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Behavioral Effects and Binding Affinities of the Fentanyl Derivative OHM3507

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FRANCE, C. P., S. C. AHN, L. L. BROCKUNIER, J. R. BAGLEY, M. R. BRANDT, P. J. WINSAUER AND J. M. MOERSCHBAECHER. *Behavioral effects and binding affinities of the fentanyl derivative OHM3507.* PHARMACOL BIOCHEM BEHAV **59**(2) 295–303, 1998.—Several fentanyl derivatives have been reported to have novel pharmacologies that might be exploited for developing alternate approaches to the treatment of pain. The purpose of the current series of studies was to evaluate OHM3507, a novel fentanyl derivative reported to have an unusual pharmacological profile in nonprimate species. Similar to several other fentanyl derivatives with clinical potential, OHM3507 had the highest affinity (IC_{50} = 10 nM) for μ[^{3}H]D-Ala^2,N-Me-Phe^4, Gly^5-OH–labeled) receptors with 6- and 176-fold lower affinity for δ ([^{3}H]D-Pen^2-D-Pen^5–labeled), and κ [^{3}H]ethylketocyclazocine–labeled) receptors, respectively. In rhesus monkeys, OHM3507 shared discriminative stimulus effects with morphine, increased tail-withdrawal latencies in a warm-water procedure of antinociception, decreased ventilation in monkeys breathing normal air or 5% CO_2, and failed to modify accuracy on acquisition and performance tasks up to doses that decreased rates of food-maintained responding. The opioid antagonists naltrexone and naltrindole antagonized the behavioral effects of OHM3507 in a manner that was consistent with μ-receptor mediation. Although OHM3507 appeared to have low efficacy opioid actions in nonprimate species, results from the current studies clearly show this compound to have strong, fentanyl-like μ agonist actions in rhesus monkeys. These results provide another example of the sometimes poor predictability in the behavioral pharmacology of fentanyl derivatives among species, in this case between monkeys and rats, mice and rabbits, and demonstrates the need for evaluating new drugs under a broad range of conditions to increase the probability of identifying novel compounds that can be used to treat pain. © 1998 Elsevier Science Inc.

Acquisition and performance Antinociception Drug discrimination Fentanyl Mirfentanil OHM3507 Opioids Rhesus monkey

MU opioid agonists continue to be the drugs of choice in the treatment of moderate to severe pain, despite the well-established toxicity and abuse liability of most compounds in this pharmacological class. Notwithstanding the need for strong analgesics that have reduced abuse liability and reduced toxicity, there has been relatively little success in the effort to develop alternate pharmacological approaches to the treatment of pain. Thus, virtually all opioid agonists that are effective in treating moderate to severe pain also have high abuse liability, produce physical dependence, and decrease ventilatory function.

Fentanyl (Fig. 1) is a morphine-like (i.e., μ-receptor selective) opioid agonist that is used widely in anesthesia and, to a lesser extent, to treat pain. Like morphine, fentanyl has a very high abuse liability, produces physical dependence, and decreases ventilatory function (1,12,13). Recently, several 4-heteroanilido-piperidine derivatives of fentanyl have been shown to have robust antinociceptive effects in nonhuman primates and not to have some of the other undesirable effects that are typical of morphine-like opioids (3,6,8). One compound in this chemical series, mirfentanil, has an especially interesting profile of effects. For example, mirfentanil has sufficiently low efficacy at μ opioid receptors that, like naltrexone, it precipitates withdrawal in morphine-dependent subjects (8); it also has antinociceptive effects and respiratory-depressant effects in rodents while reversing the antinociceptive effects of morphine in rabbits (19) and rats (3). In rhesus monkeys, the antinociceptive effects of mirfentanil are not mediated by opioid receptors and, compared to other opioid agonists, the effects

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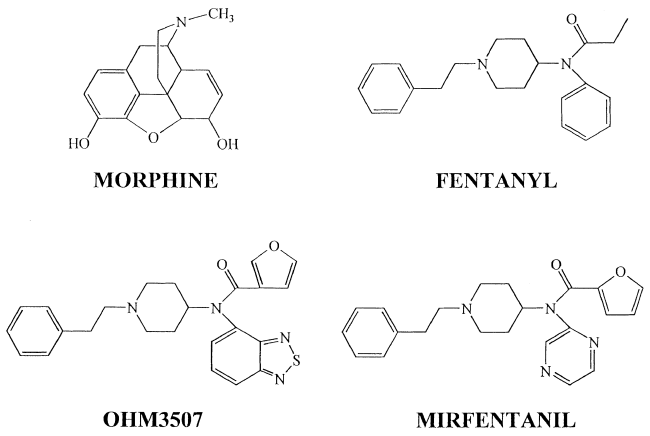

FIG. 1. Structures of morphine, fentanyl, OHM3507, and mirfentanil.

of mirfentanil on ventilatory function are very modest. Another compound in this series, OHM3295, also has nonopioid antinociceptive effects in nonhuman primates as well as low-efficacy μ agonist actions (6). These antinociceptive effects of OHM3295 and mirfentanil are not blocked by naltrexone or other opioid antagonists, suggesting that these effects (in primates) are not mediated by opioid receptors. While OHM3295 has low efficacy opioid agonist effects in both primates and rodents (6), another compound in this series, OHM3568 [compound 28 in (3)], has very low opioid efficacy in rodents and very high opioid efficacy (i.e., fentanyl-like effects) in monkeys (9). Thus, there is not necessarily a strong correlation in the behavioral pharmacology of this series of compounds between primate and nonprimate species.

