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Interaction between morphine and norketamine enantiomers in rodent models of nociception

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ABSTRACT

Ketamine, one of a few clinically-available *N*-Methyl-D-aspartate (NMDA)-receptor antagonists, is known to improve the analgesic efficacy of opioids in humans and rodents. However, the use of ketamine in combination with opioids is mainly restricted to the perioperative setting, due to severe psychotomimetic, sedative and motor side effects. Recent data from our laboratory demonstrated that a major metabolite of ketamine, norketamine, in particular the S(+) enantiomer, had a better antinociception/side effects profile than ketamine in rats. It is unknown if norketamine affects opioid antinociception. In the present study, morphine (a low dose) was combined with S(+)- and R(−)-norketamine (sub-antinociceptive doses) and characterized utilizing rodent models of pain including: thermal nociception (the tail-flick test), peripheral neuropathy (chronic constriction nerve injury) and tonic inflammatory pain (the formalin test). The data showed that: 1) Norketamine enhanced morphine antinociception and blocked tolerance to this effect; 2) Norketamine potentiated morphine effectiveness in the alleviation of symptoms resulting from injury to nerve (mechanical hyperalgesia, tactile allodynia) and peripheral tissue (formalin-induced nociceptive behavior); 3) S(+)-norketamine was more potent than R(−)-norketamine; 4) Antinociception was not confounded by significant side effects. Morphine-S(+)-norketamine combination drug therapy may prove clinically useful for the alleviation of acute and chronic pain of differing etiology.

1. Introduction

μ-Opioids (e.g. morphine, oxycodone) remain the primary drugs for the treatment of moderate to severe acute and chronic pain. Nociceptive pain, a result of activation of peripheral nociceptors by various stimuli (e.g. surgical incision, inflammatory mediators) is typically successfully treated with opioids. Although opioids are also used for neuropathic pain (e.g. radiculopathy, complex regional pain syndrome) it is often with less success and requiring higher doses. In addition to dose-limiting side effects (cognitive dysfunction, respiratory depression, constipation) the clinical utility of opioids is often hampered by the development of analgesic tolerance with long-term use and more recently appreciated, the development of opioid-induced hyperalgesia (Ballantine, 2006; Ballantine and Mao, 2003; Harden, 2002, Mao, 2002, reviews). One approach to address these issues has been to consider combining opioids with drugs from other classes (Kalso, 2005; Gilron and Max, 2005, reviews).

Evidence exists for a role of the *N*-Methyl-D-aspartate (NMDA)-receptor complex in pain processing, in particular in chronic pain states where central sensitization is involved (i.e. neuropathic pain) (Bennett, 2000, review). Interactions between μ-opioid-receptors and NMDA-receptors in antinociceptive pathways have been described (Mao 1999, review). Therefore, attention has focused on the concept of combining μ-opioids with NMDA-receptor antagonists. The potential utility of this approach has been demonstrated in rodents and humans whereby NMDA-receptor antagonists have been shown to enhance opioid antinociception and reduce development of tolerance (Mao 1999; Prince et al., 2000; Subramaniam et al., 2004; Sveticic et al., 2005; Wiesenfeld-Hallin, 1998, reviews). Preclinical studies have demonstrated that NMDA-receptor antagonists also block opioid-induced hyperalgesia (Celerier et al., 2000, Holtman and Wala, 2005, Laulin et al., 2002; van Elstraete et al., 2005).

Unfortunately, blockade of the NMDA-receptor not only causes desirable effects (i.e. enhancement of opioid antinociception, prevention of tolerance, blockade of opioid-induced hyperalgesia), but also evokes unacceptable phencyclidine (PCP)-like psychotomimetic side effects. As far as clinically-available NMDA-receptor antagonist agents are concerned, the most efficacious drug, ketamine, produces marked sedation, dysphoria and hallucinations and thus, its use as an adjuvant agent with opioids has practically been restricted to perioperative settings (Hocking and Cousins, 2003; Visser and Schug, 2006; White and Ryan, 1996, reviews). Clinical data on other drugs with NMDA-receptor antagonistic activities (dextromethorphan, memantine, amantadine) are contradictory (McCartney

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et al., 2004, review). With these factors in mind, identification of an agent that possesses the capability of antagonizing the NMDA-receptor without causing marked sedative and psychotomimetic effects would be of significant benefit. A recent study suggests that norketamine (primary metabolite of ketamine) may be useful in this regard (Holtman et al., 2007). Norketamine has a lower affinity at the PCP site of the NMDA-receptor compared to ketamine (Ebert et al., 1997) likely resulting in fewer side effects. Moreover, the fact that norketamine has enantiomers, S(+) and R(−), opens the possibility that one enantiomer could be better suited than the other for use as an adjuvant agent.

The present study was conducted to determine the effect of the norketamine enantiomers on morphine antinociception. This was done by combining morphine (low doses) and S(+)- or R(−)-norketamine (non-antinociceptive doses) and characterizing their efficacies in rodent models of nociceptive, neuropathic and inflammatory pain. These models included: 1) acute thermal nociception (tail-flick test), 2) peripheral neuropathy (chronic constriction nerve injury, CCI) and 3) tonic inflammatory pain (formalin test). In order to assess side effects, motor function (rotarod) and locomotor activity were determined.

2. Methods

2.1. Animals

Male Sprague Dawley rats (≈90 days old, ≈350 g; Harlan, IN) were used in this study. Rats were housed separately in a transparent cage, with free access to standard laboratory chow and tap water in a humidity- and temperature-controlled facility (lights on 0600–1800 h). Experiments were conducted during the light phase of the cycle. Rats were trained in the test situation before initiation of the experimental procedure. Body weights were determined on the day of experiment. A cross-over paradigm was used (whenever possible) to minimize the number of rats. Rats were euthanized with pentobarbital sodium (150 mg/kg). All testing was performed in accordance with the guidelines of the National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). The protocol was approved by the University of Kentucky Animal Care and Use Committee.

2.2. Drugs

S(+)-Norketamine hydrochloride and R(−)-norketamine hydrochloride were synthesized by Yaupon Therapeutics, Inc. (Radnor, PA). Morphine sulfate was obtained from Mallincrodt (St. Louis, MI). Drugs were dissolved in saline and administered either by the intraperitoneal (IP, 1 ml/kg) or intrathecal (IT, 10 µl) routes. Saline (vehicle) served as control. Doses refer to salt forms.

2.3. Tail-flick test

The purpose of this study was to characterize morphine-norketamine antinociception and the effect of norketamine on the development of tolerance to the antinociceptive effect of morphine. In addition, the action of the drug combinations was also examined at a spinal level to provide insight regarding site of action. The responsiveness to radiant heat was assessed in the tail-flick test (D'Amour and Smith, 1941). Tail-flick latency (TFL, s) was measured by recording the time from the onset of heat stimulus to the tail to withdrawal of the tail from the heat source using a standard tail-flick apparatus (EMDIE Instrument Co., Roanoke, VA). Power intensity was adjusted to yield baseline TFL equal to 2–3 s. A cut-off time was set to 10 s to avoid possible tissue damage. Three series of experiments were done.

First, rats (8/group) were treated with morphine, S(+)-norketamine or R(−)-norketamine alone, as well as a fixed dose of morphine (3 mg/kg) in combination with various doses of S(+)- or R(−)-norketamine (0.75, 1.5, 3 mg/kg). Drugs were administered by the IP route at weekly intervals. A double-block Latin square design (4×4) was used for randomization of doses. Responsiveness (TFL) was measured prior to (twice, 15 min apart) and at 15, 30, 60 and 120 min after injection.

Second, rats (8/group) were subjected to chronic catheterization of the spinal subarachnoid space (Yaksh and Rudy, 1976). Thereafter, they were treated with morphine and S(+)-norketamine alone or a fixed dose of morphine (0.5 µg) in combination with various doses of S(+)-norketamine (10, 50, 100 µg). Injections (10 µl) were made via IT catheter at weekly intervals (randomized doses). The TFLs were measured at 0, 15, 30, 60, 120, and 180 min after injection.

