Short communication

Single-Channel Potassium Currents in Human Melanoma Cells

A. ZAHRADNÍKOVÁ, I. ZAHRADNÍK and K. RÝDLOVÁ

Centre of Physiological Sciences, Slovak Academy of Sciences, Vlárska 5, 833 06 Bratislava, Czechoslovakia

Current development in electrophysiological techniques has provided means to approach experimentally also small and flat cells. Ionic channels of nonexcitable cells are now studied together with those of excitable ones, and many similarities in their nature have been found (MacVicar 1984; Shrager et al. 1985). Concerning melanocytes, the pigmented cells of the skin, there is practically no information available on ionic channels in their membrane. This topic is of interest in the context of recent findings (Tsokos et al. 1985) that melanocytic as well as neuronal and Schwannian cells may be products of differentiation of the same primitive neuroblastic cell.

The present communication brings preliminary information concerning ionic channels in a human neoplastic melanocytic cell. Melanoma cells of the line B-HM8 (Siracký et al. 1982) were used. Experiments were made on day 3—5 after plating.

Single ionic channels were measured using the patch-clamp technique of Hamill et al. (1981) with a List Medical EPC-7 device (Darmstadt, FRG). Borosilicate glass pipettes were fire-polished to have a resistance of ~ 10 mΩ. The pipette solution contained (in mmol/l): 140 KCl, 1 $MgCl_2$, 10 Hepes, 5 Tris, 1 Egta, pH = 7.4. The cells were bathed in the culture medium (Eagle' s medium supplemented with 10 % fetal calf serum). Currents were measured in the cell-attached mode with seal resistances of 5—10 GΩ. Records were made on a digital oscilloscope screen (Gould OS 1420) and photographed. Amplitudes of single-channel currents were determined by hand. Experiments were done at room temperature. All potentials are given relative to the resting potential.

At resting potential, single inward currents flow through the cell membrane when the pipette is filled with 140 mmol/l KCl. Currents are activated in bursts lasting up to several tens of milliseconds. The channel is most active in the vicinity of resting potential. Typical current traces at various potentials are shown in Fig. 1*A*. In the experiment illustrated, a maximum of 4 overlapping single-channel currents was observed at the resting potential; at potentials more negative than −30 mV there was only one channel active at a time, whereas at depolarized potentials periods of lower and higher activity occurred, with a

maximum of three overlapping channels. Despite small pipette openings, there was always more than one channel of this type present in the patch. A corresponding current-voltage curve is shown in Fig. 1*B*. The channel shows a slight inward rectification. At negative potentials the I—V curve is linear with a conductance of 37 pS. The conductance decreases to 16 pS at positive potentials.

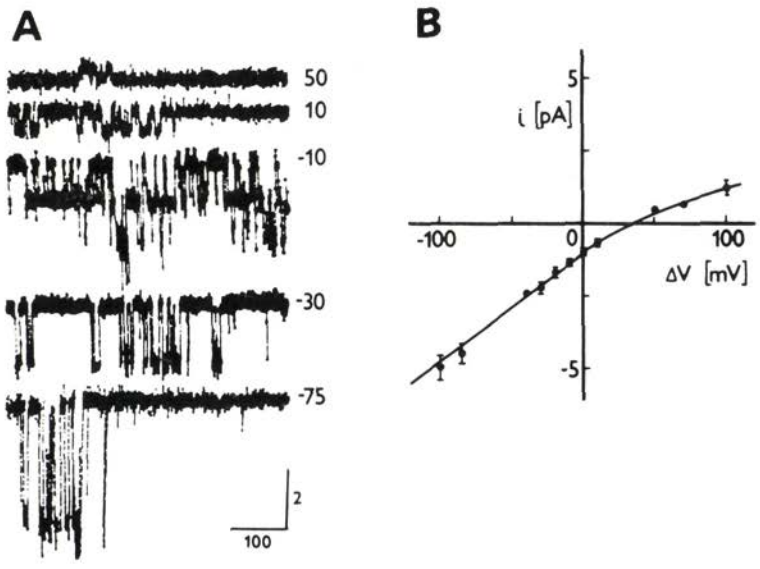

Fig. 1. Low-conductance channels. *A* — typical current traces at various membrane potentials (relative to the resting potential). The values of the membrane potential are indicated at the right side of the records. Calibration bars in pA and ms for current and time, respectively. *B* — current-voltage relationship of the low-conductance channel. The linear part gives a conductance of 37 pS.

