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ORIGINAL ARTICLE

Subanalgesic ketamine enhances morphine-induced antinociceptive activity without cortical dysfunction in rats

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Abstract
Purpose Ketamine, a noncompetitive *N*-methyl-D-aspartate receptor antagonist, has been used for the treatment of cancer pain as an analgesic adjuvant to opioids. However, ketamine is known to produce psychotomimetic side effects including cognitive impairments under a high-dose situation, presumably as the result of cortical dysfunction. Here, we investigated whether low-dose ketamine was useful as an analgesic adjuvant to morphine for pain control, focusing on frontocortical function.
Methods To assess the analgesic effects of ketamine with or without morphine, we performed behavioral and histochemical experiments, using the hot plate test and c-Fos expression analysis in rats. The effect on cortical function was also determined by prepulse inhibition (PPI) of the acoustic startle and evoked potentials in the hippocampal CA1-medial prefrontal cortex (mPFC) synapses as measures of synaptic efficacy.
Results Coadministration of ketamine as a subanalgesic dose significantly enhanced intraperitoneal morphine-induced antinociceptive response, which was measured as the increased reaction latency in the hot plate test. In addition, the noxious thermal stimulus-induced c-Fos expression in the ventrolateral periaqueductal gray matter was significantly suppressed by concomitant ketamine and morphine. In contrast, the subanalgesic dose of ketamine did not impair PPI and synaptic efficacy in the mPFC.
Conclusion The present results indicate that the morphine-induced analgesic effect is enhanced by a concomitant subanalgesic dose of ketamine without affecting cortical function. Our findings possibly support the clinical notion that low-dose ketamine as an analgesic adjuvant has therapeutic potential to reduce opioid dosage, thereby improving the quality of life in cancer pain patients.

Keywords Ketamine · Morphine · Analgesia · Cortical dysfunction · PAG

Introduction

Ketamine, a noncompetitive *N*-methyl-D-aspartate receptor (NMDAR) antagonist, has a broad spectrum of clinical effects, including anesthesia and analgesia. At subanesthetic doses, ketamine exerts a NMDAR blockade and produces an antihyperalgesic effect via modulation of central neural circuits involved in pain control [1]. Ketamine is a drug used as an analgesic adjuvant for the treatment of patients with cancer pain [2], including advanced cancer pain refractory to opioids in a palliative-care patient population [3]. Reports indicated that ketamine can potentiate and prolong the analgesic effects of morphine without increasing sedation [4, 5], while preventing the development of analgesic tolerance and physical withdrawal symptoms [6–9]. Studies on animals

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have shown that concomitant administration of ketamine and opioids produces additive or synergistic analgesic effects in neuropathic pain [10, 11] and in inflammatory pain [12]. Therefore, ketamine is considered to have a potential role in pain management without increasing or reducing the amount of opioids required, thereby decreasing side effects and improving opioid effectiveness in patients with cancer pain. More recently, the functional cross-regulation between mu-opioid receptor (MOR) and NMDAR was reported in the midbrain periaqueductal gray (PAG), a critical brain region implicated in the analgesic effects of opioids [13]. However, the synergistic effects and/or mechanisms of ketamine as an analgesic adjuvant to opioids have not been fully documented.

The potent noncompetitive NMDAR antagonists, phencyclidine (PCP) and MK-801, produce transient schizophrenia-like symptoms including cognitive dysfunction in healthy individuals and exacerbate existing symptoms in patients with schizophrenia [14, 15], which are possibly implicated in cortical dysfunction. NMDAR antagonists have been widely used in animal models to study the symptomatology of schizophrenia, because they produce psychotomimetic symptoms characterized by hyperlocomotion, abnormal social interaction, and working memory impairment [16, 17]. Considerable evidence shows the usefulness of ketamine as an analgesic adjuvant; however, there is some concern regarding its adverse effects, such as psychotomimetic symptoms [18–20], presumably caused by cortical dysfunction.

The medial prefrontal cortex (mPFC) is considered to be a key region associated with cognitive function [21–23]. Dysfunction of the PFC contributes to the pathophysiology of schizophrenia [24–26]. Numerous studies have reported on possible neural mechanisms in the mPFC underlying cognitive deficits caused by NMDAR blockade [27, 28]. We previously reported that intraperitoneal ketamine at subanesthetic doses of 5.0 or 25 mg/kg resulted in frontocortical dysfunction; synaptic efficacy in the hippocampus–mPFC pathway was suppressed and prepulse inhibition (PPI) of the acoustic startle, an operational measure of sensorimotor gating that can be modulated by forebrain structures [29], was impaired by ketamine [30].

In the present study, to elucidate whether low-dose ketamine is useful as an analgesic adjuvant to morphine for pain control, we evaluated the concomitant effects of ketamine and morphine on analgesic activity in rats, focusing on cortical function. Analgesic activity was determined by behavioral responses and c-Fos expression in the PAG to acute thermal pain in the hot plate test. Cortical function was evaluated by means of PPI of the acoustic startle and synaptic efficacy in the mPFC synapses.

