

TRANSMEMBRANE POTENTIALS OF CULTURED RECTAL GLAND CELLS
OF RAJA ERINACEA

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Elasmobranch rectal gland has become an important organ to study function of a secretory epithelium. To understand more about cell mechanisms regulating electrolyte transport, we have placed R. erinacea rectal gland cells into primary culture and have measured some electrical properties under steady-state conditions and after added dibutyryl cAMP.

R. erinacea rectal glands were removed and minced in modified elasmobranch Ringer's solution containing (in mM): 280 NaCl, 5 KCl, 5 MgCl₂, 5 CaCl₂, 300 urea, 10 HEPES-triethanolamine, pH 7.3. The suspension of minced tissue was incubated for 15 min at room temp. in sterile, modified elasmobranch Ringer's solution plus 2x penicillin-streptomycin, 0.2% collagenase, and 0.2% hyaluronidase. Isolated tubules and tissue fragments were centrifuged, washed, suspended in growth medium (Eagle's MEM, plus 2% fetal calf serum, gentamicin, and in mM: 140 NaCl, 2 MgCl₂, 2 CaCl₂, 200 urea, and 100 trimethylamine oxide), and seeded into 35 mm tissue culture dishes.

For microelectrode impalements cells were cultured on glass coverslips, which were transferred to an acrylic chamber on the stage of inverted microscope. Cells were superfused continuously

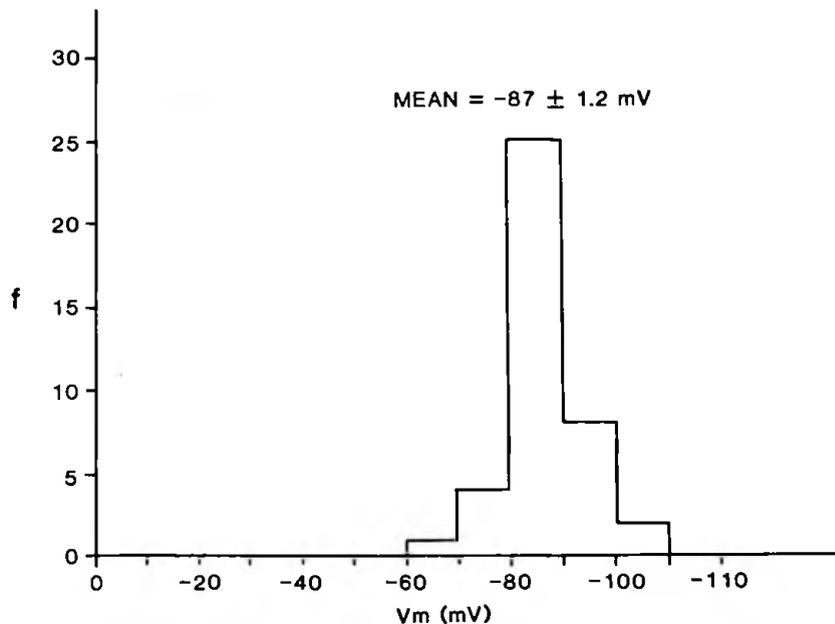


Fig. 1

Table 1. Mean transmembrane potential of *R. erinacea* rectal gland cells in primary culture on glass coverslips.

Time in culture	V_m (mean \pm SE)
8 hr	-87 \pm 1.2 mV (n=40)
24 hr	-69 \pm 4.7 mV (n=15)
48 hr	-70 \pm 2.3 mV (n=12)

n = number of impalements

with elasmobranch Ringer's solution (17-18°C, pH 7.6), and cells were impaled with glass microelectrodes filled with 0.5 M KCL (~100 megaohms).

Rectal gland tubule fragments form acinar-like structures in culture. These adhere to tissue culture substratum, and with time the cells flatten, spread, and grow out from the fragments. During this time cells are impaled readily with microelectrodes. A frequency distribution of the transmembrane potential (V_m) for cells in culture for 8 hr is shown in Fig. 1. V_m of cells at various times in culture are summarized in Table 1.

