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Astrocytes contribute to the effects of etomidate on synaptic transmission in rat primary somatosensory cortex

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ABSTRACT

Little is known about the mechanisms of unconsciousness induced by general anesthetics. Previous studies have shown that the primary somatosensory cortex (S1) is a sensitive region to a variety of intravenous general anesthetics. Etomidate is a widely used intravenous anesthetic that can influence synaptic transmission. Recently, there are some evidences suggesting that astrocytes, a type of glia cell, also contribute to information transmission in the brain, and modulate synaptic function by releasing neuroactive substances. However, it is unknown whether astrocytes influence the effects of etomidate on information transmission in S1 pyramidal neurons. In the present study, the role of astrocytes in etomidate-induced unconsciousness was investigated by using the whole-cell patch clamp technique. We observed etomidate at clinically relevant concentrations inhibited the spontaneous postsynaptic currents (sPSCs) of rat S1 pyramidal neurons in a concentration-dependent manner, and the EC50 value of etomidate for inhibiting sPSCs from the concentration-effect curve was 6.9 μM. Furthermore, in the presence of fluorocitrate, a glia-selective metabolism inhibitor that blocks the aconitase enzyme, both the amplitude and frequency of sPSCs in rat S1 pyramidal neurons were reduced, and the inhibitory effects of etomidate on sPSCs amplitude was strengthened without affecting the effects of etomidate on frequency. From these data, we deduce that etomidate suppresses synaptic activity via presynaptic and postsynaptic components. Furthermore, astrocytes participate in synaptic transmission and influence the effects of etomidate on postsynaptic receptors. This study provides new insight into the role of astrocytes in etomidate-induced unconsciousness.

1. Introduction

Loss of consciousness is the most characteristic feature of general anesthesia. Unfortunately, the mechanisms of unconsciousness induced by general anesthetics are not completely understood. Etomidate, a carboxylated imidazole, is a widely used ultrashort-acting nonbarbiturate intravenous anesthetic that produces sedative and immobilizing actions (Giese and Stanley, 1983). Considerable evidence suggests that modulation of synaptic transmission is the principal mechanism of general anesthetic action (Shahani et al., 2002).

Astrocytes, a type of glial cell, have long been considered to act as a supportive and protective network for central nervous system, with little role in information representation or processing. However, over the past few decades, accumulating evidence indicates astrocytes participate in the physiological control of synaptic transmission and the existence of a bidirectional communication between synapses and astrocytes (Agulhon et al., 2008; Halassa and Haydon, 2010; Perea et al., 2009; Perea and Araque, 2010; Volterra and Meldolesi, 2005). This bidirectional communication is based on the ability of astrocytes to respond to neurotransmitters, such as glutamate, Ach, etc (Araque et al., 2002; Latour et al., 2001; Porter and McCarthy, 1996), released from synapse, and to release neuroactive substances, such as purines, D-serine, glutamate, adenosine, ATP, called gliotransmitters (Halassa and Haydon, 2010; Perea and Araque, 2010; Volterra and Meldolesi, 2005) that can modulate synaptic transmission and neuronal activity. And it is supposed that consciousness is the result of the

Abbreviations: ACSF, artificial cerebrospinal fluid; AP5, D,L-2-amino-5-phosphonovaleric acid; DNQX, 6,7-dinitroquinoxaline-2,3-dione; EC50, the concentration for 50% of maximal effect; EGTA, ethylene glycol tetraacetic acid; HEPES, 4-(2-hydroxyethyl)−1-piperazineethanesulfonic acid; sPSCs, spontaneous postsynaptic currents; TTX, tetrodotoxin

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interaction between astrocytes and neurons (Damasio, 1999; Pereira and Furlan, 2010). However, whether the activities of astrocytes influence the effects of etomidate on synaptic transmission is unclear. To address this issue, in the present study, we analyzed the change in the effects of etomidate on sPSCs in rat S1 pyramidal neurons before and during astrocytes were inhibited. We expected that this study can help us better understand the mechanisms of unconsciousness induced by general anesthetics.

