Anesthesiology
1996; 85:1357-66

Ketamine Attenuates and Reverses Morphine Tolerance in Rodents

Naohito Shimoyama, M.D.,* Megumi Shimoyama, M.D.,* Charles E. Inturrisi, Ph.D.,† Kathryn J. Elliott, M.D.‡

***Background*: The development of tolerance complicates the use of morphine to manage persistent pain. *N*-methyl-D-aspartate receptor antagonists can attenuate or reverse morphine tolerance. The authors studied ketamine's ability to modulate morphine tolerance.**

***Method*: Tolerance was produced in mice given morphine subcutaneously and was assessed by a cumulative dose-response analysis using the tail-flick test. The ability of ketamine at 0.3, 3, or 10 mg/kg given subcutaneously before and after morphine to attenuate the development of tolerance was assessed. The ability of 10 mg/kg ketamine to reverse tolerance produced by the subcutaneous implantation of morphine pellets to mice was also assessed. Rats were made tolerant to intraspinal morphine and the effects of the coadministration of 12 μg intraspinal ketamine were assessed.**

***Results*: Morphine given subcutaneously produced a fivefold increase in the median effective (ED_{50}) dose of morphine, which was dose-dependently attenuated by subcutaneously administered ketamine. A tenfold increase in the morphine ED_{50} produced by morphine pellets was completely reversed by ketamine given subcutaneously. Intraspinal morphine produced a 46-fold increase in its ED_{50}, which was almost completely attenuated by the coadministration of intraspinal ketamine.**

***Conclusions*: Systemically administered ketamine attenuates and reverses systemically induced morphine tolerance in mice, and intraspinal ketamine attenuates tolerance produced by intraspinal morphine in rats. (Key words: Analgesics, opioid: morphine. Antagonist, *N*-methyl-D-aspartate: ketamine. Receptor: *N*-methyl-D-aspartate. Tolerance.)**

THE *N*-methyl-D-Aspartate (NMDA) receptor antagonists, both competitive or noncompetitive, have been shown to attenuate or reverse analgesic tolerance to morphine in animals. MK-801, a noncompetitive NMDA antagonist, attenuates the development of morphine tolerance as assessed by the tail-flick analgesic assay in rats[1] and in mice[2] and the by hot-plate analgesic assay in rats.[3,4] LY274614, a competitive NMDA receptor antagonist, and dextromethorphan, a noncompetitive NMDA receptor antagonist, attenuate and reverse the development of morphine tolerance as assessed by the hot-plate assay in rats or the tail-flick assay in mice.[2,4-6] Furthermore, the changes in central nervous system concentrations of the mRNA that codes for the major subunit of the NMDA receptor (NMDAR1 mRNA) that occur in morphine-tolerant rats are prevented by the coadministration of LY274614.[7] These studies indicate that the NMDA receptor system is involved in morphine tolerance and have increased our understanding of its underlying mechanism. Furthermore, these studies suggest the clinical utility of NMDA antagonists. However, MK-801 and LY274614 are experimental NMDA antagonists that are not yet clinically available. On the other hand, dextromethorphan is clinically available as an oral antitussive and has the potential for clinical use as an NMDA antagonist.

Ketamine is another clinically available drug with noncompetitive NMDA receptor antagonist activity. It is a phencyclidine (PCP) analog with many behavioral effects in common with other PCP-like drugs, including those that are anesthetic, antinociceptive, psychotomimetic, anticonvulsant, neuroprotective, and amnesic.[8] Although it acts on many neurotransmitter systems, more recent studies suggest that the locus for many of these shared behavioral effects is its activity as a noncompetitive antagonist of the NMDA receptor.[9-12]

Ketamine given by continuous subcutaneous infusion at a dose of 10 $mg \cdot kg^{-1} \cdot d^{-1}$ attenuates the develop-

* Research Fellow, Pain Research Program, Memorial Sloan-Kettering Cancer Center.

† Professor of Pharmacology, Cornell University Medical College.

‡ Assistant Professor of Neurology and Instructor of Pharmacology, Cornell University Medical College; Assistant Attending Neurologist, Pain Service, Department of Neurology, Memorial Sloan-Kettering Cancer Center.

Received from the Department of Pharmacology, Cornell University Medical College, New York, New York. Submitted for publication May 26, 1996. Accepted for publication July 18, 1996. Supported in part by National Institute of Drug Abuse (NIDA) grant DA 01457 (to Dr. Inturrisi), NIDA grant DA 00255 (to Dr. Elliott), and by the V. Z. V. Foundation (to Dr. Elliott). Dr. Inturrisi is a recipient of a Research Scientist Award from the NIDA (DA00198).

Address reprint requests to Dr. Inturrisi: Department of Pharmacology, Cornell University Medical College, 1300 York Avenue, New York, New York 10021.

ment of morphine tolerance in rats.[13] We have extended this observation to include the dose-response relationship of ketamine on systemically induced morphine tolerance in mice, the ability of ketamine to reverse established morphine tolerance in mice, and the effect of intraspinal ketamine on intraspinal morphine tolerance in the rat.

Materials and Methods

All studies were approved by the Institutional Animal Care and Use Committee of Cornell University Medical College.

Animals

Adult male CD1 mice (Charles River Laboratories, Kingston, NY) weighing 25 to 30 g were used for studies 1 and 2. Adult male Sprague-Dawley rats (Taconic Farm, Germantown, NY) weighing 350 to 375 g at the time of surgery were used for study 3. The mice were caged in groups of five and the rats were caged individually with free access to food and water; all animals were maintained on a regular light-dark cycle. Treatment groups averaged ten animals.

Tail-flick Test

To assess the analgesic effect of morphine, the tail-flick test was used. The tail-flick apparatus (EMDIE, Richmond, VA) emits radiant heat to the tail at 2 cm from the tip in mice and at 5 to 8 cm in rats. The time from the onset of heat to the withdrawal of the tail (tail-flick latency) was measured. The intensity of the radiant heat was adjusted so that the baseline latencies were between 2.5 and 3.5 s. To avoid causing tissue damage, the heat stimulus was turned off after 10 s (cut-off latency). A mean tail-flick latency was calculated from two repeated measurements. Baseline latency was obtained before opioid or saline administration. Subsequent response latencies were determined at the peak analgesia, which was 30 min after subcutaneous morphine and 10 min after intrathecal morphine were given. The latency data were converted to a quantal form by determining the percentage of analgesic responders in each group from the response latency values compared with baseline latencies.

