Single Non-inactivating K^+ Channels in the Myotubes of the Chick Embryo in Tissue Culture

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Abstract. Single-channel K^+ currents were studied in the myotubes from the chick embryo grown in tissue culture for 4—9 days by employing the patch clamp technique. The "cell attached" configuration was used and the pipette was filled with a solution containing 3 mmol.l^{-1} K^+. The channels exhibited a high conductance of ≈90 pS and the probability of finding them open increased by an e-fold factor for 13 mV depolarization for low levels of activity. The channels did not inactivate during long-lasting depolarization. These channels have been suggested to contribute to delayed rectification.

Key words: K^+ channels — Patch clamp — Myotubes — Tissue culture

Introduction

There are at least two types of potassium channels which exhibit states of high conductance and which do not inactivate during long-lasting depolarization. First, there are Ca^{2+}-dependent K^+ channels, the probability of opening of which being highly dependent on the intracellular concentration of Ca^{2+} and which are moderately sensitive to changes in membrane potential (Meech 1978; Barrett et al. 1982; Marty 1983). Another type of K^+ channels opens at membrane potentials less negative than the equilibrium potential for K^+ (Marty 1983; Benham and Bolton 1983; Benham et al. 1983). The K^+ channels with an increased probability of opening during depolarization are apparently widely distributed in excitable cells (Conti and Neher 1980) but probably not in inexcitable cells (Kettenmann et al. 1982). In this report we give an account on the kinetics of K^+ channels with high conductance in the myotubes from chick embryos in tissue culture. Evidence will be presented that this type of channel contributes to delayed rectification (Jenerick 1959).

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Materials and Methods

Myotubes form 9-day-old chick embryos were grown in Falcon dishes in a minimal essential medium supplemented with micronutrients and growth promoting α-globulin (Tolar et al. 1983). The cells were used for experiments after 4—9 days of cultivation.

The patch clamp technique (Hamill et al. 1981) was adopted to record currents from single channels (Ujec and Vyklický Jr. 1984). Borosilicate glass tubing (SIMAX) 1.65 mm (e.d.) and 0.8 mm (i.d.) was used to fabricate electrodes which were pulled in two steps to obtain tips of about 3 μm. The tips were heat polished to have orifices of about 1 μm. Single-channel currents were measured across a 1 GΩ resistor in the feedback of the current-voltage converter. Voltages to control the potential of the patch were applied between the reference electrode in the bath and the non-inverting input of the operational amplifier.

The measurements were performed in a "cell attached" configuration (Hamill et al. 1981) in a solution similar in composition to that which can be expected to be the medium under physiological conditions. Recordings lasting from several minutes to two hours were made on 12 cells. For the composition of the solution for filling the microelectrodes and the bath, see text to Figure 1. As the "cell attached" configuration does not permit direct measurement of the resting membrane potential (RMP), the voltages indicate changes of RMP. In the "cell attached" configuration positive voltages applied to the microelectrode produce hyperpolarization of the membrane of the patch and in agreement with the convention they are denoted minus while negative voltages to the pipette which produce depolarization are indicated as plus.

The output signals of the patch clamp amplifier were stored with a FM tape recorder (Bell Howell VR 3200) at a speed of 19 $cm.s^{-1}$ without filtering. The data were digitized at 150 μs intervals and histograms of the distribution of opening and closing times were obtained from digitized records on a PDP 11/03 (DEC) computer. No corrections were made for capacitance and leakage currents. All measurements were made at room temperature (21—23 °C).

Data analysis: We have developed a heuristic pattern recognition program which identifies single channel currents and interactively provides for a variety of amplitude and duration measurements. All data were replayed from an FM tape recorder digitized with sampling rate 0.15 ms (i.e. 6.67 kHz) and stored in blocks containing 512 points. The files were processed off-line by flexible semiautomatic setting of base-line and threshold levels. The files were analysed through closed and open times and through the total amplitude distribution. Usually about 150 files were processed. The histograms were stored for later statistical analysis.