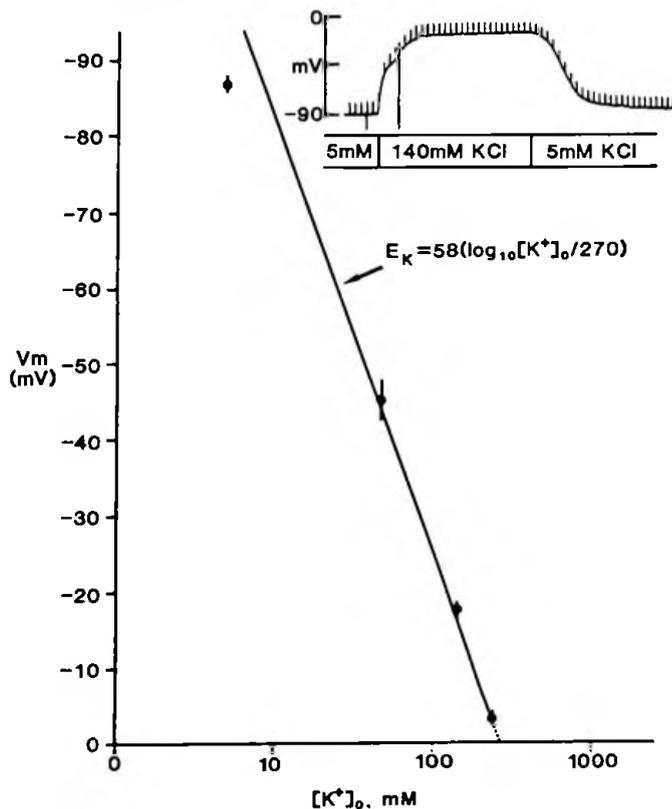


Fig. 2

V_m plotted as a function of \log_{10} of external K^+ concentration (KCl substituted for equivalent concentrations of NaCl) nearly fits the Nernstian plot of the K^+ equilibrium potential, Fig 2. Therefore, K^+ ion conductance (g_K) predominates among the total membrane ion conductances underlying V_m . Ba^{2+} (1mM), a presumed K -channel blocker, decreases V_m from -75 mV to -22 mV (not shown), which is consistent with the conclusion that g_K is a large component of total membrane conductance.

Dibutyryl cAMP (1mM) decreases V_m reversibly by 7 ± 1.8 mV, Fig 3. If the cells were pretreated with bumetanide (10^{-4} M) dibutyryl cAMP decreased V_m by 18 ± 6.0 mV (not shown).

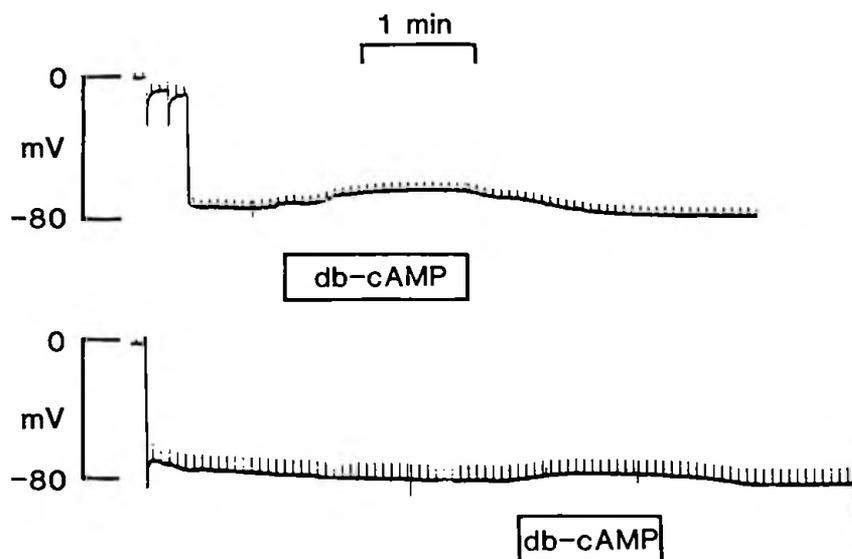


Fig. 3

Our results showing a predominant g_K and a decrease in V_m by dibutyryl cAMP are consistent with the findings of Greger and Schlatter (Pflug. Arch. 402:63, 1984) on the electrical properties of the basolateral membrane in isolated, in vitro perfused *S. acanthias* rectal gland tubules. We conclude that these membrane properties of the intact tubule are maintained by the cells of *R. erinacea* in culture. However, V_m of our cells in culture are considerably greater than that reported by Farmer et al (Bull, MDIBL 24:20, 1984) for cultured *S. acanthias* rectal gland cells. This may be a species difference in the cells' ability to adapt to tissue culture environment, a difference in culture conditions, or it may reflect sampling of different cell populations in the respective studies. In our experiments, V_m of cells flattened and spread out were approximately -40 mV, which is consistent with values reported by Farmer et al (1984).

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