ABSTRACT

The antinociceptive and ventilatory effects of morphine and other opioid agonists were determined in three rhesus monkeys during a period of morphine maintenance, as well as before and after the chronic exposure to morphine. Before the onset of the daily dosing regimen, morphine increased tail-withdrawal latencies from 50°C water, with an ED50 of 6.4 ± 2.1 mg/kg. Daily injection of 3.2 mg/kg morphine produced a rightward displacement of the morphine dose-response curve, increasing the ED50 of morphine to 28.4 ± 12.3 mg/kg. Doubling the daily morphine dose to 6.4 mg/kg resulted in a further shift to the right of the dose-response curve of morphine. After cessation of the daily dosing regimen, the morphine dose-response curve for producing antinociceptive effects returned toward baseline. The antinociceptive effects of the kappa opioid agonist, ethylketazocine, were similar during the period of daily exposure to morphine, and after cessation of the daily dosing regimen. Before the onset of the daily dosing regimen, morphine, ethylketazocine, fentanyl, butorphanol and nalbuphine decreased ventilation in the presence of air or air mixed with CO2. The baseline ED50 value of morphine for decreasing minute volume in the presence of 5% CO2 was 2.9 ± 0.8 mg/kg. The ventilatory effects of morphine and other mu opioid agonists tested were not attenuated during the daily morphine-dosing regimen. After 40 weeks of daily injections of 3.2 mg/kg morphine, the ED50 of morphine for decreasing minute volume in 5% CO2 was 2.3 ± 1.0 mg/kg, and when the daily dose was doubled to 6.4 mg/kg morphine, the ED50 of morphine was 1.5 ± 0.5 mg/kg. The ventilatory depressant effects of the daily injection 3.2 mg/kg morphine were also unchanged during morphine maintenance. The differential development of tolerance to the antinociceptive and ventilatory effects of morphine demonstrates a separation of these two mu opioid agonist effects in rhesus monkeys.

Chronic opioid use often results in the development of tolerance to the effects of opioid drugs, yet the emergence and expression of tolerance to different opioid effects is not well understood. For instance, it is widely assumed that tolerance to the respiratory depressant effects of opioids necessarily develops, based on observations that opioid-experienced addicts and cancer patients can tolerate doses of opioid drugs that are lethal in people who are opioid-naive (Reisine and Pasternak, 1995). However, tolerance to the respiratory depressant effects of opioids in humans has been directly examined in relatively few studies and these have produced mixed results. For example, in one study of morphine-dependent human subjects, morphine initially decreased respiratory rate by 15 to 20%, and this respiratory suppression was unchanged for the duration of the study, which suggests that tolerance did not develop to the ventilatory effects of opioids (Martin and Jasinski, 1969). However, the respiratory responses to 60 and 120 mg of morphine were less in both morphine-dependent subjects than were the responses to 15 and 30 mg morphine in nondependent subjects (Martin et al., 1968). Similarly, in cancer patients receiving morphine for pain relief, decreases in the effects of morphine on measures of pCO2 and minute volume were correlated to morphine dose and the duration of treatment (Pfeifer et al., 1989). These latter results suggest that tolerance may develop to some of the respiratory effects of morphine.

The development of tolerance to the antinociceptive effects of opioids in humans is similarly unclear. In a clinical study that examined analgesic dosing requirements in cancer patients, opioid-experienced patients required higher doses of morphine postoperatively than did the opioid-naive patients (de Leon-Casasola et al., 1993). In addition, dose escalation is common in the prescription of opioids to patients with termi-
nal cancer, as the doses originally prescribed fail to continuously provide adequate analgesia, which suggests that the antinociceptive effects of *mu* opioid agonists in humans are subject to tolerance (Reisine and Pasternak, 1995). In another clinical report, however, the analgesic effects of morphine were not altered in cancer patients receiving 20 to 120 mg of morphine daily (Pfeifer et al., 1989) and it has been argued that when dose escalation occurs, the higher doses of analgesics are necessitated not by the development of tolerance, but by disease progression and intensifying pain (Portenoy and Foley, 1986). Some of the difficulties in resolving whether opioid tolerance develops in humans might arise from various methodological problems or practical limitations of clinical research, and the development of analgesic tolerance to opioids in humans remains a topic of debate (e.g., Colpaert, 1996).

