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The involvement of nitric oxide in the analgesic effects of ketamine

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Abstract

We investigated the contribution of NO-cyclic GMP (cGMP) pathway to the antinociceptive effects of ketamine in mice by using the nitric oxide synthase inhibitor, nitro[g]- L-arginine methyl ester (L-NAME). Intraperitoneal (i.p.) (1, 5 or 10 mg/kg) or intrathecal (i.th.) (10, 30 or 60 μg/mouse) administration of ketamine produced dose-dependent antinociceptive effects in the acetic acid-induced writhing and formalin tests but not in the tail-flick nor in hot-plate tests. Pretreatment of mice with L-NAME (10 mg/kg, i.p.) which produced no antinociception on its own, significantly inhibited the antinociceptive effect of ketamine (1, 5 or 10 mg/kg, i.p.). However, L-NAME (30 μg/mouse) was given intrathecally, it neither modified the antinociceptive effect of i.th. ketamine (10, 30 or 60 μg/mouse) nor did it produce an antinociceptive effect alone. These data suggest that the activation of the NO-cGMP pathway probably at the supraspinal level, but not spinal level, contributes to the antinociceptive effects of ketamine. © 2002 Published by Elsevier Science Inc.

Keywords: Ketamine; Nitric oxide; Analgesia; NMDA receptors; Anaesthetics; L-N[G] nitro arginine methylester (L-NAME); Formalin; Writhing; Pain; Dissociative anesthesia

Introduction

The dissociative anesthetic agent ketamine is a noncompetitive blocker of the glutamate subtype of N-methyl-D-aspartate (NMDA) receptors which also exerts analgesic properties in rodents and humans [1,2]. Ketamine has been used as an anesthetic, rather than an analgesic in clinical practice in the past, but recently it has been introduced as an analgesic agent to be used in the management of chronic pain

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[3], neuropathic pain as well as in cancer pain [4,5]. The scientific basis for this type of use relies on data from animal studies that indicates its efficacy in various models of persistant pain. On the contrary, ketamine has been reported to elicit only weak or produce no analgesic effect in tests of thermal nociception [6]. Systemic and/or i.th. administration of ketamine potently reduces hyperalgesia, spontaneous pain-related behavior, and evoked neuronal responses of spinal cord dorsal horn neurons following an intraplantar formalin injection, likely due to the inhibition of NMDA receptors [7–10]. While ketamine is certainly able to act as an antagonist at the glutamate subtype of NMDA receptors, it also has been shown to interact with opioidergic, monoaminergic and cholinergic systems [11]. Moreover, ketamine has also been demonstrated to depresses sodium- and voltage-dependent calcium channels [12,13] which have been shown to contribute to the processing of antinociception [14–17]. Given that the mechanism(s) of ketamine-induced analgesia are not clearly understood, further investigation is therefore warranted.

The actions of glutamate, particularly on NMDA receptors, is of importance to the transmission of both acute and chronic pain [18,19]. Excitatory amino acids exert their effects via NMDA receptors and, the subsequent activation of NMDA receptors promotes an increase in intracellular calcium resulting in the production of nitric oxide (NO) [8,20]. Various studies suggest that the activation of nitric oxide synthase (NOS) with a consequent production of NO triggered by the NMDA receptor activation plays a important role in central and peripheral modulation of nociception [8,21,22]. Because ketamine blocks excitation of NMDA receptors, it is possible to suggest that it may influence NO production. Previous reports have demontrated that ketamine effects NMDA receptor-dependent NO pathway [23–25]. If the NMDA receptors that are blocked by ketamine are also linked to NO synthesis and release, then it is reasonable to predict that inhibition of NOS in central nervous system would enhance the analgesia produced by ketamine. However, the role of NO in nociceptive transmission is somewhat divergent. On the one hand, increased NO production has been shown to produce antinociception [26,27] while L-NAME, an inhibitor of NOS, elicits antinociception in some models of acute and persistant pain [28–30]. In addition, recent immunohistochemical studies have revealed the presence of intense neuronal NOS staining in nerve terminals and in interneurons located in the superficial layers of the spinal cord dorsal horn [31]. This observation has been interpreted as evidence of a facilitatory role of NO in spinal nociceptive transmission [29]. Therefore, the present study was undertaken to investigate the contribution of the NO-cGMP pathway in the antinociceptive effects of ketamine at spinal and supraspinal levels by using L-NAME in various models of pain.

