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Pharmacological Profile of a Deuterium-Substituted Mirfentanil Derivative, OHM10579, in Rhesus Monkeys

SNJEZANA LELAS,* LISA R. GERAK,* LAURA K. LANDERS,* MICHAEL R. BRANDT,*
JEROME R. BAGLEY,† LINDA L. BROCKUNIER† AND CHARLES P. FRANCE*

*Department of Pharmacology and Neuroscience Center of Excellence,
Louisiana State University Medical Center, New Orleans, LA 70112
†Ohmeda Inc., Murray Hill, NJ 07974*

LELAS, S., L. R. GERAK, L. K. LANDERS, M. R. BRANDT, J. R. BAGLEY, L. L. BROCKUNIER AND C. P. FRANCE. *Pharmacological profile of a deuterium-substituted mirfentanil derivative, OHM10579, in rhesus monkeys.* PHARMACOL BIOCHEM BEHAV **60**(3) 665–675, 1998.—The discriminative-stimulus, respiratory, and antinociceptive effects of OHM10579, an isotopic isomer of mirfentanil, were characterized in rhesus monkeys. In monkeys discriminating nalbuphine, 0.32 mg/kg of OHM10579 partially substituted for nalbuphine. In monkeys treated daily with 3.2 mg/kg of morphine and discriminating 0.01 mg/kg of naltrexone, 0.32 mg/kg of OHM10579 substituted for naltrexone. In morphine-abstinent monkeys, morphine reversed naltrexone-lever responding, an effect attenuated by OHM10579. The shift to the right in the morphine dose–effect curve was greater 2 h after 0.32 mg/kg of OHM10579 compared to 0.32 mg/kg of mirfentanil, indicating that OHM10579 has a longer duration of action than mirfentanil. In a warm-water tail-withdrawal procedure, 10 and 17.8 mg/kg of OHM10579 had antinociceptive effects that were not antagonized by naltrexone. Morphine decreased breathing in air to 48%, whereas the maximal decrease with OHM10579 was to 75% of control. OHM10579 attenuated hyperventilation induced by 5% CO_2 and partially antagonized the respiratory-depressant effects of morphine. OHM10579 can be classified as a low-efficacy μ-opioid agonist with some nonopioid actions. These results indicate that the pharmacology of the mirfentanil isotope OHM10579 is similar to that of mirfentanil, but that OHM10579 might have a longer duration of action. © 1998 Elsevier Science Inc.

OHM10579   Mirfentanil   Drug discrimination   Respiration   Antinociception   Rhesus monkey

POTENT and short-acting μ-opioid agonists, such as fentanyl, are used for anesthesia and treatment of pain following surgery (10,11,13). One limitation of these opioids is that, when large doses are necessary, they can cause severe respiratory depression, which can lead to respiratory arrest (14). Moreover, these μ-opioid agonists have been shown to have high abuse liability. For example, a tendency towards illicit use of these drugs has been seen with health professionals who have regular access to them (12). As a result of the high risks involved with the use of these μ-opioid agonists, the search continues for therapeutic compounds, such as those thought to be low-efficacy agonists, that are effective as anesthetics and analgesics with lower toxicity and abuse liability.

Mirfentanil, a structural derivative of fentanyl, has been shown to have both opioid and nonopioid effects in nonhumans (1,4). In pigeons treated chronically with morphine and discriminating between naltrexone and saline, omission of the daily morphine injection resulted in responding on the naltrexone-appropriate key; this effect was reversed by mirfentanil, suggesting that it may attenuate withdrawal following discontinuation of morphine. However, mirfentanil substituted for naltrexone in morphine-treated monkeys discriminating

between naltrexone and saline, an effect attenuated by morphine. These and other data suggest that mirfentanil has agonist actions under some conditions (following discontinuation of morphine in pigeons) and antagonist actions under other conditions (during chronic administration of morphine in monkeys) (4,17). In addition, small doses of mirfentanil reversed antinociception induced by alfentanil, another fentanyl derivative, and antagonized the respiratory-depressant effects of large doses of alfentanil in monkeys. Given by itself, mirfentanil produced much less respiratory depression than other μ-opioid agonists. This antagonism of the respiratory-depressant and antinociceptive effects of alfentanil further suggests that mirfentanil is a low-efficacy μ-receptor agonist (4). However, antinociception induced by large doses of mirfentanil was not attenuated by opioid antagonists, suggesting that the antinociceptive effects of mirfentanil are due to nonopioid effects (4,16). Thus, mirfentanil could be classified as a low-efficacy μ-opioid agonist with some nonopioid effects (4). Mirfentanil also has been shown to have a short duration of action (5). The latency to remove the tail from warm water was maximally increased at 1 h and returned to baseline at 1.5 h following the injection of 3.2 mg/kg of mirfentanil (4), whereas an equally effective dose of morphine (5.6–10 mg/kg) continued to have effects 2.75 h following the injection (unpublished observation). This short duration of mirfentanil decreases the potential utility of mirfentanil-like drugs in treatment of pain. Thus, further research on pharmacological effects of other derivatives of fentanyl is directed towards finding a compound with significant antinociceptive effects, lower toxicity and abuse liability, and a longer duration of action.

OHM10579, an isomer of mirfentanil, includes a substitution of the nonradioactive heavier deuterium at the furan ring (Fig. 1). It has been proposed that the short duration of action of mirfentanil is due to hepatic hydroxylation of the furan ring at position five (1). One possibility is that by introducing deuterium, and thereby slowing the abstraction of hydrogen and the insertion of oxygen, the duration of action could be increased. Deuterated beta-phenylethylamine (PEA) has been shown to increase intensity and duration of behaviors typically induced by PEA such as sniffing, head weaving, splayed hindlimbs, and hyperreactivity, and this is thought to be due to the fact that deuterated PEA is a poorer substrate for monoamine oxidase than the protonated amine (3).

The purpose of these experiments was to determine whether the pharmacology of mirfentanil is modified by the deuterium substitution and to fully characterize OHM10579 in rhesus monkeys by examining its discriminative-stimulus, antinociceptive, and respiratory effects. OHM10579 was studied in monkeys discriminating nalbuphine (0.178 mg/kg) from saline, to determine if it would substitute for a low-efficacy agonist (8). OHM10579 was also studied in monkeys treated daily with 3.2 mg/kg of morphine and discriminating between naltrexone (0.01 mg/kg) and saline, to see if it was similar to mirfentanil in exhibiting low-efficacy opioid agonist effects by substituting for naltrexone in morphine-treated monkeys and attenuating the effects of morphine in morphine-abstinent monkeys (4). Time course studies were also conducted to learn if OHM10579 has a longer duration of action compared to mirfentanil. In addition, a warm-water tail-withdrawal experiment was performed to determine whether the antinociceptive effects of OHM10579, like those of mirfentanil, are insensitive to naltrexone. Finally, respiration studies were carried out to see whether OHM10579 produces less respiratory depression compared to morphine. If OHM10579 showed a similar drug discrimination profile to that of mirfentanil and displayed nonopi-

oid antinociception and modest respiratory depression with a longer duration of action, OHM10579 could be clinically useful in treatment of pain outside of the hospital setting.