Materials and methods

Animals

Male Wistar rats were supplied by Sankyo Labo Service (Tokyo, Japan). Rats were housed in a room with a 12 h:12 h light/dark cycle under controlled temperature (21 ± 2 °C). All animal procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Animal Research Committee of the Health Sciences University of Hokkaido and were in accordance with National Institutes of Health guidelines.

Drugs

Ketamine hydrochloride and morphine hydrochloride were purchased from Dai-ichi Sankyo (Tokyo, Japan) and Takeda Pharmaceutical (Osaka, Japan), respectively. The doses of ketamine were selected on the basis of earlier studies performed in our laboratory [30]. Urethane (20 %; Sigma, St. Louis, MO, USA) was dissolved in 0.9 % saline, which was used to anesthetize rats. All vehicles and drugs (5 ml/kg) were intraperitoneally (i.p.) injected at 20 min before behavioral testing.

Prepulse inhibition

Prepulse inhibition of the acoustic startle is the reduction in the startle response caused by a low-intensity nonstartling stimulus (i.e., prepulse) that is presented shortly before the startle stimulus. PPI is used in animals, as well as in clinical settings, to screen the antipsychotic effects of drugs as an operational measure of sensorimotor gating. Rats were acclimatized to the startle chamber (SR-LAB; San Diego Instruments, San Diego, CA, USA) for 5 min with 65 dB background white noise that was continued throughout the session. After acclimatization, rats were subjected to only a startle pulse (120 dB, 40-ms broadband burst) or a startle pulse preceded by 68, 71, and 74 dB prepulse (20-ms broadband noise) 100 ms earlier. A piezoelectric accelerometer was used to detect startle amplitude, and the bursts of acoustic noise were delivered by loudspeakers. For each test session, 70 trials were conducted in random order: 10 trials with only a startle pulse, 10 trials with no stimulation (background noise only), and 10 trials of 68, 71, and 74 dB prepulse followed by a startle pulse. The intertrial interval was 15, 20, 25, and 30 s, which occurred in a random order. SR-LAB software controlled the delivery of all stimuli and recorded the responses. PPI was calculated as the percent inhibition of the startle amplitude evoked by the pulse alone: PPI (%) = [(magnitude of pulse alone trial − magnitude of prepulse + pulse trial)/magnitude of pulse alone

trial] × 100. Startle amplitude was expressed as the startle response by assessing the average of the last five trials of each startle pulse. Drugs were injected 10 min before the PPI procedure.

Hot plate test

The hot plate test was conducted to assess the analgesic effect of ketamine and/or morphine on thermal nociception. Drugs were injected 20 min before the hot plate test. The temperature of the plate was maintained at 52 ± 1 °C. Animals were placed in plastic funnels onto the heated surface. The reaction latency was measured as the time between placement of the animals and the onset of paw-licking or jumping behaviors.

Immunohistochemistry and cell counting

Histochemical experiments involving c-Fos expression to assess neural activation were previously reported [31]. In brief, rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) 2 h after the hot plate test and transcardially perfused via the left ventricle with 0.9 % saline followed by 4 % paraformaldehyde in 0.1 M sodium phosphate buffer (PB, pH 7.2). After removal from the skull, their brains were immersed overnight in the same fixative at 4 °C, placed in 0.1 M PB containing 30 % sucrose at 4 °C and sectioned at 30 μm thickness using Leica HM 300 cryostat. The sections were collected in 0.01 M phosphate-buffered saline (PBS, pH 7.2) containing 0.1 % Triton X-100 (PBSTx) and stored for at least 1 h before immunohistochemical staining. All immunohistochemical incubations were conducted at room temperature, and sections were washed three times in PBSTx between incubations. First, sections were successively incubated with 10 % normal donkey serum for 20 min, subsequently, with the primary antibody overnight: rabbit anti-c-Fos (1:20,000; PC38T, Calbiochem) antibody. The sections were then incubated for 1 h with the fluorophore-linked species-specific secondary antibody, Alexa 555 (Invitrogen) at a dilution of 1:200. Images were taken with a confocal laser-scanning microscope FV1000 (Olympus). For the PAG, we selected one representative section located 7.8 mm posterior to the bregma from each rat. ImageJ (NIH) was used as the fluorescent image analysis system and cell counter. The number of c-Fos-positive cells was counted in three rostrocaudal sections every 30 μm in the dorsomedial PAG (DMPAG), dorsolateral PAG (DLPAG), lateral PAG (LPAG), and ventrolateral PAG (VLPAG), and assessed by an automated selection of cells within the unit areas (675 × 675 μm). The background gray value was determined in a part of each unit area containing no nuclei. Finally, the number of c-Fos-positive cells was averaged in respective regions of the PAG.