Materials and methods

Adult male Swiss–Webster mice (25–30 g) served as the subjects for this investigation. They were placed in a quiet, temperature and humidity controlled room (22 ± 3° C and 60 ± 5%, respectively) in which a 12/12 hr light-dark cycle was maintained (08 am–08 pm light).

All drugs were freshly prepared by dissolving them in saline. Drug solutions were prepared so that the desired dose, expressed in terms of salt, was contained in a volume 5 ml/kg of body weight for intraperitoneal (i.p.) injection and in 10 μl for i.th. injection. Intrathecal injections were performed free-hand between the L5 and L6 lumbar space in unanesthetized mice according to a previously described method [32]. Control animals received saline. Ketamine or saline were administered i.p. or i.th., 15 min

prior to nociceptive testing. L-NAME was given either by i.p. or i.th. route at 15 min and 5 min prior to administration of ketamine, respectively.

Assessment of antinociceptive effect

The radiant heat tail-flick, hot plate, acetic acid-induced writhing and formalin tests were used to assess nociception.

The radiant heat tail flick test was performed using a radiant heat tail flick apparatus (Columbus, Ohio, USA; Type 812). The intensity of the beam is adjusted to produce mean control reaction times of 2–3 s. A cut-off time of 7 s was used to prevent tissue damage.

For hot plate test, each mouse was placed individually on a hot plate (maintained at 52 °C) and the reaction time was measured starting from time the mouse was placed on the plate until the mouse either demonstrated hind paw licking or jumping. The cut-off latency used was 90 sec to prevent tissue damage.

Acetic acid-induced writhing was employed via an i.p. injection of 0.9% acetic acid in a volume 10 ml/kg. The number of the writhes were tabulated between 5 and 15 min after the injection.

Formalin-induced pain was produced based on a previously reported method [33] with some minor modifications. Briefly, mice were lightly anaesthesized with ether and 20 μl of 10% formalin solution (made up in a phosphate-buffered solution containing: NaCl 137 mM; KCl 2.7 mM and phosphate buffer 10 mM) was injected s.c.under the plantar surface of the left hindpaw. The amount of time that animals spent licking the injected paw was monitored during the early phase (0–5 min) and during the late phase (35–50 min).

Effects of ketamine and L-NAME alone and in combination on motor performance:

In order to assess whether any of the observed antinociceptive effects resulted from sensory blockade or from an impairment of motor function, we assessed the perfomance of mice in the rotarod test. Prior to drug treatment, mice were placed on the rotarod apparatus (Rotamex-V-EE/85, Columbus, Ohio, USA), and after a learning period, only those mice that remained on the rotating rod (5 rev/min) for 180 sec were used. Five, 10 and 15 min after drug or saline administration, the length of time that the each animal stayed on the rotarod was recorded. The cut-off time of 180 sec was used for rotarod latency.

Drugs

The drugs used and their suppliers were as follows: L-NAME and ketamine HCl (Sigma Chemical Co.).

Statistical Analysis

The data were expressed as the mean ± S.E.M. in all cases. Groups of 8–12 mice were tested at each dose. A non-parametric method of statistical analysis was used. Statistical significance of more than two groups were evaluated by Kruskall–Wallis test ($p < 0.05$), followed by Dunnett's multiple test for individual comparisons. To compare two groups, Mann–Whitney U-test was used ($p < 0.05$).

