Reversible Activation of the ATP-Dependent Potassium Current with Dialysis of Frog Atrial Cells by Micromolar Concentrations of GDP

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Summary. We studied the effects of internal and external solutions on potassium currents in frog atrial cells. Experiments were carried out in whole cell recording in the presence of tetrodotoxin and cobalt in the bath to suppress the inward currents. In the absence of pyruvate and glucose in the external solution, a time-independent current increased progressively in a few minutes till the death of the cell. This current had the properties of the ATP-sensitive potassium current IK(ATP) in mammalian cells. In the presence of pyruvate and glucose in the external solution, the membrane current stayed low for 30 min. Addition of guanosine monophosphate (GMP, 40 μM), guanosine triphosphate (GTP, 40 to 1000 μM), adenosine diphosphate (ADP, 40 μM) or adenosine triphosphate (ATP, 3000 μM) to the internal solution had no major effect on the current amplitude. In contrast, addition of GDP (20 or 40 μM) produced a loss of rectification in a few minutes. The current activated by GDP was time independent as was the current observed in the absence of glucose and pyruvate. It was sensitive to cesium and barium, it was blocked when ATP was added to GDP in the internal solution, and it was suppressed by guanosine diphosphate. This hypothesis is supported by the fact that the current activated by GDP was rapidly suppressed when adding GTP in excess to the internal solution.

Key Words frog atrial cells · ATP · GTP · GDP · IK(ATP)

Introduction

Potassium currents play an important role in the regulation of the cardiac electrical activity.

A potassium current is known to activate in the absence of glucose (Vereecke et al., 1981) in the external (extracellular) solution, or under depletion of adenosine triphosphate (ATP) on the internal (intracellular) solution (Noma, 1983). This current, called IK(ATP), is also regulated by ADP (Dunne & Petersen, 1986a; Findlay, 1988), GTP and GDP (Dunne & Petersen, 1986b; Findlay, 1987), and other pyridine nucleotides (Dunne, Findlay & Petersen, 1988). It is specifically blocked by a class of hyperglycemic agents, the sulphonylureas (Fosset et al., 1988).

The background conductance, IK1, has also been reported to be regulated by internal ATP (Trube & Hescheler, 1984; Trube, 1985).

We studied in frog cardiocytes the effects of the composition of internal and external solutions on potassium currents, and we focused on the internal effects of nucleotides. We observed a pronounced increase of conductance when micromolar concentrations of guanosine diphosphate were present in the cells. The present paper describes this effect and our hypothesis about its mechanism.

Materials and Methods

Cell Preparation

Single-cells from Rana esculenta were obtained as described by Bonvallet (1987), except that collagenase from Clostridium histolyticum was purchased from Boehringer (see below). Cells were kept overnight in nominally calcium-free solution (see solutions). We used the patch-clamp technique in the whole cell recording mode.

Solutions

Concentrations are in millimolar, except where otherwise stated.

Control external solution contained: NaCl, 110; KCl, 2.5; MgCl₂, 2; CaCl₂, 1.8; CoCl₂, 3; tetrodotoxin, 1 μM; glucose, 10; pyruvic acid, 5; HEPES buffer, 10; pH adjusted to 7.2 with NaOH; CsCl (20 mM) and BaCl₂ (1.8 mM) were added to this solution when needed.

In one series of experiments, glucose and pyruvate were omitted. The other concentrations were unaffected.

Nominally calcium-free solution contained: same solution as above, but without CaCl₂, CoCl₂, and tetrodotoxin.

Control internal solution filling the patch pipettes contained: KCl, 20; K aspartate, 130; KH₂PO₄, 1; MgCl₂, 1; K-EGTA, 5; HEPES buffer, 10; pH adjusted to 7.3 with KOH.
When needed, CsCl (30 mM), MgCl₂ (9 mM) or LiCl (20 μM to 2 mM), and adenosine tri-, diphosphate (respectively ATP, ADP), guanosine tri-, di- and monophosphate (respectively GTP, GDP, GMP) were added to this solution as K₂ATP (3 mM), KHADP (40 μM), Na₂GTP (1 mM), Li₂GDP (20 or 40 μM), Na₂GMP (40 μM). The nonhydrolyzable and nonphosphorylable analogue of GDP, GDP-β-S (40 to 80 μM) was also added to this solution in one series of experiments.

The GDP concentrations used were three to sixfold higher than the actual concentration measured in heart (6 μM), cerebral cortex and muscle (10 μM), or liver (12 μM). Values were calculated from De Azeredo et al. (1979), assuming an average of 1 mg of protein per 4.7 mg wet wt.

Pronase was purchased from Sigma (St. Louis, MO). Other chemicals were purchased from Boehringer Mannheim France (Meylan, France).

**ELECTROPHYSIOLOGICAL EXPERIMENTS**

Experiments were carried out at room temperature (21–22°C). The bath solution was continuously flowing in the experimental chamber. The pipette solution was continuously perfused by the use of a capillary tube close to the tip of the patch electrode. A constant depression of 15 cm of water was applied to the inside of the pipette and ensured a constant flow rate of about 0.2 μl/min through the capillary and the pipette assembly. Patch pipettes were made from borosilicate glass (Clark Electromedical, Reading, UK). Their impedance was between 2–5 MΩ when filled with the internal solution, and the seals between the pipettes and the cells ranged from 1 to 10 GΩ.

A RK300 amplifier (Biologic, Echrolles, France) was used for whole cell voltage-clamp experiments. Voltage command and simultaneous signal recording were performed by the pCLAMP software (Axon Instruments, Burlingame, CA) on a PC microcomputer. Programs for measurements, computations and plots from pCLAMP files were developed in our laboratory.

After the rupture of the membrane patch, a standard double voltage-steps protocol was used at 0.1 Hz; from a holding potential of −70 mV, symmetrical pulses of ±40 mV were applied, setting the membrane potential to −30 mV for 100 msec, then to −110 mV for 100 msec. Dots on both ends of the current traces in the figures indicate the zero current level.

The currents were measured at the end of each step: −70 mV (triangles in the figures), −30 mV (lozenges) and −110 mV (squares), and plotted as function of time. Time zero referred to the rupture of the membrane patch. The control recording was taken a few tens of seconds after breaking the membrane patch. Some points were often removed from the plots in order to show the different symbols.

In some experiments, the conductance changed continuously, and the current voltage relation could not be accurately measured with a series of voltage steps. The currents appeared, however, time independent. All current voltage relations were then obtained with a ramp protocol (1 V/sec) applied at 0.1 Hz (see Fig. 6).