Electrophysiology

Procedures for electrophysiological experiments to record evoked field potentials were previously reported [30, 32]. Rats were anesthetized with urethane (1 g/kg, i.p.), and stimulating electrodes and a recording electrode were implanted in the CA1/subicular region of the ventral hippocampus (6.0 mm posterior to the bregma, 5.6 mm lateral, and 4.0–7.0 mm ventral from the cortical surface) and mPFC (3.3 mm anterior to the bregma, 0.8 mm lateral, and 3.3 mm ventral from the cortical surface), according to the Paxinos and Watson brain atlas, respectively. To stimulate the CA1/subicular region, the stimulating electrodes were connected to an electric stimulator (SEN-3301; Nihon Koden) and an isolator (SS-202J; Nihon Koden). The recording electrode was connected to an amplifier (MEG 5200; Nihon Koden: gain 0.2–2 mV, low-cut filter 50 Hz, high-cut filter 300 Hz). The potential evoked by test stimulation (frequency 0.1 Hz, pulse duration 250 μs) was monitored with an oscilloscope (VC-10; Nihon Koden) and measured with the Power Lab data analysis system connected to computers. Test stimuli were delivered every 30 s at an intensity that produced a response that was 60 % of that induced by the maximal stimulation intensity. The integrated population spike amplitude (PSA) obtained from seven successive stimuli was recorded every 5 min with data analysis systems.

Statistics

Differences between multiple group comparisons were performed using a one-way or two-way analysis of variance (ANOVA) with Bonferroni's post hoc test, if the differences in the variance of these multiple groups were not significant. The alpha level was set at 5 %. When the difference in the variance of multiple groups was significant, the Kruskal–Wallis test was performed; subsequently, multiple comparison was performed using the Mann–Whitney *U* test, setting the alpha level at 1.25 % (5 %/4) because the number of comparison was 4 (four groups).

Differences between two groups were compared using Student's *t* test when the difference in the variance of two groups was not significant. If the difference of variance in two groups was significant, comparisons were performed using the Mann–Whitney *U* test. The alpha level was set at 5 %.

Results

Effects of ketamine and/or morphine on behavioral responses in the hot plate test

First, we examined the analgesic effect of ketamine using the hot plate test. At a dose of 25 mg/kg, ketamine significantly increased the reaction latency to escape from the hot plate compared with the vehicle group. However, the low doses (1.0 and 5.0 mg/kg) of ketamine did not exert significant changes on the reaction latency, which was as follows: vehicle, 11.6 ± 1.0 s ($n = 13$); ketamine 1.0 mg/kg, 12.5 ± 0.8 s ($n = 13$); ketamine 5.0 mg/kg, 15.6 ± 0.8 s ($n = 7$); ketamine 25 mg/kg, 20.8 ± 2.1 s ($n = 6$); $F_{3,35} = 11.2$, $^{**}p < 0.01$ (Fig. 1).

Next, we determined the analgesic dose of morphine using the same behavioral test. The reaction latency significantly increased by 1.0 and 2.0 mg/kg morphine compared with the vehicle group, which was as follows: vehicle, 11.6 ± 1.0 s ($n = 13$); morphine 0.2 mg/kg, 13.4 ± 1.4 s ($n = 12$); morphine 1.0 mg/kg, 17.6 ± 0.9 s ($n = 12$); morphine 2.0 mg/kg, 25.0 ± 2.6 s ($n = 6$); $F_{3,39} = 15.3$, $^{**}p < 0.01$ (Fig. 2).

Subsequently, we examined the synergistic effects of the coadministration of ketamine and morphine on the analgesic activity using the hot plate test. We selected 1.0 mg/kg ketamine as a subanalgesic dose, because in our previous studies 5.0 mg/kg ketamine induced a PPI deficit, which was concerned with frontocortical dysfunction [30]. In the presence of 1.0 mg/kg ketamine, 0.2 mg/kg morphine did not change the reaction latency, which was as follows:

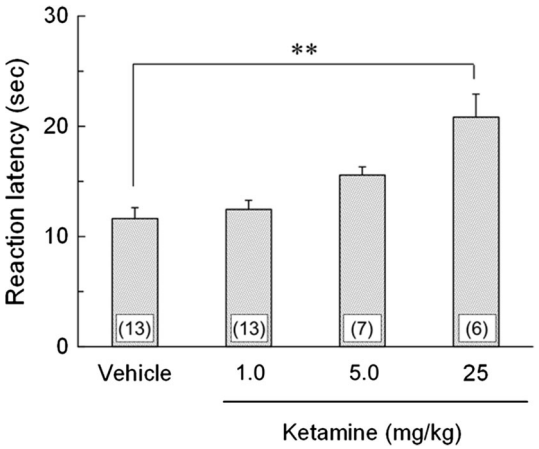

Fig. 1 Analgesic activity of ketamine or morphine. The analgesic dose of ketamine was determined in the hot plate test. At a dose of 25 mg/kg, but not 1.0 and 5.0 mg/kg, ketamine significantly increased reaction latency compared with the vehicle group. Data are expressed as mean ± SEM. $^{**}p < 0.01$ compared with vehicle groups. Number of rats tested is shown in *parentheses*

