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APICAL BICARBONATE TRANSPORT IN PANCREATIC DUCT CELLS

Hiroshi Ishiguro[1], Satoru Naruse[2], Maynard Case[3] and Martin Steward[3]

[1]Human Nutrition, Nagoya University Graduate School of Medicine, [2]Gastroenterology, Nagoya University Graduate School of Medicine, [3]Faculty of Life Sciences, The University of Manchester, Manchester, UK

Pancreatic duct epithelial cells secrete a HCO_3^--rich isotonic fluid. Recent evidence suggests that the mechanism of HCO_3^- transport across the apical membrane changes according to the anion composition of the luminal fluid. When luminal Cl^- concentration is high, intracellular HCO_3^- exits in exchange for luminal Cl^-, probably via one of the SLC26 family anion exchangers, and CFTR works as a Cl^- efflux pathway to maintain the inward Cl^- gradient. As the luminal HCO_3^- concentration rises and the luminal Cl^- concentration falls, the apical anion exchangers are no longer able to support HCO_3^- secretion, and CFTR, acting as a HCO_3^- channel, is thought to take over as the principal efflux pathway for HCO_3^-. In this study we have estimated the apical HCO_3^- permeability of guinea-pig pancreatic duct cells by measuring changes in intracellular pH when the cells were de- or hyper-polarized by manipulation of extracellular K^+. Interlobular ducts ($\sim$100 μm in diameter) were isolated by collagenase digestion and microdissection. Intracellular pH (pH_i) was measured by microfluorometry at 37°C in ducts loaded with the pH-sensitive fluoroprobe BCECF. Dihydro-4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid (H_2DIDS) was used to inhibit basolateral HCO_3^- transport. Changes in bath K^+ concentrations ($[K^+]_B$) were achieved by replacement with N-methyl-D-glucamine and extracellular Na^+ was fixed at 60 mM. Isolated ducts were superfused with the standard HCO_3^--buffered solution (25 mM HCO_3^-, 124 mM Cl^-, 5% CO_2) containing H_2DIDS (0.5 mM) and dibutyryl AMP (0.5 mM), and luminally perfused with a solution contaning 125 mM HCO_3^-, 24 mM Cl^-, and 5% CO_2. De- and hyper-polarization (by changing $[K^+]_B$ from 5 to 70 mM and 1 mM) caused increase and decrease in pHi that reflected the influx and efflux of HCO_3^- across the apical membrane. Apical membrane HCO_3^- fluxes were calculated from the rate of pHi change by taking into account the intracellular buffering capacity. The values of intracellular potential (V_m), when $[K^+]_B$ was 1, 5, and 70 mM, were estimated to be -40, -50, and -60 mV using conventional microelectrodes. From the HCO_3^- flux and Vm values (and assuming a cell height of 10 μm), the HCO_3^- permeability coefficient of the apical membrane was calculated to be $\sim$0.1 μm sec^{-1}, which is close to the value required to account for the secretion of fluid containing140 mM HCO_3^-.

Key Words: bicarbonate transport, pancreatic duct, CFTR, bicarbonate permeability

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DISORDERS OF NEUTRAL AMINO ACID TRANSPORT IN EPITHELIAL CELLS

Stefan Broer

School of Biochemistry & Molecular Biology, Australian National University, Canberra, Australia

Neutral amino acids are absorbed in the intestine by specific transporters located in the apical membrane of intestinal enterocytes. Subsequently amino acids are released across the basolateral membrane and are being delivered to all tissues. In the kidney amino acids pass through the glomerulus and are reabsorbed from the primary urine. Mutations in neutral amino acid transport systems of the apical membrane are associated with disorders such as Hartnup disorder and Iminoglycinuria. Analysis of a large number of families diagnosed with Hartnup disorder indicates that SLC6A19 is the major gene associated with this disorder. Functional analysis demonstrates that the transport activity is impaired due to the mutations. Comparison of the amino acid profile in the urine of Hartnup disorder individuals with the substrate specificity of SLC6A19 suggests the presence of additional transporters for neutral amino acids in the kidney proximal tubule and in the intestine. The neutral amino acid transporter SLC1A5 is also expressed in the proximal tubule and intestine, but it cannot mediate net uptake due to its antiport mechanism. Additional transporters for proline, glycine and methionine are expressed in epithelial cells. SLC6A20 was identified as a high-affinity proline transporter, which is expressed in the intestine and more distal parts of the proximal tubule. These and other transporters are candidates for being mutated in Iminoglycinuria and methionine malabsorption syndrome.