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Elucidation of Dose–Effect Relationships for Different Opiate Effects Using Alfentanil in the Spontaneously Ventilating Rat

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In addition to producing antinociception and mild sedation, opiates diminish spontaneous movement and produce muscle rigidity. Examination of the relationship between different opiate effects may lead to a better understanding of the mechanism and sites of action of opiate anesthesia. Previous studies have compared the dose–effect relationships for morphine and fentanyl between antinociception and loss of righting reflex. However, neither muscle rigidity nor lack of spontaneous movement (as measured by catalepsy) has been fully examined or directly compared with either antinociception or loss of righting reflex. This study, therefore, compared five clinically relevant opiate endpoints (antinociception, muscle rigidity, catalepsy, loss of righting reflex, and respiratory depression) using the μ-selective agonist alfentanil in the spontaneously ventilating rat. Rats were randomized to receive alfentanil (0–500 μg/kg) subcutaneously. For muscle rigidity, 59 rats had electromyographic activity measured with percutaneous hindlimb electrodes. After alfentanil injection, electromyographic data were recorded for 60 min. For antinociception and catalepsy, 49 rats were studied for 120 min after alfentanil. Catalepsy was measured from the time the rat's forelimbs were placed on a 10-cm-high bar until either limb was removed. Antinociception was studied by measuring tail-flick response to hot (55° C) water. For righting reflex, 40 rats were studied for 120 min. Alfentanil-induced respiratory depression was assessed in 40 rats with indwelling tail arterial catheters. Alfentanil was administered after baseline arterial blood gas measurements, and then additional samples were obtained for 45 min. For each effect, data were converted into quantal responses and were then transformed to probit-log dose–response curves for analysis. There were no significant differences in the slope of the dose–response relationships between the four endpoints studied. The effective dose in 50% of animals (ED_{50}) for antinociception (46 μg/kg) differed significantly from that for catalepsy (114 μg/kg), elevation of Pa_{CO_2} (146 μg/kg), and loss of righting reflex (215 μg/kg), and the ED_{50} for muscle rigidity (62 μg/kg) differed significantly from that for elevation of Pa_{CO_2} and loss of righting reflex. The results are discussed in the context of other data on the opioid receptors' mediation of different opiate effects. These techniques and the data obtained will aid in the elucidation of the pharmacology of different opiate effects and their underlying receptor mechanisms. (Key words: Anesthetics, intravenous: alfentanil. Animals: rodent. Pharmacology: dose–response curves. Potency, anesthetic: ED_{50}.)

A COMPLETE ANESTHETIC AGENT must produce a decreased level of consciousness, as well as amnesia, analgesia, and blunting of the cardiovascular response to surgical manipulation. Currently available opiates are used widely in anesthesia because they reliably address the latter two requirements. However, their use may be complicated by untoward effects such as respiratory depression and muscle rigidity. It has been suggested that different effects of high-dose opiates are mediated by different opioid receptor populations.[1,2] Although this hypothesis remains to be proven, the ability pharmacologically to separate the opiates' desirable effects from their undesirable ones on the basis of opioid receptor specificity could lead to the development of new clinically valuable opiate drugs. The study of the dose–effect relationships of both the desirable and undesirable effects of opiates will contribute to the understanding of opioid receptor pharmacology.

Previous studies have demonstrated the validity of evaluating the dose–effect curve of a single drug on a number of effects to illuminate possible differences in the mechanisms responsible for those effects.[1,3–5] For example, Kissin and colleagues generated dose–effect curves for morphine and fentanyl on three different end-points: antinociception (as measured by the lack of purposeful movement response to a noxious stimulus), loss of righting reflex, and prevention of the increased heart rate response to a noxious stimulus.[3] In this study, the reduction of the movement and heart rate responses to a noxious stimulus by fentanyl appeared to be mediated by different underlying mechanisms. Similarly, Ling and colleagues found different dose–effect relationships for opiate-induced antinociception compared with respiratory depression.[1]

Alfentanil, a relatively potent and μ-selective opiate agonist, has been widely used clinically as an analgesic and anesthetic because of its short duration of action. One of the factors limiting the use of high-dose alfentanil is the occurrence of significant muscle rigidity.[6] Opiate-induced rigidity, which occurs in both humans and animals,[6–8] is only one component of opiate-induced catatonia.[9] Another component of opiate catatonia is lack of spontaneous movement, also known as akinesia or catalepsy. Catalepsy, a disorder of movement, may be pharmacologically distinguished from rigidity, a disorder of muscle tone.[10–12]

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Previous studies have suggested that opiate-induced muscle rigidity, catalepsy, antinociception, and respiratory depression may be mediated by different neuroanatomic and neuropharmacologic mechanisms.[2,8,11-17] In the present study, the dose–effect relationships for subcutaneously administered alfentanil in producing these different endpoints of opiate action were evaluated to allow further insight into the nature of these opiate effects.