METHOD

Subjects

Seven female and six male adult rhesus monkeys (*Macacca mulatta*) were used in these experiments (four in the naltrexone and three in the nalbuphine discrimination experiment; four in the respiration and four in the antinociception experiment; two monkeys participating in both the respiration and antinociception experiments). The food-restricted weights of the animals were between 5 to 13 kg, and they were fed monkey food (Lab Diet, Teklad, Inc.), fruit, and peanuts daily. They were housed individually with free access to water in a colony room maintained on a 14 L:10 D cycle. The drug discrimination sessions were conducted 7 days a week, and the antinociception and respiration sessions were conducted twice a week. Some of the monkeys used in these experiments had received drugs, including opioids, in previous experiments. The animals were maintained in accordance with the Institutional Animal Care and Use Committee, Louisiana State University Medical Center and guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council (Department of Health, Education and Welfare, Publication No. (NIH) 85-23, revised 1985).
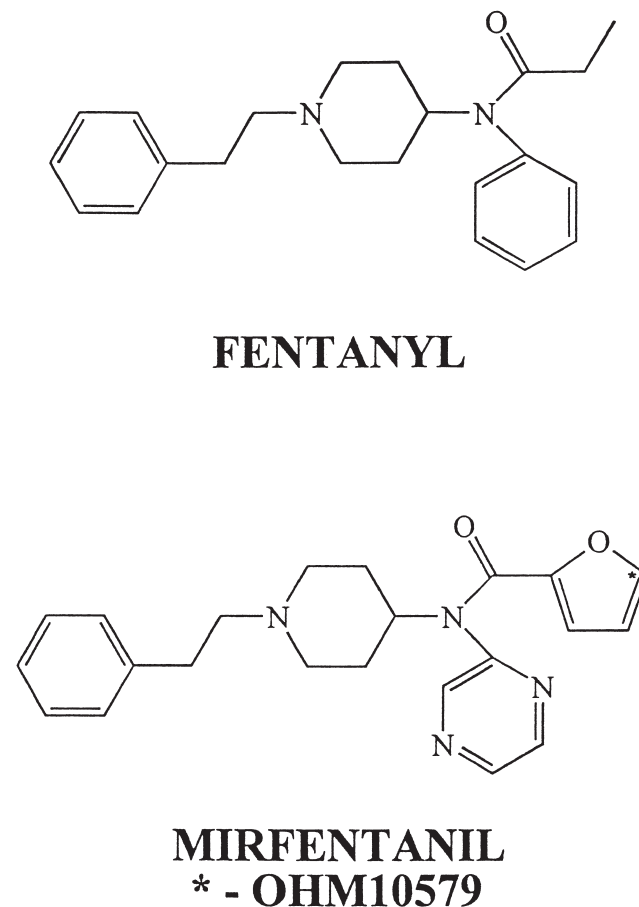
FENTANYL

MIRFENTANIL
* - OHM10579

FIG. 1. Chemical structures of fentanyl, mirfentanil, and OHM 10579.

Apparatus

Drug discrimination. The monkeys were seated in Lexan and aluminum primate restraint chairs and placed in ventilated, sound-attenuating chambers. Each chamber contained a stimulus light located above each of three response levers; during response periods, two of the stimulus lights were illuminated and two corresponding levers were active. At the front of the chair, the feet of the monkeys were restrained in a pair of shoes containing brass electrodes through which brief electric shock (3 mA, 250 ms) could be delivered. Electric shock was generated by A.C. shock generators. The experiments were controlled and data collected by a microprocessor (Dell Computer) and a commercially available interface (Med Associates, St. Albans, VT).

Antinociception. Four Lexan and aluminum primate chairs, which restrained the monkeys loosely at the neck and waist, were used. The bottom 10–12 cm of the tail were shaved and easily accessible to the experimenter. To obtain the desired temperatures used in the procedure (40, 50, and 55°C), hot and cold water were mixed in insulated mugs until the desired temperature was obtained. The latencies for the monkeys to withdraw their tails from the water were recorded by a stopwatch.

Respiration. The apparatus used to measure respiratory frequencies and volumes in rhesus monkeys has been described previously (7,9). Briefly, monkeys were seated in primate restraint chairs, and two alternating Lexan neckplates and latex sheaths were placed around their necks. To ensure a tight seal within the respiration helmet (head plethysmograph), a foam rubber pad was arranged at the base of the plethysmograph and the helmet was then fitted over the head of the monkey. The chair was then placed in a sound-proof chamber. Air or a mixture of 5% CO_2 in oxygen was pumped into the plethysmograph at a rate of 10 l/min and removed by a vacuum pump. All pressure changes were measured by a pressure transducer, and inspirations and expirations were recorded by a Grass Model 7 polygraph. The analogue signal was also converted via an analog-to-digital converter and recorded by the microprocessor (Dell Computer). The dependent variables obtained in this study were tidal volume (V_T, ml/inspiration), frequency (f, breaths/min), and minute volume (V_E, ml/min), which is a product of f and V_T.

Procedure

Nalbuphine discrimination. Monkeys were trained to discriminate 0.178 mg/kg of nalbuphine from saline under a fixed-ratio (FR) 5 schedule of stimulus-shock termination (8). Training sessions consisted of four to eight 15-min cycles. At the beginning of each cycle was a 10-min time out period during which the chamber was dark and lever pressing had no programmed consequence. The time out was followed by a 5-min response period, in which two of the lights in the box were illuminated and the schedule of stimulus–shock termination was in effect. During the response period, shocks were scheduled to occur every 10 (monkey BI) or 15 s (monkeys SH and ST). Five consecutive responses on the lever designated correct by the injection given at the beginning of the cycle extinguished the stimulus lights and postponed the shock for 30 s. The designation of saline- and drug-appropriate levers varied across monkeys. At the end of the 30-s time out period, the stimulus lights were illuminated and the schedule of stimulus–shock termination was again in effect. Response periods ended after 5 min or the delivery of four shocks, which ever occurred first.

Test sessions were conducted following two training sessions in which the following criteria were met: 1) ≥90% responding on the lever designated correct for that session; and 2) <5 responses (1 FR) on the incorrect lever before the first reinforcer (first shock postponement). During the test sessions, the procedure was the same as that used in the training sessions, except that five consecutive responses on either lever postponed the shock. Injections of the test drug were given during the first minute of the time out period of each cycle. Discriminative–stimulus effects of OHM10579 were determined using a cumulative dosing procedure (0.01–3.2 mg/kg increasing by 0.5 log unit per injection). For antagonism studies, a single dose of naltrexone (0.01 or 0.1 mg/kg) was administered during the first cycle and cumulative doses of OHM10579 were administered during subsequent cycles (0.01–3.2 mg/kg increasing by 0.5 log unit per injection). Test sessions ended when ≥90% of the total responses occurred on the drug-appropriate lever or when response rates decreased sufficiently to result in delivery of shock.

Naltrexone discrimination. The discriminative–stimulus effects of OHM10579 were examined in four monkeys treated daily with 3.2 mg/kg of morphine and discriminating between 0.01 mg/kg of naltrexone and saline while responding under a FR 5 schedule of stimulus–shock termination (6). Training sessions were conducted 3 h following administration of morphine. Training and testing sessions were similar to those described for the nalbuphine discrimination, except that during the response period, shock was scheduled to occur every 15 s for all monkeys.

The criteria for testing were the same as those used in the nalbuphine discrimination study. In the tests conducted to evaluate discriminative–stimulus effects of OHM10579, doses of the drug were administered in a cumulative fashion (0.01–3.2 mg/kg increasing by 0.5 log unit per injection). To determine whether OHM10579 could antagonize the discriminative–stimulus effects of morphine, saline was substituted for the daily morphine injection, a single dose of OHM10579 (0.32 or 1 mg/kg) was administered during the first cycle and cumulative doses of morphine (0.1–56 mg/kg increasing by 0.5 or 0.25 log unit per injection) were administered on subsequent cycles.

To determine the time course of OHM10579 and mirfentanil, a dose of 0.32 mg/kg of one of these compounds was administered at 0, 2, or 4 h prior to the determination of a morphine dose–effect curve (0.1–100 mg/kg increasing by 0.5 or 0.25 log unit per injection); three monkeys participated in the time course studies. Mirfentanil and OHM10579 are approximately equipotent because 0.32 mg/kg of mirfentanil produced >70% naltrexone-appropriate responding in morphine-treated monkeys discriminating between 0.01 mg/kg of naltrexone and saline (2) and 0.32 mg/kg of OHM10579 produced >90% naltrexone-appropriate responding in the present experiment.

