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INCREASE OF DELAYED RECTIFIER POTASSIUM CURRENTS IN LARGE ASPINY NEURONS IN THE NEOSTRIATUM FOLLOWING TRANSIENT FOREBRAIN ISCHEMIA

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Abstract —Large aspiny (LA) neurons in the neostriatum are resistant to cerebral ischemia whereas spiny neurons are highly vulnerable to the same insult. Excitotoxicity has been implicated as the major cause of neuronal damage after ischemia. Voltage-dependent potassium currents play important roles in controlling neuronal excitability and therefore influence the ischemic outcome. To reveal the ionic mechanisms underlying the ischemia-resistance, the delayed rectifier potassium currents (I_k) in LA neurons were studied before and at different intervals after transient forebrain ischemia using brain slices and acute dissociation preparations. The current density of I_k increased significantly 24 h after ischemia and returned to control levels 72 h following reperfusion. Among currents contributing to I_k, the margatoxin-sensitive currents increased 24 h after ischemia while the KCNQ/M current remained unchanged after ischemia. Activation of protein kinase A (PKA) down-regulated I_k in both control and ischemic LA neurons, whereas inhibition of PKA only up-regulated I_k and margatoxin-sensitive currents 72 h after ischemia, indicating an active PKA regulation on I_k at this time. Protein tyrosine kinases had a tonic inhibition on I_k to a similar extent before and after ischemia. Compared with that of control neurons, the spike width was significantly shortened 24 h after ischemia due to facilitated repolarization, which could be reversed by blocking margatoxin-sensitive currents. The increase of I_k in LA neurons might be one of the protective mechanisms against ischemic insult.

Key words: striatum, cerebral ischemia, neuronal death, interneuron, membrane excitability, potassium channel.

The neostriatum is one of the highly vulnerable regions in the brain to cerebral ischemia. Unlike small- to medium-sized spiny neurons, which die 24 h after 25–30 min of transient forebrain ischemia (Pulsinelli et al., 1982), large aspiny (LA) neurons in the striatum are resistant to ischemic insults (Francis and Pulsinelli, 1982; Chesselet et al., 1990). The mechanisms of this selective neuronal damage after cerebral ischemia are still unclear. Excitotoxicity has been widely accepted as one of the major causes of neuronal death after ischemia (Rothman and Olney, 1986; Choi and Rothman, 1990). Recent studies have shown that, after transient forebrain ischemia, the evoked fast excitatory postsynaptic currents in LA neurons were suppressed, suggesting that the depression of excitatory neurotransmission might be involved in post-ischemic neuroprotection (Pang et al., 2002). Decrease in membrane excitability is also believed to be neuroprotective because the membrane potentials of spiny neurons were depolarized during *in vitro* hypoxia/hypoglycemia, whereas that of LA neurons was hyperpolarized (Calabresi et al., 1997; Pisani et al., 1999; Centonze et al., 2001). While the activation of voltage-dependent Na^+ channel and Ca^{2+} entry contribute to the generation of the ischemia-induced membrane depolarization in spiny neurons, the activation of ATP- and Ca^{2+}-dependent potassium channel is responsible for the hyperpolarization in LA neurons (Centonze et al., 2001).

Voltage-dependent potassium (Kv) channels play important roles in the maintenance of neuronal excitability by regulating resting membrane potential, interspike membrane potential and spike frequency (Rudy, 1988). In particular, delayed rectifier potassium currents (I_k) are critical for controlling the action potential repolarization and spike duration (Rudy, 1988; Storm, 1990). It has been demonstrated that potassium channel activity in neurons is changed during hypoxia (Jiang and Haddad, 1994; Jiang et al., 1994; Gebhardt and Heinemann, 1999) or after ischemia (Chi and Xu, 2000, 2001). Evidence from CA1 pyramidal neurons in the hippocampus suggests that the up-regulation of potassium conductance might contribute to the decrease of neuronal excitability following ischemic insults (Chi and Xu, 2000). The post-ischemic changes of potassium currents in striatal neurons remain to be elucidated. LA neurons possess both A-type potassium currents and I_k (Song et al., 1998; Tkatch et al., 2000). Since I_k regulates Ca^{2+} influx during action potential (Du et al., 2000), alteration of I_k after ischemia might affect the intracellular Ca^{2+} concentration and the pathological process associated with intracellular Ca^{2+} (Choi, 1995). To reveal the temporal changes of I_k in LA neurons after transient forebrain ischemia and the possible mechanisms underlying such changes, whole-cell voltage-clamp recordings were performed on acutely dissociated neurons and brain slices. Dissociated neurons are generally the first choice for voltage-clamp recording because most of the dendrites have been removed and the space clamp errors are minimal. However, the mechanical and enzymatic procedures during dissociation might have adverse affects on channel