2. Results

2.1. Spontaneous PSCs recorded in rat S1 pyramidal neurons

In coronal slices, the whole-cell patch electrodes were located in rat S1 (Fig. 1A and B). Under voltage-clamp, the S1 neurons with multipolar or triangular-shaped soma, bright and smooth appearance and no visible organelles were selected for sPSCs recording (Fig. 1B). In the presence of AP5 (100 μM), an antagonist of NMDA glutamate receptors, DNQX (20 μM), an antagonist of AMPA and kainite glutamate receptors, gabazine (10 μM), an antagonist of $GABA_A$ receptor, TTX(1 μM), Na^+ channel blocker, all the spontaneous synaptic events were reversibly abolished, confirming that these currents are sPSCs (Fig. 1C).

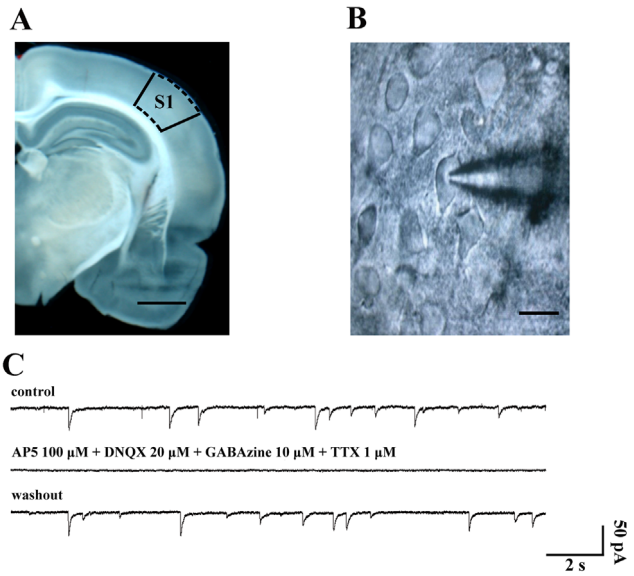

Fig. 1. Recording site and sPSCs recorded in S1 pyramidal neurons. (A) coronal brain slice in 350 μM thickness and S1 recording area. Scale bar, 1200 μM. (B) Pyramidal neuron in S1 with whole-cell recording electrode in place. Scale bar, 10 μM. (C) Original traces of sPSCs recorded in S1 pyramidal neurons in control condition, after AP5 (100 μM), DNQX (20 μM), gabazine (10 μM) and TTX (1 μM) treatment, and after washout with ACSF.

2.2. Effects of etomidate on sPSCs in rat S1 pyramidal neurons

In coronal slices, etomidate (1.5 μM, 3 μM, 6 μM, 12 μM and 24 μM) decreased the amplitude (Fig. 2A and B) and frequency (Fig. 2A and C) of sPSCs, but did not significantly change the decay time (not shown). Etomidate (1.5 μM, 3 μM, 6 μM, 12 μM and 24 μM) inhibited the amplitude of sPSCs by $8.9 \pm 1.3\%$, $10.3 \pm 2.0\%$, $24.8 \pm 3.5\%$, $47.9 \pm 3.3\%$ and $52.6 \pm 2.6\%$, respectively ($n=10$, $P<0.01$) (Fig. 2D), and reduced the frequency of sPSCs by $15.5 \pm 5.1\%$, $16.4 \pm 4.3\%$, $26.8 \pm 5.0\%$, $28.6 \pm 4.6\%$ and $56.9 \pm 6.3\%$, respectively ($n=10$, $P<0.01$) (Fig. 2E). Above data shown, etomidate at clinically relevant concentrations decreased the amplitude of sPSCs more effectively. According to the inhibitory effects of etomidate on sPSCs amplitude at different concentrations, the concentration-effect curve was drawn and the EC_{50} value of etomidate for inhibiting spontaneous postsynaptic currents in rat S1 pyramidal neurons was calculated as 6.9 μM (Fig. 2F). Thus, we used 6.9 μM etomidate in the subsequent experiments. These results indicate that etomidate can modulate postsynaptic receptors function and inhibit presynaptic neurotransmitters release.