Drugs

Morphine sulfate was obtained from Mallinkrodt (St. Louis, MO), and racemic ketamine hydrochloride was obtained from Sigma Chemical Company (St. Louis, MO). The doses of morphine and ketamine are expressed as the free base. Morphine and ketamine were dissolved in normal saline. The pH of the ketamine dosing solutions for both subcutaneous and intraspinal administration were between 5.7 and 6.2. Each subcutaneous injection of morphine, racemic ketamine, and saline was delivered in a volume of 0.1 ml/10 g mouse weight. Each intraspinal injection was delivered in a volume of 5 μl, followed by 10 μl saline to flush the intrathecal catheter.

Intrathecal Catheterization

For the intraspinal administration of drugs to the rat, a catheter was placed in the intrathecal space. Under halothane anesthesia, a PE-10 tube was inserted through a small hole made in the atlantooccipital membrane and threaded 8.5 cm down the intrathecal space to the lumbosacral level of the spinal cord.[14] The catheterized rats were observed for 24 h after operation, and those with any signs of paralysis were excluded from the study. At the end of the study, 5 μl of a 1% methylene blue solution was introduced into the catheter followed by 10 μl saline to confirm the position of the catheter and the spread of the dye in the intrathecal space. Animals with a misplaced catheter or inadequate spread of the dye, as confirmed by dissection, were excluded from the study (8.3%).

Dose-Response Studies

After measuring the baseline latencies, increasing doses of morphine were administered until each animal responded to the analgesic (cumulative dose-response assessment[2,6]). An analgesic responder was defined as one whose mean response tail-flick latency was two or more times the value of the mean baseline latency. The percentage of analgesic responders in the group for each cumulative dose was calculated, and a cumulative dose-response curve was constructed. The changes in the median effective dose (ED_{50}) of morphine determined from the curves were used to express the changes in the relative analgesic potency of morphine.

Tolerance Paradigms

Tolerance paradigms were based on previous studies of the kinetic-dynamic relationships of NMDA receptor antagonists and morphine tolerance.[2,4-6]

Study 1: Effect of Systemic Ketamine on the De-

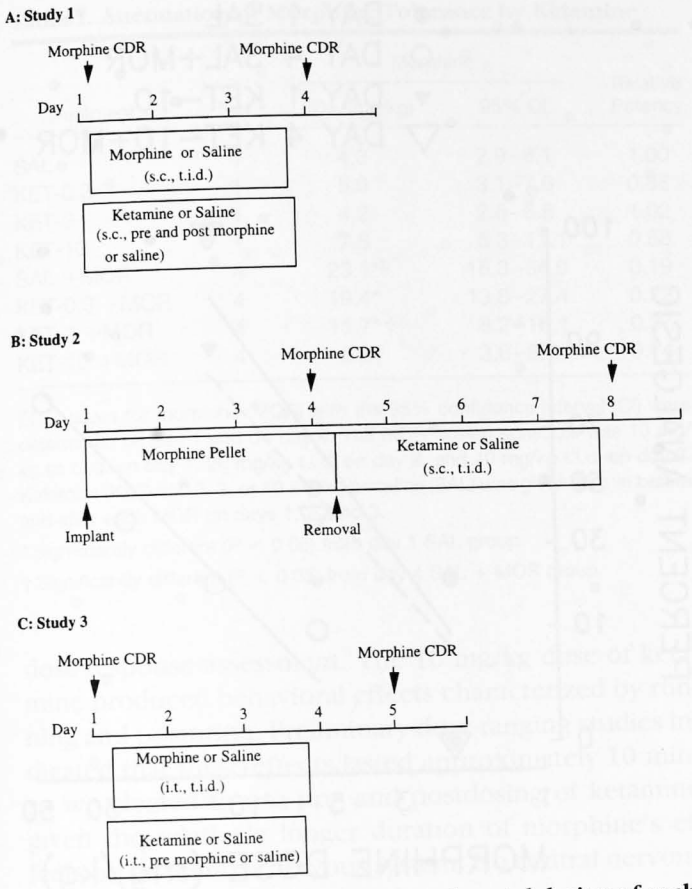

Fig. 1. A timeline that shows the experimental design of each study (see Materials and Methods section).

velopment of Morphine Tolerance. Study 1 (fig. 1A) was designed to estimate the dose-response relationship for subcutaneous ketamine in attenuating the development of morphine tolerance. An escalating schedule of morphine doses administered subcutaneously three times a day for 3 days was used to produce tolerance in mice. On the morning of day 1, each animal received 10 mg/kg morphine given subcutaneously at 08:00 A.M. or underwent a cumulative dose-response assessment using subcutaneous morphine to determine their ED_{50} value for morphine. Each analgesic responder was not subjected to further tail-flick assay but received the subsequent dose of morphine so that each animal received the same opioid dose, approximately 10 mg/kg on the morning of day 1. On that same day, 10 mg/kg morphine was administered two more times. On day 2, the animals received 20 mg/kg morphine given subcutaneously three times per day; on day 3, the animals received 40 mg/kg morphine given subcutaneously three times per day. Ketamine at a dose of 0.3, 3, or 10 mg/kg or saline was administered subcutaneously 15 min before and 15 min after each morphine injection on days 1 through 3. On day 1, the first dose of ketamine or saline was administered before the cumulative dose-response assessment. On day 4, all animals underwent the morphine cumulative dose-response assessment without first receiving ketamine or saline. The development of morphine tolerance was determined by comparing the morphine ED_{50} values obtained on day 4 to those of day 1.

