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A STUDY WITH MICROELECTRODES IN THE NECTURUS GALLBLADDER

BY

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Liquid ion-exchanger and conventional open-tip microelectrodes were used to measure intracellular Cl,K and Na activities (a^i_{Cl},a^i_K and a^i_{Na}),transmucosal membrane potential (E_m) and transepithelial electrical potential difference (E_{Tr}) in epithelial cells of Necturus gallbladder. Measurements were done at different external Na concentrations,$[Na]_o$,under steady-state conditions as determined by the constancy of E_{Tr}. Tris was substituted for Na.

The results show that E_m is not altered by Na substitution. The mean E_m value in these experiments was -52 mV (cell interior negative),whereas E_{Tr} was not significantly different from zero.

Under normal conditions ($[Na]_o$=100 mM) Cl is accumulated above the equilibrium value,a^{eq}_{Cl}. The ratio a^i_{Cl}/a^{eq}_{Cl} was 1.8. However when Na was removed from the bathing solutions, a^i_{Cl}/a^{eq}_{Cl} decreased to 1.0. The dependence of a^i_{Cl} on $[Na]_o$ showed saturation kinetics characteristics.

a^i_K (96 mM) did not change when $[Na]_o$ was varied between 100mM and 10 mM. However a^i_K decreased to 80 mM in a Na-free medium.

a^i_{Na} also increased in a saturable fashion with $[Na]_o$. Under normal conditions,a^i_{Na} was 9.7 mM. With some justified assumptions,analysis of the curve relating a^i_{Na} to $[Na]_o$ permits the calculation of the active Na efflux from the cell (about 200 p mole $cm^{-2}s^{-1}$) and of the fraction of Na entry that is coupled to Cl (about 77%). The remaining Na entry into the cell is effected by diffusion down its electrochemical potential gradient. The fraction of Na entry coupled to Cl permits a higher rate of Na transport that would be accom-

plished by a purely diffusive mechanism only. At the same time,the carrier-mediated,NaCl coupled mechanism plays a regulatory role in Na transport (and eventually in water reabsorption) by the gallbladder. This mechanism permits the rate of Na transport to remain virtually unchanged over a wide range of $[Na]_o$.

When the transmembrane electrochemical potential differences,$\Delta\bar{\mu}$,for Cl and Na,were calculated at four different $[Na]_o$ levels,a highly significant linear relation between $\Delta\bar{\mu}_{Cl}$ and $\Delta\bar{\mu}_{Na}$ was found,indicating that Na and Cl transport are energetically linked. The results support the view that the energy necessary for intracellular Cl accumulation is derived from the simultaneous dissipation of the Na chemical potential gradient across the apical membrane and that the coupled entry mechanism is electroneutral.