2001). Furthermore, various studies have now documented the in vivo effects of Ro 64-6198 in a variety of assays designed to investigate the anxiolytic, cognitive, and abuse liability of this drug class (Jenck et al., 2000; Higgins et al., 2002; Le Pen et al., 2002).

Clinically, many opioid drugs are traditionally associated with their subjective effects, which can be investigated experimentally in various animal species through drug discrimination procedures (Stolerman et al., 1987; Dykstra et al., 1997). Among the opioid subclass, the discriminative stimulus effects of μ-, κ-, and δ-selective compounds have been intensively investigated in multiple species, including rats and primates. Morphine, U50,488, and SNC-80 have each been used as training drugs to establish and characterize μ, κ, and δ opioid receptor cues, respectively (Shannon and Holtzman, 1976; Picker et al., 1990; Broqua et al., 1998; Brandt et al., 1999; Stevenson et al., 2002). Such studies suggest that each receptor subtype is associated with a pharmacologically distinct cue, consistent with a clinical literature that describes a unique subjective state induced at least by μ and κ receptor-selective ligands (Smith and Beecher, 1962; Kumor et al., 1986; Dykstra et al., 1997; Walsh et al., 2001). As yet, there is little or no information relating to whether animals can be trained to an ORL-1 receptor discriminative stimulus, and if so, how it may compare with other opioid receptor cues.

Accordingly in the present article, we trained of a group of Wistar rats to discriminate Ro 64-6198 from saline. Once discriminative control had been attained, we first established that the cue is clearly distinct from a morphine (μ) cue, and the pharmacology is most consistent with ORL-1 receptor activation. Second, because Ro 64-6198 has at least 100-fold selectivity over other opioid receptors, notably μ (Jenck et al., 2000), we trained a separate group of rats to a morphine cue to examine the effect of Ro 64-6198 in these animals. These studies were also of interest, given recent reports that OFQ/N pretreatment may block the acquisition of a morphine conditioned place preference (Murphy et al., 1999; Ciccocioppo et al., 2000), thus indicating a potential interaction between ORL-1 receptor agonism and a morphine-discriminative stimulus. Furthermore, because ORL-1 receptor agonists are considered to have therapeutic potential in indications such as anxiety (Jenck et al., 2000; Smith and Moran, 2001), a comparison between the Ro 64-6198 and morphine cue may provide insight into any potential abuse liability that selective ORL-1 agonists may have in humans (Mansbach et al., 2003).

Materials and Methods

Animals and Housing. Fourty-eight male Wistar rats (starting weight 200–250 g; Charles River Laboratories, Inc., Wilmington, MA) were randomly divided into two groups of 24. One group was assigned to receive either morphine or saline, the other Ro 64-6198 or saline (see below for details). The animals were grouped in pairs, in plastic bottomed cages containing sawdust bedding in a holding room controlled for temperature (22 ± 1°C) and humidity (45%). The animals were placed on restricted diet (ad libitum food 1 h/day) presented as a single meal at the end of the day. All testing was conducted during the light phase of the animals light/dark cycle (lights on 7:00 AM–7:00 PM).

Animal housing and subsequent experimentation was conducted in accordance with the National Institutes of Health Guide for the Health and Care of Laboratory Animals. A local Animal Users Committee also served to ensure effective implementation of standards.

Drugs and Injections. Drug form and source were as follows: morphine sulfate, naloxone HCl, codeine sulfate, and buprenorphine HCl were all from Sigma-Aldrich (St. Louis, MO); Ro 64-6198 and J-113397 were synthesized in-house by Drs. Ginny Ho and Deen Tulshian (Department of Chemistry, Schering-Plough, Kenilworth, NJ); and U50,488 and SNC-80 were both from Tocris Cookson Inc. (Ellisville, MO). Morphine, codeine, naloxone, U50,488, and buprenorphine were each dissolved in saline and injected subcutaneously at 1 ml/kg. SNC-80 was prepared in saline with drops of 0.1 M HCl and injected intraperitoneally at 3 ml/kg. Ro 64-6198 was suspended in 0.3% Tween 80 and injected intraperitoneally at 5 ml/kg. J-113397 was dissolved in 1% Tween 80 solution and injected intraperitoneally at 2 ml/kg. All drugs were injected 30 min before testing with the exception of naloxone, which was injected 45 min before testing. All drug doses refer to base.

Apparatus. Test apparatus consisted of 12 standard operant boxes (33 × 25 × 33 cm, length × width × height; MED Associates, St. Albans, VT), each equipped with a central food hopper dispensing 45-mg food pellets (Bio-Serve, Frenchtown, NJ). Two retractable levers were located 7.5 cm either side of the food tray. The equipment was under the control of Kestrel software (Conclusive Solutions, Harlow, UK).

