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Selective enhancement of fentanyl-induced antinociception by the delta agonist SNC162 but not by ketamine in rhesus monkeys: Further evidence supportive of delta agonists as candidate adjuncts to mu opioid analgesics

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ABSTRACT

Mu-opioid receptor agonists such as fentanyl are effective analgesics, but their clinical use is limited by untoward effects. Adjunct medications may improve the effectiveness and/or safety of opioid analgesics. This study compared interactions between fentanyl and either the noncompetitive N-methyl-D-aspartate (NMDA) glutamate receptor antagonist ketamine or the delta-opioid receptor agonist SNC162 [(+)-4-[(alphaR)-alpha-[(2S,5R)-2,5-dimethyl-4-(2-propenyl)-1-piperazinyl]-(3-phenyl)methyl]-N,N-diethylbenzamide] in two behavioral assays in rhesus monkeys. An assay of thermal nociception evaluated tail-withdrawal latencies from water heated to 50 and 54 °C. An assay of schedule-controlled responding evaluated response rates maintained under a fixed-ratio 30 schedule of food presentation. Effects of each drug alone and of three mixtures of ketamine + fentanyl (22:1, 65:1, 195:1 ketamine/fentanyl) or SNC162 + fentanyl (59:1, 176:1, 528:1 SNC162/fentanyl) were evaluated in each assay. All drugs and mixtures dose-dependently decreased rates of food-maintained responding, and drug proportions in the mixtures were based on relative potencies in this assay. Ketamine and SNC162 were inactive in the assay of thermal antinociception, but fentanyl and all mixtures produced dose-dependent antinociception. Drug interactions were evaluated using dose-addition and dose-ratio analysis. Dose-addition analysis revealed that interactions for all ketamine/fentanyl mixtures were additive in both assays. SNC162/fentanyl interactions were usually additive, but one mixture (176:1) produced synergistic antinociception at 50 °C. Dose-ratio analysis indicated that ketamine failed to improve the relative potency of fentanyl to produce antinociception vs. rate suppression, whereas two SNC162/fentanyl mixtures (59:1 and 176:1) increased the relative potency of fentanyl to produce antinociception. These results suggest that delta agonists may produce more selective enhancement than ketamine of mu agonist-induced antinociception.

1. Introduction

Mu-opioid agonists are effective clinical analgesics, but their use is limited by untoward effects such as sedation, respiratory depression and constipation. Therefore, the development of candidate analgesics with improved therapeutic effects and decreased untoward effects is warranted. One strategy for achieving this goal may be to combine mu opioid agonists with adjuncts that improve effectiveness and/or safety. For example, numerous preclinical studies have shown that delta-opioid receptor agonists alone have antinociceptive effects in some nociceptive assays (Brandt et al., 2001; Do Carmo et al., 2008; Heyman et al., 1987; Stewart and Hammond, 1993). Moreover, combinations of mu and delta agonists have produced synergistic antinociceptive effects while producing additive, subadditive or mutually antagonistic effects in assays of other, undesirable drug effects (Adams et al., 1993; Heyman et al., 1989; Negus et al., 2009; O'Neill et al., 1997; Stevenson et al., 2003). These results suggest that delta agonists may have clinical value as adjuncts to mu agonists in the treatment of pain; however, clinical evaluation of this hypothesis cannot proceed until either selective delta agonists or mixed-action delta/mu agonists are approved for clinical use. An alternative strategy to gauging the potential value of delta/mu interactions is to conduct preclinical studies that directly compare delta/mu interactions with interactions between mu agonists and adjuncts that have been examined clinically. Toward that end, the present study compared interactions between the mu agonist fentanyl and either an intermediate-efficacy delta agonist (SNC162; Jutkiewicz et al., 2004; Negus et al., 1998) or the noncompetitive N-methyl-D-aspartate (NMDA) glutamate receptor antagonist ketamine.

Anatomical studies have demonstrated the presence of NMDA receptors at both spinal levels within the dorsal horn and at supraspinal

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levels involved in nociceptive transmission, such as brainstem, thalamus and cortex (Kalb and Fox, 1997; Mugnaini et al., 1996; Roth et al., 1996). Functional studies also suggest that NMDA receptors participate in the transmission of nociceptive signals (for review, see Dickenson et al., 1997; Furst, 1999; Mao, 1999). Although NMDA receptor function can be modulated by antagonists acting at glutamate- or glycine-binding sites (Brown and Krupp, 2006), the only clinically available NMDA receptor antagonists are noncompetitive channel blockers such as ketamine and dextromethorphan. Previous studies using these non-competitive antagonists have provided some evidence to suggest that they can enhance the antinociceptive effects of mu agonists in rodents (Baker et al., 2002; Hoffmann et al., 2003; Holtman et al., 2008; Nadeson et al., 2002) and nonhuman primates (Allen et al., 2002). More importantly for the present study, an extensive clinical literature has investigated interactions between ketamine and mu agonists in humans (Bell et al., 2003; Carstensen and Moller, 2010; Schulte et al., 2003; Tucker et al., 2005). These studies generally agree that sedative and other untoward effects of ketamine complicate its use as an adjunct to mu agonists, but at least some studies have concluded that low-dose ketamine may be useful as a safe and effective adjunct under at least some conditions (Carstensen and Moller, 2010; Kollender et al., 2008; Subramaniam et al., 2004).

Fentanyl/ketamine and fentanyl/SNC162 interactions were compared in rhesus monkeys using procedures described previously to assess opioid interactions (Negus et al., 2008, 2009; Stevenson et al., 2003, 2005). Antinociceptive interactions were evaluated in a warm-water tail-withdrawal assay of thermal nociception. For two reasons, drug interactions were also evaluated in an assay of schedule-controlled behavior, in which operant responding was maintained by food reinforcement. First, fentanyl, ketamine and SNC162 administered alone each produce a dose-dependent and maximal effect in this procedure, and as a result, data from this procedure could be used to quantify relative potencies for determination of drug proportions in drug mixtures. Second, drug effects on schedule-controlled responding can provide one measure of non-selective behavioral depression that can confound measures of antinociception. Consequently, a comparison of drug (or drug mixture) effects in assays of thermal nociception and schedule-controlled responding can provide one useful index of antinociceptive selectivity.

