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Effects of norketamine enantiomers in rodent models of persistent pain

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ABSTRACT

NMDA-receptor antagonists are potential drugs for chronic pain treatment, in particular for neuropathic pain involving central sensitization processes. Clinical use of available NMDA antagonists, such as ketamine, is limited for this indication due to its side effects (psychotomimetic, sedative, motor). There is a need for novel NMDA-receptor antagonist(s) with better analgesia/toxicity profile(s). One such potential candidate is norketamine, a primary metabolite of ketamine. S(+) and R(−)norketamine were characterized utilizing rodent models of persistent pain: the chronic constriction nerve injury model of peripheral neuropathy (CCI) and the formalin-injection model of tonic inflammatory pain (formalin test). Side effects (motor coordination, stereotypic behaviors, locomotor activity) were also assessed. (±)Ketamine served as a reference NMDA-receptor antagonist in some studies. Norketamine alleviated, in a dose-dependent fashion, mechanical and thermal hyperalgesia (CCI), and blocked formalin-induced flinches (2nd phase). It had less effect on tactile allodynia (CCI). Efficacy was demonstrated after parenteral and oral administration. The antinociceptive properties resided primarily in the S(+) enantiomer. Antinociception was not accompanied by significant side effects. The present findings suggest that norketamine, in particular the S(+) enantiomer, might be a useful NMDA-receptor antagonist for treatment of chronic pain involving central sensitization.

1. Introduction

Chronic pain (malignant, non-malignant origin) which affects millions of people worldwide is a significant health problem. In many patients it is inadequately managed with currently available drugs (i.e. opioids, NSAIDs, and various adjuvant agents). The treatment of neuropathic pain, which occurs as a result of damage to the nervous system due to many diverse causes, is an especially challenging task (Johnson, 2000, review). Neuropathic pain is characterized by several abnormal sensations including hyperalgesia (an exaggerated response to a noxious stimulus) and allodynia (a painful response to a normally non-noxious stimulus). These sensations are associated with repetitive firing of pain fibers resulting in central sensitization likely involving an *N*-methyl-D-aspartate (NMDA)-receptor-mediated mechanism (Bennett, 2000; Parson, 2001). Hyperalgesia and allodynia, demonstrated in rodent models following injury to nerve (Bennett and Xie, 1988) or peripheral tissue (Chaplan et al., 1997), have been shown to be alleviated by NMDA-receptor antagonists (e.g. ketamine, MK-801, memantine, dextromethorphan) (Chaplan et al., 1997; Mao et al., 1993; Yamamoto and Yaksh, 1992). These preclinical findings suggest that drugs acting as antagonists at the NMDA-receptor complex are likely promising candidates for use in treatment of neuropathic pain (Parson, 2001; Sang, 2000; Fisher et al., 2000, reviews).

Ketamine, a non-competitive NMDA-receptor antagonist and an intravenous anesthetic, is the primary drug that has been studied in the above regard. At sub-anesthetic doses, ketamine produces profound analgesia for neuropathic pain syndromes in humans; however, undesirable side effects, including sedation, psychosis and motor impairment, limit its practical use for this indication (Backonja et al., 1994; Eide et al., 1994; White et al., 1980). Of interest is that ketamine is extensively metabolized to *N*-desmethylketamine, norketamine (Grant et al., 1981; Yanagihara et al., 2003), which also binds to the phencyclidine (PCP) site on the NMDA-receptor complex; however, with lower affinity than ketamine (Ebert et al., 1997). Thus, it is appealing to assume that norketamine itself may retain the analgesic activity of ketamine with perhaps less likelihood for PCP-like side effects. In the USA ketamine is used clinically as a racemic mixture; however, each enantiomer also exhibits pharmacological

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activity (Schuttler et al., 1997). The potential advantages of using an enantiomer rather than a racemate may include a better separation between efficacy and toxicity. This has been demonstrated for S-ketamine versus (±)-ketamine (hypnosis) in humans and rodents (Liu et al., 2006; Marietta et al., 1977; Ryder et al., 1978; White et al., 1980). In this respect, norketamine also exists in S(+) and R(−) enantiomeric forms (Ebert et al., 1997).

The purpose of this study was to characterize the antinociceptive properties of S(+)- and R(−)-norketamine in two well-established rodent models of persistent pain involving central sensitization: the chronic constriction nerve injury model of neuropathic pain (CCI) and the formalin model of tonic inflammatory pain (the formalin test). Side effects, including motor coordination, stereotypic behaviors and locomotor activity were also assessed. (±)-Ketamine was tested as a reference NMDA-receptor antagonist in selected experimental paradigms. This preclinical study will assist in determining the potential of norketamine as a novel NMDA-receptor antagonist for the treatment of neuropathic pain.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (~350 g; Harlan, Indianapolis, IN) were used in this study. Rats were housed in a humidity- and temperature-controlled facility (lights on 0600–1800 h). Each rat was housed separately in a transparent cage with a sawdust-covered floor and with free access to standard laboratory chow and tap water. All experiments were conducted during the light phase of the cycle. Experiments were performed by a trained observer blind to the experimental conditions. All rats were handled and trained in the test situation before initiation of the procedure. Body weights were determined on the day of experimentation. At the end of the experiment, rats were euthanized with pentobarbital sodium (150 mg/kg). A cross-over paradigm was used (if possible) to minimize the number of rats. Experiments were performed according to a protocol approved by the University of Kentucky Animal Care and Use Committee and were carried out in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No.85-23, revised 1985).

2.2. Drugs

S(+)-Norketamine hydrochloride and R(−)-norketamine hydrochoride were provided by Yaupon Therapeutics, Inc. (Radnor, PA). (±)-Ketamine hydrochloride was from Sigma-Aldrich (St. Louis, MO). Drugs were dissolved in saline and administered by the intraperitoneal (IP) or oral (PO) routes (1 ml/kg). Doses refer to salt forms.

2.3. Chronic constriction nerve injury (CCI)

Unilateral peripheral mononeuropathy was produced on the left hind limb according to the method described by Bennett and Xie (1988). Briefly, under pentobarbital anesthesia (40 mg/kg, IP) ligation to the sciatic nerve and sham surgery were performed in each rat on the left and right hind paws, respectively. Proximal to the sciatic trifurcation, the 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, barely constricting the diameter of the nerve. In sham surgery, the right sciatic nerve was exposed using the same procedure, but the nerve was not ligated. The incision was closed in layers with silk thread 3.0. Rats showed a mild aversion of the affected paw and a mild degree of foot drop. No severe motor impairment was observed.

2.3.1. Mechanical hyperalgesia

The presence of a decreased threshold to mechanical noxious stimuli (mechanical hyperalgesia) was evaluated by the Randal and Selitto (1957) method (paw pressure test) in CCI rats. Briefly, the hind paw was placed between a flat surface and a blunt pointer in the Basile Analgesimeter (UGO Basile, Italy) and increasing pressure (32 g/s) was applied to the dorsal side of the paw. Vocalization was used as the end point (vocalization threshold, VT, g). Experiments were carried out at days 7, 9, 11 and 14 after surgery. At this time the abnormal pain behavior was at a stable maximum. The nerve-injured and sham-operated paws were tested alternatively in each rat. Responses were assessed prior to (baseline, taken twice) and at 15, 30, 45, 60 and 120 min after injection. Each rat received four doses (1, 2, 4, 8 mg/kg) of a drug [S(+)-norketamine, R(−)-norketamine or (±)-ketamine] at 48 h intervals by the IP route. The order of doses was balanced by the double block Latin square design [2×(4×4)]. Additional rats received S(+)-norketamine (16 mg/kg, IP) or R(−)-norketamine (16 and 32 mg/kg, IP). Control rats were treated with saline. In addition, S(+)-norketamine (10, 20, 40, 80 mg/kg; randomized doses) was administered orally (1 ml/kg) with the use of a gavage feeding needle after overnight fasting.

2.3.2. Thermal hyperalgesia

Decreased threshold to thermal noxious stimuli (thermal hyperalgesia) was determined by the plantar test in CCI rats (Hargreaves et al., 1988). Radiant heat (60% intensity) was positioned under the glass floor directly beneath the plantar hind paw in the Plantar Stimulator Analgesia Meter (IITC, Life Science). Latency to paw withdrawal (paw withdrawal threshold, PWT, s) from the heat source was measured. A cut-off at 20 s prevented tissue damage. Each rat received four doses of S(+)-norketamine (1, 2, 4, 8 mg/kg; randomized; IP) at days 7, 9, 11 and 14 after surgery. Control rats were injected with saline.