Materials and Methods

Animals

One hundred forty-eight male Wistar rats (Harlan Laboratories, Indianapolis, IN) weighing 215–370 g were studied during a 3-month period. A second cohort of 50 rats weighing 250–400 g were studied subsequently to examine the dose–effect relationship for alfentanil-induced respiratory depression and behavioral effects. In all studies, animals were housed, two or three per cage, in a temperature-controlled environment with 12-h cycles of alternating light and darkness. Access to food and water was unrestricted. Prior to each study, animals were habituated to the handling and experimental procedures. Each animal was only studied once. The experimental protocol was approved by the Animal Care Committee of the San Diego VA Medical Center.

Drug

Alfentanil hydrochloride (15–500 μg/kg; Janssen Pharmaceutica, Piscataway, NJ) was dissolved in 0.9% sterile physiologic saline and injected subcutaneously in a volume of 1 ml/kg. In all experiments, animals were randomly assigned to treatment groups, and the observer was blind to the dose of alfentanil used.

Procedures

Muscle Rigidity

To acclimate the animals to the experimental conditions, 3 days before the experiment the animals were placed individually in barred cylindrical holding cages that permitted free movement of all extremities. Groups of three or four were then placed inside a Coulbourn soundproof chamber (Coulbourn Instrument Co., Lehigh Valley, PA) and separated by cardboard partitions for three 1-h intervals.

For the muscle rigidity experiment, animals again were placed in the barred cylindrical holding cages. Two monopolar platinum recording electrodes (Grass E2, Grass Instruments, Quincy, MA) were inserted percutaneously into the left gastrocnemius muscle, and a third, ground electrode was inserted into the right hindlimb.[7,8,18] Animals were then placed alone in a Coulbourn chamber. The raw electromyographic (EMG) signal was differentially amplified 200 times and band-pass–filtered from 10 Hz to 3 kHz (Grass P511K). The resulting signal, viewed on an oscilloscope (Tektronix 7633, Tektronix Inc., Beaverton, OR), was then converted with a root-mean-squared voltage rectifier (time constant of 3 s) to produce time-varying analog deflections on Triplet 200-mV meters. The amplified EMG signal was displayed on a four-channel strip-chart recorder calibrated to 1 mv/division and running at 5 cm/s. The signal was also full-wave–rectified and integrated by a signal processor (Grass 7P10E) calibrated such that vertical pen travel per unit time on the four-channel strip-chart recorder was proportional to the root-mean-squared power of the EMG signal.

Fifty-nine animals were randomly assigned to one of eight dose groups. Baseline EMG activity was recorded at 1-min intervals for 15 min prior to treatment with alfentanil. After administration of alfentanil, EMG activity was recorded at 5-min intervals for 60 min.

Catalepsy/Antinociception

Forty-nine animals were randomly assigned to one of eight dose groups. For the catalepsy (bar) test, the animal's two forelimbs were placed over a metal bar situated 10 cm above the ground. Catalepsy time was measured from the time that the forelimbs were placed onto the bar to the time that one of the limbs was removed from the bar. A 30-s cut-off was used. Although cut-off times have long been used to study opiate-induced catalepsy,[19,20] in the present study the primary purpose of the cut-off was to ensure uniformity of timing of the antinociceptive tests that followed immediately thereafter. Thus, after each bar test, the most distal 3 cm of each animal's tail was immersed into 55° C water. Tail-flick latency was measured from the moment of tail immersion to either the complete removal of the animal's tail from the water or a forceful movement of the tail from its original position.[21] A cut-off of 10 s was used to prevent tissue damage. Three baseline readings for catalepsy and antinociception were taken at 5-min intervals prior to alfentanil treatment. Both catalepsy time and tail-flick latency were measured 5 min and 15 min after injection and at 15-min intervals for a total of 120 min. The catalepsy test always preceded the tail-immersion test.

Righting Reflex

Forty animals were randomly assigned to one of five treatment groups. The righting reflex was measured by placing the animal onto its back and measuring how long it took to regain an upright posture. If the distal end of any limb made contact with the table surface, the righting reflex was con-

sidered absent when all four limbs remained off the table surface for at least 15 s. After a baseline righting reflex, one of the five doses of alfentanil was injected subcutaneously into the dorsal back region. After alfentanil treatment, each animal was placed on its back every 15 s for 10 min. Whenever an animal lost its righting reflex, the experimenter briskly clapped to assess the animal's ability to right with auditory stimulation. The response to hand-clapping was not used in the data analysis of the presence or absence of loss of righting reflex. Hand clapping was performed to examine whether the endpoint of loss of righting reflex after high-dose opiate administration was truly equivalent to loss of righting reflex after other anesthetic agents.[22] The time to loss of righting reflex following drug treatment and the presence or absence of loss of righting were measured for each animal.

