UREA & WATER TRANSPORT IN ISOLATED PERFUSED RENAL TUBULES OF THE DOGFISH SHARK, SQUALUS ACANTHIAS.

Peter A. Friedman and *Steven C. Hebert. Department of Pharmacology, Dartmouth Medical School, Hanover, NH 03756, *Renal Division, Brigham and Women's Hospital, Boston, MA 02115

The ability of the mammalian kidney to elaborate a urine more concentrated than plasma involves renal medullary countercurrent exchange and multiplication. The initial, so-called "single effect," is provided by the active transport of NaCl from a diluting segment which exhibits low urea permeability (Rocha and Kokko, Kidney Int. 6:379-387, 1974) and is virtually impermeable to water (Hebert and Andreoli, Am. J. Physiol. 246:F745-F756, 1984). Combined with spatially separated and distinct permeabilities for NaCl, water and urea, the transport of NaCl out of the thick ascending limb is operationally coupled to dissipative urea reabsorption from terminal nephron segments (Jamison and Kriz, Urinary Concentrating Mechanism, New York: Oxford, 1982).

Renal urea reabsorption in the cartilagenous fishes proceeds against a large chemical concentration gradient since plasma urea is some 350mM while the urea concentration of urine is less than 100mM. The mechanisms responsible for this active renal reabsorption of urea in the kidney of S. acanthias have not been identified. Several features of urea reabsorption and its relation to sodium movement in elasmobranch fishes are reviewed in the accompanying report ; and Friedman, this Bulletin). While the elasmobranch kidney is not organized into discrete cortical and medullary regions, a countercurrent arrangement of individual nephrons has been recognized (Deetjen and Antkowiak, Bull. MDIBL 10:5-7, 1970; Boylan, Comp. Biochem. Physiol. 42a:27-30, 1972; Lacy et al., Bull. MDIBL 15:54-56, 1975; Science (Washington), 227:1351-1354, 1985). Moreover, the five segments so organized are encapsulated in a peritubular sheath (Lacy et al., 1985) which may serve to create a microenvironment in which the general features of a countercurrent system could operate. Thus, urea absorption might be linked facultatively to the transport of sodium through the presence of appropriate and spatially separated urea, NaCl and water permeabilities. The basic tenets of such a model for the elasmobranch kidney were proposed by Boylan (1972). For a such a scheme to be responsible for the reabsorption of urea by the processes described above and, analogous to those in the mammalian nephron, the permeability to water, urea and sodium of the nephron segments running through the peritubular sheath would have to differ with at least one segment exhibiting active salt absorption in the absence of water or urea movement; another with high water but low urea permeability; and a segment with low water and high urea permeability. The purpose of these studies was to evaluate the feasibility of the countercurrent model for active urea reabsorption in the elasmobranch kidney by determining urea and water permeability characteristics of the nephron segments running through the peritubular sheath of the dogfish kidney. We were able to identify a putative diluting segment and to characterize in a preliminary fashion the unidirectional urea efflux coefficients and water movement in this and in two other nephron segments.

MATERIALS AND METHODS: Single shark tubules were perfused in vitro using methods similar to those described by us for the mammalian thick ascending limb (Friedman and Andreoli, J. Gen. Physiol. 80:683-711, 1982) and proximal tubule (Friedman et al., Am. J. Physiol. 240:F558-F568, 1981) and for the proximal tubule segment V of S. acanthias by Beyenbach & Fromter (Am. J. Physiol.

248:F282-F295, 1985). Kidneys from 2-4 kg male dogfish sharks were removed, sliced in coronal sections and immersed in cold shark Ringer solution. Tubule segments, 0.5-3.5 mm in length, were dissected free-hand following the anatomical descriptions by Ghouse et al. (Bull. MDIBL 8:22-29, 1968) and Lacy et al (Science 227: 1351-1354, 1985). Results are reported for tubules perfused at 16-18°C in symmetrical balanced shark Ringer solutions. Unidirectional lumen-to-bath fluxes of urea were measured by the rate of disappearance of ^{14}C-urea from the luminal perfusate. The isotope was added to the luminal perfusion solutions at an average activity of 12.5×10^{-6} Ci/cm^3. An apparent urea efflux constant, k_{urea} (cm/sec), which makes no a priori assumptions regarding the mechanism of efflux, was computed as described previously (Friedman and Andreoli, J. Gen. Physiol. 80:683-711, 1982). The rate of transepithelial fluid absorption (J_v, nl min^{-1} mm^{-1}) was measured concomitantly with the lumen-to-bath urea fluxes by determining the rate of change in concentration of ^3H-methoxy inulin added to the perfusion solutions. J_v was calculated as described previously (Friedman et al., Am. J. Physiol. 240:F558-F568, 1981).

