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Clinically relevant concentrations of ketamine mainly affect long-term potentiation rather than basal excitatory synaptic transmission and do not change paired-pulse facilitation in mouse hippocampal slices

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ABSTRACT

Ketamine, an analgesic/anesthetic drug, is increasingly popular in clinical practice due to its analgesic properties and importance for emergency procedures. The impact of ketamine on basal excitatory synaptic transmission and synaptic plasticity are not yet fully understood. Therefore we investigated the effects of different concentrations of ketamine on basal excitatory synaptic transmission and on two forms of synaptic plasticity: paired-pulse facilitation (PPF) and long-term potentiation (LTP). Evoked field excitatory postsynaptic potentials (fEPSP) were recorded in Schaffer fiber – CA1 pyramid synapses of mouse hippocampal slices and the initial slope of the fEPSP was measured to estimate the percentage of inhibition of the basal synaptic transmission. Presynaptic volley amplitude, PPF and LTP induction and maintenance were also calculated. For basal synaptic transmission and PPF increasing concentrations of ketamine (1, 3, 10, 30, 100, 200, 300 and 600 μM) were applied to each slice and for LTP individual slices were used for each concentration (3, 10, 30 or 100 μM). Clinically relevant concentrations of ketamine decreased LTP in a concentration-dependent manner without changing PPF, whereas basal excitatory synaptic transmission and presynaptic volley amplitude was affected only with high concentrations of ketamine (300 and 600 μM). These results allow dissociating

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the blockade of LTP from a reduced synaptic input in the action of clinically relevant concentrations of ketamine in the CA1 region of the mouse hippocampus. Moreover, this work shows that the effects of ketamine on LTP and on basal synaptic transmission are dependent of the concentration used.

1. Introduction

Ketamine, a purported non-competitive glutamate N-methyl-D-aspartate (NMDA) receptor antagonist (Yamamura et al., 1990), is used in veterinary and human clinical anesthesia for more than 45 years (Domino, 2010). It is popular mainly due to its analgesics properties (Adriaenssens et al., 1999; Michelet et al., 2007) and its importance for emergency procedures (Kuznetsova et al., 1984; Rice et al., 2010). However, the use of ketamine has been associated with the disruption of learning and with psychotic effects such as the post-anesthetic delirium (Sussman, 1974; Irifune et al., 1991).

Alterations in synaptic efficacy in glutamatergic pathways are documented to play a key role in psychopathology (Garcia, 2002). Moreover, activity-dependent synaptic plasticity is considered a cellular mechanism for learning and memory (Bliss and Collingridge, 1993). Synaptic plasticity encompasses both short term plasticity, such as paired-pulse facilitation (PPF) and long-term forms of plasticity (Maruki et al., 2001) such as long-term potentiation (LTP), which is proposed to represent a neurophysiological trait of learning and memory (Lynch, 2004). Hippocampal LTP is mostly dependent on the NMDA receptors (Lynch, 2004), which are considered the main molecular target of ketamine (Davies et al., 1988; Orser et al., 1997). Therefore, it is possible that ketamine may impair synaptic plasticity in the hippocampus. Indeed, an earlier study suggested that dissociative anesthetics including ketamine (30 mg/kg) abolished LTP in the rat hippocampus in vivo (Stringer and Guyenet, 1983). However, it is unclear if ketamine selectively affects synaptic plasticity rather than synaptic transmission, which would require comparing the effects of different concentrations of ketamine on synaptic transmission and on synaptic plasticity. Furthermore, LTP has multiple (pre- and post-synaptic) expression mechanisms (Lynch, 2004), which is particularly relevant since it was reported that higher concentrations of ketamine (1000 μM) can decrease the amplitude of NMDA population spikes in CA1 hippocampal neurons induced by paired-pulse stimuli (Wakasugi et al., 1999), a form of short term plasticity dependent on presynaptic mechanisms (Kamiya and Zucker, 1994; Zucker and Regehr, 2002). However, it still remains to be established if clinically relevant concentrations of ketamine indeed affect paired-pulse facilitation (PPF) in the CA1 region of the hippocampus, which would be in agreement with the proposed localization and function of presynaptic NMDA receptors in the glutamatergic terminals in the hippocampus (Musante et al., 2011).

The purpose of this study was to evaluate the effect of different concentrations of ketamine on basal excitatory synaptic transmission and on two forms of synaptic plasticity (LTP and PPF) in the CA1 area of mice hippocampus.

2. Results

2.1. *Effects of ketamine on basal synaptic transmission and on presynaptic volley amplitude*

After 20 min of stable baseline recordings with aCSF, consecutive application of increasing concentrations of ketamine (1, 3, 10, 30, 100 and 200 μM) did not modify ($p>0.05$) synaptic transmission, as gauged by the lack of alteration of the fEPSP slopes (Fig. 2A). Only at the higher concentrations tested (300 and 600 μM) did ketamine decrease synaptic transmission; thus the concentrations of 300 and 600 μM of ketamine inhibited the fEPSP slope by 22.2±5.3% and 48.1±7.9%, respectively ($n=4$; $p<0.05$) (Fig. 2A, B). It is worth noting that the inhibition of synaptic transmission by the higher concentrations of ketamine was not completely eliminated after washout. In fact, following the washout of ketamine, the fEPSPs slopes were 10.6±2.8% lower than before ketamine administration ($n=4$; $p<0.05$) (Fig. 2A).

Regarding, the effect of ketamine on the presynaptic volley amplitude, we observed that only higher concentrations of ketamine significantly decreased fiber volley amplitude: thus, 300 and 600 μM of ketamine inhibited the amplitude in 48.9±8.5% and 76.3±3.3%, respectively ($n=4$; $p<0.05$). This observation argues for a possible effect of ketamine on action potential propagation, in line with the

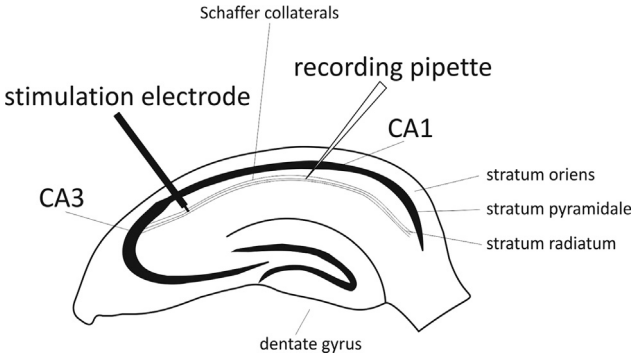

Fig. 1 – Schematic representation of a mouse hippocampal slice showing the placement of the stimulating and recording electrodes. The recording pipette was placed in the *stratum radiatum* of the CA1, and the stimulating electrode was placed in the *stratum radiatum* near the CA3/CA1 border to stimulate the afferent Schaffer collateral pathway.