Drug interactions within each procedure in the present study were analyzed using dose-addition analysis to compare experimental results with expected additivity (Stevenson et al., 2003; Tallarida, 2000). Drug interactions across procedures were analyzed using dose-ratio analysis to assess relative potencies to produce antinociception versus response-rate suppression (Negus et al., 2008, 2009). We hypothesized that SNC162, like other delta agonists studied previously, would selectively and synergistically enhance fentanyl-induced antinociception. In view of the findings with ketamine described above, we further hypothesized that SNC162 would produce a more selective enhancement of fentanyl-induced antinociception than ketamine.

2. Methods

2.1. Subjects

Six male rhesus monkeys (*Macaca mulatta*) were used in studies of schedule-controlled responding, and four monkeys (two female and two male) were used in studies of thermal nociception. Subjects weighed 4.5 to 12 kg during the course of these studies. All monkeys had prior exposure to drugs (primarily dopaminergic and opioid compounds) and to the behavioral procedures in which they were tested. The subjects were individually housed, and water was freely available. Their diet consisted of Lab Diet monkey chow (Purina, Framingham, MA). This diet was supplemented with fresh fruit twice weekly. In addition, monkeys in the assays of schedule-controlled behavior could earn additional food pellets during experimental sessions. A 12-h light/12-h dark cycle was in effect (lights on from 7:00 AM to 7:00 PM).

Animal maintenance and research were conducted in accordance with the guidelines provided by the National Institutes of Health Committee on Laboratory Animal Resources. The facility was licensed by the United States Department of Agriculture, and protocols were approved by the Institutional Animal Care and Use Committee. The health of the monkeys was monitored daily by technical staff and periodically by a veterinarian. Monkeys had visual, auditory, and olfactory contact with other monkeys throughout the study. Monkeys also had access to puzzle feeders, mirrors, and chew toys to provide environmental enrichment. Music was played daily in all housing rooms.

2.2. Behavioral procedures

2.2.1. Schedule-controlled responding assay

Experiments were conducted in each monkey's home cage (dimensions $60 \times 65 \times 75$ cm) as previously described (Negus et al., 2008, 2009; Stevenson et al., 2003, 2005). The home cages of all monkeys were modified to include an operant response panel (28×28 cm) mounted on the front wall. Three square translucent response keys (6.4×6.4 cm) were arranged 2.54 cm apart in a horizontal row 3.2 cm from the top of the operant panel. Each key could be transilluminated by red, green or yellow stimulus lights. The operant panel also supported an externally mounted pellet dispenser (Gerbrands, Model G5210; Arlington, MA) that delivered 1 gm banana-flavored food pellets (Purina Test Diet, Richmond, IN) to a food receptacle mounted on the cage beneath the operant response panel. The panel was controlled by a MED-PC interface and an IBM compatible computer programmed in MEDSTATE Notation (MED Associates, Inc., East Fairfield, VT).

Experimental sessions consisted of multiple cycles. Each of 5 cycles was 15 min long and consisted of two components: a 10-min timeout period followed by a 5-min response period. During the timeout period, no stimulus lights were illuminated and responding had no scheduled consequences. During the response period, the center key was transilluminated yellow, and the subjects could respond for up to 10 food pellets under a fixed-ratio 30 (FR30) schedule of reinforcement. If all 10 food pellets were earned before 5 min had elapsed, the lights were turned off, and responding had no scheduled consequences for the remainder of that response period. Sessions were conducted 5 days a week. Test sessions were usually conducted on Tuesdays and Fridays, and training sessions were conducted on Mondays, Wednesdays, and Thursdays. In addition, test sessions were conducted only after a training session during which the monkeys responded at rates greater than 1.0 response/s for all five cycles. During training sessions, monkeys received either no injection or saline injections at the beginning of each cycle. During test sessions, test compounds were administered using a cumulative dosing procedure, in which doses of the test drug or drug mixture were administered at the beginning of each cycle, and each dose increased the total cumulative dose by one-fourth or one-half log units.

Fentanyl/ketamine interactions were examined in a group of five monkeys, and fentanyl/SNC162 interactions were examined in a different group of three monkeys (two monkeys were included in both studies). Initially, complete dose-effect curves were determined for fentanyl (0.001 – 0.1 mg/kg) and ketamine (0.1 – 5.6 mg/kg) alone, or for fentanyl and SNC162 (0.1 – 10 mg/kg) alone, and each drug was tested twice. Tests of fentanyl and ketamine alone were conducted no more than twice a week. Tests of SNC162 alone were conducted no more than once a week to prevent tolerance to the rate-decreasing effects of this delta agonist (Brandt et al., 2001). Subsequently, three mixtures of ketamine or SNC162 in combination with fentanyl were examined, and the proportions of each drug in the

mixtures were based on the relative potency of the drugs to decrease response rates. Thus, relative potency was defined as ED50 of the combination drug (ketamine or SNC162) ÷ ED50 fentanyl, and the proportions of the combination drug to fentanyl in the three mixtures were relative potency ÷ 3, relative potency, and relative potency × 3. Each mixture was tested at least once, and test sessions were conducted no more than twice a week for fentanyl/ketamine mixtures and no more than once a week for fentanyl/SNC162 mixtures. Fentanyl, ketamine, SNC162, and all mixtures were tested up to doses that eliminated responding in most or all monkeys.

Drug interactions can be influenced not only by the relative doses of two drugs in a mixture, but also by their relative time courses; nominal drug proportions in an administered mixture are most likely to approximate actual biologically available drug proportions if the drugs have similar time courses (e.g. Kenakin, 1987). Accordingly, the relative time courses of fentanyl, ketamine and SNC162 were compared in a group of four monkeys. For these time course experiments, either saline or a single dose of fentanyl, ketamine or SNC162 was administered, and 5 min response periods identical to those described above were initiated 10, 30, 100, and 300 min after administration. The fentanyl dose was determined as the ED90 dose from cumulative dosing experiments described above. Ketamine and SNC162 doses were based on their relative potency to fentanyl.