2.3. Astrocytes participate in synaptic activities and influence the effects of etomidate on sPSCs in rat S1

Fluorocitrate (FC) is a glia-selective inhibitor of the aconitase enzyme (Clarke, 1991; Lauble et al., 1996), which has been shown to be effective in inhibiting astrocytic function both in vivo and in vitro experiments (Christian and Huguenard, 2013; Hassel et al., 1992; Nakanishi et al., 1996; Paulsen et al., 1987). FC solution was prepared as described previously (Lee et al., 2014), and was diluted to 5 μM, a dose that does not appear to alter neuronal function (Glanowska and Moenter, 2011; Hassel et al., 1992), by adding additional standard ACSF. In order to inhibit the activities of astrocytes completely, we applied the ACSF that contain FC till electrophysiological recording finished.

sPSCs recorded in S1 pyramidal neurons with different drug solutions (Fig. 3A). During bath application of FC, we observed that the amplitude of sPSCs reduced from 47.1 ± 4.2 pA to 35.9 ± 5.4 pA ($n=12$, $P<0.01$) (Fig. 3B), and the frequency of sPSCs decreased from 27.6 ± 4.7 min^{-1} to 21.8 ± 3.0 min^{-1} ($n=12$, $P<0.01$) (Fig. 3C). These results suggest that astrocytes actually participate in synaptic activities.

Next, we tested the inhibitory effects of etomidate on sPSCs in rat S1 pyramidal neurons before and during astrocytes were inhibited. In the absence of FC, etomidate (6.9 μM) decreased the amplitude and frequency of sPSCs from 47.1 ± 4.2 pA, 27.6 ± 4.7 min^{-1} to 34.2 ± 2.6 pA, 20.1 ± 3.2 min^{-1}, respectively ($n=12$) (Fig. 3B and C). While in the presence of FC, etomidate (6.9 μM) decreased the amplitude of sPSCs from 35.9 ± 5.4 pA in FC to 20.4 ± 3.7 pA in FC + etomidate ($n=12$, $P<0.01$) (Fig. 3B), and reduced the frequency of sPSCs from 21.8 ± 3.0 min^{-1} in FC to 15.4 ± 1.9 min^{-1} in FC+etomidate ($n=12$, $P<0.01$) (Fig. 3C). Moreover the inhibition rate of sPSCs amplitude increased from $27.3 \pm 3.1\%$ in etomidate to $38.3 \pm 4.9\%$ in FC+etomidate ($n=12$, $P<0.01$) (Fig. 3D), however, the inhibition rate of sPSCs frequency did not significantly changed ($n=12$, $P>0.05$) (Fig. 3E).

3. Discussion

The role of bidirectional communication between astrocytes and neurons is important in information integration (Carmignoto, 2000; Haydon, 2000). In the present experiment, we have shown that etomidate reduced the amplitude and frequency of sPSCs, and the activities of astrocytes can influence the effects of etomidate on sPSCs in rat S1 pyramidal neurons.

In the current study, we found that etomidate at clinically relevant concentrations decreased the amplitude and frequency of sPSCs in a concentration-dependent manner without altering the decay time. Generally, the change in the amplitude and frequency of sPSCs hints the alterations in postsynaptic receptor function and presynaptic neurotransmitters release respectively. Consequently, our results suggest that etomidate exerts its action, at least in part, through regulating the function of postsynaptic receptors and inhibiting presynaptic neurotransmitters release. Our findings are consistent with previous studies which have demonstrated that etomidate influences synaptic transmission by modulating postsynaptic inhibitory ligand-gated ion channels, such as $GABA_AR$ that contain the α3, α5, β2 and β3 subunit (Cheng et al., 2006; Drexler et al., 2009; Jurd et al., 2003; Kretschmannova et al., 2013;