EVIDENCE FOR HCO_3^-/Cl^--EXCHANGE MEDIATED HCO_3^- SECRETION IN THE RECTAL GLAND OF *SQUALUS ACANTHIAS*

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Last year we have analyzed the pH regulatory systems of isolated in vitro perfused rectal gland tubules of *Squalus acanthias* (Bleich, M. et al. Pfluegers Arch Eur J Physiol 436:248-254, 1998). We found that pH homeostasis is achieved by two transport systems in the basolateral membrane: Na^+/H^+ exchange which is inhibited by HOE 694 (0.1 mmol/l), and, as we have examined now, also by ethylisopropyl-amiloride (EIPA, IC_{50} 30 µmol/l, n = 5). The other basolateral transport system is probably a Na^+-$2HCO_3^-/Cl^-$ exchanger which serves for HCO_3^- import. Hence recovery from an acid load by these two systems is entirely Na^+ dependent. In the presence of HCO_3^- (20 mmol/l, 2% CO_2, pH 7.5) a large fraction of the realkalinization rate occurs via Na^+-$2HCO_3^-/Cl^-$ exchange and only a minor fraction by Na^+/H^+ exchange. We also provided preliminary evidence for the existence of luminal HCO_3^-/Cl^- exchange. Now we have examined this aspect in more detail and specifically asked whether HCO_3^- can be secreted by this system.

The experiments were performed in isolated and *in vitro* perfused rectal gland tubules (RGT) (Greger, R. and Schlatter E. Pfluegers Arch Eur J Physiol 402:63-75, 1984). In most of the experiments the RGT were loaded with BCECF-AM and 488/439 fluorescence ratio (BCFR) corresponding to cytosolic pH was monitored by a system described in detail previously (Bleich, M. et al. Pfluegers Arch Eur J Physiol 436:248-254, 1998). The calibration function was pH = (BCFR +2.93)/0.6, n = 6.

The RGT were acid loaded by the NH_4^+/NH_3 (20 mmol/l) pulse technique (Warth, R. et al. Pfluegers Arch Eur J Physiol 436:521-528, 1998). The addition of NH_4^+/NH_3 led to an initial significant increase in BCFR from 1.31 ± 0.02 to 1.46 ± 0.02 and thereafter to an exponential fall to 0.99 ± 0.01 (n = 94). BCFR fell further when NH_4^+/NH_3 was removed to 0.74 ± 0.01 (n = 93). In the absence of HCO_3^- and when in addition Na^+ was reduced to 5 mmol/l on the blood side very little pH recovery was seen. The rate of BCFR increase (rate of recovery = ΔBCFR/min) was 0.017 ± 0.007 (n = 31). This rate of recovery was increased markedly when HCO_3^- was added to the luminal perfusate: 0.16 ± 0.019 (n = 33). This effect of luminal HCO_3^- was blocked almost completely by luminal DIDS (0.1 mmol/l) and the recovery rate was reduced to 0.055 ± 0.018 (n = 15). This indicates that HCO_3^-, under these conditions can be taken up from the lumen and is compatible with the presence of HCO_3^-/Cl^- exchange.

Next we examined whether stimulation of Cl^- secretion by 0.5 mmol/l cAMP, 0.5 mmol/l adenosine and 10 µmol/l forskolin (Stim) has any impact on pH recovery. We found that Stim reduced BCFR slightly but significantly from 1.36 ± 0.04 to 1.29 ± 0.05 (n = 11). The recovery rate was again very low in the absence of HCO_3^- and with low basolateral Na^+: 0.044 ± 0.017 (n = 12). Addition of luminal HCO_3^- increased this rate markedly to 0.73 ± 0.20 (n = 12). A reduction of the luminal Na^+ concentration from 278 to 5 mmol/l had no influence on the rate of recovery:

0.60 ± 0.16 (n = 9). These data are qualitatively similar to that observed in the absence of Stim. Furthermore the data suggest that luminal HCO_3^-/Cl^- exchange is Na^+ independent.

