

Original Article

Aluminum Inhibits Delayed-rectifier K⁺ Current in *Drosophila* Neurons

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ABSTRACT

Objectives: The purpose of this study was to evaluate the effect of aluminum on the delayed-rectifier potassium current (IK_{DR}), which is important in regulating neuronal excitability.

Methods: We characterized the neurotoxic effect of aluminum on IK_{DR}. The conventional whole-cell patch-clamp technique was applied to cultured *Drosophila* neurons derived from embryonic neuroblasts. IK_{DR} was measured from neurons before and after application of

0.1 mM aluminum chloride to the external saline.

Results: IK_{DR} was smaller in the aluminum-containing saline (281 ± 58 pA) than in the control saline (549 ± 40 pA). There was less IK_{DR} inactivation in the presence of aluminum.

Conclusion: These results demonstrate that aluminum inhibits IK_{DR}, which in turn can affect neuronal excitability.

KEY WORDS: aluminum, neurotoxic, patch-clamp, potassium current

INTRODUCTION

Accumulation of aluminum has been detected within the brain tissue of patients with Alzheimer's disease and the Parkinsonian dementia complex of Guam^[1]. The increased acidification of surface waters has led to increased aluminum absorption and this has been linked to an increased incidence of Alzheimer's disease^[2]. Aluminum also induced memory impairment in experimental animals, accompanied by the formation of neuronal cytoskeletal abnormalities^[3]. However, aluminum effects on neuronal membrane currents are partially understood. In particular, no information is available about aluminum effects on the delayed-rectifier K⁺ current.

Studies on aluminum-injected rabbits and cats revealed a reduced frequency of spikes in neurons and impaired long-term potentiation in brain cells^[4,5]. Aluminum also reduced the voltage-dependent influx of calcium into synaptosomes and smooth muscle cells^[6,7]. Moreover, aluminum enhanced the release of acetylcholine at the frog motor synapse^[8]. Aluminum might exert its neurotoxic action by altering the intracellular calcium level. The inhibition of the neuronal ATPase by aluminum^[9] and aluminum interference with calcium binding to calmodulin^[10] may elevate intracellular calcium concentration in neurons. Moreover, aluminum may participate in the development of glutamate-induced excitotoxic neuronal injury^[11].

The effects of aluminum on different voltage-dependent channels have been characterized by several studies. Aluminum blocked Ca²⁺ current in rat dorsal root ganglia^[12]. Aluminum inhibited Na⁺ single channel activity in rat and rabbit hippocampal neurons^[13]. In rat dorsal root ganglia, aluminum blocked the Ca²⁺ current, but reduced K⁺ and Na⁺ currents by less than 15%^[12]. Aluminum selectively increased a slow, voltage-dependent K⁺ current in molluscan neurons^[14]. However, no information is available about aluminum effect on delayed-rectifier K⁺ current. In the present study, we used the whole-cell patch clamp technique to investigate the effect of aluminum on the delayed-rectifier K⁺ current. We have found that aluminum inhibits the delayed-rectifier K⁺ current in *Drosophila* cultured neurons.

MATERIALS AND METHODS**Cell culture:**

Eggs were collected over one and half hour period from *Drosophila melanogaster* (Oregon-R) flies maintained in pint milk bottles at 26 °C. Each culture was prepared from the cells of 1-3 gastrulating embryos in a modified Schneider's *Drosophila* medium (DM). Five hours after the beginning of egg collection, the embryos were placed in a 50% ethanol / 50% Clorox solution for two minutes to sterilize and dechorionate them. The embryos were then repeatedly washed with DM. Two or three embryos were transferred to a

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drop of DM on a 35 mm tissue culture dish (Falcon 3001). Each embryo was impaled by a hand-held micropipette (tip diameter about 100 μm); the cells were collected by suction and blown onto the surface of the dish. The cells were further dispersed by repeated passage through the tip of a smaller pipette (tip diameter 50 μm). The cells adhered to the surface of the dish within minutes of dispersal. The culture dish containing the embryonic cells (in a single drop of DM) was kept in a humid container at room temperature (23 °C). All cell cultures were studied electrophysiologically two days (43-49 hrs) later at room temperature. The culture dish was used as the recording chamber with a Sylgard form insert in the dish to confine the extracellular solution to a small volume (0.3 ml). Cells were viewed using Carl Zeiss bright-field optics.