Results

Effects of ketamine and L-NAME on rotarod performance

In order to eliminate the possibility that decreased motor function is confounding our results and subsequent data interpretation, motor performance of the mice was evaluated using the rotarod test for up to 15 min following. Administration of systemic ketamine (1, 5 and 10 mg/kg, i.p.) or i.th. ketamine (10, 30 and 60 μg/mouse) did not alter the motor performance of the mice at 5, 10 and 15 min when compared with saline groups (Table 1). However, at higher doses of the systemic or i.th. ketamine, beyond the range of used for nociception testings, impairment of motor performance was measured. The doses and time courses at which motor disfunction was measured are presented in Table 1. Ketamine at 30 mg/kg, i.p. and 300 μg/mouse, i.th. resulted in a significant motor impairment on rotarod at 5 and 10 min and motor impairment typically resolved at 15 min. However, ketamine at 50 mg/kg, i.p., mice losted the ability of staying on the rotarod for 15 min. When given ketamine at 600 μg/mouse, i.th. resulted in a significant motor impairment at 5 min and motor impairment remained at 10 and 15 min. Systemic L-NAME (10 mg/kg, i.p.) or i.th. L-NAME (30 μg/mouse) either alone or in combination with systemic ketamine (10 mg/kg, i.p.) and intrathecal ketamine (30 μg/mouse), respectively did not affect the motor performance of mice in the period of between 5 and 15 min (Table 1).

Effects of systemic and intrathecal ketamine on thermal nociception

The basal tail flick and hot plate latencies were 2.8 ± 0.17 and 25.1 ± 1.67 sec, respectively. Animals that received ketamine (1, 5 or 10 mg/kg, i.p.; 10, 30 or 90 μg/mouse, i.th.) and L-NAME (10 mg/kg, i.p.

Table 1
The effects of ketamine and L-NAME on rotarod performance time given by either intraperitoneal (i.p.) or intrathecal (i.th.). Significantly different from respectively saline controls, *$p<0.05$

Treatment	Route	Dose	Rotarod performance time (sec)		
			5 min	10 min	15 min
Saline	i.p.	0.5 ml/100 g	180	180	180
Ketamine	i.p.	1 mg/kg	180	180	180
Ketamine	i.p.	5 mg/kg	180	180	180
Ketamine	i.p.	10 mg/kg	180	180	180
Ketamine	i.p.	30 mg/kg	3.1 ± 0.4*	111.3 ± 18.9*	167.3 ± 9.6
Ketamine	i.p.	50 mg/kg	1.14 ± 0.14*	1.28 ± 0.28*	1.14 ± 0.14*
L-NAME	i.p.	10 mg/kg	180	180	180
Ketamine + L-NAME	i.p. + i.p.	10 mg/kg + 10 mg/kg	180	180	180
Saline	i.th.	10 μl/mouse	180	180	180
Ketamine	i.th.	10 μg/mouse	180	180	180
Ketamine	i.th.	30 μg/mouse	180	180	180
Ketamine	i.th.	60 μg/mouse	180	180	180
Ketamine	i.th.	300 μg/mouse	4.3 ± 0.49*	100.7 ± 27.9*	180
Ketamine	i.th.	600 μg/mouse	1.16 ± 0.16*	24.2 ± 12.3*	103.2 ± 29.1*
L-NAME	i.th.	30 μg/mouse	180	180	180
Ketamine + L-NAME	i.th. + i.th.	60 μg/mouse + 30 μg/mouse	180	180	180

or 30 μg/mouse, i.th.) had tail flick and hot plate latencies that were not significantly different from that of control mice (Table 2). L-NAME (10 mg/kg, i.p. or 30 μg/mouse, i.th.) pretreatment did not significantly alter the tail flick and hot plate latencies produced by ketamine (Table 2).

Effects of systemic and intrathecal ketamine on acetic acid-induced writhing

In saline treated animals, the number of writhes was found to be 22.7 ± 1.71 and 24.4 ± 2.5 following i.p. and i.th. administration, respectively (Figs. 1 and 2). I.p. and i.th. administration of ketamine produced dose-dependent antinociception in the acetic acid-induced writhing test (Figs. 1 and 2). Ketamine reduced significantly the number of writhes to 18.4 ± 2.4 and 14.7 ± 2.8, respectively ($P < 0.05$), when given at the lowest doses (1 mg/kg, i.p. and 10 μg/mouse, i.th.). Maximal doses of ketamine (10 mg/kg, i.p. and 60 μg/mouse, i.th.) inhibited the number of writhes to 13 ± 2.5 and 8.3 ± 2.1, respectively.