Third, rats (8/group) were repeatedly exposed to morphine alone (7 mg/kg) or in combination with S(+)-norketamine (3 mg/kg) administered IP two times/day for 12 days (approximately at 0900 and 1700). This time and dosing regimen with morphine has previously been demonstrated to produce antinociceptive tolerance (Holtman et al., 2004). Responsiveness (TFL) was assessed daily prior to (baseline) and 30 and 60 min after the morning dose.

2.4. Chronic constriction nerve injury (CCI)

2.4.1. Surgery

The rodent model of peripheral neuropathy (the chronic constriction nerve injury, CCI) (Bennett and Xie, 1988) was used to characterize the antihyperalgesic and antiallodynic effects of morphine and norketamine combination therapies. Rats were subjected to a sciatic nerve ligation (left paw) and sham surgery (right paw). Briefly, under pentobarbital anesthesia (40 mg/kg, IP) the sciatic nerve (about 7 mm) was freed from adhering tissue and four loose ligatures were tied around the nerve (1 mm apart) using 4.0 chromic catgut. In sham surgery, the sciatic nerve was exposed but was not ligated. The incision was closed with silk thread 3.0. Rats showed a mild inversion of the affected paw and a mild degree of foot drop. No severe motor impairment was observed. Mechanical hyperalgesia and tactile allodynia developed within 7 days after surgery.

2.4.2. Mechanical hyperalgesia

Enhanced sensitivity to mechanical noxious stimuli (mechanical hyperalgesia) was evaluated using the paw pressure test (Randall and Selitto, 1957). This was done prior to surgery (pre-CCI baseline) and on post-surgery days 7, 9, 11 and 14 (time of maximum hyperalgesia). Briefly, the hind paw was placed between a flat surface and a blunt pointer (Analgesimeter, UGO Basile; Italy) and increasing pressure was applied to the dorsal side of the paw (32 g/s, cut-off=300 g). Rats (8/group) were treated with morphine, S(+)- and R(−)-norketamine alone, as well as with a fixed dose of morphine (3 mg/kg) in combination with various doses of S(+)-norketamine (0.01, 0.1 and 1 mg/kg) or R(−)-norketamine (0.75, 1.5 and 3 mg/kg). Drugs were administered by the IP route (randomized doses). Vocalization threshold (VT, g) was measured prior to (baseline) and at 15, 30, 45, 60 and 120 min after injection.

2.4.3. Tactile allodynia

Responsiveness to a normally painless stimulus (allodynia) was determined prior to and on post-surgery days 7, 11, 14 and 17 (time of maximum allodynia), as previously described (Chaplan et al., 1994). Briefly, von Frey filaments (Stoelting, Wood Dale, IL) with incremental stiffness (0.4–15 g) were presented to the mid-plantar surface of the hind paw and held there for 6–8 s. A sufficient force was applied to cause slight buckling against the paw. A positive response was noted either by the sharp paw withdrawal or flinching immediately upon the removal of the filament (paw withdrawal threshold, PWT, g). Each hair was applied 10 times and the number of positive responses were recorded (the percent response). If no response was elicited by the initially selected filament a stronger stimulus was presented. The 50% PWT was calculated. Rats (4/group) were treated (IP; randomized doses) with morphine and S(+)-norketamine alone and a fixed dose of morphine (1 mg/kg) in combination with various doses of S(+)-norketamine (2, 4 and 8 mg/kg). Responses were assessed prior to (baseline) and at 15, 30, 45, 60 and 120 min after injection.

2.5. Formalin test

A rat model of tonic inflammatory pain (the formalin test) was used in this study (Wheeler-Aceto and Cowan, 1991). Fifty µl of formalin (5%) was injected subcutaneously (SC) into the dorsal surface of the left hind paw. This procedure typically produces a biphasic behavioral response consisting of flinching, lifting and licking. The first phase (0–10 min) is thought to result from direct stimulation of nociceptors (nociceptive pain) whereas the second phase (20–60 min) is thought to involve central sensitization. Rats (4–8/dose/treatment) were pretreated 15 min prior to formalin (SC) injection with morphine, S(+)-norketamine and R(+)-norketamine alone and a fixed dose of morphine (0.5 mg/kg) in combination with various doses of S(+)-norketamine (0.01, 0.1, 0.5 and 1 mg/kg) or R(−)-norketamine (0.5, 1 and 1.5 mg/kg) administered by the IP route. Saline served as control. Incidences of formalin-induced flinching were counted continuously in 5 min intervals for 60 min. Each rat received only one treatment.

2.6. Rotarod performance test

Motor coordination was determined using the rotarod test (Watzman et al., 1964). Rats (6/group) were trained to run on a rat rotarod apparatus (Ugo Basile, Comeno, Italy) at a constant speed (10 rev/min) for 180 s on two consecutive days. Thereafter, they were treated with morphine (3 mg/kg) and S(+)-norketamine (3 mg/kg) alone and in combination (IP). Each rat was placed on the rotarod prior to and 5, 15, 30, 60 and 120 min after injection and the time for which it was able to remain on the drum was recorded (cut-off = 180 s).