2.2.2. Thermal nociception assay

Monkeys were seated in acrylic restraint chairs so that their tails hung down freely. The bottom 10 cm of each monkey's shaved tail was immersed in a thermal container of warm water. If the subject did not remove its tail within 20 sec, the tail was removed by the experimenter, and a latency of 20 s was assigned to that measurement. During each cycle of measurements, tail-withdrawal latencies were measured from water heated to 38 °C, 50 °C, and 54 °C. The order in which the temperatures were presented varied from one set of measurements to the next. Experiments were conducted no more than twice a week. A stopwatch was used to measure and record time intervals. Each test session consisted of multiple 15-min cycles. Before the first drug dose was administered, baseline latencies to tail-withdrawal from 38, 50 and 54 °C water were determined. Testing continued only if tail withdrawal from 38 °C water did not occur before the 20 s cutoff, and if tail withdrawal occurred in ≤2 s from 50 and 54 °C water. These criteria were met during every session with every monkey in this study. After determination of baseline tail withdrawal latencies, a single drug dose was administered at the start of each of five sequential 15 min cycles, and each dose increased the total cumulative dose by one-fourth or one-half log units. Starting 10 min after each injection, tail-withdrawal latencies were redetermined as described above.

Fentanyl/ketamine interactions were examined in a group of three monkeys, and fentanyl/SNC162 interactions were examined in a different group of four monkeys (three monkeys were included in both studies). Initially, complete dose-effect curves were determined for fentanyl (0.001 – 0.056 mg/kg) and ketamine (0.1 – 5.6 mg/kg) alone, or for fentanyl and SNC162 (0.1 – 10 mg/kg) alone. Subsequently, three mixtures of fentanyl in combination with ketamine or SNC162 were examined, and the proportions of each drug in the mixtures were identical to those examined in the assay of schedule-controlled responding described above. Each drug mixture was tested once, and any mixtures producing a synergistic effect were tested a second time to evaluate reliability of results. As in the assay of schedule-controlled responding, test sessions were conducted no more than twice a week, and tests with SNC162 alone or in combination with fentanyl were conducted only once per week. Time courses were not compared in the assay of thermal nociception because fentanyl was the only one of the three drugs that was active in this procedure.

2.3. Data analysis

2.3.1. Dose-effect curve analysis

For the assay of schedule-controlled responding, operant response rates from each cycle were converted to percent of control using the average rate from the previous training day as the control value. The ED50 for each drug was defined as the dose that reduced response rates to 50 percent of the control rate of responding. Individual ED50s were calculated by interpolation when only two data points were available (one below and one above 50 percent control response rate) or by linear regression when at least three data points were available on the linear portion of the dose-effect curve. For drug mixtures, ED50 values were defined as the dose of each drug in the mixture that reduced response rates to 50 percent of control. In addition, a related quantity, Zmix, was also calculated for each monkey as the total drug dose that reduced response rates to 50 percent of control (i.e. ED50 for fentanyl ÷ ED50 for ketamine or SNC162). Individual fentanyl, ketamine, and SNC162 dose-effect curve slopes were calculated by interpolation when only two data points were available or by linear regression when at least three data points were available on the linear portion of the dose-effect curve, and individual slopes were averaged to determine mean values and 95% confidence limits.

For the assay of thermal nociception, drug effects were expressed as %Maximum Possible Effect (%MPE) using the following equation:

$$\%MPE = (TestLatency - BaselineLatency) / (20 - BaselineLatency) * 100$$

where test latency was the tail withdrawal latency from 50 or 54 °C water obtained after drug administration, and baseline latency was the latency obtained at that temperature at the beginning of the session prior to drug administration. ED50 and Zmix for each drug or drug mixture were defined as the dose that produced 50%MPE, and these values were determined by interpolation or linear regression as described above. For both the schedule-controlled responding and thermal nociception procedures, individual ED50 and Zmix values were averaged to determine mean values and 95% confidence limits, and values were considered to be significantly different if confidence limits did not overlap.

2.3.2. Dose-addition analysis

Drug interactions were evaluated both within and across assays. Drug interactions within a given assay were assessed using both graphical and statistical approaches to dose-addition analysis (Tallarida, 2000; Tallarida, 2007; Wessinger, 1986) as described previously (Negus et al., 2008, 2009; Stevenson et al., 2003, 2005). Graphically, data for each drug and drug mixture were plotted as isobolograms at the 50% effect level. Thus, these isobolograms plotted dose ± SEM of one drug in a mixture as function of dose ± SEM of the other drug in the mixture at the overall mixture dose that produced 50% effect. Statistical evaluation of drug interactions was accomplished by comparing the experimentally determined ED50 values for each mixture (Zmix) with predicted additivity ED50 values (Zadd) as described by Tallarida (2000). Zmix values were determined empirically as described above. When mixtures were studied in the assay of schedule-controlled responding, where each drug alone was active, Zadd values were calculated individually for each monkey from the equation $Zadd = fA + (1-f)B$, where A was the ED50 for fentanyl alone, B was the ED50 for the other drug (ketamine or SNC162) alone, and f was the fractional multiplier of A in the computation of the additive total dose. Any choice of f is related to the proportion of fentanyl (ρA) in a mixture according to the equation $\rho A = fA/Zadd$. This study tested mixtures that yielded values of $f = 0.25$, $f = 0.5$ and $f = 0.75$. When mixtures were studied in the thermal nociception assay, where only fentanyl was active, the hypothesis of additivity predicts that the inactive drug should not contribute to the effects of a mixture. As a result, the equation for Zadd reduces to

Zadd = A/ρA. Mean Zmix and Zadd values were considered to be significantly different if 95% confidence limits did not overlap.