Discrimination Training. Animals were trained to lever press on each lever for food reinforcement; the schedule requirements gradually increased to a final fixed ratio of 10, i.e., 10 responses on the designated lever was required for delivery of a single pellet, whereas responses on the second lever were recorded but not reinforced. At this point, drug discrimination training began. Rats were randomly assigned to two groups of 24. One group was treated with either morphine (2 mg/kg; MOR group) or saline by the subcutaneous route 30 min before each operant session. The second group was treated with either Ro 64-6198 (initial dose 2 mg/kg; RO group) or saline by the intraperitoneal route. Half of the animals in each group were trained to press the left lever after drug injection and the right lever after saline injection. The remainder were trained to the opposite lever designation. Training sessions were either of 20-min duration or until the delivery of 50 pellets. Care was taken to balance lever designation over consecutive sessions. Training sessions were run 5 days/week and a single session conducted per day, although occasionally two sessions/day were run, in which case the initial trial was always vehicle designation.

Training continued until the animals attained appropriate stimulus control that was defined as six consecutive sessions where animals made no more than 16 lever presses before the delivery of the first reward, and at least 95% total responses on the appropriate lever. Although appropriate stimulus control was attained with all rats from the MOR group at the initially chosen dose, in the RO group a final Ro 64-6198 dose of 4 mg/kg was required before a significant number of rats showed stable stimulus control. These training criteria were based on previously published work (Joharchi et al., 1993).

Discrimination Testing. Testing was conducted on Wednesdays and Fridays, subject to appropriate performance on intervening days. On test days, both levers were designated active, i.e., every 10th response on either lever resulted in delivery of a food pellet. Test sessions continued until 50 pellets had been obtained or 20 min had elapsed. During these sessions response rate was also measured. Tests of generalization and antagonism were conducted with drugs

given as outlined above, and doses/routes of injection based on previously published literature.

Data Analysis. The percentage of drug-appropriate lever responding was determined for each animal, and group means ± S.E.M. were determined. Response rate was also measured and expressed as percentage of most recent vehicle response rate (1–2 lever/presses/s). ED_{50} values from tests of generalization or antagonism were calculated for each test drug using Prism software (GraphPad Software, Inc., San Diego, CA).

Results

Acquisition of Ro 64-6198 and Morphine Discrimination. All rats readily acquired stable discriminative stimulus control to morphine (2 mg/kg s.c.), requiring 44 ± 3 trials to reach criteria as outlined under *Materials and Methods* (i.e., six successive correct trials). In contrast, acquisition of the Ro 64-6198 cue was considerably slower and required gradual dose escalation, such that at 26 trials the training dose was increased from 2 to 3 mg/kg i.p. and then further increased to 4 mg/kg i.p. after 83 trials. At this point, stimulus control to Ro 64-6198 began to emerge. Overall, the mean number of trials to criteria to the RO cue was 113 ± 6, excluding the 33% of animals which failed to reach criteria by 150 trials. Consequently, these animals were dropped from the study, leaving a total of 16 animals for the pharmacological characterization of this stimulus. Furthermore, to be able to use this number of animals, a less stringent set of inclusion criteria was adopted. A subject was considered to meet criteria when five of the last six trials were correct (<16 presses

before a reward, 95% appropriate responses), with each of the last three trials being classified as correct.

Tests of Substitution and Antagonism: MOR Cue. Morphine (0.1–2 mg/kg s.c.) generalized completely in MOR-trained rats, with a calculated ED_{50} value of 0.7 mg/kg. Response rate was only marginally reduced (~25%) at the 2 mg/kg dose, which was the highest dose tested in these animals (Fig. 1A; Table 1). Ro 64-6198 (0.3–10 mg/kg i.p.) was tested for potential generalization to the MOR cue, and only very modest generalization was noted at 6 (14%) and 10 mg/kg (20%). Higher doses could not be tested due to the magnitude of its rate decreasing effects (Fig. 1B; Table 1). Both codeine (0.3–20 mg/kg s.c.) and buprenorphine (0.003–1 mg/kg s.c.) produced dose-related and complete generalization to the MOR cue. Although codeine had minimal effect on response rate over the dose range tested, by the 0.3 mg/kg dose, buprenorphine suppressed response rate by 87% relative to vehicle pretreatment (Fig. 2; Table 1).

The κ opioid receptor agonist U50,488 (0.3–6 mg/kg s.c.) and the δ opioid receptor agonist SNC-80 (0.3–6 mg/kg i.p.) produced no significant generalization to the MOR cue. U50,488 was tested up to 6 mg/kg, which completely suppressed response rate. SNC-80 had no effect on responding at any dose tested (Fig. 1; Table 1).

