Cl^- AND HCO_3^- INFLUENCE PROPIONATE EXIT FROM RECTAL GLAND CELLS OF SQUALUS ACANTHIAS

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We previously observed that cells from the rectal gland of Squalus acanthias swell when bathed in elasmobranch Ringer's solution containing propionate and that the mechanism of propionate swelling involves cell accumulation of propionate (Feldman et al, Bull. MDIBL 27: 108, 1987-88). Propionate-swollen cells also shrink upon transfer to standard elasmobranch Ringer's solution, returning to their original volume. In this study, we examined the mechanism of propionate exit from cells during volume recovery, and observed that Cl^- and CO_2/HCO_3^- facilitate propionate exit.

Cell pH (pH_i) was measured in suspensions of isolated rectal gland tubules utilizing the fluorescent probe BCECF as described (Feldman and McCallum, this Bulletin). After 3 hours in propionate medium (pH 7.4), pH_i was 6.83±0.07, and upon transfer to propionate-free medium (MOPS buffered, pH 7.4) pH_i increased to 7.61±0.08. This suggests that nondissociated propionic acid rapidly diffuses from cells. Also supporting rapid propionic acid diffusion, propionate-loaded tissue released more H^+ than did control slices upon transfer to unbuffered medium: propionate-loaded, 46±6 nanomoles H^+/mg dry weight (DW), and control 11±2 nanomoles H^+/mg DW.

The anion composition of the recovery medium had little effect on the rise in pH_i. In contrast, the buffer composition of the recovery solution did affect pH_i. Replacing the MOPS buffer with 8 mM HCO_3^- and 1% CO_2 (pH 7.4) resulted in recovery of pH_i. For example, immediately upon transfer, propionate-loaded cells had pH_i of 7.54 in MOPS buffered solution and 7.39 in HCO_3^-/CO_2 buffered solution. Ten minutes later, cells in MOPS buffered solution had pH_i of 7.49, while cells in HCO_3^-/CO_2 solution had a pH_i of 7.18, a value similar to control pH_i, 7.17. The facilitation of pH_i recovery by CO_2/HCO_3^- suggests that rectal gland cells have a Cl^-/HCO_3^- exchange system.

Efflux of ^{14}C-propionate was measured in iso-osmotic solutions having a pH of 7.4; the results were partitioned into fast and slow time constants (Ziyadeh et al, Biochim. Biophys. Acta 943: 43, 1988). Solution buffer composition affected propionate efflux. At pH 7.4, replacing MOPS with CO_2/HCO_3^- accelerated the "fast" time constant from 0.25 min^{-1} to 0.7 min^{-1} and increased the "slow" time constant from 0.08 min^{-1} to 0.15 min^{-1}. These CO_2/HCO_3^- dependent increases in propionate efflux may represent CO_2's ability to diffuse into cells and simultaneously supply H^+ for intracellular generation of propionic acid and HCO_3^- for exchange for Cl^-.

In the absence of CO_2/HCO_3^-, solution anion composition did affect the "slow" time constant, but not the "fast" constant: "fast" time constant, Cl^-, 0.26 min^{-1} and gluconate, 0.25 min^{-1}; "slow" time constant, Cl^-, 0.08 min^{-1} and gluconate, 0.023 min^{-1}. The Cl^- effect on the "slow" time constant supports the existence of Cl^-/HCO_3^- (OH^-) exchange, but it does not exclude Cl^- dependent propionate movement, for example, Cl^-/propionate exchange.

This study demonstrates that propionic acid diffuses rapidly out of rectal gland cells, alkalinizing cell contents. Rectal gland cells recover pH_i during propionic acid efflux if the CO_2/HCO_3^- system is present. In addition, CO_2/HCO_3^- and Cl^- are necessary for maximal propionate efflux from cells. Therefore, these observations suggest that Cl^-/HCO_3^- exchange is present in the rectal gland of Squalus acanthias.

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