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Single Channel Currents in Adrenocortical Cells

J. López-Barneo*, L. Tabares and D. Mir

Departamento de Fisiología
Facultad de Medicina
Sevilla-9 (Spain)

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Single channel currents have been recorded from cell-attached patches of tumoral adrenocortical cells. Our experiments suggest the existence of three sets of potassium channels in the surface membrane of these cells. All channel types can be recorded in a given membrane patch but some patches have only one type of single channel currents. One channel type has a unitary conductance of about 103 pS. The other two channels have smaller conductances and opposite voltage dependence. In one case channels open on depolarization and have a single channel conductance of 31.6 pS. In the other case the probability of being in the open state increases on hyperpolarization and the single channel conductance is of 21 pS. These channels seem to be similar to the delayed and anomalous rectifying potassium channels seen in other preparations. The role of membrane ionic permeability in steroid release induced by ACTH is discussed.

Key words: Potassium channels, Adrenocortical cells, Tumoral cells, Ionic permeability, ACTH.

The patch clamp is a recently developed technique which allows precise and quantitative electrophysiological studies in small cells. With this technique it is possible to resolve the current flowing through a single ionic channel, giving information at a molecular level about the control of membrane ionic permeability (3, 16).

In many secretory cells membrane electrophysiological events seem to play an important role in stimulus-secretion coupling (7, 13). Although there is some information about the electrophysiology of cells secreting by exocytosis (13), it is not known if ionic membrane permeability plays a part in the mechanisms underlying the regulation of steroid secretion. The increase in the biosynthesis

* To whom all correspondence should be sent: Departamento de Fisiología. Facultad de Medicina. Sevilla-9 (Spain).

and release of steroid hormones evoked by ACTH in adrenocortical cells requires an appropriate concentration of extracellular cations (4, 6, 10). Therefore, it is suggestive to consider the possibility that, in addition to other phenomena, ACTH may modify membrane permeability to ions.

We have set up the patch clamp technique and recorded single channel currents in tumoral adrenocortical cells which it is known increase steroid secretion in the presence of ACTH. In these cells we have characterized three different types of single channel currents which probably correspond to different classes of potassium channels.

Materials and Methods

The technical requirements and the experimental set up needed to record single channel currents in cultured cells have been described in detail by HAMILL *et al.* (3). Here, we will briefly describe the major steps and particularities of our preparation. Tumoral adrenocortical cells of the line Y-1 (American Type Culture Collection) were cultured in Ham's F-10 medium with 5 % fetal bovine serum and antibiotics. Recordings were performed in cells 2 hours to 3 days after plating in small culture dishes. Several minutes before the beginning of the recording sessions the culture medium was replaced by a solution containing NaCl 140 mM, KCl 2.8 mM, $MgSO_4$ 2 mM, $CaCl_2$ 1-4 mM, HEPES 10 mM, pH 7.3.

Patch microelectrodes were doubled pulled from haematocrit capillaries (Hirschmann, West Germany) with a vertical puller (David Kopf, mod. 700D, USA) and the tip fire polished under microscope control. Micropipettes were filled with the solution indicated above, fixed to the head of a three-dimensional hydraulic micromanipulator (Narishige, mod. MO 103, Japan) and electrically connected to the headstage of a patch clamp amplifier built in our laboratory. The indifferent electrode was a silver chloride wire connected to ground. Culture dishes were placed in the stage of an inverted microscope (Nikon, mod. Diaphot, Japan) with Nomarski optics. Micropipettes were moved under visual control until touching the cell surface.

All recordings were done in the cell-attached configuration (3) which is schematically shown in figure 1A. Micropipettes had an initial resistance between 2-12 Mohm. This resistance was continuously monitored by applying a 0.6 mV square voltage step at the non-inverting input of the headstage amplifier (Vc). Pipette resistance increased up to 50-100 Mohm after pressing it slightly against the cell membrane. The application of a slight negative pressure at the pipette interior resulted in a sudden increase in resistance (> 1 Gohm) which was used as an indication that a gigaseal had been established between the micropipette and the membrane. In this situation background noise decreased by an order of magnitude and the small signal due to single channel current could be resolved.