Study 2: Effect of Systemic Ketamine on Established Morphine Tolerance. Study 2 (fig. 1B) was designed to determine whether ketamine could reverse established morphine tolerance. Tolerance was produced by implanting two 25-mg morphine pellets on day 1. The animals underwent a cumulative dose-response assessment on day 4. The day 4 morphine ED50 value was compared with the value obtained with morphine-naive animals to determine the degree of tolerance. The pellets were then removed and mice were separated into two groups that received either ketamine (10 mg/kg) or saline subcutaneously once on day 4 and three times per day on days 5 through 7. A repeated cumulative dose-response assessment was done on day 8 without administering ketamine. The morphine ED_{50} values of ketamine-treated mice on day 8 was compared with those of morphine-treated mice on day 4 and saline-treated mice on day 8.

Study 3: Effect of Intraspinal Ketamine on the Development of Morphine Tolerance. Study 3 (fig. 1C) was designed to determine whether intraspinal ketamine could attenuate tolerance produced by intraspinal morphine. A paradigm similar to study 1 was used to produce intraspinal morphine tolerance in rats. On the morning of day 1, each animal underwent a cumulative dose-response assessment with intraspinal morphine to determine the morphine ED_{50} value. In this study, the cumulative dose-response assessment was performed with each group before administering ketamine to preclude any difference among the groups that might have been caused by the intrathecal catheterization. After this procedure, the animals received additional doses of intraspinal morphine so that each animal received the same dose of morphine, approximately 10 μg on the morning of day 1. Ten micrograms of morphine or saline was administered two more times on day 1. Twenty and 40 μg morphine or saline were administered intrathecally three times per day at 9:00, 14:00, and 19:00 on days 2 and 3, respectively. Ketamine at 12 μg (50 nmol) or saline was administered intraspinally 10 min before each morphine injection on

days 1, 2, and 3. Ketamine was administered after the cumulative dose-response assessment on day 1, 10 min before the subsequent dose of morphine was injected. Thus the treatment arms included saline-morphine (SAL + MOR), ketamine-morphine (KET + MOR), ketamine-saline (KET + SAL), and saline-saline (SAL + SAL). Each animal was tested for its analgesic response to morphine, and the morphine ED_{50} value was determined again on day 5. This was done on day 5 because the return of tail-flick latencies to baseline values required more than 24 h after the last 40-μg intraspinal dose of morphine on day 3. The development of morphine tolerance was determined by comparing the morphine ED_{50} values obtained on day 5 to those obtained on day 1.

The acute effect of the intraspinal administration of 12 μg ketamine on tail-flick latencies was tested on another group of intrathecally cannulated rats. Twelve micrograms ketamine was given intraspinally followed by 5 μl saline. Mean tail-flick latency was obtained before ketamine injection (baseline latency), 10 min after ketamine injection (immediately before saline injection), and 10 min after saline injection.

Data Analysis

The quantal dose-response data were analyzed using the BLISS-21 computer program. This program maximized the log-likelihood function to fit a parallel set of Gaussian normal sigmoid curves to the dose-response data and provides ED_{50} values, 95% confidence limits (CI), and relative potency estimates.[15]

Results

Study 1 (fig. 2) shows that the escalating subcutaneous dosing schedule for 3 days results in a shift to the right of the morphine dose-response curve on day 4 (SAL + MOR). However, when 10 mg/kg ketamine is coadministered subcutaneously with morphine, no significant shift of the curve is observed. The rightward shift of the dose-response curve indicates the development of tolerance to morphine, the magnitude of which can be expressed as an increase in the ED_{50} value and a decrease in the relative potency of morphine. Table 1 shows these values for each group. On day 4, the SAL + MOR group had a morphine ED_{50} value of 23.1 mg/kg, more than five times greater than the ED_{50} value obtained with saline pretreatment on day 1 (SAL group). Coadministration of ketamine subcutaneously at a dose of 0.3 mg/kg (KET − 0.3 + MOR) did not reduce the

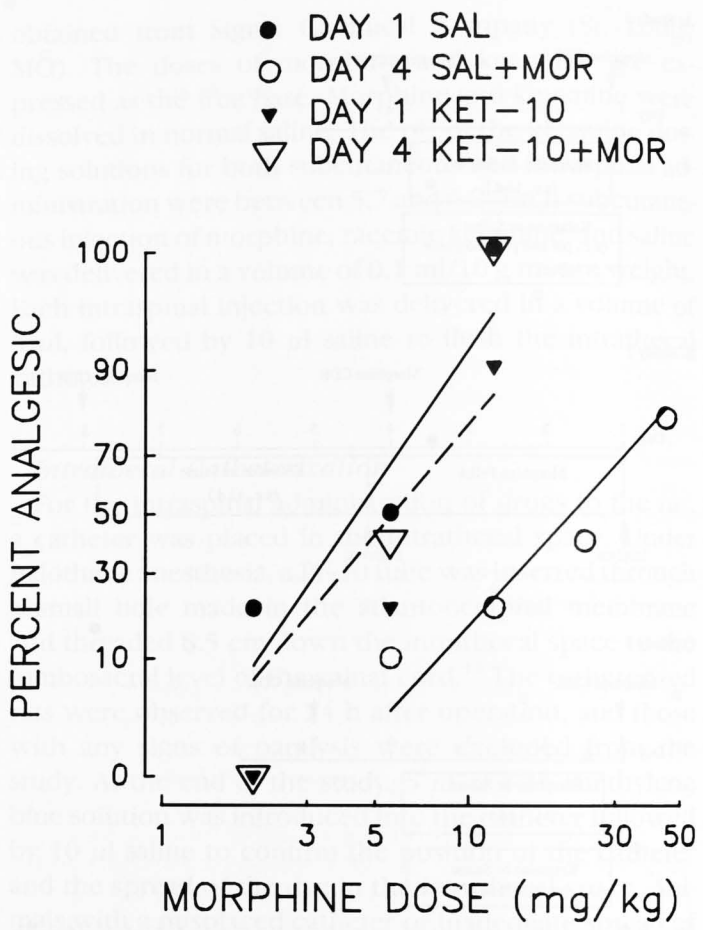

Fig. 2. Ketamine given in a subcutaneous dose of 10 mg/kg (KET − 10) prevents the rightward shift in the morphine (MOR) dose-response curve on day 4. Tolerance, as assessed by a rightward shift in the cumulative dose-response curve, was produced by administering MOR three times a day subcutaneously at 10 mg/kg on day 1, at 20 mg/kg on day 2, and at 40 mg/kg on day 3. KET − 10 or saline (SAL) was administered 15 min before and after each MOR dose. On day 1, the first dose of KET − 10 or SAL was followed by a cumulative MOR dose-response assessment. On day 4, each animal underwent cumulative dose-response assessment without previous KET − 10 or SAL administration. On day 4, the SAL + MOR curve shifted more than five times, but the curves for the day 1 KET − 10 and day 4 KET − 10 + MOR groups were not significantly different from the day 1 SAL group (see table 1 for ED_{50} values).