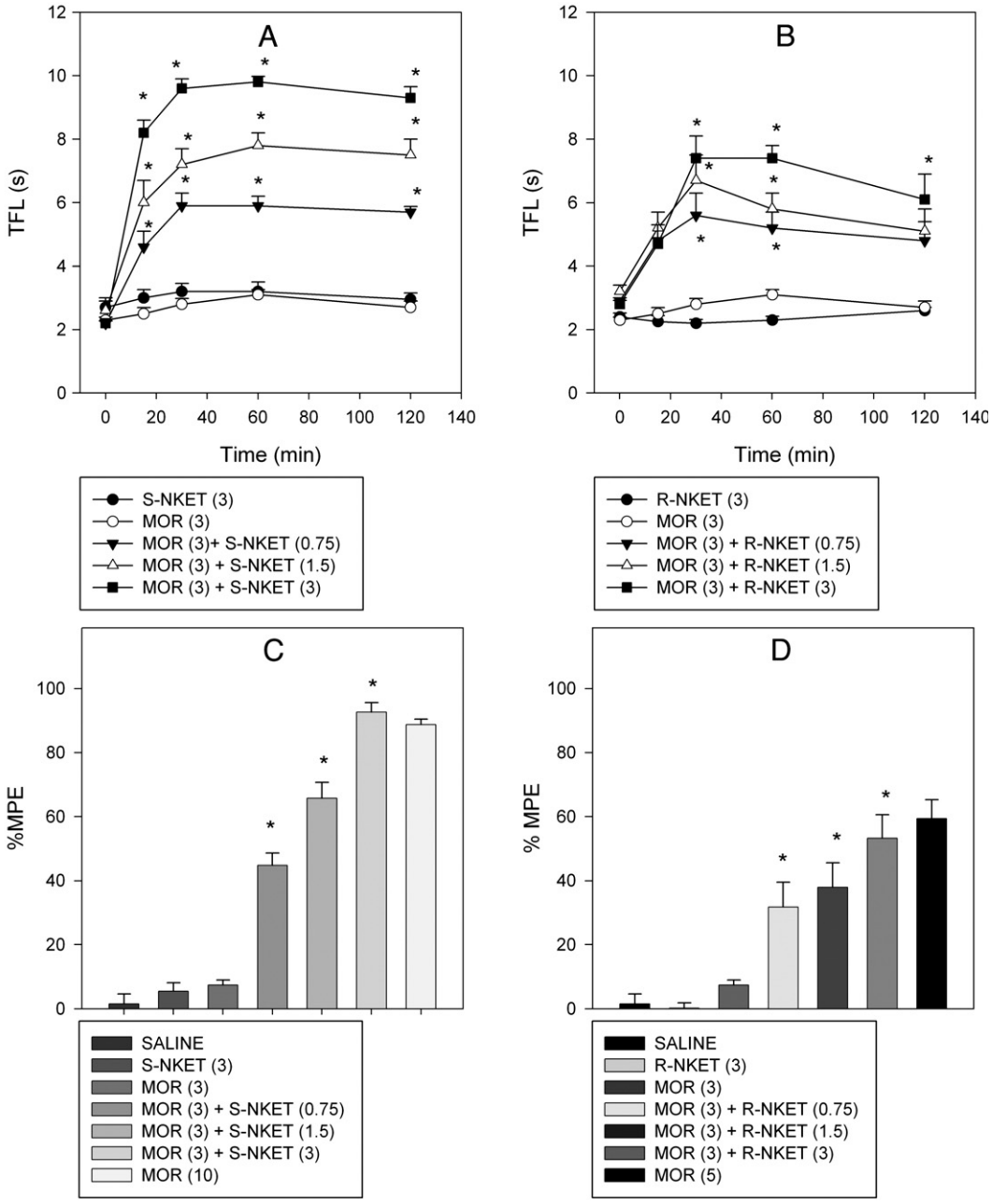

Fig. 1. Time courses of responsiveness to thermal noxious stimulus (tail-flick latency, TFL, s) following intaperitoneal (IP) administration: [Panel A] morphine (MOR) and S(+)-norketamine (S-NKET) alone and MOR in combination with S-NKET; [Panel B] MOR and R(−)-norketamine (R-NKET) alone and MOR in combination with R-NKET (the tail-flick test). Percent maximum possible effect (%MPE, at peak time): [Panel C] MOR and S-NKET alone and in combinations; [Panel D] MOR and R-NKET alone and in combinations. Saline (IP) served as control. Doses are in mg/kg, IP. Data are mean ± SEM (n = 8 rats). *Significantly different from MOR (3 mg/kg) alone (P < 0.05, post-hoc SNK).

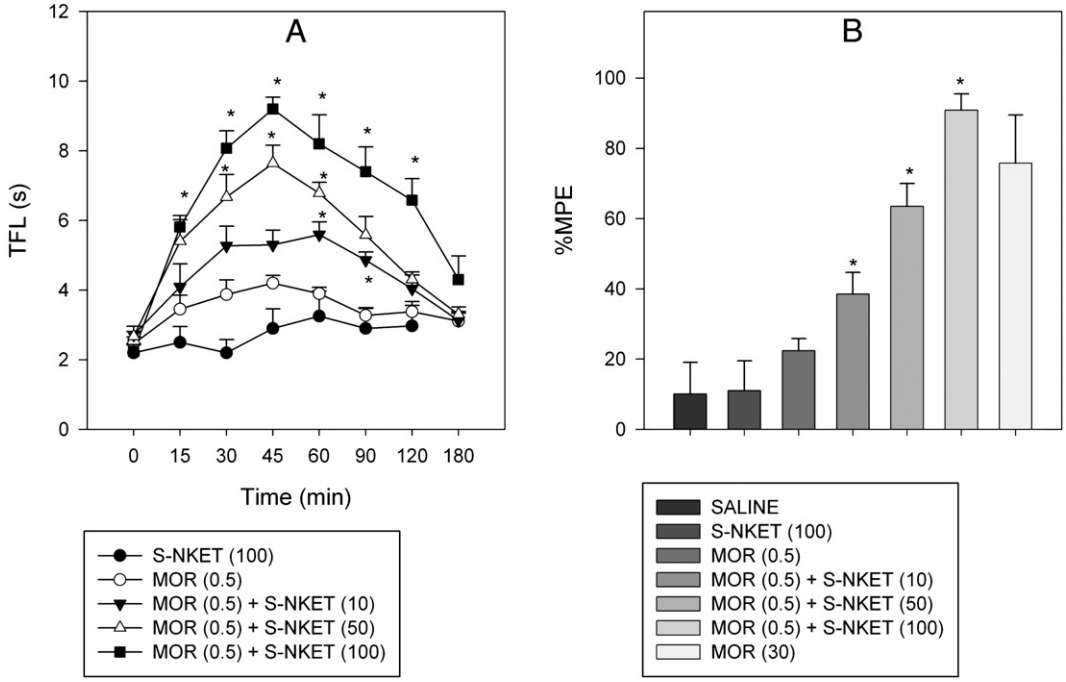

Fig. 2. [Panel A] Time courses of responsiveness (tail-flick latency, TFL, s) to thermal noxious stimulus following intrathecal (IT) administration of morphine (MOR) and S(+)-norketamine (S-NKET) alone and MOR in combination with S-NKET (the tail-flick test). [Panel B] Percent of maximum possible effect (%MPE, at peak time): MOR and S-NKET alone and in combination. Saline (IT) served as control. Doses are in μg, IT. Data are mean±SEM (n=7–9 rats). *Significantly different from MOR (0.5 μg) alone (P<0.05, post-hoc SNK).

2.7. Locomotor activity

Effects of morphine (7 mg/kg, IP) and S(+)-norketamine (3 mg/kg, IP) alone and in combination on locomotor activity (ambulation and vertical movement; 5 min epoch) were determined utilizing an Opto-Varimex infrared photocell-based activity monitor (Life Science, Columbus, OH). Rats were tested prior to (baseline) and 60 min after injection.

2.8. Statistical analysis

Responses (TFL, VT, PWT) were normalized for baseline values. Percent maximum possible effect (%MPE; antinociception) was calculated at the time of peak response: %MPE=(TFL−baseline)/(cut-off−baseline)*100. The overall effects (antihyperalgesia, antiallodynia) were presented as areas under the time curves (AUC_{0-t} calculated by the trapezoidal rule) for

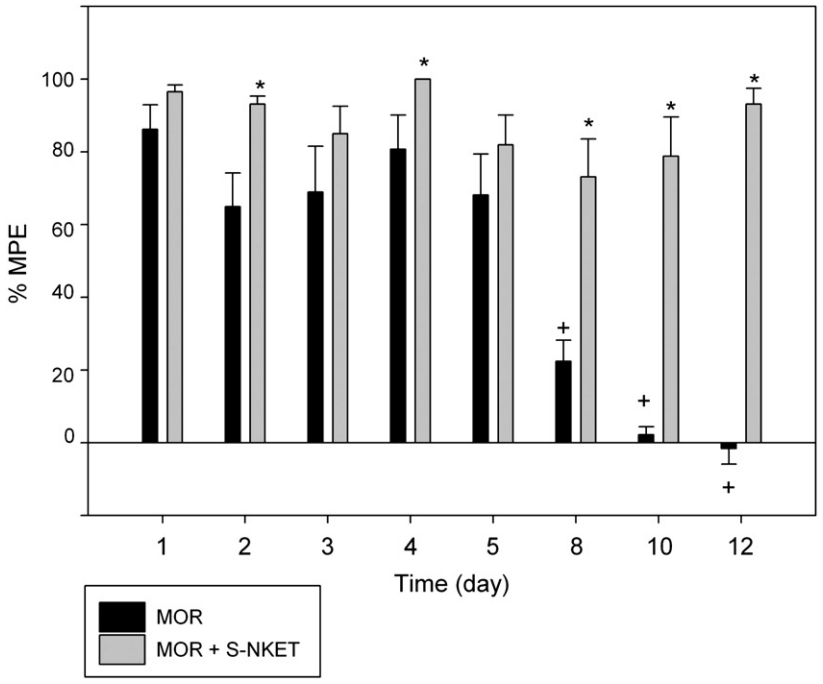

Fig. 3. Responsiveness to a thermal noxious stimulus across the time of repeated administration of morphine (MOR, 7 mg/kg) alone and in combination with S(+)-norketamine (S-NKET, 3 mg/kg) in the tail-flick test. Drugs were administered by the intraperitoneal (IP) route twice daily for 12 days. Data are presented as percentage of maximum possible effect (%MPE) and are mean±SEM (n=8 rats). *Significantly different from MOR alone (P 0.05; post-hoc SNK); +significantly different from Day 1 (P<0.05; post-hoc SNK).

baseline-normalized responses (VT, PWT). Data were analyzed using regression analysis, analysis of variance (ANOVA), post-hoc Student Newman Keuls test (SNK) and *t*-test. Level of significance was $P \leq 0.05$. All data are mean ± SEM (*n* rats).