The purpose of the current study was to assess the binding selectivity and behavioral effects of another fentanyl derivative, OHM3507, that had an unusual pharmacological profile in nonprimate species (2,19). In rabbits, OHM3507 appeared to have low-efficacy opioid agonist actions. For example, when administered alone, OHM3507 produced only modest antinociception (39% of the maximum possible effect), and when administered in combination with morphine, OHM3507 partially reversed morphine-induced antinociception and completely reversed morphine-induced respiratory depression (19). Thus, like mirfentanil and OHM3295, OHM3507 had very limited agonist actions and also attenuated the actions of more efficacious μ agonists (e.g., morphine). In light of the novel and interesting effects that were obtained with other compounds in this series, particularly those that appeared to be low efficacy opioid agonists in nonprimate species (e.g., mirfentanil), the present studies were initiated in the hopes of identifying another fentanyl derivative that might have some potential for the treatment of pain. Thus, OHM3507 was evaluated for its binding affinity to each of the three major types of opioid receptors (μ, κ, δ) and also for its behavioral effects in rhesus monkeys using procedures that have been used to assess the effects of other opioids and nonopioids [e.g., (8,15)].

METHOD

Subjects

For binding assays, adult male Hartley guinea pigs (300–350 g, certified viral antibody free; Hilltop Laboratory Animals, Scottdale, PA) were housed individually and maintained on a 12 L:12 D cycle with free access to food (Agway Guinea Pig Maintenance Ration) and water. For behavioral studies, 14 adult rhesus monkeys (*Macaca mulatta*; antinociception, two male, two female; respiration, three male, one female; drug discrimination, three female; and acquisition and performance, three female) were housed individually with free access to water. Subjects used in studies on acquisition and performance were maintained at 85% of their free-feeding weights by banana-flavored food pellets received during experimental sessions, supplemental feeding (Purina Monkey Chow) in the home cage, fresh fruit, and vitamins. All other subjects had free access to food (Purina Monkey Chow) in the home cage and received fresh fruit twice weekly. Monkeys in the drug discrimination study had received 3.2 mg/kg/day (SC) of morphine for several years prior to these experiments and all subjects had received opioids (chronically for monkeys in the naltrexone discrimination study and acutely for other monkeys) in previous studies.

APPARATUS

Drug discrimination. Subjects were seated in Lexan primate chairs that provided restraint at the neck, waist, and feet; during experimental sessions, chairs were located in sound-attenuating, ventilated operant chambers that were equipped with two or three response levers and accompanying red stimulus lights. Chairs were also equipped with a pair of shoes containing brass electrodes, to which brief, 250-ms, electric shocks (3 mA) could be delivered from an AC generator located outside the chamber. Experimental sessions were controlled and data recorded by microprocessors using Med-PC software as well as commercially available interfacing.

Antinociception. For studies of antinociception, monkeys were seated in chairs that provided minimal restraint at the neck, thereby allowing free access to the tails that hung unimpeded from the bottom of the seat. Thermos bottles filled with water (40, 50, or 55 ± 1°C) were used to assess tail-withdrawal latencies. Latency was measured and recorded by an investigator using a push-button switch connected to a microprocessor.

Acquisition and performance. A removable response panel (76 × 71 × 97 cm; Research Equipment Co., Inc., Byran, TX; model LC-1004) was attached to the side of the home cage during experimental sessions. Three translucent response keys (BRS/LVE, press plate model PPC-012) were located on the response panel 50 cm from the cage floor and 11.5 cm apart. Food pellets could be delivered to an aperture (5.5 cm in diameter) located to the right of the right-most key. Experimental sessions were controlled and data recorded by a microprocessor located in an adjacent room.

Ventilation. Subjects were seated in primate chairs that provided restraint at the neck, waist, and arms; during experimental sessions, the chair was located within a sound-attenuating, ventilated chamber. Alternating layers of Lexan plates and latex collars (two of each), as well as a foam cushion, formed the base and minimized gas leakage from the plethysmograph. Air or 5% CO_2 was pumped into the plethysmograph at a rate of 10 l/min and removed with a vacuum pump. Changes in air pressure were measured using a pressure transducer and recorded through a microprocessor. According to calibration with known standards, changes in pressure were transformed to estimates of ventilation: inspirations/min (f); tidal volume in ml/inspiration (V_T); and minute volume in ml/min (V_E).

Procedures

Opioid receptor binding. The procedures for evaluating binding at μ receptors with ^{3}H-[D-Ala^2, N-Me-Phe^4, Gly^5-OH] (DAGO), κ receptors with ^{3}H-ethylketocyclazocine (EKC), and δ receptors with [^{3}H-[D-Pen^2-D-Pen^5] (DPDPE) were similar to those described elsewhere [e.g., (11)]. Briefly, whole brains (including cerebellum) were homogenized in cold 50 mM TRIS HCl pH 7.4 at 50 mg/ml for 40 s using a polytron. The homogenates were preincubated for 40 min at 37°C and centrifuged at 30,000 × g for 20 min. The pellets were resuspended in buffer at 50 mg/ml and incubated for 2 h at 37°C with 1 nM concentration of tritiated ligand ([^{3}H]DAGO, [^{3}H]EKC, or [^{3}H]DPDPE) and 3–7 concentrations of unlabeled ligand (total volume = 500 μl). Homogenates were then diluted in 4.5 ml of buffer, filtered, and washed with 4.5 ml of cold buffer. Membrane-bound radioactivity was measured using a scintillation counter.