Antinociception. Monkeys were seated in primate restraining chairs and the bottom 10–12 cm of their tails were immersed in water of either 40, 50, or 55°C contained within various thermoses. The tail-withdrawal latency was then measured; if the monkey did not remove its tail within 20 s, the experimenter removed the tail from the water and the maximum latency (20 s) was recorded. The session ended if the maximum latency was obtained in 50°C water.

Control latencies in the tail-withdrawal procedure were obtained by injecting saline and recording tail-withdrawal latencies in 40, 50, and 55°C water. A cumulative dosing procedure was implemented to determine dose–effect curves for OHM10579 (1–17.8 increasing by 0.5 or 0.25 log unit per in-

jection). Each cycle lasted 30 min, with the injection given during the first minute of the cycle. Tail-withdrawal latencies were then measured starting 25 min after the injection, with the order in which each water temperature was presented varying nonsystematically among monkeys. During the first cycle of the antagonism study, one dose of naltrexone (0.1 mg/kg) was administered and the tail-withdrawal latencies were measured. Cumulative doses of OHM10579 (1–17.8 mg/kg increasing by 0.5 or 0.25 log unit per injection) were administered during subsequent cycles.

Respiration. Experimental sessions consisted of four to eight cycles, with each cycle lasting 30 min. Each cycle was divided into an air exposure for 23 min followed by 5% CO_2 in oxygen exposure for 7 min. Saline or drug injections were given during the first minute of each cycle. To assess the effects of OHM10579, following the first cycle, a cumulative dosing procedure was implemented (0.1–17.8 mg/kg increasing by 0.5 or 0.25 log unit per injection). The session was terminated when several doses of the drugs used did not produce any further change in ventilation. To determine if OHM10579 antagonized the effects of morphine, one dose of OHM10579 (0.1, 1, or 10 mg/kg) was given 30 min prior to a morphine dose–effect curve determination (0.1–32 mg/kg increasing by 0.5 or 0.25 log unit per injection).

Data Analyses

Drug discrimination data are expressed as the percentage of total responses occurring on the drug-appropriate lever averaged across monkeys and plotted as a function of dose (mean $\pm$ SEM). Drugs that produced $\geq$90% responding on the nalbuphine-appropriate lever or on the naltrexone-appropriate lever in morphine-treated monkeys were considered to have substituted completely for the training stimulus. ED_{50} values were calculated for each test by linear regression when more than two appropriate data points were available or, when drug-appropriate responding did not reach 90%, by interpolation. One-way repeated-measures analysis of variance was performed on the difference between more than two conditions, and paired *t*-tests were performed on the difference between two conditions. If a normality test failed, then a Wilcoxon signed rank test was performed. In the nalbuphine discrimination study, control response rates were the average of 10 saline training sessions prior to the test calculated for each monkey. In the naltrexone discrimination study, control response rates were determined in the same manner as control rates in the nalbuphine experiment, except that on the saline training days in this experiment, morphine had been administered 3 h before the session. Response rates were expressed as a percentage of control rates for individual animals, averaged across animals, and plotted as a function of dose (mean $\pm$ SEM). Discrimination data for an individual animal were not included in analyses when response rates were less than 25% of control.

In the antinociception studies, latency was expressed as the percent of maximum effect using the following formula: % Maximum Effect = [(test latency − control latency)/(20 s − control latency)] × 100%, where the control latency was defined as the average latency of tail withdrawal determined in the absence of drug. The latencies for individual monkeys were averaged to obtain a group mean, and then the percent maximum effect was calculated. Only the data from the 50°C condition are shown.

In the respiration study, V_T and f were recorded throughout the entire session, with the data obtained during the last 3 min of each component (air or 5% CO_2) of the cycle used for analyses. V_E was calculated from V_T and f ($V_E = V_T * f$). The first cycle of the day was used to determine the average respiratory measures for that day in each animal. The mean values for V_E, V_T, and f in each cycle were calculated for individual animals. The averages were then expressed as a percentage of the values obtained during the air exposure in the saline cycle. Next, the percentages for the individual animals were averaged to obtain a group mean and SEM. Paired *t*-tests were performed on the maximum decrease obtained for each monkey for each of the variables.
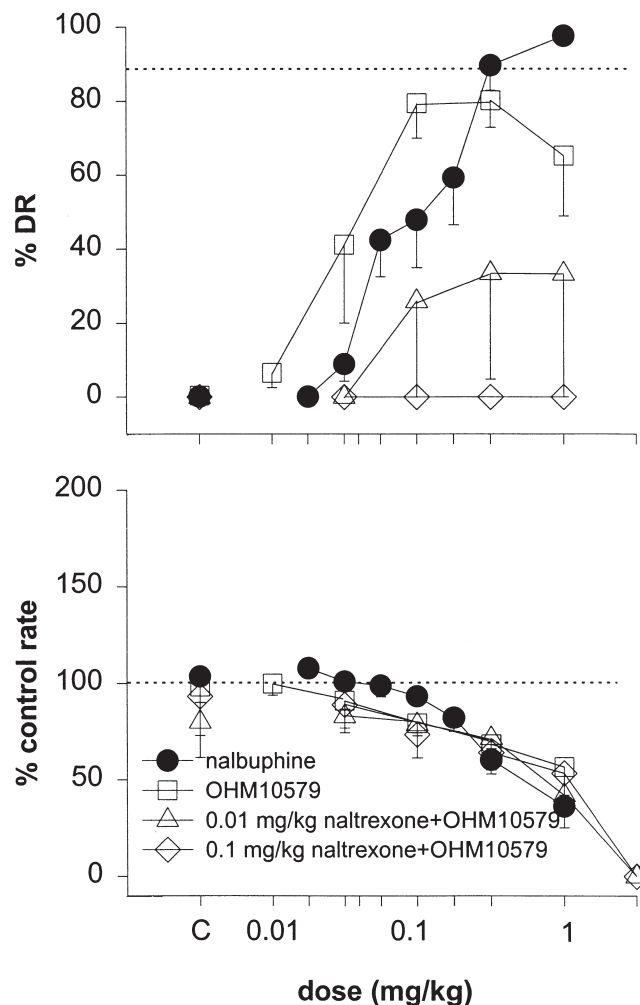

FIG. 2. Discriminative-stimulus and rate effects of nalbuphine, OHM10579 alone, and OHM10579 in combination with naltrexone in monkeys discriminating between 0.178 mg/kg of nalbuphine and saline. Abscissae: dose of nalbuphine or OHM10579 in mg/kg body weight; C = control. Ordinates: mean ($\pm$SEM) percent of responding on the nalbuphine-appropriate lever (%DR = drug responding; upper panel) and mean ($\pm$SEM) response rate expressed as percent of control (saline training days) rates (% control rate; lower panel). The nalbuphine dose–effect curve data represent the average of 8, 4, and 5 determinations for monkeys BI, SH, and ST, respectively. The OHM10579 dose–effect curve data represent the average of three determinations for monkey BI and one determination each for monkeys SH and ST. The naltrexone antagonism data represent one determination for each monkey averaged across monkeys.

Drugs

OHM10579 and mirfentanil hydrochloride (both synthesized by LLB), naltrexone hydrochloride, and morphine sulfate (National Institute on Drug Abuse, Rockville, MD), and nalbuphine hydrochloride (Mallinckrodt, Inc., St. Louis, MO) were all dissolved in sterile water. The injections were given SC in the back with volumes ranging from 0.1 to 3 ml.