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Abbreviations: ACSF, artificial cerebrospinal fluid; H-89, *N*-[2-((*p*-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide; I_k, delayed rectifier potassium current; ISI, interspike interval; LA, large aspiny; MgTX, margatoxin; OGD, oxygen/glucose deprivation; TEA, tetraethylammonium; TTX, tetrodotoxin; 4-AP, 4-aminopyridine; 8-Br-cAMP, 8-bromoadenosine 3′,5′-cyclic monophosphoate.

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properties. These traumatic effects could be more severe in neurons after ischemia. On the other hand, neurons in the brain slice preparation are preserved relatively better than the dissociated neurons. But the extended dendrites of neurons in the slice compromise the voltage clamping of the cell and might result in a space clamp error. Therefore, both techniques were used in the present study to validate the results.

EXPERIMENTAL PROCEDURES

Male Wistar rats (100–180 g; Charles River Laboratories, Wilmington, MA, USA) were used in the present study. Experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Indiana University School of Medicine in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the suffering and the number of animals used.

Transient forebrain ischemia

Transient forebrain ischemia was induced using the four-vessel occlusion method (Pulsinelli and Brierley, 1979) with modifications (Ren et al., 1997). Briefly, the animals were fasted overnight to provide uniform blood glucose levels. For surgical preparation, the animals were anesthetized with a mixture of 1–2% halothane, 33% O_2 and 66% N_2 via a gas mask placed around the nose. The common carotid arteries were isolated after which a silicon-tube loop was placed loosely around each common carotid artery to allow subsequent occlusion of these vessels. The animal was then placed on a stereotaxic frame, and the vertebral arteries were electrocauterized. A very small temperature probe (0.025-inch diameter; Physitemp, Clifton, NJ, USA) was inserted beneath the skull in the extradural space, and the brain temperature was maintained at 37 °C with a heating lamp using a temperature control system (BAT-10; Physitemp). Glass microelectrodes (5–8 μm in diameter of tip) filled with 2 M NaCl were used to record ischemic depolarization, which is an indication of complete ischemia (Ren et al., 1997). A burr hole was drilled at 9.5 mm anterior to the interaural line, 3.0 mm from the midline. The microelectrode was advanced 3.0 mm below dura into the neostriatum. The recordings were performed with a neuroprobe amplifier (Model 1600; A-M Systems, Carlsborg, WA, USA). The duration of ischemic depolarization was determined by measuring the period from the beginning of the extracellular direct current potential reaching −20 mV to the point where the potential started to repolarize after recirculation. Transient forebrain ischemia was produced by occluding both common carotid arteries to induce ischemic depolarization for approximately 22 min. Cerebral blood flow resumed immediately upon release of the carotid artery clasps. Animals were returned to the cages after recovering from ischemia and allowed free access to water and food.

Brain slice and acute dissociation preparation

Brain slices were prepared from animals before ischemia and at 6 h, 24 h, 48 h and 72 h after reperfusion using procedures similar to those previously described (Pang et al., 2002). Briefly, the animals were anesthetized with ketamine–HCl (80 mg/kg, i.p.) and decapitated. The brains were quickly removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF), which was composed of the following (in mM): 130 NaCl, 3 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10 glucose, pH 7.4, 295–305 mOsm/L. Transverse striatal slices of 280–300 μm thickness were cut using a vibratome (VT 1000; Leica, Nussloch, Germany) and incubated in ACSF for ≥1 h at room temperature (approximately 24 °C) before being transferred to the recording chamber. The slice was submerged beneath the fluid surface and superfused continuously with oxygenated ACSF. The flow rate was adjusted to 2–3 ml/min. Unless otherwise stated, recordings were carried out at room temperature.

