The Influence of K^+ Diffusion Potentials on the Fluorescence of a Cyanine Dye in Brush Border Membrane Vesicles

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3,3'-diethylthiadicarbocyanine iodide, a positively charged cyanine dye is tested as a fluorescent indicator of membrane potential changes in renal brush border membrane vesicles. Changes in fluorescence observed after addition of various L-amino acids are in agreement with results obtained by microelectrode measurements in intact kidneys indicating a Na^+-coupled electrogenic transport of these substrates. Since these results proved the dye to be a qualitative monitor of membrane potential, the relationship between membrane potential and fluorescence is investigated.

Membrane potential is varied by imposition of various K^+ concentration differences across the brush border membrane in the presence of valinomycin. A sudden increase in extravesicular K^+ concentration ($(K^+)_o$) causes a rapid increase in fluorescence (λ_{ex} = 622 nm, λ_{em} = 669 nm) with a maximum after a few seconds. During this time the intravesicular K^+ concentration ($(K^+)_i$) is treated to be constant.

With vesicles equilibrated with 0.5 mmol/l K_2SO_4 and 74.5 mmol/l Na_2SO_4 the maximum changes in fluorescence after a sudden increase in $(K^+)_o$ are proportional to 1 g $(K^+)_o$ for $(K^+)_o > 3$ mmol/l. For smaller $(K^+)_o$ deviations from linearity are observed. Whenever the vesicles are preloaded with higher K^+ concentrations fluorescence change is still proportional to 1 g $(K^+)_o$ but the slope is affected by $(K^+)_i$. Thus for the same ratio $(K^+)_o/(K^+)_i$ the fluorescence signal increases with increasing $(K^+)_i$. This is not expected when a linear relationship between fluorescence and membrane potential calculated from the Nernst equation is assumed.

When vesicles preloaded with various $(K^+)_i$ are exposed to the same extravesicular K^+ concentration, a decrease in fluorescence answer should occur with increasing $(K^+)_i$, since the ratio $(K^+)_o/(K^+)_i$ decreases. Experimental results show a decrease which is, however, not proportional to 1g $(K^+)_i$ as would be the membrane potential predicted by the Nernst equation. The curvilinear relationship under this condition as well as the deviations for small $(K^+)_o$ mentioned above could be due to a noticeable permeability of the membrane for Na^+. In this case membrane potentials calculated from the Nernst equation would be overestimated. Unfortunately the actual membrane potential can not be calculated since the permeabilities for K^+ and Na^+ are not known. Thus at the moment it is not possible to describe properly the relationship between fluorescence and membrane potential in brush border membrane vesicles.