RESULTS

Nalbuphine Discrimination

In rhesus monkeys discriminating between 0.178 mg/kg of nalbuphine and saline, control response rates (responses/s ± SEM) were 1.69 ± 0.05 for monkey Bl, 2.07 ± 0.06 for monkey SH, and 2.32 ± 0.05 for monkey ST. Nalbuphine dose dependently increased responding on the nalbuphine-appropriate lever with a cumulative dose of 0.32 mg/kg completely generalizing to the training dose of nalbuphine (>90% nalbuphine-lever responding; Fig. 2, filled circles). Doses of nalbuphine up to 0.1 mg/kg produced modest decreases in response rates (response rates were still >80% of control), with a dose of 0.32 mg/kg reducing response rates to 60% of control.

OHM10579 dose dependently increased responding on the nalbuphine-appropriate lever up to the dose of 0.1 mg/kg and decreased response rates (Fig. 2, squares). A dose of 0.32 mg/kg of OHM10579 produced the greatest substitution level (80% of responding on the nalbuphine-appropriate lever) and decreased response rates to only 68% of control. A larger dose of OHM10579 (1 mg/kg) produced only 65% nalbuphine-lever responding and further decreased response rates to 57% of control. The ED_{50} values for OHM10579 and nalbuphine in producing 50% nalbuphine-appropriate responding were not significantly different ($p > 0.05$), 0.099 (±0.019) mg/kg for nalbuphine and 0.082 (±0.019) mg/kg for OHM10579.

In the absence of other treatment, single doses of naltrexone (0.01 and 0.1 mg/kg) resulted in responding on the saline-appropriate lever and did not alter response rates (Fig. 2,
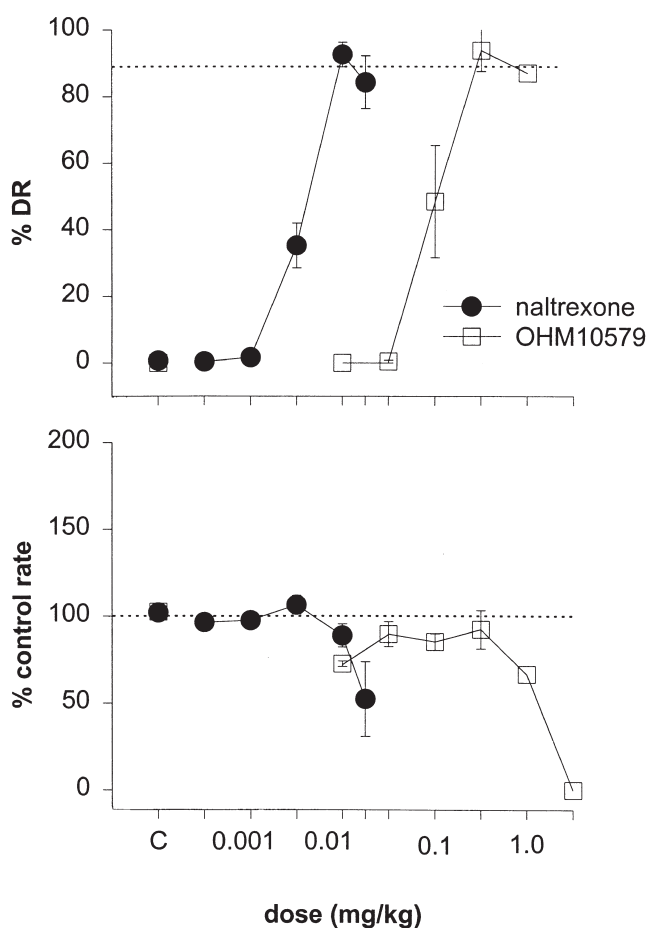

FIG. 3. Discriminative-stimulus and rate effects of naltrexone and OHM10579 in morphine-treated (3.2 mg/kg 3 h prior to session) rhesus monkeys discriminating between 0.01 mg/kg of naltrexone and saline. Abscissae: dose of naltrexone or OHM10579 in mg/kg body weight; C = control. The naltrexone dose–effect curve data represent the average of 11, 9, 3, and 13 determinations for monkeys WI, PI, LO, and GR, respectively. The OHM10579 dose–effect curve data represent the average of two determinations for each monkey. For other details, see Fig. 2.
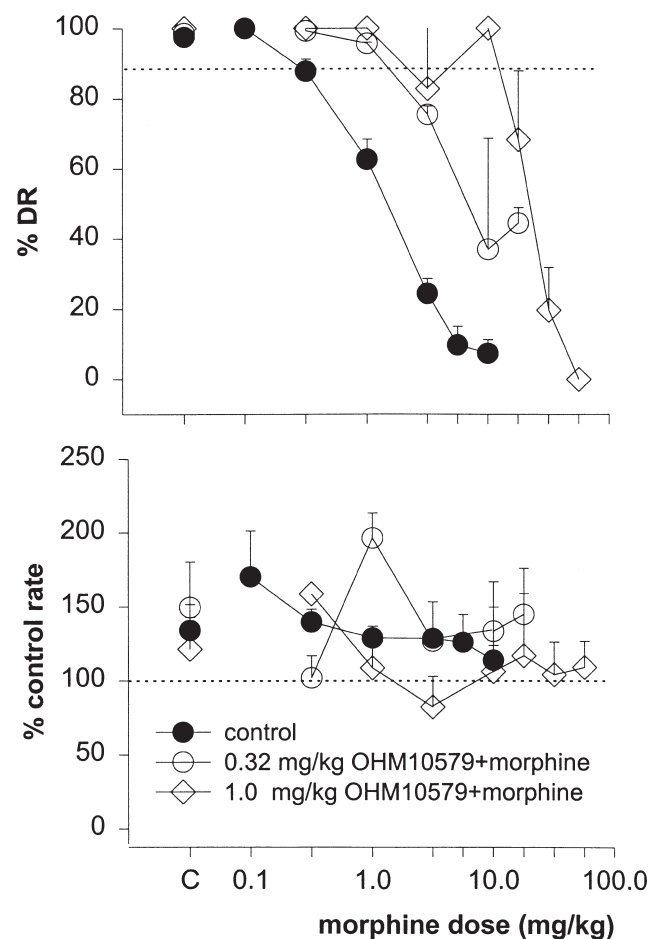
FIG. 4. Discriminative-stimulus and rate effects of morphine alone and in combination with several doses of OHM10579 in morphine-abstinent monkeys. Abscissa: dose of morphine in mg/kg body weight; C = control. The morphine dose–effect curve data represent the average of 14, 12, 9, and 14 determinations for monkeys GR, LO, PI, and WI, respectively. The OHM10579 antagonism data represent one determination per monkey, with three monkeys contributing to the data at the 0.32 mg/kg dose and four monkeys contributing to the data at the 1 mg/kg dose of OHM10579. See Fig. 2 for other details.

points above C). A dose of 0.01 mg/kg of naltrexone shifted the OHM10579 dose–effect curve to the right, with a decrease in the maximum nalbuphine-lever responding from 80 to 33% (Fig. 2, upper panel, triangles). When a larger dose of naltrexone (0.1 mg/kg) was administered, OHM10579 produced responding on the saline-appropriate lever up to doses of OHM10579 that eliminated responding (Fig. 2, both panels, diamonds). At both doses tested, naltrexone did not antagonize the rate-decreasing effects of OHM10579 (Fig. 2, bottom panel, triangles and diamonds).

Naltrexone Discrimination

In monkeys treated daily with 3.2 mg/kg of morphine 3 h prior to sessions and discriminating between 0.01 mg/kg of naltrexone and saline, control (saline training days) response

rates (responses/s ± SEM) were 1.53 ± 0.06 for monkey PI, 1.17 ± 0.04 for monkey LO, 0.88 ± 0.03 for monkey GR, and 0.76 ± 0.04 for monkey WI. The training dose of naltrexone (0.01 mg/kg) produced >90% naltrexone-lever responding and only modestly reduced response rates (Fig. 3, circles). In morphine-treated monkeys, a dose of 0.32 mg/kg of OHM10579 substituted for naltrexone, and this dose had no rate-decreasing effects; larger doses of OHM10579 decreased response rates to less than 75% of control (Fig. 3, squares). OHM10579 was approximately 20 times less potent than naltrexone in producing naltrexone-appropriate responding; the ED$_{50}$ values were 0.007 (±0.002) mg/kg for naltrexone and 0.135 (±0.026) mg/kg for OHM10579 ($p < 0.05$).