2.3.3. Dose-ratio analysis

To evaluate drug interactions across assays, the relative potency of each drug and drug mixture in the two behavioral procedures was quantified using dose-ratio analysis as described previously (Negus et al., 2008, 2009). Specifically, Dose Ratio = ED50 in the assay of schedule-controlled responding/ED50 in the assay of thermal nociception. Thus, if a drug or drug mixture was equipotent in the two procedures, then Dose Ratio = 1. A Dose Ratio > 1 indicates that the drug or drug mixture was more potent in the assay of thermal nociception. Conversely, Dose Ratio < 1 indicates that the drug or drug mixture was more potent in the assay of schedule-controlled responding.

2.3.4. Time-course analysis

Time-course data in the assay of schedule-controlled responding were analyzed by two-way repeated-measures analysis of variance, with drug (saline, fentanyl, ketamine, SNC162) as one factor and time after administration (10, 30, 100 and 300 min) as the second factor. A significant analysis of variance was followed by the Bonferroni post-hoc test for multiple comparisons. The criterion for significance was set *a priori* at the 95% confidence level.

2.4. Drugs

Fentanyl HCl (National Institute on Drug Abuse; Bethesda, MD) was dissolved in sterile water. Ketamine HCl (100 mg/ml) was obtained from a commercial supplier (KetaVed™, Vedco Inc, Saint Joseph, MO). SNC162 base (Dr. K. Rice) was dissolved in 3% lactic acid in sterile water to 50 mg/mL. Dilutions of ketamine and SNC162 were made with sterile water. Drugs were administered intramuscularly in the thigh, and doses were expressed as the forms listed above.

3. Results

3.1. Fentanyl and ketamine interactions

3.1.1. Assay of schedule-controlled responding

The average control response rate (± SEM) throughout the study was 2.5 (± 0.4) responses/s. Fentanyl and ketamine produced dose-dependent decreases in response rates, and slopes were not significantly different [slopes (95% confidence limits) of -163.0 (-245.7 to -80.3) and -161.2 (-221.6 to -100.7) for fentanyl and ketamine, respectively]. ED50 values are shown in Table 1, and based on relative potencies, three mixtures of ketamine + fentanyl were examined (22:1, 65:1, 195:1 ketamine/fentanyl). Table 1 also shows the ED50 values for each drug in each mixture, and Table 2 shows the predicted Zadd values and the empirically determined Zmix values for each drug mixture as determined by dose-addition analysis. All drug mixtures produced effects that were consistent with additivity (i.e. Zadd was not significantly different from Zmix). The isobologram for ketamine/fentanyl interactions in the assay of schedule-controlled responding is shown in Fig. 1 (upper left panel).

3.1.2. Assay of thermal nociception

Average baseline tail-withdrawal latencies (±SEM) throughout the study were 1.2 ± 0.4 s and 0.9 ± 0.3 s from 50 and 54 °C water, respectively. Only fentanyl produced a dose-dependent antinociceptive effect at either thermal intensity, and the ED50 values for fentanyl at the two stimulus intensities are shown in Table 1. Ketamine did not produce an antinociceptive effect up to the highest doses tested (maximum % MPE was 25.5 ± 25.3 and 14.8 ± 8.5 after 3.2 mg/kg at 50 and 54 °C, respectively). Table 1 also shows ED50 values for each drug in the three ketamine/fentanyl mixtures, and Table 2 shows the predicted Zadd values and empirically determined Zmix values for each drug mixture.

Table 1
ED50 values (95%CL) for fentanyl and ketamine alone or in combination in assays of schedule-controlled responding and thermal nociception (50 and 54 °C). * Indicates that ED50 value for fentanyl or ketamine in a drug mixture was different from ED50 value for fentanyl or ketamine alone.

Drug or drug mixture	Fentanyl	Ketamine
Schedule-controlled responding		
Fentanyl alone	0.023 (0.015-0.036)	
Ketamine alone		1.5 (0.8-2.7)
22:1 Ketamine/fentanyl	0.012 (0.004-0.035)	0.3 (0.1-0.8)*
65:1 Ketamine/fentanyl	0.009 (0.004-0.021)	0.6 (0.3-1.3)
195:1 Ketamine/fentanyl	0.004 (0.001-0.012)*	0.8 (0.2-2.4)
Thermal nociception (50 °C)		
Fentanyl alone	0.009 (0.007-0.012)	
Ketamine alone		Inactive
22:1 Ketamine/fentanyl	0.006 (0.003-0.014)	0.1 (0.1-0.3)
65:1 Ketamine/fentanyl	0.009 (0.008-0.009)	0.6 (0.5-0.6)
195:1 Ketamine/fentanyl	0.006 (0.002-0.019)	1.2 (0.4-3.6)
Thermal nociception (54 °C)		
Fentanyl alone	0.042 (0.020-0.089)	
Ketamine alone		Inactive
22:1 Ketamine/fentanyl	0.024 (0.017-0.033)	0.5 (0.4-0.7)
65:1 Ketamine/fentanyl	0.024 (0.013-0.046)	1.6 (0.8-3.0)
195:1 Ketamine/fentanyl	0.021 (0.004-0.100)	4.0 (0.8-19.5)

All drug mixtures produced effects at both thermal intensities that were consistent with additivity. The isobolograms for ketamine/fentanyl interactions in the assay of thermal nociception are shown in Fig. 1 (upper center and right panels).

3.2. Fentanyl and SNC162 interactions

3.2.1. Assay of schedule-controlled responding

The average control response rate (± SEM) throughout the study for this group of monkeys was 3.0 (± 0.2) responses/s. Fentanyl and SNC162 produced dose-dependent decreases in response rates, and slopes of the dose-effect curves were not significantly different [slopes (95% confidence limits) were -131.5 (-186.3 to -76.8) for fentanyl and -119.2 (-162.1 to -76.2) for SNC162]. ED50 values are shown in Table 3, and based on relative potencies, three mixtures of SNC162 + fentanyl were examined (59:1, 176:1, 528:1 SNC162/fentanyl). Table 3 also shows the ED50 values for each drug in each mixture, and Table 4 shows the predicted Zadd values and the empirically determined Zmix values for each drug mixture as determined by dose-addition analysis. All drug mixtures produced effects that were

Table 2
Experimentally determined Zmix values and predicted Zadd values (95% CL) for mixtures of fentanyl and ketamine in assays of schedule-controlled responding and thermal nociception (50 and 54 °C). All mixtures produced additive effects in that 95% CL for Zmix values overlapped with 95%CL of Zadd values.