Respiratory Depression

Forty halothane-anesthetized animals were implanted with dorsal tail arterial catheters using a method described previously.[23] For the experiment, the rats were placed in holding cages inside a soundproof chamber (see above) and were permitted at least 60 min of recovery following catheter placement. Baseline room air arterial blood gas samples were collected twice during a 15-min period. One of five doses of alfentanil was then injected (100–500 μg/kg), and arterial samples were obtained 5, 15, 30, and 45 min postinjection. Arterial blood samples, 200 μl, were drawn through a side port in the arterial catheter and analyzed for *p*H, Pa_{CO_2}, and Pa_{O_2} with an Instrumentation Laboratories *p*H/gas analyzer (IL-1306, Lexington, MA) run at 37° C and calibrated each day. No more than 15 min is required to attain steady-state respiratory parameters in conscious animals habituated to the experimental apparatus,[23,24] and the minimal restraint has no effect on baseline minute ventilation or arterial blood gas values.[23,25]

Behavior

Ten previously handled animals were randomly assigned to a treatment group and then placed in an open field environment for 15 min and scored for a number of discrete well-described behaviors. A 24-cm-high open cardboard box (48.3 cm by 66 cm) with a single 2 cm diameter hole in one side 3 cm above the floor was used. Each rat was scored by a blinded observer (0 = absent, 1 = reduced, 2 = normal, and 3 = increased), based on normal rat behavior in such an environment, for overall spontaneous activity; rearing; grooming; sniffing; nose pokes into the 2-cm hole; corneal reflex; and startle reflex in response to hand-clapping. After baseline scoring, each animal was removed from the box, given a dose of alfentanil (100–500 μg/kg subcutaneously) and immediately returned to the box. Blinded behavioral scoring then resumed at 1-min intervals for the first 5 min and then at 5-min intervals for a total of 30 min.

Statistical Analysis

Data from each of the five studies were analyzed individually. Raw data values (EMG [microvolts], catalepsy [seconds], antinociception [seconds], loss of righting reflex [seconds], Pa_{CO_2} [mmHg], Pa_{O_2} [mmHg], and *p*H) were used for graphic presentation, and, for most of the effects, were used as the variables in statistical analysis. However, for statistical analysis of data in the two effects using cut-offs (antinociception and catalepsy), the raw data for each effect were adjusted to obtain the percent maximum attainable effect by using the standard formula: maximum attainable effect = ([result − baseline]/[cut-off − baseline]) × 100.[15] Statistical differences between treatment groups (raw or maximum attainable effect) for each effect over time were then determined using two-way analyses of variance followed by Newman-Keuls *a posteriori* tests. A *P* value of less than 0.05 was considered statistically significant.

Raw data for each effect *for each animal* were also transformed to a quantal response (presence or absence of expected drug effect) to generate uniform quantal dose–response graphs to permit direct comparison of different effects. For muscle rigidity, catalepsy, antinociception, and righting reflex, the occurrence of any raw data value of greater than four times the baseline value was determined to be a positive response (based on preliminary data). For example, if the mean baseline antinociceptive response latency was 2 s, and, after a particular dose of alfentanil, the mean latency was 9 s, then this would be considered a positive response. Four times baseline was selected to ensure a strong and definitive positive response. This criteria was chosen because it permitted a *positive* response for each endpoint to be "clinically relevant." It should be noted that reanalysis of the data using a criteria of either three or five times baseline did not appreciably alter the results. Because the baseline values of arterial Pa_{O_2}, Pa_{CO_2}, and *p*H are nonzero values, a multiple of the baseline could not be used in the quantal analysis. Instead, the arterial blood gas data were converted to percentage of animals responding using a criteria of four times the standard deviation of the baseline values in each dose group.

The percentage of animals with positive responses for each treatment group for each effect was determined. A computer program[26] was used to convert the percentage of animals with positive responses to probit values[3] and to obtain the effective dose in 50% of the animals (ED_{50}) and the slope of the dose–effect curve in each group. For each effect, the maximal dose that produced minimal or

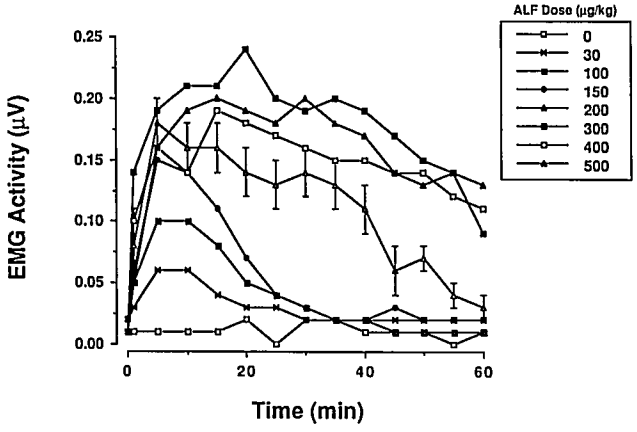

FIG. 1. The effect of alfentanil (ALF) treatment on muscle rigidity. Hindlimb muscle tone, presented on the y-axis, is measured by recording electromyographic (EMG) activity (millivolts root-mean-squared). Time is presented along the x-axis (in minutes). In the interest of clarity in this and the subsequent figures, error bars (±SEM) are shown only for an intermediate dose of alfentanil (200 μg/kg). In rats treated with saline or 15 μg/kg alfentanil, no significant muscle rigidity was recorded. In rats given 100, 150, or 200 μg/kg alfentanil, significant muscle rigidity was observed for 20, 30, and 40 min, respectively. For alfentanil doses greater than 200 μg/kg, significant muscle rigidity was recorded for the entire 60-min experimental period. Note that the same symbol has been used throughout all of the figures for each specific dose of alfentanil to facilitate comparison of time–effect relationships among the different opiate effects studied.

