PROXIMAL TUBULAR ACIDIFICATION IN THE ISOLATED PERFUSED KIDNEY OF THE LITTLE SKATE, *RAJA ERINACEA*

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Although macroscopically quite different, the kidneys of the little skate (*Raja erinacea*) and of the spiny dogfish (*Squalus acanthias*) are very similar in the microscopic structure as well as the function of their nephrons (R.T.Kempton, J. Morphol.*73*: 247-263, 1943; P.Deetjen and D.Antkowiak, Bull. MDIBL, *10*: 5-10, 1970; H.Stolte et al., J.Exp.Zool.*199*:403-410,1977). The urine has a fixed pH of around 5.8 and the renal acidification, although carbonic anhydrase-independent, is nearly inexhaustible (J.W.Boylan et al. Bull. MDIBL, *13*: 17-20, 1973; E.R.Swenson and T.H.Maren, Amer. J. Physiol. *250*: F288-F293, 1986). In the dogfish, Kempton localized the onset of tubular acidification at the beginning of the proximal tubule (Bull. MDIBL, 34-36, 1940), and Deetjen and Maren quantified the acidification rate of this nephron section in the skate by the microinjection of buffer dyes (Pflügers Arch. *346*: 25-30, 1974).

In the present study, we wanted to investigate the cellular mechanims involved in tubular acidification in the little skate. The macrovasculature of the kidney was studied by means of Microfill injections, and an isolated-perfused kidney preparation was developed. The luminal pH of the proximal tubule (sinus zone) was monitored continuously with proton-sensitive microelectrodes in the presence and absence of several inhibitors that are known to block ion transport mechanisms possibly involved in tubular acidification.

Methods and Materials

The experiments were performed in the MDIBL in the summer of 1986. Ten female and two male skates, *Raja erinacea*, weighing between 0.7 and 1.8 kg, were anaesthetized by injecting sodium pentobarbital (20 to 25 mg · kg^{-1}) into one of the lateral tail veins. 500 units · kg^{-1} heparin were also injected, at this time. The gills of the fish were perfused with fresh seawater through the dorsal spiracles into which two inflatable Foley catheters, size 23 F, were inserted. The abdomen was opened by a ventral midline incision. All pelvic organs covering the kidneys were removed after ligation of their vessels. The left kidney was dissected free by an initial incision of the capsule-like connective tissue along its lateral edge. Use of the male skates was soon abandoned because ventral access to the kidney is hindered by the seminiferous duct. The big caudal branch and about 5 to 7 small segmental branches of the portal venous system were dissected. Shortly before removing the kidney, we closed the branches with small clips in order to find them again during the catheterization of the isolated organ.

The isolated kidney was placed on a small table cooled by fresh sea water. Four polyethylene (PE) catheters (outer diameter about 0.4 mm) were glued into a 1cm piece of PE tubing (inner diameter 4 mm) by a methyl metacrylate glue (Agomet, D-6450 Hanau 1, FRG). As described similarly by Oberleithner et al. (Pflügers Arch. 402: 272-280, 1984), the ends of up to eight pieces of PE 50 tubing, connected by a multi-valve system to the different perfusion fluids, were then glued into the other opening of the 1 cm tubing. The dead space between ingoing and outgoing catheters was kept as low as about 30 ul. This type catheter connected the perfusion solution selected (1 out of 8; hydrostatic pressure 35-40 cm H_2O) with the big caudal branch and three small branches of the portal venous system entering the dorsal side of the kidney. The rest of the portal branches remained clipped or were tightly bound because these vessels communicate with each other within the kidney before they enter their renal lobe. Small bolusses of lissamine green or phenol red were injected to check for proper renal perfusion and leaks. The small and highly variable renal arteries (J. Hyrtl, Denkschr. Akad. Wiss., Wien 1857) were usually not catheterized. Care was taken to maintain an unhindered venous outflow. The kidney was perfused with O_2- and CO_2-bubbled (99:1) Forster's solution which had a pH of 7.4. The drugs mentioned below were solved in the same solution.