The data were analysed to give the number of events corresponding either to open or closed states within a given interval. This is closely proportional to the integral of the probability density function over the time interval taken and can be used as an experimental estimate of probability density. The data which exhibited two exponentials could be fitted to the function

$$N_{c1} \exp(-t/\tau_{c1}) + N_{c2} \exp(-t/\tau_{c2}) \quad (1)$$

where τ_{c1}, τ_{c2} are the time constants for the fast and slow components, and N_{c1}, N_{c2} are the amplitudes of the intercepts of the individual curves with zero time axis (N_{c1} and N_{c2} are interpreted as the extrapolated numbers of closings with zero duration, see Fig. 3).

The relative areas under the two exponential functions of this empirical density function are obtained by integrating (1):

$$\int_0^\infty (N_{c1} \exp(-t/\tau_{c1}) + N_{c2} \exp(-t/\tau_{c2})\, dt = N_{c1}\tau_{c1} + N_{c2}\tau_{c2} \quad (2)$$

This quantity represents an approximate number of all closings at a given time interval. If the

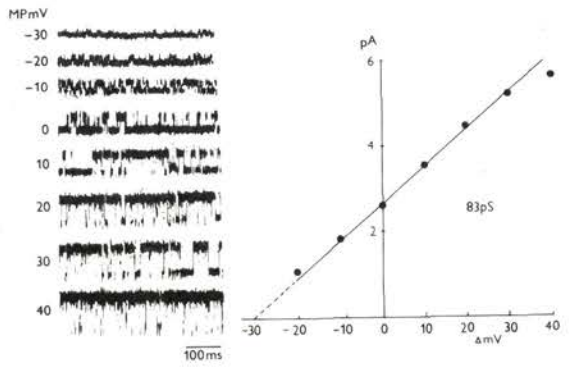

Fig. 1. The effects of membrane potential changes on the activity of a single potassium channel in the myotube of the chick embryo in tissue culture. "Cell attached" configuration. Left: Oscilloscope traces of outward current steps. The pipette contained (in mmol.l^{-1}): 120 NaCl, 3 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, pH adjusted to 7.3. The same solution to which 20 mmol.l^{-1} glucose was added was used in the bath during the experiment. MP — membrane polarization by extracellular pipette. Room temperature (22—23 °C). No filtering was applied. Right: Current-voltage relation. The amplitude of current steps (ordinate) was plotted against changes of the patch membrane potential (abscissa). Minus and plus values mean hyperpolarization and depolarization, respectively.

distribution has no slow component of closing, the second addend in (2) is zero.

The ratio

$$r = \frac{N_{c1} \cdot \tau_{c1}}{N_{c1} \cdot \tau_{c1} + N_{c2} \cdot \tau_{c2}} \tag{3}$$

was used in the formula for computing the average probability of finding the channel in open state:

$$\frac{\tau_o}{\tau_o + r \cdot \tau_{c1} + (1-r) \cdot \tau_{c2}} \tag{4}$$

This term is one of the simplest parameters that can be determined and gives a way of checking the consistency of the results of the statistical data.

Results

In one patch one or two distinct levels of the current steps were usually observed. A typical example of outward current steps produced by single channel openings in the "cell attached" configuration is shown in Fig. 1. At the resting membrane level (no potential applied to the pipette) the rectangular outward going current steps had an average amplitude of 2.6 pA. The longest opening did not exceed 10 ms and the shortest one was less than 1 ms. Depolarization of the patch by applying minus voltage to the pipette increased both the amplitude of the current pulses and the probability of the channel's opening.