2.3.3. Tactile allodynia

The presence of a painful response to a normally non-noxious stimulus (tactile allodynia) was determined in CCI rats, as previously described (Chaplan et al., 1994). Each rat was confined under a Plexiglas box on a raised platform of wire mesh. Von Frey filaments (Stoelting, Wooddale, IL) with incremental stiffness (0.4–15 g) were presented to the ventral surface of the injured and sham-operated paw and held there for 6–8 s. A positive response was noted by sharp paw withdrawal or flinching. If no response was elicited by the initially selected filament, a stronger stimulus was presented. S(+)-Norketamine (2, 8, 16, 32 mg/kg; randomized doses) was administered by the IP route on days 7, 11,

Table 1
Behavioral rating scale (Sturgeon et al., 1979, with minor modification)

Rating	Description of behavior
Activity level	
0	Activity no different from control
−1	Moderate activity (25% stationary; walking with intermittent passes)
−2	Low activity (75% stationary)
−3	No activity (100% of the time stationary)
Stereotypic behaviors	
0	Repetitive movement no different from control
1	Head bobbing, sniffing
2	Swinging head side to side, mouth chattering
3	Shaking, twitching, weaving
Ataxia	
0	Coordinated movement, no different from control
1	Loss of balance when rearing, jerky movement
2	Cannot move beyond a restricted area, frequent falling with attempted walking
3	Unable to walk

Table 2
The development of mechanical hyperalgesia, thermal hyperalgesia, and tactile allodynia after chronic constriction nerve injury (CCI)

CCI Model	Response	Time after surgery (day)				
		Baseline	7	9	11	14
Mechanical hyperalgesia	VT (g)	209.1±5.69	113.5*±17.65	86.7*±11.23	82.8*±5.33	92.1*±6.44
Thermal hyperalgesia	PWT(s)	6.5±0.57	4.1*±0.32	4.0*±0.24	3.7*±0.25	3.9*±0.26
CCI Model	Response	Paw	Time after surgery (day)			
			7	11	14	17
Tactile allodynia	VFT (g)	CCI	1.24**±0.09	1.14**±0.16	0.98**±0.08	0.86**±0.03
		Sham	7.59±0.36	7.73±0.38	7.38±0.64	7.54±0.072

Decrease in vocalization threshold (VT, g), paw withdrawal threshold (PWT, s), and von Frey threshold (VFT, g) in response to mechanical and thermal noxious stimuli and non-noxious mechanical stimulus, respectively (control rats). Data are mean±SEM (n=4–8 rats). *Significantly different than pre-CCI baseline (P<0.05; post-hoc SNK); **significantly different than sham paw (P<0.001; t-test).

14 and 17 of recovery from surgery. The von Frey thresholds (VFT, g) were assessed in CCI and sham-operated paws prior to and 5, 15, 30, 45, 60 and 120 min after injection. Saline served as control.

2.4. Formalin-induced flinches (the formalin test)

The formalin test was performed in intact rats as previously described (Wheeler-Aceto and Cowan, 1991). A 50 µl volume of

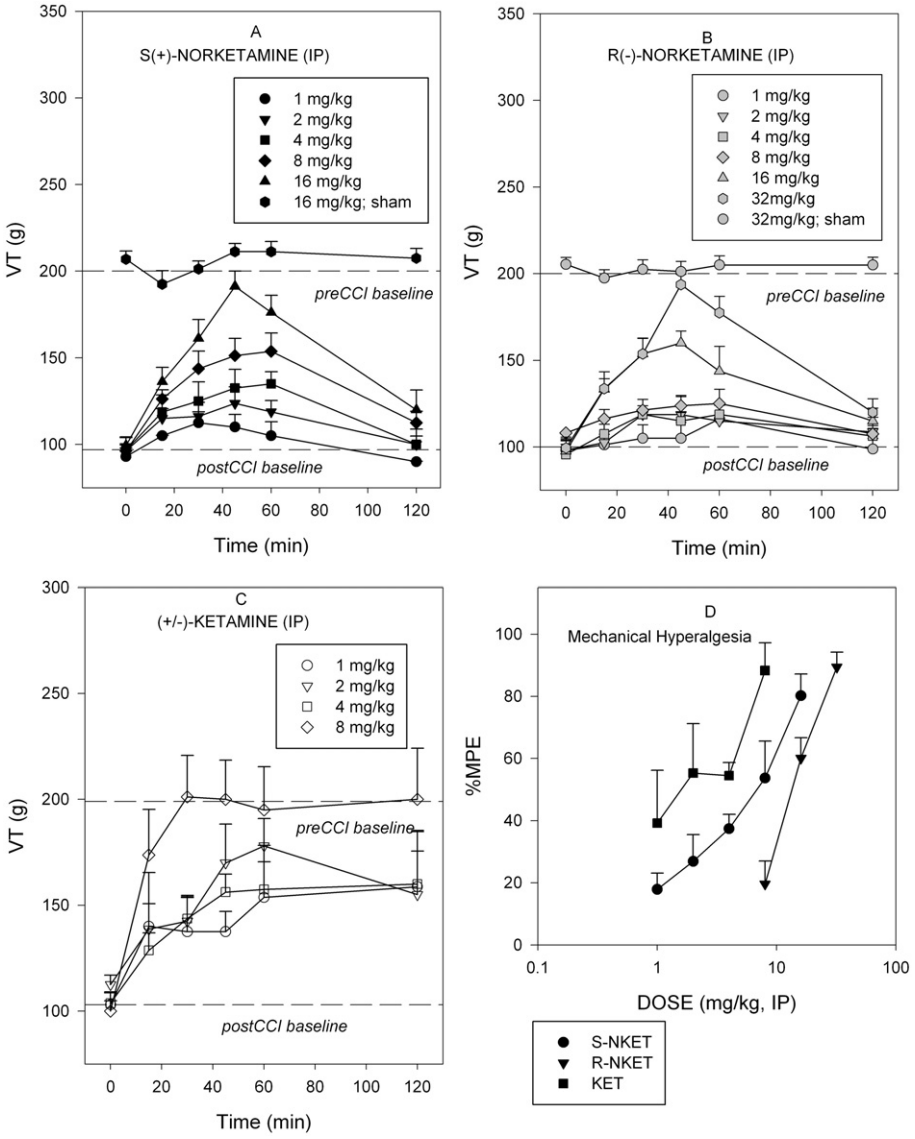

Fig. 1. Time course of responsiveness to mechanical noxious stimuli (vocalization threshold, VT, g) in nerve-injured paw (chronic constriction nerve injury, CCI) after intraperitoneal administration (IP): [Panel A] S(+)-norketamine (S-NKET), [Panel B] R(−)-norketamine (R-NKET), [Panel C] (±)-ketamine (KET). [Panel D] Dose–response curves: S-NKET, R-NKET, and KET (IP). Percent maximum possible effect (at peak time), %MPE=(VT−postCCI baseline/preCCI baseline−postCCI baseline)*100; preCCI baseline≈200 g. Mean±SEM (n=8 rats).