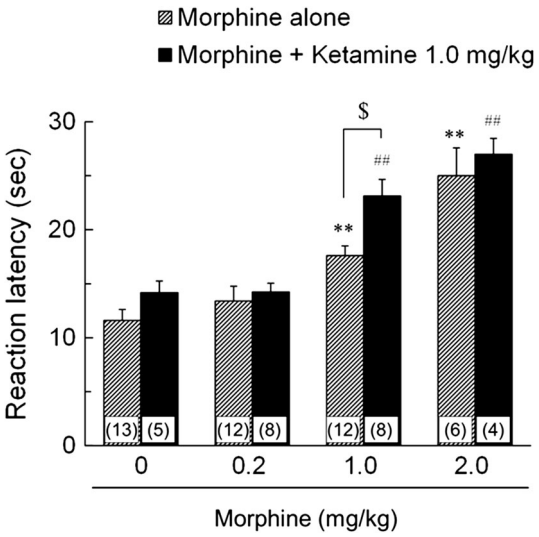

Fig. 2 Synergistic effects of ketamine and morphine on analgesic activity. First, the analgesic dose of morphine was assessed. Morphine significantly increased reaction latency at doses of 1.0 and 2.0 mg/kg compared with the vehicle group (morphine 0 mg/kg, $^{**}p < 0.01$). Next, the concomitant effects of ketamine and morphine were determined in the hot plate test. Coadministration of 1.0 or 2.0 mg/kg morphine with 1.0 mg/kg ketamine produced a significant increase in reaction latency compared with the vehicle + ketamine group (ketamine alone, $^{\#\#}p < 0.01$). Moreover, the morphine-induced analgesic effect was significantly enhanced by the subanalgesic dose of ketamine ($^{\$}p < 0.05$). Data are expressed as mean ± SEM. $^{**}p < 0.01$ compared with the vehicle groups (morphine 0 mg/kg). $^{\#\#}p < 0.01$ compared with the vehicle + ketamine groups (ketamine alone). $^{\$}p < 0.05$ compared with morphine (1.0 mg/kg) alone. Number of rats tested is shown in *parentheses*

vehicle + ketamine (ketamine alone) 14.2 ± 1.1 s ($n = 5$); morphine 0.2 mg/kg + ketamine, 14.3 ± 1.5 s ($n = 8$). In contrast, coadministration of 1.0 or 2.0 mg/kg morphine with 1.0 mg/kg ketamine significantly increased the reaction latency compared with the vehicle + ketamine group (ketamine alone), which was as follows: morphine 1.0 mg/kg + ketamine, 23.9 ± 1.6 s ($n = 8$); morphine 2.0 mg/kg + ketamine, 27.0 ± 1.5 s ($n = 4$); $F_{3,21} = 15.6$, $^{\#\#}p < 0.01$. Moreover, the increased reaction latency by coadministration of morphine (1.0 mg/kg) and ketamine (1.0 mg/kg) was different from that in morphine (1.0 mg/kg) alone ($F_{1,18} = 4.3$, $^{\$}p < 0.05$, Fig. 2).

Effects of ketamine and/or morphine on c-Fos expression in the PAG

We evaluated the synergistic effects of ketamine as an analgesic adjuvant to morphine by histochemical c-Fos expression in the PAG 2 h after the noxious thermal stimulus.

In the VLPAG, a subdivision of the PAG, the density of c-Fos-positive cells significantly increased after the

thermal stimulus, but not DMPAG, DLPAG, and LPAG. The number of c-Fos-positive cells (cells/675 μm^2, $n = 4$) was as follows: VLPAG, non-stimulus: 40.0 ± 1.7; stimulus: 60.5 ± 4.9, $F_{1,6} = 26.0$, $^*p < 0.05$; DMPAG, non-stimulus: 27.8 ± 1.4; stimulus: 28.0 ± 2.2, $F_{1,6} = 0.02$, not significant (n.s.); DLPAG, non-stimulus: 52.0 ± 3.1; stimulus: 50.8 ± 4.1, $F_{1,6} = 0.007$, n.s.; LPAG, non-stimulus: 52.0 ± 5.1; stimulus: 42.8 ± 5.0, $F_{1,6} = 0.002$, n.s. (Fig. 3a, b). Thus, these results demonstrated that the VLPAG is a critical region for pain control.

Subsequently, we administered ketamine (1.0 mg/kg) and/or morphine (1.0 mg/kg) 20 min before the hot plate test and analyzed c-Fos expression in the VLPAG. Coadministration of ketamine and morphine significantly reduced thermal stimulus-induced-c-Fos expression in the VLPAG, although a single administration of ketamine or morphine had no effect on c-Fos expression: number of c-Fos positive cells (cells/675 μm^2, $n = 4$): vehicle + vehicle, 60.5 ± 4.9; ketamine + vehicle, 61.25 ± 5.9; vehicle + morphine, 56.8 ± 1.6; ketamine + morphine, 34.5 ± 4.7; $F_{3,12} = 2.0$, $^*p < 0.05$ (Fig. 3c, d).

