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Full Paper

Effect of Cu^{2+} on K^+ Current in Acutely Isolated Rat Hippocampal Neurons by Whole Cell Patch Clamp Technique

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Using the whole cell patch clamp technique, the effect of Cu^{2+} on transient outward K^+ current (I_{to}) and delayed rectifier K^+ current (I_{dr}) was studied in acutely isolated rat hippocampal neurons. I_{to} and I_{dr} were increased when the concentration of Cu^{2+} was lower than 2×10^{-5} and 10^{-5} mol/L, respectively, and increased ratio was decreased with increasing Cu^{2+} concentration in the bath solutions. When the concentration continued to increase to 5×10^{-5} and 2×10^{-5} mol/L, the currents were hardly changed, while the concentration was more than 10^{-4} and 5×10^{-5} mol/L, the currents were inhibited remarkably. Cu^{2+} (10^{-5} mol/L) did not affect the activation and inactivation process of I_{to}. The activation curve of I_{dr} was shifted toward positive potential, but 10^{-5} mol/L Cu^{2+} did not affect slope factor. According to these results, it was considered that Cu^{2+} at low concentration in the bath solution could promote I_{to} and I_{dr} while at high concentration could inhibit them, and change of amplitude was different with different membrane voltage. Conclusion was drawn: Cu^{2+} may be involved in the pathophysiologic mechanism of diseases with neuropathological components.

Keywords whole cell patch clamp technique, hippocampal neurons, copper(II), potassium current, transient outward potassium current, delayed rectifier potassium current

Introduction

Copper enters the body from the environment and from diet and medication, and is an essential trace metal that plays a fundamental role in the biochemistry of the human nervous system.[1,2] Recent insights into the molecular physiology of copper metabolism also have led to a better understanding of the role of copper in normal brain development and function.[3] Large quantities of data have shown that copper, in excess, is toxic for cells, and its toxic effect and mechanism are different with different cells.[4,5] Disruption of this tightly regulated cellular copper homeostasis affects normal tissue development and can cause severe neurodegeneration.[6-8] Copper has also been implicated in diseases with neuropathological components, including Alzheimer's disease *etc*., and found at particularly high concentrations in the olfactory bulb OB and hippocampus.[9,10]

Ion channels play very important roles in the growth development and the process of cell corresponding to the outside, and those in cell membrane are target for many kinds of toxin and drug. Depolarization activating outward K^+ current in neurons is main components of action potential repolarization course. Interrupting the functions of ion channels causes many neuronal damages of central nervous system. Hence to study effect of toxin and drug on these K^+ channels is base of discussing their action mechanisms.[11-13] However, no body has reported yet that patch clamp techniques were used to study how copper affects potassium channel currents. With the whole cell patch clamp technique, we studied the effect of Cu^{2+} on outward K^+ current in acutely isolated rat hippocampal neurons, in order to provide powerful proof for researching further the effects of Cu^{2+} on physiological activities of nerve cells and its toxicity to nerve cells.

Experimental

Preparation of single rat hippocampal neuron

Single rat hippocampal neuron was acutely isolated from about 7 d old Wistar rats (male or female, from the Animal House of Institute of Traditional Chinese Medicine of Shanxi Province) as previously described.[14] In brief, approximate 500 μm thick brain slices were cut from hippocampal region and incubated for at least 30 min at 32 ℃ in artificial cerebrospinal solution (ACS), successively transferred into ACS containing 0.5 mg/mL protease and digested for 35 min at 32 ℃. After digestion, the tissue pieces were washed 3 times with ACS, and then triturated through a series of Pasteur pipettes with decreasing tip diameter in external solution. The cell suspension was transferred into a 35 mm culture dish, filled with 1 mL of external solution. Cell with a pyramidal shape remained viable for the patch clamp recording.