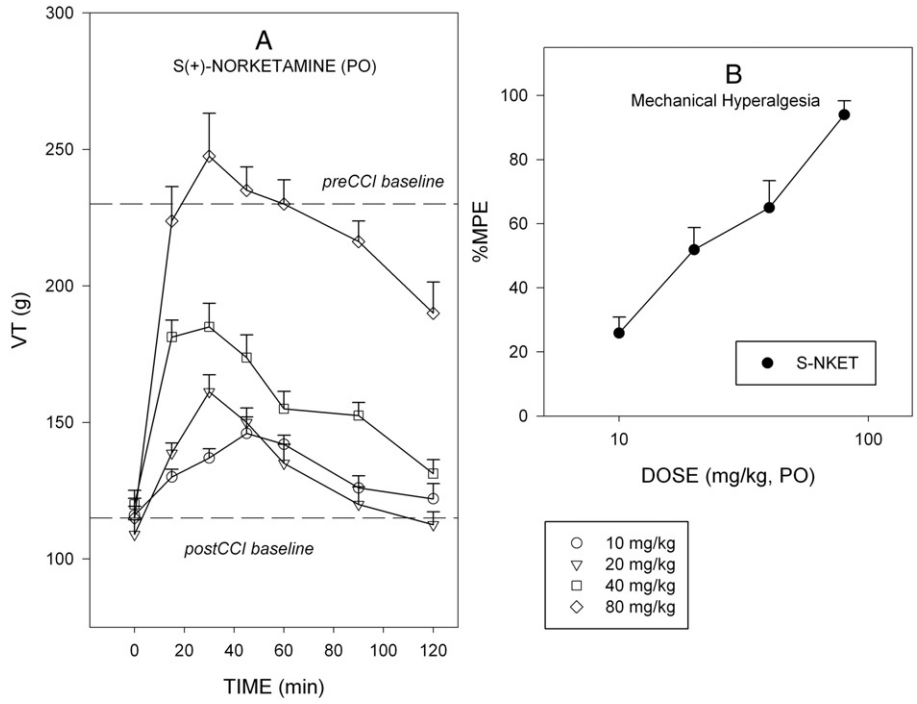

Fig. 2. [Panel A] Time course of responsiveness to mechanical noxious stimuli (vocalization threshold, VT, g) in nerve-injured paw (chronic constriction nerve injury, CCI) after oral administration (PO): S(+)-norketamine (S-NKET). [Panel B] Dose–response curve S-NKET (PO). Percent maximum possible effect (at peak time), %MPE = (VT − postCCI baseline/preCCI baseline − postCCI baseline) * 100; preCCI baseline ≈ 225 g. Mean ± SEM (n = 8 rats).

formalin (5%) was injected subcutaneously (SC) into the dorsal surface of the left hind paw. S(+)- and R(−)-norketamine (0.5, 1, 5, 10 mg/kg), as well as (±)-ketamine (0.25, 0.5, 1 and 5 mg/kg) were administered by the IP route 15 min before SC formalin injection. Control rats were treated with saline. The incidences of spontaneous flinches, defined as quick shakes of the injected paw, were counted continuously in 5 min intervals for 60 min after drug administration. In addition, S(+)-norketamine (2.5, 10, 20, 40, 80,

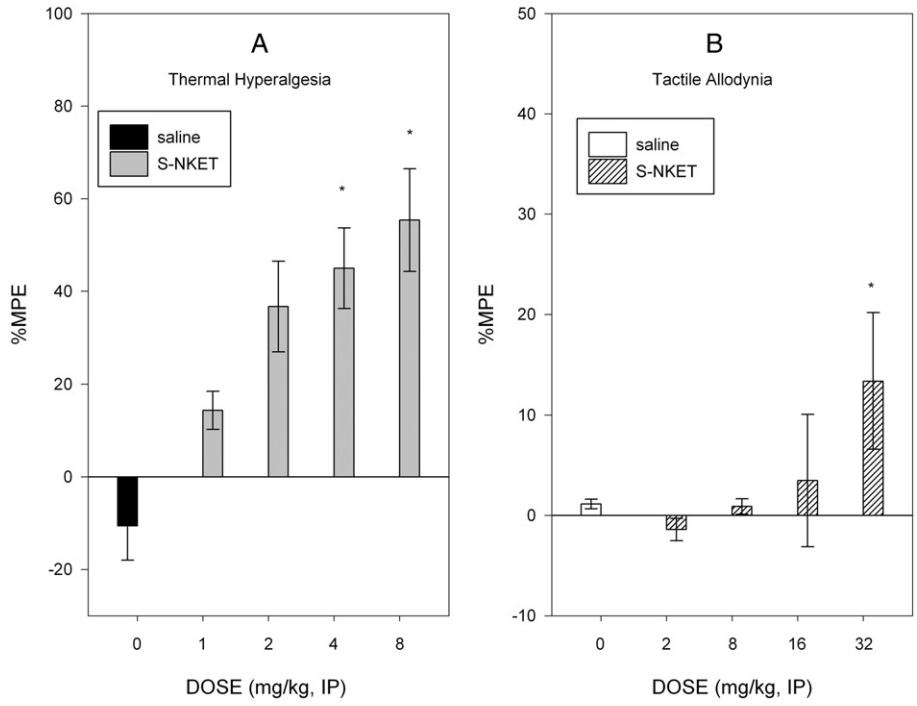

Fig. 3. [Panel A] Dose-related antihyperalgesic effect of S(+)-norketamine (S-NKET) after intraperitoneal administration (IP). Responsiveness to thermal noxious stimuli (paw withdrawal threshold, PWT, s) was assessed in a nerve-injured paw (chronic constriction nerve injury, CCI). Percent maximum possible effect (at peak time), %MPE = (PWT − baseline/cut off − baseline) * 100; cut off = 20 s. Mean ± SEM (n = 8 rats). [Panel B] Dose-related antiallodynic effect: S-NKET (IP). Responsiveness to non-noxious mechanical stimuli (50% von Frey paw withdrawal threshold, VFT, g) was assessed in a CCI paw. %MPE = (VFT − baseline / cut off − baseline)*100; cut off = 15 g. Mean ± SEM (n = 4 rats). Saline served as control in both experiments. *Significantly different from saline (P < 0.05; post-hoc SNK).

160 mg/kg) and R(−)-norketamine (80, 160, 320 mg/kg) were administered by the PO route and evaluated as described above. Each rat was used in only one trial.

2.5. Motor coordination (rotarod test)

The motor function was determined using a Rat Rotarod (Ugo Basile, Comeno, Italy) apparatus in intact rats. Each rat was trained to run on the rotarod at a constant speed (10 rev/min) until it could remain there for 180 s without falling (for two consecutive days). Thereafter, the rats received one of the following IP treatments: S(+)-norketamine (8, 16, 24, 32 mg/kg), R(−)-norketamine (24, 32, 48, 56, 64 mg/kg) and (±)-ketamine (4, 8, 16, 24 mg/kg). In addition, S(+)-norketamine was administered by the PO route (80, 160, 240, 320 mg/kg). Rats were placed in the rotarod prior to (baseline, taken twice 15 min apart) and at 5, 15, 30, 60 and 120 min after drug administration. The length of time the rat was able to remain on the drum was recorded up to 180 s (cut off). Saline served as control.

2.6. Behavioral rating scale

Ataxia, stereotypic behaviors and activity level were determined prior to (baseline) and at 5, 10 and 15 min after IP administration of S(+)-norketamine (16 mg/kg), R(−)-norketamine (32 mg/kg) and (±)-ketamine (8 mg/kg) in intact rats. These doses were found to be approximately equipotent in reversing mechanical hyperalgesia (CCI). The behavioral rating scale developed for quantification of PCP-induced effects was used for quantification (Sturgeon et al., 1979, with minor modification) (Table 1).

2.7. Locomotor activity

Locomotor activity was determined using the Opto-Varimex infrared photocell-based activity monitor (Columbus Instrument). Horizontal (ambulation) and vertical (rearing) activities were recorded during 5 min sessions prior to (baseline) and at 15, 60 and 120 min following administration of S(+)-norketamine, R(−)-norketamine and (±)-ketamine (1, 2, 4, 8, 16 mg/kg; IP; randomized