Effects of ketamine and/or morphine on frontocortical function

To examine the effects of the subanalgesic dose of ketamine (1.0 mg/kg) and/or the analgesic dose of morphine (1.0 mg/kg) on sensorimotor gating mechanisms (frontocortical function), behavioral analysis assessment by PPI of the acoustic startle response was performed. As shown in Fig. 4a, b, a single administration of the subanalgesic dose of ketamine (1.0 mg/kg) or the analgesic dose of morphine (1.0 mg/kg) exerted acoustic startle reactivity to test stimuli (120 dB) and produced inhibition of the startle acoustic response (PPI) at every prepulse stimulus tested, almost identical to the vehicle-administered group. Furthermore, the coadministration of ketamine and morphine did not induce a PPI deficit, which was as follows: PPI (%),

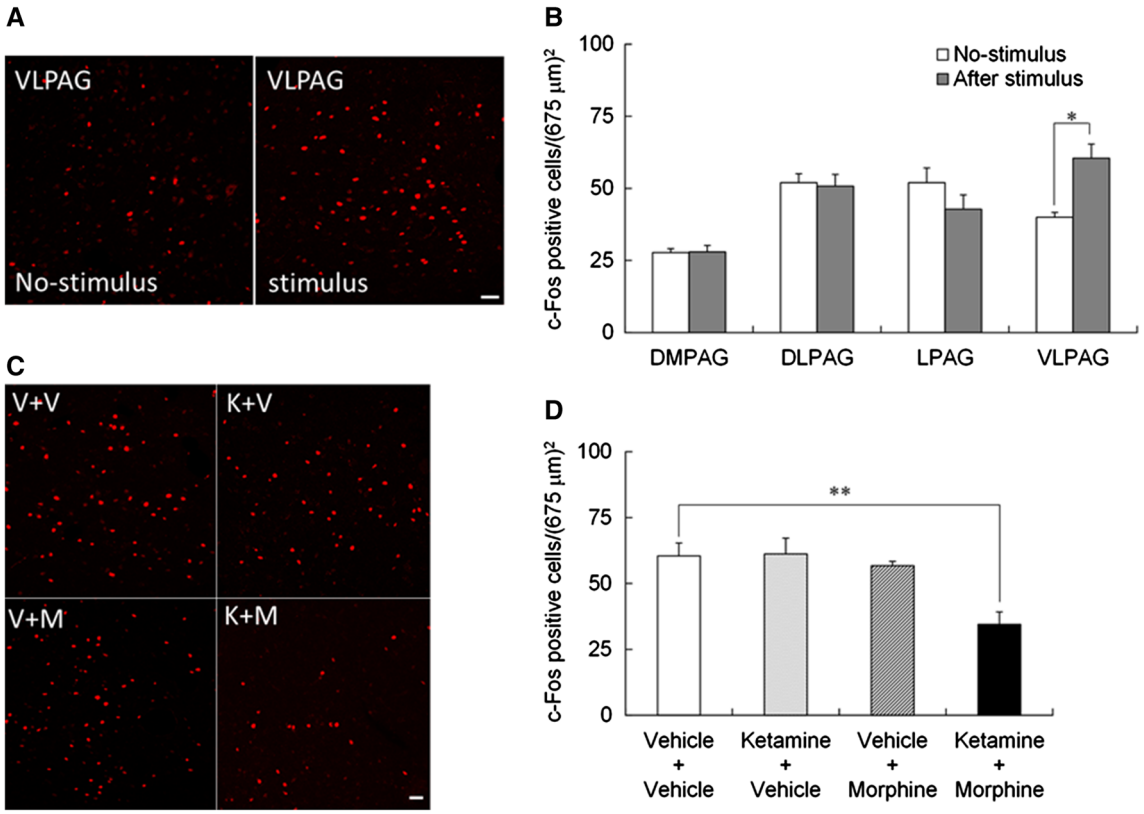

Fig. 3 Effects of ketamine and/or morphine on c-Fos expression induced by noxious thermal stimulus in the periaqueductal gray (PAG). **a** High-power photographs show basal c-Fos expression of no-stimulus (*left*) and thermal stimulus-induced c-Fos expression (*right*) in the ventrolateral PAG (*VLPAG*). **b** Number of thermal stimulus-induced c-Fos-positive neurons in the dorsomedial PAG (*DMPAG*), dorsolateral PAG (*DLPAG*), lateral PAG (*LPAG*), and VLPAG. The expression of c-Fos-positive neurons significantly increased in the VLPAG, but not DMPAG, DLPAG, and LPAG. **c** High-power fluorescent photographs shows the effect of ketamine and/or morphine administration on thermal stimulus-induced c-Fos expression. Coadministration of ketamine (1.0 mg/kg) and morphine (1.0 mg/kg) suppressed c-Fos expression in the VLPAG. **d** The number of thermal stimulus-induced c-Fos-positive neurons after coadministration of ketamine and morphine in the VLPAG. Data are expressed as mean ± SEM. $^*p < 0.05$ compared with no-stimulus groups (**b**), $^{**}p < 0.01$ compared with vehicle + vehicle groups (**d**). *V* vehicle, *K* ketamine 1.0 mg/kg, *M* morphine 1.0 mg/kg. *Bar* 30 μm