In contrast to studies of opioid tolerance in humans, experiments with various animal models have consistently demonstrated that chronic exposure to morphine-like drugs results in the development of tolerance to many opioid effects. In rodents, tolerance has been observed repeatedly in both behavioral and physiological responses to opioids. After chronic exposure to morphine, the dose-response curves for effects of morphine and morphine-like drugs on schedule-controlled responding (Adams and Holtzman, 1990; Picker et al., 1991), antinociception (Lange et al., 1980; Paronis and Holtzman, 1992) and respiration (McGilliard and Takemori, 1978) are shifted to the right. The tolerance induced by chronic exposure to morphine appears pharmacological in that it is restricted to *mu* opioid agonists (Craft et al., 1989), and the magnitude of the rightward displacement of the dose-response curves is dependent on the dose of morphine administered chronically (Paronis and Holtzman, 1992). However, the degree of tolerance can vary according to the response that is measured. For example, implantation of 50-mg morphine pellets in mice resulted in a 2- to 3-fold shift to the right of the respiratory dose-response function for morphine, but a 5- to 6-fold shift to the right in the dose-response curve of morphine-induced antinociception (McGilliard and Takemori, 1978). Thus, although various rodent models of opioid effects may be used to measure morphine tolerance, the development of tolerance to these effects is not uniform.

Most studies involving chronic administration of morphine in monkeys have focused on the effects of opioid dependence; comparatively few studies have examined opioid tolerance in non-human primates. However, some studies have indicated that morphine tolerance will develop in monkeys. Daily injections of morphine, however, did not result in tolerance to the ventilatory depressant effects of morphine. In animals maintained on a regimen of food-maintained, schedule-controlled behavior, the morphine dose-response curve for food-maintained, schedule-controlled behavior was shifted 5- to 10-fold to the right relative to the position of the morphine curve before the onset of the daily injections (Woods and Carney, 1978; Bergman and Schuster, 1985). Similarly, studies of other characteristic effects of *mu* opioid agonists in rhesus monkeys demonstrated that daily administration of morphine produced tolerance to the muscle relaxing and stupor-inducing effects of *mu* opioid agonists, but did not alter the overt behavioral effects of *kappa* opioid agonists (Gmerek et al., 1987). The results of these studies demonstrate that after repeated morphine administration tolerance will develop to at least some of the behavioral effects of opioids in monkeys. To date, however, no studies have directly examined the development of tolerance to the antinociceptive and respiratory depressant effects of morphine in rhesus monkeys.

In an effort to further characterize opioid tolerance in non-human primates, the present studies examined ventilatory effects of morphine and other *mu* opioid agonists in rhesus monkeys receiving daily injections of 3.2 or 6.4 mg/kg morphine. The *mu* opioid agonists used in these studies covered a range of efficacies, from the partial agonist, nalbuphine, to the full agonist, fentanyl, and included the mixed *mu/kappa* opioid agonist, ethylketazocine. The antinociceptive effects of morphine and ethylketazocine were also assessed in the same group of monkeys. These studies were completed in conjunction with another series of experiments that characterized ventilatory effects of morphine withdrawal in rhesus monkeys (Paronis and Woods, 1997). The results of the present experiments demonstrate that tolerance does develop to the antinociceptive effects of morphine in monkeys. Daily injections of morphine, however, did not result in tolerance to the ventilatory depressant effects of morphine.

### Methods

#### Subjects

Subjects were two adult male (909B and S67) and one adult female (F2) rhesus monkeys. The weights of the subjects remained relatively stable throughout the study and were 6.5 kg (F2), 7.5 kg (S67) and 9 kg (909B). Subjects 909B and F2 had been used previously in experiments involving opioids. Monkeys were housed singly in a temperature-controlled colony room with a 12-hr light/dark cycle (lights on at 6:00 A.M.). Water was available ad libitum, and the monkeys were fed approximately 30 biscuits (Purina Monkey Chow) daily, supplemented twice weekly with fresh fruit.