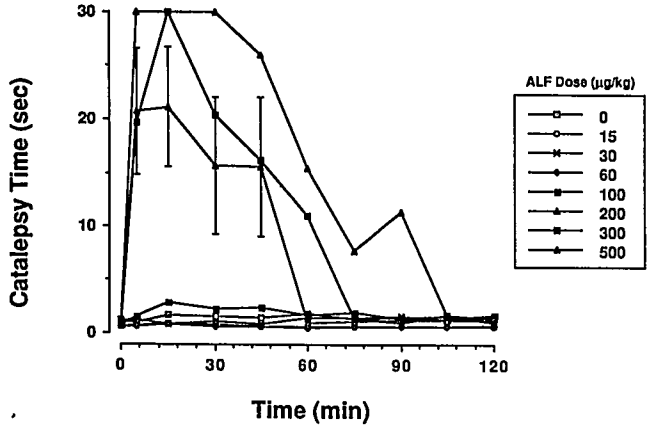

FIG. 2. The effect of alfentanil (ALF) treatment on catalepsy. The duration of catalepsy (30-s cut-off), as measured with the bar test, is presented on the y-axis. Time (in minutes) is presented along the x-axis. Treatment with 100 μg/kg or less of alfentanil did not produce catalepsy. Alfentanil in doses of 200, 300, or 500 μg/kg produced the onset of significant catelepsy within 5 min ($P < 0.05$ compared with saline control) that persisted for 45, 60, and 90 min, respectively. In addition, an alfentanil dose of 500 μg/kg produced higher maximal responses than did smaller doses.

no response, the minimal dose that produced maximal response, and all of the doses in between were used in this analysis. The slopes and ED_{50} values for each effect were analyzed and compared using the Litchfield and Wilcoxon II procedure in the same computer program.[26] With this technique, ED_{50} values can be statistically compared by calculating potency ratios (the ratios of ED_{50} values for two effects). If the 95% confidence interval for a potency ratio fails to include 1.0, then the two effects have significantly different ED_{50} values.

Results

Time–Effect Relationships

Alfentanil produced a dose-dependent increase in both the magnitude and duration of muscle rigidity (fig. 1), catalepsy (fig. 2), and antinociception (fig. 3). In addition, alfentanil produced a dose-dependent increase in the percent of rats displaying a loss of righting reflex (fig. 4). The lowest dose of alfentanil producing an effect significantly different from saline differed across these four endpoints. For antinociception, the most sensitive endpoint, a dose of 30 μg/kg alfentanil produced a significant increase in tail-dip latency at 15 min postinjection. For muscle rigidity, the lowest effective dose was 100 μg/kg, which produced a significant increase in muscle rigidity for 20 min. For catalepsy, 200 μg/kg alfentanil was the lowest effective dose, and this dose produced a significant increase in catalepsy for 45 min. One half of the animals lost their righting reflex at 250 μg/kg alfentanil, and all of the animals did so with alfentanil doses of 300 μg/kg or greater. There was also a dose-dependent decrease in the time to loss of righting.

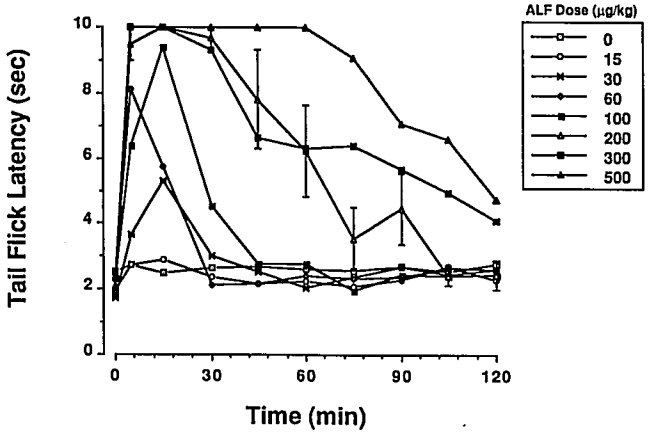

FIG. 3. The antinociceptive effects of alfentanil (ALF) treatment. Antinociception as measured by tail-flick latency (10-s cut-off) is presented on the y-axis, and time (in min) is presented on the x-axis. Rats given alfentanil 15 μg/kg had no alteration in tail-flick latency compared with baseline. Alfentanil in doses of 30, 60, and 100 μg/kg resulted in less than 30 min of significant antinociception. Alfentanil 200 μg/kg produced 60 min of significant prolongation of tail-flick latency, whereas doses of 300 μg/kg and greater produced 120 min of antinociception. In addition, at higher doses of alfentanil (≥300 μg/kg), there was a greater incidence of maximal response.