Current flowing through the electrically isolated membrane patch was recorded with a FET operational amplifier (Burr Brown 3523, USA) wired as a current to voltage converter with a 8.5 Gohm feed-back resistor (fig. 1A). Pipette voltage could be modified by applying a DC potential to the non-inverting input of the operational amplifier (Vc), this circuit was used to control the membrane potential of the isolated membrane patch. In all figures outward current is shown above and inward current below the zero current level. The preparation was electrically isolated by a metallic shield and placed on a heavy granite table sitting on inner

tubes for shock absortion. After gigaseal formation the peak to peak noise of the recording was about 0.25 pA at a bandwidth of 3 kHz. All experiments were done at room temperature (20-23° C).

Results

At a given membrane potential single channel currents appear as discrete current steps of fixed amplitude. Figure 1 (B and C) illustrates two different types of single channel currents repeatedly observed in adrenocortical cells. Both types of channel currents could be recorded from a given membrane patch but the records have been selected from patches containing only one channel type to make easier their comparison. Figure 1B is a continuous recording of outward current from a membrane patch. It is apparent the existence of two different current levels (1 and 2 in the figure) with a fixed amplitude of 3 pA as result of the opening and closing of two channels included in this patch. At this membrane potential (depolarization of 20 mV) one or two channels are open most of the time. When both channels are closed the recording is at zero current level (0 in the figure). An estimate of the single channel conductance gives a value of 103 pS (see below).

Figure 1C is a continuous recording of outward current through a different membrane patch. As in the case illustrated before there are two different current levels of the same amplitude indicating the existence of at least two channels in the membrane patch. At this membrane potential (depolarization of 10 mV) the single channel amplitude is of about 0.5 pA. Besides its amplitude, this channel also differs from the one described above because the switching between the open and closed state is more frequent.

The characteristics of this second channel type had been better studied in experiments where the patch current

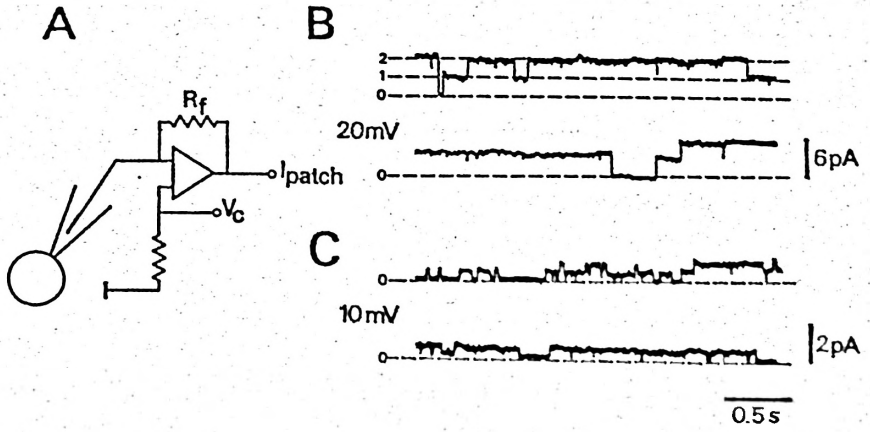

Fig. 1. *Recordings of single channel current in adrenocortical cells.*
A. Basic electronic circuit used to record single channel currents in the cell-attached configuration. R_f = feedback resistance (8.5 Gohm in our amplifier). V_c = command voltage. B. Continuous recordings of single channel currents at a depolarization of 20 mV. The elementary events appear as discrete steps (1 and 2) of about 3 pA of amplitude above the zero current level (0 in the figure). C. Continuous recordings of single channel currents at a depolarization of 10 mV in a different membrane patch. The current steps appear above the zero current level (0 in the figure) and have a smaller amplitude than those shown in B.