Omission of the daily injection of morphine produced >90% naltrexone-lever responding (Fig. 4, points above C). In these monkeys, morphine dose dependently reversed naltrexone-lever responding while not markedly altering re-
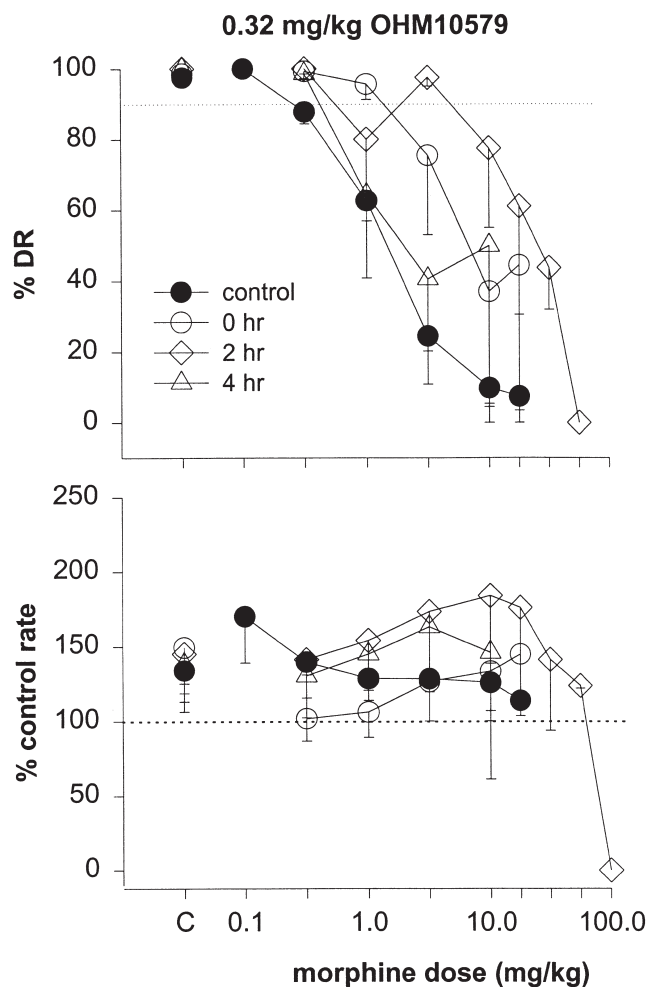

FIG. 5. Discriminative-stimulus and rate effects of morphine in morphine-abstinent monkeys following pretreatment with 0.32 mg/kg of OHM10579 0, 2, or 4 h prior to session. Abscissae: dose of morphine in mg/kg body weight; C = control. The morphine dose–effect curve data represent the average of 14, 12, 9, and 14 determinations for monkeys GR, LO, PI, and WI, respectively. The morphine dose–effect curve data with OHM10579 injection 0 h prior to session represent the average of two determinations for each monkey; at 2 h the average of two determinations for monkey GR and one determination each for monkeys LO, PI, and WI; at 4 h one determination per monkey, averaged across monkeys. See Fig. 2 for other details.
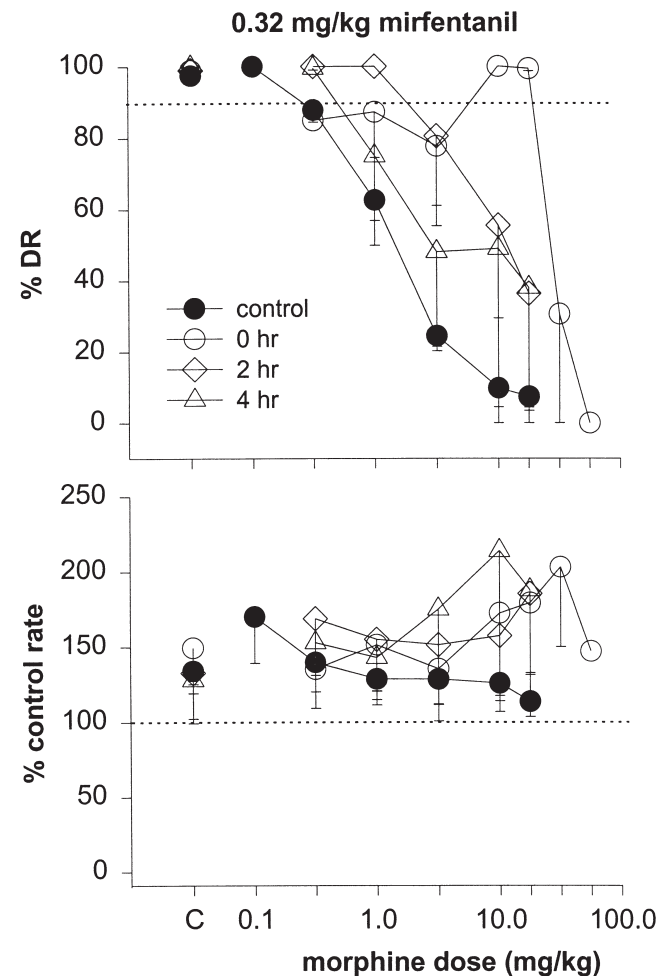
FIG. 6. Discriminative-stimulus and rate effects of morphine in morphine-abstinent monkeys following pretreatment with 0.32 mg/kg of mirfentanil 0, 2, or 4 h prior to session. Abscissae: dose of morphine in mg/kg body weight; C = control. The morphine dose–effect curve data represent the average of 14, 12, 9, and 14 determinations for monkeys GR, LO, PI, and WI, respectively. The morphine dose–effect curve data with mirfentanil injection at 0, 2, and 4 h prior to session represent one determination for each monkey, averaged across monkeys. See Fig. 2 for other details.

sponse rates (Fig. 4, filled circles). Only 7% naltrexone-lever responding was obtained with the largest dose of morphine tested (10 mg/kg). A dose of 0.32 mg/kg of OHM10579 shifted the morphine dose–effect curve two-fold to the right ($p > 0.05$), especially at lower doses of morphine (Fig. 4, upper panel, open circles). The ED_{50} values for morphine and morphine in combination with 0.32 mg/kg of OHM10579 were 1.98 (±0.22) and 5.58 (±1.6) mg/kg, respectively. A larger dose of OHM10579 (1 mg/kg) produced a 24-fold shift to the right in the morphine dose–effect curve ($p < 0.05$) (Fig. 4, upper panel, diamonds); the ED_{50} values for morphine and morphine in combination with OHM10579 were 1.98 (±0.22) and 24.52 (±3.75) mg/kg, respectively. The dose of 0.32 mg/kg of OHM10579 did not markedly alter response rates in the presence of morphine (Fig. 4, lower panel, open circles), whereas 1 mg/kg of OHM10579 slightly decreased response rates relative to morphine alone (Fig. 4, lower panel, diamonds).

The time-course data, also obtained in morphine-abstinent monkeys, show that an injection of 0.32 mg/kg of OHM10579 or mirfentanil immediately prior to determination of a morphine dose–effect curve shifted the curve threefold ($p < 0.1$) and 12-fold ($p < 0.1$) to the right, respectively. The ED_{50} for morphine was 23.43 (±8.61) mg/kg in the presence of mirfentanil and 5.58 (±1.6) mg/kg in the presence of OHM10579 compared to 1.98 (±0.22) mg/kg for morphine alone (Fig. 5 and Fig. 6, upper panels, filled and open circles). At 2 h following administration of 0.32 mg/kg of OHM10579 or mirfentanil, the dose–effect curve for morphine continued to shift in the presence of OHM10579 ($p < 0.1$), but did not shift further in the presence of mirfentanil ($p > 0.05$). At 2 h, the ED_{50} value for morphine in the presence of mirfentanil was 7.96 (±3.32) mg/kg, whereas the ED_{50} value in the presence of OHM10579 was 16.69 (±6.35) mg/kg (Figs. 5 and 6, upper panels, filled circles and diamonds). However, at 4 h following administration of either of these two drugs the morphine dose–effect curve was similar to that obtained following an injection of saline ($p > 0.05$) (Figs. 5 and 6, filled circles and triangles).