Effects of systemic and intrathecal ketamine on formalin-induced paw licking

In saline treated mice, the duration of paw licking across the early phase was 32.4 ± 4.4 sec and across the late phase it was 106.3 ± 24.4 sec. Systemic (1, 5 or 10 mg/kg) and i.th. (10, 30 or 60 μg/mouse) administration of ketamine caused a dose related reduction in the paw licking time across the second phase of testing without altering the early phase (Figs. 3 and 4). Higher doses of ketamine (10 mg/kg, i.p. or 60 μg/mouse, i.th.) reduced paw licking time to 49.2 ± 8.0 and 5.1 ± 1.8, respectively, across the late phase.

Effects of L-NAME on the antinociceptive effects of ketamine

Pretreatment with the NO synthesis inhibitor L-NAME (10 mg/kg, i.p. or 30 μg/mouse, i.th.) failed to produce antinociception alone in the radiant heat tail-flick, hot plate and acetic acid-induced writhing

Table 2
The effects of ketamine and L-NAME on tail-flick and hot-plate latencies given by either intraperitoneal (i.p.) or intrathecal (i.th.)

Treatment	Route	Dose	Hot plate latency (15 min)	Tail-flick latency (15 min)
Saline	i.p.	0.5 ml/100 g	25.3 ± 2.6	2.2 ± 0.1
Ketamine	i.p.	1 mg/kg	23.8 ± 3.7	2.3 ± 0.2
Ketamine	i.p.	5 mg/kg	28.2 ± 1.4	2.4 ± 0.3
Ketamine	i.p.	10 mg/kg	27.5 ± 2.3	2.1 ± 0.2
L-NAME (10 mg/kg)	i.p.	10 mg/kg	29.1 ± 1.3	2.2 ± 0.2
Ketamine + L-NAME	i.p. + i.p.	10 mg/kg + 10 mg/kg	31.1 ± 3.2	2.3 ± 0.1
Saline	i.th.	10 μl/mouse	28.7 ± 1.9	2.3 ± 0.2
Ketamine	i.th.	10 μg/mouse	32.1 ± 4.2	2.1 ± 0.2
Ketamine	i.th.	30 μg/mouse	30.1 ± 3.1	2.5 ± 0.3
Ketamine	i.th.	60 μg/mouse	24.3 ± 3.3	2.4 ± 0.1
L-NAME	i.th.	30 μg /mouse	29.4 ± 2.9	2.5 ± 0.1
Ketamine + L-NAME	i.th. + i.th.	60 μg/mouse + 30 μg/mouse	26.4 ± 2.8	2.7 ± 0.4