In tests of antagonism to the MOR (2 mg/kg s.c.) cue, naloxone produced a dose-related inhibition with an ED_{50} value of 0.005 mg/kg s.c. (Fig. 3A; Table 1). In contrast, the ORL-1 antagonist J-113397 (1–50 mg/kg i.p.) had no effect against this cue (Fig. 3B; Table 1). In separate studies,
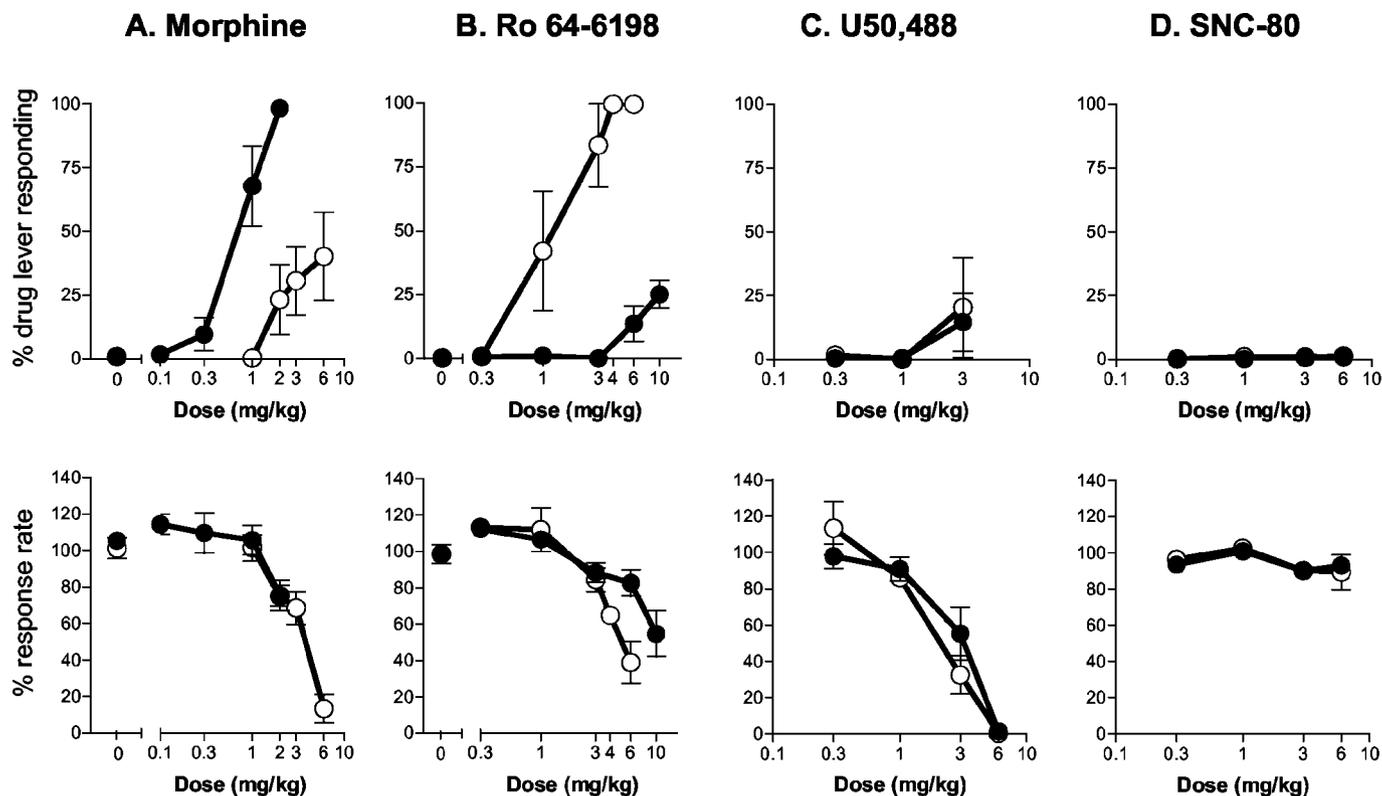
Fig. 1. Results of generalization tests in rats trained to discriminate either the ORL-1 agonist Ro 64-6198 (4 mg/kg i.p.) from saline (○) or morphine (2 mg/kg s.c.) from saline (●) in a two-lever operant drug discrimination procedure. Rats were injected with different doses of either morphine (4–8 animals/dose) (A), Ro 64-6198 (4–8 animals/dose) (B), U50,488 (4 animals/dose) (C), or SNC-80 (4 animals/dose) (D). Top, percentage of responding on the drug-associated lever. Bottom, rate of responding during this test, expressed as a percentage of the vehicle rate from the trial immediately preceding this test. The normal rate averaged over each experiment presented was 1 to 2 lever presses/s.

TABLE 1
Summary of generalization and antagonist studies for test compounds in rats trained to discriminate an Ro 64-6198 cue (4 mg/kg i.p.) from saline or a morphine cue (2 mg/kg s.c.) from saline

Effect of each drug on suppression of response rate is also summarized. Data are expressed as ED_{50} values as calculated using Prism, version 5.0.

| Test Drug	Ro 64-6198 Cue			Morphine Cue		
	Generalization	Antagonism	Response Rate	Generalization	Antagonism	Response Rate
Ro 64-6198	1.1 mg/kg		5.8 mg/kg	20% @ 10 mg/kg	>6 mg/kg	~10 mg/kg
Morphine	40% @ 6 mg/kg		4.3 mg/kg	0.7 mg/kg		>2 mg/kg
U50,488	>6 mg/kg		1.7 mg/kg	>6 mg/kg		2.9 mg/kg
SNC-80	>6 mg/kg		>6 mg/kg	>6 mg/kg		>6 mg/kg
Codeine	>20 mg/kg		>20 mg/kg	5.8 mg/kg		>20 mg/kg
Buprenorphine	>0.1 mg/kg		0.08 mg/kg	0.03 mg/kg		0.09 mg/kg
Naloxone		> 0.3 mg/kg	>0.3 mg/kg		0.005 mg/kg	>0.3 mg/kg
J-113397		9.7 mg/kg	>30 mg/kg	>30 mg/kg	>50 mg/kg	14.7 mg/kg