Unidirectional lumen-to-bath urea efflux coefficients and net volume movement of several of the nephron segments of the dogfish shark were determined and are shown in the following table.

segment	$K^{l,b}$ cm/sec x 10^5	J_v nl/min mm	n
sac "diluting segment"	0.37 ± 0.17	0.5 ± 0.5	4
proximal V	2.08 ± 0.74	-0.3 ± 1.4	6
collecting tubule	0.91	1.7	2

These preliminary results suggest a hybrid pattern of urea and water permeabilities. The sac "diluting segment" exhibits a low urea efflux coefficient and insignificant volume movement. On the other hand, proximal segment V reveals a rather different pattern of water movement and urea efflux in which there is a high urea efflux coefficient but negligible water movement. Finally, in two observations in collecting tubules from outside the peritubular sheath, the urea efflux coefficient had a value intermediary between that in the sac "diluting segment" and that in the collecting tubule while at the same time demonstrating a value for J_v consistent with net fluid absorption.

In order to determine if inhibition of the Na^++K^+ATPase would alter the efflux coefficient for urea, we examined, in paired observations in single perfused tubule segments, the action of ouabain addition to the serosal bathing solution. Clearly, a positive result from such an experiment would be consistent with the notion that urea movement was linked directly to that of sodium in single cells, perhaps through the presence of a Na-urea cotransport mechanism. In the sac "diluting segments", addition of 10^{-4}M ouabain to the bathing solution abolished the spontaneous transepithelial voltage, consistent with an inhibition of the Na^++K^+ATPase and electrogenic salt absorption. No effect on the rate of unidirectional lumen-to-bath efflux was observed. It should be noted, however, that the sac "diluting segment" exhibits a low basal urea efflux coefficient and, therefore, it is perhaps to be expected that

passive urea permeation in this segment is not coupled to sodium movement. Such an interpretation would be consistent with a role of this sac segment to dissociate NaCl movement from that of urea.

An action of ouabain was also explored in proximal segment V. In several preliminary experiments, we could find no evidence for an effect of ouabain on unidirectional urea efflux in this nephron segment. Based on these pilot studies, it would appear that even in a segment in which appreciable urea efflux occurs, urea movement is not coupled directly to the hydrolysis of ATP through the Na^++K^+ATPase.

Finally, we also investigated, in a preliminary fashion, the possibility that phloretin, an agent known to interfere with facilitated diffusion of urea in epithelial and nonepithelial cells alike, had an inhibitory action on urea efflux in isolated shark nephrons. In two experiments on proximal segment V, addition of 10^{-6}M phloretin caused a 75-80% reduction of unidirectional urea efflux.

Together with the data on salt transport in the distal sac "diluting segment" (cf. companion report, Hebert and Friedman), the present observations are consistent with the notion that this nephron segment, located in the bundle zone and encapsulated by the peritubular sheath, has the characteristic properties necessary for diluting luminal fluid. Thus, one of the requirements of the proposed model appears to be satisfied. Moreover, it does not appear that unidirectional urea efflux, in any of the nephron segments thus far examined, is coupled directly to that of sodium and, finally, the cellular ·t of urea appears to involve facilitated diffusion. Evidently, these tentative conclusions require further studies before they can be accepted.

ACKNOWLEDGEMENTS: P.A. Friedman and S.C. Hebert were recipients of Markey Fellowships made available by the Lucille Markey Charitable Trust during the summer of 1985. This work was also performed during the tenure as Established Investigators of the American Heart Association awarded to P.A.F. and S.C.H. Support was also provided by Dartmouth College BRSG 2S07 RR05392-24G.