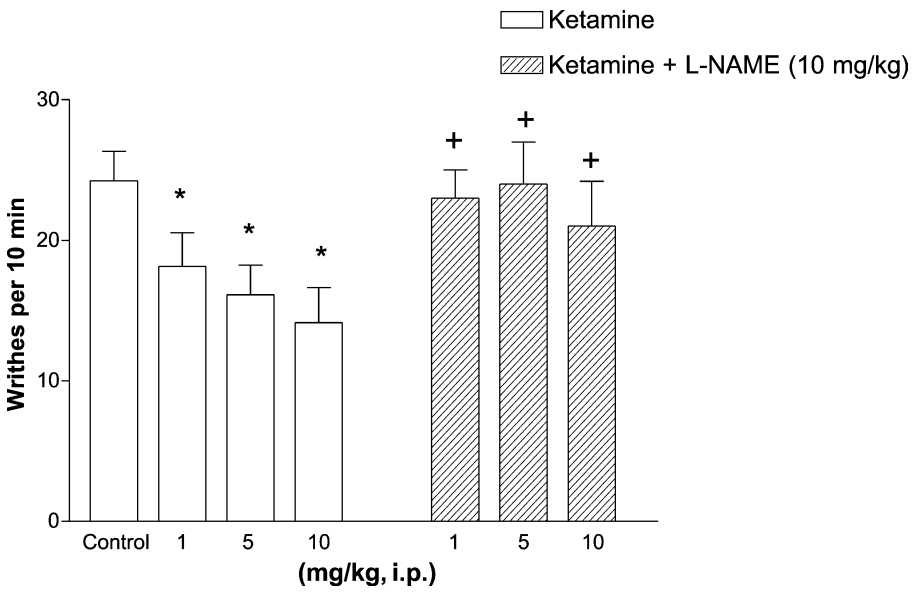

Fig. 1. Blockade of the antinociceptive effect of ketamine (i.p.) by L-NAME (10 mg/kg, i.p.) pretreatment in acetic acid-induced writhing test. Results indicate the mean number of writhes in a 10 min period (n = 8–12). Open colums represent increasing doses of ketamine in saline pretreated animals and striped columns indicate increasing doses of ketamine in L-NAME pretreated animals. Significantly different from saline controls, *$p < 0.05$. Significantly different from the groups that received equal ketamine doses alone, $^{+}p < 0.05$.

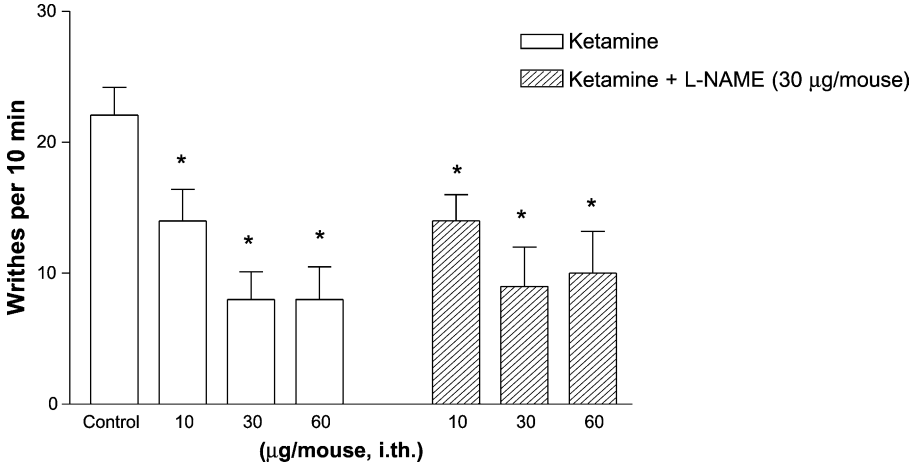

Fig. 2. L-NAME (30 μg/mouse, i.th.) pretreatment failed to alter the antinociceptive effects of i.th. ketamine in the acetic acid-induced writhing test. Results indicate the mean number of writhes in a 10 min period (n = 8–12). Open bars represent increasing doses of ketamine in saline pretreatment mice whereas, striped bars indicate increasing doses of ketamine in L-NAME pretreated mice. Significantly different from the saline controls, *$p < 0.05$. Significantly different from the groups that received equal ketamine doses alone, $^{+}p < 0.05$.

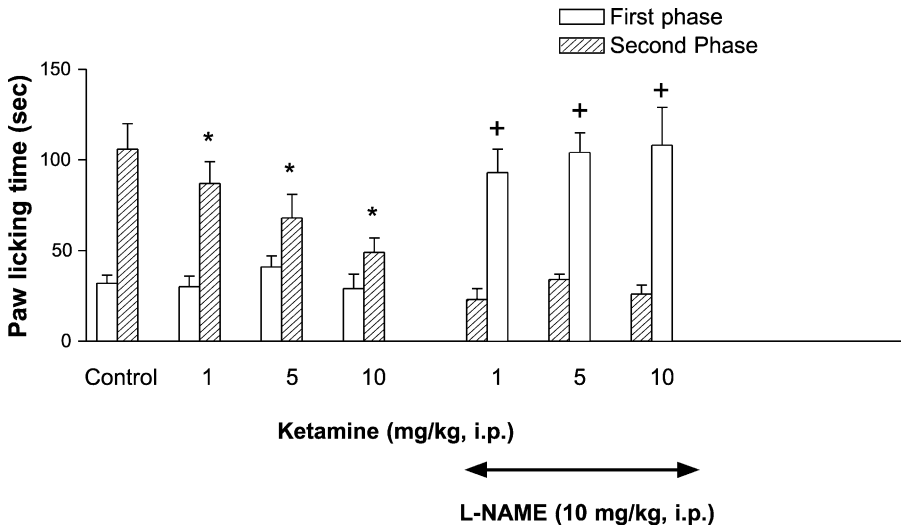

Fig. 3. Pretreatment with systemic L-NAME (10 mg/kg, i.p.) blocks the antinociceptive effects of systemic ketamine (i.p.) in the formalin-induced paw licking test. Open bars represent paw licking time across the early phase (0–5 min) and striped bars indicate licking time across the late phase (35–50 min) of the formalin test. Significantly different from the saine controls, *$p < 0.05$. Significantly different from the groups that received equal ketamine doses alone, $^{+}p < 0.05$.