day 4 morphine ED_{50} value significantly from control. The 3 mg/kg dose of ketamine (KET − 3 + MOR) significantly attenuated tolerance, whereas the 10 mg/kg dose of ketamine (KET − 10 + MOR) completely blocked morphine tolerance. On day 1, ketamine at 0.3, 3, or 10 mg/kg did not significantly alter the morphine ED_{50} value (table 1). These data demonstrate a dose-dependent attenuation of morphine tolerance by ketamine. None of the treatments altered the baseline tail-flick latencies assessed before the morphine cumulative

Table 1. Attenuation of Morphine Tolerance by Ketamine

Treatment	Day	Morphine ED_{50} (mg/kg)	Morphine 95% CI	Relative Potency
SAL	1	4.3	2.9–6.1	1.00
KET-0.3	1	5.0	3.1–7.6	0.86
KET-3	1	4.2	2.6–6.6	1.02
KET-10	1	7.6	5.3–11.1	0.58
SAL +MOR	4	23.1*	16.3–34.0	0.19
KET-0.3 +MOR	4	19.4*	13.8–27.4	0.22
KET-3 +MOR	4	11.7*†	8.2–16.1	0.37
KET-10 +MOR	4	5.8†	3.8–8.7	0.74

ED_{50} values for morphine (MOR) with the 95% confidence interval (CI) were determined on day 1 and on day 4. The MOR dosing schedule was 10 mg/kg sc t.i.d. on day 1, 20 mg/kg t.i.d. on day 2, and 40 mg/kg t.i.d. on day 3. Ketamine (KET) at 0.3, 3, or 10 mg/kg or saline (SAL) was given 15 min before and after each MOR on days 1, 2, and 3.

* Significantly different ($P < 0.05$) from day 1 SAL group.

† Significantly different ($P < 0.05$) from day 4 SAL + MOR group.

dose-response assessment. The 10 mg/kg dose of ketamine produced behavioral effects characterized by running and posturing. Preliminary dose-ranging studies indicated that these effects lasted approximately 10 min, so we elected to use pre- and postdosing of ketamine given the relatively longer duration of morphine's effects on the central nervous system. No central nervous system sedation was observed.

In study 2 (table 2), mice in which two 25-mg morphine pellets were implanted developed a significant degree of morphine tolerance when tested on day 4. Cumulative dose-response assessment showed a tenfold shift in the morphine ED_{50} values (46.3 and 42.3 mg/kg) compared with naive controls (4.3 mg/kg). After the pellets were removed on day 4, the mice received 10 mg/kg ketamine subcutaneously on day 4 after the pellets were removed, the same dose three times per day on days 5 through 7, or saline. A cumulative dose-response study on day 8 revealed that in the ketamine-treated group the morphine ED_{50} value decreased significantly to 8.7 mg/kg (not significantly different from the SAL group on day 1), indicating a complete reversal of tolerance. In the control group receiving saline subcutaneously, the morphine ED_{50} value on day 8 (46.7 mg/kg) showed no change compared with day 4, demonstrating the persistence of a significant degree of morphine tolerance. None of the treatments altered the baseline tail-flick latencies assessed before the morphine cumulative dose-response assessment.

In study 3, the intrathecal administration of morphine in escalating doses for 3 days (SAL + MOR group) resulted in a large rightward shift of the dose-response curve for morphine on day 5 (fig. 3), indicating a significant degree of analgesic tolerance. The magnitude of this tolerance can be expressed as a 46-fold increase in the ED_{50} value for intraspinal morphine from 0.8 μg (0.5 to 1.3 μg; 95% CI) in morphine-naive rats to 38.5 μg (26.9 to 54.9 μg; 95% CI) in morphine-treated rats (table 3). In contrast, the rats that had been given ketamine and morphine (KET + MOR group) concurrently showed only a slight rightward shift on day 5 (fig. 3). The intraspinal morphine ED_{50} value increased two times from 1.1 μg (0.8 to 1.5 μg; 95% CI) on day 1 to 2.1 μg (1.6 to 2.7 μg; 95% CI) on day 5 (table 3). The intraspinal morphine ED_{50} for the KET + MOR group on day 5 was significantly different from that of the SAL + MOR group on day 5, indicating an attenuation in the development of tolerance to intraspinal morphine (from a 46-fold increase in the ED_{50} value to a twofold increase in the ED_{50} value of intraspinal morphine). Control groups (KET + SAL; SAL + SAL) showed no difference in the ED_{50} value of intraspinal morphine on day 1 or day 5 compared with that of day 1 in the SAL + MOR group (table 3). No differences were observed in baseline tail-flick latencies assessed before the morphine cumulative dose-response assessment between days 1 and 5 in any of the treatment groups.

No changes in tail-flick latencies were seen 10 min after the intrathecal administration of 12 μg ketamine and 10 min after the subsequent administration of saline (table 4), confirming that intraspinal ketamine at this dose did not affect baseline tail-flick latencies. No behav-

Table 2. Reversal of Morphine Tolerance by Ketamine

Treatment	Day	Morphine ED_{50} (mg/kg)	Morphine 95% CI	Relative Potency
SAL	1	4.3	2.9–6.1	1.00
MOR-1	4	46.3*	33.0–66.3	0.10
MOR-2	4	42.7*	30.1–60.8	0.09
MOR-1 + KET-10	8	8.7†	5.9–12.6	0.49
MOR-2 + SAL	8	46.7*	33.2–66.9	0.09

ED_{50} values for morphine (MOR) with the 95% confidence interval (CI) were determined on days 1, 4, and 8. The MOR pretreatment was two 25-mg morphine pellets implanted on day 1 and removed on day 4. Ketamine (KET) treatment was 10 mg/kg sc given once on day 4 following the removal of pellets and t.i.d. on days 5, 6, and 7. Controls received parallel saline (SAL) injections. The notations −1 and −2 identify separately treated MOR groups.