The microelectrodes filled with H^+-sensitive liquid ion-exchanger (D. Ammann et al., Anal. Chem. 53: 2267, 1981; Fluka, CH-9470 Buchs, Switzerland) were made according to Oberleithner et al. (vide supra). The borosilicate glass capillaries (outer diameter 1.5 mm) with internal fiber were purchased from Hilgenberg, D-3509 Malsfeld, FRG, and pulled on a horizontal puller (Narishige PD-5). Measurements with the microelectrodes were performed using a two-channel high input impedance amplifier (FD 223, WPI instruments, Hamden, Ct., USA). A large Ag(AgCl) reference electrode was brought in direct contact to the kidney surface. For details see Oberleithner et al. (vide supra). The ventral side of the kidney was used for micropuncture. The thick tubules found in this 'sinus zone' are part III of the proximal tubule according to Lacy and Reale (Anat. Embryol. 173: 23-34 and 163-186, 1985). These tubules are bathed in a space perfused by the portal venous system. Thus it is the basolateral side of the tubule which is reached by the perfusion solutions used in this study.
DNDS (4,4'-dinitro stilbene-2,2'-disulfonate) was obtained from Pfaltz and Bauer Inc., Stamford, Ct.

Results

In several pilot experiments, the transepithelial voltage in the proximal tubules of the sinus zone was measured. Confirming the data obtained in the isolated shark proximal tubule (K.W.Beyenbach and E.Frömter, Amer. J. Physiol. 248: F282-F295, 1985), we found it to be only 1-3 mV (lumen negative). It did not change significantly in presence of the inhibitors used in this study. Therefore it was neglected during the measurements of luminal pH. The luminal pH in the proximal tubule III was found to be 5.8 $\pm$ 0.43 SD (n = 27). Application of the K^+-$2Cl^-$-Na^+-cotransport blocker (R.Greger and E.Schlatter, Klin.Wochenschr.61:1019-

1027,1983) furosemide (10^{-3} or 10^{-4}M) led to a mean ΔpH in the lumen of +0.8 ± 0.15 SEM (n=12). 10^{-4}M DNDS, a reversible blocker of Cl^-/HCO_3^- exchange in erythrocytes (O.Fröhlich, J.Membr.Biol. 65: 111-123, 1982), increased the luminal pH as well: ΔpH = +0.66 ± 0.08 SEM (n= 8). $BaCl_2$ (3mM), a well known blocker of K^+-channels, and amiloride (10^{-4}M), an inhibitor of Na^+/H^+ exchange, had only very small effects on luminal pH.

In one experiment, the intracellular electro-chemical gradient for H^+ across the baso-lateral membrane of a single proximal tubule cell was recorded for more than 4 hours. The H^+-sensitivity of the electrode used in this case, however, was extremely small (about 7 mV/pH unit). Therefore, the voltage .pa recorded reflects primarily the baso-lateral membrane potential of this cell. It amounted to -40mV (control) for more than 4 hours. DNDS (10^{-4} M) in the perfusion fluid led to an immediate overshoot hyperpolarization of about 45 mV (within 15 s) that settled down to a steady-state hyperpolarization of 24± 1.7 mV (SEM; n = 3) within 1 min. Furosemide (10^{-4} M) had nearly the same effect (steady-state hyperpolarization of 22.5 mV). $BaCl_2$ (3 mM) or amiloride (10^{-4}M) reversed this hyperpolarization to a great extent, but had only a small depolarizing effect (3-5 mV) if applied at the control voltage of -40mV. The Cl^- channel blocker (H.Oberleithner et al., Pflügers Arch.398:172-174,1983) anthracene-9-carboxylic acid (10^{-4}M) caused a much slower hyperpolarization (within 1 min and without overshoot) of 17 mV, whereas the carbonic anhydrase inhibitor methazolamide (10^{-5} M) did not affect the voltage.

Conclusion

It can be concluded from these results that (a) it is possible to study tubular acidification by microelectrodes in the isolated-perfused skate kidney; (b) the proximal tubule III of Raja erinacea is sensitive to furosemide and a Cl^--channel blocker in agreement with the data of Frömter and Beyenbach obtained in the dogfish (vide supra); (c) a K^+-$2Cl^-$-Na^+-cotransport system and a Cl^-/HCO_3^- exchanger both seem to be involved directly or indirectly in proximal tubular acidification; (d) amiloride, if applied from the peritubular side, does not greatly influence this acidification and (e) as expected, in fish, carbonic anhydrase inhibitors do not affect the membrane potential. The side of the tubule at which the drugs act is not fully clear because they are all likely to be secreted into the lumen. However, the fast effects of furosemide and DNDS suggest a peritubular effect (not excluding an additional luminal one), whereas the slower effect of the Cl^--channel blocker suggests a luminal action. Although further studies are needed to fully clarify tubular acidification in the skate proximal tubule, it is already clear that the process is greatly different from that in the mammalian proximal tubule.

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