FURTHER EVALUATION OF NaCl TRANSPORT IN THE IN VITRO PERFUSED DILUTING SEGMENT FROM THE PERITUBULAR SHEATH OF SQUALUS ACANTHIAS KIDNEY

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Previously, we tentatively identified a nephron segment present in the peritubular sheath of the dogfish, Squalus acanthias, kidney as a diluting segment (Hebert & Friedman, Bull. MDIBL, 25:128-131, 1985). This was based both on the similarity of the basic electrophysiological characteristics of the dogfish segment to other mammalian and amphibian diluting segments (Hebert & Andreoli, Am.J.Physiol. 246:F745-F756, 1984) and on the finding that this peritubular sheath segment had a low water permeability (Friedman & Hebert, Bull. MDIBL, 25:24-26, 1985). In addition, we suggested that NaCl absorption by this nephron segment might provide the energy for a countercurrent system of urea reabsorption consistent with the morphologic observations of Lacy and coworkers (Lacy et al. Science 227:1351-1354, 1985; Lacy & Reale, Anat. Embryol. 173:23 -34 & 163-186, 1985). This system would account for the long-standing observations of the direct correlation between the rates of sodium and urea reabsorption by elasmobranch kidneys (Schmidt-Nielsen, Truniger & Rabinowitz, Comp. Biochem. Physiol. 42A:13-25, 1972) and of furosemide's ability to increase the renal excretion of both sodium and urea (Meyers et al. Bull. MDIBL 11:71-72, 1971). Since this model of urea reabsorption depends on active NaCl absorption by the peritubular sheath diluting segment in the dogfish, the purpose of the present studies was to establish that this nephron segment actively reabsorbs this salt. In particular, we tested in the in vitro perfused dogfish (Squalus acanthias) diluting segment from the peritubular sheath (i) whether the transepithelial voltage (Ve, mV) and the equivalent short circuit current, Isc ($\mu A/cm^2$) were dependent on the presence of Na^+ and Cl^-, (ii) the effects of several transport inhibitors on these electrical events, and (iii) the rate of chemical Cl^- absorption.

MATERIALS AND METHODS: In vitro tubule perfusion was performed using electrical and net chemical flux measurements similar to those described by us for the mammalian thick ascending limb (Friedman & Andreoli, J.Gen.Physiol. 80:683-711, 1982; Hebert et al. J.Membr.Biol. 80:201-219, 1984) and for the dogfish nephron (Friedman & Hebert, Bull. MDIBL 25:24-26, 1985; Hebert & Friedman, Bull. MDIBL 25:128-131,1985). Kidneys were removed from 1.5-3.0 kg male dogfish shark after anesthetizing the animal by cord transection and cross-sectional slices immersed in cold shark Ringers. Tubule segments , 0.2-1.2 mm in length, were dissected freehand from peritubular sheaths and mounted on concentric glass pipets. This segment was identified by the characteristic "cobblestone" appearance of its epithelium and the lumen-positive Ve (Hebert & Friedman, Bull MDIBL 25:128-131, 1985). All tubules were perfused in symmetrical shark Ringers at 16-18°C. N-methyl-d-glucamine was used to substitute for sodium (sodium-free) and isethionate replaced chloride (chloride-free) where indicated. The electrical properties of the nephron segment were determined using the same electrical arrangement described by us previously (Hebert & Friedman, Bull. MDIBL 25:128-131, 1985). Biphasic command current pulses 400 nA in magnitude and 400 ms in duration were used to calculate the transepithelial conductance (Ge, mS/cm^2) by terminated cable equations. Isc was then calculated as Ve•Ge.

The net rate of chemical chloride transport (J_{Cl}, picomoles/sec•cm^2) was determined from the disappearance of chloride from the perfusate as:

$$J_{Cl} = (V_L([Cl^-]^o - [Cl^-]^L))/A$$

where $[Cl^-]^o$ and $[Cl^-]^L$ are the concentrations of chloride in the perfused and collected fluid, respectively, V_L is the fluid collection rate (equals fluid perfusion rate since there is negligible fluid absorption by this nephron segment; Friedman & Hebert, Bull. MDIBL 25:24-26, 1985), and A is the apparent luminal surface area. $[Cl^-]^o$ and $[Cl^-]^L$ were determined by electrotitrametric methods using a computer-based titrator (IDEA, Inc., Houston, TX). All results are expressed as mean ± SEM for the indicated number of tubules (n).

RESULTS AND DISCUSSION: As indicated above these segments could be easily identified in the peritubular sheaths as the largest tubule segment and by their "cobblestone" appearance. The average inside and outside diameters of this diluting segment were 46.9 ± 1.6 µm and 74.4 ± 2.1 µm, respectively. From these dimensions this segment would correspond to Intermediate segment IV in the morphologic description of Lacy & Reale (Anat Embryol. 173:163-186, 1985).