Current recording:

Neurons were identified by the presence of one or more thin processes. Neurons with clearly visible cell bodies, usually alone were selected for study. The typical cell studied throughout this paper had one process with a cell body 4-7 μm in diameter. Occasionally, cells with 2 or 3 process were studied. The conventional whole-cell (CWC) patch-clamp technique was used to study the membrane currents of neurons. Electrodes were pulled from 100 μl micropipettes (VWR, Cerritos, CA, USA), coated with Sylgard resin near the tip, and polished to a bubble number^[15] of 3.0-4.0. When filled with potassium aspartate solution, these electrodes had resistances of 6-12 M Ω . The application of CWC patch-clamp to cultured embryonic *Drosophila* neurons has been described in detail previously^[16]. Typically, pipette potential was nulled, gigaohm seal was formed using gentle suction, pipette capacitance was compensated, and the whole-cell configuration was obtained with the application of further suction.

A patch-clamp amplifier measures the membrane current while keeping the membrane potential at a specific level. Experiments were performed with an Axopatch 200 A-patch-clamp amplifier (Axon Instruments, Foster City, CA, USA). Data acquisition and analysis were performed using Digidata 1200 (Axon Instruments) and pCLAMP software (version 5.5, Axon Instruments) on a 486 HP personal computer. Current recordings were filtered (four-pole Bessel) at 5 kHz (capacitive currents) or 1 kHz (ionic currents) and digitized at 20 or 200 μs intervals, respectively. Passive (leakage) currents, determined from negative pulses of one-quarter the amplitude of the test pulse (-P/4), were subtracted from all of the ionic currents. The series resistance, which was the resistance of the patch electrode during the whole-cell recording, was estimated by dividing the magnitude of the 50 mV voltage step by the

amplitude of the capacitive transient current. There are errors due to uncompensated series resistance of about 5-mV/100 pA.

Internal and external solutions:

K⁺ currents were measured in an external 6K/0Ca Tris *Drosophila* saline, which contained 6 KCl, 10 MgCl₂, 140 TrisHCl, 10 Hepes, and 10 glucose in mmol/L. The pH was adjusted to 7.4 with TrisOH. The osmolarity was about 296 mOsm /L (measured using a 3 MO micro-osmometer, Advanced Instruments, Needham Heights, MA). The external solution was changed during experiments by pipetting three milliliters of the new solution into the 0.3 ml bath; excess solution was removed by a continuous, vacuum-powered exhaust. The new solution contained 0.1 mM aluminum chloride in addition to the above. The pipette internal solution was potassium aspartate, it contained (in mmol /L) 3 KCl, 139 L-aspartic acid, 1 MgCl₂, 10 Hepes, 0.1 CaCl₂, and 1 EGTA (final Ca²⁺ concentration is about 10 nmol/L). The internal solution was adjusted to pH 7.3 with KOH (final K⁺ concentration is about 156 mmol/l). The osmolarity of the internal solutions was about 10 % lower than that of the external solution, to improve seal formation.

The protocol of investigating IK_{DR} included two types of studies, the population studies and the single-cell studies. The population studies included IK_{DR} measurement from neurons bathed in the aluminum-containing (0.1 mM) solution, and from other neurons bathed in the control solution. The purpose of the population studies is to determine whether the aluminum effect on IK_{DR} is observed despite the variability in current amplitude among neurons. The single-cell studies included IK_{DR} measurement from neurons before and after aluminum (0.1 mM) addition to the bath. The purpose of the single-cell study is to determine precisely the aluminum effects on IK_{DR}, without the variability in current amplitude among neurons.

Statistical analysis and data presentation:

Throughout the results, population data are presented as the mean \pm S.E.M. The means of two populations were compared using a two-tailed Student's *t*-test for independent samples. A difference was considered statistically significant if the probability that both samples came from the same distribution was at least less than 0.01. Graphics were generated with Excel (Microsoft) and SigmaPlot (Jandel Scientific) software packages.