#### Apparatus

The apparatus used to measure ventilation was similar to that described by Howell et al. (1988). Subjects were seated in a standard primate restraint chair enclosed within a sound-attenuating chamber. A Plexiglas helmet that was placed over the head of the subject served as a pressure-displacement plethysmograph. Customized Plexiglas plates and rubber dams were used to provide an airtight seal around the monkey’s neck. A continuous flow, 10 liters/min, of either air or a mixture of 5% CO2 in air (hereafter referred to only by the CO2 concentration) was introduced through a port in the front of the helmet, and was extracted at the same rate through a port in the back of the helmet. Changes in flow within the plethysmograph were measured by a pressure transducer connected to a polygraph (model 7E, Grass Instrument Co., Quincy, MA) and computer (IBM/PCjr). Pressure displacement was converted to a volume measure by a polygraph integrator (model 7P122E, Grass Instrument Co.). Minute volume (V′E) was determined by integration of changes in flow through the plethysmograph, frequency of ventilation (f) was directly determined and tidal volume (V′T) was calculated as the quotient V′E / f.

Experimental sessions generally consisted of four to six consecutive cycles, each comprising a 23-min exposure to air followed by a 7-min exposure to 5% CO2. Data from the first air/CO2 cycle were used for session-control values. Cumulative dosing procedures similar to those described by Howell et al. (1988) were used. At the start
of each test cycle, graded doses of drug were administered i.m. so that the total dose increased by 0.25 or 0.5 log unit increments throughout the session. When ventilatory responses to single injections of morphine were measured, experimental sessions consisted of two 63-min cycles in which 7-min exposures to increasing concentrations of CO₂ alternated with 14-min exposures to air. Animals received an i.m. injection of drug after the end of the first cycle. Data obtained from the first cycle served as session-control values.

Antinociception was measured by a tail-withdrawal procedure described by Dykstra and Woods (1986). Monkeys were seated in standard primate restraint chairs and the distal 10 cm of the shaved tail was immersed in a thermos flask containing water at 40°C, 50°C or 55°C. Tail-withdrawal latencies were manually timed by a hand-held stopwatch, and the maximal withdrawal latency was set at 20 sec to prevent damage to the tail. At least 1 min intervened between observations at the different temperatures; one latency measure was recorded per temperature per drug dose. Drugs were administered i.m. with a cumulative dosing procedure. Injections were given at 30-min intervals, starting 15 min after control tail-withdrawal latency determinations. Subsequent withdrawal latencies were determined 15 to 20 min after each drug injection.

**Experimental Design**

Drug tests were conducted during four phases, baseline, single-dosing, double-dosing and abstinence, during which the subjects were exposed repeatedly to morphine and other mu opioid agonists, to the opioid antagonist, naltrexone, and to several nonopioid drugs. Experimental sessions were conducted at least twice a week to ensure stable patterns of ventilation, and at least 2 days intervened between drug tests. Five to seven days intervened between tests when the daily dosing schedule was interrupted to assess abstinence-associated withdrawal effects, or when the monkeys received large doses of drugs. The order of the drug tests was randomized between subjects within each phase of the study to minimize the influence of duration of exposure to the maintenance dose of morphine on the responses to the test drug.

**Baseline phase.** During the baseline phase, the ventilatory effects of morphine, naltrexone, nalbuphine, ethylketazocine, butorphanol and fentanyl were determined in all monkeys. Dose-response curves for the antinociceptive effects of morphine were determined in all monkeys, and the antinociceptive effects of ethylketazocine were determined in 909B and S67. This phase of the study lasted 14 weeks in S67 and 16 weeks in 909B and F2. Data obtained during this phase are referred to throughout this paper as baseline values, and should be distinguished from the session-control values obtained at the start of individual test sessions.

**Single-dosing phase.** During the single-dosing phase, the monkeys received injections of 3.2 mg/kg morphine every morning, either in the test chamber or, on days when they were not tested, in the home cage. Except where noted, tests occurred 24 hr after injection of the maintenance dose of morphine. Only the ventilatory effects of morphine were studied during the first 4 weeks of the single-dosing phase. Subsequently, the ventilatory effects of a range of opioid agonists and the antinociceptive effects of morphine and ethylketazocine, were examined in all monkeys. Except where noted, tests occurred 24 hr after morphine.