3. Results

3.1. Effect of norketamine on morphine antinociception and tolerance (tail-flick test)

The antinociceptive effects of morphine alone and in combination with various doses of S(+)- and R(−)-norketamine (0.75–3 mg/kg) were characterized after the IP route of administration in the tail-flick test. Norketamine, in doses that did not produce measurable effects, dose-dependently enhanced the antinociceptive effect of morphine (dose: $F_{3,127} = 147.8$ and 11.7, $P < 0.0001$; time: $F_{3,127} = 18.3$ and 15.5, $P < 0.0001$; dose × time: NS and $F_{9,127} = 2.2$, $P < 0.05$ for S(+)- and R(−)-norketamine, respectively). The enhancement is evident at several points of the time course (Fig. 1A,B), as well as in the %MPE (Fig. 1C,D). The effect was more pronounced with the S(+) (Fig. 1A,C) compared to the R(−) enantiomer (Fig. 1B,D). For example, morphine (3 mg/kg) in combination with S(+)-norketamine (3 mg/kg) produced maximum possible effect (%MPE ≈ 100%). An effect of equal magnitude was achieved only after administration of an approximately three-fold higher dose (10 mg/kg) of morphine alone.

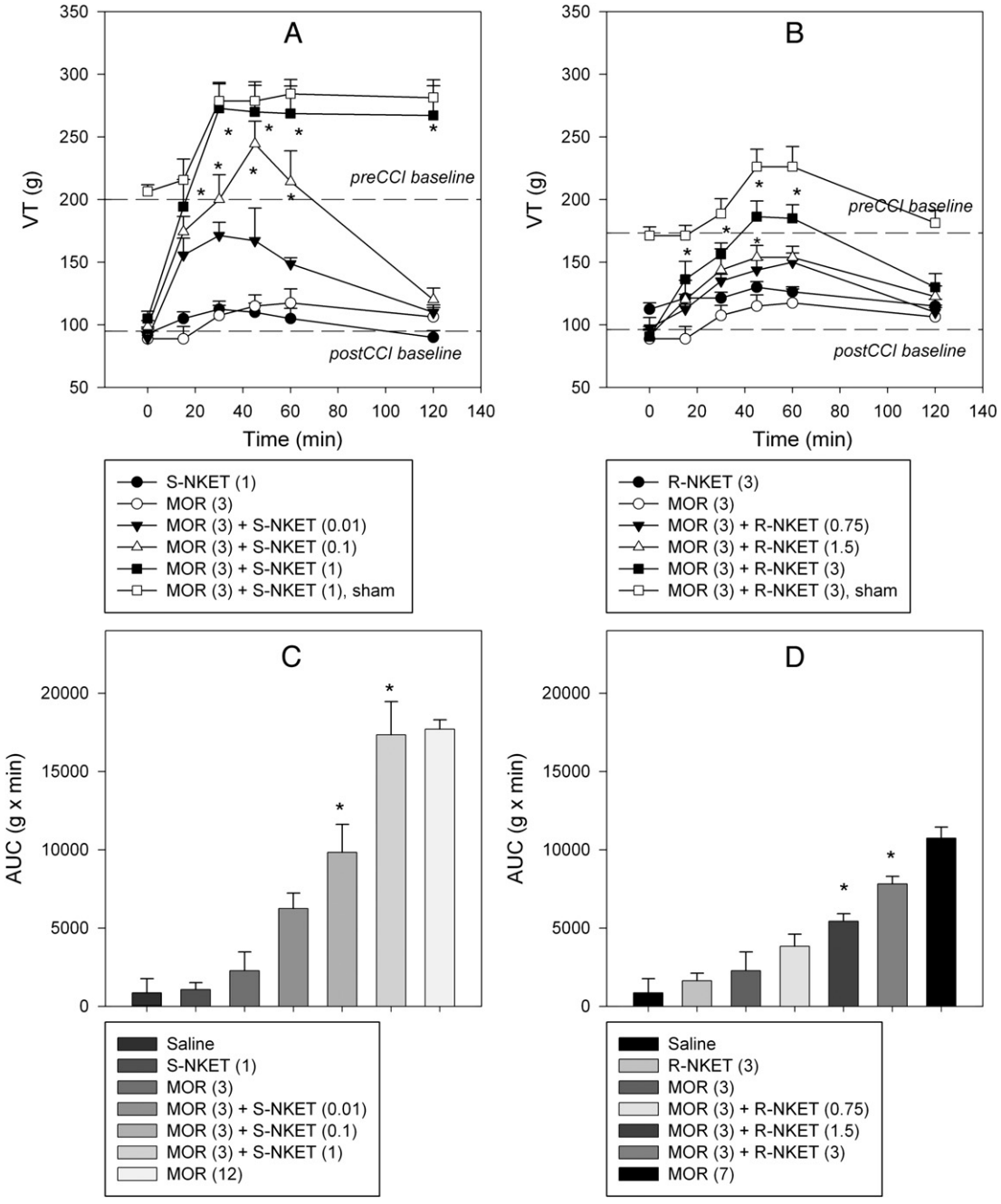

Fig. 4. Time courses of responsiveness to a mechanical noxious stimulus (vocalization threshold, VT, g) after intraperitoneal (IP) administration: [Panel A] morphine (MOR) and S(+)-norketamine (S-NKET) alone and MOR in combination with S-NKET; [Panel B] MOR and R(−)-norketamine (R-NKET) alone and MOR in combination with R-NKET (chronic constriction nerve injury, CCI, the paw pressure test). The overall effect (area under the curve 0–120 min, AUC, g × min): [Panel C] MOR and S-NKET alone and in combination; [Panel D] MOR and R-NKET alone and in combinations. Saline (IP) served as control. Doses are in mg/kg, IP. Data are mean ± SEM (*n* = 8 rats). *Significantly different from MOR (3 mg/kg) alone ($P < 0.05$, post-hoc SNK).

Morphine (3 mg/kg) in combination with R(−)-norketamine (3 mg/kg) was less effective and only produced approximately 60% MPE.

The effect of morphine in combination with S(+)-norketamine was also examined at the spinal level (IT). The time course of the effect and the %MPE are shown in Fig. 2A and B, respectively. The data showed that the antinociceptive effect of morphine was significantly enhanced, in a dose-related fashion, by S(+)-norketamine (dose: $F_{3,139}=27.4$, $P<0.001$; time: $F_{6,139}=46.6$, $P<0.001$; dose×time: $F_{18,139}=8.1$, $P<0.001$). The effect of morphine (0.5 μg) in combination with the highest dose of S(+)-norketamine (100 μg) tested produced approximately 100% MPE. This % MPE could only be achieved after administration of a sixty-fold higher dose of morphine alone (30 μg).

The effects of repeated dosing of morphine alone (IP) or in combination with S(+)-norketamine were determined, as well. These findings are presented in Fig. 3. Tolerance developed to morphine antinociception (<5% MPE) by Day 10 with repeated dosing (7 mg/kg/2×day), as shown in our previous studies (Holtman et al., 2004). Tolerance to morphine was blocked when combined with S(+)-norketamine (3 mg/kg) [treatment: $F_{1,121}=70.7$, $P<0.001$; time: $F_{7,121}=21.7$, $P<001$; treatment×time: $F_{7,121}=12.7$, $P<0.001$]. Of note, neither morphine alone nor in combination with S(+)-norketamine had an effect on the baseline responsiveness across time of chronic treatment as evidenced by preinjection values; morphine alone: TFL=2.21±0.14 s and 2.75±0.2 s; morphine and S(+)-norketamine: TFL=2.29±0.08 s and 2.53±0.09 s on Day 1 and Day 12, respectively.