The sodium and chloride dependence of Ve and Isc in this nephron segment is presented in Table 1. Either sodium-free or chloride-free symmetrical solutions reversibly abolished both the transepithelial voltage and the equivalent short circuit current. Thus, the electrical parameters of transport in this segment are dependent on the presence of both sodium and chloride, observations consistent with the effects of ion deletion studies in other diluting segments (Hebert & Andreoli, Am.J.Physiol. 246:F745-F756, 1984).

The effects of luminal 10^{-4} M furosemide or amiloride or hydrochlorothiazide (HCTZ) on Ve and Isc are shown in Table 2. The data in this table indicate that these NaCl-dependent electrical parameters are reversibly abolished by furosemide and that both amiloride and HCTZ are without effect. These observations suggest that luminal entry of NaCl into cells may be mediated by the "loop" diuretic-inhibitable $Na^+:K^+:2Cl^-$ cotransporter rather than by a sodium conductance pathway or $Na^+:H^+$ exchanger (presumable which would be blocked by amiloride) or by an Na:Cl cotransporter (presumably sensitive to the diuretic agent, HCTZ).

The average net rate of chemical chloride transport in the Intermediate IV segment of the dogfish shark is presented in Table 3. Chloride transport was in the absorptive direction and averaged 1379 ± 270 pmoles/sec•cm^2. Since this process proceeds against an electrochemical gradient (symmetrical solutions and a lumen-positive transepithelial voltage), chloride transport is active.

In summary, the largest nephron segment in the dogfish peritubular sheath which has a "cobblestone" appearance was identified as Intermediate segment IV. The electrical and chemical flux data indicate that this segment actively absorbs chloride (associated with sodium) by a process that is specifically blocked by the "loop" diuretic furosemide, and thus, likely involves a $Na^+:K^+2Cl^-$ cotransport process located on luminal membranes. The present observations, when taken together with the negligible water permeability of this nephron segment, indicate that it may function as a diluting segment in vivo, and therefore, in analogy with mammalian countercurrent processes, may provide the necessary conditions for passive urea reabsorption in some more distal sheath segment.

ACKNOWLEDGEMENTS: S.C. Hebert and P.A. Friedman were recipients of Markey Fellowships made available from the Markey Charitable Trust. This work was also performed during the tenure as Established Investigators of the American Heart Association awarded to S.C.H. and P.A.F. Support was also provided by MFBS FCOR at MDIBL (NIH #1-P30EF03828-01) through the kind efforts of Dr. Frank Epstein.

TABLE 1. THE EFFECTS OF SODIUM AND CHLORIDE REMOVAL FROM PERFUSATE AND BATH ON Ve AND Isc IN THE DOGFISH INTERMEDIATE IV SEGMENT

Ion	Ion Concentration (mM) Perfusate	Bath	Ve (mV)	Isc ($\mu A/cm^2$)
	270	270	5.7 ± 1.7	490 ± 120
Na^+	0	0	*0.6 ± 0.4	*30 ± 20
	270	270	5.6 ± 1.7	520 ± 100
	270	270	5.1 ± 0.6	630 ± 130
Cl^-	0	0	*0.3 ± 0.3	*20 ± 20
	270	270	5.3 ± 0.8	640 ± 160

Tubules were perfused in vitro at 16-18°C. n=5 and 6 for sodium and chloride replacement studies, respectively. * indicates $p<0.05$ compared to initial values obtained with 270 mM ions present.

TABLE 2. THE EFFECTS OF FUROSEMIDE, AMILORIDE AND HCTZ ON Ve AND Isc

Agent	Concentration (M)	Ve (mV)	Isc ($\mu A/cm^2$)	n
	0	9.2 ± 2.2	780 ± 120	
Furosemide	10^{-4}	*0.1 ± 0.2	*4 ± 10	11
	0	9.0 ± 2.4	720 ± 130	
Amiloride	0	14.5 ± 3.7	1113 ± 210	6
	10^{-4}	10.5 ± 2.6	940 ± 140	
HCTZ	0	11.2 ± 1.8	1190 ± 260	5
	10^{-4}	11.6 ± 1.7	1190 ± 250	

Tubules were perfused in vitro at 16-18°C. * indicates $p<0.05$ compared to control in the absence of agent.

TABLE 3. NET CHEMICAL CHLORIDE TRANSPORT BY INTERMEDIATE SEGMENT IV

Vo (nl/min)	Ve (mV)	J_{Cl} (pmoles/sec•cm²)
8.15 ± 0.94	5.2 ± 1.2	1380 ± 270

Tubules perfused in vitro at 16-18°C. n=7