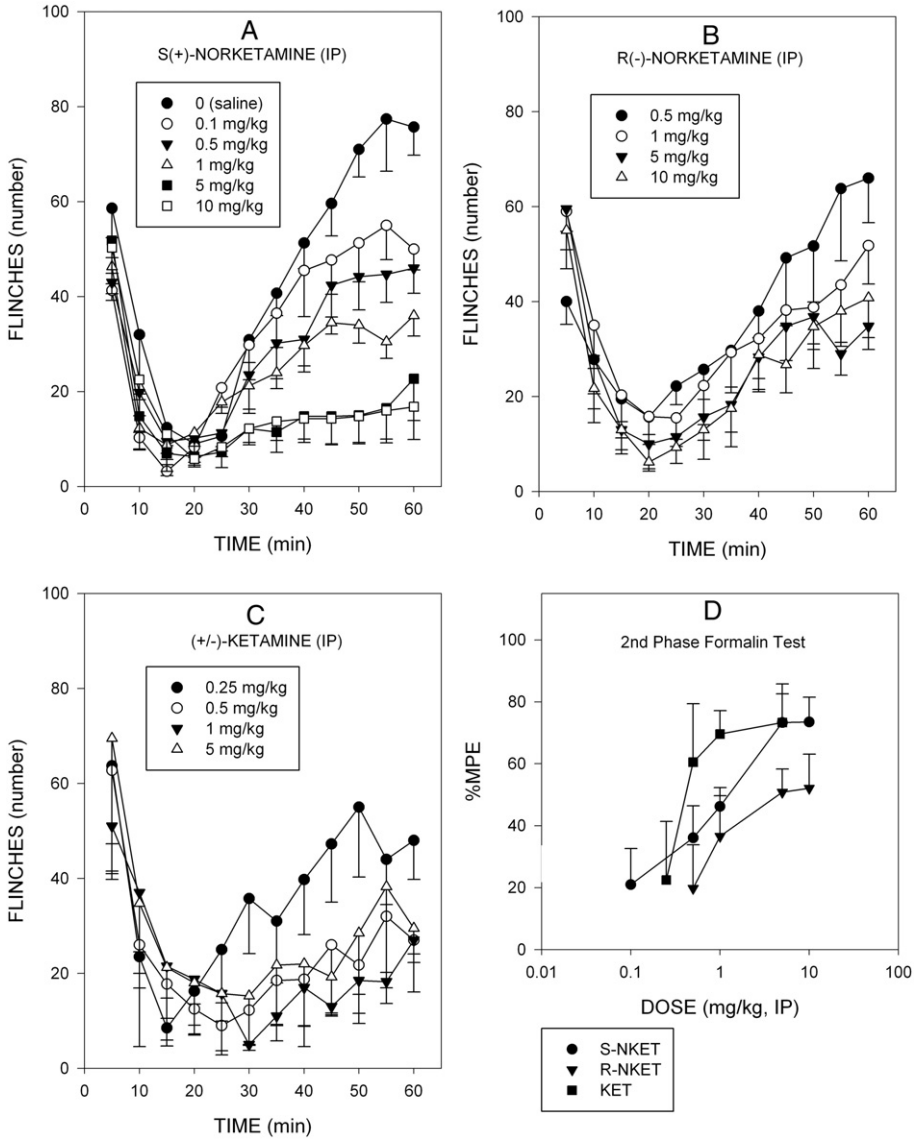

Fig. 4. Time course of formalin-induced flinching behavior (number of flinches; the 1st and 2nd phases) after intraperitoneal administration (IP): [Panel A] S(+)-norketamine (S-NKET), [Panel B] R(−)-norketamine (R-NKET), [Panel C] (±)-ketamine (KET). Saline served as control [see Panel A]. [Panel D] Dose–response curves: S-NKET, R-NKET, and KET (IP). Percent maximum possible effect, %MPE = $(AUC_{saline} - AUC_{drug}/AUC_{saline}] * 100$; the AUC_{drug} and AUC_{saline} are areas under the curve (20–60 min; the formalin test) for drug and vehicle (saline), respectively. Mean ± SEM (n = 4–8 rats).

doses, 48 h intervals). Saline served as control. In addition, locomotor activity was assessed at 5, 10 and 15 min after IP administration of S(+)-norketamine (16 mg/kg), R(−)-norketamine (32 mg/kg) and (±)-ketamine (8 mg/kg), at doses that produce approximately maximum effects in a CCI model.

2.8. Data presentation and statistics

Data were normalized for baseline values. Areas under the curve (AUC_{0-t}) were calculated for the normalized data by the trapezoidal rule. Percent maximum possible effects (%MPE) were calculated as described in the figure legends. Dose–response curves were generated (%MPE as a function of log dose). The effective doses for a 50% maximum possible effect (ED_{50}) and 95% confidence limits (95% CL) were calculated using the method of Tallarida and Murray (1987). Data are the mean±SEM for (n) rats. Statistical analyses were performed using regression, analysis of variance (ANOVA), post-hoc Student Newman Keuls (SNK) and t-test. The level of significance was chosen at $P \leq 0.05$.

3. Results

3.1. Development of mechanical hyperalgesia, thermal hyperalgesia and tactile allodynia (a model of chronic constriction nerve injury, CCI)

Chronic constriction injury of the sciatic nerve (CCI) resulted in development of mechanical and thermal hyperalgesia (enhanced sensitivity to mechanical and thermal noxious stimuli) (Table 2). This was evident by a decrease in vocalization threshold (VT, g; paw pressure test) and paw withdrawal threshold (PWT, s; plantar test) compared to pre-surgical values ($F_{4,32}=23.2$ and $F_{4,37}=10.5$ for VT and PWT, respectively; $P<0.001$). Responsiveness was stable at days 7, 9, 11 and 14 after surgery. Sham operation did not affect the VT and the PWT. Injury to the sciatic nerve (CCI) also resulted in tactile allodynia. This was evident by decreased von Frey threshold (VFT, g) to a normally non-noxious mechanical stimulus in the nerve-injured paw (CCI) compared to sham-operated paw on days 7, 11, 14 and 17 after surgery. No changes in pain thresholds were observed after administration of saline (control).

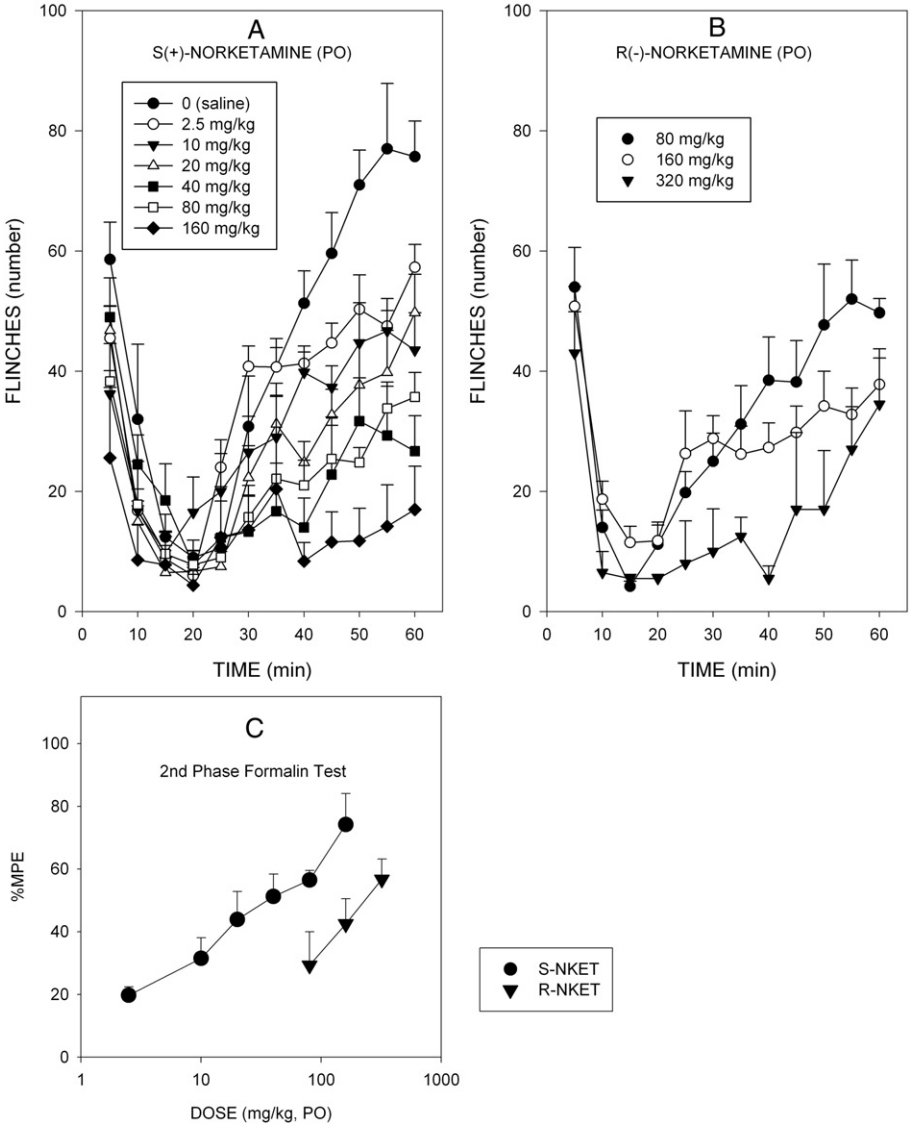

Fig. 5. Time course of formalin-induced flinching behavior (number of flinches; the 1st phase and 2nd phase, the formalin test) after oral administration (PO): [Panel A] S(+)-norketamine (S-NKET) and [Panel B] R(−)-norketamine (R-NKET). Saline served as control [Panel A]. Mean±SEM (n=4–6 rats). [Panel C] Dose–response curves (the 2nd phase, formalin test): S-NKET (PO) and R-NKET (PO). Percent maximum possible effect, $\%MPE = (AUC_{saline} - AUC_{drug}/AUC_{saline}) * 100$; the AUC_{drug} and AUC_{saline} refer to areas under the curve (the 2nd phase, 20–60 min) for drug and vehicle (saline), respectively. Mean±SEM (n=4–6 rats).

