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Research Report

Methamphetamine modulates glutamatergic synaptic transmission in rat primary cultured hippocampal neurons

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ABSTRACT

Methamphetamine (METH) is a psychostimulant drug. Abuse of METH produces long-term behavioral changes including behavioral, sensitization, tolerance, and dependence. It induces neurotoxic effects in several areas of the brain via enhancing dopamine (DA) level abnormally, which may cause a secondary release of glutamate (GLU). However, repeated administration of METH still increases release of GLU even when dopamine content in tissue is significantly depleted. It implies that some other mechanisms are likely to involve in METH-induced GLU release. The goal of this study was to observe METH affected glutamatergic synaptic transmission in rat primary cultured hippocampal neurons and to explore the mechanism of METH modulated GLU release. Using whole-cell patch-clamp recordings, we found that METH (0.1–50.0 μM) increased the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) and miniature excitatory postsynaptic currents (mEPSCs). However, METH decreased the frequency of sEPSCs and mEPSCs at high concentration of 100 μM. The postsynaptic NMDA receptor currents and P/Q-type calcium channel were not affected by the use of METH (10,100 μM). METH did not present visible effect on N-type Ca^{2+} channel current at the concentration lower than 50.0 μM, but it was inhibited by use of METH at a 100 μM. The effect of METH on glutamatergic synaptic transmission was not revered by pretreated with DA receptor antagonist SCH23390. These results suggest that METH directly modulated presynaptic GLU release at a different concentration, while dopaminergic system was not involved in METH modulated release of GLU in rat primary cultured hippocampal neurons.

1. Introduction

Methamphetamine (METH) is a psychostimulant drug. METH abuse throughout the world has markedly increased during recent years (Lezcano and Bergson, 2002; Chirwa et al., 2005) and represents a significant public health concern (Reiner et al., 2009). Drug abuse, including METH, can cause long-lasting changes in neuronal systems (Cadet et al., 2005;

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Nestler, 2001) and alter synaptic plasticity (Kauer and Malenka, 2007; Thompson et al., 2002). Sub-acute METH administration increased the expression of the plasmalemmal glial-glutamate transporter EAAT3 in the hippocampal (Smith et al., 2007). METH increases extracellular concentrations of DA and glutamate in the striatum (Ernst and Chang, 2008), while the combined effects of DA and GLU releases are thought to produce oxidative stress and glutamate-mediated excitotoxicity to DA nerve terminals. Although there are reports addressing the effects of METH on glutamate synaptic transmission in the striatum and hippocampus (He et al., 2014), the specific nature and pharmacology of METH-evoked changes in hippocampal glutamate-mediated excitatory postsynaptic transmission is not clearly understood. METH-induced increases in corticostriatal GLU release occur via an indirect polysynaptic mechanism that is mediated by D_1 DA receptors in the substantia nigra (Mark et al., 2004). Our study shows that extracellular concentrations of GLU remain elevated at 24 h after METH, while METH-induced DA release is no longer present 4 hours after METH (Mark et al., 2007). These results suggest that the METH-facilitated release of GLU may involve not only the dopaminergic systems, but also other unknown mechanisms.

