ATP-Inhibited and Ca\textsuperscript{2+}-Dependent K\textsuperscript{+} Channels in the Soma Membrane of Cultured Leech Retzius Neurons

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Abstract. The properties of one ATP-inhibited and one Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channel were investigated by the patch-clamp technique in the soma membrane of leech Retzius neurons in primary culture. Both channels rectify at negative potentials. The ATP-inhibited K\textsuperscript{+} channel with a mean conductance of 112 pS is reversibly blocked by ATP (K\textsubscript{i} = 100 \textmu M), TEA (K\textsubscript{i} = 0.8 mM) and 10 mM Ba\textsuperscript{2+} and irreversibly blocked by 10 nM glibenclamide and 10 \mu M tolbutamide. It is Ca\textsuperscript{2+} and voltage independent. Its open state probability (P\textsubscript{o}) decreases significantly when the pH at the cytoplasmic face of inside-out patches is altered from physiological to acid pH values. The Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channel with a mean conductance of 114 pS shows a bell-shaped Ca\textsuperscript{2+} dependence of P\textsubscript{o} with a maximum at pCa 7-8 at the cytoplasmic face of the membrane. The P\textsubscript{o} is voltage independent at the physiologically relevant V range. Ba\textsuperscript{2+} (10 mM) reduces the single channel amplitude by around 25\% (ATP, TEA, glibenclamide, tolbutamide, and Ba\textsuperscript{2+} were applied to the cytoplasmic face of the membrane).

We conclude that the ATP-dependent K\textsuperscript{+} channel may play a role in maintaining the membrane potential constant—independently from the energy state of the cell. The Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channel may play a role in generating the resting membrane potential of leech Retzius neurons as it shows maximum activity at the physiological intracellular Ca\textsuperscript{2+} concentration.

Key words: Leech — Potassium channel — Patch clamp — ATP — pH — Sulfonylureas

Introduction

Various types of K\textsuperscript{+} channels with distinctive biophysical properties have been identified and characterized in excitable and nonexcitable cells. These channels differ with respect to their pharmacological specificities, kinetics and ion selectivity (for review see, e.g., Latorre & Miller, 1983; Schwarz & Passow, 1983; Ashcroft, 1988; Rudy, 1988; Ashcroft & Ashcroft, 1990; Kolb, 1990). In nervous systems, K\textsuperscript{+} channels are involved in generating the resting membrane potential and they play an important role in regulating the level of neuronal excitability, information coding and integration. The requirement for a large repertoire of neuronal firing patterns may therefore underlie the need for a diversity of K\textsuperscript{+} channels (cf. Rudy, 1988).

In the membrane of leech neurons different types of voltage-dependent K\textsuperscript{+} channels have been characterized on the macroscopical level, but very little is known about the properties of K\textsuperscript{+} channels on the single channel level. In growth cones of leech Retzius neurons three K\textsuperscript{+} currents were detected (García et al., 1990): A rapidly activating and inactivating A-type K\textsuperscript{+} current, a more slowly activating and inactivating delayed K\textsuperscript{+} current, and a Ca\textsuperscript{2+}-activated K\textsuperscript{+} current. In the axonal stump of Retzius neurons Bookman and Dagan (1987) have already described a 35 pS and a 20 pS K\textsuperscript{+} channel.

The present study was undertaken to investigate K\textsuperscript{+}-selective ion channels in the soma membrane of leech Retzius neurons in more detail. Retzius neurons have the largest cell bodies within the leech central nervous system and are easily identifiable. They can be isolated from single ganglia and maintained in tissue culture for up to two weeks (Dietzel,
Drapeau & Nicholls, 1986). Some functional roles of these neurons in the integrative behavior of the leech have been suggested, e.g., the control of muscle secretions from the skin (Lent, 1973), the effects on the tension of body wall muscle (Mason & Kristan, 1982), and a probable role in the regulation of swimming (Willard, 1981).

We recorded single channel activity in the cell-attached and inside-out configuration (Hamill et al., 1981). We characterized two types of K⁺ channels: one type is sensitive to internal adenosine-5'-triphosphate (ATP) and the other type is dependent on the intracellular Ca²⁺ concentration.

Materials and Methods

Retzius neurons from the central nervous system of the medicinal leech, Hirudo medicinalis, were isolated from single ganglia as follows (for leech preparation see Schlue & Deitmer, 1980): After opening the ganglion capsule with a miniature needle, the ganglia were enzyme treated with collagenase/dispase (Boehringer-Mannheim; 0.5 mg/ml in modified Leibovitz-15 medium) for three hours. Retzius neurons, which were identified by their size and position in the ganglion were then removed by means of a fire-polished suction pipette (diameter 100-150 µm) (Dietzel et al., 1986). The Retzius neurons were kept in culture dishes (Nunc) in modified Leibovitz-15 medium at 20°C for up to two weeks. The modified Leibovitz-15 medium (abbreviated as L-15) contained: 5 mM Na⁺, 5.8 K⁺, 1.4 Ca²⁺, 1.8 Mg²⁺; GIBCO), 25 mM a-D-glucose, 1 ml gentamicin (GIBCO), 2 ml heat-inactivated fetal calf serum (GIBCO), 10 mM HEPES (N-2 hydroxyethylpiperazine-N'-2-ethanesulfonic acid, Roth) adjusted to pH 7.4 with NaOH.