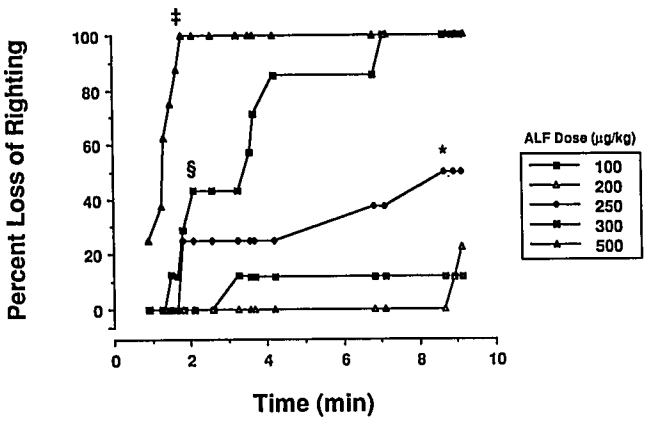

FIG. 4. The effect of alfentanil (ALF) treatment on righting reflex. The incidence of loss of righting reflex (percent of animals in each dose group losing their righting reflex) is presented on the y-axis, and time (in minutes) is presented on the x-axis. Alfentanil, in a dose-dependent manner, produced a more rapid onset and an increased incidence of loss of righting. At doses of 300 μg/kg and greater, all animals lost their righting reflex. The time of onset of loss of righting was significantly faster after 500 μg/kg than after any lower dose, and this highest dose of alfentanil produced a higher incidence of loss of righting than did doses of less than 300 μg/kg (‡$P < 0.05$). Alfentanil 300 μg/kg produced a more rapid onset and a higher incidence of loss of righting than did doses of 200 μg/kg or less (§$P < 0.05$). Alfentanil 250 μg/kg was significantly more likely to produce loss of righting than were lower doses (*$P < 0.05$).

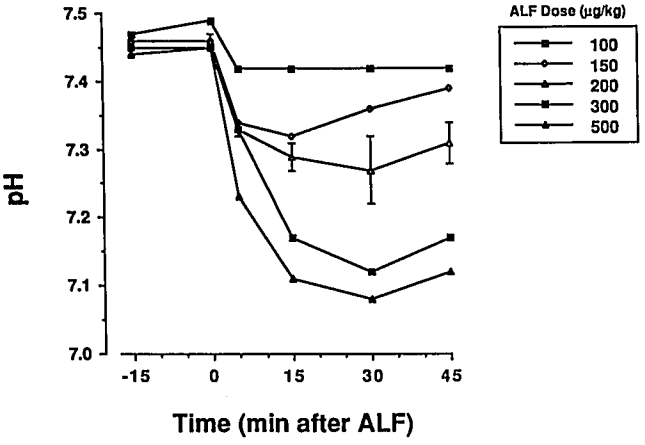

FIG. 5. The effect of alfentanil (ALF) treatment on arterial *p*H. The *p*H (displayed on the y-axis) was measured at 5–15-min intervals (time on the x-axis) before and after subcutaneous alfentanil administration (100–500 μg/kg). Rats given alfentanil 100 μg/kg showed no change in arterial *p*H. Although the *p*H values at 5 and 15 min after 150 μg/kg were significantly lower than baseline or control values, persistent acidosis was not seen until alfentanil 200 μg/kg. *p*H was profoundly decreased after both 300 and 500 μg/kg alfentanil. Note that even at the highest alfentanil dose, there was at least partial recovery by 45 min after opiate injection.

ARTERIAL BLOOD GASES

Subcutaneous alfentanil produced predictable dose-related changes in arterial blood gas values consistent with opiate-induced respiratory acidosis (fig. 5), hypercapnia (fig. 6), and hypoxemia (fig. 7). Arterial *p*H (fig. 5) was not significantly affected by an alfentanil dose of 100 μg/kg. Although the *p*H values at 5 and 15 min after alfentanil 150 μg/kg were significantly lower than baseline values, persistent acidosis did not occur until 200 μg/kg was administered, and profound acidosis occurred with 300 μg/kg. Except at 5 min, there were no significant differences in *p*H between the 300- and 500-μg/kg doses.

As might be expected, the pattern of response in Pa_{CO_2} (fig. 6) was quite similar to that described above for arterial *p*H. A significant elevation in Pa_{CO_2} did not occur until a dose of 150 μg/kg was administered, and the effects of this dose were not different from that caused by the next higher dose (200 μg/kg). Profound hypercapnia occurred at 300 μg/kg, and these effects were not appreciably different from those at the highest dose studied.

In contrast to ventilation, it appeared that oxygenation was affected at a lower alfentanil dose (fig. 7). Alfentanil 100 μg/kg produced a mild but significant decrease in Pa_{O_2}. There was then a further dose-dependent decrease in Pa_{O_2} up to an alfentanil dose of 300 μg/kg. The highest dose of alfentanil produced no additional change in Pa_{O_2}. It should also be noted that, for all three arterial blood gas indices, by 45 min the animals showed significant recovery from alfentanil-induced respiratory depression.

