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Research Report

Enhancement of an outwardly rectifying chloride channel in hippocampal pyramidal neurons after cerebral ischemia

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ABSTRACT

Cerebral ischemia induces delayed, selective neuronal death in the CA1 region of the hippocampus. The underlying molecular mechanisms remain unclear, but it is known that apoptosis is involved in this process. Chloride efflux has been implicated in the progression of apoptosis in various cell types. Using both the inside-out and whole-cell configurations of the patch-clamp technique, the present study characterized an outwardly rectifying chloride channel (ORCC) in acutely dissociated pyramid neurons in the hippocampus of adult rats. The channel had a nonlinear current-voltage relationship with a conductance of 42.26 ± 1.2 pS in the positive voltage range and 18.23 ± 0.96 pS in the negative voltage range, indicating an outward rectification pattern. The channel is Cl^- selective, and the open probability is voltage-dependent. It can be blocked by the classical Cl^- channel blockers DIDS, SITS, NPPB and glibenclamide. We examined the different changes in ORCC activity in CA1 and CA3 pyramidal neurons at 6, 24 and 48 h after transient forebrain ischemia. In the vulnerable CA1 neurons, ORCC activity was persistently enhanced after ischemic insult, whereas in the invulnerable CA3 neurons, no significant changes occurred. Further analysis of channel kinetics suggested that multiple openings are a major contributor to the increase in channel activity after ischemia. Pharmacological blockade of the ORCC partly attenuated cell death in the hippocampal neurons. We propose that the enhanced activity of ORCC might contribute to selective neuronal damage in the CA1 region after cerebral ischemia, and that ORCC may be a therapeutic target against ischemia-induced cell death.

1. Introduction

Stroke is the fourth leading cause of death and is a major cause of serious, long-term disability and functional cognitive impairment (Taxin et al., 2014). The vulnerability of neurons to cerebral ischemia varies widely among the different regions in the central nervous system. Different neuronal populations within a specific brain region also show different susceptibilities to ischemic insult. Studies have shown that CA1 pyramidal neurons in the hippocampus are particularly vulnerable to ischemic insult and die 3–7 d after transient forebrain ischemia (this phenomenon is also known as delayed neuronal death, or DND), whereas CA3 neurons are relatively resistant to transient ischemia and remain viable (Pulsinelli et al., 1982). A number of studies suggest that ischemic neuronal death involves apoptosis, an active and genetically controlled cell suicide process (Love, 2003). Histological and biochemical characteristics of apoptosis are present in dying neurons after ischemia, and inhibition of new protein synthesis protects CA1 neurons from apoptosis after ischemia (Namura et al., 1998). Several ion channels have been implicated to be involved in and essential for apoptosis (Kondratskyi et al., 2015). Induction of apoptosis is tightly associated with activation of K^+ and Cl^- efflux pathways, which mediate apoptotic volume decrease (AVD) (Bortner and Cidlowski, 2004). K^+ and Cl^- channel blockers prevent apoptotic cell death in a variety of cell types (Maeno et al., 2000). The K^+ current is increased in hippocampal CA1 pyramidal neurons after transient forebrain ischemia (Chi and Xu, 2000; Gong et al., 2000). The K^+ channel blocker TEA prevents ischemic cell death in hippocampal CA1 neurons (Huang et al., 2001). Additionally, several Cl^- channel blockers have been shown to suppress neuronal apoptosis induced by a variety of apoptotic stimuli (Inoue et al., 2007; Wei et al., 2004).

Among the various Cl^- channel types, outwardly rectifying Cl^- channel (ORCC) has been characterized in a variety of cells by the patch clamp technique (Demion et al., 2006; Marino et al., 2010;

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Martins et al., 2011), although it has not yet been identified at the molecular level. The induction of apoptosis in T lymphocytes by Fas (CD95) crosslinking activates ORCC, and channel blockers inhibit this apoptosis, suggesting that ORCC may regulate apoptosis (Szabo et al., 1998).

In the present study, we characterize an outwardly rectifying Cl^- channel in acutely dissociated hippocampal CA1 pyramid neurons of adult rats. In addition, we examine the change in this channel after transient forebrain ischemia, and explore the protective effects of ORCC blockers against apoptosis of hippocampal neurons.

2. Results

2.1. Biophysical properties of channel activity recorded from inside-out patches

Single-channel activity was investigated in excised inside-out patches from freshly isolated hippocampal neurons. Under symmetrical Cl^- conditions, ORCC was either spontaneously active or was activated by applying a depolarizing voltage pulse to ≥ 60 mV lasting for several minutes in 32% (134/415) of the inside-out patches. Once activated, the channel openings were observed at both positive and negative membrane potentials.

Fig. 1A shows the typical channel activity observed from an inside-out membrane patch under symmetrical 150 Cl^- conditions at several membrane holding potentials. The corresponding current-voltage relationship showed a strong outward rectification (Fig. 1B). The average slope conductance of the channel was 18.23 ± 0.96 pS over the range of -60 mV to 0 mV (inward currents) and 42.26 ± 1.20 pS (n=20) in the range of 0 mV to 60 mV (outward currents).