Drug mixture	Zmix	Zadd
Schedule-controlled responding		
22:1 Ketamine/fentanyl	0.27 (0.09-0.81)	0.39 (0.22-0.70)
65:1 Ketamine/fentanyl	0.59 (0.26-1.35)	0.75 (0.41-1.38)
195:1 Ketamine/fentanyl	0.76 (0.23-2.45)	1.12 (0.61-2.06)
Thermal nociception (50 °C)		
22:1 Ketamine/fentanyl	0.14 (0.06-0.31)	0.20 (0.15-0.27)
65:1 Ketamine/fentanyl	0.56 (0.52-0.61)	0.58 (0.43-0.78)
195:1 Ketamine/fentanyl	1.25 (0.43-3.63)	1.71 (1.27-2.31)
Thermal nociception (54 °C)		
22:1 Ketamine/fentanyl	0.55 (0.40-0.77)	0.97 (0.46-2.05)
65:1 Ketamine/fentanyl	1.59 (0.84-3.01)	2.78 (1.32-5.87)
195:1 Ketamine/fentanyl	4.05 (0.84-19.59)	8.26 (3.92-17.43)

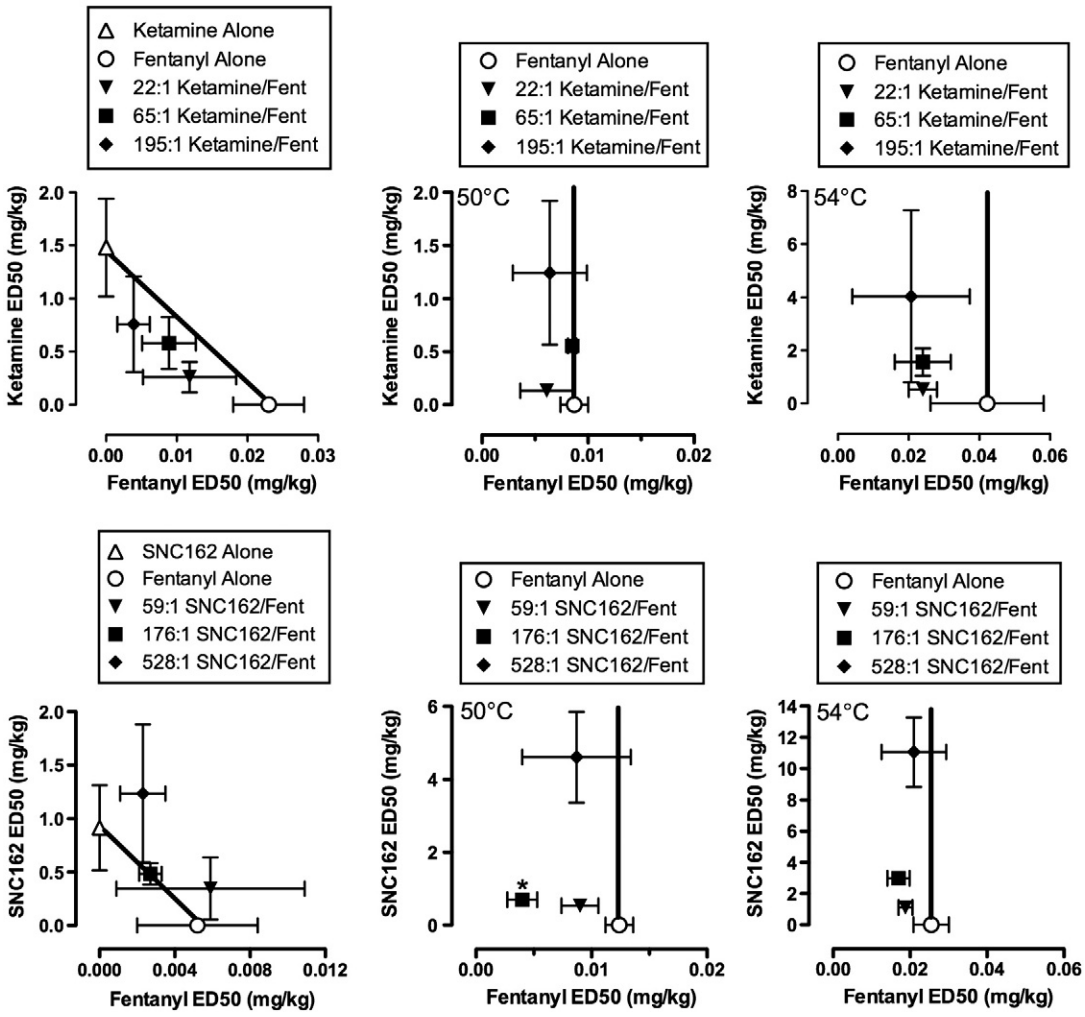

Fig. 1. Effects of the mu-opioid agonist fentanyl alone or in combination with the noncompetitive NMDA antagonist ketamine (top panels) or in combination with the delta-opioid agonist SNC162 (bottom panels) on rates of schedule-controlled responding (left panels) and thermal nociception at 50 °C (center panels) and 54 °C (right panels). All panels show isobolograms for each behavioral endpoint at the ED50 effect level for fentanyl, ketamine, or SNC162 alone or as part of a mixture. Abscissae: ED50 values for fentanyl alone or in a mixture in mg/kg (linear scale). Ordinates (top panels): ED50 values for ketamine alone or in a mixture in mg/kg (linear scale). Ordinates (bottom panels) ED50 values for SNC162 alone or in a mixture in mg/kg (linear scale). Each point represents mean ± SEM of 3-5 monkeys. * Asterisk indicates that the 176:1 SNC162/fentanyl mixture produced a synergistic antinociceptive effect as determined by dose-addition analysis (see Table 4).

consistent with additivity. The isobologram for SNC162/fentanyl interactions in the assay of schedule-controlled responding is shown in Fig. 1 (lower left panel).

