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Streptozotocin inhibits synaptic transmission and edaravone attenuates streptozotocin-induced electrophysiological changes in CA1 pyramidal neurons of rat hippocampal slices

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ABSTRACT

The purpose of this study was to investigate the acute and chronic effects of streptozotocin (STZ) upon synaptic transmission and the effects of edaravone (EDA, a free radical scavenger) on STZ-induced electrophysiological changes in CA1 pyramidal neurons of rat hippocampal slices. To accomplish this goal, spontaneous excitatory postsynaptic current (sEPSC), miniature excitatory postsynaptic current (mEPSC), spontaneous inhibitory postsynaptic current (sIPSC) and miniature inhibitory postsynaptic current (mIPSC) were recorded within hippocampal slices using whole-cell patch clamp techniques. The results showed that the amplitudes and frequencies of sEPSC, mEPSC, sIPSC and mIPSC were inhibited by 1000 μM STZ, while treatment of EDA (1000 μM) attenuated these STZ-induced changes. The degree of these neurotoxic effects of STZ and effects of EDA increased as a function of drug duration as assessed at 2, 4 or 8 h of exposure. Taken together, our results demonstrate that STZ induces neurotoxicity within these hippocampal slices through its capacity to alter synaptic transmission and these STZ-induced alterations in electrophysiological responses are attenuated by EDA.

1. Introduction

Alzheimer's disease (AD) is the most common cause for dementia among the aging population. According to the World Alzheimer's Report, 46.8 million people were affected by AD worldwide in 2015, and this number is expected to rise to 131.5 million in 2050. Therefore, this condition will result in a huge social and economic burden for families as well as society in general (Kalaria et al., 2008; Collaborators USBoD, 2013).

Although the exact etiology of AD remains unknown, there exists considerable evidence which indicates that oxidative imbalances may play an important role in the development and neuronal deterioration associated with AD (Reddy, 2006, 2007; Mangialasche et al., 2009). One approach to investigate the mechanisms of AD involves an intracerebroventricular (ICV)-injection of streptozotocin (STZ) in rats. ICV-STZ results in a deregulation of brain insulin signaling and abnormalities in cerebral glucose utilization/metabolism which are then accompanied by energy deficits (Grünblatt et al., 2007; Deng et al., 2009; Chen et al., 2013). ICV-STZ was also used for preclinical testing of pharmacological therapies for AD. Specifically, the neuropathological and biochemical changes induced by ICV-STZ in rats, including hyperphosphorylation of tau protein, decreased choline acetyltransferase activity, impaired brain glucose and energy utilization, nitrite/nitrate producrion and increased oxidative stress, are similar to those observed in AD (Sharma and Gupta, 2001; Grünblatt et al., 2007; Ishrat et al., 2009; Salkovic-Petrisic et al., 2011, 2014; Zhou et al., 2013; Gutierres et al., 2014; Nayebi et al., 2014; Xu et al., 2014; Zamani et al., 2015; Wang et al., 2016).

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, EDA) is a novel radical scavenger which serves as a safe and effective agent for clinical use in ischemic stroke (Yoshida et al., 2006). Moreover, EDA can function as a neuroprotectant as demonstrated in disease models of amyotrophic lateral sclerosis and Parkinson's disease (Schultz and Rolls, 1999; Yoshino and Kimura, 2006; Ito et al., 2008;

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Ji Yuan et al., 2008; Kikuchi et al., 2011, 2012; Xiong et al., 2011). Work within our laboratory has revealed that EDA can also exert neuroprotective effects in the STZ-induced model of AD, as we found that EDA ameliorated behavioral impairments of memory deficits, attenuated oxidative stress and blocked tau hyperphosphorylation in ICV-STZ rats (Zhou et al., 2013). Although the neurotoxic effects of STZ and the protective effects of EDA against STZ-induced neurotoxicity have been observed in animal models, these STZ/EDA effects have not been investigated using brain slices as a means for understanding the potential electrophysiological mechanisms involving synaptic transmission.

Synaptic dysfunction or loss contributes to clinical signs of dementia, apparently by disrupting neuronal communication (Coleman et al., 2004). Findings from a number of studies have indicated that glutamate (especially AMPA and NMDA) and GABA, along with their receptors may play critical roles in AD pathogenesis (Hsieh et al., 2006; Alberdi et al., 2010; Revett et al., 2013; Wang et al., 2014; Maite et al., 2015; Ulrich, 2015). Moreover, the early symptoms appear to correlate with deficits in neurotransmitters prior to neuronal atrophy and synaptic function seems to be affected in major brain areas involved in learning and memory, which result in cognitive impairments (Selkoe, 2002; Parameshwaran et al., 2008). Therefore, synaptic dysfunction and failure underlie the early cognitive features of AD and thus represent important targets for protective treatments to slow AD progression and preserve cognitive and functional capacities.

The purpose of the present study was to evaluate the acute and chronic effects of STZ upon synaptic transmission and the effects of edaravone (EDA, a free radical scavenger) on STZ-induced electrophysiological changes as assessed using brain slices. In this study we focused on hippocampal CA1 pyramidal neurons, a brain area which is critically responsible for certain types of memory and most vulnerable in AD patients (Mattson and Magnus, 2006). To evaluate these effects of STZ and its modulation by EDA, we recorded spontaneous excitatory postsynaptic current (sEPSC), miniature excitatory postsynaptic current (mEPSC), spontaneous inhibitory postsynaptic current (sIPSC) and miniature inhibitory postsynaptic current (mIPSC) using the whole-cell patch clamp technique. By collating the data obtained from these parameters it is possible to gain an understanding of the effects of STZ and EDA as related to their role in modulating the efficacy of synaptic transmission.