3.2. Chronic constriction nerve injury (CCI)

Chronic constriction nerve injury (CCI) resulted in significantly decreased thresholds to mechanical noxious stimuli (hyperalgesia) compared to pre-surgical threshold: VT (pre-CCI)=196.2±6.3 g versus VT (post-CCI)=116.9±6.8; 97.5±4.4; 90.6±5.2 and 105.1±4.5 g on days 7, 9, 11 and 14 after CCI, respectively ($F_{4,39}=63.9$, $P<0.0001$). Sham operation had no effect on the pain threshold: VT=193.7±7.0; 196.9±5.0; 207.5±3.3; 183.1±7.9 and 181.9±6.4 g prior to and on days 7, 9, 11 and 14 after sham surgery, respectively. In addition, ligation of the sciatic nerve resulted in decreased thresholds to normally non-noxious mechanical stimulus tactile allodynia) compared to sham surgery: PWT (CCI paw)=1.05±0.15; 0.92±0.11; 0.9±0.26 and 1.08±0.75 g versus PWT (sham paw)=8.69±1.15; 8.38±1.72; 6.72±1.53 and 6.98±0.71 g on days 7, 11, 14, and 17 after surgery, respectively ($P<0.001$; *t*-test).

3.3. The antihyperalgesic effects of norketamine-morphine combination (CCI model)

The antihyperalgesic effect of morphine alone and in combination with various doses of S(+)-norketamine (0.01–1 mg/kg) and R(−)-norketamine (0.75–3 mg/kg) were characterized after the IP route of administration in the CCI model (Fig. 4). Both S(+)- and R(−)-norketamine, at doses that did not produce significant effects of their own, enhanced in dose-related manner the antihyperalgesic effect of a low dose of morphine in the CCI paw (dose: $F_{3,139}=21.4$ and $F_{3,159}=10.9$, $P<0.0001$; time: $F_{4,139}=17.4$ and $F_{4,159}=19.9$, $P<0.0001$; dose×time: $F_{12,139}=5.9$; $P<0.0001$ and NS for S(+)- and R(−)-norketamine, respectively). The enhancement is evident at several points of the time curves (Fig. 4A,B), as well as in the overall effect (AUC) (Fig. 4C,D). The effect was more pronounced with the S(+) enantiomer (Fig. 4A,C) compared to the R− enantiomer (Fig. 4B,D). For example, combining morphine (3 mg/kg) with S(+)-norketamine (1 mg/kg) resulted in an effect approximately equal to the effect produced by a four-fold higher dose of morphine alone (12 mg/kg). Morphine (3 mg/kg) in combination with R(−)-norketamine (3 mg/kg) was less effective.

3.4. The antiallodynic effects of norketamine-morphine combination (CCI model)

The antiallodynic effect of morphine in combination with S(+)-norketamine was assessed by von Frey hair (Fig. 5). S(+)-Norketamine, at doses that were not effective against tactile allodynia (2–8 mg/kg), enhanced in a dose-related manner the antiallodynic effect of a low dose morphine in the CCI paw (dose: $F_{4,174}=6.4$, $P<0.001$; time: $F_{5,174}=20.4$, $P<0.001$; dose×time: NS). This was evident at several point along the time course (Fig. 5A) and in the overall effect (AUC) (Fig. 5B). For

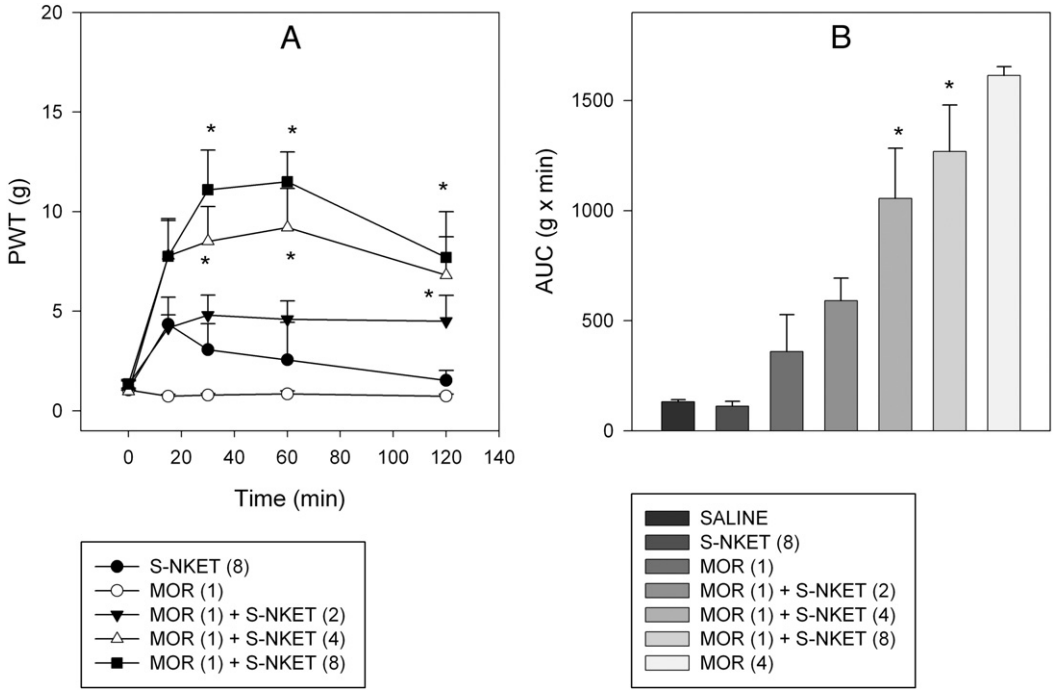

Fig. 5. [Panel A] Time courses of the responsiveness (paw withdrawal threshold, PWT, g) to initially non-noxious mechanical stimuli (tactile allodynia) following intraperitoneal administration (IP) of morphine (MOR) and S(+)-norketamine (S-NKET) alone and in combination. [Panel B] The overall effect (area under the curve 0–120 min, AUC, g×min): MOR and S-NKET alone and in combination. Saline (IP) served as control. Doses are in mg/kg, IP. Data are mean±SEM ($n=4$ rats). *Significantly different from MOR (1 mg/kg) alone ($P<0.05$, post-hoc SNK).