was recorded at several membrane voltages (fig. 2). Part A of the figure illustrates ionic currents at different membrane potentials. The current is outward at the resting potential and increases in amplitude with depolarizing voltages. These results suggest that they are potassium currents. Depolarization increases the driving force for potassium ions and therefore single channel current increases in amplitude according with the formula

$$i = \gamma (V_m - E_k) \qquad [1]$$

were i is the single channel current, γ the single channel conductante, V_m is the membrane potential and E_k the potassium equilibrium potential. When V_m is equal to E_k single channel current is zero. Current is inward at hyperpolarizing potentials more negative than E_k. γ can be calculated from the slope of the curve relating the amplitude of single channel current as a function of the voltage at the pipette interior (fig. 2B). The plot in figure 2B shows that E_k is about 9 mV more negative than V_m. For the channel type (figure 1B), γ can be calculated from [1]. In this case i = 3pA and the driving force $(V_m - E_k) = 29$ mV, giving a single channel conductance of 103 pS.

Records shown in figure 2A also illustrate the voltage dependence of channel opening. Membrane depolarization increases the channel open time and the number of channels that open simultaneously. In the record obtained at a depolarization of 30 mV there are at least five channels (indicated by the five current levels in the figure) which contribute to the total current flowing out of

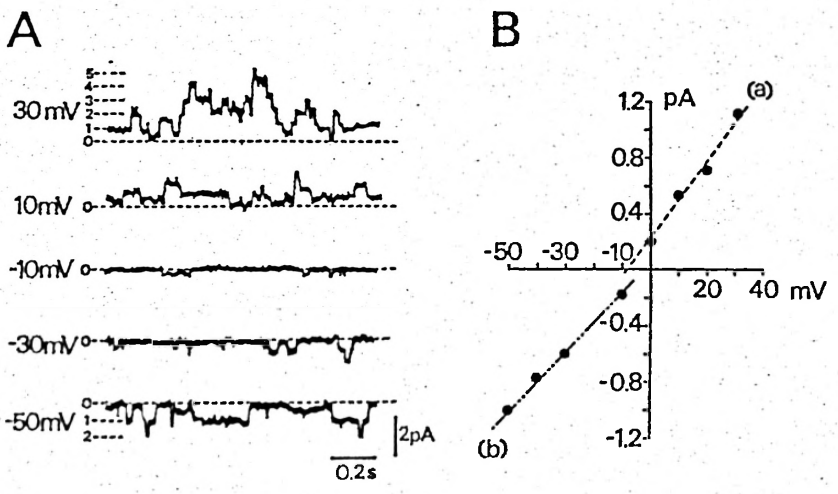

Fig. 2. *Single channel currents recorded at several patch membrane potentials.*
A. Ionic current recorded at different depolarizing and hyperpolarizing voltages. The amplitude of the displacement from the cell resting potential is indicated at the left of each record. In all cases outward current is represented above and inward current below zero current level (0 in the figure). The outward current at depolarizing voltages is probably due to the opening of voltage dependent potassium channels (delayed rectifying potassium channels). Note the increase in channel open time and the number of open channels with depolarization. The inward current at hyperpolarizing voltages is probably carried by potassium ions flowing through inward rectifying channels (anomalous rectifying potassium channels). B. Current-voltage curves corresponding to the records shown in A. Straight lines (a) and (b) have been fitted by eye. The slope is in each case the value of the single channel conductance (31.6 and 21 pS).

the membrane patch. Therefore, it can be concluded that for this channel type the probability of being in the open state increases with membrane depolarization. It is surprising to observe that with large hyperpolarizations (see the record at —50 mV in fig. 2A) channel open time and the number of open channels increase. This behavior is not expected for a channel that opens with depolarization and it is probably a consequence of the existence of two separate sets of potassium channels in the membrane patch. One type opens with depolarization (delayed rectifying channel) while for the other type the probability of being in the open state increases with hyperpolarization (anomalous rectifying channel). The data points in figure 2B has been separated in two populations, one at each side of the voltage axis, that can be best fitted by two straight lines with different slopes. The straight line (a) is the current-voltage relationship for the delayed rectifying channel, being its slope 31.6 pS. The straight line (b) represents the current-voltage relationship of the anomalous rectifying channel. The value of the single channel conductance in our experimental conditions is 21 pS. We have recently observed in some membrane patches a complete absence of channel openings at depolarizing voltages. In these patches single channel current only appeared on hyperpolarization and had current-voltage curves similar to curve (b) in figure 2B, which suggests that all channels were of the anomalous rectifying type.