Patch-clamp recordings were performed on the soma membrane of Retzius neurons according to the method of Neher and Sakmann (cf. Hamill et al., 1981). Ionic currents from single potassium channels were recorded in the cell-attached and inside-out configuration. All experiments were carried out at room temperature (20-25°C). During experiments in the inside-out configuration the bathing solution was exchanged by means of a single-barreled perfusion pipette (up to 12 solutions could be handled by a valve; the exchanging time was less than 15 sec).

The standard bathing solution (120 mM K⁺ solution) and the pipette solution contained in mM: 120 KCl, 1 CaCl₂, 10 HEPES adjusted to pH 7.4 with KOH. In ion selectivity measurements KCl was replaced by NH₄Cl and RbCl in equimolar amounts. Solutions with Ca²⁺ levels below 1 mM were prepared according to Ammann et al. (1987) [120 mM K⁺ solution pH X]. The solutions with altered pH values contained 120 mM KCl, 1 mM CaCl₂ and 10 mM buffer (see 120 mM K⁺ solution) [10 mM PIPES (piperazine-N,N'-bis[2-ethane-sulfonic acid], Sigma) for pH 6.1 and 6.5, 10 mM HEPES for pH 7.0 and 7.5 or 10 mM TAPS (N-tris[hydroxyethyl]methyl]ammonium chloride, Merck) for pH 8.0, 8.5 and 9.0; 120 mM K⁺ solution pH X]. TEA (tetrathylammonium chloride, Merck-Schuchardt), Cs⁺, Ba⁴⁺, ATP (adenosine 5'-triphosphate dipotassium salt, vanadium-free, Sigma), and ATP-γ-S (adenosine 5'-O-[3-thiotriphosphate], Sigma) were added to the 120 mM K⁺ solution shortly before use. These ATP-containing solutions were nominally Mg²⁺-free. Stock solutions of tolbutamide and glibenclamide were prepared in 0.1 M KOH (20 mM) and added to the 120 mM K⁺ solution to provide the desired final concentrations.

Patch pipettes were pulled from borosilicate glass capillaries (outer diameter 1.5 mm, Clark GC150F-10) using a two-stage horizontal microelectrode puller (Mecanex SA BB-CH) (pipette resistance 30-50 MΩ). A standard patch-clamp setup as described by Hamill et al. (1981) was used. The signal was amplified by an L/M-EPCL7 list amplifier and filtered at 2 kHz by an 8-pole low-pass filter (Rockland). Data were digitized at a 4 kHz rate by a Labmaster TL-1 interface, recorded and analyzed on a computer using the pCLAMP 5.5 program (Axon Instruments). Amplitude histograms and idealized traces for kinetic analysis were constructed from recordings stored on the hard disk of an IBM-compatible computer. The open state probabilities (Pₒ) were determined as follows: Integral amplitude histograms were constructed from the recordings. The peaks belonging to the open and closed states were defined and the area beneath each peak was integrated. The ratio of the area of each open state and of the total area was defined as Pₒ. Pₒ was calculated on an IBM-compatible computer using a program written by the authors. Channel-state lifetimes were evaluated by fitting sums of exponentials in the lifetime distribution as obtained from the idealized traces. The single channel conductances were calculated from the linear portion of the corresponding I/V plots with 120 mM K⁺ solution on both sides of the membrane patch. The potentials are denoted according to the physiological definition. In all figures cation fluxes from pipette to bath are shown as upward deflections and negative in sign. The closed states are marked by bars.

Results

Patch-clamp experiments were carried out on the soma membrane of leech Retzius neurons in primary culture. In the following we characterize the ATP, Ca²⁺, pH and voltage dependence, the ion selectivity and pharmacology of one ATP-inhibited and one Ca²⁺-dependent K⁺ channel in the cell-attached and inside-out configuration. The mean channel conductance is 112 pS of the ATP-inhibited and 114 pS of the Ca²⁺-dependent K⁺ channel in symmetrical 120 mM K⁺ solution. These K⁺ channels did not "run down" when changing from the cell-attached to the cell-free configuration. Furthermore, they showed no inactivation in 30 min at our experimental conditions.

ATP-Inhibited K⁺ Channel

The properties of an ATP-inhibited K⁺ channel are shown in Fig. 1: (A) shows recordings, (B) the amplitude histogram, (C) and (E) the lifetime distributions of the closed states and (D) and (F) the lifetime distributions of the open states (inside-out configuration, −40 mV holding potential, symmetrical 120 mM K⁺ solution). We separated fast (C, D) and slow (E, F) kinetics of the bursting behavior. The mean state lifetimes are tₒ,f = 1.3 msec, tₒ,s = 0.2 msec, t₁,f = 28.8 msec, t₁,s = 3.8 msec and t₂,s = 49.3 msec (s = slow bursting kinetics; f = fast kinetics...