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Expression of K channels in *Xenopus laevis* oocytes injected with poly(A^+) mRNA from the insulin-secreting β-cell line, HIT T15

Frances M. Ashcroft, S.J.H. Ashcroft[+], P.O. Berggren[°], C. Betzholz[†], P. Rorsman[△], G. Trube[*] and M. Welsh[×]

University Laboratory of Physiology, Oxford, [+]Nuffield Department of Clinical Biochemistry, John Radcliffe Hospital, Headington, Oxford, England, [°]Department of Endocrinology, Karolinska Institute, Stockholm, [†]Department of Pathology, Uppsala University, [△]Department of Medical Physics, Gothenburg, Sweden, [*]Max-Planck-Institut für biophysikalische Chemie, Göttingen, FRG and *[×]Department of Medical Cell Biology, Uppsala University, Sweden*

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Two types of exogenous K channel were identified in *Xenopus laevis* oocytes injected with poly(A^+) mRNA from the insulin-secreting cell line HIT T15. One of these was the ATP-regulated K channel (G channel) as evidenced by its conductance and inhibition by tolbutamide. The other resembled the Ca-activated K channel from β-cells.

ATP-sensitive K channel; K channel; (Pancreatic β-cell)

1. INTRODUCTION

The electrical activity of the pancreatic β-cell is controlled by several types of K channel [1]. The ATP-regulated K channel (G channel) constitutes the major K permeability of the unstimulated β-cell: its closure in response to glucose metabolism depolarises the β-cell and triggers a series of events that culminate in insulin secretion [2]. The G channel is directly inhibited by intracellular ATP and changes in $[ATP]_i$ are believed to couple metabolic events to channel activity. Sulphonylureas, such as tolbutamide, specifically block this channel with high affinity [2–4]. Ca-activated K channels are thought to be important in the regulation of the slow wave activity generated by glucose concentrations above 7 mM [1,5–8]. Both of these channels are also found in the insulin-secreting cell line HIT T15 [9].

Correspondence address: F.M. Ashcroft, University Laboratory of Physiology, Parks Rd, Oxford OX1 3PT, England

* Present address: F. Hoffmann-La Roche Ltd, Basel, Switzerland

In this paper we show that poly(A^+) mRNA from an insulin-secreting cell line (HIT T15) expresses functional G channels and Ca-activated K channels when injected into *Xenopus laevis* oocytes.

2. MATERIALS AND METHODS

HIT T15 cells were cultured as described previously [10]. RNA (~3 mg) was extracted from approx. 25×10^9 cells using the guanidinium/cesium chloride method [11]. Poly(A^+) mRNA was purified by oligo(dT) cellulose chromatography [12] and stored in frozen aliquots at a concentration of 1 μg/μl in ethanol/sodium acetate at −70°C. Methods of culture and injection of *X. laevis* oocytes were as described previously [13]. Oocytes were pressure injected with 50 nl (0.1–0.2 μg) of poly(A^+) mRNA in water and kept at 19°C in Barth's medium [13] supplemented with 100 U/ml of penicillin, 100 U/ml of streptomycin and 4 μg/ml of cefuroxin.

Oocytes injected 4–7 days previously were defolliculated and skinned of their vitelline membrane [13]. Standard patch-clamp methods were used to record single channel currents from cell-attached and inside-out membrane patches. Patch pipettes were pulled from aluminosilicate glass and had resistances of 0.4–1.2 MΩ when filled with extracellular solution. The reference potential for all recordings was the zero current potential before the establishment of the seal. Inside-out patches were formed by withdrawing the pipette from the

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oocyte surface: in almost all cases this resulted in a vesicle which was then opened by touching the vesicle against a Sylgard ball. We verified that each patch was open by switching to a solution containing Ca^{2+}, which causes activation of endogenous Ca-dependent chloride channels [14].

Single-channel current amplitudes were measured directly from a digital oscilloscope. Open and closed time distributions were calculated as described previously [15], using a 50% threshold crossing routine written by Drs M.B. Jackson and K. Schroter to automatically detect events and the maximum likelihood method to fit the exponential functions. The constant field equation was used to fit the current-voltage relations, and to estimate P_K, the K permeability coefficient. P_K was calculated for each individual data point and the mean value for all data points was used to calculate the current-voltage relations shown in the figures.