* Significantly different ($P < 0.05$) from day 1 SAL group.

† Significantly different ($P < 0.05$) from day 4 MOR-1 and MOR-2 and day 8 MOR-2 + SAL groups.

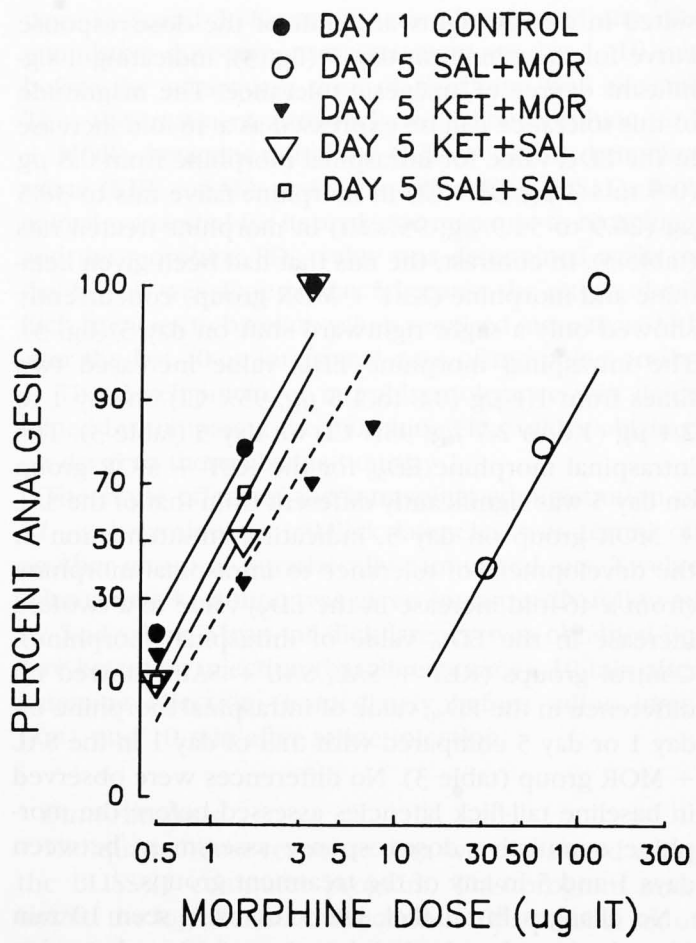

Fig. 3. Intraspinal ketamine (KET) given in a dose of 12 μg (50 nmol) prevents the rightward shift in the intraspinal morphine (MOR) dose-response curve on day 5. Tolerance was produced by administering MOR three times a day at 10 μg on day 1, at 20 μg on day 2, and at 40 μg on day 3. KET or saline (SAL) was administered 10 min before each MOR dose. On day 1, cumulative MOR dose-response assessment preceded each treatment. On day 5, the SAL + MOR curve shifted 46 times, whereas curves for day 5 KET + MOR, KET + SAL, and SAL + SAL groups were not significantly different from the curve for day 1 before treatment (see table 3 for ED_{50} values).

ioral effects were observed on days 1 through 5 after intraspinal ketamine (KET + SAL) was given.

Discussion

Study 1 showed that coadministration of systemic ketamine with morphine dose-dependently attenuates the development of morphine tolerance (table 1). Study 2 found that the systemic administration of 10 mg/kg ketamine could reverse established morphine tolerance (table 2). This is perhaps a more clinically relevant issue, because many patients have some degree of opioid tolerance. These data correlate with the earlier finding that various NMDA receptor antagonists can attenuate and reverse morphine tolerance.[2,4-6] Trujillo and Akil[13] previously studied the effect of systemic ketamine on the attenuation and reversal of morphine tolerance in rats, and they attenuated but did not reverse morphine tolerance. These authors described "reversal" as the inhibition of the expression of morphine tolerance, and they tested this by administering a single bolus dose of ketamine (10 mg/kg^{-1} given intraperitoneally) after morphine tolerance had been established to determine the effect on morphine tolerance 60 min after ketamine administration. In our study, we administered ketamine subcutaneously at 10 mg/kg for 4 days (once on day 4 and 3 times per day on days 5 through 7) after established morphine tolerance was demonstrated by determining morphine ED_{50} values (table 2). We assessed the reversal of morphine tolerance by repeating the ED_{50} determination on day 8 without ketamine pretreatment. The reversal of established morphine tolerance that we observed was not a result of the inhibition of expression of tolerance but rather the actual decrease in the magnitude of the acquired analgesic tolerance as expressed by a direct comparison of quantitative morphine ED_{50} shifts. Studies of morphine tolerance suggest that the adaptive changes that occur require days to develop.[13,16] The development of complete tolerance to 10 mg/kg morphine given subcutaneously twice daily, evaluated by the hot-plate test, required more than 8 days.[13] Continuous infusion of intraspinal morphine at rates of 2, 6, and 20 nmol/h elevated the hot-plate latencies in a concentration-dependent manner on day 1, and these latencies gradually returned to saline-infused values by 3 to 5 days for each dose of morphine.[16] Furthermore, the subcutaneous infusion of LY274614 to morphine-tolerant animals that continue to receive morphine showed a gradual return of morphine sensitivity over 2 to 3 days.[4] Thus reversal of established morphine tolerance probably requires repeated administration of ketamine over time. For this reason, we repeatedly administered ketamine for 4 days after morphine tolerance had been established.