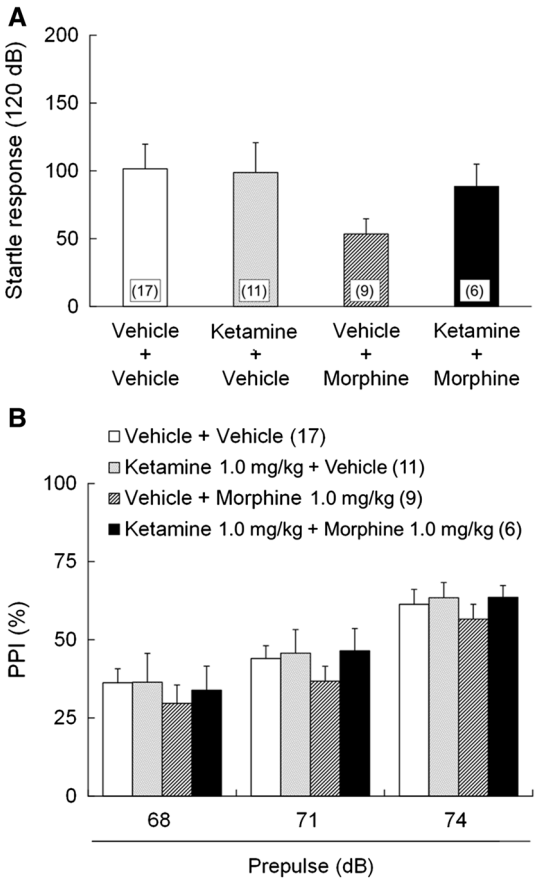

Fig. 4 Effects of coadministration of ketamine and/or morphine on prepulse inhibition (*PPI*) of the acoustic startle response: the acoustic startle reactivity to test stimuli (120 dB) (**a**) and PPI (%) (**b**). No significant change was noted in the absence and presence of ketamine (1.0 mg/kg) and morphine (1.0 mg/kg), respectively. Data are expressed as mean ± SEM. Number of rats tested is shown in *parentheses*

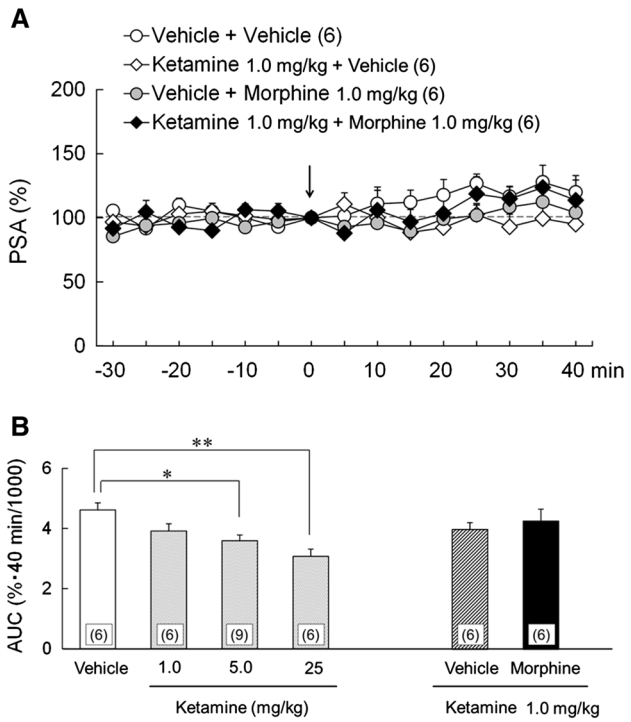

Fig. 5 Effects of ketamine and/or morphine on synaptic efficacy in the hippocampal–mPFC pathway. Hippocampal stimulation-evoked field potential in the mPFC was measured as the peak population spike amplitude (*PSA*): time-course of the PSA changes (**a**) and the area under the curve (*AUC*) (**b**) of the PSA for 40 min before and after vehicle, ketamine (1.0 mg/kg), and morphine (1.0 mg/kg) administration. Stimulation of the hippocampal CA1/subicular region induced a characteristic negative synaptic potential in the mPFC. Each time-course of response changes was expressed as the percentage of the PSA obtained immediately before vehicle, ketamine and/or morphine administration. Data are expressed as mean ± SEM. $*p < 0.05$ or $**p < 0.01$ compared with vehicle group. Number of rats tested is shown in *parentheses*

vehicle + vehicle ($n = 7$), ketamine + vehicle ($n = 8$), vehicle + morphine ($n = 7$), ketamine + morphine ($n = 6$): 68 dB, 44.3 ± 11.6, 58.5 ± 4.8, 29.0 ± 6.7, 33.9 ± 7.7, respectively, $F_{3,24} = 1.31$, n.s.; 71 dB, 51.7 ± 5.3, 62.4 ± 6.0, 37.3 ± 5.4, 46.5 ± 7.1, respectively, $F_{3,24} = 0.07$, n.s.; 74 dB, 68.5 ± 6.5, 74.0 ± 3.1, 58.4 ± 4.9, 63.6 ± 3.8, respectively, $F_{3,24} = 0.73$, n.s.

