EVIDENCE FOR Na^+/H^+ EXCHANGE IN RECTAL GLAND CELLS OF SQUALUS ACANTHIAS

George M. Feldman and Scott McCallum
Depart. of Med., Univ. of Penn. and Med. Cntr., Phila., PA 19104

In previous studies, slices of rectal gland from Squalus acanthias swelled when bathed in elasmobranch Ringer's medium containing propionate, and amiloride, an inhibitor of Na^+/H^+ exchange, inhibited propionate-induced swelling (Feldman et al, Bull. MDIBL 27: 108, 1987-88). These results suggested that the rectal gland has Na^+/H^+ exchange activity. To further characterize Na^+/H^+ exchange, we evaluated cell pH during exposure to propionate and the Na^+ requirement for cell swelling.

Rectal gland tubules in suspension were utilized for cell pH (pH_i) measurements, utilizing the pH sensitive fluorescent probe (2',7')-bis(carboxyethyl)-(5,6)-carboxyfluorescein (BCECF, Molecular Probes, OR). Tubules were prepared by incubating tissue slices in elasmobranch saline that contained collagenase, 2 mg/ml (Type II, Sigma), and 1% bovine serum albumen. At 15° C tubules were: loaded with membrane permeable BCECF-AM (2 µg/ml for 1 hour), washed, resuspended, and studied in a spectrofluorometer. Fluorescence emission was measured at 526 nm, while excitation was alternately 504 nm and 440 nm in order to obtain the pH dependent and the concentration independent fluorescence intensity ratio, F_{504}/F_{440}. To calibrate, tubules were in medium containing 2 µg/ml of nigericin and approximating intracellular cation content (50 mM Na^+, 130 mM K^+ and a poorly permeable cation, N-methylglucamine) while medium pH was altered (Thomas et al, Biochem. 18: 2210, 1979). The F_{504}/F_{440} ratio correlated with medium pH from pH 6.5 to 7.9. BCECF leak was reduced with probenecid; in preliminary studies, 4.5 mM probenecid reduced leak from > 2 %/min to < 0.2 %/min. Cell volume was measured in tissue slices as previously described. Data is mean ±S.E.

In medium of pH 7.4, rectal gland tubules maintained pH_i at 7.12±0.02 (6 preparations). Exposure of tubules to propionate medium (pH 7.4) rapidly decreased pH_i to 6.60±0.04 ($p < 0.01$), suggesting that propionic acid diffuses into the cell. Following initial acidification, pH_i returned toward normal attaining a value of 6.85±0.03 after 8 minutes. Although 1 mM amiloride did not inhibit propionate-induced cell acidification, amiloride inhibited the recovery of pH_i, and pH_i remained less than 6.5 (below the calibration range), suggesting that Na^+/H^+ exchange was responsible for the recovery of pH_i. In support of propionic acid diffusion, ^{14}C-propionate accumulated in tissue slices more rapidly at acid pH. After 5 minutes in 10 mM propionate at pH 6.5, 7.4 and 7.8, apparent cell propionate concentrations were 9.1±0.9, 7.7±0.4 and 5.8±0.4 mM, respectively.

The Na^+ requirement for swelling was examined. Replacing medium Na^+ with N-methylglucamine (NMG) prevented swelling: Na^+ propionate, 3.95±0.05 kg H_2O/kg DW vs NMG propionate, 2.77±0.05 kg H_2O/kg DW, $p<0.001$, $n=5$. However, Li^+, a Na^+ congener for Na^+/H^+ exchange activity, but not Na^+-K^+ ATPase activity, did not prevent swelling: Na^+ propionate 3.97±0.04 kg H_2O/kg DW vs Li^+ propionate 4.07±0.04 kg H_2O/kg DW. In Li^+ and Na^+ media, 1 mM amiloride inhibited swelling comparably, 32% and 40%, respectively, as did another inhibitor of Na^+/H^+ exchange, 10^{-4} M dimethylamiloride. Thus, propionate-induced cell swelling requires Na^+ and Na^+ entry is via Na^+/H^+ exchange.

These results support the existence of Na^+/H^+ exchange in the rectal gland of Squalus acanthias. Upon exposure to propionate, Na^+/H^+ exchange activity is required for pH_i regulation and cell swelling, and inhibition of Na^+/H^+ exchange activity inhibits both pH_i homeostasis and cell swelling.

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