The results of our study (see figs. 2 and 3 and tables 1 and 3) support the concept that functional NMDA receptors are necessary for the development of morphine tolerance. However, the exact role that they play has not been clearly defined. *In vitro*, mu receptor activation increases NMDA receptor-gated calcium currents and is mediated by protein kinase C.[17] This poten-

Table 3. Attenuation of Intraspinal Morphine Tolerance by Intraspinal Ketamine

Group	Day	Morphine		Relative Potency
		ED_{50} (μg)	95% CI	
SAL + MOR	1 (before treatment)	0.83	0.53–1.27	1.00
	5	38.5*	26.9–54.9	0.02
KET + MOR	1 (before treatment)	1.10	0.85–1.52	0.72
	5	2.09*,†	1.59–2.75	0.34
KET + SAL	1 (before treatment)	0.99	0.57–1.70	0.84
	5	1.41	0.83–2.41	0.58
SAL + SAL	1 (before treatment)	1.20	0.70–2.04	0.69
	5	1.20	0.70–2.04	0.69

ED_{50} values for intraspinal morphine (MOR) with the 95% confidence interval (CI) were determined for all groups on day 1 before any treatment and on day 5. The MOR dosing schedule was 10 μg t.i.d. on day 1, 20 μg t.i.d. on day 2, and 40 μg t.i.d. on day 3. Intraspinal ketamine at 12 μg (50 nmol) (KET) or saline (SAL) was given 10 min prior to each MOR or SAL injection on days 1, 2, and 3.

* Significantly different ($P < 0.05$) from day 1 SAL + MOR group.

† Significantly different ($P < 0.05$) from day 5 SAL + MOR group.

tiation of the NMDA receptor-mediated response by protein kinase C is a result of an increase in the probability of opening and a reduction of the Mg^{2+} block of the NMDA receptor channels.[18] This may be one mechanism by which morphine interacts with the NMDA receptor in the development of tolerance. Activation of the NMDA receptors increases Ca^{2+} influx, thereby increasing intracellular Ca^{2+} concentration. An increase in basal free intracellular calcium is seen in brain synaptosomes from morphine-tolerant mice.[19] The increase in intracellular Ca^{2+} may then initiate a cascade of intracellular events leading to the development of morphine tolerance, such as the production of nitric oxide. N^G-nitro-L-arginine, a nitric oxide synthase inhibitor, attenuates morphine tolerance in mice.[2,20,21] In addition, rats tolerant to intraspinal morphine show an increase in membrane-bound protein kinase C (translocated protein kinase C) in dorsal horn neurons that is not seen after a single dose of morphine. GM1 ganglioside, a substance that blocks the translocation of protein kinase C, prevents morphine tolerance and the increase in membrane-bound protein kinase C.[22] These findings suggest that nitric oxide production and translocation of protein kinase C are some of the important intracellular events involved in the development of tolerance. Furthermore, changes in central nervous system levels of NMDAR1 mRNA are seen in morphine-tolerant rats,[7] and NMDA receptors are down-regulated in specific regions of the central nervous system in rats subjected to long-term treatment with morphine,[23] suggesting that changes in gene expression are involved in the development of morphine tolerance. Ketamine has been shown to block NMDA-evoked currents in voltage- and use-dependent manners in cultured mouse hippocampal neurons[11] and to attenuate an NMDA-evoked increase in intracellular Ca^{2+} levels in rat cortical slices.[12] Ketamine probably modulates morphine tolerance by blocking the NMDA receptor-dependent ion channel and thereby prevents the subsequent intracellular events that lead to morphine tolerance.

Table 4. Acute Effect of Intraspinal Ketamine on Tail-flick Latencies

Treatment	Mean Tail-flick Latency (s)
Baseline	3.2 ± 0.22
Ketamine 12 μg	3.87 ± 0.33
Ketamine 12 μg + saline 5 μl	3.74 ± 0.68

A total of 12 μg of ketamine was given intraspinally followed by a 5-μl saline injection with an interval of ~10 min. Mean tail-flick latencies were obtained prior to ketamine (baseline latency), 10 min after ketamine (immediately prior to saline), and 10 min after saline. Mean tail-flick latencies were calculated from two repeated latency measurements. Mean tail-flick latency values are expressed in mean ± SD. No significant difference among the three groups was seen.

The spinal cord is probably a major site of action of NMDA receptor antagonists in attenuating morphine tolerance.[24] Intraspinal administration of the NMDA receptor antagonist MK-801 attenuates morphine tolerance development produced by the intraspinal administration of morphine.[25,26] We found in study 3 that intraspinal administration of increasing doses of morphine produced tolerance, as shown by a 46-fold increase in the intraspinal morphine ED_{50} value. This morphine tol-

erance was almost completely attenuated by the coadministration of intraspinal ketamine (fig. 3 and table 3). Ketamine, a compound with high lipid solubility, is rapidly accumulated by nervous system tissue.[27] Ketamine's site of action when given intraspinally probably is local, within the spinal cord. Thus, if ketamine acted spinally in attenuating tolerance to intraspinal morphine, spinal cord NMDA receptors are probably important in the development of tolerance to intraspinal morphine. The dose of intraspinal ketamine that we used, 12 μg or 50 nmol, although it had no effect on baseline tail-flick latencies (table 4), has been associated with antinociceptive effects in rat models of central sensitization.[28-30]

Subcutaneous ketamine given at 0.3, 3, or 10 mg/kg to morphine-naive mice did not affect the analgesic sensitivity to morphine, as demonstrated by no difference in the morphine ED_{50} value after ketamine was given subcutaneously compared with saline (table 1). Trujillo and Akil[13] also showed that in morphine-tolerant rats, 10 mg/kg ketamine given intraperitoneally did not change morphine sensitivity. We did not evaluate the acute effects of intraspinal ketamine on analgesic sensitivity to intraspinal morphine. However, the long-term intraspinal administration of ketamine did not change analgesic sensitivity to intraspinal morphine (table 3). Furthermore, the ED_{50} value of intraspinal morphine was evaluated more than 24 h after the last dose of ketamine was given, suggesting that the attenuation of tolerance to intraspinal morphine that we observed was not a result of the change in intraspinal morphine sensitivity caused by intraspinal ketamine.

