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Short communication

NMDA receptor-mediated mechanism of ketamine-induced facilitation of glutamatergic excitatory synaptic transmission

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Abstract

The effect of ketamine on CA1-field EPSPs (fEPSPs) in rat hippocampal slices was investigated. Ketamine (100 μM) facilitated fEPSPs at 0.05 Hz. The fEPSP facilitation was suppressed completely by AP-5 and partially by propranolol, and also by an increase in stimulation frequency. These results indicate that ketamine facilitates excitatory synaptic transmission by activating NMDA receptors via β-adrenoceptors under conditions in which NMDA receptor channel block is slight.

Theme: Neurotransmitters, modulators, transporters, and receptors

Topic: Excitatory amino acids: pharmacology

Keywords: Adrenergic receptor; Hippocampus; Ketamine; Synaptic transmission

Ketamine, a dissociative intravenous anesthetic, inactivates the thalamo-neocortical projection system and the medial medullary reticular formation but activates the limbic system simultaneously while inducing an anesthetic state [5,18]. Since activation of the limbic system may contribute to the disadvantageous excitation and hallucination characteristics of ketamine anesthesia, the mechanisms by which this activation is induced should be investigated in order to establish a method for more favorable anesthetic and sedative management using ketamine.

In the hippocampus, a main constituent of the limbic system, it was found that ketamine facilitates orthodromic stimulation-induced excitatory responses of pyramidal cells without enhancement of their excitability in the CA1 region [24]. This finding suggests that ketamine may facilitate the excitatory synaptic transmission on CA1-pyramidal cells that seems to support the activation of the limbic system.

The aim of this study was to elucidate the effect of ketamine on excitatory synaptic transmission. We investigated the effect of ketamine on CA1-field excitatory postsynaptic potentials (fEPSPs) in hippocampal slices, which exclusively reflect glutamatergic excitatory synaptic transmission and are independent of GABAergic transmission at low stimulation frequency [8,14,17,25].

All of the protocols were approved by the Animal Use Committee of Sapporo Medical University. From brains removed from isoflurane-anesthetized male Wistar rats (5–7 weeks old), transverse hippocampal slices (400 μm thick) were prepared with a vibratome in oxygenated and cooled artificial cerebrospinal fluid (ACSF). Each slice was placed in a submerged recording chamber (2 ml vol) constantly superfused (3 ml/min) with oxygenated ACSF at 26±0.5 °C (pH 7.40±0.05). The fEPSPs, elicited by orthodromic inflamaximal electrical stimulation on CA1-Schaffer collateral at 0.05, 0.2, or 0.5 Hz with a tungsten bipolar microelectrode, were recorded from the CA1-stratum radiatum using an extracellular tungsten microelectrode (1.0±0.1 MΩ). The stimulation intensity eliciting fEPSP for each slice was set at about 20–30% of that of threshold eliciting population spikes (range: 0.21–0.96 mV). As an index of synaptic strength, the fEPSP amplitudes, which were averaged in groups of three, were analyzed using an on-line computer program. Drug effects were investigated following stabilization of the fEPSP amplitude. All of the drugs were continuously applied to

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the slices through the superfusing ACSF. The fEPSP amplitude is expressed as mean±S.D. (n=6–13) and as a % of the control value, defined as 'mean field EPSP amplitude (averaged for 3 min) immediately before the application of ketamine'. Statistical analysis was performed using paired Student's t-test and one- or two-way repeated measures ANOVA. Data were considered to be statistically significant when $P<0.05$.

Ketamine (100 μM) increased the fEPSP amplitude at 0.05 and 0.2 Hz but not at 0.5 Hz (155.0±28.8, 118.6±21.5, and 99.6±12.2% of the control at 30 min, n=9, 11 and 8, respectively). With an increase in stimulation frequency, the ketamine-induced fEPSP facilitation was depressed ($P<0.01$ by ANOVA; Fig. 1A). At 0.05 Hz, the fEPSP amplitude was increased by 10 μM ketamine (147.1±30.1% of the control at 30 min, n=6) but not by 1 μM ketamine (n=6; Fig. 1B). The increases in amplitude with concentrations of 10 and 100 μM ketamine at 0.05 Hz were not significantly different. A subsequent washout with normal ACSF decreased the amplitude increased by ketamine almost to the control level within 60 min.