A. Buprenorphine

B. Codeine
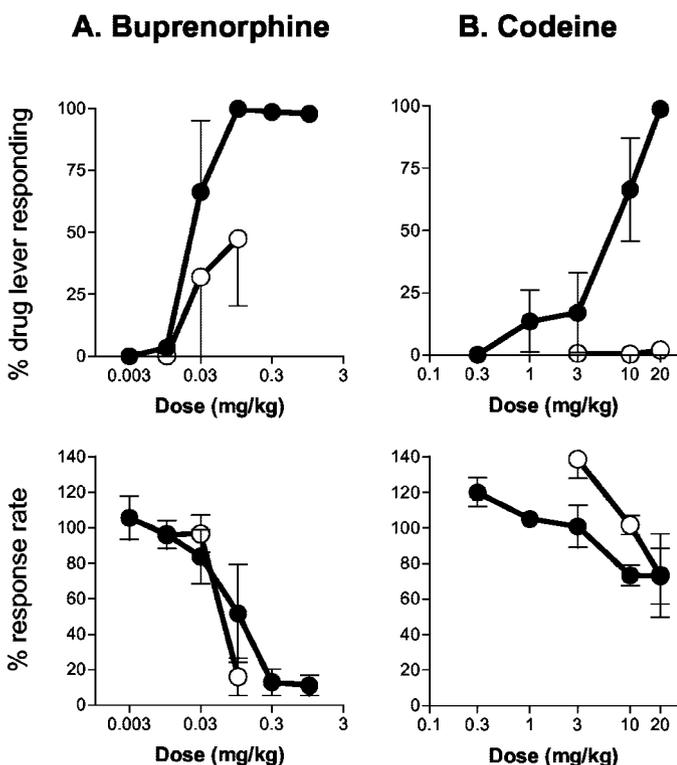
Fig. 2. Results of generalization tests in rats trained to discriminate either the ORL-1 agonist Ro 64-6198 (4 mg/kg i.p.) from saline (○) or morphine (2 mg/kg s.c.) from saline (●) in a two-lever operant drug discrimination procedure. Rats were injected with different doses of either buprenorphine (4 animals/dose) (A) or codeine (4–8 animals/dose) (B). Top, percentage of responding on the drug-associated lever. Bottom, rate of responding during this test, expressed as a percentage of the vehicle rate from the trial immediately preceding this test. The normal rate averaged over each experiment presented was 1–2 lever presses/s.

J-113397 also did not significantly generalize to MOR (0% at 3 mg/kg, 22% at 10 mg/kg, and 21% at 30 mg/kg; n = 4/dose). At the highest dose of 50 mg/kg, J-113397 reduced response rate by 90%. In addition to tests of generalization, Ro 64-6198 was also administered before MOR treatment to examine whether it could block the MOR cue. At doses up to 6 mg/kg, Ro 64-6198 only minimally affected the morphine cue, reducing generalization to approximately 80% of baseline response. Interpretation of these data is also compromised by a parallel decrease in response rate (Fig. 3C).

Tests of Substitution and Antagonism: RO Cue. Ro 64-6198 (0.3–6 mg/kg i.p.) produced a dose-related generalization to the RO cue with an ED_{50} value of 1.1 mg/kg. Above the 3 mg/kg dose, Ro 64-6198 reduced response rate; indeed,

at the training dose of 4 mg/kg, this measure was approximately 35% lower than response rate after vehicle pretreatment (Fig. 1B; Table 1). At a low dose of 1 mg/kg, morphine elicited no RO generalization. However, at 2 mg/kg and above, some modest generalization to the RO lever was apparent, being in the order of 40% at the 6 mg/kg dose, although at this dose, response rate was only 13% of vehicle control (Fig. 1A; Table 1). U50,488 (0.3–6 mg/kg s.c.), codeine (3–20 mg/kg s.c.), and SNC-80 (0.3–6 mg/kg i.p.) failed to elicit any significant RO generalization (Figs. 1 and 2B; Table 1). Buprenorphine (0.03–0.1 mg/kg s.c.), on the other hand, evoked a modest generalization, although in common with morphine this was at doses that markedly suppressed rate (Fig. 2A; Table 1).

In tests of antagonism to the RO (4 mg/kg) cue, the ORL-1 antagonist J-113397 (1–30 mg/kg) produced a dose related inhibition with an ED_{50} value of 9.7 mg/kg i.p. (Fig. 3B; Table 1). Alternately, naloxone (0.0003–0.3 mg/kg) produced only modest inhibition (40%) at the highest dose (Fig. 3A). J-113397 (3–10 mg/kg) also reversed the rate decreasing effect of Ro 64-6198; however, at 30 mg/kg J-113397 decreased response rate itself. Naloxone failed to reverse the rate decreasing effects of the 4 mg/kg dose of the training compound (71% at 0.3 mg/kg naloxone).

Discussion

There are two novel observations from the present studies. First, rats may be trained to discriminate the ORL-1 receptor agonist Ro 64-6198 from saline, with a pharmacology most consistent with ORL-1 receptor activation and clearly distinct from μ, δ, or κ opioid receptor pharmacology. Second, there seems to be little evidence for cross-generalization between the Ro 64-6198 cue and a morphine cue; thus, Ro 64-6198 did not engender significant morphine substitution except at high rate decreasing doses, and the selective ORL-1 receptor antagonist J-113397 did not block this cue.