After skinning, oocytes were immersed in an extracellular solution containing 115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 10 mM Hepes (pH 7.2 with NaOH). This was switched to an intracellular solution containing 105 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$ (free Ca^{2+} = 0.06 μM), 10 mM EGTA, 10 mM Hepes (pH 7.2 with KOH) and 1 mM ATP, after obtaining a seal. Inside-out patches were excised into this solution. The high Ca^{2+} solution contained 100 mM KCl, 10 $MgCl_2$, 10 mM EDTA, 2 mM $CaCl_2$ (free Ca^{2+} = 20 μM), 10 mM Hepes (pH 7.2 with KOH). Tolbutamide was dissolved in DMSO; final concentration of DMSO < 1%. The pipette was filled with a 5 mM K extracellular solution (K^+ replaced Na^+) and in later experiments 100 μM $GdCl_3$ was included to block endogenous stretch-activated channels [16]; Gd^{3+} does not affect G channels at this concentration (unpublished). Experiments were carried out at room temperature (20°C).

3. RESULTS

3.1. *G channels*

We encountered several difficulties when attempting to record exogenous G-channel currents. Firstly, the density of these channels was low, so that we had to use pipettes of large diameter. Secondly, channel activity disappeared soon after the establishment of the seal, even in cell-attached patches. Thirdly, endogenous stretch-activated channels present in the oocyte membrane are easy to mistake for G channels, as they have similar kinetics and are permeable to K^+. They can be distinguished, however, by the fact that stretch-activated channels are non-selective to cations and activated by negative pressure applied to the patch. We therefore used pipettes filled with 5 mM K^+, as in this case the reversal potential for a non-selective channel will be close to 0 mV whereas that of a K^+-selective channel will be around −70 mV. The oocyte was depolarised with a high K^+ solution (intracellular solution, see section 2) so that at a patch membrane potential of 0 mV, K^+ currents were outward and non-selective channel currents too small to resolve. The oocyte resting potential was not always 0 mV in high K^+ solution, probably because of pump currents.

Under these conditions, we recorded single-channel currents with a mean amplitude of 1.3 ± 0.1 pA from 8 cell-attached patches on 4 injected oocytes (fig.1). This amplitude is close to that found for the G channel under similar conditions (1–1.7 pA; [8,117–20]). The 1.3 pA currents were insensitive to negative pressure and were never seen in control (uninjected) oocytes. In addition, these currents were seen only in oocytes injected at least 5 days previously; no currents were found on day 4 and the probability of seeing channels declined considerably on day 6. Two separate series of injections gave similar results.

Although the presence of the stretch-activated channels made it difficult to examine the current-voltage relation of the channel, we were successful in two patches (fig.1B). The best linear fit to the data points gave single-channel conductances of 22 pS and 31 pS, values not very different from those reported for the G channel in β-cells, which lie between 17 pS and 25 pS [8,17–20]. The extrapolated reversal potentials were around −50 mV, consistent with the channel being primarily permeable to K^+. Indeed, because of the expected rectification of the *I-V* relation under these ionic conditions, the reversal potential is likely to be even more negative. If we assume that the internal K^+ concentration of the oocyte equilibrates with that of the external solution (~135 mM) and that the oocyte resting potential is 0 mV, K-permeability coefficients of 1.16 and 0.97 $\times$ 10^{-13} cm^3/s are predicted by constant field theory (fig.1B), close to that found for the G channel [2,17].

The kinetics of the 1.3 pA channel resemble those found for the G channel [2] in showing bursts of openings separated by relatively long silent periods (figs 1A,3). Mean open and closed time histograms for burst of openings recorded at 0 mV are given in fig.2. The open time distribution (B) was best fit with a single exponential having a time constant of 277 ms at 0 mV. Two exponentials (at least) were required to fit the closed time distribution (A), with time constants of 15 ms and 131 ms.