**Abstinence phase.** During the abstinence phase, daily morphine injections were discontinued and the monkeys were drug-free for 4 weeks. Experiments continued throughout this time to assess any effects of abstinence-associated withdrawal. After at least 28 days, the ventilatory effects of morphine and naltrexone and the antinociceptive effects of morphine and ethylketazocine were redetermined in all monkeys.

**Data Analysis**

Antinociceptive data are presented as a percentage of the M.P.E. according to the following equation: [(test latency – control latency/ (20 sec – control latency)] × 100%. Drug effects on ventilation were determined with data from the last 3 min of exposure to air or CO₂ within each cycle and are presented as a percentage of the session-control values. ED₅₀ values were calculated by linear regression of individual dose-response curves, and the individual ED₅₀ values were averaged to determine group means and S.E. For ventilatory measures, ED₅₀ values represent the dose required to decrease minute volume in 5% CO₂ to 50% of session-control values. For antinociceptive measures, ED₅₀ values represent the dose required to increase tail withdrawal latencies in 50°C water to 50% of the M.P.E. Dose-effect curves were compared by repeated measures one-way or two-way ANOVA, followed by Student-Neuman-Keuls multiple comparison test. Significance was set at P < .05.

**Drugs**

Morphine sulfate (Mallinckrodt, Inc., St. Louis, MO), nalbuphine HCl (DuPont Pharmaceuticals, Garden City, NY), butorphanol tartrate (Bristol Myers Squibb, Wallingford, CT), fentanyl HCl (Research Technology Branch, National Institute on Drug Abuse, Rockville, MD) and ethylketazocine (Sterling Winthrop, Rensselaer, NY) were dissolved in sterile water and injected i.m. in a volume of 0.1 to 1.0 ml. Drug doses are expressed as the weight of the salt.

**Results**

**Antinociception.** Morphine dose-dependently increased tail-withdrawal latencies in all monkeys under baseline conditions (fig. 1, top panel). Session-control tail-withdrawal latencies from 50°C and 55°C water were 1.6 ± 0.3 and 1.1 ± 0.1 sec, respectively. Morphine produced only 25% of the M.P.E. when the monkeys were tested with 55°C water. In contrast, when the monkeys were tested with 50°C water, 18 to 32 mg/kg morphine produced a full antinociceptive effect and the baseline ED₅₀ of morphine was 6.4 ± 2.1 mg/kg. Daily injections of 3.2 mg/kg morphine did not consistently alter session-control tail-withdrawal latencies; however, the cumulative morphine dose-response curve was increasingly shifted to the right during the single-dosing phase of the study. After 12, 19 and 40 weeks of daily morphine administration, the ED₅₀ values of morphine for producing antinociception were 8.9 ± 0.7, 14.4 ± 2.6 and 28.4 ± 12.3 mg/kg, respectively. After 40 weeks of daily morphine administration, the mean antinociceptive effect in 50°C water produced by 32 mg/kg morphine was 60 ± 12% of the M.P.E. (fig. 1). The morphine dose-response curve was displaced further to the right during the double-dosing phase of the study. ED₅₀ values for antinociception could not be calculated reliably during the double-dosing phase. Twenty-four hours after morphine during the double-dosing phase, a cumulative dose
of 32 mg/kg morphine produced only 23% of the M.P.E., and a dose of 56 mg/kg failed to produce greater than 70% analgesia in any of the three monkeys. Two-way ANOVA revealed significant effects for drug dose ($F_{3,6} = 92.88, P < .001$), phase of testing ($F_{3,6} = 7.31, P < .02$) and dose × phase interaction ($F_{9,18} = 5.41, P < .01$). Post hoc analysis indicated that the morphine dose-effect curve for antinociceptive effects during the double-dosing phase was significantly different from the curves obtained during the baseline phase, and that the effects of 10 and 32 mg/kg morphine during the last determination of the single-dosing phase and during the double-dosing phase differed from the baseline effects of these doses.