We selected the ORL-1 agonist Ro 64-6198 as the training drug in an attempt to establish a cue based at this receptor. There are very few centrally acting, nonpeptide ORL-1 receptor agonists available with acceptable bioavailability after systemic injection. Presently, Ro 64-6198 (Jenck et al., 2000) likely represents the best characterized drug of this class, because many of its behavioral effects after intraperitoneal injection are absent in ORL-1 receptor knockout mice (Higgins et al., 2001). In rats, Jenck and coworkers have characterized Ro 64-6198 as having efficacy across a broad range of anxiety tests, over the dose range 0.3 to 3 mg/kg i.p., whereas
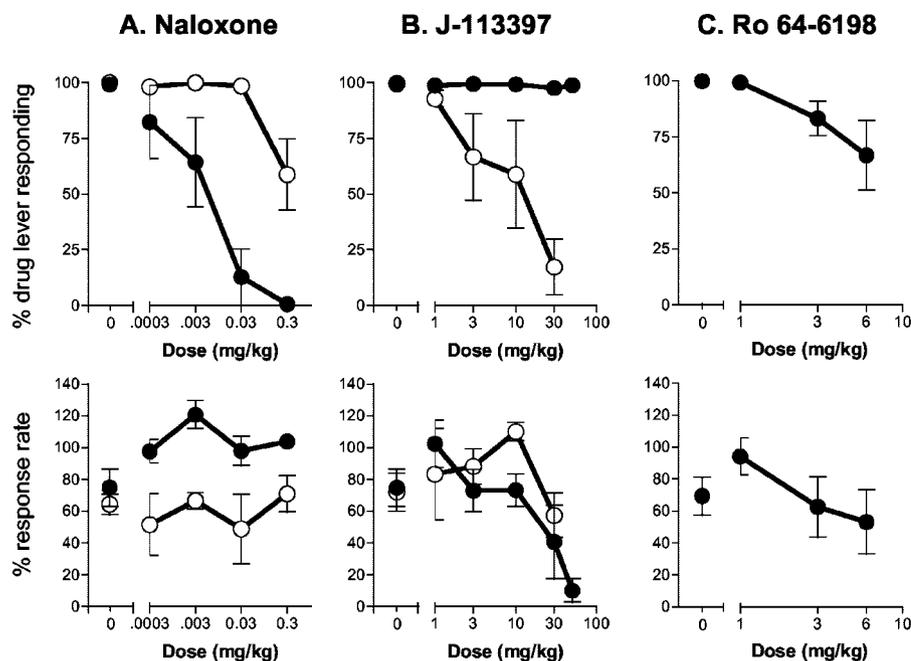
A. Naloxone

B. J-113397

C. Ro 64-6198

Fig. 3. Results of tests to antagonize either the ORL-1 agonist Ro 64-6198 (4 mg/kg i.p.) cue (○) or morphine (2 mg/kg s.c.) cue (●). Test compound was administered prior to the relevant training dose of Ro 64-6198 or morphine (see *Materials and Methods* for pretreatment times). Rats were injected with different doses of either naloxone (4–8 animals/dose) (A), J-113397 (4–8 animals/dose) (B), or Ro 64-6198 (4 animals/dose; MOR study only) (C). Top, percentage of responding on the drug-associated lever. Bottom, rate of responding during this test, expressed as a percentage of the vehicle rate from the trial immediately preceding this test. The normal rate averaged over each experiment presented was 1–2 lever presses/s.

at higher doses of 6 to 10 mg/kg i.p., neurological effects such as hypolocomotion and reduced responding for food under a variable interval 20-s schedule of reinforcement emerge (Jenck et al., 2000; Higgins et al., 2001). Accordingly, we initially selected a 2 mg/kg i.p. training dose of Ro 64-6198. However, by 26 trials no robust discrimination was manifest, and it was only after extended training with a higher dose of 4 mg/kg that the drug elicited reliable stimulus control in the majority of animals. It was interesting to note the slow acquisition of this stimulus, suggesting at least by comparison with morphine that it was relatively weak. Consistent with some of the aforementioned neurological effects seen at higher doses of Ro 64-6198 in rats, we noted some reduction in the rate of operant responding confirming the doses used were pharmacologically relevant. Thus, at the 4 mg/kg training dose, Ro 64-6198 reduced rates of responding to about 60 to 70% of that after vehicle pretreatment. Despite repeated treatment, tolerance did not seem to develop to this effect, as evidenced by rate decreasing effects of Ro 64-6198 maintained throughout these studies.