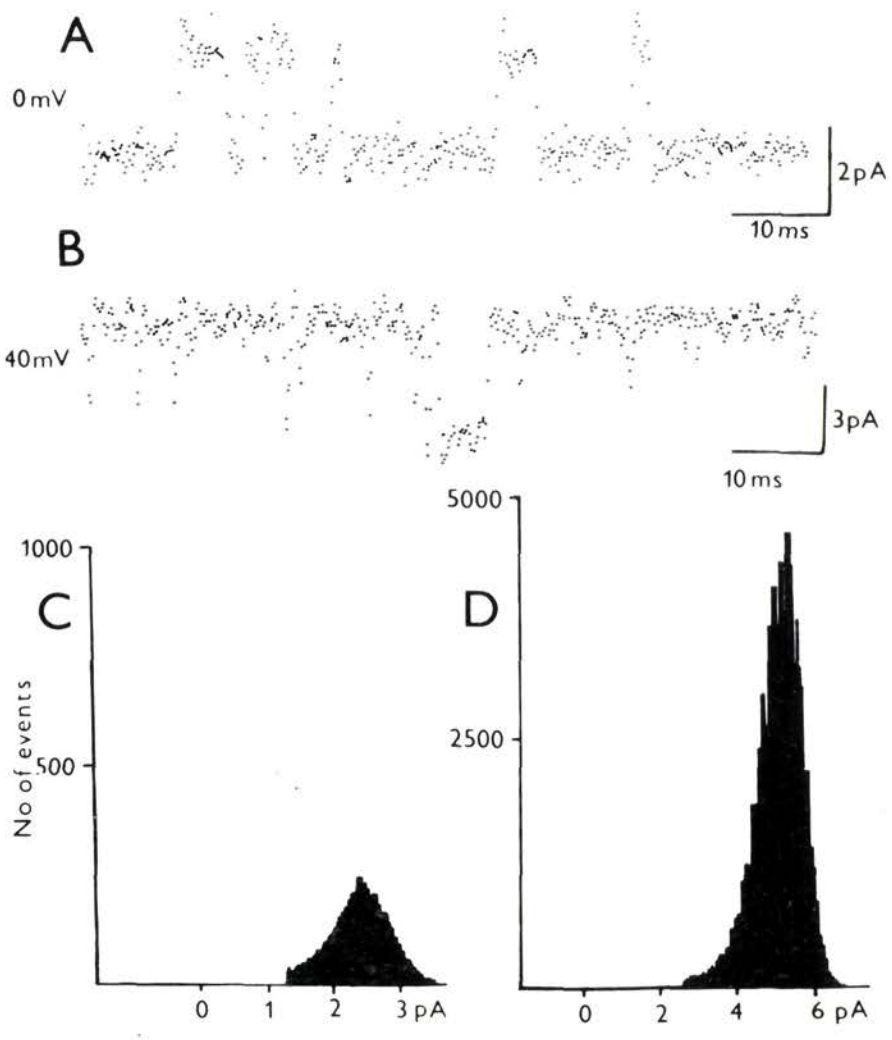

Fig. 2. The distribution of single channel current amplitudes. More than one hundred 512-points records such as shown in *A* and *B* were digitized with a sampling rate of 0.15 ms (i.e. 6.67 kHz). Amplitude histograms *C* and *D* obtained from data points of type *A* (0 mV) and *B* (40 mV) gave distributions with peaks of 2.4 pA and 5.6 pA respectively.

At depolarization by 40 mV the channels were open for most of the time. The long-lasting openings were interrupted by frequent closings, which were often shorter than 1 ms. The shortest duration could not be measured because of the limitation of the bandwith of the recording system. At patch potentials more negative than the RMP the outward current steps decreased in amplitude and