3.2. Effect of norketamine on mechanical hyperalgesia

Responses to S(+)- and R(−)-norketamine were examined by paw pressure test in CCI rats. Both S(+)- and R(−)-norketamine (IP) reversed, in a dose-related manner, the mechanical hyperalgesia in the nerve-injured paw (Fig. 1A and B). This was evident as responses in the presence of norketamine were similar to those observed prior to nerve injury (VT≈200 g). The antihyperalgesic effect lasted less than 2 h, and was more pronounced for S(+)-norketamine than for R(−)-norketamine. The %MPE was related to dose ($F_{4,39}$=10.4, P<0.0001; $F_{5,47}$=24.5, P<0.0001) but not to time of testing (days 7–14 after surgery). The ED_{50} (95% CL) values were 5.4 (3.9–7.4) and 12.1 (10.0–14.4) mg/kg, IP for S(+)- and R(−)-norketamine, respectively (Fig. 1D). Neither drug had an effect on the sham-operated paw (Fig. 1A and B). (±)-Ketamine was also tested in this paradigm. The time course of (±)-ketamine was of longer duration (>2 h) (Fig. 1C). This may perhaps be explained by metabolic conversion of ketamine to norketamine. The ED_{50} (95% CL) value for (±)-ketamine was 2.2 (0.9–5.1) mg/kg, IP. In addition, oral efficacy of S(+)-norketamine was demonstrated in a CCI model (Fig. 2A and B). The effect was dose-related ($F_{3,31}$=32.8; P<0.0001) with an ED_{50} (95% CL) value of 21.2 (17.2–25.9) mg/kg, PO. Interestingly, the onset of antihyperalgesia appeared similar after the IP and the PO routes of administration (t_{max}≈30–60 min).

3.3. Effect of norketamine on thermal hyperalgesia

S(+)-Norketamine (IP) attenuated in a dose-related manner the thermal hyperalgesia in nerve-injured paw ($F_{4,36}$=3.5; P<0.025) (Fig. 3A). The ED_{50} (95% CL) value was 6.2 (3.2–11.8) mg/kg, IP. No effect was observed on the sham-operated paw after administration of the highest dose (8 mg/kg) tested (data not shown).

3.4. Effect of norketamine on tactile allodynia

Significant attenuation of tactile allodynia was seen only with the highest dose of S(+)-norketamine tested (32 mg/kg, IP) (Fig. 3B). No effect was observed on the sham-operated paw (data not shown).

3.5. Effect of norketamine on formalin-induced flinches

Formalin injection into the paw caused a biphasic reaction (the 1st phase, 0–10 min; the 2nd phase, 20–60 min) consisting of flinching behavior in control rats (saline) (Fig. 4A). Both S(+)- and R(−)-norketamine (IP) attenuated flinching during the second phase, which is thought to involve central sensitization (Fig. 4A and B). S(+)-Norketamine produced a dose-related effect ($F_{4,29}$=8; P<0.0005) with the ED_{50} (95% CL) value of 1.2 (0.7–2.1) mg/kg, IP. The ED_{50} for R(−)-norketamine was not determinable due to limited efficacy (%MPE≈50% at 5–10 mg/kg, IP) (Fig. 4D). Neither enantiomer produced a dose-related effect in the first phase of the formalin test, which is thought to represent nociception (data not shown). (±)-Ketamine also decreased flinching in the 2nd phase of the formalin test; ED_{50}=0.6 (0.4–0.9) mg/kg, IP (Fig. 4C and D). The ability of S(+)- and R(−)-norketamine to block formalin-induced flinching (the 2nd phase) was also demonstrated after the oral route of administration (Fig. 5A and B). The effects were dose-related ($F_{5,35}$=9.7; P<0.0001 and $F_{2,17}$=6.9; P<0.01) with ED_{50} (95% CL) values of 35.1 (23.5–52.5) and 230.5 (132.6–414.5) mg/kg, PO for S(+)- and R(−)-norketamine, respectively (Fig. 5C).

3.6. Effect of norketamine on motor coordination

Both S(+)- and R(−)-norketamine (IP) produced a transient (<30 min) impairment of rotarod performance in intact rats. In contrast, (±)-ketamine-produced motor incoordination that lasted longer than the time of testing (>2 h). Motor incoordination was dose-related ($F_{3,23}$=6.3, P<0.005; $F_{3,23}$=6.3, P<0.0025; $F_{3,27}$=25.8, P< 0.0001) for drugs tested (Fig. 6A). The ED_{50} (95% CL) values were 16.2 (10.3–25.6), 57.0 (48.6–66.8) and 5.9 (4.1–8.4) mg/kg, IP for S(+)-norketamine, R(−)-norketamine and (±)-ketamine, respectively. Oral S(+)-norketamine also caused a dose-related motor effect ($F_{3,23}$=13.4, P<0.0001) with an ED_{50} (95% CL) value of 206.8 (151.1–275.8) mg/kg, PO (Fig. 6B). Saline had no effect in the rotarod test.

3.7. Behavioral effects of norketamine

The behavioral rating scale (Table 1) was used for quantitation of ataxia, stereotypic behaviors and activity levels. Both S(+)- and R(−)-norketamine (IP) produced significantly less ataxia and stereotypic behaviors compared to (±)-ketamine at doses maximally effective against mechanical hyperalgesia (16, 32 and 8 mg/kg, respectively) (Fig. 7A and B). Activity levels were similar for all drugs tested (Fig. 7C). Of note, (±)-ketamine was observed to evoke an early (within 5 min) PCP-like behavior (i.e. head weaving, turning).

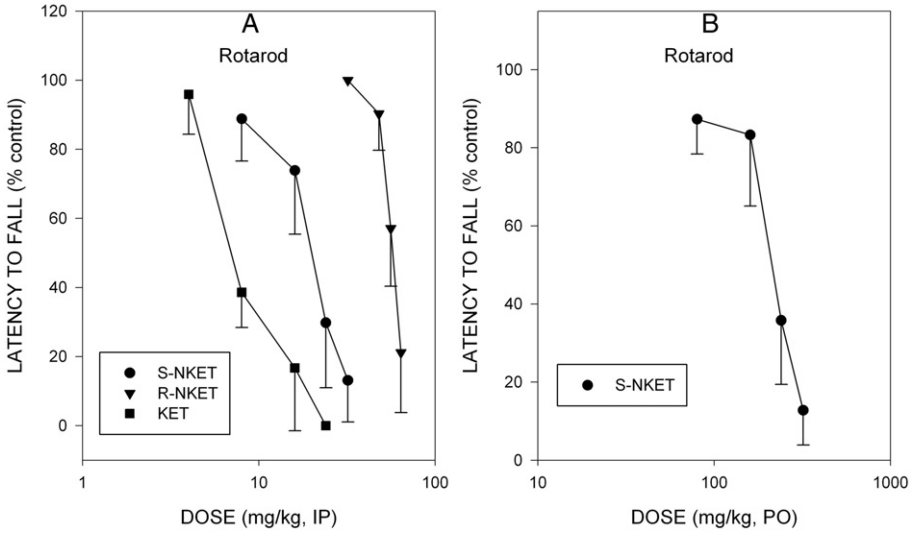

Fig. 6. Dose–response curves for motor effects (rotarod performance test) [Panel A] after intraperitoneal administration (IP): S(+)-norketamine (S-NKET), R(−)-norketamine (R-NKET), and (±)-ketamine (KET) and [Panel B] after oral administration (PO): S-NKET. Latency to fall (at 5 min after drug administration) was expressed as a percentage of vehicle control value (cut off=180 s). Mean±SEM (n=6–8 rats).