Four to six weeks after termination of the daily morphine injections, the morphine dose-response curve for antinociceptive effects was shifted back toward the baseline curve. The $ED_{50}$ of morphine for antinociceptive effects was 9.8 ± 1.7 mg/kg during the abstinence phase, and 32 mg/kg morphine produced 100% of the maximum possible antinociceptive effect in 50°C water; these effects were not significantly different from baseline.

The antinociceptive effects of ethylketazocine were determined in two monkeys before the onset of the daily morphine injections. During the baseline phase, ethylketazocine dose-dependently increased tail-withdrawal latencies from 50°C water, and a dose of 0.1 mg/kg ethylketazocine produced 100% of the maximum possible antinociceptive effect in both monkeys. Antinociception dose-response curves for ethylketazocine were determined in all three monkeys during the single- and the double-dosing phases, as well as during the abstinence phase (fig. 1, bottom panel). In all instances, ethylketazocine produced dose-dependent antinociception and 0.1 mg/kg ethylketazocine produced 100% of the M.P.E.; a two-way ANOVA revealed a significant effect for dose of ethylketazocine ($F_{2,4} = 93.61, P < .001$) but not for the experimental phase ($F_{2,4} = 0.647, P = .57$). The mean $ED_{50}$ values for the antinociceptive effects of ethylketazocine during the single-dosing, double-dosing and abstinence phases were 0.028, 0.024 and 0.026 mg/kg, respectively. These values are consistent with previously reported $ED_{50}$ values of ethylketazocine in morphine-free rhesus monkeys (Butelman et al., 1993).
Ventilatory effects. Morphine (0.32–32.0 mg/kg) dose-dependently decreased all measures of ventilation in both air and 5% CO₂ during the baseline phase of the study (fig. 2). The ventilatory depressant effects of morphine were most evident in measures of minute volume, and ventilation was more readily suppressed by morphine in the presence of CO2 than in the presence of normal air. During the baseline phase, the mean ED₅₀ value (± S.E.) of morphine for ventilatory depressant effects in air was 13.5 ± 1.3 mg/kg; in the presence of 5% CO₂ the mean ED₅₀ value of morphine was 2.9 ± 0.8 mg/kg. The effects of 3.2 mg/kg morphine given as a single injection decreased minute volumes in the presence of air and 5% CO₂ to 75 ± 8% and 44 ± 3% of session-control values, respectively.

Butorphanol (0.0032–0.32 mg/kg), fentanyl (0.001–0.032 mg/kg) and ethylketazocine (0.0032–0.032 mg/kg) dose-dependently decreased ventilation during the baseline phase of the study. The highest doses of each drug decreased minute volume in the presence of CO₂ to 20 to 30% of session-control values and decreased minute volume in air to 30 to 40% of session-control values. The effects of nalbuphine were dose-dependent up to a dose of 3.2 mg/kg, which decreased minute volume in 5% CO₂ to 38% of session-control values; further increases in the dose of nalbuphine did not produce greater ventilatory effects.

The ventilatory depressant effects of 3.2 mg/kg morphine did not decrease as a result of the daily administration of morphine. During weeks 38 to 40 of the single-dosing phase, 3.2 mg/kg morphine given as a single injection decreased minute volume in the presence of air and 5% CO₂ to 48 ± 2% and 34 ± 6% of session-control values, respectively. Cumulative morphine dose-response curves were determined 24 hr after 3.2 mg/kg morphine seven times, at approximately weeks 1, 3, 4, 10, 20, 30 and 40 of the single-dosing phase. Comparisons of the ED₅₀ values calculated from the dose-response curves determined in the presence of 5% CO₂ demonstrate that the potency of morphine did not change during

