Delayed Rectifier Potassium Current in Dissociated Bullfrog Primary Afferent Neurons

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Summary: Cultured bullfrog dorsal root ganglion cells were voltage-clamped in the whole-cell configuration. The classical delayed rectifier potassium current (I_K) was separated from other ionic currents. Tetraethylammonium (1-50 mM) depressed the amplitude of I_K in a concentration-dependent manner, a complete block occurring with 30 mM. With the concentration of potassium ions in the superfusate at 20 mM, the reversal potential of I_K amounted to about -30 mV. I_K was activated between -30 and +70 mV. The half activation of I_K occurred at +15 mV. The amplitude of I_K was increased e-fold with 13.6 mV depolarization. The time constant of I_K de-activation was shortened with membrane hyperpolarization (τ=4 ms at -100 mV). Finally, reciprocal time constant (τ^-1) of the de-activating I_K was increased e-fold with ≈13 mV hyperpolarization. It appears that the properties of I_K in amphibian afferent neurons are comparable to those which have been observed with respect to the I_K of the squid giant axons (Hodgkin and Huxley, 1952).

Key words: tissue culture—whole-cell patch clamp—dorsal root ganglion cells—delayed rectification—potassium current—potassium accumulation

Introduction

A classical delayed rectifier potassium current of Hodgkin and Huxley (1952) has been suggested to occur in bullfrog dorsal root ganglion cells (Ito, 1959; Koketsu et al. 1959). Ito and Maeno (1986) have recently reported that step depolarizations from the holding potential close to the resting membrane potential evoke a rapidly inactivating potassium current followed by a sustained potassium current in isolated bullfrog dorsal root ganglion cells. These authors have suggested that the sustained potassium current may be analogous to the classical delayed rectifier potassium current since the current is insensitive to 4-aminopyridine and the current is not a family of a calcium-activated current. However, voltage-dependence of the sustained potassium current has not been systemically quantified as yet (Ito and Maeno, 1986). The main purpose of the present study was to examine voltage- and time-dependence of the delayed rectifier potassium current in dissociated bullfrog dorsal root ganglion cells. We found an outward rectifier current with properties similar to those which have been reported in the

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Materials and Methods

All the experiments in the present study were carried out at 22-24°C. Statistics are expressed as means ±SE of the mean for the cells tested.

Tissue culture. Dorsal root ganglia (DRG) were dissected from the bullfrogs (Rana Catesbiana) and minced with forceps into small pieces. DRG neurons were enzymatically dissociated with collagenase (Sigma type IA, 0.5 mg/ml) and trypsin (Sigma type XI, 2.5 mg/ml); they were then stocked for up to 11 days at 4°C in Leibovitz's L-15 medium (Gibco 320-1415; 20% fetal bovine serum (Gibco 200-6140 AG) added and then diluted to 80% with H2O). The stock medium was changed every three days.

Whole-cell clamp. The cells were pipetted into the recording chamber (1.5 ml total volume) which was continuously superfused (1-3 ml/min) with Ringer solution. Pipettes for the whole-cell recordings had a tip resistance of 2-5 MΩ when filled with a solution of the following composition (mM): KCl, 100; ethylene glycol-bis (β-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA), 1; adenosine 5'-triphosphate disodium salt (Na2ATP), 5; MgCl2, 4; HEPES (sodium salt), 2.5. The pH was adjusted with KOH to 7.1. Sample-and-hold/voltage-clamp amplifier (Axoclamp 2A) was used at 10-20 kHz with a 70-30 duty cycle. The headstage current gain used was 0.1. This indicated the clamping current as large as ±20 nA could be applied to the cells.

As the spheroidal cells of the DRG have the diameter of 60 μm, can their surface area be estimated as being about 11300 × 10⁻⁸ cm². Thus, the density of total current of 1 nA was about 9 μA/cm².