2. Material and methods

2.1. Solutions and drugs

The sucrose artificial cerebrospinal fluid (sucrose-ACSF) contained (in mM): 213 Sucrose, 2.5 KCl, 2 $CaCl_2$, 2 $MgSO_4$, 10 D-glucose, 26 $NaHCO_3$, and 1.25 NaH_2PO_4 (pH 7.4–7.5) and the normal ACSF contained (in mM): 126 NaCl, 2.5 KCl, 2 $CaCl_2$, 2 $MgSO_4$, 10 D-glucose, 26 $NaHCO_3$, and 1.25 NaH_2PO_4 (pH 7.4–7.5). Streptozotocin (STZ, from Sigma, St. Louis, MO, USA) was dissolved in oxygenated ACSF and prepared immediately prior to use in the experiments. Edaravone (EDA, 3-methyl-1-phenyl-2-pyrazolin-5-one, from Calbiochem, Germany) was dissolved in 1 N NaOH, diluted with ACSF and pH adjusted to 7.4 with 1 N HCl according to the manufacturer's instructions. The pipette solution for recording EPSCs contained (in mM): 140 K-Gluconate, 10 HEPES, 2 Na_2ATP, 3 KCl, 2 $MgCl_2$ and 10 EGTA, with pH adjusted to 7.3-7.4 using KOH. The pipette solution for recording IPSCs contained (in mM): 125 Cs-methanesulfonate, 10 CsCl, 2 $MgCl_2$, 5 NaCl, 10 HEPES, 1 EGTA, 5 Mg-ATP and 0.3 Na_3GTP, with pH adjusted to 7.3–7.4 using CsOH. Tetrodotoxin (TTX, 1 μM, from the Research Institute of the Aquatic Products of Hebei, China) was added to ACSF to block spontaneous action potentials of miniature postsynaptic currents. Bicuculline (BMI, 10 μM, from Sigma, St Louis, MO, USA) was used to block the gamma-aminobutyric acid type A ($GABA_A$) receptor mediated synaptic currents. 2-amino-5-phosphonovaleric acid (APV, 50 μM, from Sigma, St Louis, MO, USA) was used to block *N*-methyl-D-aspartic acid (NMDA) glutamate receptor mediated synaptic currents. 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX, 10 μM, from Sigma, St. Louis, MO, USA) was used to block non-NMDA glutamate receptor mediated synaptic currents. TTX, BMI, APV and CNQX were prepared as stock solutions and diluted directly in pre-gassed (95% O_2–5% CO_2) ACSF to generate the concentrations described. The final dimethylsulfoxide (DMSO) concentration was $<0.1\%$.

2.2. Slice preparation

All experiments were carried out in accordance to the guidelines of the Medical Experimental Animal Administrative Committee of China and all efforts were made to minimize the number of animals used and their suffering. Hippocampal slices from Wistar rats of both genders (14–18 days) were prepared as described previously (De Simoni and Lily, 2006; Chen et al., 2014). The animals were decapitated and brains were placed in cold sucrose-ACSF (0–4 °C, saturated with 95% O_2–5% CO_2) for 1 min. The brains were then cut into 300 μm thick slices with a vibratome (NVSLM1, WPI, USA) and slices were incubated with ACSF (saturated with 95% O_2–5% CO_2) at 35 °C for at least 1 h.

2.3. Electrophysiological recordings

A conventional whole cell patch clamp technique was used in the present study. One slice from the hippocampus was transferred to the recording chamber (1 ml), continually perfused with oxygenated ACSF and viewed under an infrared differential interference microscope (40× water immersion lens, Nikon, Japan). Hippocampal neurons were visualized on a television monitor connected to a low light sensitive CCD camera (1300-QD, VDS, Germany). Activity of hippocampal CA1 neurons were recorded using an amplifier (Axon 700B, Foster City, CA) and an analogue/digital interface (CED Power 1401, Cambridge, UK) in the voltage-clamp mode with 1 kHz filtration and 10 kHz digitization. The electrode puller (P-97, Sutter, USA) was used to make patch electrodes with 4–8 MΩ resistance when filled with the pipette solution. Postsynaptic current recordings began 5 min after membrane rupture when the current achieved a steady state. Agents were then administered to the slices for at least 5 min to detect the effects on the properties of all the physiological indexes in the voltage-clamp mode. Acute exposure experiments involved application of drugs to slices by switching the perfusion solution with varying concentrations of STZ (10, 100 or 1000 μM) or STZ (1000 μM) +EDA (10, 100 or 1000 μM). Currents were recorded for a single given concentration within each slice. For the chronic exposure experiments, slices from one rat brain were divided into 3 groups (control, STZ, STZ +EDA). After 1 hour of incubation at 35 °C, slices were transferred to room temperature and incubated with the normal medium (control) or media containing STZ or the co-treatment of STZ and EDA for varying durations (2, 4 or 8 h). STZ was added to ACSF after 20 min at room temperature within the STZ and STZ +EDA conditions, to avoid a decrease of drug efficacy that could result from temperature alteration. For the STZ +EDA group, a pre-treatment of STZ was administered for a period of 30 min prior to the application of EDA to avoid the potential for a direct interaction between STZ and EDA. One slice was transferred to the recording chamber to perform the electrophysiological recordings at the given time point. Both EPSCs and IPSCs were observed under basal conditions (without any stimulus) by setting the holding potential at −70 mV for EPSCs and 0 mV for IPSCs.