Antinociception

The average tail-withdrawal latencies ($\pm$SEM) under control (no drug) conditions were 20 ± 0, 0.95 ± 0.13, and 0.75 ± 0.03 s, at 40, 50, and 55°C, respectively. OHM10579 increased tail-withdrawal latencies at doses of 10 and 17.8 mg/kg such that
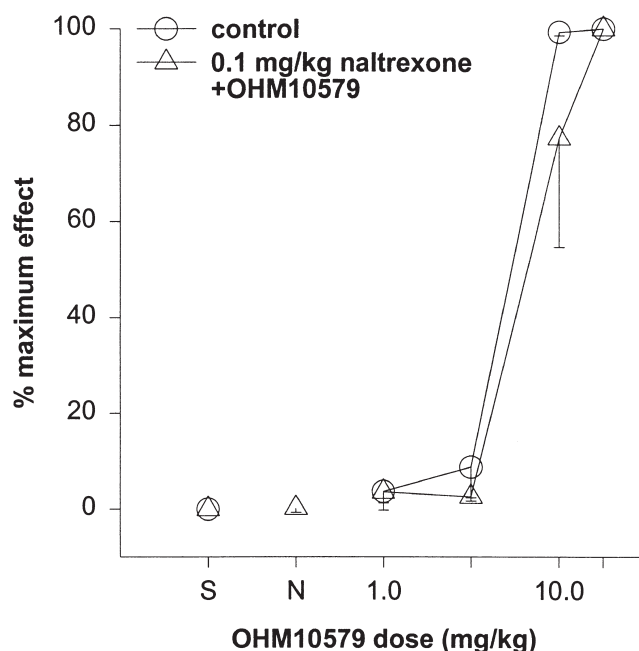

FIG. 7. Antinociceptive effects of OHM10579 alone and in combination with naltrexone in a warm-water tail withdrawal procedure in rhesus monkeys. Abscissa: dose of OHM10579 in mg/kg body weight; S = saline, n = naltrexone. Ordinates: percent of maximum possible effect (%MPE) $\pm$ SEM for 50°C water.

monkeys failed to remove their tails from 50°C water within 20 s (Fig. 7, circles). However, in 55°C water, the greatest effect obtained with 10 and 17.8 mg/kg of OHM10579 was 60% of the maximum possible effect (data not shown). Naltrexone, at a dose of 0.1 mg/kg, did not affect tail-withdrawal latencies (Fig. 7, points above n) and did not antagonize the antinociceptive effects of OHM10579 in 50° water (Fig. 7, triangles).

Respiration

Table 1 lists means $\pm$ SEM for V_E, V_T, and f for individual monkeys and the percent change between the air and 5% CO_2

TABLE 1

MINUTE VOLUME (ML/MIN; V_E), TIDAL VOLUME (ML/INSPIRATION; V_T), FREQUENCY (INSPIRATIONS/ MIN; f) (MEAN $\pm$ SEM) AND PERCENT CONTROL FOR INDIVIDUAL MONKEYS

Subject	Condition	N/Days	Minute Volume (V_E)	Tidal Volume (V_T)	Frequency (f)
GO	AIR	10	1222.1 ± 128	58.09 ± 6.66	21.43 ± 0.85
	5% CO_2	10	2183 ± 162	58.69 ± 6.59	39.25 ± 2.26
	% control		178.67	101.03	183.15
LI	AIR	9	1154.6 ± 133	50.97 ± 4.87	22.4 ± 1.28
	5% CO_2	9	2018.1 ± 180	49.02 ± 4.49	41.96 ± 2.59
	% control		174.79	96.17	187.32
MA	AIR	10	1218.8 ± 105	32.69 ± 3.36	38.19 ± 2.5
	5% CO_2	10	1849.5 ± 181	39.05 ± 4.03	48.06 ± 1.89
	% control		151.75	119.46	125.84
PR	AIR	9	860.2 ± 91	36.67 ± 3.38	23.52 ± 1.32
	5% CO_2	9	1706.5 ± 134	38.14 ± 3.4	45.44 ± 2.49
	% control		198.38	104.01	193.2

conditions. When the animals were exposed to 5% CO_2, respiration increased rapidly and reached a maximum over the first 4 min and then remained constant during the next 3 min (data not shown). During this period, V_E reached between 152 and 198% of control levels, V_T varied from 96 to 119%, and f increased to between 126 and 193% of control levels in air.

OHM10579 decreased V_E in air at doses of 1.78, 3.2, and 5.6 mg/kg, with the maximal decrease to 72% of control at 1.78 mg/kg (Fig. 8, upper panel, circles). Doses larger than 1.78 mg/kg produced no further decrease in V_E, which returned to control levels at the dose of 10 mg/kg. The decrease in overall V_E was primarily a result of a reduction in f obtained at all doses tested and not a reduction in V_T, which was increased at some doses (Fig. 8, lower and center panels, circles).

OHM10579 decreased V_E in 5% CO_2 with the maximum decrease at the dose of 1.78 mg/kg, when V_E decreased from 186 to 109% of control respiration in air. Similar to the effects obtained in air, doses larger than 1.78 mg/kg produced no further depression and V_E in 5% CO_2 was greater at larger doses compared to 1.78 mg/kg (Fig. 8, upper panel, squares). The decrease in V_E was due to a decrease in f, which was also maximal at 1.78 mg/kg (Fig. 8, lower panel, squares). OHM10579 had no effect on V_T in the presence of 5% CO_2 (Fig. 8, middle panel, squares).

Morphine dose dependently decreased V_E in air, with the maximum effect occurring at the largest dose tested (17.8 mg/kg; Fig. 9, upper panel, circles). This decrease in respiration was due to a decrease in f (Fig. 9, bottom panel, circles), which was as low as 48% of control at the largest dose tested. For two of the animals, the dose that decreased f to approximately 50% of control (and was, therefore, the last dose administered) was 10 mg/kg and for another two animals it was 17.8 mg/kg. The V_T value was increased at the largest morphine dose tested and was generally unchanged for other doses. Morphine-induced decreases in V_E in CO_2 ($p < 0.05$), V_T in air ($p < 0.05$), and CO_2 ($p = 0.1$), and f in air ($p < 0.05$) and CO_2 ($p < 0.05$) were greater than OHM-induced changes in these parameters (compare Fig. 8 and solid circles in Fig. 9 and Fig. 10).

The dose of 0.1 mg/kg of OHM10579 given in the first drug cycle produced 92% of V_E control breathing in air and 90% of f in air with no change in V_T. This dose of OHM10579 did not affect the decrease in breathing produced by morphine except at the largest doses of morphine tested (10 and 17.8) (Fig. 9, squares).

When given alone, 1 mg/kg of OHM10579 produced a decrease in V_E to 90%, a decrease in f to 71%, and an increase in V_T to 121% (Fig. 9, diamonds). When given prior to a morphine dose–effect curve determination, this dose of OHM10579 did not affect the morphine dose–effect curve for any of the variables.

The dose of 10 mg/kg of OHM10579 alone reduced V_E to 75%, V_T to 88%, and f to 86% of control (Fig. 9, triangles). When administered prior to a morphine dose–effect curve determination, it attenuated the effects of morphine on V_T, especially at the higher doses (17.8 and 32) and it had no effect on V_E or f. At this dose of OHM10579, the final dose of morphine was 17.8 mg/kg for two animals and 32 mg/kg for the other four. The dose of 32 mg/kg of morphine decreased f to 50% of control (Fig. 9, lower panel, triangles).