This was not observed with S(+)- and R(−)-norketamine at any dose tested.

3.8. Effect of norketamine on locomotor activity

S(+)- and R(−)-norketamine, as well as (±)-ketamine affected locomotor activity in intact rats. The effect (%MPE) was dose-related at 15 min ($F_{5,46}$=4.8, P<0.0025; $F_{5,45}$=4.6, P<0.025; $F_{5,46}$=6.8, P<0.0001 for S(+)-norketamine, R(−)-norketamine and (±) -ketamine, respectively) (Fig. 8A) but not at 60 or 120 min after IP administration. In addition, S(+)- and R(−)-norketamine had similar effects on locomotor activity as (±)-ketamine (5, 10, and 15 min) at approximately equipotent antihyperalgesic doses (16, 32 and 8 mg/kg, IP) (Fig. 8B).

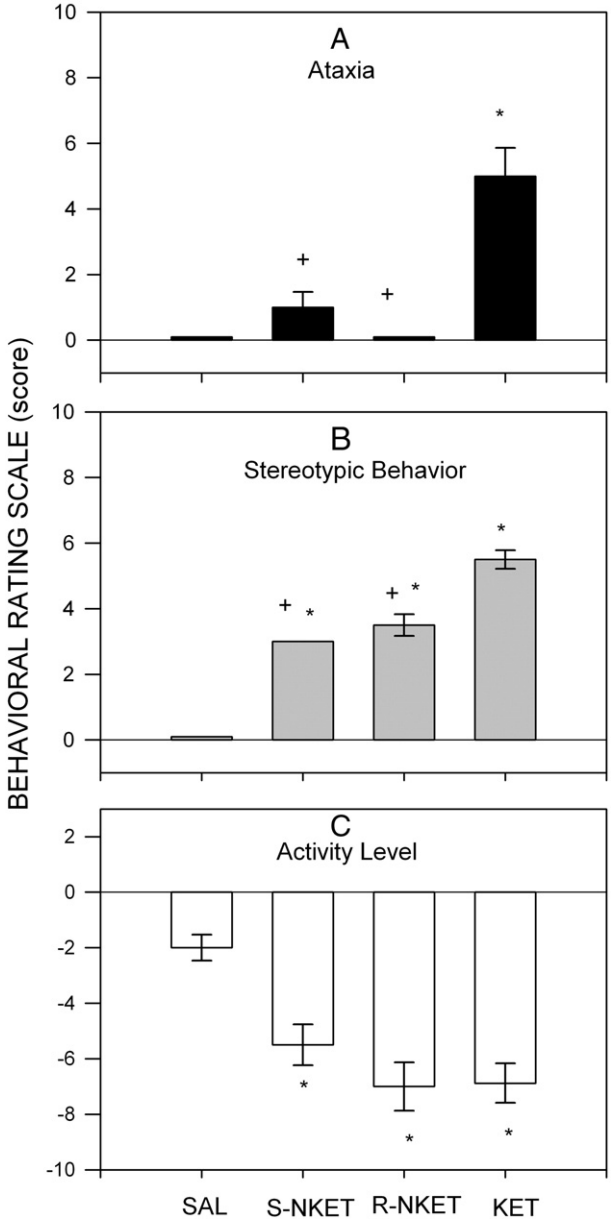

Fig. 7. Ataxia [Panel A], stereotypic behavior [Panel B], and activity level [Panel C] following intraperitoneal administration (IP): S-norketamine (S-NKET, 16 mg/kg), R-norketamine (R-NKET, 32 mg/kg), (±)-ketamine (KET, 8 mg/kg), at approximately maximum antihyperalgesic doses, see Fig. 1. Data are presented as total scores (Behavioral Rating Scale; see Table 1). Saline served as control. Mean±SEM (n=4–8 rats). * Significantly different from saline; + significantly different from KET (P<0.05; post-hoc SNK).

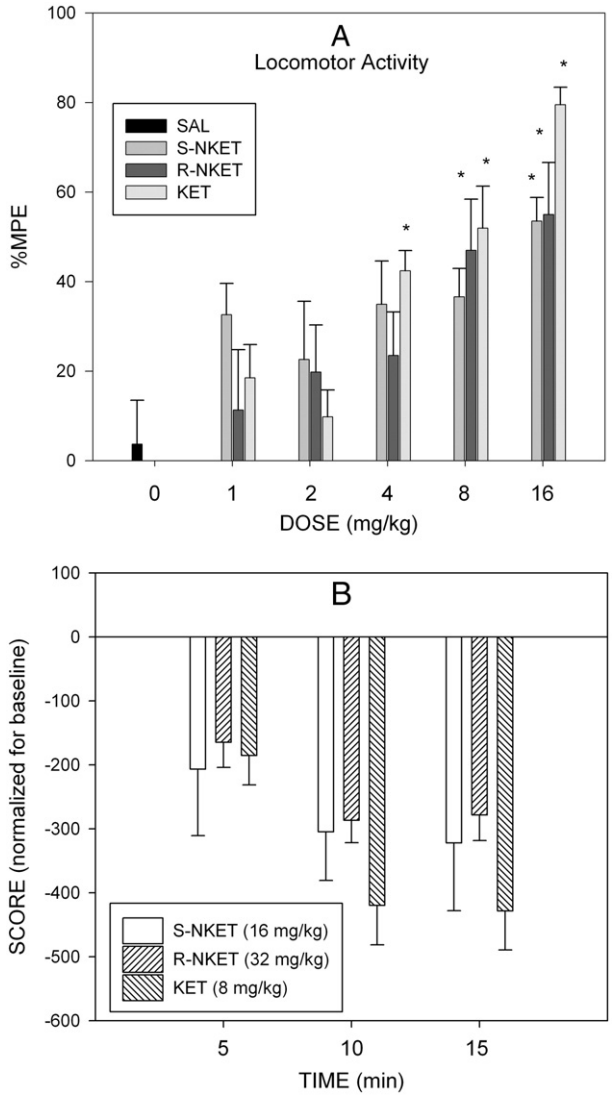

Fig. 8. [Panel A] Dose–response curves locomotor activity (LOC, at 15 min) after intarperitoneal (IP) administration: S(+)-norketamine (S-NKET), R(−)-norketamine (R-NKET), and (±)-ketamine (KET). Saline served as control. Percent maximum possible effect, %MPE=(baseline−LOC/baseline)*100. Mean±SEM (n=8 rats). [Panel B] LOC (normalized for baseline) at 5, 10, and 15 min after administration (IP) of S-NKET (16 mg/kg), R-NKET (32 mg/kg), and KET (8 mg/kg) at approximately maximum anihyperalgesic doses (see Fig. 1). Mean± SEM (n=6–8 rats). *Significantly different from saline (P<0.05; post-hoc SNK).

4. Discussion

The S(+) and R(−) enantiomers of norketamine were studied in rats in an effort to identify a novel NMDA-receptor antagonist drug. Norketamine, a major metabolite of ketamine, binds to a non-competitive site at the NMDA-receptor complex (Ebert et al., 1997). The results of this study indicate several important findings. First, norketamine efficacy was demonstrated in two well-established rodent models of persistent pain with a component of central sensitization (sensitive to NMDA-receptor antagonists): i.e. the chronic constriction nerve injury rodent model of peripheral neuropathy (CCI) and the formalin model of tonic inflammatory pain (the formalin test). Second, the antinociceptive properties were found to be more prominent in the S(+) enantiomer. Third, the antinociceptive effect of norketamine could be separated from significant side effects. Thus,

norketamine is likely acting as an antagonist at the NMDA-receptor complex in blocking NMDA-mediated central sensitization occurring as a result of nerve or peripheral tissue injury in rats. The preclinical data suggest that norketamine, in particular the S(+) enantiomer, may be an effective agent for the treatment of chronic pain where central sensitization is involved.