In these studies in mice and rats, the investigators were not blinded to the treatments, and thus bias could have affected the results. However, these results correlate with previous blinded studies using other NMDA receptor antagonists.[1-6]

Ketamine is a clinically available noncompetitive NMDA antagonist that has been used in the operating room as an intravenous anesthetic for almost 30 y. Ketamine, like other PCP-like drugs, blocks the NMDA-dependent ion channel as a consequence of binding to the PCP receptor. The PCP binding site may be located within the lumen of the ion channel, and PCP-like drugs can exert their effects on the channel most readily when the channel is open as a result of activation by agonists (*i.e.*, they are open channel blockers).[11,31] The clinical significance of open channel blockers of the NMDA receptor channel may be that their effect is maximal with pathologic activation of NMDA receptors, as in disease states, and they may have a lesser effect in steady-state physiologic conditions.[32] Ketamine, which is effective as an NMDA receptor antagonist at doses lower than currently used as an anesthetic,[9] deserves consideration for clinical use in blocking pathologic NMDA receptor-mediated events, such as morphine tolerance. Ketamine at subanesthetic doses has been reported to have analgesic effects in humans[33-35] and may be of clinical use in managing neuropathic pain[36-38] and cancer pain.[39,40]

The most probable side effects that may be encountered with the clinical use of ketamine at subanesthetic doses are dose-dependent psychotomimetic effects.[41] The coadministration of a benzodiazepine attenuates ketamine's postanesthetic emergence reactions[42] and may be useful in controlling this side effect. The use of S(+)-ketamine, which produces fewer psychotomimetic effects in humans compared with R(−)-ketamine at equipotent doses,[43] may also be an approach to minimize these psychotomimetic side effects in patients.

Neuropathologic evaluation after large doses of both competitive and noncompetitive NMDA receptor antagonists in the adult rat reveal neuronal vacuolation in the cingulate cortex and retrosplenial cortex and other neuronal regions suggestive of a neurotoxic effect of excessive NMDA receptor blockade.[44] These findings have not been replicated in the dose range that modulates nociceptive processing and the development of tolerance in rodents, nor have they been reproduced in primate models. We are unaware of any reported cases of neurotoxicity or permanent psychosis from the established clinical experience with ketamine. The safety of repeated administration of systemic ketamine has been demonstrated in patients with burns, in whom it is used for analgesia for daily debridement and dressing changes.[45-47]

After repeated administration of intraspinal ketamine to rabbits, postmortem examination showed no evidence of histologic damage to the spinal cord.[48] However, in primates, only single-dose administration of intraspinal ketamine has been reported.[49] Ketamine has been given to patients in single doses for surgical anesthesia without any obvious sequela.[50] However, the safety of repeated administration of intrathecal ketamine in humans has not been determined.

We presented evidence of ketamine's efficacy in attenuating and reversing morphine tolerance and of the presence of a spinal site of action for ketamine. The clinical utility and safety of ketamine for modulating

morphine tolerance in patients with chronic pain syndromes should be evaluated.

References

1. Trujillo KA, Akil H: Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. Science 1991; 251:85-7

2. Elliott K, Minami N, Kolesnikov YA, Pasternak GW, Inturrisi CE: The NMDA receptor antagonists, LY274614 and MK-801, and the nitric oxide synthase inhibitor, NG-nitro-L-arginine, attenuate analgesic tolerance to the mu-opioid morphine but not to kappa opioids. Pain 1994; 56:69-75

3. Marek P, Ben-Eliyahu S, Gold M, Liebeskind JC: Excitatory amino acid antagonists (kynurenic acid and MK-801) attenuate the development of morphine tolerance in the rat. Brain Res 1991; 547:77-81

4. Tiseo PJ, Inturrisi CE: Attenuation and reversal of morphine tolerance by the competitive N-methyl-D-aspartate receptor antagonist, LY274614. J Pharmacol Exp Ther 1993; 264:1090-6

5. Tiseo PJ, Cheng J, Pasternak GW, Inturrisi CE: Modulation of morphine tolerance by the competitive N-methyl-D-aspartate receptor antagonist LY274614: Assessment of opioid receptor changes. J Pharmacol Exp Ther 1994; 268:195-201

6. Elliott K, Hynansky A, Inturrisi CE: Dextromethorphan attenuates and reverses analgesic tolerance to morphine. Pain 1994; 59:361-8

7. Brodsky M, Elliott K, Hynansky A, Minami N, Inturrisi CE: NMDA receptor and proenkephalin gene expression are modulated by chronic morphine administered alone or in combination with NMDA antagonist [Abstract]. Soc Neurosci 1993; 19:1352

8. Church J, Lodge D: N-methyl-D aspartate (NMDA) antagonism is central to the actions of ketamine and other phencyclidine receptor ligands, Status of Ketamine in Anesthesiology. Edited by EF Domino. Ann Arbor, NPP Books, 1990, pp 501-19

9. Anis NA, Berry SC, Burton NR, Lodge D: The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-aspartate. Br J Pharmacol 1983; 79:565-75

10. Martin D, Lodge D: Ketamine acts as a non-competitive N-methyl-D-aspartate antagonist on frog spinal cord *in vitro*. Neuropharmacology 1985; 24:999-1003

11. MacDonald JF, Miljkovic Z, Pennefather P: Use-dependent block of excitatory amino acid currents in cultured neurons by ketamine. J Neurophysiol 1987; 58:251-66

12. O'Shaughnessy CT, Lodge D: N-methyl-D-aspartate receptor-mediated increase in intracellular calcium is reduced by ketamine and phencyclidine. Eur J Pharmacol 1988; 153:201-9

13. Trujillo KA, Akil H: Inhibition of opiate tolerance by non-competitive N-methyl-D-aspartate receptor antagonists. Brain Res 1994; 633:178-88

14. Yaksh TL, Rudy TA: Chronic catheterization of the spinal subarachnoid space. Physiol Behav 1976; 17:1031-6

15. Umans JG, Inturrisi CE: Pharmacodynamics of subcutaneously administered diacetylmorphine, 6-acetylmorphine and morphine in mice. J Pharmacol Exp Ther 1981; 218:409-15

16. Stevens CW, Yaksh TL: Time course characteristics of tolerance development to continuously infused antinociceptive agents in rat spinal cord. J Pharmacol Exp Ther 1989; 251:216-23

17. Chen L, Huang LY: Sustained potentiation of NMDA receptor-mediated glutamate responses through activation of protein kinase C by a μ opioid. Neuron 1991; 7:319-26

18. Chen L, Huang LY: Protein kinase C reduces Mg^{2+} block of NMDA-receptor channels as a mechanism of modulation. Nature 1992; 356:521-3