### TABLE 1

<table>
<thead>
<tr>
<th>Phase</th>
<th>ED₅₀*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base line</td>
<td>2.9 ± 0.8</td>
</tr>
<tr>
<td>Single-dosing phase</td>
<td>4.2 ± 1.1</td>
</tr>
<tr>
<td>First determination</td>
<td>3.4 ± 1.0</td>
</tr>
<tr>
<td>Second determination</td>
<td>5.1 ± 2.9</td>
</tr>
<tr>
<td>Fourth determination</td>
<td>2.8 ± 0.8</td>
</tr>
<tr>
<td>Fifth determination</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>Sixth determination</td>
<td>2.8 ± 0.5</td>
</tr>
<tr>
<td>Seventh determination</td>
<td>2.3 ± 1.0</td>
</tr>
<tr>
<td>Double-dosing phase</td>
<td>1.5 ± 0.5</td>
</tr>
</tbody>
</table>

* Values given are group means ± S.E. in milligrams per kilogram and were determined from linear regression of individual morphine dose-response curves.

### TABLE 2

<table>
<thead>
<tr>
<th>Drug</th>
<th>Base Line</th>
<th>Single Dosing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fentanyl</td>
<td>0.020 ± 0.012</td>
<td>0.011 ± 0.001</td>
</tr>
<tr>
<td>Nalbuphine</td>
<td>0.961 ± 0.215</td>
<td>0.847 ± 0.313</td>
</tr>
<tr>
<td>Butorphanol</td>
<td>0.056 ± 0.018</td>
<td>0.035 ± 0.010</td>
</tr>
<tr>
<td>Ethylketazocine</td>
<td>0.015 ± 0.001</td>
<td>0.014 ± 0.003</td>
</tr>
</tbody>
</table>

* Values given are group means ± S.E. in milligrams per kilogram and were determined from linear regression of individual dose-response curves.

Fig. 3. Effects of different agonists on minute volumes in the presence of 5% CO₂ during the baseline (□) and single-dosing (■) phases. Mean session-control values ranged from 5.5 to 6.9 l/min during the baseline phase, and from 7.2 to 8.8 liters/min during the single-dosing phase. Abscissae: cumulative drug dose; ordinates: percentage of the session-control values. Each point represents the group mean, based on one observation in three subjects, vertical lines are ± 1 S.E.

Fig. 4. Morphine effects on minute volume in the presence of 5% CO₂ during the baseline phase (○), single-dosing phase (■, seventh determination), double-dosing phase (▲) and abstinence phase (□). Mean session-control values were 6.9 liters/min (baseline), 9.0 liters/min (single-dosing), 8.6 liters/min (double-dosing), 5.2 liters/min (abstinence). Other details are as in figure 3.
the single-dosing phase (table 1), and statistical analysis indicated that the dose-effect curves did not differ from one another ($F_{9,18} = 0.636, P = .75$). The ED$_{50}$ values for decreasing ventilation in the presence of normal air could not be calculated as minute volume in air was rarely decreased to less than 50% of session-control at the doses tested. Higher doses of morphine were not tested to avoid exposing the animals to doses of morphine more than a log unit higher than the maintenance dose. Daily injections of 3.2 mg/kg morphine also did not alter the dose-response curves of fentanyl, nalbuphine, butorphanol or ethylketazocine (fig. 3). The mean ED$_{50}$ values of these four drugs under baseline conditions and during the single-dosing phase are listed in table 2.

The ventilatory effects of 0.32 to 10.0 mg/kg morphine during the double-dosing phase were similar to morphine effects during the baseline and single-dosing phases (fig. 4), and the ED$_{50}$ values of morphine during the different phases of the study were not significantly different (table 1). During the double-dosing phase, the morphine dose-response determination was extended to include doses up to 56 mg/kg. Under baseline conditions, ventilation in two monkeys was markedly reduced by 32 mg/kg morphine, and naltrexone was administered to reverse the effects of this dose. In contrast, during the regimen of two daily injections of 3.2 mg/kg morphine all three monkeys appeared to tolerate the effects of the cumulative 56 mg/kg dose.

Redetermination of the morphine dose-response curve 4 to 6 weeks after termination of the daily morphine injections showed that morphine continued to dose-dependently decrease minute volume (fig. 4). The morphine ED$_{50}$ during the abstinence phase was 5.5 ± 3.0 mg/kg.