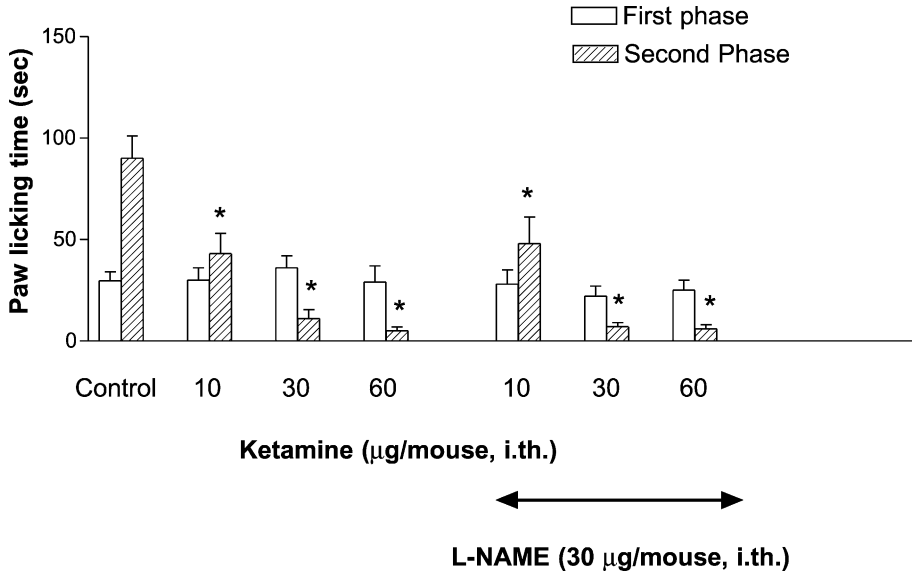

Fig. 4. I.th. L-NAME (30 μg/mouse) pretreatment failed to alter the antinociceptive effects of i.th. ketamine assessed by the formalin-induced paw licking test. Open bars represent paw licking time across the early phase (0–5 min) whereas, striped bars indicate licking time across the late phase (35–50 min) of the formalin test. Significantly different from the saline controls, *$p < 0.05$. Significantly different from the groups that received equal ketamine doses alone, $^{+}p < 0.05$.

tests or across either the early or late phases of the formalin test. However, L-NAME (10 mg/kg, i.p.) pretreatment reversed the antinociception produced by systemic ketamine (1, 5 or 10 mg/kg, i.p.) in the acetic acid-induced writhing test (Fig. 1). L-NAME (10 mg/kg, i.p.) pretreatment also significantly reversed the antinociceptive effects of systemic ketamine (1, 5 or 10 mg/kg) across the late phase of formalin test without altering the responses across the early phase (Fig. 3). However, pretreatment with i.th. L-NAME (30 μg/mouse) did not alter the antinociception produced by i.th. ketamine in the acetic acid-induced writhing test (Fig. 3) and across the late phase of the formalin test (Fig. 4).

Discussion

In the present study, systemic and intrathecal ketamine produced antinociceptive effects in the acetic acid-induced writhing and the formalin tests, but not in the radiant heat tail-flick and hot plate tests. We also observed that systemic administration of L-NAME inhibited the antinociceptive effects of systemic ketamine. However, intraspinal L-NAME did not alter the antinociceptive effects of intrathecal ketamine. Taken together, these data suggest that systemic ketamine activates the NO-cGMP pathway to produce antinociception at supraspinal but not at the spinal level.

