## CELLULAR ELECTROPHYSIOLOGY OF HOMOLOGOUS DILUTING SEGMENT FROM <u>SQUALUS</u> ACANTHIAS KIDNEY.

S.C. Hebert and P.A. Friedman

Renal Division and Department of Physiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115; Department of Pharmacology & Toxicology Dartmouth Medical School, Hanover, NH 03756

In previous studies we identified a nephron segment from within the peritubular sheath that exhibited transport characteristics typical of diluting segments found in mammalian and amphibian species (Bull. MDIBL 25: 24, 128, 1985; 26: 61, 1986). The present studies used intracellular microelectrodes to assess further the electrophysiological properties of this segment. Single intermediate IV segments were perfused and bathed with shark Ringer's at 16-19°C. Microelectrodes were filled with 1M KCl and had tip resistances of 70-100 Mohm. Basolateral membrane voltage (V<sub>bl</sub>, mV) and fractional resistance of apical membranes  $(f_a)$  were -64.0 ± 2.5 and 0.892 ± 0.011, respectively (n=17). Baseline transepithelial voltage (Ve, mV), conductance (G<sub>e</sub>, mS/cm<sup>2</sup>) and equivalent short-circuit current ( $I_{SC}$ ,  $\mu A/cm^2$ ) averaged 5.4 ± 0.8, 111.3 ± 14.5, and 486 ± 43, respectively. Luminal addition of 0.1 mM furosemide hyperpolarized  $V_{b1}$  by -16.1 ± 1.8 mV (n=15, P<0.001) and abolished V<sub>e</sub> and I<sub>sc</sub>.  $F_a$  decreased significantly from 0.889 ± 0.013 to 0.854 ± 0.018 (n=9, paired P<0.001). Replacement of luminal K with 1.0 mM Ba depolarized apical membranes by  $-10.4 \pm 2.7 \text{ mV}$  (n=7, paired P<0.01) and increased  $f_a$  from 0.862 ± 0.019 to 0.918 ± 0.016 (paired P< 0.03). Individual cellular (apical,  $R_a$ , and basolateral,  $R_{bl}$ ) and paracellular resistances ( $R_s$ ) were calculated from the linear relation between the temporal changes in  $G_e$ and  $f_a$  immediately following Ba addition ( $G_e = 1/R_{bl}[1-f_a] + 1/R_p$ ).  $R_a$ ,  $R_{bl}$  and  $R_s$  (ohm cm<sup>2</sup>) were 40.6 ± 4.9, 6.0 ± 0.7 and 35.9 ± 5.5, respectively. Cellular conductance averaged  $43.0 \pm 4.9\%$  of Ge. We conclude: 1) the hyperpolarization of  $V_{bl}$  and fall in  $f_a$  associated with abolition of transepithelial NaCl absorption by furosemide in intermediate segment IV is consistent with a reduction in conductive Cl efflux across basolateral membranes; 2) the depolarization of  $V_a$  and rise of  $f_a$  with Ba is consistent with the presence of potassium conductive pathways in apical membranes; and, 3) the electrical leakiness of this nephron segment, like that of other diluting segments, is due not only to the cation selective shunt pathway, but also to a large transcellular conductive pathway (Bull. MDIBL 25:128, 1985). (These studies were supported grant DCB 87-02159 from the National Science Foundation).