It has been speculated that the analgesic activity of ketamine was due, at least in part, to the presence of its major metabolite, norketamine (Bushnell and Craig, 1995; Grant et al., 1981). Nevertheless, direct evidence for the antinociceptive activity of norketamine was limited to one study showing that (±)-norketamine attenuated formalin-induced nociceptive behavior after neuroaxial (intrathecal) administration in a rat model of persistent inflammatory pain (the 2nd phase of the formalin test) (Shimoyama et al., 1999). Utilizing the formalin test we have demonstrated norketamine efficacy following both intraperitoneal and oral routes of administration in rats. Formalin injection resulted in typical biphasic nociceptive behaviors in control (saline) rats. Both S(+)- and R(−)-norketamine attenuated, in a dose-related fashion, formalin-induced flinches in the late phase (which is thought to represent central sensitization) but not in the early phase (which is thought to reflect nociceptive pain) in the formalin test. This pattern of action is a common feature of NMDA-receptor antagonists, including MK-801, ketamine, memantine and dextromethorphan (Berrino et al., 2003; Chaplan et al., 1997, Coderre and Melzack, 1992; Medvediev et al., 2004; Vaccarino et al., 1993).

To the best of our knowledge, no prior study has examined the ability of norketamine to alleviate pain occurring as the result of nerve injury. Herein, using a rat model of peripheral neuropathy (chronic constriction nerve injury, CCI) we have demonstrated that S(+)- and R(−)-norketamine attenuated, in a dose-related fashion, an enhanced sensitivity to noxious stimuli (mechanical and thermal hyperalgesia) in the nerve-injured paw but not in the sham-operated paw. The present finding on the antihyperalgesic property of norketamine is consistent with previous data on other NMDA-receptor antagonists (Davar et al., 1991, Mao et al., 1993, Yamamoto and Yaksh 1992; Eisenberg et al., 1995; Qian et al., 1996). Furthermore, the present data showed that S(+)-norketamine had an effect on tactile allodynia in the CCI paw at the highest dose tested. Similar to the NMDA-receptor antagonists, dextrophan and ketamine (Christoph et al., 2006; Kim et al., 1997; Tal and Bennett, 1994), the antiallodynic action of norketamine is less robust than the antihyperalgesic effect. An important finding in the present study was that norketamine-mediated antinociception was not confounded by significant impairment of motor function (rotarod test). Doses at which this undesirable side effect was noted were beyond the range of doses that were required to either reverse nerve-injury-induced hyperalgesia (CCI) or block propagation of formalin-induced flinching behavior (the 2nd phase in the formalin test).

The present study has shown that the antinociceptive properties of norketamine reside primarily in the S(+) enantiomer. The potency of R(−)-norketamine was approximately half that of S(+)-norketamine (CCI, formalin). The S(+) enantiomer also demonstrated greater motor effect (rotarod) than the R(−) enantiomer. In addition, S(+)- and R(−)-norketamine were qualitatively similar with regard to stereotypic behaviors, ataxia and locomotor activity (the behavioral rating scale) when used at approximately maximum antihyperalgesic doses in rats. In an early study, it was shown that the duration of the loss of righting reflex was greater with (+)- than (−)-norketamine in mice (Hong and Davisson, 1982). Overall, these in vivo data in rodents appear to be in agreement with previous binding and in vitro electrophysiological studies showing that S(+)-norketamine has higher affinity for non-competitive sites at the NMDA-receptor complex than R(+)-norketamine (Ebert et al., 1997).

Although a number of studies have demonstrated that norketamine is present in high concentration in plasma and brain following ketamine administration (Cohen et al., 1973; Cohen and Trevor, 1974; Grant et al., 1981; Malinovsky et al., 1996) data directly comparing the effects of these two drugs are scarce (Leung and Baillie, 1986; Shimoyama et al., 1999). Herein, (±)-ketamine was tested as a reference NMDA-receptor antagonist in some experimental paradigms. The present data do not allow a thorough comparison between norketamine and ketamine; however, it is plausible to conclude from this study the following points. First, each norketamine enantiomer had lower potency than (±)-ketamine in attenuation of nociception due to injury to nerve (CCI) or peripheral tissue (formalin injection). This can likely be explained by differences in the affinities of norketamine and ketamine for the NMDA-receptor complex (Ebert et al., 1997). It is noteworthy that ketamine, in addition to its action at NMDA-receptors, interacts with other systems involved in pain modulation, including opioid, monoaminergic and cholinergic systems (Hustveit et al., 1995). Little is known about norketamine in this regard. Furthermore, one cannot rule out the possibility that pharmacokinetics (i.e. brain accumulation) and/or metabolism may also play a role (Edwards and Mather 2001; Kharasch and Labroo, 1992; Leung and Baillie, 1988; Theurillat et al., 2005; Yanagihara et al., 2003). Second, the stereoselectivity of norketamine [the S(+)>the R(−)] closely resembled that of ketamine in rodents (Marietta et al., 1977; Ryder et al., 1978). Clinical superiority of S- versus (±)-ketamine (anesthesia) also has been suggested (Arendt-Nielsen et al., 1996; Engelhardt, 1997; Vranken et al., 2005; Vollenweider et al., 1997; White et al., 1980, 1985). In this regard, the S-enantiomers of norketamine and ketamine have higher affinities for non-competitive sites at the NMDA-receptor complex than their R-enantiomeric counterparts (Ebert et al., 1997). Third, the time courses of both antihyperalgesia (CCI rats) and motor dysfunction (intact rats) were of shorter duration for norketamine than ketamine. This finding is consistent with previous speculation that the activity of ketamine is attributed, at least in part, to the presence of the active metabolite, norketamine (Cohen et al., 1973; Cohen and Trevor, 1974; Leung and Baillie, 1986; Malinovsky et al., 1996). Low oral bioavailability of ketamine was also thought to be due to the first-pass effect, and to the formation of norketamine (Grant et al., 1981; Clements et al., 1982). Oral efficacy of norketamine was demonstrated in the present study. Fourth, both S(+)- and R(−)-norketamine produced significantly less stereotypic behavior and ataxia compared to (±)-ketamine at maximum IP antihyperalgesic doses. A previous study also demonstrated that (±)-ketamine, at an oral dose that was effective in the formalin test, caused a significant ataxia and loss of the righting reflex in rats (Shimoyama et al., 1999). This was not observed with oral S(+)- and R(−)-norketamine in the present study. These findings on norketamine may be of importance in the context that ketamine, due to its marked side effects (hallucinations, sedation, vivid dreams and motor dysfunction) is not widely used for the treatment of chronic pain. Norketamine may likely represent a better choice than ketamine for this indication.

In conclusion, the present study has provided evidence that norketamine (IP, PO) is effective in preventing central sensitization (the 2nd phase in the formalin test) and is also effective in reversing an established hyperalgesia (a CCI model of neuropathy) in rats. This preclinical study suggests the feasibility that norketamine, specifically the S(+) enantiomer, may have potential as a novel, orally effective, NMDA-receptor antagonist agent for treatment of chronic neuropathic pain (e.g. diabetic peripheral neuropathy, AID's-related neuropathy).

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References

Arendt-Nielsen L, Nielsen J, Petersen-Felix S, Schnider TW, Zbinden AM. Effect of racemic mixture and the S(+)-isomer of ketamine on temporal and spatial summation of pain. Br J Anesth 1996;77:625–31.