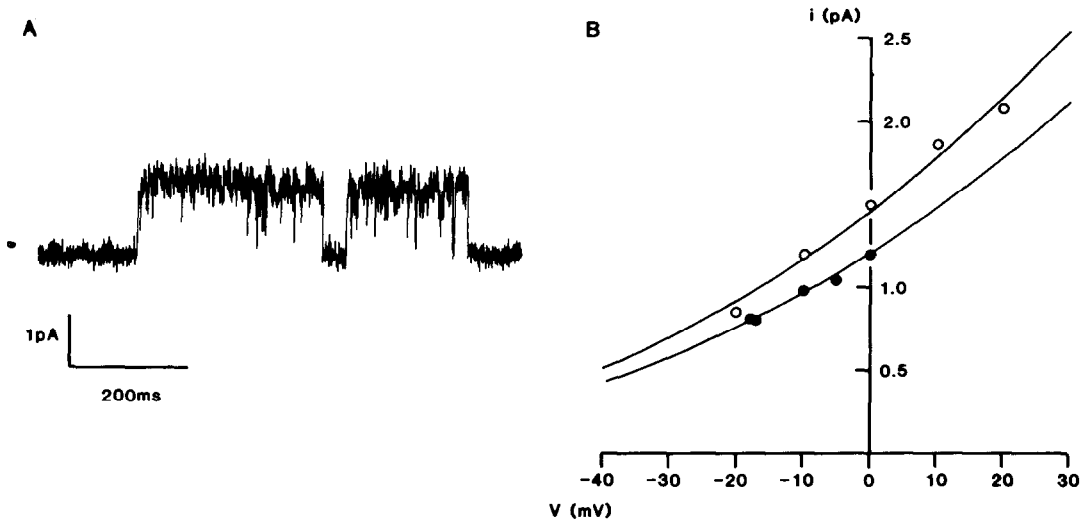

Fig.1. (A) Single-channel currents recorded from a cell-attached patch on an oocyte injected 5 days previously with HIT T15 poly(A^+) mRNA. Filter, 500 Hz; sample rate, 5 kHz; pipette potential, 5.4 mV. (B) Single-channel current-voltage relations. The lines are drawn to the constant field equation with $P_K = 1.16 \times 10^{-13}$ cm^3/s (○) and 0.97×10^{-13} cm^3/s (●) as described in the text.

G channels are selectively blocked by sulphonylureas such as tolbutamide [3,4]. Fig.3 shows that 100 μM tolbutamide rapidly and reversibly inhibits channel activity in the cell-attached patch. This result strongly suggests that the 1.3 pA currents flow through ATP-regulated K channels.

In β-cells, G-channel activity declines rapidly after excision of inside-out membrane patches but can be restored by exposure to phosphorylating solutions containing MgATP [2]. We therefore excised patches into solutions containing 1 mM ATP and looked for channel activity on withdrawal of the nucleotide. No channel activity was seen in ATP solution. Upon removal of ATP, channel ac-

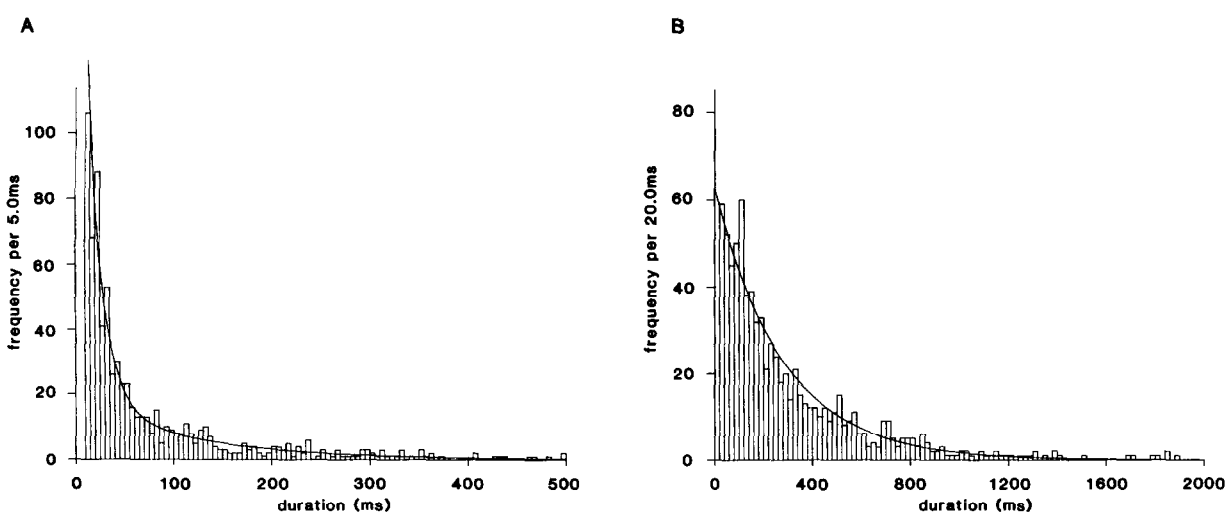

Fig.2. Frequency histograms of closed (A) and open (B) times. Events shorter than 9 ms were neglected. Filter, 60 Hz; sample rate, 500 Hz. The closed time distribution was fit with a sum of 2 exponentials with time constants of 15 ms and 113 ms and relative areas of 0.63 and 0.37, respectively. The open time distribution was fit with a single exponential having a time constant of 277 ms.