**Discussion**

Opioid tolerance is generally described as a decreased effect of a given dose of a drug or, more fully, as a shift to the right of the dose-response curve. In the present study, daily administration of 3.2 mg/kg morphine to rhesus monkeys resulted in the development of tolerance, which was observed in the rightward displacement of the dose-response curve of morphine for producing antinociceptive effects. The antinociceptive effects of ethylketazocine were similar during the single-dosing, double-dosing and abstinence phases of the study, and are in line with published reports of the antinociceptive effects of ethylketazocine in rhesus monkeys that did not receive morphine daily (Dykstra et al., 1987; Butelman et al., 1993; France et al., 1994). Earlier studies of the effects of ethylketazocine in rhesus monkeys demonstrated that ethylketazocine-induced antinociception is mediated by kappa opioid receptors (Dykstra et al., 1987). The present observation that the morphine dose-response curve for antinociceptive effects was displaced to the right and the ethylketazocine dose-response curve was unchanged indicates that the tolerance that developed in the morphine-maintained monkeys was mu opioid selective. These results are in agreement with previous studies of opioid tolerance in monkeys, in which daily morphine injections produced rightward displacements of the dose-response curves of morphine, but not ethylketazocine, in a shock-titration procedure or in measures of overt behavioral responses (Craft and Dykstra, 1990; Gmerek et al., 1987). In the present experiments, tolerance to the antinociceptive effects of morphine increased when the daily dose of morphine was doubled and returned toward baseline after termination of the daily dosing regimen. The time- and dose-dependent nature of tolerance to the antinociceptive effects of opioids has been previously demonstrated in rodents (Adams and Holtzman, 1990). The present work extends these results by demonstrating that the development of tolerance to the antinociceptive effects of morphine in monkeys also is time- and dose-dependent.

In contrast to the tolerance that developed to the antinociceptive effects of morphine, daily morphine administration did not produce tolerance to the ventilatory effects of morphine or other mu opioid agonists. Among the drugs tested, nalbuphine and butorphanol have been characterized as low to intermediate efficacy mu opioid agonists in monkeys (Gerak et al., 1994; Butelman et al., 1995). Based on previous results (Paronis and Holtzman, 1992) it was expected that tolerance to the effects of lower efficacy agonists would develop more readily; however, the ventilatory effects of nalbuphine and butorphanol were unaltered by the daily dosing regimen with morphine. Likewise, the dose-response curve of morphine was not shifted to the right, nor were the ventilatory responses to 3.2 mg/kg morphine attenuated despite daily exposure to this dose of morphine for more than 40 weeks. The lack of tolerance to the ventilatory effects of morphine was a surprising result, especially in light of the tolerance that developed to its antinociceptive effects.

The present results directly contrast with those of a previous investigation in human subjects, in which tolerance developed to the respiratory, but not the antinociceptive, effects of morphine (Pfeifer et al., 1989). Studies in rodents also have demonstrated that tolerance will develop to the respiratory effects of opioids. For example, the ED$_{50}$ values of morphine, heroin and etorphine for decreasing respiratory rate in mice were increased by 5- to 7-fold after 3-day exposures to morphine pellets (Roerig et al., 1987). Similarly, respiratory responses to sufentanil were attenuated after 7-day infusions of 4 $\mu$g/hr sufentanil in rats (Ayesta and Florez, 1989). It is unclear why tolerance to the ventilatory effects of opioids did not develop in monkeys.

A lack of uniformity in the development of tolerance to the respiratory and antinociceptive effects of morphine has been reported previously and it was suggested that these two mu opioid effects might be mediated by different receptor subtypes, each associated with different regulatory mechanisms (Pfeifer et al., 1989; Roerig et al., 1987). In support of this idea, the mu opioid antagonist, naloxonazine, was shown to antagonize the antinociceptive, but not the respiratory effects of morphine in rats, leading to the proposal that the antinociceptive effects of morphine are mediated by the mu-1 receptor type, whereas the respiratory depressant effects are mediated by mu-2 receptors (Ling et al., 1985). In more recent studies, however, naloxonazine was found to antagonize the antinociceptive and the ventilatory depressant effects of the mu opioid agonist, levorphanol, to the same degree, consistent with the notion that both of these opioid effects in monkeys are mediated by the same type of mu opioid receptor (Gatch et al., 1996). Therefore tolerance might be expected to develop equally to both of these mu opioid effects.