Once trained, tests of substitution were conducted and at pharmacologically relevant doses, the κ opioid receptor agonist U50,488 (Vonvoightlander et al., 1983), the δ opioid receptor agonist SNC-80 (Calderon et al., 1994; Stevenson et al., 2002), and the μ opioid receptor agonists morphine and codeine each failed to evoke significant generalization to the RO cue. Each drug was tested up to doses that markedly suppressed responding, except SNC-80, which was tested up to a dose reported as a training cue, characterized as δ opioid receptor-mediated, in the rat and primate (Brandt et al., 1999; Stevenson et al., 2002). We were limited by solubility issues and drug supply from testing higher doses of this compound. Naloxone was similarly ineffective in blocking the RO cue, with only partial (40%) blockade evident at a dose 100-fold greater than the ED_{50} value necessary to block a morphine cue (Shannon and Holtzman, 1976; Joharchi et al., 1993; present study). Naloxone also failed to block the rate decreasing effects of Ro 64-6198. Aside from the relative

selectivity of Ro 64-6198 for the ORL-1 receptor, the main line of evidence to support a likely ORL-1 receptor involvement to the RO cue was the demonstration that the selective ORL-1 antagonist J-113397 blocked this cue with a potency consistent with the in vivo literature on this compound (Ozaki et al., 2000; Lutfy et al., 2003). J-113397 also seemed to reverse the rate-decreasing effect of R0 64-6198 , although at 30 mg/kg this drug itself reduced response rate. The inclusion of OFQ/N or other peptidergic ORL-1 receptor agonists (Calo et al., 2000) in these generalization studies would have been a useful addition to further characterize this cue; however, a limited supply of Ro 64-6198 prevented such studies.

Buprenorphine is a partial μ opioid receptor agonist with a potency some 25 to 50 times greater than morphine (Reisine and Pasternak, 1996) that has been reported to have ORL-1 receptor agonist properties in vitro (Wnendt et al., 1999). Recently, Lutfy et al. (2003) have reported that its limited antinociceptive effects may reflect ORL-1 receptor agonist properties. Thus, in ORL-1 receptor knockout mice, or wild-type mice pretreated with J-113397, buprenorphine elicits an enhanced antiniociceptive effect measured using the tail-flick assay, equivalent to that of morphine (Lutfy et al., 2003). In the present drug discrimination studies, at doses that generalized completely to a morphine cue, buprenorphine produced a partial Ro 64-6198 substitution. However, rate-decreasing effects of this drug prevented us from testing this compound at doses higher than 0.1 mg/kg, which engendered approximately 50% drug lever responding. Nonetheless, the κ opioid receptor agonist U50,488 reduced responding to an equivalent extent, yet no Ro 64-6198 generalization was evident, suggesting the partial generalization of buprenorphine to this cue likely reflects ORL-1 receptor agonism. In the morphine-trained group, buprenorphine generalized completely by the 0.1 mg/kg dose with an ED_{50} value of 0.03 mg/kg, consistent with other reports demonstrating cross-generalization between these agents (Holtzman, 1997).

As far as we are aware, the present data are the first to

demonstrate that an ORL-1 agonist may elicit a unique interoceptive cue in rodents using a drug discrimination procedure. However, other recent studies might have predicted this outcome. First, whereas μ and δ opioid receptor agonists tend to elicit conditioned place preferences and κ opioid agonists conditioned place aversions (Mucha and Herz, 1985; Bals-Kubik et al., 1993), both OFQ/N and Ro 64-6198 seem neutral in tests of place conditioning (Devine et al., 1996; Ciccocioppo et al., 2000; Le Pen et al., 2002). In tests of intracranial self-stimulation, both μ and δ opioid agonists generally lower response threshold (Esposito and Kornetsky, 1977; Jenck et al., 1987), whereas κ opioid agonists seem to increase threshold (Todtenkopf et al., 2004). On the other hand, Ro 64-6198 seems inert in intracranial self-stimulation threshold experiments (Jenck et al., 2000). These findings, together with the place conditioning studies, suggest that ORL-1 agonists may be devoid of appetitive properties, at least in rodents (Jenck et al., 2000). Because μ opioid receptor agonists produce subjective effects generally described as "euphoric" in humans, and κ opioid receptor agonists subjective effects generally described as "dysphoric" (Smith and Beecher, 1962; Kumor et al., 1986; Dykstra et al., 1997; Walsh et al., 2001), the available evidence further suggests that ORL-1 receptor agonists may be devoid of such interoceptive properties. However, this will only be established on clinical evaluation.

Indeed, rather than elicit a conditioned place preference, OFQ/N pretreatment has actually been reported to block the acquisition of a morphine CPP (Murphy et al., 1999; Ciccocioppo et al., 2000), which would imply that an ORL-1 receptor agonist may reduce the rewarding effects of morphine. Accordingly, we investigated interactions between Ro 64-6198 pretreatment and the morphine cue. We failed to detect any robust evidence for an antagonism of the morphine generalization after Ro 64-6198 pretreatment. This profile is shared by 5-hydroxytryptamine$_3$ receptor antagonists, which have been reported to block a morphine CPP without affecting its discriminative stimulus properties (Carboni et al., 1989; Higgins et al., 1992; Joharchi et al., 1993) and would suggest the MOR cue is a complex of multiple stimuli of which only a subset contributes to CPP induction (Bechara and Van der Kooy, 1985). Thus, blockade of the cueing effects that may underlie place conditioning may not necessarily diminish the compound cue that underlies the overall discriminative state as measured in a drug discrimination paradigm.