In the 5% CO_2 mixture, morphine dose dependently reduced V_E, with the maximal decrease produced by the largest dose tested (17.8 mg/kg), which reduced V_E from 179 to 67% of control breathing in air (Fig. 10, upper panel, circles). This decrease in V_E produced by morphine was due to a reduction in both V_T and f, especially at higher doses (10 and 17.8 mg/

kg). All doses of OHM10579 studied (0.1, 1, and 10 mg/kg) decreased V_E in CO_2 (Fig. 10, upper panel). None of the doses of OHM10579 tested had any effect on V_T (Fig. 10, middle panel), and they decreased f.

When 0.1 mg/kg of OHM10579 was administered prior to morphine, it had no effect on morphine-induced decreases in V_E and f at any dose of morphine (Fig. 10, squares). When 1 mg/kg of OHM10579 was given prior to morphine, it produced only a slight increase in V_T relative to morphine alone, and only at the two intermediate doses of morphine (3.2 and 10 mg/kg), whereas it had no effect on morphine-induced decreases in V_E and f (Fig. 10, diamonds). OHM10579 at the dose of 10 mg/kg antagonized morphine-induced decreases in V_E at the higher doses (3.2, 10, and 32 mg/kg), so that even the largest dose of morphine (32 mg/kg) in combination with 10 mg/kg of OHM10579 maintained V_E at 92% of control breathing in air. This effect of 10 mg/kg of OHM10579 in combination with morphine was due to an increase in V_T rather than a change in f.
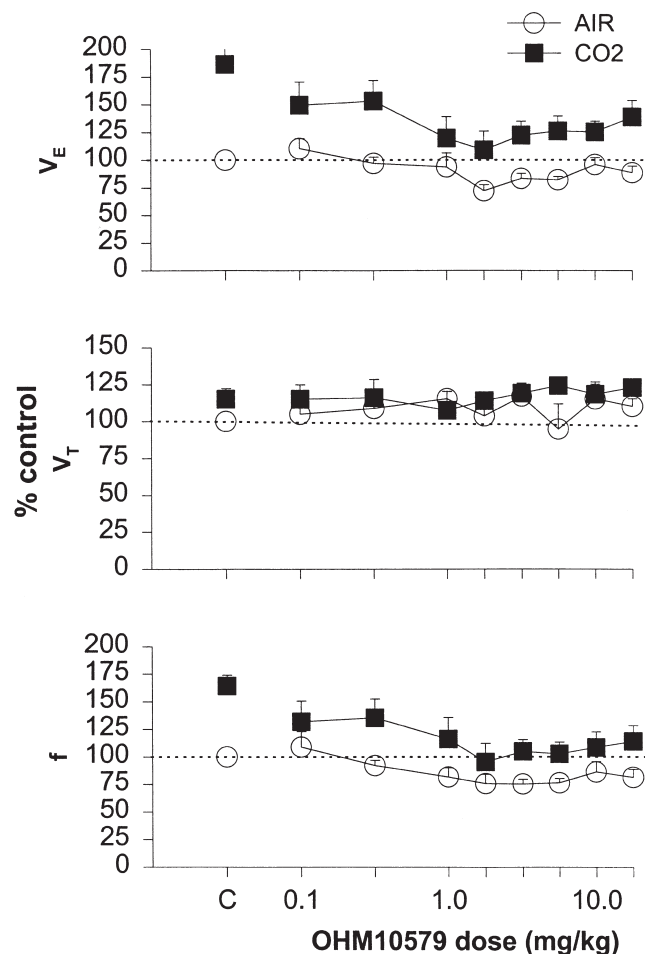

FIG. 8. Effects of OHM10579 on respiration in air and in 5% CO_2. Abscissae: dose of OHM10579 in mg/kg body weight; C = control. Ordinates: percent of control breathing in air for V_E (ml/min), V_T (ml/inspiration), and f (breaths/min). The data represent the average of two determinations in monkeys LI and PR and three determinations in monkeys GO and MA.
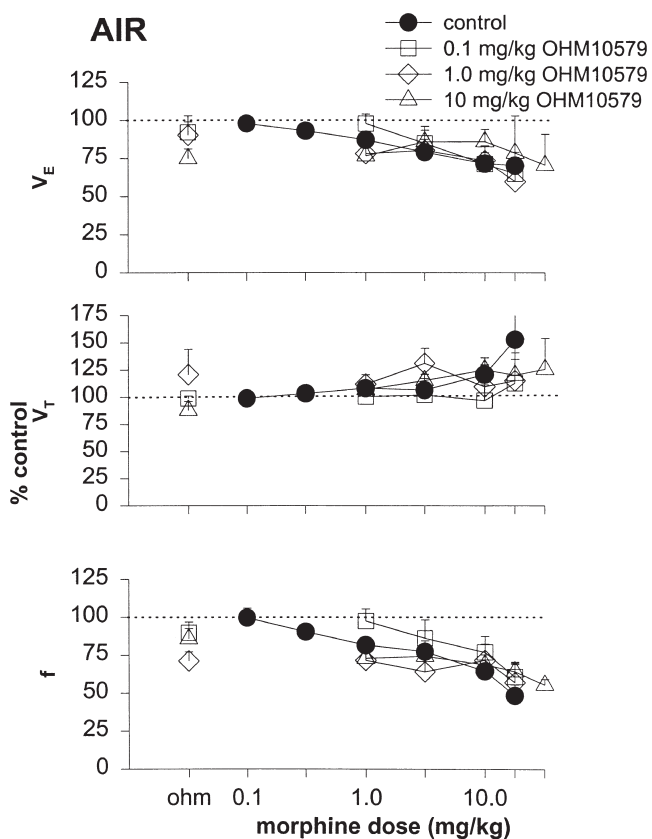
FIG. 9. Effects of morphine alone and in combination with different doses of OHM10579 on respiration in air. Abscissae: dose of morphine in mg/kg body weight; C = control. The morphine dose–effect curve data represent the average of four determinations for each monkey. The OHM10579 antagonism data represent one determination for each monkey at each of the doses averaged across monkeys. See Fig. 8 for more details.

FIG. 10. Effects of morphine alone and in combination with different doses of OHM10579 on respiration in 5% CO_2. See Figs. 8 and 9 for more details.

DISCUSSION

Fentanyl and other potent, short-acting μ-opioid agonists are used for anesthesia and treatment of pain following surgery (10,11,13). However, these drugs have also been shown to have significant respiratory-depressant effects (14) and high abuse liability (12). Mirfentanil, a fentanyl derivative, was demonstrated to be a low-efficacy μ-opioid agonist (4), which could indicate reduced abuse liability compared to morphine-like opioids that are commonly used to treat pain. In rhesus monkeys, it also has antinociceptive effects that are comparable to those of morphine, but it differs from morphine in that these effects are nonopioid; moreover, mirfentanil produces less respiratory depression compared to morphine (4). Unfortunately, mirfentanil has a short duration of action (5), which could limit its clinical usefulness.