RESULTS

Examples of the types of cells studied are indicated by arrows in the photos of Fig. 1. Cells

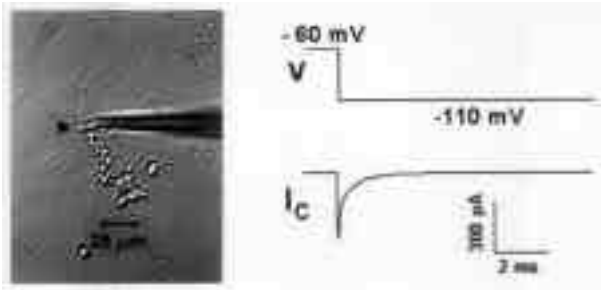


Fig. 1: General properties of neurons studied

were studied between 43 and 49 hr from the time of egg laying (23 °C). In the single-cell studies, $I_{K_{DR}}$ was measured from 20 neurons before and after aluminum addition to the external bath. In the population studies, $I_{K_{DR}}$ was recorded from 19 neurons bathed in the aluminum-containing solution, and from 17 neurons bathed in the control solution. Aspartate was found to be the best anion to use in the intracellular solution during whole cell experiments. We calculated the total capacitance (C) for each cell by integrating the capacitive current flowing in response to a 50 mV hyperpolarizing step. The cell capacitance was 6.69 ± 1.24 pF ($n = 15$), and it gives a measure of cell membrane area. The resting membrane potential (RMP) was 79.7 ± 0.3 mV ($n = 16$). The whole-cell resistance (R_{in}) was measured by stepping the membrane potential from -60 mV to -110 mV and dividing this 50 mV step by the measured current amplitude between 90 and 100 msec. R_{in} was 6.92 ± 1.21 G ($n = 16$).

$I_{K_{DR}}$ properties:

Potassium current was measured in a Ca^{2+} - free *Drosophila* external solution because $I_{K_{DR}}$ is smaller in Ca^{2+} - containing solutions than in Ca^{2+} - free solutions due to contamination of the outward $I_{K_{DR}}$ by the inward Ca^{2+} current^[17]. Moreover, neuronal K⁺ current in *Drosophila* does not have a Ca^{2+} - dependent component^[16]. $I_{K_{DR}}$ was recorded from neurons at potentials from -40 to +60 mV. $I_{K_{DR}}$ was calculated between 490 and 500 msec (steady state) of the pulse to exclude any possibility of A-current contribution to the measured amplitude. All neurons displayed the delayed-rectifier (non-inactivating) K⁺ current. We applied a voltage protocol that maximizes the delayed-rectifier K⁺ current and diminishes the A-type (inactivating) K⁺ current (holding potential, -80 mV; test pulses, -40 to +60 mV)^[17,18]. In the control solution, $I_{K_{DR}}$ was clearly activated at 0 mV, but only weakly activated at -20 mV. The time course of inactivation was qualified by calculating the percentage of the peak current that had inactivated at 500 msec.

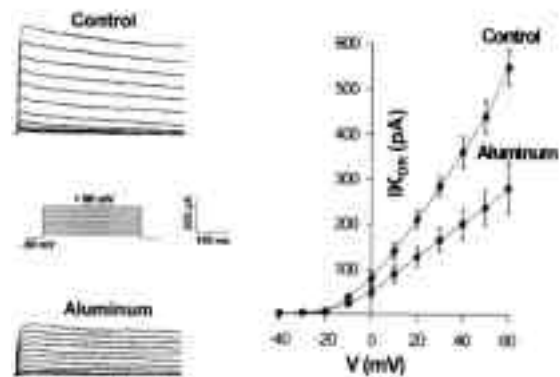


Fig. 2: Current-Voltage (I-V) relations of *Drosophila* neurons

Single cell studies of Al^{3+} effect on $I_{K_{DR}}$:

To exclude variability due to sampling, $I_{K_{DR}}$ was measured from single neurons before and after the addition of aluminum chloride to the external solution. In the aluminum-containing (0.1 mM) solution, $I_{K_{DR}}$ was smaller (281 ± 58 pA, $n = 20$) than that measured in the control 6K/0Ca Tris solution (549 ± 40 pA, $n = 20$) ($p < 0.001$) (Fig. 2). This represents about 50% reduction of $I_{K_{DR}}$ amplitude. The time course of inactivation was quantified by calculating the percentage of the current that had inactivated at 100 msec. There was a significant difference in values of $I_{K_{DR}}$ steady-state inactivation, which were $19 \pm 3\%$ ($n = 8$) for control solution and $8 \pm 2\%$ ($n = 8$) ($p < 0.01$) for aluminum-containing solution (60 mV pulse). Figure 2 shows the I-V relations of $I_{K_{DR}}$ measured in control 6K/0Ca Tris solution and in aluminum-containing 6K/0Ca Tris solution. At each of the clamp voltages between 0 and +60 mV, the current amplitude was significantly smaller in the aluminum-containing solution than in the control solution. The current was considered substantially activated if it reached an amplitude of at least 80 pA^[17]. In the aluminum-containing solution, $I_{K_{DR}}$ was activated (90 ± 18 pA, $n = 20$) at 10 mV. In the control solution, $I_{K_{DR}}$ was activated (80 ± 12 pA, $n = 20$) at 0 mV. There was statistically no significant difference in $I_{K_{DR}}$ activation constant between the control solution (15 ± 3 msec, $n = 20$) and the aluminum-containing solution (17 ± 3 msec, $n = 20$). Fig. 3 shows the concentration-response relationship for the inhibition of $I_{K_{DR}}$ by Al^{3+} at 60 mV. Total blockade (98%) was obtained with a concentration of 260 μ M. Each point is mean \pm S.E.M for 4-6 experiments. Points were fitted to a sigmoid curve.

Population studies of Al^{3+} effect on $I_{K_{DR}}$:

In other experiments, $I_{K_{DR}}$ was recorded from neurons bathed in the aluminum-containing (0.1 mM) solution, and from other neurons bathed in the control-solution. $I_{K_{DR}}$ amplitude was smaller in the aluminum containing (308 ± 47 pA, $n = 19$) than

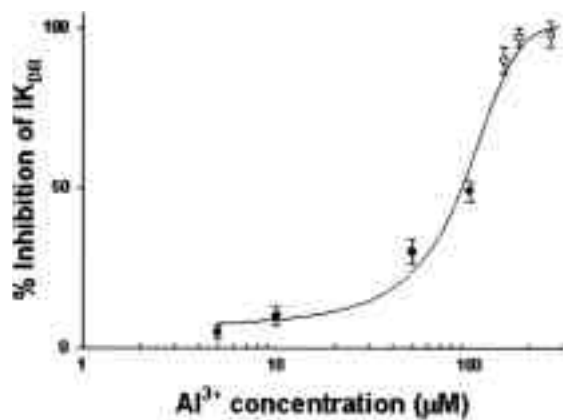


Fig. 3: Dose-response relationship of IK_{DR} inhibition by Al^{3+}

in the control solution (553 ± 39 pA, $n = 17$) ($p < 0.001$). This was observed despite the variability in current amplitude among neurons. Al^{3+} blockade of the K^+ current was not easily reversed upon washing. Recovery of the K^+ current from the Al^{3+} block did not exceed 20%.

Sensitivity of IK_{DR} to TEA :

IK_{DR} was measured from single neurons before and after application of 20 mM TEA - 6K/0Ca Tris. *Drosophila* saline (10 min). TEA reduced IK_{DR} by $91 \pm 1\%$, IK_{DR} was significantly smaller in the TEA-containing solution (53 ± 4 pA, $n = 12$) than in the control solution (560 ± 49 pA, $n = 12$) ($p < 0.001$) (Fig. 4).

DISCUSSION

Numerous studies reported on several issues of aluminum toxicity such as effects of aluminum on mechanical properties of frog atrial muscle^[19], mammalian calcium channel^[22], mouse brain acetylcholinesterase^[20], and synaptic plasticity in rat dentate gyrus^[21]. In light of neuropathological studies which have suggested a possible link between the neurotoxicity of aluminum and the pathogenesis of Alzheimer's disease^[22] and aluminum participation in the development of glutamate-mediated excitotoxic neuronal injury^[23], we characterized the neurotoxic effect of aluminum on the delayed-rectifier current in *Drosophila* neurons. Such information is essential to understand aluminum toxic effects on CNS function especially because K^+ currents modulate neuronal excitability. Despite anticipated differences between *Drosophila* neurons and human neurons (e.g., *Drosophila* $[Ca^{2+}]_i = 45$ nM, mammalian $[Ca^{2+}]_i = 64$ nM^[24], similarities between the two neuronal species could lead to important medical therapies. For example, transgenic *Drosophila* cells increase the survival of neural grafts when cotransplanted with embryonic neural tissue in the mammalian peripheral nervous system^[25].