Solutions. The normal Ringer solution had the following composition (mM): NaCl, 112; KCl, 2; CaCl2, 1.8; NaHCO3, 2.4; the pH was 7.2. I_K was separated from other membrane currents in a HEPES-TRIS buffered solution having the following composition: NaCl (88 mM), KCl (20 mM), MgCl2 (10 mM), CoCl2 (2 mM), BaCl2 (2mM), CsCl (2 mM), tetrodotoxin (3 μM), apamin (10 nM) and 4-aminopyridine (1mM) (pH 7.2). This solution is referred to as a standard solution in the present study.

Drugs. Drugs used were tetrodotoxin (Sankyo), 4-aminopyridine (Sigma) and apamin (Sigma).

Results

Whole-cell voltage recordings were made from bullfrog dorsal root ganglion (DRG) cells maintained in primary culture (Day 2-11). About 5 min after the establishment of the whole-cell configuration the resting membrane potential of the cells tested ranged from -60 to -80 mV (-73.1 ±2.1 mV, n = 40). The action potential evoked by depolarizing current pulses (≤1 nA, 5-20 ms) had the peak amplitude of about 100 mV (n = 25) (Fig. 1A). The steady-state current-voltage (I/V) curve in the normal Ringer solution containing tetrodotoxin (TTX) (3 μM) is shown in Fig. 1B. Tetraethylammonium (TEA) (20 mM) only weakly depressed outward rectification (Fig.1B), whereas it prolonged markedly the action potential as observed earlier by Koketsu et al. (1959). This TEA-insensitive outward rectification was eliminated by barium (1-3 mM) (n = 6); thus, it appears to be due to a potassium current analogous to the M-current (Adams et al. 1982).
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Fig. 1. The action potential and outward rectification in dissociated DRG neurons in the normal Ringer solution. A: four superimposed action potentials. Resting potential was -70 mV. B: the steady-state I/V curve measured at the end of depolarization step commands (pulse duration was 1s for the depolarizing direction and 5s for the hyperpolarizing direction). Holding potential (Vh) was -64 mV. Open circles, squares and filled circles denote the I/V curve before, during and after wash out of TEA (20 mM), respectively. TTX (3 μM) was present in B.

Isolation of Ik

All the experiments described in this and the following sections were carried out in the calcium-free standard solution (cf. Materials and Methods). It was expected that a variety of membrane currents such as a sodium current, calcium currents, a transient or the A-current, a calcium-activated potassium current and a calcium-activated chloride current have been totally or at least substantially blocked in this solution (Mayer and Westbrook, 1983; Ito and Maeno, 1986; Fox et al. 1987; Inoue et al. 1987).

The concentration of potassium ions ([K]o) in the standard solution was elevated to 20 mM in order to reduce the amplitude of a delayed rectifier potassium current (IK) to 20 nA or less (cf. Materials and Methods). Typical recordings of IK and resultant outward rectification in the standard solution are shown in Fig. 2 and Fig. 3, respectively. Clamping current between -40 and -80 mV was completely leaky (Fig. 2B). The activation of IK could be completed within 150 ms at +50 mV, as judged by the envelop of the tail current (Fig. 2C). Since [K]o was 20 mM, the polarity of the tail current of IK was always inward at the holding potential between -50 and -70 mV (Figs. 2-5).

IK was blocked by tetraethylammonium (TEA; 1-50 mM) in a concentration-dependent manner (Fig. 3A, B). The effectiveness of TEA was quantitated in terms of the a percent depression of control tail current of IK upon repolarization from +15 mV (approximately the half-activation voltage of IK, see below) to the holding potential. The minimum concentration of TEA which produced a complete block of IK was 30 mM (n=4).
Fig. 2. $I_K$ in the standard solution. A: leak current at $-40$ mV and $I_K$ at $-20$, zero and $+20$ mV are superimposed. B: leak currents at $-60$ and $-80$ mV are superimposed. C: $I_K$ activation at $+50$ mV. Pulse duration was 30, 50, 100, 200, 300 and 500 ms. Circles denote the envelope of the tail current.