In the electrophysiological experiment, we previously reported that ketamine (5.0 and 25 mg/kg) decreased synaptic transmission in the mPFC [30]. Moreover, we also suggested that the decreases in the evoked response in the mPFC induced by subanesthetic doses of ketamine (5.0 and 25 mg/kg) correlated well with PPI impairment. Based on our previous results, we investigated the effect of ketamine (1.0 mg/kg) and/or morphine (1.0 mg/kg) on synaptic efficacy in the hippocampal–mPFC neural circuit to assess the influence on frontocortical function. As shown in Fig. 5a, ketamine (1.0 mg/kg) did not significantly change the evoked potential in the mPFC. In contrast, 5.0 and 25 mg/kg of ketamine did, which was consistent with our previous results [30]. AUC (% 40 min/1,000) was as follows: vehicle, 4.6 ± 0.2 ($n = 6$); ketamine 1.0 mg/kg, 3.9 ± 0.2 ($n = 6$); ketamine 5.0 mg/kg, 3.6 ± 0.2 ($n = 9$); ketamine 25 mg/kg, 3.1 ± 0.2 ($n = 6$); $F_{3,23} = 7.7$, $*p < 0.05$, $**p < 0.01$. Moreover, no significant difference of PSA was noted by morphine (1.0 mg/kg) with/without subanalgesic ketamine [AUC (% 40 min/1,000)]: vehicle + morphine, 4.0 ± 0.2 ($n = 6$); ketamine + morphine, 4.3 ± 0.4 ($n = 6$); $F_{1,10} = 2.6$, n.s. (Fig. 5b).

Discussion

In the present study, we evaluated therapeutic potentials of low-dose ketamine as an analgesic adjuvant to morphine in experimental pain models. At a dose of 1.0 mg/kg, ketamine did not produce significant increases in reaction

latency in the hot plate test as compared with the vehicle-treated group. This subanalgesic dose of ketamine enhanced the morphine-induced (1.0 mg/kg) analgesic activity to thermal pain. In addition, the thermal stimulus-induced c-Fos expression in the VLPAG was significantly suppressed by the concomitant administration of ketamine and morphine. However, ketamine (1.0 mg/kg) and/or morphine (1.0 mg/kg) did not impair PPI and synaptic efficacy in the mPFC. These results indicate that the morphine-induced analgesic effect on acute thermal pain is synergistically enhanced by the concomitant subanalgesic dose of ketamine without affecting frontocortical function.

Ketamine, a dissociative anesthetic with analgesic properties, is characterized as a noncompetitive NMDAR antagonist. In general, potent noncompetitive NMDAR antagonists, such as PCP and MK-801, are well known to produce psychotomimetic symptoms characterized by hyperlocomotion, abnormal social interaction, and working memory impairment [16, 17]. Ketamine also produces hyperlocomotion, cortical dysfunction, and schizophrenia-like behaviors [18]. PPI is widely used as a cross-species test battery for evaluating cortical function [33]. Acoustic startle response, a defensive reaction that is mediated via a simple neural circuit in the lower brainstem structures, can be modulated by forebrain structures [29]. Moreover, the cortico-limbic system is known to be crucial for PPI control [34]. Deficits in PPI manifest in the inability to filter out the unnecessary information and have been linked to abnormalities of sensorimotor gating. Such deficits are noted in patients suffering from psychiatric disorders with cortical dysfunction, such as schizophrenia disease [35]. In addition, some studies have shown that PPI impairment induced by administration of NMDAR antagonists is ameliorated by atypical antipsychotics but not by typical antipsychotics [36, 37], indicating that NMDAR blockade-induced PPI deficits may be involved in the negative symptoms and/or cognitive deficits associated with schizophrenia. In our previous study [30], behavioral analysis using PPI showed that subanalgesic or subanesthetic doses of ketamine (5.0 and 25 mg/kg) caused PPI deficits, which was consistent with previous reports [38, 39]. For this reason, we selected a lower subanalgesic dose of ketamine (1.0 mg/kg) as an analgesic adjuvant to morphine, although 5.0 mg/kg ketamine was a subanalgesic dose as well.