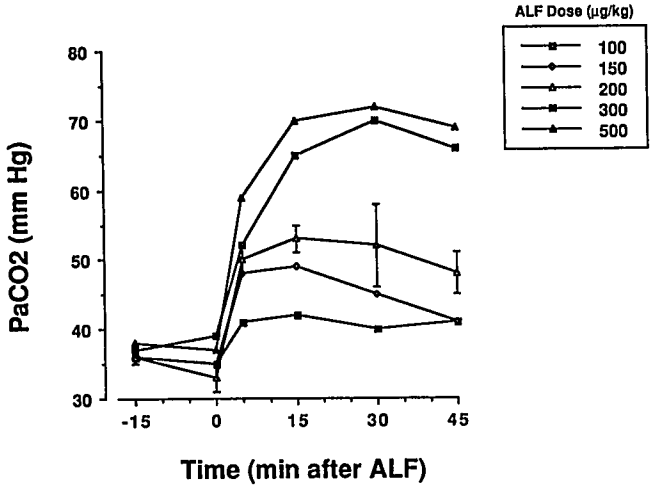

FIG. 6. The effect of alfentanil (ALF) treatment on arterial Pa_{CO_2}. Arterial samples were obtained at 5–15-min intervals before and after alfentanil (100–500 μg/kg subcutaneous). The dose–effect pattern for Pa_{CO_2} was quite similar to that for arterial *p*H. A significant elevation in Pa_{CO_2} did not occur until alfentanil 150 or 200 μg/kg was administered. The effects of these two doses were not significantly different from each other. Profound hypercarbia occurred with alfentanil 300 μg/kg, and the effects at this dose were not significantly different from those seen at 500 μg/kg.

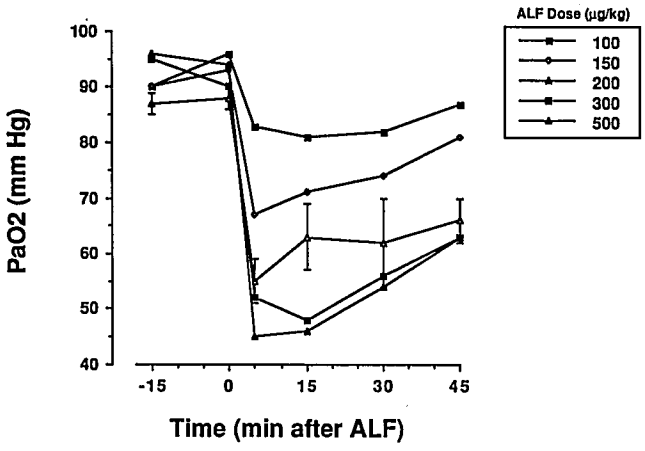

FIG. 7. The effect of alfentanil (ALF) treatment on arterial Pa_{O_2}. Arterial samples were obtained at 5–15-min intervals before and after alfentanil (100–500 µg/kg subcutaneous). In contrast to ventilation, it appeared that oxygenation was affected at a lower alfentanil dose. Alfentanil 100 µg/kg produced a mild but significant decrease in Pa_{O_2}. There was then a further dose-dependent decrease in Pa_{O_2} at an alfentanil dose of up to 300 µg/kg. The highest dose of alfentanil produced no additional change in Pa_{O_2}.

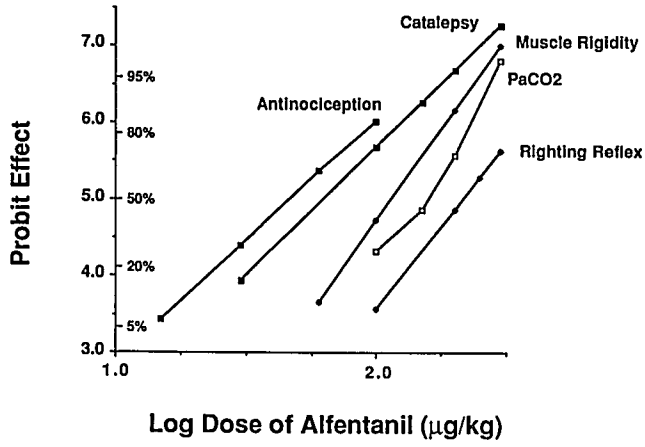

FIG. 8. The relationship of the (log) dose of alfentanil (ALF) to different end-effects. Percentage positive response for each end-effect was converted to probit values and is presented on the y-axis. The log dose of alfentanil is presented on the x-axis. There were no differences in the slopes of the dose–effect curves for the five effects studied. The ED_{50} of antinociception was significantly different from that of catalepsy, Pa_{CO_2}, and righting reflex, whereas the ED_{50} of muscle rigidity differed significantly from that of Pa_{CO_2} and righting reflex.

BEHAVIOR

Overall, the behavior of animals receiving even very low doses of alfentanil (*e.g.*, 50 µg/kg) was mildly obtunded, as manifested by slightly decreased exploratory behavior and locomotion. Nose-poke activity appeared to be appreciably reduced at alfentanil 100 µg/kg. Rearing and grooming were obtunded with an alfentanil dose of 200 µg/kg, whereas sniffing behaviors, though diminished at lower doses, were not seriously affected until the animals had received alfentanil 300 µg/kg. Eye reflexes appeared relatively more resistant to alfentanil. Corneal responses persisted for up to 15 min after alfentanil 300 µg/kg but were rapidly (within 2 min) and completely ablated by an alfentanil dose of 500 µg/kg.