Drug discrimination. Subjects received 3.2 mg/kg/day of morphine SC 3 h prior to daily sessions and discriminated between SC injections of saline and 0.01 mg/kg of naltrexone (10). Training sessions consisted of multiple, discrete 15-min cycles with each cycle comprising a 10-min timeout, during which the chamber was dark and responses had no programmed sequence, followed by a 5-min response period, during which stimulus lights were illuminated and a fixed-ratio schedule of stimulus-shock termination was in effect. During the response period, shocks were scheduled to occur every 15 s. Five consecutive responses on the appropriate lever (determined by an injection administered within the first minute of the timeout period) terminated the shock-associated stimulus and postponed impending shock for 30 s. Cycles ended after either 5 min or the delivery of four shocks, whichever occurred first. Responses on the injection-inappropriate (incorrect) lever reset the response requirement on the correct lever. During saline training days, saline was administered during the first minute of each cycle; during drug training days, zero to four saline or sham (no injection) cycles preceded a cycle in which naltrexone was administered.

Testing sessions were identical to training sessions, except that: 1) for some tests, saline was substituted for morphine 3 h prior to the session; 2) five consecutive responses on either lever postponed the shock schedule (i.e., no designated correct lever); and 3) increasing doses of drug (morphine, OHM3507, or naltrexone) were administered across cycles so that the cumulative dose increased by 0.25 log unit per cycle. For antagonism studies, monkeys received saline (not morphine) 3 h prior to the session, a single dose of naltrexone during the timeout of the first cycle, and increasing doses of an agonist during the timeout of subsequent cycles.

Antinociception. The latency for monkeys to remove their tails from a thermos containing warm (50 or 55°C) water was used as a measure of antinociceptive effect (4,7); monkeys also were tested with a 40°C stimulus; this stimulus typically does not elicit tail withdrawal within 20 s. While subjects were seated in chairs, the bottom 10–12 cm of the shaved tail was placed in the thermos of water, and tail withdrawal latency was measured. If the tail was not removed within 20 s, it was removed manually by the investigator and a latency of 20 s was recorded for that cycle.

Control (predrug) latencies were measured after subjects had been seated in the chairs for a minimum of 10 min. Single-dose time-course studies were determined for OHM3507 using discrete 15-min cycles (10 min timeout; 5 min tail-withdrawal latency measurement period) for a total session time of 90 min (six cycles). All other experimental sessions consisted of discrete 30 min cycles (25 min timeout; 5 min latency measurement period) and utilized a cumulative-dosing procedure whereby injections were administered during the first minute of pretreatment periods. Sessions were terminated when the maximum possible effect (i.e., 20 s latency) was observed in all subjects at 50°C (with the exception that for time course studies with single doses of OHM3507, drug was administered up to doses that produced the maximum possible effect with 55°C), or after 90 min, whichever occurred first.

A cumulative-dosing procedure was used whereby the dose of agonist increased by 0.25 or 0.5 log unit per cycle. In antagonism studies, a single dose of antagonist was administered 10 (naltrindole) or 15 (naltrexone) min prior to the first injection of agonist. Because the antagonist effects of 0.01 mg/kg of naltrexone (SC) decline markedly after 2.5 h [e.g., (5)], sessions with antagonists were limited to 90 min, or a maximum of five doses of agonist. Control latencies were determined immediately before the administration of antagonist, and again immediately prior to the first agonist injection. Tests were administered no more than twice weekly, with an intervening period of at least 48 h between tests. Other responses (e.g., flushing, pupillary dilation, decreased activity) were also noted and recorded immediately prior to the latency measurement period in the antinociception studies.

Acquisition and performance. A multiple schedule comprising a series of alternating acquisition and performance components (15) was used to evaluate the effects of OHM3507 on a two-member conditional discrimination. Within each component, subjects could respond on the right or left key, with the correct response (key) determined by stimuli that were displayed on a center key (i.e., a combination of four different colors and four different geometric shapes). A correct response resulted in a continuation to the second link of the component, during which a different combination of stimuli were displayed on the center key. A completion of the two-member discrimination resulted in the delivery of a 50 mg food pellet; an incorrect response resulted in a 5-s time out, during which responses had no programmed consequence. During the acquisition component, the correct stimuli varied across days; during the performance component, the stimuli were the same across days. Experimental sessions began with an acquisition component and alternated with a performance component after 20 food presentations or 15 min, whichever occurred first. Consecutive components were separated by a 5 s timeout, during which all stimuli were extinguished and responses had no programmed consequence. Sessions terminated after the delivery of 200 reinforcers, or 120 min, whichever occurred first. Sessions were conducted 5 days per week, with drug administered generally on Tuesdays and Fridays (no more than twice per week), and saline (control session) administered on Thursdays. Drug or saline was administered (SC) 30 min prior to the session (i.e., the first acquisition component); for antagonism studies, naltrexone was administered (SC) 40 min prior to the session.

Ventilation. The procedure that was used to study ventilation was similar to procedures described previously (7,14). Subjects were seated in a primate restraining chair that was fitted with the head plethysmograph and located in a sound-attenuating chamber. Experimental sessions consisted of a series of discrete, 30-min cycles, beginning with a saline (control) cycle and followed by two to six cycles during which either drug or saline was administered during the first minute of each cycle. Each cycle comprised a 23-min exposure to air, followed by a 7-min exposure to 5% CO_2. Data were recorded continuously throughout the cycle, and reported as the mean of the last 3 min of exposure either to air or to 5% CO_2. Drug was ad-

ministered no more than twice weekly and with an intervening period of at least 48 h between consecutive drug tests.

A multiple-dosing procedure was used for morphine and for OHM3507, whereby the cumulative dose of drug increased by 0.25 or 0.5 log unit per cycle. Test sessions were terminated when VE was decreased to at least 50% of control in air, or eight cycles (4 h), whichever occurred first. During antagonism studies, a single injection of 0.01 mg/kg of naltrexone was administered one cycle (i.e., 30 min) prior to the cycle during which the first dose of agonist was administered.