The different results in the present studies and previous investigations of tolerance to the ventilatory effects of opioids
may be caused, in part, by procedural differences. In studies of tolerance to the respiratory effects of opioids in rodents, either drug pellets or drug-filled osmotic minipumps were used to administer the tolerance-inducing agent continuously, and drug effects were determined while the pellets or osmotic minipumps remained implanted. In contrast, the current experiments used a daily injection procedure in which drug effects were measured 24 hr after morphine, a time at which the subjects likely experienced some degree of opioid withdrawal (Paronis and Woods, 1997; France and Woods, 1989). If the appearance of opioid withdrawal did alter the ventilatory effects of morphine, then the degree of tolerance to the ventilatory effects of opioids that could be measured might have been compromised. This suggestion is speculative, however, because studies in rodents that have addressed the issue of whether opioid tolerance is best revealed in the presence or absence of the tolerance-inducing drug have produced ambiguous results. For example, in mice implanted for 3-days with 75-mg morphine pellets, tolerance to the antinociceptive effects of s.c. injected morphine was much greater when subjects were tested with the morphine pellets still implanted than in subjects tested 3 hr after removing the pellets (Lange et al., 1980; Paktor and Vaught, 1984). In contrast, however, to the results obtained with s.c. injected morphine, when the morphine-pelleted mice were tested with intracerebroventricularly injected morphine, tolerance was more pronounced after removal of the pellets than in the presence of the morphine pellets (Lange et al., 1980; Paktor and Vaught, 1984). The results obtained in the antinociceptive studies indicate that morphine tolerance can be assessed at 24 hr after administration of the maintenance dose; nonetheless, the conditions under which opioid tolerance is best measured in monkeys remain to be determined.

It is possible that the maintenance doses of morphine used in the present study simply were not high enough to induce tolerance to the ventilatory effects of mu opioid agonists. The dose of 3.2 mg/kg/day morphine is relatively low in comparison with previous studies of morphine tolerance in rhesus monkeys, in which the subjects received 12 to 15 mg/kg morphine daily (Bergman and Schuster, 1985; Gmerek et al., 1987). However, daily administration of 3.2 mg/kg morphine was adequate to produce opioid dependence in the monkeys used in the present studies (Paronis and Woods, 1997). Moreover, the maintenance dose of 3.2 mg/kg morphine clearly produced tolerance to the antinociceptive effects of morphine, which demonstrates that this relatively low dose of morphine, administered daily, is indeed large enough to induce tolerance to at least some behavioral effects of opioids in monkeys.

In conclusion, the results presented here, together with the results presented in the companion paper (Paronis and Woods, 1997), indicate that daily dosing regimens with low doses of morphine are adequate to produce both opioid tolerance and dependence in rhesus monkeys. The tolerance and dependence that developed after chronic exposure to morphine were not apparent within the same behavioral measures. The ventilatory responses that permitted orderly, quantitative assessments of precipitated and abstinence-associated opioid withdrawal provided little evidence for opioid tolerance. In contrast, tolerance did develop in a dose-dependent fashion to the antinociceptive effects of morphine. The lack of tolerance to the ventilatory effects of morphine in the present study was surprising, given the clinical and anecdotal evidence that tolerance does develop to the respiratory depressant effects of opioids in humans. The expression of tolerance to the ventilatory effects of mu opioid agonists in the present experiments may have been hindered by the timing and dosing parameters used, or by influences of non-opioid compensatory mechanisms. Alternatively, it may be that the mu opioid receptors that mediate the antinociceptive effects of morphine are differentially regulated from those that mediate the ventilatory effects. These explanations are speculative, however, and the mechanisms underlying the differential development of tolerance to the antinociceptive and ventilatory effects of opioids are unclear at this point. Nonetheless, the apparent differences in the tolerance and dependence that developed to antinociceptive and ventilatory effects of morphine within the same subjects suggest that, in whole animals, not all mu opioid receptors are affected in the same manner by chronic drug administration.


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