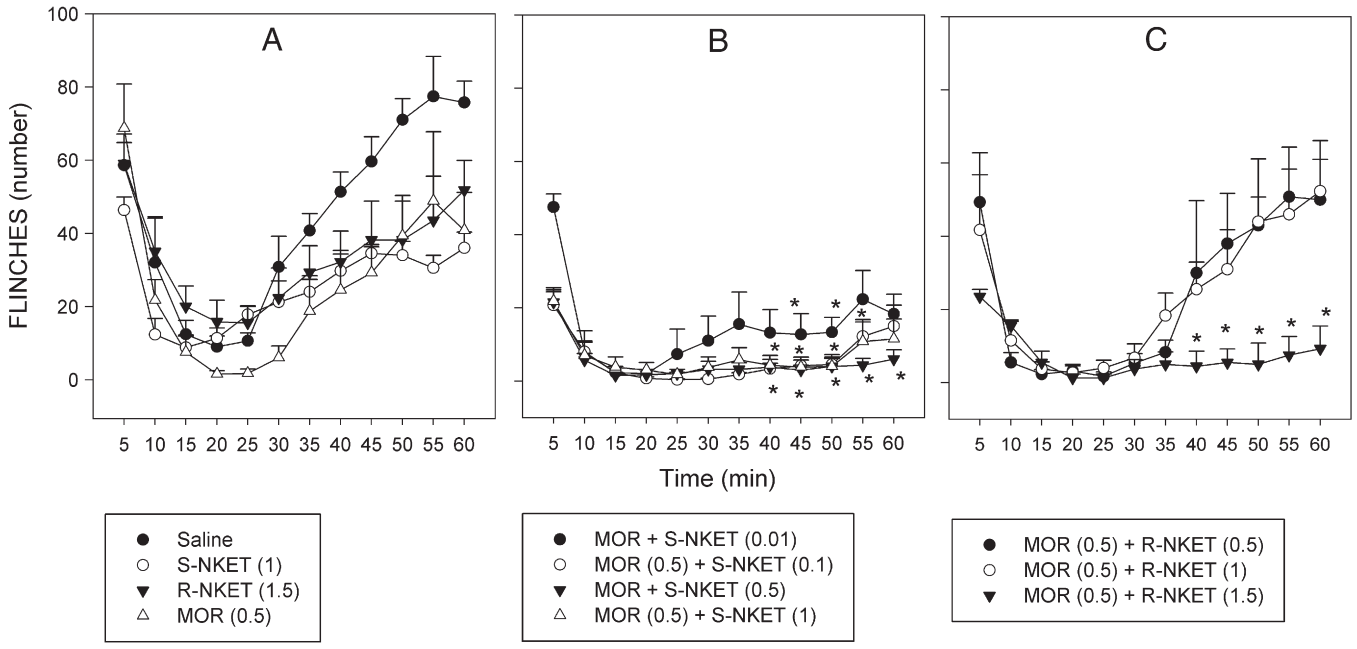

Fig. 6. Time courses of formalin-induced behavior (number of flinches/5 min epoch): [Panel A] saline (control), morphine (MOR), S(+)-norketamine (S-NKET) and R(−)-norketamine (R-NKET); [Panel B] MOR in combination with S-NKET; [Panel C] MOR in combination with R-NKET. Drugs were administered by the intraperitoneal (IP) route. Doses are in mg/kg, IP. Data are mean±SEM (n=4–7 rats/dose). *Significantly different from MOR (0.5 mg/kg) alone (post-hoc SNK, $P<0.05$).

example, the effect of morphine (1 mg/kg) in combination with S(+)-norketamine (8 mg/kg) was similar to the effect achieved by a four-fold higher dose of morphine alone (4 mg/kg).

3.5. Effect of norketamine-morphine combination in rats with formalin-induced hindpaw inflammation (the formalin test)

The formalin injection paradigm was used to assess efficacy of morphine alone and in combination with S(+)- or R(−)-norketamine in a rat model of tonic inflammatory pain (Fig. 6). Formalin-induced nociceptive behavior (flinches) consisted of two typical phases: an early phase (0–10 min; nociceptive pain) and a late phase (20–60 min; central sensitization) in control rats (see saline; Fig. 6A). The effects of morphine, S(+)-norketamine and R(−)-norketamine alone were also shown in Fig. 6A. S(+)-Norketamine (0.01–1 mg/kg) enhanced, in a dose-related fashion, the ability of morphine to block flinches in the second phase of the formalin test (dose: $F_{4,251}$=24.0, $P<0.0001$; time: $F_{8,251}$=7.5, $P<0.0001$; dose×time=$F_{32,251}$=1.6; $P<0.05$) (Fig. 6B versus A). Only the high dose of R(−)-norketamine (1.5 mg/kg) tested significantly potentiated morphine activity (dose: $F_{3,179}$=10.3, $P<0.0001$; time:

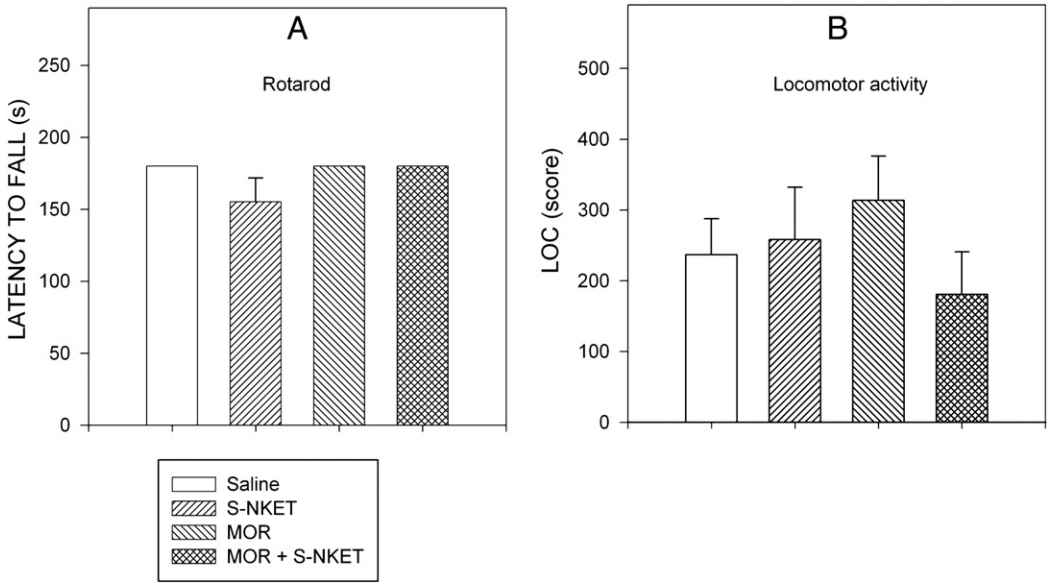

Fig. 7. [Panel A] The motor effect (latency to fall, s) of morphine (MOR, 3 mg/kg) and S(+)-norketamine (S-NKET, 3 mg/kg) alone and in combination (rotarod test). [Panel B] Locomotor activity (LOC, score/5 min) following administration of MOR (7 mg/kg) and S-NKET (3 mg/kg) alone and in combination. Drugs were administered by the intraperitoneal (IP) route. Saline (IP) served as control. Data are mean±SEM (n=6–8 rats).

$F_{8,179}$ = 10.7, P < 0.0001; dose × time: NS) (Fig. 6C versus A). Effect was less pronounced in the first phase in this test.

3.6. Effect of norketamine and morphine combination on motor coordination (rotarod performance test)

Morphine (3 mg/kg) in combination with S(+)-norketamine (3 mg/kg) had no effect on motor coordination in rats. This was evident by the time spent on rotarod equal to cut-off value (180 s) (Fig. 7A). Of note, at these (or lower) doses of morphine and S(+)-norketamine combination therapy maximum effects were achieved in the tail-flick, CCI and formalin models (see Figs. 1, 4 and 6).

3.7. Effect of norketamine on the locomotor effect of morphine

Locomotor activity was not significantly affected by morphine (7 mg/kg) in combination with S(+)-norketamine (3 mg/kg) (Fig. 7B).

4. Discussion

The major findings of the present study are that a primary metabolite of ketamine, norketamine [the S(+) enantiomer to a greater extent than the R(−) enantiomer]: 1) enhances morphine antinociception in an acute pain model 2) attenuates the development of tolerance to this effect; and 3) increases the antinociceptive effectiveness of morphine in rodent pain models involving central sensitization (i.e. injury to nerve or tonic inflammatory pain). Importantly, maximum analgesia is achieved without the side effects commonly seen with higher doses of morphine and/or NMDA-receptor antagonist. The present data suggest that norketamine, in particular the S(+) enantiomer, may be useful in combination with morphine for treatment of pain of differing etiologies including acute nociceptive, neuropathic and persistent inflammatory pain.