Next we examined whether HCO_3^- is secreted by this luminal exchanger under normal conditions. To this end BCFR was measured in the presence of luminal and basolateral Na^+. BCFR was 1.25 ± 0.04 (n = 16) in the absence of basolateral HCO_3^- and was increased significantly by the basolateral addition of HCO_3^- to 1.36 ± 0.04 (n = 14). The addition of DIDS (0.1 mmol/l) to the luminal perfusate increased BCFR further to 1.43 ± 0.04 (n = 14). These data suggest that inhibition of luminal HCO_3^-/Cl^- exchange alkalinizes the cells because HCO_3^- secretion is inhibited.

To measure this HCO_3^- secretion more directly the lumen was perfused with anionic BCECF (membrane impermeable) and luminal fluorescence ratio was monitored. A typical experiment is shown in Fig. 1. It is evident that the addition of HCO_3^- to the bath leads to a marked increase in BCFR, directly showing the secretion of HCO_3^-. This experiment also shows that this alkalinization really occurs in the lumen an was not caused by contaminating cytosolic fluorescence, because the addition of NH_4^+/NH_3 to the bath only led to a rather slow alkalinization and not to the usual transient consisting of an initial alkalinization and a marked secondary acidification (cf. above). In a larger series of experiments the addition of HCO_3^- to the bath increased BCFR from 1.28 ± 0.03 to 1.39 ± 0.04 (n = 26).

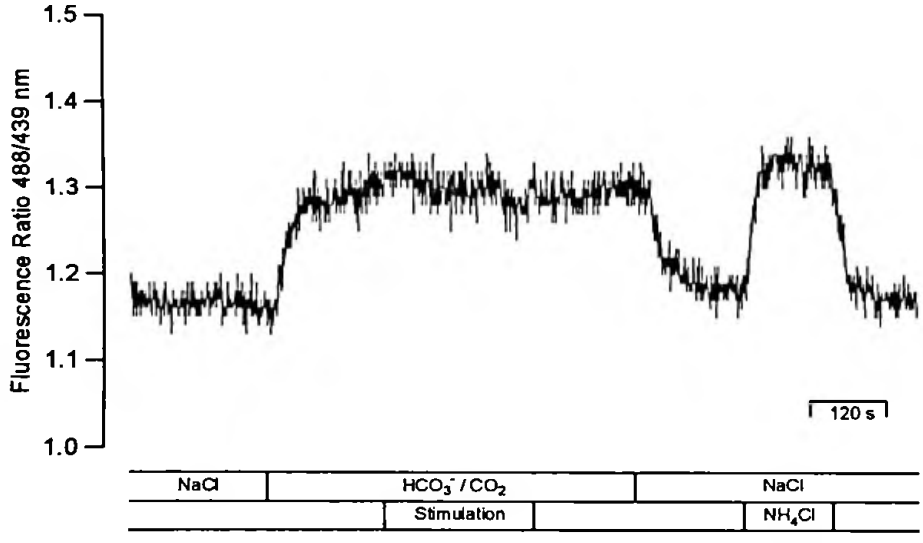

Figure 1: BCECF-fluorescence measurement in the lumen of an isolated perfused recal gland tubule of *Squalus acanthias*.

These data can be summarized as follows. In addition to the pH homeostatic systems in the basolateral membrane (Na^+/H^+ exchange and Na^+-$2HCO_3^-/Cl^-$ exchange) rectal gland tubules possess a HCO_3^-/Cl^- exchanger in the luminal membrane. This exchange is Na^+ independent and is inhibited by DIDS. The HCO_3^-/Cl^- exchanger can, depending on the experimental conditions, operate as a HCO_3^- uptake and a HCO_3^- extrusion system. Physiologically it will mainly serve HCO_3^- secretion. In fact, Swenson and Maren (Swenson, E. and Maren, T. Am J Physiol

253:R450-R458, 1987) have shown many years ago that RG cells secrete HCO_3^- during metabolic alkalosis.

The constellation of CFTR-type Cl^- conductance in the luminal membrane in conjunction with HCO_3^-/Cl^- exchange is reminiscent of the pancreatic duct (Novak, I. and Greger, R. Pfluegers Arch Eur J Physiol 411:546-553, 1988) where the parallel arrangement of both transporters als serves HCO_3^- secretion.

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