Data Analyses

Specific binding was determined to be: (total binding measured) − (binding in the presence of 1 μl of cold ligand). IC_{50}s were estimated by plotting the percentage of specific binding as a function of the −log (inhibitor concentration).

Drug discrimination data are presented as the percentage of responses on the drug-associated lever (% DR [number of responses on the naltrexone-associated lever]/[total number of responses] × 100) and are plotted as a mean value ± 1 SEM as a function of dose. Drugs that produced at least 90% responding on the drug-associated lever were considered to have substituted for the training drug (naltrexone); conversely, drugs that reversed naltrexone-lever responding (i.e., in monkeys that were acutely deprived of morphine) to less than 10% were considered to have substituted for morphine.

Tail-withdrawal latencies are presented as a percentage of the maximum possible effect (% MPE; 20 s) and were calculated as: % MPE = [(experimental latency − baseline latency)/(20 − baseline latency)]. These values were calculated individually for each subject then averaged among all subjects; mean values ± 1 SEM are plotted as a function of dose or time after drug administration.

The effects of drugs on acquisition and performance were determined by calculating the overall rate (i.e., responses/min) and accuracy (i.e., percentage of errors [incorrect responses]/[total number of responses] × 100) for each component. A drug was considered to have an effect when the range of values obtained with a dose of OHM3507 fell outside of the range of values obtained with vehicle. Data are plotted as a mean ± range as a function of dose.

The ventilatory parameters that were monitored and reported were *f* (frequency), V_T (tidal volume, ml) and V_E (minute volume, ml [*f* multiplied by V_T]). Measures of ventilation in air and in 5% CO_2 are presented as a percentage of values determined in the absence of drug (% control) during the first cycle of each session and are plotted as a function of dose.

Potency differences among drugs were estimated by comparing ED_{50} (drug discrimination and antinociception) or ED_{80} [acquisition and performance (because response rates were not decreased to less than 50% of control rates under all conditions) and ventilation (because ED_{50} doses were the same or nearly the same as doses that decreased ventilation to the extent that subjects had to be rescued with opioid antagonists)] values that were determined by linear regression, when three or more appropriate data points were available, or otherwise by interpolation. The apparent affinity of antagonists (pA_2 and pK_B) was estimated using the methods of Arunlakshana and Schild (2) as well as Schild analyses with the slope constrained to −1 (18) on the assumption of a simple, competitive interaction at a single receptor type. For some studies, Student–Newman–Keuls *t*-tests and ANOVA were conducted on ED_{50} values to identify statistically significant differences between conditions.

Drugs

The drugs used in these studies were morphine sulfate, naltrexone hydrochloride, naltrindole hydrochloride, fentanyl citrate (National Institute on Drug Abuse, Rockville, MD), mirfentanil hydrochloride, and OHM3507 hydrochloride [synthesized by L. L. Brockunier according to (3)]. Drugs were dissolved in sterile 0.9% saline, water (OHM3507) or a propylene glycol vehicle (40% propylene glycol, 50% physiological saline, and 10% ethanol; OHM3507 in concentrations greater than 10 mg/ml). OHM3507 was made fresh daily as needed. Drugs were administered IM (acquisition and performance study) or SC (all other studies), typically in a volume of 0.1 ml/kg body weight.

RESULTS

Opioid Receptor Binding

The IC_{50} values obtained for OHM3507 in displacing [^{3}H]DAGO, [^{3}H]DPDPE, and [^{3}H]EKC were 10, 63, and 1764 nM, respectively. Thus, OHM3507 displayed the highest affinity for μ opioid receptors and the lowest affinity for κ opioid receptors. OHM3507 had a sixfold selectivity for μ receptors over δ receptors and a 28-fold selectivity for δ receptors over κ receptors.

Drug Discrimination

In morphine-treated monkeys, increasing doses of naltrexone occasioned a progressively greater percentage of responding on the naltrexone-associated lever (ED_{50} = 0.009 ± 0.002 mg/kg) with greater than 90% drug-lever responding occurring with doses of naltrexone larger than 0.01 mg/kg (left panel, Fig. 2). When saline was substituted for the daily injection of morphine, monkeys responded at least 90% on the naltrexone lever (point above C, right panel, Fig. 2); under these conditions, morphine dose-dependently reversed naltr-

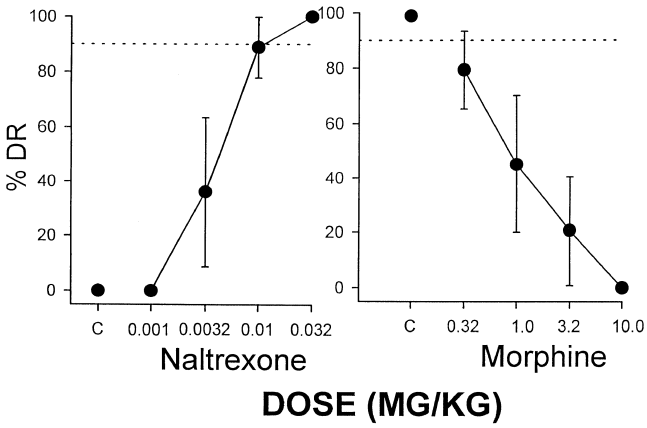

FIG. 2. Discriminative stimulus effects of naltrexone (left panel) and morphine (right panel) in four rhesus monkeys treated daily with 3.2 mg/kg of morphine 3 h prior to sessions in which they discriminated between saline and 0.01 mg/kg of naltrexone. For the naltrexone dose–effect determination (left panel), monkeys received the normal daily dose of morphine 3 h prior to the session; for the morphine dose–effect determination (right panel), saline was substituted for the daily dose of morphine 3 h prior to the session (i.e., monkeys had not received morphine for 28 h). Data are expressed as means ± 1 SEM. Ordinates: percentage of responses emitted on the drug-associated lever (% DR); abscissae: dose in mg/kg of body weight. C = values observed under saline (control) conditions.

exone-lever responding (ED_{50} = 0.94 ± 0.35 mg/kg) with a dose of 10.0 mg/kg of morphine occasioning exclusively saline-lever responding (right panel, Fig. 2). Response rates were not different from control (saline) rates for any dose of naltrexone or morphine (data not shown).