Ketamine failed to produce antinociception when given either systemically or spinally in the radiant heat tail flick or hot plate test. These results are concordant with the numerous reports that also show that systemic and intrathecal ketamine do not have antinociceptive activity in hot plate and radiant heat tail flick tests [34–37]. However, there are a few studies that demonstrate that systemic or intrathecal ketamine can produce a small degree of antinociception in both the tail-flick and hot-plate tests [38–40,51]. The reason for the observed differences of ketamine is unclear. However, in these studies, higher doses of systemic (25 mg/kg to 160 mg/kg) and i.th. ketamine (500 μg to 1600 μg/per animal) were used as compared to the present investigation [38–41]. We have reported in the present investigation that ketamine at the doses of higher than 10 mg/kg (i.p.) and 60 μg (i.th.) impaired motor function. Therefore, there is a possibility that the ketamine-induced motor impairment effected the withdrawal latency to the noxious heat and this possibly confounded the interpretation of the results obtained in these studies.

In agreement with previous studies, systemic and spinal administration of ketamine produced dose dependent antinociception in the acetic acid-induced writhing [42,43] and formalin tests [40]. It has been previously shown that following peripheral tissue injury such as those produced by formalin and acetic acid, glutamate is released from primary afferent and dorsal horn neurons [23,44–46]. The subsequent NMDA receptor activation leads to a Ca^{2+} -influx and initiates an enzymatic cascade which finally evokes the release of NO [8,25,45]. NMDA receptor activation is essential for central sensitization after repeated nociceptive stimulation and it is a key factor in the generation and maintenance of persistant pain [19]. NMDA receptor activation does not depolarize dorsal horn neurons in the resting state but it prolongs action potentials if excitatory impulses have been initiated by other neurotransmitters or neuromodulators [47,48]. Consequently, in our study, blockade of the NMDA receptors by ketamine did not elicit antinociception againsts acute noxious thermal stimuli. However, ketamine is efficacious against tonic/persistent types of pain produced by the acetic acid writhing and formalin tests. Our results are in agreement with the observation that NMDA receptor antagonists have a role in mediating persistant pain, but not in acute pain. NMDA receptor antagonists have been shown to preferentially reduce the the nociceptive responses during the late or second phase of the formalin test [42,49]. In the present study, systemical and spinal administration of ketamine inhibited the late phase of the formalin

test without having an effect on the early phase. Since ketamine is a non competetive NMDA receptor antagonist, the antinociceptive effects of spinal and systemic ketamine in the present study are a likely result of NMDA receptor antagonism.

The activation of NMDA receptors initiates an influx of Ca^{2+}, inducing Ca^{2+}-dependent intracellular processes. NOS is a enzyme that is stimulated by the activation of NMDA receptors and synthesizes NO from L-arginine. We expected that ketamine might decrease NO production by blocking NMDA receptors, so inhibition of NOS in central nervous system by L-NAME would be expected to enhance the analgesic effects of systemic and spinal ketamine. However, in the present study, systemic administration of L-NAME prevented the antinociceptive effects of systemic ketamine in formalin and acetic acid-induced writhing tests rather than potentiates the antinociceptive effects of ketamine. But, spinal L-NAME did not alter the antinociceptive effects of spinal ketamine in formalin and acetic acid-induced writhing tests. These results suggest that the activation of the NO-cGMP pathway may have an important role in the antinociceptive effects of systemic ketamine. Interestingly, Mueller and Hunt (1998) showed that L-NAME antagonized, rather than potentiating, the anesthetic effects of ketamine using 45 mg/kg and higher dose of ketamine [50]. In addition, although some studies have showed that ketamine inhibits NO production [24,51], Wu et al (2000) recently have reported that ketamine induced a dose dependent increase in the concentation of NO oxidation products in the rat hippocampus and striatum and this was an effect that was blocked by systemic L-NAME pretreatment [25]. A variety of NOS inhibitors have been used to explore the role of NOS in pain transmission. L-NAME is a non-selective antagonist that inhibits both neuronal NOS and endotelial NOS. In mice, a 94% inhibition of cortical nitric oxide synthase activity have been reported following i.p. administration of L-NAME at the dose of 10 mg/kg [52]. So, we assumed that systemic L-NAME at the used dose in this study (10 mg/kg, i.p.) inhibits sufficiently of brain NOS activity at the used dose. Therefore, the activation of L-arginine–NO-cGMP pathway and NO release in the central nervous system appears to be required in the antinociceptive effects of systemic ketamine.