The mPFC is a critical brain region for cognitive function [21–23]. Numerous studies have concentrated on the possible neural mechanisms in the mPFC underlying cognitive deficits caused by NMDAR blockade [27, 28]. We have previously reported that synaptic efficacy in the hippocampal–mPFC pathway is a measure of frontocortical function because cognitive interventions using physical and pharmacological manipulations were accompanied with changes in the evoked potentials in the mPFC synapses [30, 40]. Thus, we speculated that cognitive impairment associated with NMDAR blockade may be at least partly related to the downregulation of synaptic transmission in the mPFC. The present data indicate the possibility that synaptic suppression in the hippocampal–mPFC pathway observed in ketamine-treated groups (5.0 and 25 mg/kg) is implicated in the psychotomimetic aversive effects, based on frontocortical dysfunction. In other words, the present results showing that the subanalgesic dose (1.0 mg/kg) of ketamine did not elicit significant changes in synaptic suppression in the mPFC as well as PPI support our idea that subanalgesic ketamine did not impair cortical functions in rats.

The PAG, a critical brain region implicated in the analgesic effects of opioids [13], plays a pivotal role in the integration of behavioral, somatic, and autonomic responses of an animal to threat, stress, and pain [41]. Anatomically, the PAG is divided into longitudinal columns, i.e., dorsal, lateral, and ventral PAG, charged with distinct physiological and behavioral functions, and wired by distinct neural pathways [42]. For example, stimulation of the dorsal PAG produces fear and anxiety, increases blood pressure, and causes vocalizations that are normally associated with rage. In contrast, stimulation of the VLPAG produces analgesia and freezing [43]. The PAG along with its descending projections to the rostral ventromedial medulla (RVM) is one of the primary anatomical substrates mediating opioid analgesia. Opioid microinjections have an antinociceptive effect only when targeted at the VLPAG [44]. Thus, the VLPAG is a major site of opioid analgesia, which regulates opioid-mediated analgesic actions. In the present study, noxious thermal stimulus induced c-Fos expression in the VLPAG, but not DMPAG, DLPAG, and LPAG, which was suppressed by a combination of morphine and ketamine. The induction of the Fos family transcription factors has been widely used as a tool to evaluate neural activation in response to a wide range of stimuli [45]. Our findings in histochemical c-Fos expression indicate that the neural modulation of VLPAG activity underlies the synergistic analgesic effects of the coadministration of morphine and ketamine.

The neuronal and/or molecular bases of ketamine as an analgesic adjuvant to morphine have not fully been understood. Recently, functional cross-regulation between MOR and NMDAR via protein kinase C (PKC)/PKA-regulated association was reported in the PAG, and the postsynaptic association of these receptors has been implicated in the transmission and modulation of nociceptive signals [13, 46]. The functional relationship between MOR and NMDAR is bidirectional; however, persistent activation of NMDAR is responsible for neural consequences that accompany neuropathic pain. Opioids, including morphine, possibly disrupt

the MOR and NMDAR complex to stimulate the activity of NMDAR, which may result in opposing MOR signaling and the development of opioid analgesia tolerance. Therefore, the blockade of NMDAR activity, which negatively regulates the ability of morphine, may partly explain the mechanism of ketamine as an analgesic adjuvant. We should also consider the possibility that morphine and/or ketamine may modulate neural activity differently in normal versus pain states as previously reported [47]. Indeed, in our preliminary electrophysiological experiments showing the possible involvement of the PAG by monitoring the neuronal activity, we found that ketamine (5.0 and 25 mg/kg) by itself did not affect ongoing neuronal activity in the PAG. In contrast, the subanesthetic/analgesic dose (25 mg/kg), but not the high subanalgesic dose (5.0 mg/kg), of ketamine abolished the neuronal activity evoked by noxious mechanical stimuli (i.e., hind-paw pinching). To clarify the neural mechanisms underlying the synergistic effect of morphine and ketamine or the functional interaction between MOR and NMDAR, further experiments are required focusing on the neural activity within the PAG as well as the functional connections between the cortico-PAG circuits.

Pain management in patients with cancer is often limited by the adverse effects of opioids, including constipation, somnolence, nausea and vomiting, and acute tolerance that further impairs pain control when used alone in large doses for an extensive period. In our behavioral experiments, the reaction latency in the hot plate test was significantly increased by 1.0 and 2.0 mg/kg morphine, indicating that 1.0 mg/kg is the minimum dose producing analgesic effects on acute thermal pain. The present findings, therefore, indicate that the subanalgesic dose of ketamine enhanced the antinociceptive activity induced by the minimum analgesic dose of morphine, which raises the possibility that low-dose ketamine can minimize the opioid dosage for pain control without affecting cortical function. This study may provide further supportive evidence for the potential therapeutic advantages of the analgesic adjuvant ketamine.

In conclusion, the present results revealed the synergistic effects of coadministration of morphine and a subanalgesic dose of ketamine on acute thermal pain, without frontocortical dysfunction. Further understanding of the precise mechanisms underlying the synergistic effects of ketamine and opioids on antinociceptive activity is of importance for providing rational evidence of the therapeutic advantages of low-dose ketamine as an analgesic adjuvant for pain management in patients with cancer.

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Conflict of interest The authors declare no conflict of interest associated with this manuscript.

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