OHM3507 also reversed naltrexone-lever responding in monkeys that were acutely deprived of morphine (circles, Fig. 3); OHM3507 was 6.7-fold more potent than morphine in this regard (OHM3507 ED_{50} = 0.14 ± 0.03). Naltrexone dose-dependently antagonized the discriminative stimulus effects of OHM3507, as evidenced by dose-related shifts to the right in the OHM3507 dose–effect curve (Fig. 3). A Schild analysis for naltrexone in combination with OHM3507 yielded a pA_2 of 8.41 ± 0.02 and a slope of −1.20 ± 0.02 (r^2 = 0.99). When the slope of the Schild plot was constrained to −1, the pA_2 for naltrexone was 8.24 ± 0.02.

Antinociception

In the absence of drug, monkeys never removed their tails within 20 s from a thermos containing 40°C water. In contrast, the average control (baseline) latencies from 50 and 55°C water were 1.62 ± 0.33 s and 1.06 ± 0.19 s, respectively. Single doses of OHM3507 produced time- and dose-related increases in the latency for monkeys to withdraw their tails from 50 and 55°C water (Fig. 4). A dose of 0.01 mg/kg of OHM3507 did not have any consistent effect on tail-withdrawal latency for 90 min after SC administration. A dose of 0.32 mg/kg of OHM3507 maximally increased latencies from 50 and 55°C water; the effects of this larger dose of OHM3507 reached a maximum 15 (50°C) or 30 (55°C) min postinjection and persisted either for the duration of the 90-min test (50°C) or for less than 45 min (55°C). Tail-withdrawal latencies were within the range of control values 24 h after administration of OHM3507 (data not shown).

All four of the agonists studied under a cumulative-dosing procedure increased tail withdrawal latencies in a dose-related manner (data not shown) with the following order of potency: fentanyl (ED_{50} = 0.12 ± 0.01 mg/kg) = OHM3507 (ED_{50} = 0.14 ± 0.05 mg/kg) > morphine (ED_{50} = 1.77 ± 0.64 mg/kg) > mirfentanil (ED_{50} = 7.44 ± 0.59 mg/kg). Naltrexone dose-dependently antagonized the effects of OHM3507 on tail-withdrawal latency from 50°C water (left panel, Fig. 5) as evidenced by dose-related shifts to the right in the OHM3507 dose–effect curve. A the Schild analysis for naltrexone in combination with OHM3507 in the antinociception study yielded a pA_2 of 7.81 ± 0.03 and a slope of −1.33 ± 0.05 (r^2 = 0.99). When the slope of the Schild plot was constrained to −1, the pA_2 for naltrexone was 8.41 ± 0.02. Naltrindole also antagonized the effects of OHM3507 on tail-withdrawal laten-

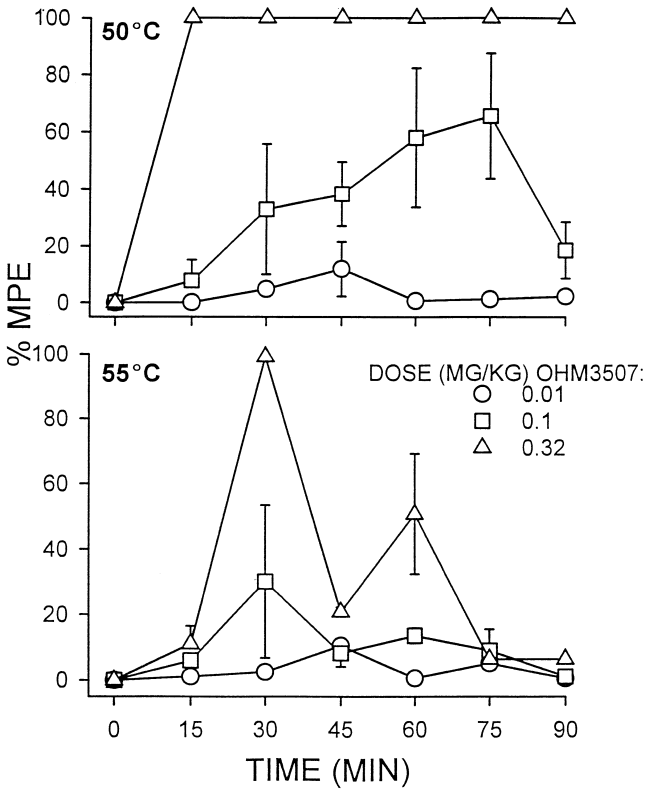

FIG. 4. Time course of effects for single doses of OHM3507 on tail-withdrawal latency from 50° (upper panel) and 55°C (lower panel) water. Ordinates: percent of the maximum possible effect (% MPE) expressed as a mean of four monkeys (except 0.01 mg/kg where n = 3) ± 1 SEM. Abscissae: time, in minutes, after sc administration of OHM3507.