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Patch clamp recording

The whole cell patch clamp recording mode was adopted on the basis of Hamill patch clamp methods.[15] Patch clamp pipettes (1—2 μm) were prepared from glass capillaries [BJ-40, diameter (1.5±0.1) mm, Beijing], and pulled on a multistage programmable puller (Narishige, Japan). Before the experiment, they were filled with internal solutions (pipette resistance of about 10 MΩ). During 20—25 ℃, after Giga Ω seals between electrode and the cell membrane were obtained, the cell membrane was broken by gentle suction, and the whole-cell recording mode was formed. In voltage clamp mode, currents were recorded with Axopatch 200B patch clamp amplifier (Axon Instrument, USA) and amplified by an amplifier (Digidata 1200), and PCLAMP 6.0.4 software was used to produce protocols, acquire and process data. Data were analyzed and then figures were plotted with Clampfit 6.0 and Origin 5.0.

I_{to} was estimated as the peak current and I_{dr} determined as late current at 300 ms step depolarization.[16]

Preparation of experimental solutions

Protease, TEA Cl and HEPES were purchased from Sigma, other reagents were of A.R. grade produced in China. ACS were data at mmol/L: NaCl 124, KCl 5, KH_2PO_4 1.2, $MgSO_4$ 1.3, $CaCl_2$ 2.4, glucose 10, $NaHCO_3$ 26, and pH adjusted to 7.4 with tris, while external solution: NaCl 150, KCl 5, $MgCl_2$ 1.1, $CaCl_2$ 2.6, glucose 10, HEPES 10, TEA-Cl 50, Cd^{2+} 0.2, and pH also adjusted to 7.4 with tris, and internal solution: KCl 65, KOH 5, KF 80, $MgCl_2$ 2, HEPES 10, EGTA 10, Na_2ATP 2, and pH adjusted to 7.3 with KOH. The solutions were bubbled with 100% O_2, before the experiment was conducted.

Drug application and data analysis

After the whole cell mode was obtained, signals were firstly recorded in natural state, and 15 min later after Cu^{2+} was added into external solution, signals were secondly recorded. All values were represented mean ±S.D., and statistical comparisons were made by the student's t-test.

Results and discussion

Outward K^+ current

Outward K^+ current was held at a holding potential E of –100 mV and a series of 300 ms depolarizing steps to 90 mV (10 mV increment at each step) were applied at a frequency of 0.5 Hz (Figure 1a). The same experiments were repeated (neuron $n>20$) and the results were similar, but there was a tiny difference in the amplitude of currents. When Cs^+ was substituted for K^+ in internal solution, the outward current could not be recorded, indicating that the outward current was K^+ current. Figure 1b shows the current-voltage relationship with the data acquired from experiments performed as in Figure 1a, where outward K^+ current chiefly included I_{to} and I_{dr}.[17] As shown in Figure 1, the outward current had a quickly ascendant starting peak, and then it slowly descended to a steady state.

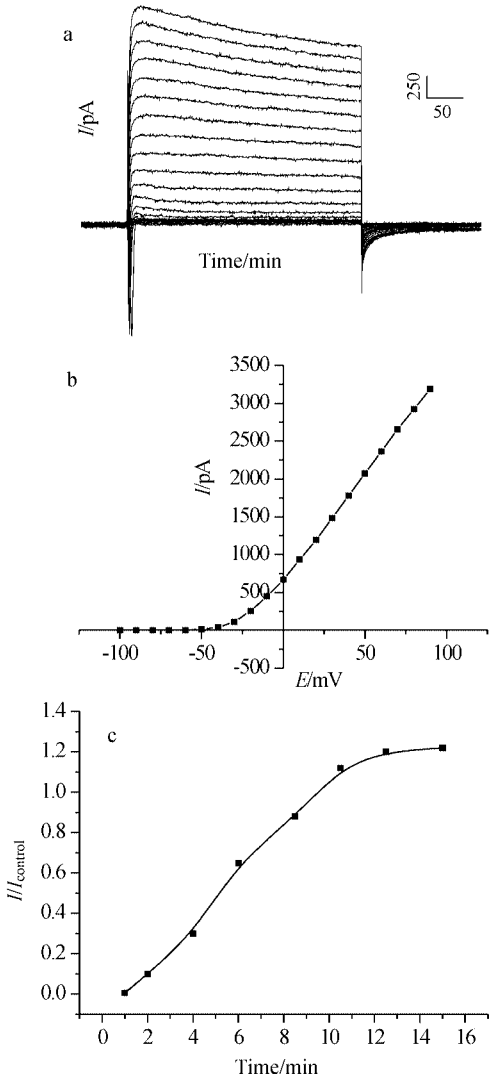

Figure 1 Outward K^+ current (a), the current-voltage relationship curve (b) and time curve of the effect of Cu^{2+} on K^+ current (c).

