TRANSMEMBRANE POTENTIAL OF *GLYCERA DIBRANCHIATA* RED BLOOD CELLS

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Body wall cells in the bloodworm, *Glycera dibranchiata*, maintain intracellular amino acid concentrations of approximately 200 mM, concentrating them one-million fold the extracellular concentration. This uptake mechanism is predominantly by secondary active transport, where amino acid influx is coupled to the transmembrane electrochemical Na^+ gradient. The electrical component of this gradient, the transmembrane potential (V_m) of *Glycera* body wall cells or red blood cells, is unknown. Preston and Stevens (Amer. Zool.,22:709, 1982) predict it to be at least -75 mV by computations based on steady-state transmenbrane Na^+ and alanine concentrations plus a coupling ratio of 3:1 (alanine:Na) for the influx rates. Now we report the V_m of *Glycera* red blood cells measured with glass microelectrodes.

Glycera red blood cells in artificial sea water (ASW) were placed onto plastic coverslips and then into an acrylic chamber on the stage of an inverted microscope. The sea water had the following composition (in mM): 440 NaCl, 9 KCl, 9.3 $CaCl_2$, 23 $MgCl_2$, 26 $MgSO_4$, and 2.2 $KHCO_3$. The final pH was 7.8. In some instances, to minimize movement of the cells during micropuncture, the coverslips were covered with a thin layer of agar (4% in ASW) and the cells were placed onto the agar as it cooled. This treatment did not affect the value of V_m. Microelectrodes were pulled from borsosilicate glass capillaries (Kwik-Fil), and they had tip resistances of approximately 100 M ohms when filled with 0.5 M KCl. Cell input resistance was

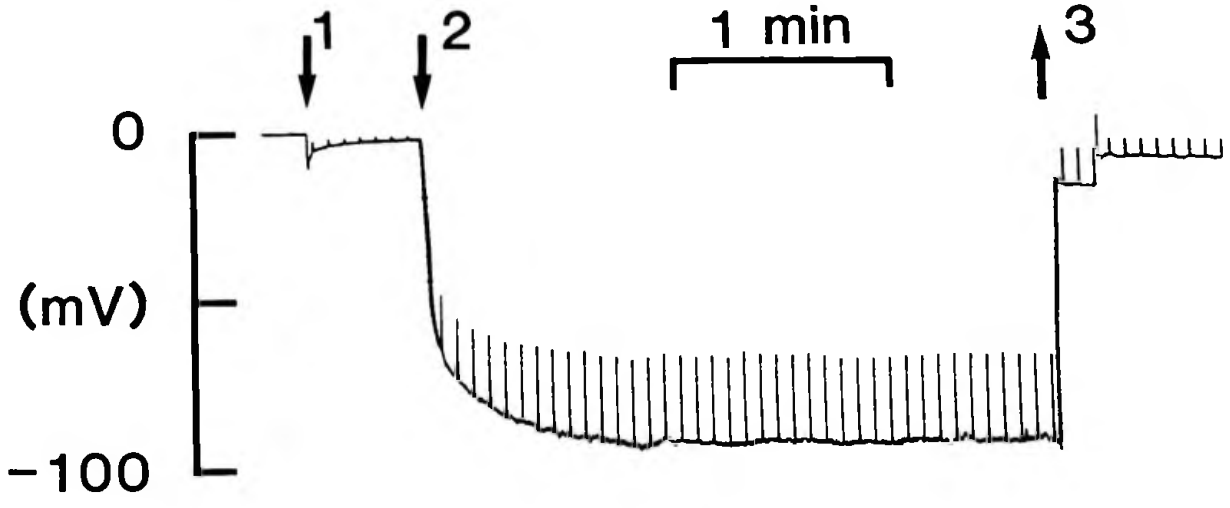

Fig. 1

measured by passing intermittent current pulses (0.1 nA, 300 ms) through the microelectrode after compensating resistance of the latter.

A recording of V_m and R_i is shown in Fig. 1. At the first arrow the microelectrode was advanced against the cell's surface, which is inferred from slight movement of the cell as observed through the microscope and from a 5 mV shift in offset voltage accompanied by an increase in R_i. At the second arrow, the cell was punctured by carefully tapping the table supporting the microscope, which resulted in a sudden increase in recorded voltage. We interpret the subsequent increase in V_m and R_i to result from membrane sealing around the the microelectrode. V_m anb R_i stabilized after 1 min when sealing was complete. At the third arrow, the microelectrode was withdrawn, but the cell remained clinging to the microelectrode resulting in an offset V_m and R_i. After the cell the was removed an -6 mV offset remained, which was subtracted from the apparent value of V_m. Five similar recordings yielded V_m ranging from -80 to -98 mV and mean $V_m \pm$ SE of -92 ± 3.5 mV.

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