To summarize, the present studies demonstrate that rats can be trained to discriminate Ro 64-6198 from saline and that this cue is distinct in character from a μ, κ, and δ opioid receptor-mediated cue and is most likely ORL-1 receptor-mediated. However, it should be noted that these distinctions are based solely on a single training dose of Ro 64-6198. Because training dose can be a critical determinant of patterns of stimulus generalization and antagonism, it is possible that the selection of alternative training doses of Ro 64-6198 or morphine may have revealed a greater similarity between the cues. However, in our opinion this is unlikely; the morphine training dose was comparatively low, and in general, it seems that higher training doses of morphine reveal greater μ opioid receptor selectivity with less cross-generalization to other drug cues (Shannon and Holtzman 1979; Picker et al., 1990; Young et al., 1992). Given the

potential clinical utility of ORL-1 receptor agonists in areas as diverse as cough, treatment of substance abuse and anxiety (Smith and Moran, 2001), the present studies suggest that drugs belonging to this class may lack the euphorigenic or dysphorigenic properties associated with certain opioid-based treatments used for these conditions.

References

Bals-Kubik R, Albeitner A, Herz A, and Shippenberg TS (1993) Neuroanatomical sites mediating the motivational effects of opioids as mapped by the conditioned place preference paradigm in rats. *J Pharmacol Exp Ther* **264:**489–495.

Bechara A and Van der Kooy D (1985) Opposite motivational effects of endogenous opioids in brain and periphery. *Nature (Lond)* **314:**533–534.

Brandt MR, Negus SS, Mello NK, Furness MS, Zhang X, and Rice KC (1999) Discriminative stimulus effects of the nonpeptide δ-opioid agonist SNC80 in rhesus monkeys. *J Pharmacol Exp Ther* **290:**1157–1164.

Broqua P, Wettstein JG, Rocher MN, Riviere PJ, and Dahl SG (1998) The discriminative stimulus properties of U50,488 and morphine are not shared by fedotozine. *Eur Neuropsychopharmacol* **8:**261–266.

Calderon SN, Rothman RB, Porreca F, Flippen-Anderson JL, McNutt RW, Xu H, Smith LE, Bilsky EJ, Davis P, and Rice KC (1994) Probes for narcotic receptor mediated phenomena. 19. Synthesis of (+)-4-[(alpha R)-alpha-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-N,N-diethylbenzamide (SNC 80): a highly selective, nonpeptide delta opioid receptor agonist. *J Med Chem* **37:**2125–2128.

Calo G, Guerrini R, Rizzi A, Salvadori S, and Regoli D (2000) Pharmacology of nociceptin and its receptor: a novel therapeutic target. *Br J Pharmacol* **129:**1261–1283.

Carboni E, Acquas E, Leone P, and Di Chiara G (1989) 5-HT$_3$ receptor antagoniss block morphine- and nicotine- but not amphetamine-induced reward. *Psychopharmacology* **97:**175–178.

Ciccocioppo R, Angeletti S, Sanna PP, Weiss F, and Massi M (2000) Effect of nociceptin/orphanin FQ on the rewarding properties of morphine. *Eur J Pharmacol* **404:**153–159.

Devine DP, Reinscheid RK, Monsma FJ, Civelli O, and Akil H (1996) The novel neuropeptide orphanin FQ fails to produce conditioned place preference or aversion. *Brain Res* **727:**225–229.

Dykstra LA, Preston KL, and Bigelow GE (1997) Discriminative stimulus and subjective effects of opioids with mu and kappa activity: data from laboratory animals and human subjects. *Psychopharmacology* **130:**14–27.

Esposito R and Kornetsky C (1977) Morphine lowering of self-stimulation thresholds: lack of tolerance with long-term administration. *Science (Wash DC)* **195:**189–191.

Hashiba E, Lambert DG, Jenck F, Wichmann J, and Smith G (2002) Characterisation of the non-peptide nociceptin receptor agonist, Ro 64-6198 in Chinese hamster ovary cells expressing recombinant human nociceptin receptors. *Life Sci* **70:**1719–1725.

Higgins GA, Grottick AJ, Ballard TM, Richards JG, Messer J, Takeshima H, Pauly-Evers M, Jenck F, Adam G, and Wichmann J (2001) Influence of the selective ORL1 receptor agonist, Ro 64-6198, on rodent neurological function. *Neuropharmacology* **41:**97–107.

Higgins GA, Joharchi N, Nguyen P, and Sellers EM (1992) Effect of the 5-HT$_3$ receptor antagonists, MDL72222 and ondansetron, on morphine place conditioning. *Psychopharmacology* **106:**315–320.

Higgins GA, Kew JNC, Richards JG, Takeshima H, Jenck F, Adam G, Wichmann J, Kemp JA, and Grottick AJ (2002) A combined pharmacological and genetic approach to investigate the role of orphanin FQ in learning and memory. *Eur J Neurosci* **15:**911–922.

Holtzman SG (1997) Discriminative stimulus effects of buprenorphine in the rat. *Psychopharmacology* **130:**292–299.

Jenck F, Gratton A, and Wise RA (1987) Opioid receptor subtypes associated with ventral tegmental facilitation of lateral hypothalamic brain stimulation reward. *Brain Res* **423:**34–38.

Jenck F, Wichmann J, Dautzenberg FM, Moreau J-L, Ouagazzal AM, Martin JR, Lundstrom K, Cesura AM, Poli SM, Roever S, et al. (2000) A synthetic agonist at the orphanin FQ/nociceptin receptor ORL1: anxiolytic profile in the rat. *Proc Natl Acad Sci USA* **97:**4938–4943.

