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Neurosteroid dehydroepiandrosterone sulphate inhibits persistent sodium currents in rat medial prefrontal cortex via activation of sigma-1 receptors

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Abstract

Dehydroepiandrosterone sulphate is one of the most important neurosteroids. In the present paper, we studied the effect of dehydroepiandrosterone sulphate on persistent sodium currents and its mechanism and functional consequence with whole-cell patch clamp recording method combined with a pharmacological approach in the rat medial prefrontal cortex slices. The results showed that dehydroepiandrosterone sulphate inhibited the amplitude of persistent sodium currents and the inhibitory effect was significant at 0.1 μM, reached maximum at 1 μM and decreased with the increase in the concentrations of above 1 μM. The effect of dehydroepiandrosterone sulphate on persistent sodium currents was canceled by the Gi protein inhibitor and the protein kinase C inhibitor, but not by the protein kinase A inhibitor. The effect of dehydroepiandrosterone sulphate on persistent sodium currents was also canceled by the sigma-1 receptor blockers and the sigma-1 receptor agonist could mimic the effect of dehydroepiandrosterone sulphate. Dehydroepiandrosterone sulphate had no significant influence on neuronal excitability but could significantly inhibit chemical inhibition of mitochondria-evoked increase in persistent sodium currents. These results suggest that dehydroepiandrosterone sulphate inhibits persistent sodium currents via the activation of sigma-1 receptors–Gi protein–protein kinase C-coupled signaling pathway, and the main functional consequence of this effect of DHEAS is presumably to protect neurons under ischemia.

Keywords: Neurosteroid; Dehydroepiandrosterone sulphate; Medial prefrontal cortex; Whole-cell patch clamp; Persistent sodium currents; Sigma-1 receptors; Gi protein; Protein kinase C; Excitability; Chemical inhibition of mitochondria

Introduction

Neurosteroids are a new family of active steroids that are referred to those steroids synthesized de novo in the brain (Baulieu, 1998; Baulieu, 1997; Robel and Baulieu, 1995; Tsutsui et al., 2000). Dehydroepiandrosterone sulphate (DHEAS) is one of the most important neurosteroids (Compagnone and Mellon, 2000). Since the discovery of high DHEAS concentration in the brain (Corpechot et al., 1981), a number of effects of DHEAS such as memory enhancing, anxiolytic and anti-depression effect have been described (Maurice et al., 1997; Reddy et al., 1998; Flood and Roberts, 1988; Urani et al., 1998; Urani et al., 2001). Especially, increasing evidence suggests that DHEAS is neuroprotective in a variety of models of brain ischemia (Kimonides et al., 1998; Kaasik et al., 2001; Li et al., 2001; Lapchak et al., 2000). Moreover, it has been proposed that the decrease in the concentration of DHEAS in the brain with age in humans may contribute to increased vulnerability of the aging or stressed human brain to ischemia (Kimonides et al., 1998). However, the mechanism through which DHEAS exerts its protective effect under ischemia remains to be studied.

Previous studies showed that DHEAS might provide neuronal protection by antagonizing glutamate-induced neurotoxicity (Kimonides et al., 1998; Mao and Barger, 1998) or by activating $GABA_A$ receptors (Lapchak et al., 2000). In addition, there was evidence suggesting that other mechanisms such as preserving neuronal mitochondria from calcium overload might also be involved in the protective effect of DHEAS under ischemia (Kaasik et al., 2003). Here, we propose that the action of DHEAS on persistent sodium currents, especially on the increase in this current under ischemia, may constitute another

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mechanism for the protective effect of DHEAS. This is because it has been shown that an early event under ischemia in neurons is an increase in intracellular sodium concentration, which is followed by an elevation of intracellular calcium concentration and then leads to neuronal damage and death (Friedman and Haddad, 1994). Moreover, this increase in intracellular sodium concentration under ischemia has been proposed to be mediated by an increase in persistent sodium currents rather than by that in classical transient sodium currents (Taylor and Meldrum, 1995; Hammarstrom and Gage, 1998).Thus, if DHEAS has an inhibitory effect on persistent sodium currents, it may constitute one mechanism for its neuronal protective effect under ischemia. However, it is still not known whether or not DHEAS has actions on persistent sodium current. Therefore, in the present paper, we studied the effect of DHEAS on persistent sodium currents and further investigated its mechanism and functional consequence.