Activity of the second channel type could be observed sparsely. At negative potentials the probability of opening was very low. It increased with depolarization, but even at +100 mV the channel spent less that 10 % of the time in the open state. This voltage dependence of opening probability is illustrated on typical current traces in Fig. 2*A*. The current-voltage curve shown in Fig. 2*B* gives a conductance of 240 pS for the linear range (+20 — +100 mV).

Both channel types have the same reversal potential of +35 mV relative to the resting potential. This indicates that the channels are cation-selective. Some resemblance to ionic channels studied in other cell types suggests that these channels may be potassium-selective. The channel with 37 pS conductance is

similar to the channel found in heart myocytes (Sakmann and Trube 1984) by its conductance, inward rectification, and high openig probability at the resting potential. The last two properties may suggest its role in the generation of membrane potential. The high-conductance channel has several properties in common with the high-conductance Ca^{2+}-activated potassium channel (for a review see Latorre et al. 1984; Petersen and Maruyama 1984): a large conductance of 240 pS, low probability of opening in the cell-attached configuration, and an increased opening probability at depolarized potentials.

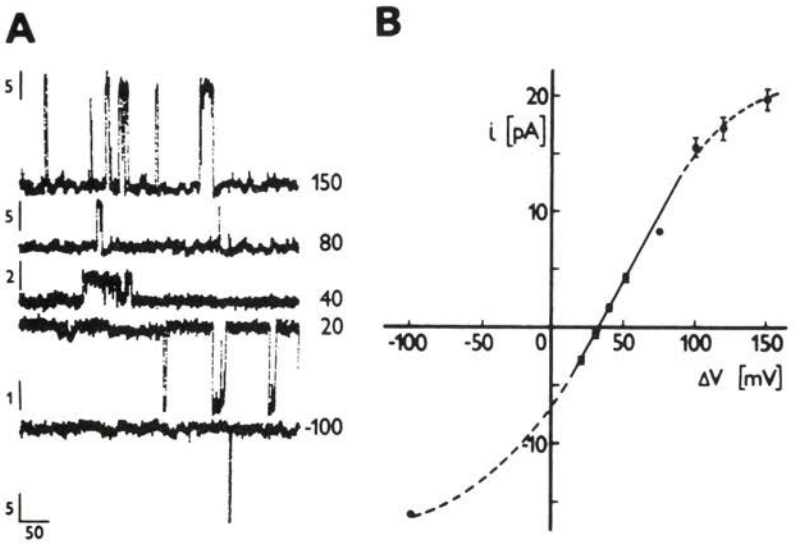

Fig. 2. High-conductance channel. *A* — examples of single-channel currents at various potentials. Numbers on the right are voltages relative to the resting potential (in mV). Calibration bars in pA and ms for current and time, respectively. Note the different current calibrations. The baseline is distorted due to the activity of the low-conductance channel. *B* — current-voltage curve of the high-conductance channel. In the linear range the channel has a conductance of 240 pS.

It may be concluded that the membrane of melanoma cells contains ionic channels with activities depending on membrane potential. First experimental results suggest similarities with two types of potassium channels present also in the membrane of excitable cells.

References

Hamill O. P., Marty A., Neher E., Sakmann B., Sigworth F. J. (1981): Improved patch clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch. **391**, 85—100

Latorre R., Coronado R., Vergara C. (1984): K^+ channels gated by voltage and ions. Annu. Rev. Physiol. **46**, 485—495

MacVicar B. A. (1984): Voltage-dependent calcium channels in glial cells. Science **226**, 1345—1347

Petersen O. H., Maruyama Y. (1984): Calcium-activated potassium channels and their role in secretion. Nature **307**, 693—696

Sakmann B., Trube G. (1984): Conductance properties of single inwardly rectifying potassium channels in ventricular cells from guinea-pig heart. J. Physiol. (London) **347**, 641—657

Shrager P., Chiu S. Y., Ritchie J. M. (1985): Voltage-dependent sodium and potassium channels in mammalian cultured Schwann cells. Proc. Nat. Acad. Sci. USA **82**, 948—952

Siracký J., Blaško M., Borovanský J., Kovařík J., Švec J., Vrba M. (1982): Human melanoma cell lines: Morphology, growth and α-mannosidase characteristics. Neoplasma **29**, 661—668

Tsokos M., Ross R. A., Triche T. J. (1985): Neuronal, Schwannian and melanocytic differentiation in human neuroblastoma cells in vitro. In: Advances in Neuroblastoma Research (Eds. A. E. Evans, G. J. Dangio, R. C. Seeger), pp. 55-69, Alan R. Liss, Inc., New York

Final version accepted October 26, 1987