3.2.2. Assay of thermal nociception

Average baseline tail-withdrawal latencies (±SEM) throughout the study for this group of monkeys were 0.8 ± 0.1 s and 0.7 ± 0.1 s from 50 and 54 °C water, respectively. Fentanyl produced a dose-dependent antinociceptive effect at both thermal stimulus intensities, and the ED50 values for fentanyl at the two stimulus intensities are shown in Table 3. SNC162 did not produce antinociception up to the highest doses tested (maximal % MPE was 24 ± 14.4 and 9.7 ± 8.2 after 10 mg/kg at 50 and 54 °C, respectively). Table 3 also shows ED50 values for each drug in the three SNC162/fentanyl mixtures, Table 4 shows the predicted Zadd values and empirically determined Zmix values for each drug mixture, and Fig. 1 shows the isobolgrams (bottom center and left panels). The ED50 values for fentanyl and SNC162 in the 176:1 mixture were significantly lower than the ED50 values for either drug alone at 50 °C (Table 3). Moreover, the 176:1 SNC162/fentanyl mixture produced a synergistic antinociceptive effect at 50 °C as indicated by the empirically determined Zmix value being significantly lower than the predicted Zadd value (Table 4). Graphically, this drug-mixture point was located to the left of the dose-additivity line in the isobologram (Fig. 1, bottom center panel). This synergistic effect with the 176:1 SNC162/fentanyl mixture was replicated in a subsequent experiment (data not shown). The 59:1 and 528:1 SNC162/fentanyl mixtures produced only additive effects at a stimulus intensity of 50 °C, and all SNC162/fentanyl mixtures produced additive effects at a stimulus intensity of 54 °C.

3.3. Dose-ratio analysis

Fig. 2 shows dose ratios for ketamine + fentanyl and SNC162 + fentanyl to produce rate suppression in the assay of schedule-controlled responding (SCR) vs. thermal antinociception (50 and 54 °C). Ketamine produced only a proportion-dependent decrease in the dose ratios at both stimulus intensities, indicating that antinociceptive doses of the ketamine/fentanyl mixtures produced greater rate suppression than antinociceptive doses of fentanyl alone. Conversely, SNC162 increased the dose ratio relative to fentanyl alone at 50 °C (both the 59:1 and 176:1 mixtures) and at 54 °C (the 59:1 mixture). Thus, antinociceptive doses of some SNC162/fentanyl mixtures produced less rate suppression than antinociceptive doses of fentanyl alone.

Table 3
ED50 values (95%CL) for fentanyl and SNC162 alone or in combination in assays of schedule-controlled responding and thermal nociception (50 and 54 °C). * Indicates that ED50 value for fentanyl or SNC162 in a drug mixture was different from ED50 value for fentanyl or SNC162 alone.

Drug or drug mixture	Fentanyl	SNC162
Schedule-controlled responding		
Fentanyl alone	0.0053 (0.0016-0.0171)	
SNC162 alone		0.92 (0.39-2.15)
59:1 SNC162/fentanyl	0.0059 (0.0011-0.0307)	0.35 (0.07-1.8)
176:1 SNC162/fentanyl	0.0027 (0.0018-0.0041)	0.48 (0.32-0.73)
528:1 SNC162/fentanyl	0.0023 (0.0008-0.0065)	1.24 (0.44-3.44)
Thermal nociception (50 °C)		
Fentanyl alone	0.012 (0.010-0.015)	
SNC162 alone		Inactive
59:1 SNC162/fentanyl	0.009 (0.006-0.013)	0.53 (0.37-0.75)
176:1 SNC162/fentanyl	0.004 (0.002-0.008)*	0.70 (0.36-1.33)
528:1 SNC162/fentanyl	0.009 (0.003-0.025)	4.61 (1.60-13.29)
Thermal nociception (54 °C)		
Fentanyl alone	0.026 (0.018-0.036)	
SNC162 alone		Inactive
59:1 SNC162/fentanyl	0.019 (0.016-0.023)	1.11 (0.93-1.34)
176:1 SNC162/fentanyl	0.017 (0.012-0.024)	3.01 (2.15-4.21)
528:1 SNC162/fentanyl	0.021 (0.010-0.046)	11.06 (5.03-24.3)

3.4. Time courses

Fig. 3 shows the time courses of equieffective doses of fentanyl (0.021 mg/kg), ketamine (1.4 mg/kg) and SNC162 (3.7 mg/kg) in the assay of schedule-controlled responding. All three drugs produced a significant decrease in response rates relative to saline treatment. Peak effects of all three drugs were observed after 10 min, and effects of all three drugs dissipated after 300 min. Using the duration of significant differences from saline treatment as a criterion, durations of action were ketamine < fentanyl = SNC162. However, effects of ketamine, fentanyl and SNC162 were not different from each other at any time point.

4. Discussion

The main finding of this study was that the noncompetitive NMDA antagonist ketamine failed to enhance the antinociceptive effects of fentanyl or improve the relative potency of fentanyl to produce antinociception vs. rate suppression in rhesus monkeys. In contrast, some combinations of fentanyl with the delta-opioid agonist SNC162 did produce synergistic antinociceptive effects and/or improve the potency to produce antinociception vs. rate suppression. These results suggest that delta agonists may be more effective than noncompetitive NMDA antagonists as adjuncts to mu agonist analgesics for the treatment of pain.

Table 4
Experimentally determined Zmix values and predicted Zadd values (95% CL) for mixtures of fentanyl and SNC162 in assays of schedule-controlled responding and thermal nociception (50 and 54 °C). * Zmix significantly lower than Zadd as determined by non-overlapping confidence limits and indicates synergism.

