Dear Sir,

A great deal of interest has been paid to the regulation of proton and ion transport systems. The control of renal epithelial permeability must perform the twofold task of regulating both physiologic ionic and acid-base balance of the organism as a whole as well as maintaining an intracellular environment compatible with efficient cellular function. Agents known to inhibit ion transport through $K_+^+$ channels in non-epithelial tissues, such as nerve, also block similar pathways in epithelial membranes. This report describes a unique method for the study of membrane channels using a purified membrane vesicle preparation and a voltage-sensitive dye.

Purified membrane vesicles were obtained from rabbit renal cortex using a series of homogenates and differential centrifugations with Ca$^{2+}$ precipitation. Membrane voltage measurements were made using the potential sensitive dye 3,3-dipropylthiodicarbocyanine iodide [dis-C$_3$-(5)] [1]. The voltage response of the dye was calibrated with standard K$^+$ diffusion potentials. The dye fluorescence was measured using a Fluorolog II spectrophotometer with temperature regulated using a circulating water bath [2]. Ionic permeabilities were determined from the observed membrane potentials using the constant field equation [1]. The membrane potentials of five ions were measured at temperatures ranging from 25 to 55 °C (table 1). At 40 °C, the ionic permeabilities were measured in the presence and absence of 5 mM tetraethylammonium (TEA$^+$), a specific K$^+$ channel inhibitor, and tetramethylammonium (TMA$^+$), a noninhibiting analog of TEA$. Ionic permeabilities were found to vary relative to Glu when temperatures were increased from 25 °C. A significant increased permeability was found at both 40 and 55 °C for most ions examined. At 40 °C, the average increase in relative permeability for the four ions evaluated was 140% while at 55 °C the average increase was 50% compared to observations made at 25 °C. In table 1 the four ions are observed to demonstrate different responses to temperature with K$^+$ being the most sensitive to temperature changes while H$^+$ shows significantly less. At 40 °C, TEA$^+$ significantly decreased K$^+$ permeation by nearly 60% in renal membrane vesicles while having little effect on the permeability of Na$^+$, Cl$^-$ or H$^+$. The nonactive analog TMA$^+$ had no significant effect on any permeabilities examined. These results suggest that ion channels and the regulation of ionic permeability can be studied using membrane vesicle preparations and potential-sensitive dyes. These channels appear to be not only specific, but quite sensitive to temperature changes.

References


Table 1. Ionic and proton permeabilities of purified renal membrane vesicles