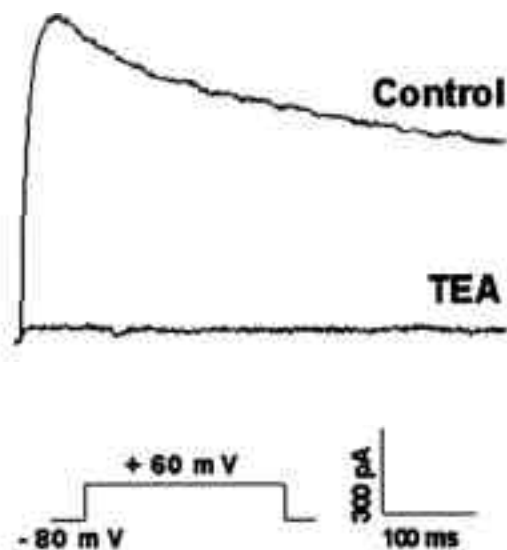


Fig. 4: Sensitivity of delayed-rectifier current to TEA in *Drosophila* neurons

Mechanism of IK_{DR} inhibition by aluminum:

The amplitude of IK_{DR} and the slope of the I-V relation were both reduced in the aluminum-containing saline compared with the control saline. Al^{3+} possibly interacts with the IK_{DR} channel; for example, Al^{3+} may bind at a specific site and obstruct the pore. The change of the voltage-dependence of IK_{DR} (Fig. 2) may result from an electrostatic interaction between gating charges and the ion when bound to a selective site in the pore. This was proposed by previous studies of cations on voltage-gated channels^[26]. On the other hand, the observation that Al^{3+} blockade of IK_{DR} is not easily reversed upon washing is suggestive of an intracellular blocking mechanism. Essentially, Al^{3+} may bind at the cytoplasmic side of the pore causing its obstruction.

Functional implications of IK_{DR} inhibition by aluminum:

IK_{DR} reduction should be considered in the context of neuronal activity. Our study is the first to reveal a potent block of the delayed-rectifier K^+ current in *Drosophila* neurons by Al^{3+} and provide a dose-response curve. A decrease in IK_{DR} is likely to have a wide range of functional consequences^[27]. For example, a decreased IK_{DR} in the soma can affect synaptic function at the axon ending. The sustained IK_{DR} current is responsible for repolarization during the falling phase of the action potential. Thus the inhibition of IK_{DR} can produce a prolonged action potential, that can lead to increased calcium influx via voltage-gated calcium channels. An increase of calcium influx into the presynaptic nerve terminal can directly enhance neurotransmitter release. Indeed the present

results of aluminum inhibition of IK_{DR} (which can prolong the action potential) coupled with previous reports of inhibition of neuronal ATPase by aluminum^[9], and aluminum interference with calmodulin binding to calcium^[10], suggest that aluminum neurotoxicity could be via elevated intracellular calcium concentration.

Al³⁺ block of voltage-dependent Na⁺ channels in rat and rabbit hippocampal neurons^[13] and K⁺ channels in the present study collectively demonstrate that Al³⁺ can easily impair neuronal function. Al³⁺ intoxication could eventually lead to a neuronal activity profile with a prolonged duration and a reduced frequency of action potentials. Such a neuronal activity profile could account for impaired long-term potentiation^[5] and memory impairment^[3] in conditions of Al³⁺ intoxication. The present data documented an acute effect of aluminum, by contrast, human intoxication with aluminum may be a chronic one through environmental pollution and the food chain. Chronic inhibition of IK_{DR} by aluminum could possibly lead to elevated [Ca²⁺]. It may be postulated that, in the case of neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease, neuronal damage is the result of relatively small changes in Ca²⁺ homeostasis that are sustained over long periods of time.

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