Drug mixture	Zmix	Zadd
Assay of schedule-controlled responding		
59:1 SNC162/fentanyl	0.35 (0.07-1.84)	0.23 (0.1-0.55)
176:1 SNC162/fentanyl	0.49 (0.32-0.73)	0.46 (0.2-1.08)
528:1 SNC162/fentanyl	1.24 (0.45-3.45)	0.69 (0.29-1.62)
Assay of thermal nociception (50 °C)		
59:1 SNC162/fentanyl	0.55 (0.38-0.76)	0.74 (0.61-0.90)
176:1 SNC162/fentanyl	0.70 (0.37-1.34)*	2.19 (1.81-2.65)
528:1 SNC162/fentanyl	4.62 (1.60-13.31)	6.54 (5.40-7.92)
Assay of thermal nociception (54 °C)		
59:1 SNC162/fentanyl	1.13 (0.94-1.36)	1.53 (1.08-2.18)
176:1 SNC162/fentanyl	3.01 (2.15-4.21)	4.52 (3.18-6.43)
528:1 SNC162/fentanyl	11.08 (5.04-24.39)	13.58 (9.55-19.33)

4.1. Effects of fentanyl, ketamine and SNC162 alone

Effects of fentanyl, ketamine and SNC162 alone were consistent with previous studies. Thus, the mu-selective opioid analgesic fentanyl has been shown to produce antinociception in both rodents and nonhuman primates, and it is well-established as an opioid analgesic in humans (Baker et al., 2002; Dambisya and Lee, 1994; Gatch et al., 1998; Hoffmann et al., 2003; Nadeson et al., 2002; Negus et al., 2008, 2009; Stevenson et al., 2003; Tucker et al., 2005). Fentanyl also produced a dose-dependent suppression of responding that was consistent with previous studies from our laboratory (Negus et al., 2008, 2009; Stevenson et al., 2003). As reported previously, SNC162 was ineffective in the tail withdrawal assay but produced a dose-dependent decrease in rates of schedule-controlled responding (Negus et al., 1998). Like SNC162, ketamine also failed to alter thermal nociception in the tail-withdrawal assay while producing dose-dependent decreases in rates of schedule-controlled responding. These findings generally agree with previous reports that ketamine and other noncompetitive NMDA antagonists do not alter thermal nociception up to doses that eliminate rates of schedule-controlled responding or produce other signs of marked sedation and motor impairment in rhesus monkeys (Butelman et al., 2003; Dykstra and Woods, 1986; France et al., 1989; Negus et al., 1993) or other species (Dambisya and Lee, 1994; Hoffmann et al., 2003; Tucker et al., 2005). At high, sedative doses, ketamine and other noncompetitive NMDA antagonists may impair withdrawal responses (Dykstra and Woods, 1986; France et al., 1989), but in the present study, tail-withdrawal responses were preserved across the dose range examined, and higher doses were not tested to avoid severe motor impairment.

4.2. Interactions between fentanyl and ketamine

Data from this study suggest that ketamine across a range of proportions failed to produce a selective enhancement of fentanyl-induced thermal antinociception in rhesus monkeys, and this conclusion adds to a growing preclinical literature reporting equivocal results with ketamine and other noncompetitive NMDA antagonists as adjuncts to mu agonists. For example, in agreement with the present study, ketamine produced only additive antinociception with mu agonists in a mouse tail-flick test and a rat test of mechanical nociception (Dambisya and Lee, 1994; Pelisser et al., 2003). Similarly, in another study conducted in rats, ketamine produced only a limited enhancement of mu agonist-induced antinociception in a warm-water tail-withdrawal test while enhancing mu agonist-induced respiratory depression (Hoffmann et al., 2003). Conversely, ketamine and/or dextromethorphan more reliably enhanced mu agonist-induced antinociception in a mouse hot plate test, an assay of neuropathy-related mechanical allodynia in rats, and a shock titration procedure in squirrel monkeys (Allen et al., 2002; Baker et al., 2002; Pelisser et al., 2003).

These variable preclinical data are paralleled by variable clinical findings. For example, in a clinical laboratory study, ketamine enhanced the analgesic effect of fentanyl against an electrical noxious stimulus but not against thermal or mechanical stimuli (Tucker et al., 2005). However, in a model of burn-induced mechanical hypersensitivity, ketamine did enhance morphine analgesia (Schulte et al., 2004). A recent review of randomized, double-blind clinical trials concluded that ketamine failed to enhance opioid analgesia in about half the trials reviewed; however, ketamine was effective in boosting opioid analgesia in the remaining trials, and the authors concluded that "…small dose ketamine is a safe and useful adjuvant to standard practice opioid-analgesia" (Subramaniam et al., 2004). Overall, ketamine enhancement of mu agonist-induced antinociception/analgesia appears weakest in assays using acute thermal or mechanical stimuli and strongest in assays