In some records there are current steps of smaller amplitude than the ones considered as channel openings. This is probably due either to the existence of different conductance states in a given channel or to the opening of other channel types (to calcium and sodium ions) in the surface membrane of adrenocortical cells. Current flowing through these channels are difficult to see unless potassium channels are completely blocked.

Discussion

The present paper describes the existence of at least three different sets of potassium channels in the surface membrane of tumoral adrenocortical cells. As far as we know, this is the first time that single channel currents have been recorded in steroid secreting adrenocortical cells. This preparation seems to be suitable for electrophysiological studies of membrane ionic channels and for experimental research on the participation of membrane ionic permeability in the control of cell secretion. One of the channel types described has a large unitary conductance. The other two channels have smaller conductances and opposite voltage dependence. The latter two channels probably represent the delayed and anomalous rectifying potassium channels observed in other preparations (2, 11, 12, 14, 15).

In many secretory cells membrane electrophysiological events seem to be important in the control of cell secretion. In this respect, there is a great deal of evidence supporting the idea that calcium entry through voltage dependent channels triggers exocytosis (13). The ionic dependence of ACTH-induced steroid release in adrenocortical cells is not well known. It has been reported that an appropriate concentration of monovalent cations in the external solution and a minimum amount of extracellular calcium are needed for this process (4, 5, 10). On the other hand, in an electrophysiological study on adrenocortical cells carried out by MATTHEWS and SAFFRAN (10) with intracellular microelectrodes, they reported the appearance in some cells of action potentials after the addition of ACTH. The action potentials were TTX resistant

and had a slow rising phase, which suggested that they were calcium dependent.

Based on previous information and the existence of different types of potassium channels in the surface membrane of adrenocortical cells as shown in this paper, it is attractive to hypothesize that ionic membrane permeability participates in the control of ACTH-induced steroid secretion. After binding to specific membrane receptors ACTH could, in parallel to activating the biochemical machinery of the cell, decreases potassium permeability causing depolarization, which in turn would open voltage dependent calcium channels. A sudden increase in intracellular calcium may accelerate the rate of steroid release and biosynthesis. We do not know for the moment which potassium channels are associated to the action of ACTH. The delayed rectifying channels are most probably the responsible for the repolarizing phase of the action potential. The other two channels (the anomalous rectifying and the one with large unitary conductance) are, in principle, good candidates as targets for the action of ACTH on membrane permeability. It has been recently reported that in isolated pacemaker cells of mammalian heart the acetylcholine dependent potassium channel is very similar to the inward rectifying potassium channel, which is responsible for the resting potassium conductance of these cells (15). On the other hand there are evidences obtained in several preparations about the existence of potassium channels with large unitary conductance modulated by intracellular calcium concentration (1, 8), intracellular ATP (11) and hormones (5, 9). The complete characterization of ionic channels in the surface membrane of adrenocortical cells and the modulation of membrane permeability by ACTH are the subjects of our current research.

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Resumen

Se registra la corriente a través de canales de potasio en células adrenocorticales tumorales mediante la técnica de *patch clamp*. Los resultados sugieren la existencia de tres tipos distintos de canales de potasio en la membrana de estas células. Un tipo de canal tiene una conductancia de 103 pS. Los otros dos tipos tienen una conductancia menor y dependen en forma opuesta del potencial de membrana. En un caso, los canales se abren al despolarizar la membrana y el valor de la conductancia de un solo canal es de 31,6 pS. En el otro, los canales se abren al hiperpolarizar la membrana y la conductancia de un solo canal es de 21 pS. Estos dos últimos parecen ser similares a los canales de potasio de rectificación retardada y anómala que se han descrito en varias preparaciones. Se discute el papel de los cambios de permeabilidad de la membrana en la liberación de esteroides inducida por la ACTH.

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