Materials and methods

Preparation of medial prefrontal cortical slices and visualization of cells

Sprague–Dawley rats weighing 30–50 g (14–16 days after birth) were anesthetized with chloral hydrate (400 mg/kg, i.p.). All experimental procedures conformed to Fudan University as well as international guidelines on the ethnics of experimental animal and all efforts were made to minimize the number of them and their suffering. Brain slices were prepared according to procedures described previously (Wang and Zheng, 2001). Briefly, following decapitation, the brain was quickly removed and submerged in ice-cold artificial cerebrospinal fluid (ACSF) containing the following (in mM): 126 NaCl, 3.5 KCl, 1.3 $MgSO_4$, 2 $CaCl_2$, 10 D-glucose, 1.25 NaH_2PO_4, 26 $NaHCO_3$ (pH 7.4 by HCl). A block of tissue containing medial prefrontal cortex (mPFC) was cut and placed on a layer of moistened filter paper glued to the cutting stage of a vibratome (VT1000M/E, Leica, Wetzlar, Germany). Serial coronal slices (300–400 μm) were cut and transferred to an incubating chamber (30–32 °C) where they stayed for at least 1 h before recordings were begun. To visualize cells, the slice was placed in a recording chamber and viewed with a fixed stage, upright microscope equipped with Nomarski optics and a 40× long working distance, water immersion objective (3 mm, N. A:0.7, Olympus, Tokyo, Japan). To increase the clarity of the image, infrared light was used to illuminate during recording. The resultant infrared differential interference contrast (DIC) image was visualized on a black-white TV monitor through the use of a low light sensitive CCD camera. Recording was made from pyramidal cells located in mPFC. Pyramidal cells were identified by their pyramidal shape and the presence of apical dendrites.

Whole-cell recording in slices

The slice was continuously perfused with ACSF. Electrodes were pulled from glass capillaries using a Narishige micropipetter puller (model PB-7; Narishige, Japan). They had a resistance of 3–5 MΩ when filled with the patch pipette solution. To pharmacologically isolate persistent sodium currents, the patch pipette solution was slightly modified from that used by Fleidervish et al. (1996). The internal pipette solution contained (in mM) 140 CsCl, 1 $CaCl_2$, 2 $MgCl_2$, 1 EGTA, 2 ATP.K_2, 0.1 GTP.Na_3 and 10 HEPES, adjusted to pH 7.25 by CsOH. In addition, 200 μM Cd^{2+} was added to ACSF to block Ca^{2+} currents. To achieve adequate space clamp of persistent sodium currents, Cs^+ was used as a main cation to block most of K^+ currents and to make the neuron electronically more compact to minimize "space clamp" error (Gorelova and Yang, 2000). Under these recording conditions, a depolarizing voltage step (from −70 to −30 mV, 1-s duration) at the holding potential of −70 mV was applied to activate persistent sodium current. Persistent sodium current was demonstrated by the experiment that TTX (0.5 μM) could completely abolish this current. The current–voltage relationship of persistent sodium currents was determined in the range from −70 to −20 mV using step pulse commands (1-s duration) with 10-mV increments from a holding potential of −70 mV. It was similar to those reported by other authors (French et al., 1990; Alzheimer et al., 1993). There was a significant persistent sodium current at −60 mV, which increased in amplitude with further depolarization to reach a maximum at −40 mV. However, as described by Alzheimer (1994) and confirmed by our experiment, even in the presence of Cs^+, an outward current was still present at potentials more positive than −20 mV, which not only influenced the reversal potential of the persistent sodium currents but also complicated the effect at these potentials, so in the present study, when constructing the current–voltage curve, we discard the currents at potentials more positive than −20 mV. In addition, we monitored the access resistance and rejected all recordings with the access resistance >20 MΩ. The current signals were recorded with an Axopatch 200B amplifier (Axon, Union City, USA) connected to a Digidata1200 interface (Axon, Union City, USA). The data were digitized and stored on disks using pClamp (v.6; Axon, Union City, USA). All experiments were conducted at room temperature (21–24 °C). The amplitude of persistent sodium currents was measured at the end of a 1-s voltage pulse. Mean current–voltage relationships were calculated by averaging the individual current–voltage relationship obtained from each cell in the study. The action potentials were recorded under current clamp mode with the internal pipette solution containing (in mM) 140 K-gluconate, 0.1 $CaCl_2$, 2 $MgCl_2$, 1 EGTA, 2 ATP.K_2, 0.1 GTP.Na_3 and 10 HEPES, adjusted to pH 7.25 by KOH.

Data analysis

Statistic significance between two groups was evaluated by Student's two-tailed test for paired data. The significance of the difference between multiple groups at different time points was evaluated by one-way ANOVA with a post hoc Tukey test. All values were expressed as means±SEM (standard errors of means) and the number of cells in each group was given.

Drugs

ATP.K_2, GTP.Na_3, CsCl, CsF, CsOH, $CdCl_2$, chelerythrine, haloperidol, *N*-(2-[p-bromocinnamylamino]ethyl)5-isoquinoline-