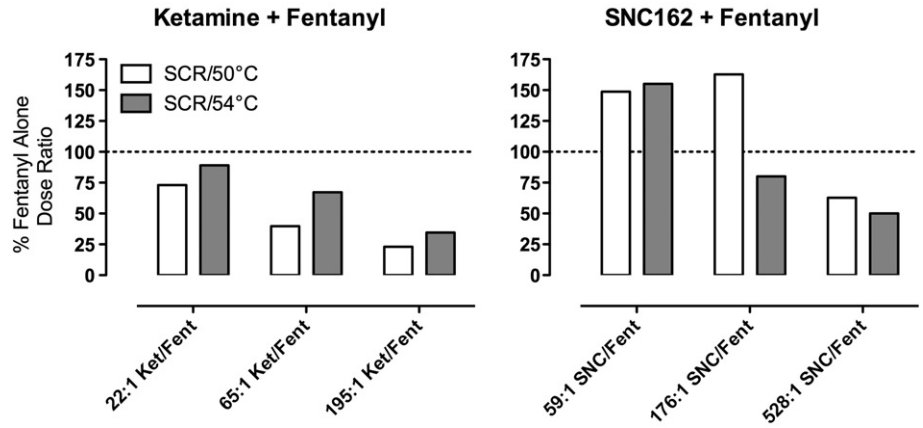

Fig. 2. Effects of ketamine and SNC162 on the relative potency of fentanyl to produce rate suppression vs. thermal nociception. Abscissae: Proportions of ketamine + fentanyl (left panel) or SNC162 + fentanyl (right panel) in the mixtures. Ordinates: Percent of the dose ratio for fentanyl alone. Dose ratios were calculated as the potency in the assay of schedule-controlled responding (SCR) ÷ potency in the assay of thermal nociception at stimulus intensities of 50 °C (open bars) or 54 °C (gray bars). In the groups used to assess the fentanyl/ketamine interactions, the dose ratios for fentanyl alone were 2.64 (SCR/50 °C) and 0.55 (SCR/54 °C). In the groups used to assess SNC162/fentanyl interactions, the dose ratios for fentanyl alone were 0.43 (SCR/50 °C) and 0.20 (SCR/54 °C).

using acute electrical stimuli or in assays involving inflammatory or neuropathic hypersensitivity.

For the purposes of the present study, these collective findings are important for two reasons. First, they illustrate the substantial preclinical and clinical research effort that has been dedicated to the investigation of interactions between mu agonists and noncompetitive NMDA channel blockers such as ketamine. Although equivocal, results of this research have been sufficiently promising to encourage continued consideration of ketamine as an adjunct to mu agonists for at least some clinical applications (Carstensen and Moller, 2010). Second, these findings provide an empirical framework against which delta/mu interactions can be compared.

4.3. Interactions between fentanyl and SNC162

In contrast to ketamine, some proportions of SNC162 produced a selective enhancement in fentanyl antinociception. These results are consistent with previous studies demonstrating that delta agonists can produce a selective and delta receptor-mediated enhancement of the antinociceptive effects of mu agonists in rodents and rhesus monkeys (Negus et al., 2009; O'Neill et al., 1997; Stevenson et al., 2003, 2005). The present results extend these earlier findings in two ways. First, this study provides a direct comparison of interactions between a mu agonist and either a delta agonist or ketamine. Under the conditions studied here, only the delta agonist was able to produce a selective enhancement in mu agonist-induced antinociception. Consequently, these results provide one source of evidence to suggest that delta/mu interactions may yield greater clinical benefit than the more widely studied and potentially useful interactions between mu agonists and ketamine. Second, the present study extends to SNC162 the range of delta agonists that have been found to selectively enhance mu opioid antinociception in rhesus monkeys. The relatively modest antinociceptive synergy observed with fentanyl in combination with SNC162 may be related to the efficacy of SNC162 at delta receptors. We have reported previously that relatively high delta agonist efficacy is required to produce antinociceptive synergy in combination with mu agonists, and high-efficacy delta agonists such as SNC80 and SNC243A selectively enhanced mu agonist antinociception across a range of proportions and noxious stimulus intensities (Negus et al., 2009; Stevenson et al., 2003, 2005). SNC162 appears to have lower efficacy in both in vitro functional assays and in vivo behavioral assays than other delta agonists such as SNC80 (Jutkiewicz et al., 2004; Negus et al., 1998). Consequently, the weak enhancement of mu agonist-induced antinociception by SNC162 is consistent with its low efficacy at delta receptors.

4.4. Drug time course as factor in drug interactions

Time course data in the assay of schedule-controlled responding suggest that SNC162 had a slightly longer duration of action than ketamine. However, three findings argue against an important role for time course as a determinant of drug interactions in this study. First, all three drugs produced peak effects within 10 min, which was the pretreatment time used in assays of both schedule-controlled responding and thermal nociception. Thus, drug interactions in both assays were evaluated at the time of peak effect for all drugs. Second, cumulative dosing studies were conducted using half-log or quarter-log dose increments. Consequently, any cumulative drug dose was composed predominantly of the most recent drug dose, and residual drug from earlier doses made a smaller contribution. This suggests that minor differences in duration of action would be expected to result in relatively

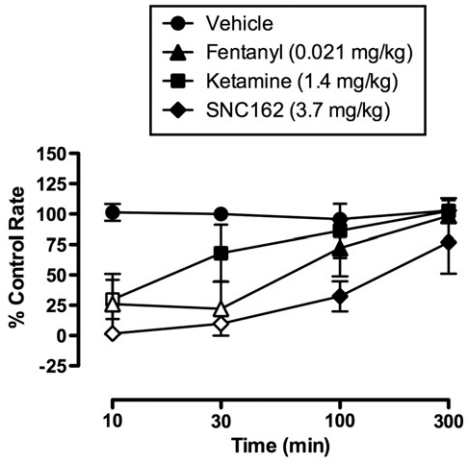

Fig. 3. Time courses of equieffective doses of fentanyl (0.021 mg/kg), ketamine (1.4 mg/kg) and SNC162 (3.7 mg/kg) in the assay of schedule-controlled responding. Abscissae: time in min. post administration. Ordinate: Percent control rate of responding. Each point represents mean ± SEM of four rhesus monkeys. Two-way repeated-measures analysis of variance revealed a significant main effect of time $F(3,9) = 22.4$, $p<0.05$, drug $F(3,9) = 6.5$, $p<0.05$ and a significant time × drug interaction $F(9,27) = 2.3$, $p<0.05$. Post-hoc analysis using the Bonferroni method for multiple comparisons demonstrated that all three drugs were significantly different ($p<0.05$) from vehicle at 10 min, and fentanyl and SNC162 were significantly different from vehicle at 30 min. However, there were no significant differences at any time point between fentanyl, ketamine and SNC162. Open symbols represent points significantly different ($p<0.05$) from vehicle.

minor changes in actual drug proportions over the course of cumulative dosing. Finally, although drugs displayed different time courses when using differences from saline treatment as a criterion, they did not display different time courses relatively to each other. This again suggests that differences in time course were modest.

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