Norketamine, in a dose range with no antinociceptive activity, significantly enhanced the antinociceptive effect of low doses of morphine in the tail-flick test. The spinal cord is an important site of action as shown by the pronounced enhancement of morphine antinociceptive effects by S(+)-norketamine following intrathecal administration of the drugs. This finding is consistent with the report of Joo et al. (2000) showing a greater enhancement of morphine (IT) antinociception by S- versus R-ketamine in rats. Previously we found an ED_{50} value for morphine of 2.63 ± 0.72 mg/kg (IP) in the tail-flick test (Holtman and Wala, 2005). In the present study, maximum antinociception was achieved when morphine (3 mg/kg, IP) was combined with a dose of S(+)-norketamine (3 mg/kg, IP) that had no antinociceptive activity by itself. Whether this effect is synergistic will need to be resolved with isobolographic analysis.

Another important aspect of combining morphine with S(+)-norketamine was evident as inhibition of the development of antinociceptive tolerance with repeated morphine dosing (the tail-flick test). This finding is in agreement with the notion that the NMDA-receptor plays a significant role in the mechanism of opioid tolerance (Mao, 1999, review). A number of studies, both from our laboratory and others, present abundant evidence that blockade of the NMDA-receptor (i.e. MK-801, ketamine, dextromethorphan) results in enhancement and prolongation of opioid antinociception in rodents (Belozertseva et al., 2000; Craft and Lee, 2005; Grass et al., 1996; Kozela et al., 2001; Holtman et al., 2003; Nadeson et al., 2002; Nemmani et al., 2004; Plesan et al., 1998; Shimoyama et al., 1996). However, data from clinical studies with ketamine indicate that it (sub-anesthetic dose) reduces opioid consumption in the perioperative setting but is often poorly tolerated due to the side effects (Elia and Tramer, 2005; Subramaniam et al., 2004; Sveticic et al., 2005, reviews).

Both hyperalgesia and allodynia demonstrated in rodents following nerve injury (i.e. chronic constriction injury of sciatic nerve, CCI) (Bennett and Xie, 1988) or tonic inflammation (i.e. formalin injection) (Chaplan et al., 1997) likely result from central sensitization which is thought to be dependent on sustained activation of the NMDA-receptor complex (Dickenson, 1997). The decreased efficacy of opioids in neuropathic pain states is also thought to be related to the NMDA-receptor-mediated cascade of events triggered by nerve/tissue injury (Ballantine and Mao, 2003, review). The present data showed that norketamine, at doses which are not antihyperalgesic or antiallodynic alone, increased the effects of morphine against mechanical hyperalgesia and tactile allodynia in rats with peripheral neuropathy (CCI). In addition, it also enhanced morphine action to block formalin-induced flinching behaviors in rats with tonic inflammatory pain (2nd phase of the formalin test). Again, such interactions were more pronounced for S(+)- than R(−)-norketamine. To the best of our knowledge, no similar studies have been carried out on optical isomers of ketamine in combination with opioids in the above rodent models of persistent pain with a component of central sensitization. Nevertheless, previous data showed that NMDA-receptor antagonists [i.e. MK-801, (±)-ketamine, dextromethorphan] improve the effectiveness of opioids in the CCI and formalin models of pain (Kauppila et al., 1998; Nishiyama, 2000; Pelissier et al., 2003; Yamamoto and Yaksh, 1992), as well as recently, that dextromethorphan enhances morphine antihyperalgesia in a capsaicin model of persistent pain (Lomas et al., 2008). Limited clinical data also showed the benefits of using ketamine (sub-anesthetic dose) in the course of opioid therapies in patients with chronic pain (cancer-related, non-malignant) (Bell et al., 2003; Hocking and Cousins, 2003; Lauretti et al., 1999, Lossignol et al., 2005, Leung et al., 2001), as well as experimentally-induced pain involving central sensitization (i.e. skin burn) (Lemming et al., 2007; Schulte et al., 2004).

An important finding with the present study was that the S(+)-norketamine and morphine combination therapy did not cause motor dysfunction (rotarod performance) at doses that achieved maximum effectiveness against thermal nociception, mechanical hyperalgesia and formalin-induced flinches. This suggests that there may be a potentially acceptable therapeutic index for the combination therapy. In addition, no overt toxicity was observed with the rats shown by lack of sedation, PCP-like stereotypic behaviors (i.e. head-weaving, turning) or lethality with this combination drug therapy at the antinociceptive doses tested. In contrast, a morphine and ketamine combination at doses that were not effective for either drug alone (loss of righting reflex), resulted in marked catalepsy in rats (Benthuysen et al., 1989; Campos et al., 2006). In addition, the S(+)-norketamine and morphine combination therapy had no significant effect on locomotor activity; an effect similar to morphine and dextromethorphan (Manning et al., 1996). Psychotomimetic activity which can be an important dose-limiting side effect of NMDA-receptor antagonists (e.g. ketamine) was not specifically tested in the present study.

In conclusion, the present data provide evidence that combining morphine (low dose) with S(+)-norketamine (non-effective dose) significantly enhances morphine effectiveness against acute nociception, peripheral neuropathy and tonic inflammatory pain without causing measurable side effects (rotarod, locomotor activity) in rodents. These preclinical findings may have potential clinical application utilizing S(+)-norketamine and morphine combination therapy for treatment of a broad spectrum of pain. This may be of importance in view of the lack of viable NMDA-receptor antagonists (efficacy/side effect profile) that are currently available for clinical use. However, this will require verification through clinical studies.