Fig. 3. TEA sensitivity of $I_K$. A: effects of TEA (20 mM) on the steady-state I/V curve measured at the end of 300 ms step commands. B: sample recordings plotted in A. Upper traces show control values for $I_K$ at $+10$, $+20$ and $+50$ mV. Lower three traces constitute the corresponding set of $I_K$ in the presence of TEA (20 mM). Arrows show an inward (de-activating tail of $I_K$) current at $-50$ mV.
Properties of $I_K$

Current-voltage relation. The steady-state $I/V$ curve for $I_K$ was studied in the standard solution. The maximum outward current at the end of 100-200 ms step depolarizations was plotted as a function of membrane potential. The threshold of $I_K$ (and resultant outward rectification) was about $-30 \text{ mV}$ ($n=8$) (Fig. 3A). The density of $I_K$ plus leak currents ranged from 70 to 180 $\mu \text{A/cm}^2$ at $+50 \text{ mV}$ ($n=7$).

Reversal potential. The reversal potential of $I_K$ ($E_K$) was determined by using a conventional twin-pulse protocol, the duration of the first (activating) pulse ranging from 50 to 300 ms. The peak amplitude of the $I_K$ tail current was plotted as a function of the level of the second pulse (Fig. 4). $E_K$ was thus estimated as $-29 \pm 3 \text{ mV}$ when the duration of the first pulse was 200-300 ms ($-34 \pm 3 \text{ mV}$ when the duration of the first pulse was 50 ms) (Fig. 4).

The linear voltage-dependence of the tail current of $I_K$ (Fig. 4A) indicated that the fully opened $I_K$ channels may lack an intrinsic voltage-dependence.

![Fig. 4. Reversal potential of $I_K$. A: reversal potential of $I_K$ was measured by using conventional paired pulse protocol in which the first (activating) pulse was +65 mV with a duration of 200 ms. The levels of the second pulses are plotted in abscissa (in mV). Ordinates denote the amplitude of the tail current of $I_K$. Arrows represent the reversal potentials. B: sample recordings plotted in A. The tail current indicated by an asterisk was obtained with the twin-pulse (see Fig. 5).](image-url)
Activation curve. The conductance of $I_K$ ($G_K$) was calculated according to the formula: $G_K = \frac{I(E)}{(E_K - V_h)}$, where $I(E)$ denotes the peak amplitude of the inward tail current of $I_K$ on repolarization to the holding potential ($V_h$) from various membrane potentials ($E$) ranging from -40 to +80 mV. The maximum conductance ($G_K$) ranged from 100 to 300 nS at +80 mV.

The steady-state activation curve was obtained by calculating $I/I_{\text{max}}$ as a function of membrane potential where $I$ and $I_{\text{max}}$ denote the amplitude of the inward tail current of $I_K$ at given potentials and its maximum value, respectively. $I_{\text{max}}$ was obtained usually at +70 or +80 mV (Fig. 6). The curve thus obtained showed half-activation at +15 mV (Fig. 6). The activation curve could be described by the equation: $I/I_{\text{max}} = \frac{1}{1 + \exp((V_o - V)/k)}$, where $V_o = +15$ mV and $k$ denotes the slope factor. In three cells, the value of $k$ ranged from 12 mV to 15 mV, with an average of $13.6 \pm 0.3$ mV.

Time course of activation and de-activation. The time course of $I_K$ activation became shorter with membrane depolarization (Fig. 2). The activation of $I_K$ was completed within 150 ms at +50 ms.

$I_K$ de-activated mono-exponentially (Fig. 7). The time constant of the exponentials became smaller with membrane hyperpolarization.