In addition, as shown in Figure 1, the current has a fast inward current in starting part, which is Na^+ current. Because activation and inactivation of Na^+ current are very fast, it does not affect K^+ current. Therefore, in the present study, the outward K^+ current was recorded in existence of Na^+ current.

After application of 10^{-5} mol/L Cu^{2+} to the bath solution, the peak amplitudes of the outward current were increased steadily within 10 min (Figure 1c).

Effect of different Cu^{2+} concentration on I_{to} and I_{dr}

When 10^{-7}, 10^{-6}, 10^{-5}, 2×10^{-5}, 5×10^{-5}, 10^{-4} and 10^{-3} mol/L Cu^{2+} were respectively added into external solution, outward K^+ currents were recorded correspondingly. When the membrane potential was 90 mV, the respective currents were compared with corre-

sponding control currents. At every concentration, the data were the average of those recorded for at least 5 cells. Figure 2 displays the relationship curve of I_{to} and I_{dr} vs. logarithm of Cu^{2+}concentration in the bath solutions.

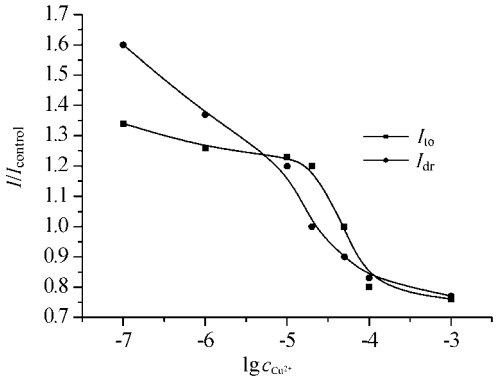

Figure 2 Effect of different Cu^{2+} concentration on I_{to} and I_{dr}.

As showed in Figure 2, I_{to} was increased when the concentration of $Cu^{2+}$was lower than 2×10^{-5} mol/L, and increased ratio was decreased with increasing Cu^{2+} concentration in the bath solutions. When the concentration continued to increase to 5×10^{-5} mol/L, the current was hardly changed, while the concentration was more than 10^{-4} mol/L, the current was inhibited remarkably. Analogously, I_{dr} increased when the concentration of $Cu^{2+}$was lower than 10^{-5} mol/L, and increased ratio was decreased with increasing Cu^{2+}concentration in the bath solutions. When the concentration continued to increase to 2×10^{-5} mol/L, the current was hardly changed, while the concentration was more than 5×10^{-5} mol/L, the current was inhibited remarkably. According to these results, it was concluded that Cu^{2+}at low concentration in the bath solutions could promote I_{to} and I_{dr} while at high concentration could inhibit I_{to} and I_{dr}.

Effect of Cu^{2+}on the current-voltage relationship of I_{to} and I_{dr}

Current-voltage curves of I_{to} and I_{dr} were obtained by plotting the currents against test pulse. However, the amplitudes of I_{to} and I_{dr} were increased differently at different membrane potential and different concentration (10^{-7}—10^{-3} mol/L, Figure 3).

