ISO-OSMOTIC CELL SWELLING INDUCED BY PROPIONATE IN RECTAL GLAND OF SHARK (Squalus acanthias)

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Under iso-osmotic conditions cells usually maintain volume if they are bathed in solutions of normal Na^+ and K^+ concentrations, but recently Grinstein et al (Am. J. Physiol. 247: C293-C298, 1984) and Law (Biochim. Biophys. Acta 773: 246-252, 1984) demonstrated that incubation in sodium salts of weak acids can cause cells to swell. To study this form of volume regulation we incubated slices of dogfish rectal gland in modified elasmobranch solutions in which 286 mM propionate replaced Cl^-; in general, solution pH was 7.4 and was buffered with either Hepes or Tris. The remainder of the experimental methods has been described (Kleinzeller and Goldstein, J. Comp Physiol B 154: 561-571, 1984).

Incubating slices in propionate medium for three hours increased tissue content Na^+ 27%, from 369±9 meq/kg dry weight (DW) to 469±23 ($p < 0.005$), and K^+ 126%, from (266±3 meq/kg DW to 602±24 ($p < 0.001$), while Cl^- content decreased 79% from 489±18 meq/kg DW to 104±12 ($p < 0.001$). As indicated by the increase in tissue K^+, the gain in tissue H_2O was primarily intracellular, with H_2O_i increasing 64% from 2.07±0.06 kg/kg DW to 3.40±0.03. Moreover, apparent cell concentration of Na_i^+ increased from 56±7 mM to 76±5 ($p < 0.05$) and K_i^+ increased from 126±3 mM to 175±6 ($p < 0.001$). Presumably, Cl_i^- was displaced by anionic propionate as Cl_i^- decreased 76% from 110±10 mM.

In terms of the time course of propionate swelling, after two hours of exposure the increment in tissue H_2O was 97% of the value at three hours, and with exposures of 30 and 60 minutes the increments were 63% and 78%, respectively. Intracellular H_2O gain, however, was slower: two hour H_2O_i, 3.09±0.02 kg H_2O/kg DW versus three hour H_2O_i, 3.40±0.03, $p < 0.001$). In addition, cation concentrations changed from hour 2 to 3, as Na_i^+ decreased from 104±6 mM to 76±5 ($p < 0.01$) and K_i^+ increased from 137±5 mM to 175±6 ($p < 0.001$). Thus, after two hours of exposure to propionate, compensatory ion movement continues and K_i^+ appears to exchange for Na_i^+. Propionate swelling is reversible; two hours after return to normal medium swollen slices had H_2O contents that were within 5% of control.

As opposed to the disrupting effect of K^+ on the cytoskeleton, propionate induced swelling did not affect the actin distribution at the apical and basolateral membrane. Thus, cell swelling per se or intracellular pH alteration does not produce changes in the cytoskeleton.

Because propionate induced K_i^+ accumulation, ^{86}Rb uptake was measured to examine Na^+/K^+ATPase activity during acute exposure to propionate and after exposure for 1 hour: control uptake, 1.8±0.1 umole/g/min, acute exposure, 2.0±0.2 and 1 hour exposure, 1.7±0.2. The similarity in ^{86}Rb uptake indicates that propionate does not alter Na^+ pump activity. Moreover, after three hours of exposure to propionate, the increased K_i^+/K_o^+ ratio, an estimate of transmembrane potential difference (Kleinzeller and Goldstein), suggests that propionate increases voltage from 75 mV to 83 mV. The role of the Na^+ pump in propionate

swelling was evaluated by exposing slices to ouabain. To assure effective inhibition slices were preincubated in either saline or saline + 0.5 mM ouabain for 2 hours. Although ouabain caused swelling, increased Na^+ and decreased K^+, in the absence of ouabain propionate induced swelling was greater (4.18±0.02 kg/kg DW versus 3.66±0.02, $p < 0.001$). Slices incubated in propionate plus ouabain swelled to the same volume as did tissues incubated in propionate alone (4.17±0.04 kg/kg DW), but the ouabain treated slices had 71% higher Na^+ ($p < 0.001$) and 75% lower K^+ ($p < 0.001$) contents. These results demonstrate that propionate swelling does not require Na^+/K^+ATPase activity and is independent of intracellular K^+ sequestration and suggest that cell Na^+ entry participates in propionate swelling.

Na^+ entry via Na^+/H^+ exchange may be especially important during propionate swelling if, as suggested by Grinstein et al, propionate enters in its nondissociated form. At medium pH 6.6, 1 mM amiloride inhibited propionate swelling: amiloride 3.53±0.04 kg H_2O/kg DW versus no amiloride 3.92±0.07, $p < 0.0025$. Also confirming participation of Na^+/H^+ exchange in propionate swelling, amiloride reduced accumulation of Na^+ (amiloride 447±13 meq/kg DW vs no amiloride 600±18, $p < 0.0025$) and K^+ (amiloride 328±4 meq/kg DW vs no amiloride 376±15, $p < 0.025$).

As noted above, in the presence of normal Na^+/K^+ATPase activity K_i^+ increased. Thus we examined the effect of propionate on K^+ leak by measuring ^{86}Rb efflux: propionate, 0.30±0.02 umole/min/g, versus control, 0.73±0.04 ($p < 0.001$). This reduction in efflux is consistent with a possible influence of intracellular pH on K^+ efflux (Cemerikick, D. et al, J. Membr. Biol. 69: 159, 1982). To explore if intracellular alkalinization accelerates K^+ efflux, ^{86}Rb efflux was assessed in slices acutely exposed to NH_4^+, an intracellular alkalinizing agent: NH_4^+, 1.40±0.20 umole/min/g versus propionate, 0.31±0.02 ($p < 0.001$) and versus control, 0.68±0.04 ($p < 0.01$). These results support the hypothesis that intracellular pH influences the leak rate of K^+ from cells. An alternative explanation is independent of pH and remains untested: that is, anionic propionate, once in the cell, exits slowly and, thus, cation retention is required to maintain electroneutrality. NH_4^+, once in the cell, substitutes for K^+ and facilitates K^+ exit.

Since Law observed that renal cortical slices swell when incubated in salts of weak acids, such as formate and acetate, we explored the possibility that HCO_3^-, the ubiquitous biological anion, induces cells to swell, too. At pH 7.4 incubating slices in 7 mM HCO_3^- and 1% CO_2 for 3 hours induced a 20% increment in H_2O_i as compared to MOPS: 2.49±0.07 kg H_2O/kg DW versus 2.07±0.06, $p < 0.002$. In HCO_3^- medium, Na_i^+ was higher (88±5 mM vs 56±7, $p < 0.01$) and K_i^+ was lower (107±5 mM vs 126±3, $p < 0.01$). Thus, like salts of other weak acids, HCO_3^- can induce cell volume to increase.

These studies are in agreement with the hypothesis that propionic acid diffuses into cells while H^+ exits in exchange for Na^+. Although Na^+/K^+ATPase activity does not participate in this form of cell swelling, it increases K^+ content. The retention of intracellular K^+ (and Na^+) is most likely due to charge obligations imposed by the burden of intracellular propionate, a poorly permeable anion. By affecting intracellular K^+ stores as well as cell volume, weak acid anions appear to have an important role in K^+ homeostasis.

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