OHM10579 was synthesized, in part, as an attempt to increase the duration of action of mirfentanil by replacing the protonium on the furan ring of mirfentanil with a heavier deuterium (1). It was hypothesized that the incorporation of the deuterium might slow the abstraction of the hydrogen and the insertion of the oxygen during hepatic hydroxylation of the furan ring at position 5, thus lengthening the duration of action. In the present experiments, the effects of OHM10579 on drug discrimination in untreated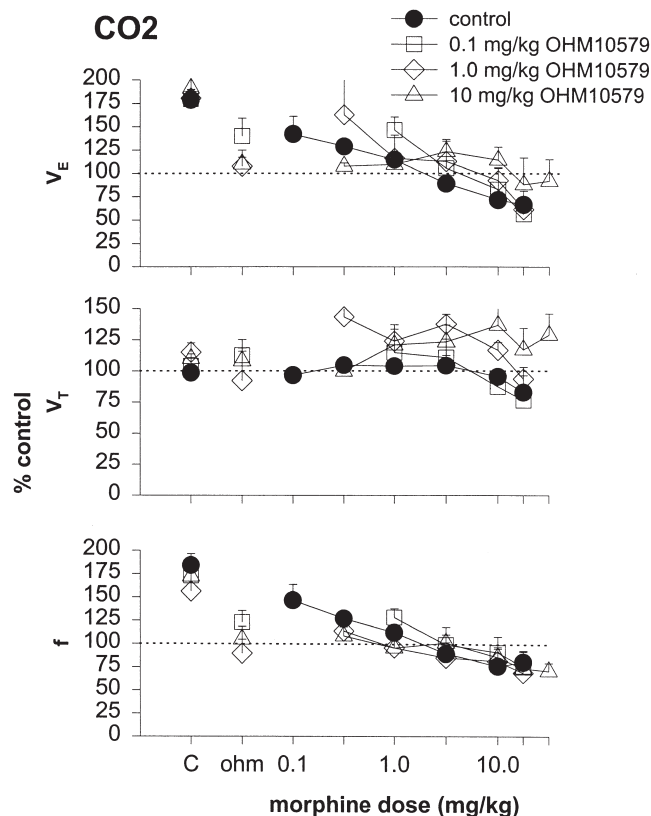, morphine-treated, and morphine-abstinent monkeys, and respiration and antinociception in untreated monkeys were examined to determine if it retained mirfentanil-like properties. Time-course studies were conducted to determine if the duration of action was prolonged compared to mirfentanil.

OHM10579 dose dependently increased responding on the nalbuphine-appropriate lever in monkeys trained to discriminate between 0.178 mg/kg of nalbuphine and saline, with the dose of 0.32 mg/kg resulting in the greatest substitution (80%). This effect was antagonized by naltrexone (0.01 and 0.1 mg/kg); for example, 0.1 mg/kg of naltrexone resulted in saline-appropriate responding at all doses of OHM10579 tested. This result suggests that OHM10579 has μ-agonist effects, because discriminative-stimulus effects of nalbuphine have been shown to be mediated by the μ-opioid receptor (8).

In monkeys treated daily with morphine, OHM10579 dose dependently substituted for naltrexone, with the dose of 0.32 mg/kg resulting in >90% naltrexone-lever responding. When saline is given instead of the daily morphine injection, all animals respond on the naltrexone-appropriate lever. In these animals, morphine dose dependently reduced responding on the naltrexone-appropriate lever, an effect attenuated by pretreatment with 0.32 and 1 mg/kg of OHM10579. These effects suggest an antagonist action of OHM10579 at the opioid receptor. Thus, the current data demonstrate that OHM10579 shows an agonist profile under some conditions (nalbuphine discrimination) and an antagonist profile under other conditions (morphine-induced reduction in naltrexone-lever re-

sponding). In fact, in both of these discrimination experiments, the same dose of OHM10579 (0.32 mg/kg) produced >80% drug-appropriate responding. Because nalbuphine discrimination is a behavioral assay that requires at stimulation relatively low amounts of μ receptors to produce drug-appropriate responding (8), these data suggest that OHM10579 is a low-efficacy μ-opioid receptor agonist. This pharmacological profile is very similar to that of mirfentanil (4,17), thus suggesting that substitution of a protonium with deuterium on the furan ring of mirfentanil did not affect its activity at the μ receptor.

OHM10579 is also similar to mirfentanil in that it decreased respiration to a lesser extent compared to morphine. The maximal decrease in respiration in monkeys breathing air was 75% of control with 1.78 mg/kg of OHM10579, with larger doses producing no further decrease; in contrast, morphine continues to suppress breathing with increasing doses until respiratory arrest (3,15) (50% of control in the present experiments). Similarly, in monkeys breathing the 5% CO_2 mixture, 1.78 mg/kg of OHM10579 produced a maximum decrease in respiration to 95% of control, whereas with morphine the maximum decrease was to 67% of control in air. When given together with morphine, OHM10579 (0.1, 1, and 10 mg/kg) did not affect the respiratory-depressant effects of morphine in monkeys breathing air and partially reversed morphine-induced decrease in respiration in monkeys breathing 5% CO_2. These effects are similar to those of mirfentanil, which also had little effect on respiration in monkeys breathing air and reduced the hypercapnia produced by 5% CO_2 (4). The effects of OHM10579, producing a smaller reduction in respiration compared to morphine and partially reversing morphine-induced respiratory depression in animals breathing 5% CO_2, further suggest that OHM10579 retained the mirfentanil-like low-efficacy μ-opioid agonist activity.

In addition to opioid agonist effects, OHM10579 appears to have some nonopioid effects that are responsible for its antinociceptive and rate-decreasing effects. In the present study, OHM10579 increased the latency to withdraw the tail from 50°C water, an effect not antagonized by 0.1 mg/kg of naltrexone. Thus, the same dose of naltrexone that antagonized nalbuphine-like discriminative-stimulus effects of OHM10579 did not antagonize its antinociceptive effects. This antinociceptive profile is similar to that of mirfentanil, whose antinociceptive effects are also not antagonized by naltrexone at doses that shift the dose–effect curves for other μ-opioid agonists 10- to 30-fold to the right compared to the control (4,16). OHM10579 also failed to produce any of the behavioral effects (e.g., stupor) that are characteristic of κ agonists in rhesus monkeys, indicating that its actions are not mediated by either μ or κ opioid receptors. These results suggest that the antinociceptive effects of OHM10579, like those of mirfentanil, are not mediated through opioid receptors. In addition, in the nalbuphine discrimination study, OHM10579 was found to have rate-decreasing effects, with the dose of 1 mg/kg reducing the response rate to 57% of control. This effect of OHM10579 was not antagonized by naltrexone (0.01 and 0.1 mg/kg), suggesting that the rate-decreasing effects of OHM10579 are not mediated by opioid receptors.

The time-course data suggest that OHM10579 has a longer duration of action compared to that of mirfentanil, in that, at 2 h following administration of 0.32 mg/kg of either OHM10579 or mirfentanil, the morphine dose–effect curve continued to shift to the right in the presence of OHM10579, but did not shift any further for mirfentanil (was shifted to the left compared to the 0 h morphine dose–effect curve). The ED_{50} values for morphine immediately following administration of OHM10579 or mirfentanil were 5.58 ± 1.6 and 23.43 ± 8.61 mg/kg, respectively, suggesting that mirfentanil had a more rapid onset of action compared to OHM10579. However, the ED_{50} values for morphine at 2 h after administration of OHM10579 or mirfentanil were 16.69 ± 6.35 and 7.96 ± 3.32 mg/kg, respectively, suggesting a longer duration of action for OHM10579. At 4 h following administration of OHM10579 or mirfentanil, the dose–effect curve for morphine was the same as when morphine was given alone.

In summary, the present study showed that OHM10579, a fentanyl derivative, has a pharmacological profile consistent with that of a low-efficacy μ-opioid agonist, suggested by the results showing that OHM10579: substitutes for nalbuphine in untreated monkeys and for naltrexone in morphine-treated monkeys; antagonizes the effects of morphine in morphine-abstinent monkeys; has little effect on respiration in monkeys breathing air; reduces 5% CO_2-induced hypercapnia; and has some nonopioid actions, such as antinociception and rate-decreasing effects. This pharmacological profile is similar to that of mirfentanil (4). Furthermore, OHM10579 seems to have a longer duration of action compared to mirfentanil. Thus, it appears that incorporation of the deuterium at the furan ring produces a compound with an almost identical pharmacological profile with that of mirfentanil with a longer duration of action.

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