Effect of Cu^{2+} on activation kinetics of I_{to} and I_{dr}

The steady-state activation curves for I_{to} and I_{dr} under control and after exposure to 10^{-5} mol/L Cu^{2+}were showed in Figure 4. I_{to} and I_{dr} were converted into conductance by use of the equation $G=I/(E-E_k)$, where G=conductance, E=membrane electromotive potential, E_k=reversal potential. With the least squares fit procedure, the normalized conductance was well fitted with a Boltzmann equation: $G/G_{max}=1/\{1+\exp[(E-E_h)/k]\}$, where E_h=membrane electromotive potential at half-activation, k=slope factor. Before and after application of 10^{-5} mol/L Cu^{2+}, the values of E_h for I_{to} were (24.255±0.772) and (23.981±0.934) mV (n=5, $P>0.05$), with k of (19.726±0.739) and (18.588±0.885)

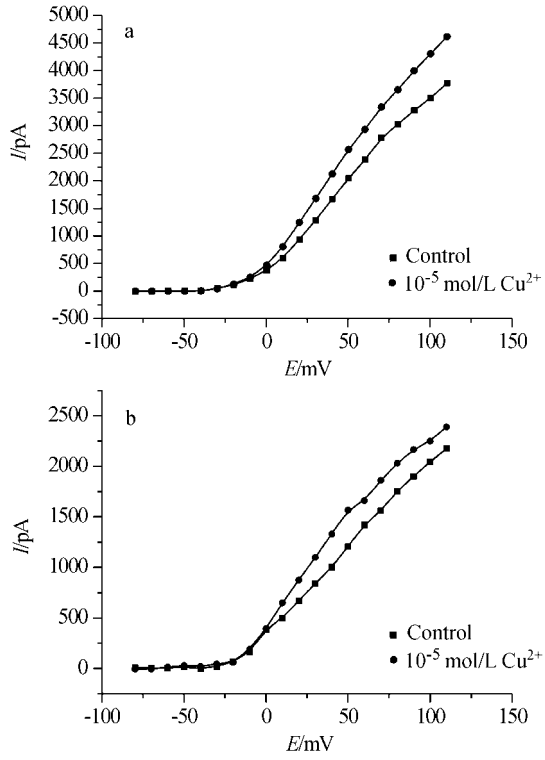

Figure 3 Effect of Cu^{2+} on the current-voltage relationship of I_{to} (a) and I_{dr} (b).

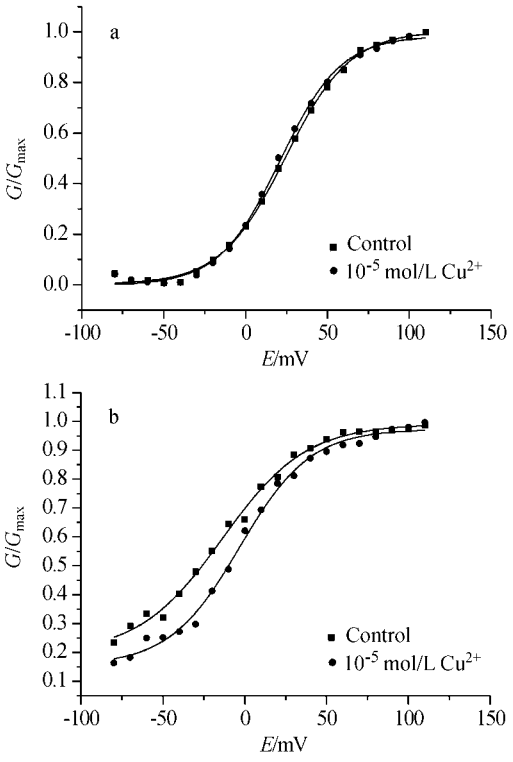

Figure 4 Effect of 10^{-5} mol/L Cu^{2+} on activation kinetics of I_{to} (a) and I_{dr} (b).

mV ($n=5$, $P>0.05$), indicating that the activation process of I_{to} was not affected by 10^{-5} mol/L Cu^{2+}. Before and after application of 10^{-5} mol/L Cu^{2+}, the values of E_h for I_{dr} were (13.689±2.15) and (3.121±1.66) mV ($n=5$, $P<0.01$), with k of (25.512±1.92) and (21.514±1.58) mV ($n=5$, $P>0.05$), indicating that the activation curve of I_{dr} was shifted toward positive potential, but slope factor was not affected by 10^{-5} mol/L Cu^{2+}.