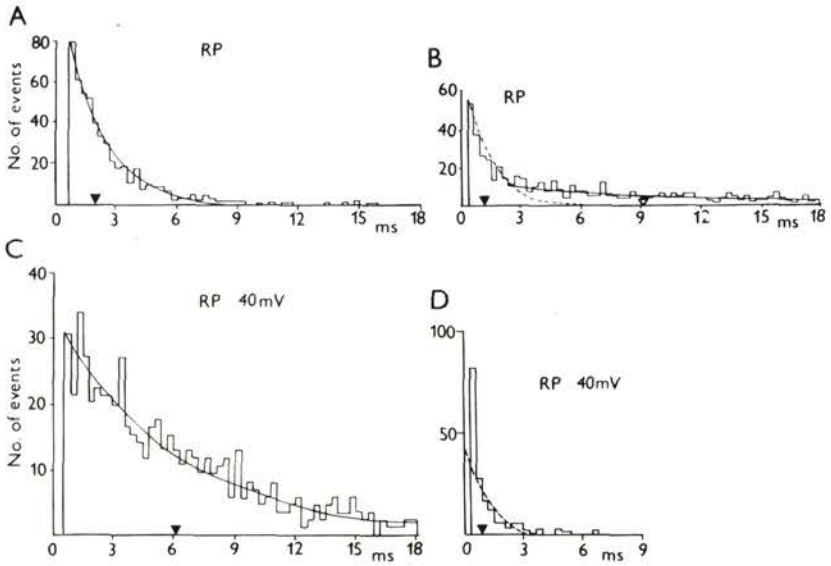

Fig. 3. Kinetics of the K^+ channel at various membrane potentials (the channel from the patch in Fig. 1). *A* and *C* represent the distribution of open times at the RMP and depolarization by 40 mV, respectively. *B* and *D* are distributions of closed times at RMP and at depolarization by 40 mV, respectively. In *A* and *C* the distributions were fitted by a single exponential with a decay constant 1.9 ms and 6.2 ms, respectively (▼). In *B* the distribution is fitted by the sum of two exponentials with decay constants of 1.2 ms and 9.5 ms (▼, ▽). In *D* only the fast decay constant 0.9 ms could be distinguished.

exhibited an apparent reversal around −30 mV which is about the value expected for the equilibrium potential for K^+. Real reversal, however, was never observed, not even when the patch was hyperpolarized by as much as 70 mV. The conductance calculated from the current-voltage plot in Fig. 1 was 83 pS. For other cells under similar experimental conditions the conductances were between 83—120 pS. It was of interest that when 2 or more channels were found in the same patch (up to 4 were found), all of them exhibited similar conductances.

For further analysis the records were digitized at 0.15 ms intervals at different holding potentials of the patch membrane as shown in Fig. 2 for RMP (*A*) and for depolarization by 40 mV (*B*). *C* and *D* represent amplitude histograms at RMP and depolarization by 40 mV, respectively. The effects of the patch polarization on the channel kinetics were examined by analysing the distribution of open and closed times. The histograms of the distribution of channel open (*A*, *C*) and closed times (*B*, *D*) for the RMP (*A*, *B*) and for depolarization by 40 mV (*C*, *D*) are shown in Fig. 3. The distribution of the open times was fitted by a single exponential, the time constants of decay were 1.9 ms at the RMP and 6.2 ms at depolarization by 40 mV.

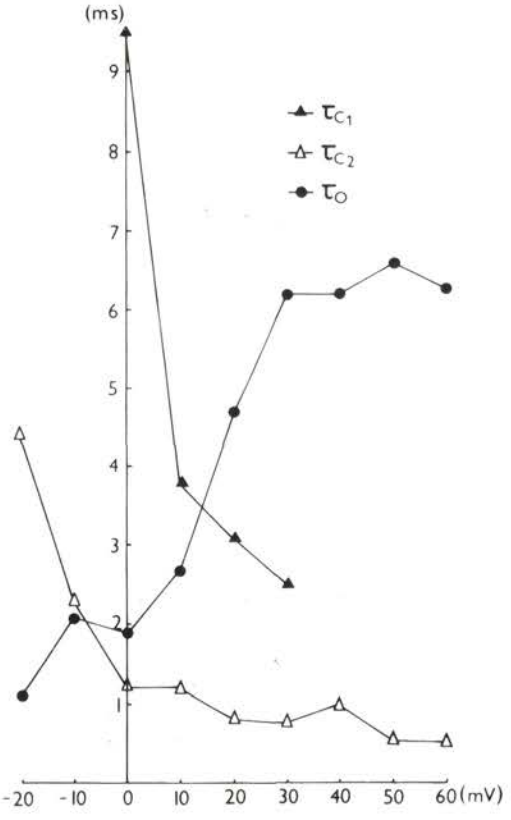

Fig. 4. Decay constants of the distribution of the closed times and open times of the K^+ channel at different holding potentials. τ_{c1} — decay constant of fast closings, τ_{c2} — decay constant of slow closings, τ_o — decay constant of openings. (The same data as in Fig. 1—3).