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References

Ballantine JC. Opioids for chronic nonterminal pain. South Med J 2006;99:1245–55.
Ballantine GJ, Mao J. Opioid therapy for chronic pain. N Eng J Med 2003;13:1943–53.
Belozertseva IV, Dravolina OA, Neznanova ON, Danysz W, Bespalov AY. Antinociceptive activity of combination of morphine and NMDA receptor antagonists depends on the inter-injection interval. Eur J Pharmacol 2000;396:77–83.
Bell R, Eccleston C, Kalso E. Ketamine as an adjuvant to opioids for cancer pain. A qualitative systemic review. J Pain Symptom Manage 2003;26:867–75.
Bennett G. Update on the neurophysiology of pain transmission and modulation: focus on the NMDA receptor. J Pain Symptom Manage 2000;19:2–9.
Bennett G, Xie Y. A peripheral mononeuropathy in rat that produced disorders of pain sensation like those seen in man. Pain 1988;33:87–107.
Benthuysen JL, Hance AJ, Quam DD, Winters WD. Comparison of isomers of ketamine on catalepsy in the rat and electrical activity of the brain and behavior in the cat. Neuropharmacology 1989;28:1003–9.
Campos AR, Santos FA, Rao VS. Ketamine-induced potentiation of morphine analgesia in rat tail-flick test: role of opioid-, alpha2-adrenoceptors and ATP-sensitive potassium channels. Biol Pharm Bull 2006;29:86–9.
Celerier E, Rivat C, Jun Y, Laulin JP, Larcher A, Reynier P, et al. Long-lasting hyperalgesia induced by fentanyl in rats: preventive effect of ketamine. Anesthesiology 2000;92:465–72.
Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994;53:55–63.
Chaplan SR, Malberg AB, Yaksh TL. Efficacy of spinal NMDA receptor antagonism in formalin hyperalgesia and nerve injury evoked allodynia in the rat. J Pharm Exp Ther 1997;280:829–38.
Craft RM, Lee DA. NMDA antagonist modulation of morphine antinociception in female and male rats. Pharm Biochem Behav 2005;80:639–40.
D'Amour FE, Smith DL. A method of determining loss of pain sensation. J Pharmacol Exp Ther 1941;27:74–9.
Dickenson A. Mechanism of central hypersensitivity; excitatory amino acids mechanisms and their control. In: Dickenson AH, Besson JM, editors. The pharmacology of pain. Berlin: Springer Verlag; 1997. p. 167–210.
Ebert B, Mikkalsen S, Thorkildsen C, Borgbjerg F. Norketamine, the main metabolite of ketamine, is non-competitive NMDA receptor antagonist in the rat cortex and spinal cord. Eur J Pharmacol 1997;333:99–104.
Elia N, Tramer MR. Ketamine and postoperative pain — a qualitative systemic review of randomized trials. Pain 2005;113:61–70.
Gilron I, Max MB. Combination pharmacotherapy for neuropathic pain: current evidence and future directions. Expert Rev Neurotherap 2005;5:823–30.
Grass S, Hoffman O, Xu XJ, Wiesenfeld-Halin Z. *N*-methyl-D-aspartate receptor antagonists potentiate morphine antinociceptive effect in the rat. Acta Physiol Scand 1996;158:269–73.
Harden RN. Chronic opioid therapy: another reappraisal. ASP Bull 2002;12:8–12.
Hocking G, Cousins MJ. Ketamine in chronic pain management: an evidence-based review. Anesth Analg 2003;97:1730–9.
Holtman Jr JR, Wala EP. Characterization of morphine-induced hyperalgesia in male and female rats. Pain 2005;114:62–70.
Holtman Jr JR, Jing X, Wala EP. Sex-related differences in the enhancement of morphine antinociception by NMDA antagonists in rats. Pharm Biochem Behav 2003;76:285–93.
Holtman Jr JR, Sloan JW, Wala EP. Morphine tolerance in male and female rats. Pharm Biochem Behav 2004;77:517–23.
Holtman Jr JR, Crooks PA, Johnson J, Wala EP. NMDA receptor antagonists for chronic pain, can we do better than ketamine? PGA NYSSA; 2007. p. 9015.
Kalso E. Improving opioid effectiveness: from ideas to evidence. Eur J Pain 2005;9:131–5.
Kauppila T, Xu XJ, Yu W, Wiesenfeld-Hallin Z. Dextromethorphan potentiates the effect of morphine in rats with peripheral neuropathy. NeuroReport 1998;9:1071–4.
Kozela E, Danysz W, Popik P. Uncompetitive NMDA receptor antagonist potentiate morphine antinociception recorded from the tail but not from the hind paw in rats. Eur J Pharmacol 2001;423:17–26.
Joo G, Horvath G, Klimscha W, Kekesi G, Dobos I, Szikszay M, et al. The effects of ketamine and its enantiomers on the morphine- or dexmedetomidine-induced antinociception after intrathecal administration in rats. Anesthesiology 2000;93:231–41.
Laulin JP, Maurette P, Corcuff JB, Rivat C, Chauvin M, Simonnet G. The role of ketamine in preventing fentanyl-induced hyperalgesia and subsequent acute morphine tolerance. Anesth Analg 2002;94:1263–9.
Lauretti GR, Gomes JM, Reis MP, Pereira NL. Low doses of epidural ketamine or neostygmine but not midazolam improve morphine analgesia in epidural terminal cancer pain therapy. J Clin Anesth 1999;11:663–8.
Lemming D, Sorensen J, Graven-Nielsen T, Lauber R, Arendt-Nielsen L, Gerdle B. Managing chronic whiplash associated with a combination of low dose opioid (remifentanil) and NMDA-antagonist (ketamine). Eur J Pain 2007;11:719–32.
Leung A, Wallace MS, Rigeway B, Yaksh T. Concentration-effect relationship of intravenous alfentail and ketamine on peripheral neurosensory thresholds, allodynia and hyperalgesia of neuropathic pain. Pain 2001;91:177–87.
Lomas LM, Terner JM, Picker MJ. Sex differences in NMDA antagonist enhancement of morphine antihyperalgesia in a capsaicin model of persistent pain: comparisons to two models of acute pain. Pharmacol Biochem Behav 2008;89:127–36.
Lossignol DA, Obiols-Portis M, Body JJ. Successful use of ketamine for intractable cancer pain. Support Care Cancer 2005;13:188–93.
Mao J. NMDA and opioid receptors: their interaction in antinociception, tolerance and neuroplasticity. Brain Research Rev 1999;30:289–304.
Mao J. Opioid-induced abnormal pain sensitivity: implications in clinical therapy. Pain 2002;100:213–7.
Manning BH, Mao J, Frenk H, Price DD, Mayer DJ. Continuous co-administration of dextromethorphan or MK-801 with morphine: attenuation of morphine dependence and naloxone-reversible attenuation of morphine tolerance. Pain 1996;67:79–88.
McCartney CJ, Sinha A, Katz J. A quantitative systemic review of the role of *N*-methyl-D-aspartate receptor antagonists in preventive analgesia. Anesth Analg 2004;98:1385–400.
Nadeson R, Tucker A, Bajunaki E, Goodchild CS. Potentiation by ketamine of fentanyl antinociception. An experimental study in rats showing that ketamine administered by non-spinal routes targets spinal cord antinociceptive systems. Br J Anesth 2002;88:685–91.
Nemmani KV, Grisel JE, Stowe JR, Smith-Carliss R, Mogil JS. Modulation of morphine analgesia by site-specific *N*-methyl-D-aspartate receptor antagonists: dependence on sex, site of antagonism, morphine dose, and time. Pain 2004;109:274–83.
Nishiyama T. Interaction between intrathecal morphine and glutamate receptor antagonists in formalin test. Eur J Pharmacol 2000;305:203–10.
Pelissier T, Laurido C, Kramer V, Hernandez A, Paeile C. Antinociceptive interactions of ketamine with morphine or methadone in mononeuropathic rats. Eur J Pharmacol 2003;477:23–8.
Plesan A, Hedman U, Xu XJ, Wissenfeld-Hallin Z. Comparison of ketamine and dextromethorphan in potentiating the antinociceptive effect of morphine in rat. Anesth Analg 1998;86:825–9.
Prince DD, Mayer DJ, Mao J. NMDA-receptor antagonists and opioid receptor interactions as related to analgesia and tolerance. J Pain Symptom Manage 2000;19:S7–11.
Randall L, Selitto J. A method for measurement of analgesic activity on inflamed tissue. Arch Int Pharmacodyn Ther 1957;111:409–19.
Schulte H, Sollevi A, Segerdahl M. The synergistic effect of combined treatment with systemic ketamine and morphine on experimentally induced windup-like pain in humans. Anesth Analg 2004;98:1574–80.
Shimoyama N, Shimoyama M, Intrussi CE, Elliott KJ. Ketamine attenuates and reverses morphine tolerance in rodents. Anesthesiology 1996;85:1357–66.
Subramaniam K, Subramaniam B, Steinbrook R. Ketamine as adjuvant analgesic to opioids: a quantitative and qualitative systematic review. Anesth Analg 2004;99:482–95.
Sveticic G, Eichenberger U, Curatolo M. Safety of morphine with ketamine for postoperative patient-controlled analgesia: an audit with 1026 patients. Acta Anesthosiol Scand 2005;49:870–5.
Van Elstraete AC, Sitbon P, Trabold F, Mazoit JX, Benhamou D. A single dose of intrathecal morphine in rats induces long-lasting hyperalgesia: the protective effect of prior administration of ketamine. Anesth Analg 2005;101:1750–6.
Visser E, Schug SA. The role of ketamine in pain management. Biomed Pharmacotherapy 2006;60:341–8.
Watzman N, Barry H, Buckley JP, Kinnard WJ. Semiautomatic system for timing rotarod performance. J Pharm Sci 1964;53:14–29.
Wheeler-Aceto H, Cowan A. Standardization of the rat paw formalin test for evaluation of analgesics. Psychopharmacol 1991;104:35–44.
White JM, Ryan CF. Pharmacological properties of ketamine. Drug Alcohol Rev 1996;15:145–55.
Wiesenfeld-Hallin Z. Combined opioid-NMDA antagonist therapies. Drugs 1998;56:1–4.
Yaksh TL, Rudy TA. Chronic catheterization of the spinal subarachnoid space. Physiol Behav 1976;17:1031–6.
Yamamoto T, Yaksh TL. Studies on the spinal interaction of morphine and the NMDA antagonist MK-801 on the hyperesthesia observed in a rat model of sciatic mononeuropathy. Neurosci Lett 1992;135:67–70.