TRANSMEMBRANE POTENTIAL OF HEPATOCYTES ISOLATED FROM LIVERS OF LITTLE SKATES (RAJA ERINACEA)

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Hepatocyte transmembrane potential (V_m) measured in isolated rat hepatocytes in primary culture varies with added hormones, with cell metabolism, and with rates of cell amino acid transport (Wondergem and Harder, J. Cell. Physiol. 104:53-60, 1980; Wondergem, Am. J. Physiol 244 (Cell Physiol. 13):C17-C23, 1983). As a step toward determining if this is characteristic of hepatocytes from all vertebrates, we have measured V_m and cell input resistance (R_i) of skate hepatocytes, in vitro.

Cells were obtained by perfusing isolated skate liver with elasmobranch Ringer's solution maintained at 15°C and containing 0.1% collagenase (Smith, et al., Bull. MDIBL 24:82-83, 1984). Hepatocytes were selected from cell suspensions by differential sedimentation, and they were plated in elasmobranch Ringer's solution into tissue culture plates containing glass coverslips. Cells adhered to the cover slips for 4 hr. to 6 hr. prior to micropuncture and electrical measurements.

A trace characteristic of recordings of V_m and R_i measured with glass microelectrodes filled with 0.5 M KCl (80 to 240 MΩ in Ringer's solution) is shown in Fig. 1. Similar measurements in cells from the same animal (n = 8 impalements) resulted in V_m = -30 ± 1.7 mV and $R_i = 701 \pm 31$ MΩ. The frequency distribution of all V_m measured at 4-6 hr. following isolation ranged from -20 to -90 mV (n = 45). The mean $V_m = -39 \pm 2.3$ mV; however, the sample population did not distribute normally. Instead, it was skewed toward V_m larger than the mode of -30 to -40 mV. V_m larger than -50 mV often resulted from increases in potential after impalement. We do not know if these reflected membrane sealing the micropipette, or whether they were artifactual, resulting from changes intracellularly of microeletrode tip-potential. Nonetheless, sample population mode (-30 to -40 mV) agreed well with rat hepatocyte V_m measured in cultured cells 4-8 hr. after isolation; however, the R_i of skate cells was approximately tenfold greater than that of rat cells (Graf, Gautam, and Boyer, Proc. Natl. Acad. Sci. USA 81:6516-6520, 1984).

V_m of skate hepatocytes in culture decreased when external K^+ concentration was increased by subsituting external Na^+ with K^+. The slope of the linear portion of V_m vs. $\log_{10} [K^+]_o$ was 25 mV/tenfold change in $[K^+]_o$. This was less than 60 mV/tenfold change in $[K^+]_o$ expected for a K^+-selective membrane. Thus, membrane permeability to ions in addition K^+ must contribute to the steady-state hepatocyte V_m. Finally, ouabain (1 mM) immediately decreased hepatocyte V_m 9 ± 0.7 mV, which indicated that an electrogenic Na-K pump also contributed to V_m.

We conclude that isolated skate hepatocytes are suitable for micropuncture and electrophysiological study. Since skate hepatocyte R_i is approximately tenfold greater than that of rat cells, we infer that plasma membranes of isolated skate cells are less permeable to ions compared with isolated rat cells. Elasmobranch cells maintain larger transmembrane ion gradients than do mammalian cells. Therefore, less permeable plasma membranes in the former cells may function to maintain these steady-state ion gradients.

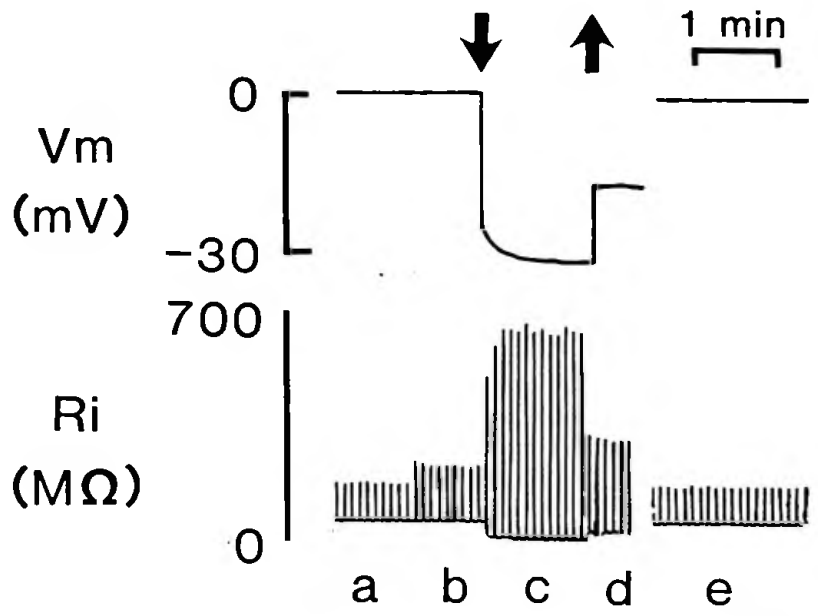

Fig. 1. Traces representative of transmembrane potential (V_m) measurements and input resistance (R_i) measurements in isolated skate hepatocytes. Measurements were made 4 hr. after cell isolation. Arrows indicate voltage changes when microelectrodes were advanced into or withdrawn from the cells. a indicates microelectrode resistance in the bath, b indicates resistance when the microelectrode was against the cell surface, c indicates resistance when the microelectrode was in the cell, d indicates resistance after the microelectrode had been withdrawn but the cell remained clinging to it, e indicates microelectrode resistance in the bath after the cell had been removed from the microelectrode. Time lag between d and e ocurred when the cell was removed from the microelectrode. Trace of V_m was redrawn to show only DC voltages without large deflections resulting from the intermittent current passed through the microelectrode.

R. Wondergem was a recipient of a grant from MDIBL and the Lucille P. Markey Charitable Trust.