The hippocampus is a limbic structure that is involved in learning and memory (Kensinger et al., 2001), and is a target for psychostimulants (Vorel et al., 2001). It represents a critical structure in methamphetamine modulation of the reward circuit (Hori et al., 2010; Keleta and Martinez, 2012; Onaivi et al., 2002; Ricoy and Martinez, 2009). Since hippocampus is implicated in the reinstatement of psychostimulant self-administration, and may even contribute to the rewarding properties of METH, it is important to study and understand the potential effects of METH in this brain region. A recent discovery shows that rats will self-administer METH directly into their hippocampus (Ricoy and Martinez, 2009). Chronic stress resulted in an upregulation of glutamate function and an enhanced glutamate response to METH may have implications for glutamate responsiveness in chronically stressed animals exposed to other challenges or stressors (Raudensky and Yamamoto, 2007). To observe a possible direct effect of METH on GLU release, the cultured hippocampal neurons are used in the present study as the hippocampus has been increasingly recognized as the brain region that is highly vulnerable to METH-induced changes in neuronal signaling and is rich in glutamatergic neurons (Maze et al., 2011). The acute effects of METH on glutamate baseline synaptic transmission were examined by recording EPSCs. EPSCs are recorded by the whole-cell recording technique as the main outcome measurement for glutamate neurotransmitter release in hippocampal neurons. It is found that METH increases the frequency and the amplitude of spontaneous excitatory postsynaptic currents (sEPSCs) at the concentration ranging from 0.1 to 50.0 μM. In the presence of tetrodotoxin (TTX), miniature spontaneous excitatory postsynaptic currents (mEPSCs) can be recorded, while METH still enhances the frequency of mEPSCs, revealing METH-enhanced release of glutamate. Our study also addressed whether dopamine involved in METH affects the glutamate-mediated excitatory postsynaptic transmission in rat primary cultured hippocampal neurons. We found that less than 0.2% TH-positive cell (dopaminergic neuron) lie in primary cultured hippocampal neurons from postnatal rats 10–12 hours. This is likely due to the fact that cultured neurons from the rats we applied were postnatal within 12 h, and the TH^+ cells could not survive. This finding is consistent with a previous report (Shimodu, 1992) where it was shown that D_1/D_5 dopamine receptors are expressed in rat primary cultured hippocampal neurons (Lezcano and Bergson, 2002). We further found that the effect of METH on GLU release is not affected by the use of dopamine D_1/D_5 receptor antagonist SCH23390. These results suggest that dopamine was most likely not involved in METH-mediated increase in the release of glutamate in rat primary cultured hippocampal neurons. METH did not display remarkable effects on NMDA and AMPA receptor-mediated currents and the voltage-gated calcium channels. Our results suggest that METH may modulate GLU release directly through a given presynaptic mechanism.

2. Results

2.1. *METH affected the frequency and the amplitude of sEPSCs*

After 10–14 days, the cultured hippocampal neurons were recorded. After the whole-cell recording configuration was established, the sEPSCs could be recorded in most neurons. The sEPSCs were blocked by a combined AMPA receptor antagonist CNQX (20 μM), NMDA receptor antagonist (L-AP5 50 μM), and KA receptor antagonist kynurenic acid (10 μM) (data not shown) in the presence of $GABA_A$ antagonist bicuculline, which revealed that the sEPSCs were mediated by neurotransmitter GLU. It was found that METH (10 μM) significantly increased the frequency of sEPSCs (K-S statistic, $n=10$, $p<0.05$), which was reversed by washing out for about 5 min (Fig. 1A and B). The frequency of sEPSCs was 1.43±0.52 Hz for control, 2.73±0.32 Hz in the presence of METH, 1.70±0.31 Hz after washout ($n=10$, $p<0.05$). The amplitude of sEPSCs was enhanced by the use of METH (10 μM) (K-S statistic, $n=10$, $p<0.05$, Fig. 1C). The median amplitude of sEPSCs was 72.43±8.78 pA for control, 89.45±9.90 pA in the application of METH (10 μM), 77.93±8.90 pA after washout ($n=10$, $p<0.05$). At concentrations 0.1, 1.0, 5.0, 10.0, and 50.0 μM, METH increased the frequency of sEPSCs for 223±40%, 318±46%, 246±62%, 225±39%, and 176±41%, respectively ($n=10$, $p<0.05$). However, METH, at concentration of 100 μM, decreased the frequency of sEPSCs by 87±13% ($n=10$, $p<0.05$, Fig. 1D). METH enhanced the amplitude of sEPSCs for 126.3±9.80%, 145.6±12.80%, 135.5±7.15%, 132.8±6.92%, 126±4.70% at concentrations of 0.1, 1.0, 5.0, 10.0, and 50.0 μM, respectively ($n=10$, $p<0.05$, Fig. 1E). METH decreased the amplitude of sEPSCs by 59±7.39% at concentration of 100 μM.

2.2. *METH regulated the frequency and amplitude of mEPSCs*

We further examined the METH-mediated alteration of presynaptic GLU release by observing the effect of METH on mEPSCs, which were action potential-independent spontaneous GLU release from presynaptic terminals. The bath solution included