The open probability (P_o) increased with depolarization from -60 mV to 60 mV, indicating that channel activity was voltage-dependent (Fig. 1C). Within this voltage range, the relationship between open probability and membrane potential is fitted by the Boltzmann function $P_o/P_{max}=1/\{(1+\exp(V-V_{1/2})/K)\}$, where K is the membrane depolarization for an *e*-fold increase in P_o, and $V_{1/2}$ is the membrane potential at which P_o is one-half of the maximum P_o. The values of $V_{1/2}$ and K were -59.68 ± 2.72 mV and 30.49 ± 3.50 (n=20), respectively. Dwell time analysis was performed on patches containing only one channel. Both open time and closed time were fitted well by two exponential distributions. As shown in Table 1, the closed time at -60 mV was longer than that at 60 mV. On the contrary, the open time at -60 mV was shorter than that at 60 mV. Thus, the increase in open probability may be due to both a decrease in the closed time and an increase in the open time.

2.2. Ionic selectivity

Ionic selectivity was assessed by ion-substitution experiments. At symmetrical 150 mM Cl^- conditions, the reversal potential of the channels was 0 mV (n=20). When patches were exposed to 30 mM Cl^- in the bath and 150 mM Cl^- in the pipette, the current-voltage relationship curve was shifted to the negative voltage range and the reversal potential changed to -34.23 ± 4.86 mV (n=5) (Fig. 2A, C). When patches were exposed to the opposite Cl^- gradient, the current-voltage relationship curve was shifted to the positive voltage range and the reversal potential changed to 29.6 ± 2.51 mV (n=5) (Fig. 2B, C). These changes in reversal potential are very close to the theoretically predicted values for Cl^- channels. When reversal potential is plotted as a function of $\log([Cl^-]_i/[Cl^-]_o)$, the points fall on a line (r=0.99), indicating Cl^- selectivity of the observed channel (Fig. 2D). It is obvious that the predicted slope of a perfect chloride channel is steeper than that of the recorded channel, suggesting that the ORCC in hippocampal neurons may also have some permeability to other ions. The relative permeability ratio (P_{Cl}/P_{Na}) derived from the Goldman-Huxley-Katz voltage equation was 12.42, which confirms the Cl^- selectivity of the channel.

2.3. Pharmacological inhibitors

Channel blockers are widely used as a means of discriminating between different channel types. DIDS, NPPB, SITS and glibenclamide were used to block ORCC. At a holding potential of 40 mV, 1 mM DIDS in the bath solution led to a flickering block of ORCC activity over 3 min (Fig. 3A). The open probability was reduced from 0.83 ± 0.06 to 0.12 ± 0.05 (n=5, $P<0.01$). Subsequent washout of DIDS from the bath solution led to a partial recovery of the channel open probability. Another type of channel blocker, NPPB, had a similar effect as DIDS. Specifically, 0.1 mM NPPB in the bath solution reduced open probability from 0.89 ± 0.06 to 0.11 ± 0.04 (n=5, $P<0.01$) (Fig. 3B). Partial blockade was also obtained with other ORCC blockers. Administration of 1 mM SITS or 0.1 mM glibenclamide to the bath solution led to $87.06 \pm 0.08\%$

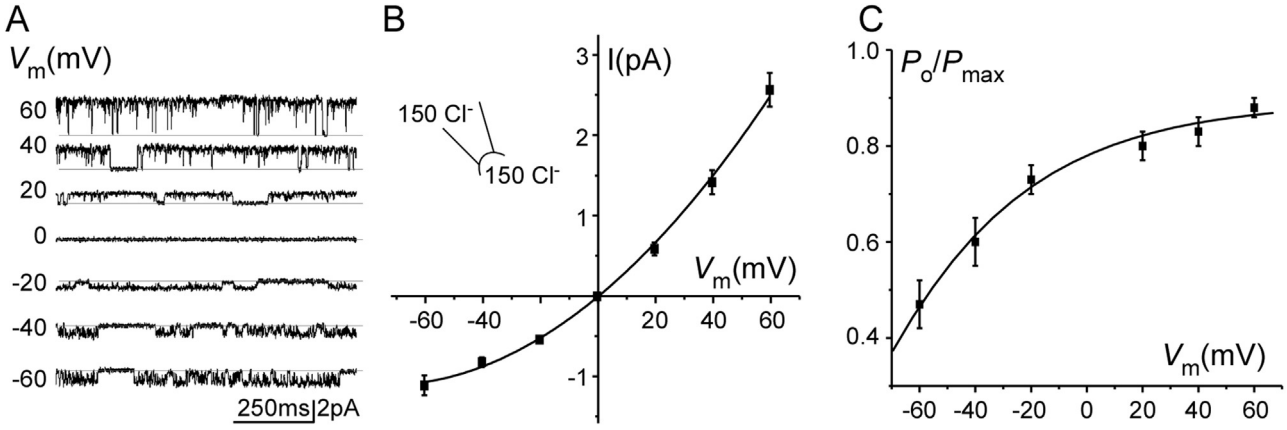

Fig. 1. Conductive properties of the outwardly rectifying chloride channel (ORCC) in CA1 pyramidal neurons. A. Representative current traces from acutely dissociated CA1 pyramidal neurons from an adult rat hippocampus bathed in symmetrical 150 mM Cl^- solution. Traces were recorded using the inside-out patch clamp configuration at various holding potentials. Gray lines indicate the current level of the closed channel, and voltages given are membrane potentials (V_m) in all figures. B. Corresponding current-voltage relationship is averaged over 20 patches. The shape of the curve, which is derived from the quadratic function, indicates a significant outward rectification of the current. Insert is the schematic illustration of an inside-out patch clamp. C. Voltage dependency of the open probability (P_o). Data points are the average P_o of the channel in inside-out patches at various holding potentials (mean ± SEM, n=20). The smooth curve is derived from the Boltzmann function.