Some of the histograms of the distribution of closed times were difficult to fit with one exponential and at least two were needed. This can clearly be seen in the histogram of the closed time distribution at RMP which could be fitted by two exponentials with $\tau_{c1} = 1.2$ ms and $\tau_{c2} = 9.5$ ms. At depolarization by 40 mV, only one exponential was required ($\tau_c = 0.9$ ms). This, however, does not rule out the presence of closed times of longer duration, implying that long duration closing were rare if present, so that a second exponential might be required if enough events were observed. The decay constants of closing τ_{c1} and τ_{c2} and openings τ_o plotted against different membrane potential are shown in Fig. 4.

The probabilities of channel opening at various levels of membrane potential calculated from either the mean open and closed times (see Materials and Methods) or directly from the times spent in the open and closed state are shown in

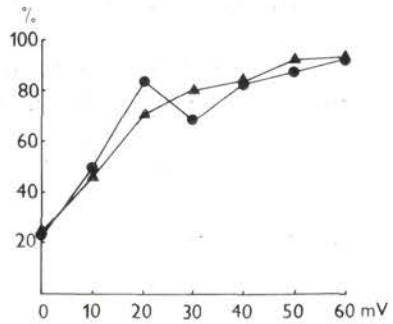

Fig. 5. The probability of finding the channel open at different levels of depolarization. The values in the plot were calculated either from the decay constants of the distribution histograms of open and closed times — triangles (see Methods) or by calculating them directly from 0.15 ms intervals during which the channel was either open or closed (filled circles) during a period of 140 s.

Fig. 5. Both calculations gave similar results. The probability of opening was low at the RMP and steeply increased with depolarization, the probability increased by a factor of *e* for 13 mV depolarization. The effects of depolarizations of more than 30 mV on the probability of opening were small, because the channel was open most of the time.

Discussion

After the introduction of the patch clamp technique to membrane physiology (Hamill et al. 1981), K^+ channels with high conductances were found in many excitable and non-excitable cells (see Marty 1983). In all preparations studied so far the conductance varied depending on the K^+ in the pipette and the highest values ($\approx$200 pS) were found with identical high K^+ solutions on both sides of the patch. The value of $\approx$90 pS found in the chick myotubes with a presumably "normal" potassium concentration gradient is not far from the values reported for myotubes of the rat (Barrett et al. 1982) or those for dispersed muscle cells of the rabbit jejunum (Benham and Bolton 1983). The probability of finding the channel open increased e-fold for 13 mV depolarization from the RMP in our experiments, and this corresponds to the value obtained in rat myotubes (Barret et al. 1982), in which it could directly be shown that this type of channels is also dependent on $[Ca^{2+}]_i$. As our experiments were performed in the "cell attached" configuration, under experimental conditions which would be expected *in vivo* (i.e. high Ca^{2+} and low K^+ in both the bath solution and the pipette) direct evidence on the Ca^{2+} dependence could not be obtained. However, in later experiments in which the "inside out" configuration was used to test the effects of various drugs on the side of the membrane facing the interior of the cell we found that only some of the K^+

channels which exhibit a high conductance are sensitive to Ca^{2+} (unpublished observations).

From our experiments it can be concluded that the membrane of myotubes of the chick embryo contains potassium channels which have a large conductance of ≈90 pS. The probability of opening increases by depolarization and the channels do not incativate during long-lasting depolarization. As the probability of opening increases with depolarization it is suggested that these channels may be responsible for delayed rectification (Jenerick 1959).

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