In the first phase of the study, with the exception of one animal in the 500-µg/kg dose group, every animal that met the criteria for loss of righting reflex at any alfentanil dose still exhibited a detectable startle response to hand-clapping. On more formal behavioral examination using the separate cohort of animals, it was ascertained that startle reflex exhibited a complex biphasic dose–effect relationship. While control and low-dose (< 100 µg/kg) alfentanil-treated animals showed minimal if any response to hand-clapping, animals given 200–300 µg/kg generally had markedly exaggerated acoustic startle responses manifested by persistent forward locomotion (so-called "festinating" locomotion[27]). However, at the highest dose of alfentanil studied, this response was no longer seen, and instead the animals exhibited only a modest flinch with hand-clapping.

DOSE–EFFECT RELATIONSHIPS

As a further means of comparing alfentanil's effects on muscle rigidity, catalepsy, antinociception, ventilatory depression, and loss of righting reflex, the log dose of alfentanil was compared to the probit conversion of the quantal responses for each of the five endpoints (fig. 8). There were no significant differences between the slopes for any of the dose–effect curves (table 1). Based on potency ratio analysis (table 2), the ED_{50} for antinociception

TABLE 1. Probit Slope Values, ED_{50} Values, and ED_{50} Confidence Intervals

Experiment	ED_{50} (µg/kg)	Probit Slope of Dose–Effect Curve
Antinociception	46.0 (26.1–80.9)*	3.24
Muscle Rigidity	62.2 (35.0–110.4)†	3.33
Catalepsy	114.3 (66.4–196.7)	4.77
Pa_{CO_2}	145.9 (113.9–186.7)	4.05
Righting Reflex	215.0 (159.1–290.6)	4.30

Numbers in parentheses represent 95% confidence intervals.
* Significantly ($P < 0.05$) different from catalepsy, Pa_{CO_2}, and righting reflex.
† Significantly ($P < 0.05$) different from Pa_{CO_2} and righting reflex.

TABLE 2. Potency Ratios with 95% Confidence Intervals

Comparison	Potency Ratio	Confidence Intervals
AN/MR	0.74	0.16–3.37
AN/CT	0.42	0.18–0.88*
ANPa_{CO_2}	0.32	0.17–0.58*
AN/RR	0.21	0.11–0.41*
MR/CT	0.54	0.25–1.20
MR/Pa_{CO_2}	0.43	0.23–0.80*
MR/RR	0.29	0.15–0.55*
CT/Pa_{CO_2}	0.78	0.43–1.42
CT/RR	0.53	0.12–2.33
Pa_{CO_2}/RR	0.68	0.45–1.00

AN = antinociception; MR = muscle rigidity; CT = catalepsy; RR = righting reflex.

* Significantly ($P < 0.05$) different from 1.00, indicating that the two effects have significantly different dose–response curves.

was significantly different from the ED_{50}s for catalepsy, Pa_{CO_2}, and loss of righting reflex. In addition, the ED_{50} for muscle rigidity was significantly different from the ED_{50}s for Pa_{CO_2} and loss of righting reflex.

Discussion

These results corroborate earlier work demonstrating that μ-opioid agonists produce antinociception, muscle rigidity, catalepsy, and loss of righting reflex in rats.[8,11–14,16,28] The present study extends these results by examining in detail the relative potency of the clinically important opiate agonist alfentanil in producing each of these effects. Specifically, the results of this study indicate that the order of sensitivity of these endpoints to alfentanil was antinociception > muscle rigidity > catalepsy > elevation of Pa_{CO_2} > loss of righting reflex.

These results corroborate previous work showing that different opioid agonists produce different effects with different potencies. Similar to the present findings, the results of Kissin *et al.* demonstrated that, for both fentanyl and morphine, the prevention of purposeful movement to a noxious stimulus had a lower ED_{50} than did the loss of righting reflex.[3] The potency ratio of antinociception/righting reflex was 0.13 for morphine and 0.42 for fentanyl. In the present study, the potency ratio of antinociception/righting reflex for alfentanil was 0.21. These different potency ratios between opioid agonists could reflect the agonists' differential affinity and efficacy for various opioid receptor types or subtypes. Alternatively, the different responses with different opioids may be related to different affinities at a single receptor. In either case, because alfentanil is a fentanyl derivative and has a similar (primarily μ) opioid receptor affinity profile, one might have expected the antinociception/righting reflex potency ratio of alfentanil to be more similar to that of fentanyl than to that of morphine.

Analogous to the findings of Kissin *et al.*,[3] Ling *et al.* reported that morphine was more potent in producing antinociception than in producing respiratory depression.[1] In both the Kissin *et al.* and Ling *et al.* studies, the investigators concluded from their findings that differences in the opiates' potency in producing their effects reflected differences in the populations of receptors mediating those effects. Similarly, the results of the present study could be interpreted to suggest that alfentanil-induced antinociception is mediated by a receptor population different (either anatomically or pharmacologically) from that mediating catalepsy, respiratory depression, or loss of righting reflex, and that alfentanil-induced muscle rigidity is mediated by a receptor population different from that mediating respiratory depression and loss of righting reflex.