Acutely dissociated neurons were prepared from rats before ischemia and at 24 h and 72 h after ischemia using procedures similar to those previously reported (Yan and Surmeier, 1997). In brief, rats were anesthetized and decapitated. The brains were quickly removed and immersed in an ice-cold low Ca^{2+} solution containing (in mM): 140 Na isethionate, 2 KCl, 4 $MgCl_2$, 0.1 $CaCl_2$, 23 glucose, 15 HEPES, pH 7.4, 300–305 mOsm/L. The brain tissue containing neostriatum was cut in 400 μm slices while bathed in the low Ca^{2+} solution. Slices were incubated at room temperature in oxygenated ACSF. Then, the slices were transferred into the low Ca^{2+} solution and regions of striatum were dissected and placed in an oxygenated Hanks' balanced salt solution containing 1 to approximately 3 mg/ml protease. After approximately 30 min of enzyme digestion at 35 °C, tissue was rinsed three times in the low Ca^{2+} buffer and mechanically dissociated with a graded series of fire-polished Pasteur pipettes. The cell suspension was plated onto a 12 mm coverslip (Fisherbrand Coverglass, Pittsburgh, PA, USA), which was then placed in the recording chamber.

Whole-cell patch-clamp recording

Recording electrodes were prepared from borosilicate glass (Warner Instruments, Hamden, CT, USA) using a horizontal electrode puller (P-97; Sutter Instruments, Novato, CA, USA) to produce tip openings of 1 to approximately 2 μm (3–5 MΩ). Electrodes were filled with an intracellular solution containing (in mM): 145 KCl, 1 $MgCl_2$, 10 EGTA, 0.2 $CaCl_2$, 10 HEPES and 2% neurobiotin (Vector Laboratories, Burlingame, CA, USA), pH 7.4, 290–295 mOsm/l. Neurons were visualized with an infrared-differential interference contrast microscope (BX50WI; Olympus Optical, Tokyo, Japan) and a CCD camera. Only those cells with large somata (>20 μm in diameter) were selected for recording. Whole-cell patch-clamp recordings were performed with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA, USA). After tight-seal (>1 GΩ) formation, the electrode capacitance was compensated. Immediately after establishment of whole-cell configuration, the resting membrane potential was obtained by direct reading from the amplifier. The membrane capacitance, series resistance and input resistance of the recorded neurons were measured by applying a 5 mV (10 ms) hyperpolarizing voltage pulse from a holding potential of −60 mV. The series resistance was 8–12 MΩ. Neurons with a series resistance >10% of the input resistance were discarded. The membrane capacitance reading was used as the value for whole cell capacitance. For all measurements, capacitance and series resistance compensation (60–80%) were used to minimize voltage errors. During the experiment, the membrane capacitance and series resistance were periodically monitored. Neurons with a series resistance change >20% during the experiment were excluded from the analysis. Signals were filtered at 2 kHz and digitized at a sampling rate of 5 kHz using a data-acquisition program (Axograph 4.6; Axon Instruments). To isolate the voltage-dependent outward potassium currents, tetrodotoxin (TTX; 1 μM) and $CdCl_2$ (300 μM) were added in the perfusate to block voltage-activated Na^+ and Ca^{2+} currents, as well as Ca^{2+}-activated potassium currents. In some experiments, 4-aminopyridine (4-AP, 1 mM) and tetraethylammonium (TEA, 20 mM) were applied to examine the pharmacological characteristics of I_k. The chemical agents were obtained from Sigma (St. Louis, MO, USA).

The current density of I_k for each neuron was obtained by dividing the total current by the membrane capacitance. The current amplitude of I_k was measured as an average amplitude at 340–390 ms after the onset of the command voltage pulses. The steady-state activation or inactivation curves were established