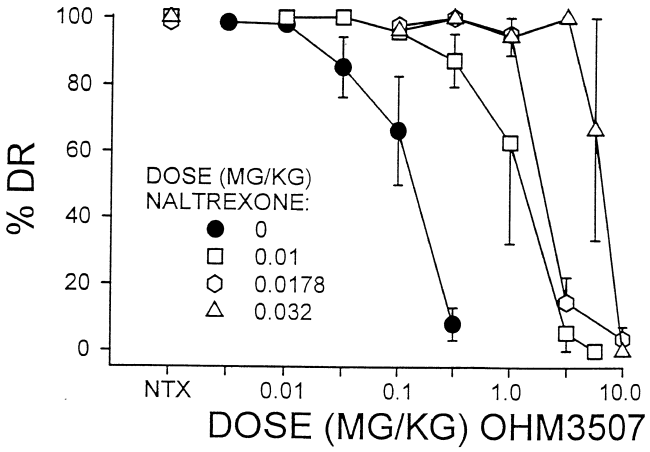

FIG. 3. Discriminative effects of OHM3507 in subjects acutely deprived of morphine. OHM3507 was administered alone (circles) and beginning 15 min after an acute injection of naltrexone. See Fig. 2 and the Method section for other details.

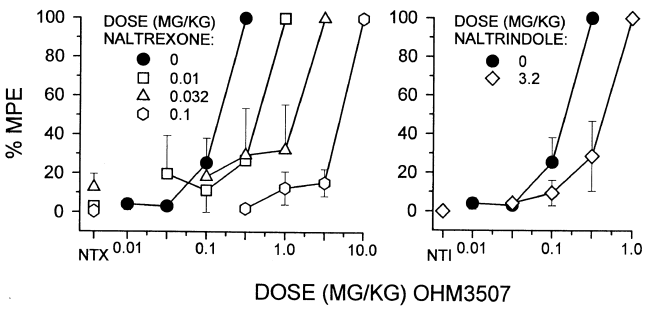

FIG. 5. Effects of cumulative doses of OHM3507 on tail withdrawal from 50°C water. OHM3507 was studied alone (circles) and in the presence of various doses of naltrexone (left panel) or in the presence of 3.2 mg/kg of naltrindole (right panel). See Fig. 4 for other details.

cies with a dose of 3.2 mg/kg of naltrindole shifting the OHM3507 dose–effect curve three-fold to the right (right panel, Fig. 5); the pK_B for naltrindole in combination with OHM3507 was 6.5.

Acquisition and performance

Under control (no drug) conditions, incorrect responses in the performance component averaged less than 1 (mean percentage = 0.06 ± 0.03) and in the acquisition component they varied among subjects from 6–23; rates of responding averaged 38.2 ± 0.6 responses per minute in the performance component and 33.3 ± 5.1 responses per minute in the acquisition component (Fig. 6). OHM3507 decreased response rates in a dose-related manner at doses that did not significantly affect accuracy in either the acquisition or performance component of the multiple schedule (Fig. 6). In the acquisition component, response rates were decreased to less than four responses per minute at doses of 0.056–0.178 mg/kg. For monkeys CO and BU, OHM3507 was less potent in decreasing responding in the performance component compared to the acquisition component (compare open and closed circles, upper left and upper center panels, Fig. 6); for monkey PA, the potency of OHM3507 in decreasing responding in the two components was the same. With the exception of 0.18 mg/kg in monkey CO (a dose that markedly decreased response rate), the percentage of errors in each of the schedule components was not changed by OHM3507 (open circles, lower panels, Fig. 6).

Naltrexone antagonized the rate-decreasing effects of OHM3507 in both the acquisition and the performance components. Acute administration of 0.032 mg/kg of naltrexone shifted the OHM3507 dose–effect curves for response rate to the right; for all three subjects, the potency of OHM3507 in decreasing responding in the two components was the same when OHM3507 was studied in combination with naltrexone (compare open and closed triangles, upper panels, Fig. 6). The pK_B for naltrexone in combination with OHM3507 was 8.02 for the performance component and 8.23 for the acquisition component. Similar to results obtained with OHM3507 administered alone, the accuracy of responding was not significantly altered by any dose of OHM3507 in combination with 0.032 mg/kg of naltrexone.

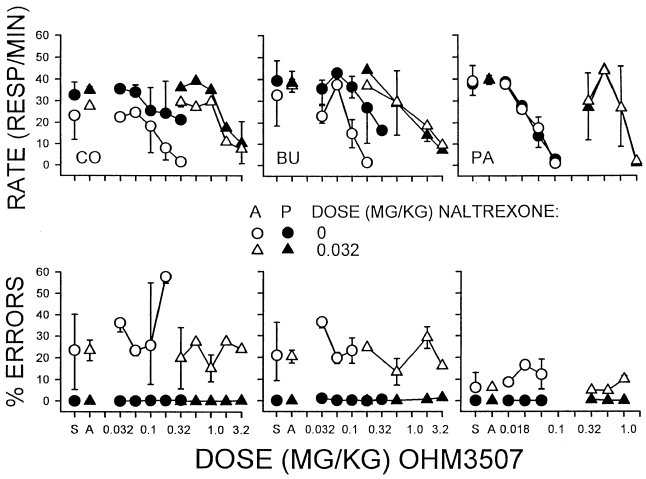

FIG. 6. The effects of OHM3507 on acquisition (A, open symbols) and performance (P, closed symbols). Subjects received either OHM3507 or vehicle 30 min prior to the experimental session. Each set of upper and lower panels shows the effects obtained in individual subjects (CO, BU, and PA). Each data point represents the mean of three determinations in each of the three subjects. OHM3507 was studied alone (circles) and when 0.032 mg/kg of naltrexone had been administered 40 min prior to session (triangles). Ordinates: rate in responses per minute (upper panels) and percentage of incorrect responses (errors) throughout the session. Error bars represent the range of determinations for each condition. Abscissae: dose in mg/kg of body weight. Points above C represent the effects obtained under vehicle control (saline) conditions and points above A represent the effects obtained with naltrexone administered alone.