There are several observations indicating that the activation L-arginine–NO-cGMP pathway participates in the antinociceptive effects of morphine [27] and in some of the non-steroidal antiinflammatory drugs [26]. Ketamine has been shown to interact not only with NMDA receptors, but also with opioid, monoaminergic and muscarinic receptors [11,53,54]. Therefore, a possible mechanism of antinociceptive effects of ketamine may be related to increase of NO concentrations via an increase in acetylcholine release in brain. It has been shown that ketamine produces a dose dependent increase in acetycholine release from the rat hippocampus [55] and striatum [56]. It is possible that ketamine elicits antinociception by increasing acetylcholine in the brain and pretreatment with L-NAME blocks the stimulatory effects of ketamine on NO production via acetylcholine. This implies that inhibition of NOS activity attenuates ketamine antinociception. Another possible mechanism is a pharmacokinetic interaction. Although we did not exclude this possibility, Mueller and Hunt (1998) showed that systemic L-NAME reduced both the rate of absorption of ketamine into blood as well as the uptake of ketamine from blood into brain [50]. They found that the distribution ketamine and its proximate metabolites in blood and brain were 75% and 36% of control values, respectively [50]. The decreased delivery of ketamine into brain is perhaps due to L-NAME-induced alterations in blood flow and may explain the inhibition of ketamine antinociception by L-NAME.

In contrast to systemic interaction of ketamine with L-NAME, it is difficult to explain why L-NAME did not alter the antinociceptive effects of ketamine at the spinal level in both the formalin and acetic acid-induced writhing tests. The site of action of ketamine when given spinally is probably local, within

the spinal cord. It has been shown that formalin injection into the paw increases glutamate concentrations in the dorsal horn of the spinal cord. This increased release of glutamate, in turn, is thought to activate NMDA receptors which are associated with activation of NOS thus resulting in increased production of NO in the spinal cord [23]. The activation of NMDA receptors in the spinal cord is believed to play a critical role in the development and maintenance of the pathologic pain states. It is well known that the activation of spinal NMDA receptors is achieved in formalin test [7]. So, inhibition of the NMDA receptor activation by ketamine at the spinal level can produce antinociception in the formalin and acetic acid-induced writhing tests. There is a lot of evidence that suggest a facilitatory role of NO in spinal nociceptive transmission. However, the antinociceptive effects of different dose of spinally administered ketamine was not changed by pretreatment with L-NAME (30 μg/mouse, i.th.) It is possible that L-NAME did not reach to high enough concentration to be effective in spinal cord at used dose. However, this is not likely, since the smaller dose than this dose of L-NAME (nearly 15 μg) has been demonstrated to cause a nearly 75% reduction in spinal NOS activity in mice [52]. It has been shown that the spinal cord has pharmacologically or functionally distinct pools of NOS and inhibition of a pool that is relatively insensitive to L-NAME appears to be responsible for delayed antinociception [57]. Therefore, it is possible that ketamine blocks NMDA receptors that may be coupled to L-NAME insensitive NOS pools at the spinal cord level. In agreement with this hypothesis, Yamada and Omote (1999) have shown that D, L-2-amino-5-phosphonovaleric acid, a competetive NMDA receptor antagonist do not have any effect on basal NOS activity when given spinally, while N^G-monomethyl-L-arginine, a nonselective inhibitor of NOS, inhibits NOS and reduces basal NOS activity approximately 70% [8]. This point needs to be further investigated.

In summary, systemic and spinal ketamine produces antinociception in the acetic acid-induced writhing and formalin tests in mice. The antinociceptive effects of systemic ketamine was blocked by L-NAME. However, the antinociceptive effects of spinal ketamine were not altered by spinal NOS inhibition. These results suggest that, besides the inhibitory action on NMDA receptors, the activation of the NO-cGMP pathway probably at supraspinal levels may play an important role in the antinociceptive effects of systemic ketamine.

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