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**Fig. 5.** The tail current of $I_K$ at various potentials. These recordings also show a complete set of samples plotted in Fig. 4. Membrane potentials at which the tail current was recorded is indicated besides each trace. Clamping current with (indicated by arrows) and without the first in the paired pulses are superimposed. The presence of inward tail currents even after the termination of the second pulses to +20 mV, 0 mV and -20 mV indicates that the de-activation of $I_K$ occurred very slowly. Tail currents indicated by asterisks show that the currents were associated with that indicated by arrows (see Fig. 4B).
Fig. 6. The steady-state activation curve of $I_K$. Ordinates show the relative amplitude of the tail current ($I$) with respect to its maximum amplitude ($I_{\text{max}}$). $I_{\text{max}}$ was obtained at potentials between $+70$ and $+80$ mV (indicated by a horizontal line at the top of the activation curve). Arrows denote a half-activation voltage of $I_K$. Continuous line denotes $I/I_{\text{max}} = \{1 + (\exp(15-V)/13.6)\}^{-1}$.

Fig. 7. Voltage-dependence of the deactivating time course of $I_K$. A: ordinates represent semi-log plot of $(I_{\text{max}} - I)/I_{\text{max}}$, where $I_{\text{max}}$ and $I$ denote the amplitude of de-activating tail current at given potentials at $t=\infty$ and $t=t$, respectively. Membrane potentials are indicated besides each line. B: sample recordings plotted in A. Inward tail currents at $-50$, $-60$ and $-80$ mV (indicated besides each trace) are superimposed after leak current subtraction.

Discussion

In the present study we described the voltage-dependence of a conventional delayed rectifier potassium current ($I_K$) in dissociated bullfrog DRG neurons. Properties of $I_K$ appear to be comparable to those which have been observed for $I_K$ of the squid axons (Hodgkin and Huxley, 1952).

Isolation of $I_K$

An inward calcium current (Fox et al. 1987) activates a chloride current in bullfrog DRG neurons (Inoue et al. 1987). Endogenous substances, neurotoxins or drugs which selectively and completely block this particular chloride current have not been reported as yet, although 4-acetamido-4'-isothiocyanostilbene-2, 2'-disulfonic acid (SITS) seems to exert a partial ($\leq 50\%$ with 1-3 mM) blocking action on the chloride current in other preparations (Bader et al. 1987; Korn and Weight, 1987). In the present study, the calcium current and subsequent activation of the chloride current have been blocked in a calcium-free solution which containing cobalt (2 mM) so as to isolate $I_K$. This procedure may have resulted in some unknown modifications on the kinetics and/or the maximum conductance of $I_K$ described here since calcium ions could significantly affect the potassium current of Hodgkin and Huxley (Armstrong and Lopez-Barneo, 1987).
Potassium accumulation

Previous studies of the bullfrog sympathetic ganglion cells in situ have demonstrated that the accumulation of potassium ions at the perineuronal space significantly affects the properties of $I_K$ (Adams et al. 1982; Lancaster and Pennefather, 1987).

The results of the present study indicate that the accumulation of potassium ions may be less severe in dissociated cells. However, the reversal potential of the tail current of $I_K$ was about $-80$ mV when $[K]_0$ was $2$ mM (unpublished data). This level was about $15$ mV less negative than the expected equilibrium potential for potassium ions indicating that the potassium accumulation may still occur even in dissociated DRG neurons. Comparable phenomenon but with inverted polarity (depletion of potassium ions) occurs in dissociated cardiac Purkinje fibres; in this case, the activation of the background potassium current leads to a depletion of potassium ions from the extracellular space causing subsequently the depression of the f-current (DiFrancesco, 1981a, b; DiFrancesco et al. 1986).

In summary, we have demonstrated that a conventional delayed rectifier potassium current could be studied in dissociated bullfrog primary afferent neurons in conditions such that there was a minimum influence of potassium accumulation. Reconstruction of changes in the membrane potentials during the excitations of the cells in situ needs further studies in dissociated neurons of other membrane currents.

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References