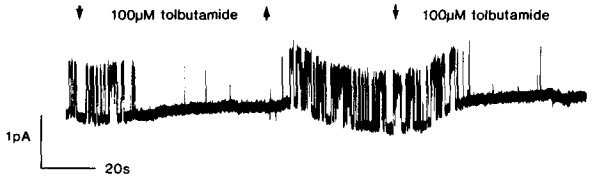

Fig.3. Effect of tolbutamide on single-channel currents recorded from a cell-attached patch. Pipette potential 0 mV. The arrows indicate the onset (↓) and withdrawal of 100 μM tolbutamide (↑). Chart recorder traces.

tivity was found in only two patches, but it decayed very rapidly and could not be restored by a subsequent addition of ATP. Therefore, we were unable to test further the effect of ATP. Probably a factor or enzyme allowing refreshment of channel activity by ATP in the β-cell membrane is missing in the oocyte.

3.2. *Ca-activated K channels*

In two oocytes we observed a large conductance Ca-activated K-channel resembling that described in β-cells [5–8]. Fig.4A illustrates that the activity of this channel, recorded in an inside-out patch, was reversibly abolished when the solution bathing the intracellular membrane face was switched from one containing 20 μM Ca^{2+} to 0.06 μM Ca^{2+}. The shift in the baseline current results from the closing of endogenous Ca-dependent chloride channels [14]: these channels are of low conductance so that individual openings are not resolved at this gain. The relationship between single-channel current amplitude and pipette potential for the large conductance channel is plotted in fig.4B; as expected for a K-selective channel exposed to asymmetric K^+ concentrations, the relation shows outward rectification. The line is drawn to the constant field equation assuming a P_K of 4.18×10^{-13} cm^3/s and a reversal potential of −78 mV. The single-channel conductance, estimated by the slope of the line between −10 and +30 mV was 144 pS. Values of P_K and of conductance measured in another patch were 4.42×10^{-13} cm^3/s and 120 pS, respectively. Similar values have been reported for the Ca-activated K channel of β-cells [5–8].

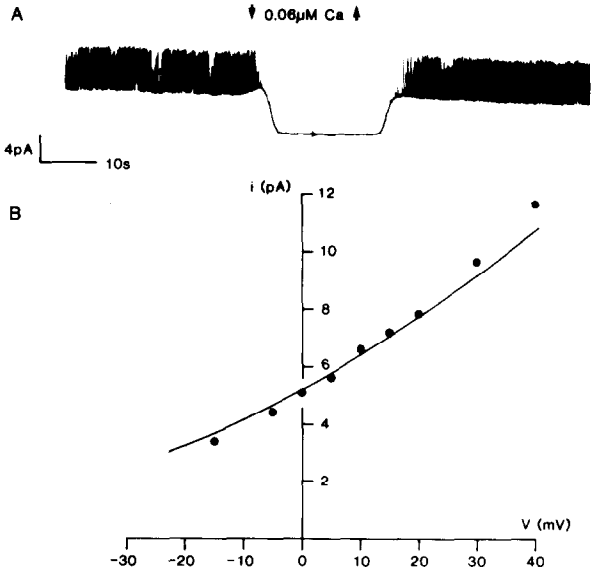

Fig.4. (A) Single-channel currents recorded at a membrane potential of 0 mV from an inside-out patch. The bath (intracellular) solution was changed from one containing 20 μM Ca^{2+} to one containing 0.06 μM Ca^{2+} for the period indicated by the arrows. The pipette solution contained 100 μM Gd^{3+}. Chart recorder traces. (B) Current-voltage relation for the channel illustrated in A. The line is drawn to the constant field equation as indicated in the text.

4. DISCUSSION

A functional expression system is an essential prerequisite for the cloning of β-cell K channels. Expression in *Xenopus* oocytes has been successfully used as a functional assay for poly(A^+) mRNA and cDNA coding for a number of ion channels [13,21]. The results reported here demonstrate the applicability of this technique to β-cell K channels.

Two types of K channel were found. The following evidence suggests that one of these is the G channel (ATP-regulated K channel): (i) the channel appears to be K selective, (ii) the mean amplitude at 0 mV and the single channel conductance are close to those reported for the β-cell G channel under the same conditions; (iii) the kinetics are similar and (iv) the channel is inhibited by the sulphonylurea tolbutamide. The Ca sensitivity and the conductance of the other channel are consistent with its identity as the Ca-activated K channel of the β-cell.

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