Effect of Cu^{2+} on inactivation kinetics of I_{to}

Figure 5 showed the steady-state inactivation curves for I_{to} under control and after exposure to 10^{-5} mol/L Cu^{2+}, where peak amplitudes for I_{to} currents were normalized and plotted versus prepulse potentials. With a least square fit method, the curves were well fitted with a Boltzmann equation: $I/I_{max}=1/\{1+\exp[(E-E_h)/k]\}$, where E_h=membrane electromotive potential at half-inactivation, k=slope factor. Before and after application of 10^{-5} mol/L Cu^{2+}, the values of E_h for I_{to} were (−35.125±3.27) and (−33.317±2.38) mV ($n=5$, $P>0.05$), with k of (15.792±3.32) and (16.139±2.42) mV ($n=5$, $P>0.05$), indicating that the inactivation process of I_{to} was not affected by 10^{-5} mol/L Cu^{2+}.

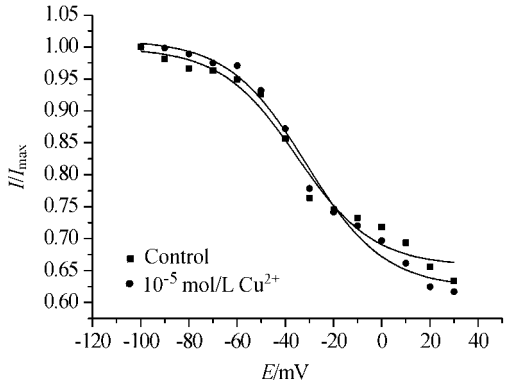

Figure 5 Effect of 10^{-5} mol/L Cu^{2+} on inactivation kinetics of I_{to}.

Voltage-gated potassium currents play crucial roles in modifying neuronal cellular and network excitability and activity.[18,19] Potassium currents are important for the regulation of the maintenance of baseline membrane potential, too. They can control action potential duration, release of neurotransmitters and hormones, Ca^{2+} dependent synaptic plasticity and epileptiform burst activity.[20] While membrane is depolarized, I_{to} and I_{dr} will appear. Since I_{to} is transient, repolarization is mainly related with I_{dr} in central nervous system.

Copper is required for the catalytic activity of enzymes that play an essential role in neurobiology and disease, including tyrosinase for melanin synthesis *etc*.[21] For this reason, specific mechanisms were evolved for the compartmentalization and trafficking of this metal within cells.[22] Copper is endogenous transition metals that can be synaptically released during neuronal activity. Synaptically released copper probably functions to modulate neuronal excitability under normal conditions,[23] this metal may produce adverse physiological effects. The interaction of metals with ion channels, on the other hand, is a well described phenomenon: $GABA_A$ receptors, for example, were modulated by lanthanum, zinc, mercury or copper.[24,25]

Our results demonstrated that Cu^{2+} at low concentration in the bath solutions could promote I_{to} and I_{dr} while at high concentration could inhibit I_{to} and I_{dr}, and change of amplitude was different with different membrane voltage. It was consistent with the results of previous experiments examining the effects of copper on hippocampal and cortical neurons.[26] In addition, it appeared to have different sensitivity for I_{to} and I_{dr}. I_{dr} seemed to be more sensitive to Cu^{2+}, and a depolarizing shift of the steady-state activation curve of I_{dr} by Cu^{2+} would account for the inhibition of I_{dr}. Cu^{2+} affected normal sensory physiology of neurons, and these neurons might display aberrant properties that resulted in abnormal sensations. This variation caused by Cu^{2+} could underlie the toxic modulation of sensory input to the central nervous system. It was also noticed that the maximum increase of Cu^{2+} on I_{to} and I_{dr} only reached near 160% even at 0.1 μm. Because voltage-activated potassium channels in hippocampal neurons are composed of various potassium channel subtypes, Cu^{2+} is probably selective toward some potassium channel subtypes.[27]

Anyway, the research on the action mechanism of copper to potassium channel is a very complicated process, which needs to be studied and solved from different angles with many different methods.

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