Ventilation

The control (no drug) values for f, V_T, and V_E in air and in 5% CO_2 are shown in Table 1. On average, exposure to 5% CO_2 increased f, V_T, and V_E to 157, 111, and 171%, respectively, of the values determined in normal air. OHM3507 and morphine (Fig. 7) decreased ventilation in a dose-related manner in all subjects, although there was considerable variability in the potency of both agonists among the four sub-

TABLE 1

VENTILATION IN AIR AND IN 5% CO_2 IN INDIVIDUAL SUBJECTS

	Air			5% CO_2		
Monkey	f (resp/min)	V_T (ml/resp)	V_E (ml/min)	f (resp/min)	V_T (ml/resp)	V_E (ml/min)
MA	37.1 ± 3.7*	35.4 ± 3.9	1169 ± 130	44.9 ± 2.6 (121%)†	38.9 ± 2.9 (110%)	1762 ± 130 (151%)
GO	20.3 ± 1.6	47.4 ± 3.0	942 ± 17	34.6 ± 1.1 (170%)	45.1 ± 3.6 (95%)	1587 ± 118 (168%)
LI	24.2 ± 0.4	45.5 ± 4.1	1108 ± 80	40.0 ± 1.9 (165%)	49.5 ± 1.8 (109%)	1982 ± 130 (179%)
PR	25.9 ± 2.0	29.4 ± 4.5	845 ± 70	44.2 ± 1.9 (171%)	38.4 ± 2.7 (131%)	1577 ± 60 (187%)

*Each entry is the average of nine determinations in each subject.
†Change, in percentage, relative to air.

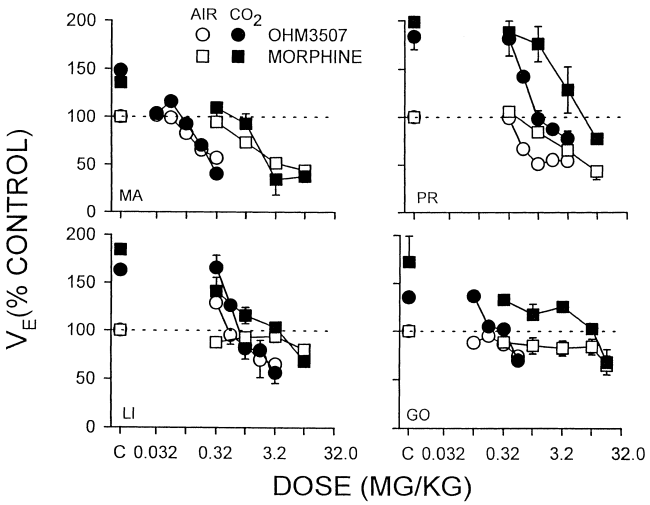

FIG. 7. Dose–effect curves for cumulative doses of morphine (squares) and OHM3507 (circles) on ventilation (V_E) in air (open symbols) and in 5% CO_2 (closed symbols) in four monkeys. Ordinates: averaged V_E expressed as a percentage of V_E under control conditions. Abscissae: dose in mg/kg of body weight. Points above C represent the measures of ventilation obtained under control (no drug) conditions.

jects. OHM3507 decreased ventilation (i.e., V_E) in air and in 5% CO_2 to less than 80% of control at doses between 0.178 and 3.2 mg/kg. In some monkeys (MA, LI, and GO), OHM3507 had a similar potency for decreasing ventilation in air and in 5% CO_2; however, in one monkey (PR), OHM3507 was sixfold more potent in decreasing V_E in air compared to V_E in 5% CO_2 (Fig. 7 and Table 2). The ventilatory-depressant effects of OHM3507 were attenuated by naltrexone, as evidenced by a 2–11-fold increase in the ED_{80} of OHM3507 for decreasing V_E when 0.01 mg/kg of naltrexone had been administered (Table 2). Single-dose affinity estimates (pK_B) for naltrexone in combination with OHM3507 varied from 6.6 to 7.4 (mean = 7.1).

Morphine decreased V_E in air and 5% CO_2 to less than 80% of control at doses between 0.56 and 17.8 mg/kg (squares, Fig. 7). The potency of morphine was similar in decreasing ventilation in air and in 5% CO_2 for three (MA, LI, and GO) of the four subjects, whereas in a fourth subject larger doses of morphine were needed to decrease ventilation in CO_2 compared to ventilation in air.

DISCUSSION

The purpose of the current study was to characterize the binding and behavioral effects of a fentanyl derivative that displayed uncharacteristic effects in preliminary studies conducted in nonprimates species (19). Previous studies with a variety of other compounds in this series, some of which showed novel pharmacological profiles in nonprimate species, have provided strong support for the clinical potential of novel fentanyl derivatives. One of the more compelling results that supports the further consideration of these compounds is that some of them appear to have reduced abuse liability, dependence potential and toxicity, compared to fentanyl, morphine, and related μ agonists.

Overall, the behavioral and pharmacological profile of OHM3507 in these studies in nonhuman primates demonstrates that this fentanyl derivative has both high affinity and high efficacy at μ opioid receptors. In binding studies, OHM3507 had high affinity and selectivity for μ opioid receptors, and its overall binding profile resembled the opioid receptor binding profile of mirfentanil under the same experimental conditions (8). The binding affinities of mirfentanil were (IC_{50}s) 27 nM for μ ([^{3}H]DAGO), 262 nM for δ ([^{3}H]DPDPE), and 12000 nM for κ ([^{3}H]EKC) receptors. Thus, the relative affinities of OHM3507 and mirfentanil for δ receptors were only 6.3- and 10-fold less, respectively, than their affinities for μ receptors. Based on results of the binding study, the novel pharmacological actions of OHM3507 might be mediated by δ opioid receptors; however, functional in vivo studies failed to clearly support the involvement of δ receptors (see below). Moreover, the possibility of significant differences between the binding profile of OHM3507 in guinea pig brain and its effects in rhesus monkeys cannot be rejected.