19. Welch SP, Olson KG: Opiate tolerance-induced modulation of free intracellular calcium in synaptosomes. Life Sci 1991; 48:1853-61

20. Kolesnikov YA, Pick CG, Pasternak GW: NG-nitro-L-arginine prevents morphine tolerance. Eur J Pharmacol 1992; 221:399-400

21. Kolesnikov YA, Pick CG, Ciszewska G, Pasternak GW: Blockade of tolerance to morphine but not kappa opioids by a nitric oxide synthase inhibitor. Proc Natl Acad Sci USA 1993; 90:5162-6

22. Mayer DJ, Mao J, Price DD: The development of morphine tolerance and dependence is associated with translocation of protein kinase C. Pain 1995; 61:365-74

23. Bhargava HN, Reddy PL, Gudehithlu KP: Down-regulation of N-methyl-D-aspartate (NMDA) receptors of brain regions and spinal cord of rats treated chronically with morphine. Gen Pharmacol 1995; 26:131-6

24. Gutstein HB, Trujillo KA: MK-801 inhibits the development of morphine tolerance at spinal sites. Brain Res 1993; 626:332-4

25. Kest B, Mogil JS, Ben-Eliyahu S, Kao B, Liebeskind JC, Marek P: The NMDA receptor antagonist MK-801 protects against the development of morphine tolerance after intrathecal administration. Proc West Pharmacol Soc 1993; 36:307-10

26. Mao J, Price DD, Mayer DJ: Thermal hyperalgesia in association with the development of morphine tolerance in rats: Roles of excitatory amino acid receptors and protein kinase C. J Neurosci 1994; 14:2301-12

27. Cohen ML, Trevor AJ: On the cerebral accumulation of ketamine and the relationship between metabolism of the drug and its pharmacological effects. J Pharmacol Exp Ther 1974; 189:351-8

28. Ren K, Williams GM, Hylden JLK, Ruda MA, Dubner R: The intrathecal administration of excitatory amino acid receptor antagonists selectively attenuated carrageenan-induced behavioral hyperalgesia in rats. Eur J Pharmacol 1992; 219:235-43

29. Yamamoto T, Yaksh TL: Spinal pharmacology of thermal hyperesthesia induced by constriction injury of sciatic nerve. Excitatory amino acid antagonists. Pain 1992; 49:121-8

30. Mao J, Price DD, Hayes RL, Lu J, Mayer DJ, Frenk H: Intrathecal treatment with dextrorphan or ketamine potently reduces pain-related behaviors in a rat model of peripheral mononeuropathy. Brain Res 1993; 605:164-8

31. Huettner JE, Bean BP: Block of N-methyl-D-aspartate-activated current by the anticonvulsant MK-801: selective binding to open channels. Proc Natl Acad Sci USA 1988; 85:1307-11

32. Lipton SA, Rosenberg PA: Excitatory amino acids as a final common pathway for neurologic disorders. N Engl J Med 1994; 330:613-22

33. Sadove MS, Shulman M, Hatano S, Fevold N: Analgesic effects of ketamine administered in subdissociative doses. Anesth Analg 1971; 50:452-7

34. Slogoff S, Allen GW, Wessels JV, Cheney DH: Clinical experience with subanesthetic ketamine. Anesth Analg 1974; 53:354-8

35. Maurset A, Skoglund LA, Hustveit O, Øye I: Comparison of ketamine and pethidine in experimental and postoperative pain. Pain 1989; 36:37-41

36. Eide PK, Jørum E, Stubhaug A, Bremnes J, Breivik H: Relief of

post-herpetic neuralgia with the N-methyl-D-aspartic acid receptor antagonist ketamine: A double-blind, cross-over comparison with morphine and placebo. Pain 1994; 58:347–54

37. Backonja M, Arndt G, Gombar KA, Check B, Zimmermann M: Response of chronic neuropathic pain syndromes to ketamine: A preliminary study. Pain 1994; 56:51–7

38. Max MB, Byas-Smith MG, Gracely RH, Bennett GJ: Intravenous infusion of the NMDA antagonist, ketamine, in chronic posttraumatic pain with allodynia: A double-blind comparison to alfentanil and placebo. Clin Neuropharmacol 1995; 18:360–8

39. Oshima E, Tei K, Kayazawa H, Urabe N: Continuous subcutaneous injection of ketamine for cancer pain. Can J Anaesth 1990; 37:385–6

40. Clark JL, Kalan GE: Effective treatment of severe cancer pain of the head using low-dose ketamine in an opioid-tolerant patient. Journal of Pain Symptom Management 1995; 10:310–14

41. Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers MB Jr, Charney DS: Subanesthetic effects of noncompetitive NMDA antagonist, ketamine, in humans. Arch Gen Psychiatry 1994; 51:199–214

42. Cartwright PD, Pingel SM: Midazolam and diazepam in ketamine anaesthesia. Anaesthesia 1984; 39:439–42

43. White PF, Ham J, Way WL, Trevor AJ: Pharmacology of ketamine isomers in surgical patients. Anesthesiology 1980; 52:231–9

44. Olney JW, Labruyere J, Price MT: Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. Science 1989; 244:1360–2

45. Corssen G, Oget S: Dissociative anesthesia for the severely burned child. Anesth Analg 1971; 50:95–102

46. Demling RH, Ellerbe S, Jarrett F: Ketamine anesthesia for tangential excision of burn eschar: A burn unit procedure. J Trauma 1978; 18:269–70

47. Cederholm I, Bengtsson M, Björkman S, Choonara I, Rane A: Long term high dose morphine, ketamine and midazolam infusion in a child with burns. Br J Clin Pharmacol 1990; 30:901–5

48. Borgbjerg FM, Svensson BA, Frigast C, Gordh T: Histopathology after repeated intrathecal injections of preservative-free ketamine in the rabbit: A light and electron microscopic examination. Anesth Analg 1994; 79:105–11

49. Brock-Utne JG, Kallichurum S, Mankowitz E, Maharaj RJ, Downing JW: Intrathecal ketamine with preservative—Histological effects on spinal nerve roots of baboons. S Afr Med J 1982; 61:440–1

50. Bion JF: Intrathecal ketamine for war surgery. A preliminary study under field conditions. Anaesthesia 1984; 39:1023–8