The 100-μM ketamine-induced increase in fEPSP amplitude was counteracted completely ($P<0.01$ by ANOVA, each) by pretreatment with 50 μM AP-5 (an NMDA-type glutamate receptor antagonist) at 0.05 and 0.2 Hz (107.1±19.0 and 103.0±18.1% of the control at 30 min, n=13 and 7, respectively; Fig. 2A) and partially ($P<0.01$ by ANOVA) by pretreatment with 10 μM propranolol (a β-adrenergic receptor antagonist, 113.1±14.5% of the control at 30 min, n=12) at 0.05 Hz (Fig. 2B). However,

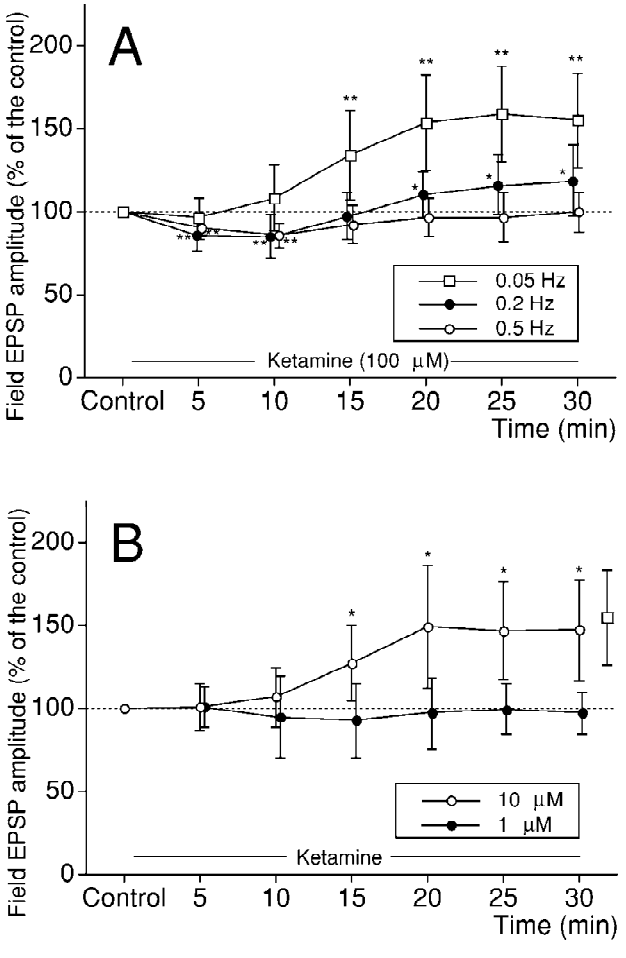

Fig. 1. Time-courses of the effects of ketamine on field excitatory postsynaptic potential (EPSP) amplitude in slices superfused with normal artificial cerebrospinal fluid (ACSF). (A) 100 μM ketamine in normal ACSF at 0.05, 0.2 and 0.5 Hz (n=9, 11 and 8, respectively). (B) 10 and 1 μM ketamine in normal ACSF at 0.05 Hz (n=6, each). Mean field EPSP amplitude (averaged for 3 min) immediately before the application of ketamine is defined as the control value (100%). Mean±S.D., * $P<0.05$ and ** $P<0.01$ vs. control. The open square with error bars at the far right of graph B indicates the effect of 100 μM ketamine in normal ACSF at 30 min for comparison.