There is considerable evidence to suggest that the effects of alfentanil evaluated in the present study are mediated by populations of receptors that are anatomically distinct. Whereas catalepsy appears to be mediated by receptors in the nucleus accumbens,[11,29,30] previous studies[11,13] have not supported a role for this site in the expression of opiate rigidity. In fact, using intracranial microinjections of the opiate antagonist methylnaloxonium (125 ng), the regions of the nucleus raphe pontis and the periaqueductal gray have been shown to mediate alfentanil rigidity in the rat.[13] In contrast, rigidity could not be consistently reversed after methylnaloxonium injections into the basal ganglia.

Antinociception appears to be mediated primarily by receptors in subcortical periventricular and spinal sites.[14,16] Animal data suggest that the respiratory actions of exogenous opiates like morphine appear to be mediated not just by brainstem periventricular nuclei[17,31] but also by more distant brain sites as well.[32] The neuroanatomic substrates of opiate-induced loss of righting reflex are likely complex and have not been systematically examined.

Receptor populations could differ from one another in at least two ways. First, the function relating the alfentanil–opioid receptor interaction to the alfentanil-induced effect may be different for some or all of these neuroanatomic sites. Such a variability in the capacity of different tissues to transduce an opiate stimulus into an opiate-induced effect has already been documented in various smooth muscle opiate assays.[33] For example, Miller *et al.*[33] demonstrated that the selective μ-opioid agonist *d*-Ala^2-MePhe^4-Gly-ol^5-enkephalin (DAGO) reduced electrically induced contractions of both the mouse vas deferens and the rat vas deferens. However, DAGO was approximately eight times more potent in the mouse vas deferens. Since naloxone was equally potent in reversing DAGO's effects in both tissues, the authors concluded that the differential potency of DAGO between tissues did not reflect differ-

ential affinity for different receptor types. Rather, they concluded that the tissues differed in their ability to transduce the DAGO stimulus into an effect.

A second possibility is that the receptor populations mediating different alfentanil-induced effects may be composed of distinct types of receptors with different affinities for alfentanil. Alfentanil is a selective μ agonist, suggesting that all of the effects described in the present study are mediated by μ-opioid receptors and not by other opioid receptor types. However, subtypes of the μ receptor have been proposed,[34,35] raising the possibility that different alfentanil-induced effects may be mediated by different subtypes of the μ receptor.

Agonists for different opioid receptors have differential effects on respiration.[36,37] Martin *et al.*, based on work on the spinal dog,[38] was the first to suggest that the μ receptor was responsible for opiate-induced respiratory depression, whereas the δ and κ receptor activation produced, respectively, no effect or respiratory stimulation. Others have since theorized that opiate-induced analgesia could be separated pharmacologically from respiratory depression on the basis of opioid receptor specificity.[17,39–41] Ling *et al.*[1] demonstrated that whereas pretreatment with naloxonazine, a noncompetitive μ antagonist, blocked morphine analgesia, there was no effect on morphine-induced respiratory depression. Wood *et al.*[42] reported that some κ agonists antagonized morphine respiratory depression without affecting analgesia.

Arterial blood gas sampling was chosen as the measure of opiate-induced respiratory depression because it is relatively easy to perform and correlates well with clinically significant respiratory depression in rats.[23,43] Arterial blood gases have been shown to be sensitive indicators of impaired ventilation and oxygenation in rats exposed to anesthetic drugs[44] as well as to hypoxia and hypercapnia (in a manner quite similar to that in the dog and the human).[40,45] Many previous studies have examined the effects of opiates on arterial blood gases.[23,25,46,47] Importantly, arterial blood gases provide information regarding the contribution of dead-space ventilation when alterations in breathing pattern (respiratory rate *vs.* tidal volume) affect alveolar ventilation.[24,48] Nevertheless, additional studies, perhaps using the ventilatory response to carbon dioxide challenge, should be performed to validate the present data.

In this study, alfentanil was administered subcutaneously. With another route of administration, both the pharmacokinetics and pharmacodynamics of alfentanil would be altered, and it is possible that different results might have been obtained. However, we believe that the relationships among the various dose–effect curves after subcutaneous alfentanil administration may not be appreciably different from that after intravenous administration, for several reasons: 1) there is a quite rapid onset of action after subcutaneous alfentanil; 2) over the steepest portion of the dose–effect curves (which primarily determines the ED_{50} values), the cardiac depression and metabolic acidosis caused by alfentanil are less pronounced than at higher doses; and 3) preliminary data for intravenous alfentanil-induced muscle rigidity suggests a similar dose–effect relationship for this one opiate effect.

In conclusion, the present investigation further characterized an important model of opiate effects using alfentanil dose–response relationships for muscle rigidity, catalepsy, antinociception, loss of righting reflex, and respiratory depression. An additional benefit of these data is that they provide normative curves using a standardized animal model against which other opiate drugs or opiate-adjuvant drug combinations can be compared. Further studies to elucidate these effects and their underlying receptor mechanisms are underway.

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