Backonja M, Arndt G, Gombar KA, Check B, Zimmermann M. Response of chronic pain syndromes to ketamine: a preliminary study. Pain 1994;56:51–6.
Bennett G. Update on the neurophysiology of pain transmission and modulation: focus on the NMDA receptor. J Pain Symptom Management 2000;19:S2–6.
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.
Berrino L, Oliva P, Massimo F, Aurill C, Maione S, Grella A, Rossi F. Antinociceptive effect in mice of intraperitoneal *N*-methyl-D-aspartate receptor antagonists in the formalin test. Eur J Pain 2003;7(2):131–7.
Bushnell TG, Craig J. Response to chronic neuropathic syndromes to ketamine. A role of norketamine? Pain 1995;60:115.
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, Malmberg AB, Yaksh TL. Efficacy of spinal NMDA receptor antagonism in formalin hyperalgesia and nerve injury evoked allodynia in the rat. J Pharmacol Exp Ther 1997;280:829–38.
Christoph T, Sciene K, Englberger W, Parsons CG, Chizh BA. The antiallodynic effect of NMDA antagonists in neuropathic pain outlast the duration of the in vivo NMDA antagonism. Neuropharmacology 2006;51:12–7.
Clements JA, Nimmo WS, Grant IS. Bioavailability, pharmacokinetics and analgesic activity of ketamine in humans. J Pharmacol Sci 1982;71:539–42.
Coderre TJ, Melzack R. The contribution of excitatory amino acids to central sensitization and persistent nociception after formalin-induced tissue injury. J Neurosci 1992;12:3665–70.
Cohen ML, Trevor AJ. On the cerebral accumulation of ketamine and the relationship between metabolism of drug and its pharmacological effects. J Pharmacol Exp Ther 1974;189:351–8.
Cohen ML, Chan SL, Way WL, Trevor AJ. Distribution in the brain and metabolism of ketamine in the rat after intravenous administration. Anesthesiology 1973;39:370–6.
Davar G, Hama A, Deykin A, Vos K, Maciewicz R. MK-801 blocks the development of thermal hyperalgesia in a rat model of experimental painful neuropathy. Brain Res 1991;553:327–30.
Edwards SR, Mather LE. Tissue uptake of ketamine and norketamine enantiomers in the rat. Indirect evidence for extrahepatic metabolic inversion. Life Sci 2001;69:2051–66.
Ebert B, Mikkalsen S, Thorkildsen C, Borgbjerg F. Norketamine, the main metabolite of ketamine, is a non-competitive NMDA receptor antagonist in the rat cortex and spinal cord. Eur J Pharmacol 1997;333:99–104.
Eide P, Stubhaug A, Jorum E. Relief of post-herpetic neuralgia with the *N*-methyl-D-aspartate (NMDA) receptor antagonist ketamine: a double-blind, cross-over comparison with morphine and placebo. Pain 1994;58:347–54.
Eisenberg E, LaCross S, Strassman AM. The clinically tested *N*-methyl-D-aspartate receptor antagonist memantine blocks and reverses thermal hyperalgesia in a rat model of painful mono-neuropathy. Neurosci Letters 1995;187:17–20.
Engelhardt W. Aufwachverhalten und psychomimetishe reactionen nach S(+)-ketamin. Anesthesist 1997;46:38–42.
Fisher K, Coderre T, Hagen N. Targeting the *N*-methyl-D-aspartate receptor for chronic pain management: preclinical animal studies, recent clinical experience, and future research directions. J Pain Symptom Management 2000;20:358–73.
Grant IS, Nimmo WS, Clemments JA. Pharmacokinetics and analgesic effects of i.m. and oral ketamine. Br J Anesth 1981;53:805–10.
Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 1988;32:77–88.
Hustveit O, Maurset A, Oye I. Interaction of chirial forms of ketamine with opioid, phencyclidine, sigma and muscarinic receptors. Pharmacol Toxicol 1995;77:355–9.
Hong SC, Davisson JN. Stereochemical studies of demethylated ketamine enantiomers. J Pharm Sci 1982;71:912–4.
Johnson BW. Pain mechanism: anatomy, physiology, and neurochemistry. In: Raja PP, editor. Practical management of pain. St. Louis: Mosby, Inc; 2000. p. 117–43.
Kharasch ED, Labroo R. Metabolism of ketamine stereoisomers by human liver microsomes. Anesthesiology 1992;77:1201–7.
Kim YI, Na HS, Yoon YW, Han HC, Ko KH, Hong SK. NMDA receptors are important for both mechanical and thermal allodynia from peripheral nerve injury in rats. NeuroReport 1997;8:2148–53.
Leung LY, Baillie TA. Comparative pharmacology in the rat of ketamine and its two principal metabolites, norketamine and (*Z*)-6-hydroxynorketamine. J Med Chem 1986;29:2396–9.
Leung LY, Baillie TA. Stereoselective metabolism of norketamine, the *N*-demethylated metabolite of ketamine, in rat liver microsomes. In: Domino EF, Kamenka JM, editors. Sigma and phencylidine-like compounds as molecular probes in biology. Ann Arbor: NPP Books; 1988. p. 607–17.
Liu J, Ji XQ, Zhu XZ. Comparison of psychic emergence reactions after (±)-ketamine and (+) -ketamine in mice. Life Sci 2006;78:1839–44.
Malinovsky JM, Cozian SA, Lapage JY, Pinaud M. Ketamine and norketamine plasma concentrations after IV and nasal and rectal administration in children. Brit J Anesth. 1996;77:203–7.
Mao J, Price DD, Haves RL, Lu J, Mayer DJ. Intrathecal treatment with dextrophan or ketamine potently reduced pain-related behaviors in a rat model of peripheral mononeuropathy. Brain Res 1993;605:164–8.
Marietta MP, Way WL, Castagnoli N, Trevor AJ. On the pharmacology of ketamine enantiomers in the rat. J Pharmacol Exp Ther 1977;2002:157–65.
Medvediev IO, Malyshkin AA, Belozertseva IV, Sukhotina IA, Sevostianova NY, Aliev K, et al. Effects of low-affinity NMDA receptor channel blockers in two rat models of chronic pain. Neuropharmacology 2004;47:175–83.
Qian J, Brown SD, Carlton SM. Systemic ketamine attenuates nociceptive behaviors in a rat model of peripheral neuropathy. Brain Res 1996;715:51–62.
Parson C. NMDA receptors as targets for drug action in neuropathic pain. Eur J Pharmacol 2001;429:71–8.
Randal L, Selitto J. A method for measurement of analgesic activity on inflamed tissue. Arch Int Pharmacodyn Ther 1957;111:409–19.
Ryder S, Way WL, Trevor AJ. Comparative pharmacology of the optical isomers of ketamine in mice. Eur J Pharmacol 1978;49:15–23.
Sang CN. NMDA-receptor antagonists in neuropathic pain: experimental methods to clinical trials. J Pain Symptom Management 2000;19:S21–25.
Schuttler J, Zsigmond EK, White PF. Ketamine and its isomers. In: White PF, editor. Textbook of intravenous anesthesia. Baltimore: Williams and Wilkins; 1997. p. 171–88.
Shimoyama M, Shimoyama N, Gorman AL, Elliott KJ, Intrussi CE. Oral ketamine is antinociceptive in the rat formalin test: role of the metabolite, norketamine. Pain 1999;81:85–93.
Sturgeon R, Fessler R, Meltzer H. Behavioral rating scales for assessing phencyclidine-induced locomotor activity, stereotyped behavior and ataxia in rats. Eur J Pharmacol 1979;59:169–79.
Tal M, Bennett GJ. Neuropathic pain sensations are differentially sensitive to dextrorphan. NeuroReport 1994;5:1438–40.
Tallarida RJ, Murray RB. Manual of pharmacological calculations with computer program. New York: Springler-Verlag; 1987.
Theurillat R, Knobloch M, Levionnois O, Larenza P, Mevissen M, Thormann W. Characterization of the stereoselective biotransformation of ketamine to norketamine via determination of their enantiomers in equine plasma by capillary electrophorese. Electrophoresis 2005;26:3942–51.
Vaccarino AL, Marek P, Kest B, Weber E, Keana JF, Liebeskind JC. NMDA receptor antagonists, MK-801 and ACEA-1011, prevent the development of tonic pain following subcutaneous formalin. Brain Res 1993;615:331–4.
Vollenweider FX, Leenders KL, Qye L, Hell D, Angst J. Differential psychopathology and patterns of cerebral glucose utilization produced by S- and R-ketamine in healthy volunteers using positron emission tomography (PET). Eur Neuropharmacol 1997;7:25–38.
Vranken JH, Troost D, Wefener JT, Kruist MR, van der Vegt MT. Neuropathological findings after continuous intrathecal administration of S(+)-ketamine for management of neuropathic cancer pain. Pain 2005;117:231–5.
White PF, Ham J, Way WL, Trevor AJ. Pharmacology of ketamine isomers in surgical patients. Anesthesiology 1980;52:2312–39.
White PF, Schuttler J, Shafer A, Stanski DR, Honai Y, Trevor AJ. Pharmacology of ketamine isomers in surgical patients. Br J Anaesth 1985;57:197–203.
Wheeler-Aceto H, Cowan A. Standardization of the rat paw formalin test for evaluation of analgesics. Psychopharmacol 1991;104:35–44.
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.
Yanagihara Y, Ohtani M, Kariya S, Uchino K, Hiraishi T, Ashizawa N, et al. Plasma concentration profiles of ketamine and norketamine after administration of various ketamine preparations to healthy Japanese volunteers. Biopharm Drug Disp 2003;24:37–43.