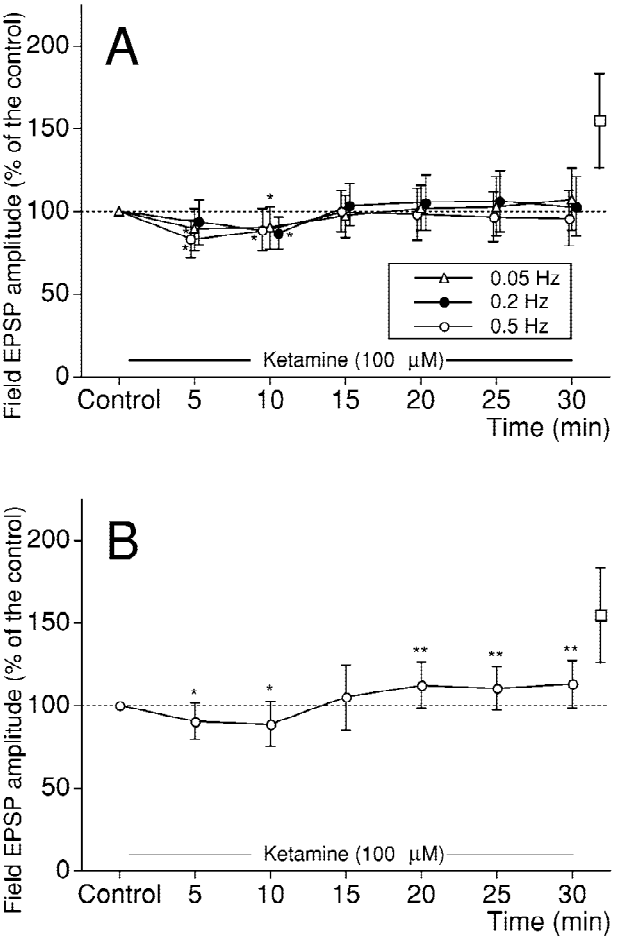

Fig. 2. Time-courses of the effects of ketamine on field excitatory postsynaptic potential (EPSP) amplitude in slices pretreated with AP-5 ((±)-2-amino-5-phosphonopentanoic acid, an NMDA receptor antagonist) or propranolol hydrochloride (a β-adrenergic receptor antagonist). After the pretreatment for 30 min, ketamine was concomitantly applied. (A) 100 μM ketamine with 50 μM AP-5 at 0.05, 0.2, or 0.5 Hz (n=13, 7 and 7, respectively). (B) 100 μM ketamine with 10 μM propranolol at 0.05 Hz (n=12). Mean field EPSP amplitude (averaged for 3 min) immediately before the application of ketamine is defined as the control value (100%). Mean±S.D., * $P<0.05$ and ** $P<0.01$ vs. control. The open square with error bars at the far right of each graph indicates the effect of 100 μM ketamine in normal ACSF at 30 min for comparison.

pretreatment with 50 μM phentolamine (an α-adrenergic receptor antagonist, $n=6$), 1 μM methysergide (a serotonin 5-HT_2/5-HT_{1C} receptor antagonist, $n=7$), 5 μM atropine (a muscarinic acetylcholine receptor antagonist, $n=6$), 10 μM naloxon (an opiate receptor antagonist, $n=6$), or 10 μM bicuculline (a $GABA_A$ receptor antagonist, $n=7$) did not significantly influence the 100-μM ketamine-induced fEPSP facilitation at 0.05 Hz (Fig. 3).

When 100 μM ketamine-induced fEPSP facilitation was suppressed by relatively high stimulation frequency (0.2 or 0.5 Hz) or pre-application of AP-5 or propranolol, a transient decrease in fEPSP amplitude (to about 85% of the control) also appeared at 5–10 min, a period immediately before the fEPSP facilitation should begin.

Ketamine at 100 μM facilitated fEPSP at 0.05 Hz, indicating that this dose of ketamine, which is within the anesthetic range for rats [15], facilitates glutamatergic excitatory synaptic transmission at a low stimulation frequency. At 0.05 Hz, 1 μM ketamine did not affect fEPSP, but 10 μM ketamine enhanced fEPSP as intensely as 100 μM ketamine did, indicating that 100 μM ketamine is sufficient to elicit E_{max}.

At 0.05 and 0.2 Hz, pre-application of AP-5 completely suppressed the 100 μM ketamine-induced fEPSP facilitation, indicating that ketamine facilitates excitatory synaptic transmission through NMDA receptors, but not through non-NMDA receptors. However, ketamine-induced NMDA receptor block, including channel block [6,13,19], can not theoretically explain the fEPSP facilitation.