Joharchi N, Sellers EM, and Higgins GA (1993) Effect of 5-HT$_3$ receptor antagonists on the discriminative stimulus properties of morphine in rats. *Psychopharmacology* **112:**111–115.

Kumor KM, Haertzen CA, Johnson RE, Kocher T, and Jaszinski D (1986) Human psychopharmacology of ketocyclazocine as compared to cyclazocine, morphine and placebo. *J Pharmacol Exp Ther* **238:**960–968.

Le Pen G, Wichmann J, Moreau J-L, and Jenck F (2002) The orphanin receptor agonist Ro 64-6198 does not induce place conditioning in rats. *Neuroreport* **13:**451–454.

Lutfy K, Eitan S, Bryant CD, Yang YC, Saliminejad N, Walwyn W, Kieffer BL, Takeshima H, Carroll FI, Maidment NT, et al. (2003) Buprenorphine-induced antinociception is mediated by mu-opioid receptors and compromised by concomitant activation of opioid receptor-like receptors. *J Neurosci* **23:**10331–10337.

Mansbach RS, Feltner DE, Gold LH, and Schnoll SH (2003) Incorporating the assessment of abuse liability into the drug discovery and development process. *Drug Alcohol Depend* **70:**S73–S85.

Mogil JS and Pasternak GW (2001) The molecular and behavioural pharmacology of the orphanin FQ/nociceptin peptide and receptor family. *Pharmacol Rev* **53:**381–415.

Mucha RF and Herz A (1985) Motivational properties of kappa and mu opioid

receptor agonists studied with place and taste preference conditioning. *Psychopharmacology* **86:**274–280.

Murphy NP, Lee Y, and Maidment NT (1999) Orphanin FQ/nociceptin blocks acquisition of morphine place preference. *Brain Res* **832:**168–170.

Ozaki S, Kawamoto H, Itoh Y, Miyaji M, Azuma T, Ichikawa D, Nambu H, Iguchi T, Iwasawa Y, and Ohta H (2000) In vitro and in vivo pharmacological characterisation of J-113397, a potent and selective non-peptidyl ORL1 receptor antagonist. *Eur J Pharmacol* **402:**45–53.

Picker MJ, Doty P, Negus SS, Mattox SR, and Dykstra LA (1990) Discriminative stimulus properties of U50,488 and morphine: effects of training dose on stimulus substitution patterns produced by mu and kappa opioid agonists. *J Pharmacol Exp Ther* **254:**13–22.

Reisine T and Pasternak GW (1996) Opioid analgesics and antagonists, in *Goodman and Gilman's The Pharmacological Basis of Therapeutics,* 9th ed. (Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, and Gilman AG eds) pp 521–556, McGraw-Hill Book Company, New York.

Shannon HE and Holtzman SG (1976) Blockade of the discriminative effects of morphine in the rat by naltrexone and naloxone. *Psychopharmacology* **50:**119–124.

Shannon HE and Holtzman SG (1979) Morphine training dose: a determinant of stimulus generalisation to narcotic antagonists in the rat. *Psychopharmacology* **61:**239–244.

Snyder SH and Pasternak GW (2003) Historical review: opioid receptors. *Trends Pharmacol Sci* **24:**198–205.

Smith GM and Beecher HK (1962) Subjective effects of heroin and morphine in normal subjects. *J Pharmacol Exp Ther* **136:**47–58.

Smith PA and Moran TD (2001) The nociceptin receptor as a potential target in drug design. *Drug News Perspect* **14:**335–345.

Stevenson GW, Canadas F, Gomez-Serrano M, Ullrich T, Zhang X, Rice KC, and Riley AL (2002) Delta opioid discrimination learning with the selective delta agonist SNC80. *Pharmacol Biochem Behav* **71:**291–300.

Stolerman IP, Stephenson JD, and Rauch RJ (1987) Classification of opioid and 5-hydroxytryptamine receptors by means of discriminative stimulus drug effects. *Neuropharmacology* **26:**867–875.

Todtenkopf MS, Marcus JF, Portoghese PS, and Carlezon WA (2004) Effects of κ-opioid receptor ligands on intracranial self-stimulation in rats. *Psychopharmacology* **172:**463–470.

Vonvoightlander PF, Lahti RA, and Ludens JH (1983) U50,488: a selective and structurally novel non-mu (kappa) opioid agonist. *J Pharmacol Exp Ther* **224:**7–12.

Walsh SL, Strain EC, Abreu ME, and Bigelow GE (2001) Enadoline, a selective kappa opioid agonist: comparison with butorphanol and hydromorphone in humans. *Psychopharmacology* **157:**151–162.

Wnendt S, Krüger T, Janocha E, Hildebrandt D, and Englberger W (1999) Agonistic effect of buprenorphine in a nociceptin/OFQ receptor-triggered reporter gene assay. *Mol Pharmacol* **56:**334–338.

Young AM, Masaki MA, and Geula C (1992) Discriminative stimulus effects of morphine: effects of training dose on agonist and antagonist effects of mu opioids. *J Pharmacol Exp Ther* **261:**246–257.

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