The uncharacteristic behavioral effects of some of the compounds in this series (mirfentanil, OHM3295) results, in part, from these compounds having very low efficacy at μ opioid receptors (e.g., they precipitate withdrawal in morphine-dependent subjects). Moreover, these compounds have limited effects on respiration (in fact, they attenuate the respiratory depressant effects of morphine-like opioids), and antinociceptive effects that are not mediated by opioid receptors (i.e., not blocked by large doses of naltrexone). In contrast, the high-efficacy μ agonist fentanyl has pronounced effects on respiration, and its antinociceptive effects are mediated by

TABLE 2

POTENCY OF OHM3507 IN DECREASING VENTILATION IN AIR AND IN 5% CO_2 AND ANTAGONISM OF THE EFFECTS OF OHM3507 BY NALTREXONE

	Air + 0.01 mg/kg			5% CO_2 + 0.01 mg/kg		
Monkey	Control	Naltrexone	Ratio (pK_B)	Control	Naltrexone	Ratio (pK_B)
MA	0.1*	0.3	3.1 (7.3)	0.1	0.4	2.6 (7.4)
GO	0.4	1.0	2.3 (7.4)	0.4	2.3	5.3 (6.9)
LI	1.1	6.5	5.8 (6.9)	1.4	5.0	3.5 (7.2)
PR	0.4	4.4	11.1 (6.6)	2.4	11.0	4.5 (7.0)

*ED_{80} (mg/kg) for OHM3507 in decreasing V_E in individual subjects.

μ opioid receptors. Although OHM3507 has the same unusual profile of effects in nonprimate species as mirfentanil and OHM3295, in rhesus monkeys this compound appears to be qualitatively identical to fentanyl. In drug discrimination studies, OHM3507 had morphine-like discriminative stimulus effects in reversing naltrexone-lever responding in monkeys acutely deprived of morphine. Like morphine, OHM3507 also had antinociceptive effects in a warm-water tail-withdrawal assay and it decreased ventilatory function in a dose-related manner. Finally, like morphine and other μ agonists, OHM3507 failed to reliably alter accuracy up to doses that substantially decreased the overall response rate in the acquisition or performance component of a complex learning task. Collectively, these in vivo studies fail to show any novel behavioral effects for OHM3507.

One general approach for identifying specific receptor systems that mediate drug effects involves parametric studies with receptor-selective antagonists (8,16). In the current studies, the μ-selective opioid antagonist naltrexone and the δ-selective antagonist naltrindole were used to assess the likelihood that: 1) the effects of OHM3507 are mediated by opioid receptors (i.e., were these effects modified by either antagonist); and 2) the effects of OHM3507 are mediated by a specific type of receptor (i.e., μ or δ). Thus, naltrexone was administered prior to OHM3507 and the dose ratios [i.e., ED_{50} (or ED_{80}) in the presence of an antagonist divided by the ED_{50} in the absence of antagonist] were evaluated using a Schild analysis (1). When the method of drug delivery for the antagonist remains constant across conditions and within a single species, the potency of an antagonist [e.g., apparent affinity (pA_2 or pK_B)] will be the same for attenuating all of the actions of an agonist that are mediated by the same (single) receptor (e.g., μ). Conversely, differences in the affinity of an antagonist among receptor types (e.g., μ and δ) will be indicated by a differential potency (i.e., different pA_2s or pK_Bs) in attenuating the actions of agonists that act at different receptors [e.g., (4)]. Naltrexone dose-dependently antagonized the discriminative stimulus, antinociceptive, and response rate-decreasing effects of OHM3507 and the affinity estimates that were obtained from Schild analyses were consistent with μ receptor mediation. For example, the apparent affinity estimates for naltrexone in attenuating the discriminative stimulus effects of other established μ agonists [e.g., (9); pA_2 = 8.2–8.6] are not different from the affinity estimates obtained with naltrexone in the current study. However, single-dose affinity estimates (pK_B) obtained with the δ receptor selective antagonist naltrindole failed to clearly support the exclusive role of μ opioid receptors in mediating the antinociceptive effects of OHM3507. The apparent affinity of naltrindole in attenuating the antinociceptive effects of OHM3507 is not different from its affinity in attenuating the rate-decreasing effects of the δ receptor selective agonist BW373U86 (17). Collectively, the qualitative effects of OHM3507 in these well-established behavioral procedures (e.g., reversal of naltrexone-lever responding in monkeys acutely deprived of morphine) as well as the quantitative effects of naltrexone in modifying the actions of OHM3507 (e.g., pA_2 values) provide strong evidence for μ agonist actions of this fentanyl derivative and fail to demonstrate any novel pharmacological features for this compound in rhesus monkeys. Despite the relatively high affinity of OHM3507 for δ opioid receptors in vitro, the behavioral effects of this compound appear to be mediated predominantly, if not exclusively, through μ receptors.

In other species, OHM3507 had novel behavioral actions that were not identical to the effects of fentanyl. There are other examples of a poor predictability between primate and nonprimate species with regard to behavioral effects of opioids. For example, OHM3568 had low efficacy opioid effects as well as nonopioid actions in nonprimate species (3); however, in rhesus monkeys this compound had high efficacy μ agonist actions and was essentially identical to fentanyl (9). Despite the failure of the current studies to confirm a potentially interesting pharmacology for OHM3507, as suggested by previous studies in nonprimate species, additional investigation of other compounds in this series appears warranted in light of the novel, and potentially clinically useful, actions of some compounds in this series (e.g., mirfentanil).

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