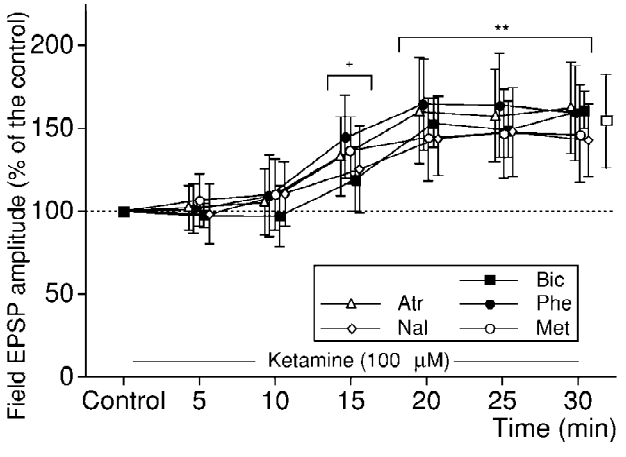

Fig. 3. Time-courses of the effects of 100 μM ketamine on field excitatory postsynaptic potential (EPSP) amplitude in slices pretreated with 50 μM phentolamine methylate (Phe, an α-adrenergic receptor antagonist, $n=6$), 1 μM methysergide maleate (Met, a serotonin 5-HT_2/5-HT_{1C} receptor antagonist, $n=7$), 5 μM atropine sulfate (Atr, a muscarinic acetylcholine receptor antagonist, $n=6$), 10 μM naloxon hydrochloride (Nal, an opiate receptor antagonist, $n=6$), or 30 μM bicuculline methiodide (Bic, a $GABA_A$ receptor antagonist, $n=7$) at 0.05 Hz. After the pretreatment for 30 min, ketamine was concomitantly applied. Mean field EPSP amplitude (averaged for 3 min) immediately before the application of ketamine is defined as the control value (100%). Mean±S.D.; ** $P<0.01$ vs. control in all experimental groups and $^{+}$ $P<0.05$ vs. control in groups pretreated with bicuculline and naloxon and $P<0.01$ vs. control in the other groups. The open square with error bars at the far right of the graph indicates the effect of 100 μM ketamine in normal ACSF at 30 min for comparison.

The ketamine-induced fEPSP facilitation was also reduced by an increase in stimulation frequency, strongly suggesting that the ketamine-induced channel block on NMDA receptors, which acts in a use-dependent manner [13], attenuates the ketamine-induced facilitation of excitatory synaptic transmission through NMDA receptors. Thus, ketamine has both facilitative and depressive effects on NMDA receptor-mediated transmission, and the facilitative effect is elicited only under the condition in which there is almost no or only a weak channel-blocking effect of ketamine on NMDA receptors.

It is possible that ketamine indirectly modulates the NMDA receptor functions through adrenergic [10,21,22], serotonergic [1,16], opioid [3,23] and muscarinic acetylcholine [2,7] receptors. The ketamine-induced fEPSP facilitation was partially suppressed by pre-application of propranolol but was not influenced by pre-application of phentolamine, methysergide, atropine or naloxon. These results indicate that the ketamine-induced facilitation of excitatory synaptic transmission is at least partially mediated by the β-adrenergic mechanism [10] from noradrenaline [11] but not significantly by the α-adrenergic [20], serotonergic, muscarine-cholinergic and opioid mechanisms.

Pre-application of bicuculline did not change the ketamine-induced fEPSP facilitation, indicating that the GABAergic effect of ketamine [9,12] does not significantly contribute to the fEPSP facilitation. Mechanisms other than the β-adrenergic mechanism by which ketamine facilitates excitatory synaptic transmission through NMDA receptors have not been revealed.

Transient fEPSP depression appeared even when NMDA receptors were antagonized by 50 μM AP-5, which is usually recognized to be sufficient to competitively block all NMDA receptors. Since the effect of a combination of ketamine and AP-5 appears to be greater than the addition of their effects alone [4], it is possible that 50 μM AP-5 may almost, but not completely, block NMDA receptors and that the ketamine-induced block of the remaining NMDA receptors enhanced by AP-5 may be the origin of the small fEPSP depression. The recovery part of the depression may be due to the development of remnants of a facilitatory effect of ketamine. The fEPSP depression disappeared when ketamine-induced fEPSP facilitation was not suppressed. As the mechanism, it is thought that the depressive and the facilitative effects of ketamine on fEPSP offset each other.

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