MINI-REVIEW

Apical Cl^-/HCO_3^- exchanger stoichiometry in the modeling of HCO_3^- transport by pancreatic duct epithelium

Makoto Yamaguchi[1], Hiroshi Ishiguro[1], Martin Steward[2], Yoshiro Sohma[3], Akiko Yamamoto[1], Akito Shimouchi[4], and Takaharu Kondo[1]

[1]*Department of Human Nutrition, Nagoya University Graduate School of Medicine, Nagoya, Japan ;* [2] *Faculty of Life Sciences, University of Manchester, Manchester, UK ;* [3]*Department of Pharmacology, School of Medicine, Keio University, Tokyo, Japan ; and* [4]*Department of Etiology and Pathogenesis, National Cardiovascular Center Research Institute, Osaka, Japan*

Abstract : Pancreatic duct cells secrete a HCO_3^--rich (~140 mM) fluid. Using a computer model of the pancreatic duct, Sohma, *et al.* have demonstrated that the activity of a Cl^-/HCO_3^- exchanger with a 1 : 1 stoichiometry at the apical membrane would have to be suppressed in order to achieve such a HCO_3^--rich secretion. Recently the apical exchanger in pancreatic ducts has been identified as SLC26A6 and this probably mediates most of Cl^--dependent HCO_3^- secretion across the apical membrane. SLC26A6 is reported to mediate electrogenic $Cl^-/2HCO_3^-$ exchange when expressed in *Xenopus* oocytes. To assess the implications of this 1 : 2 stoichiometry for HCO_3^- secretion, we have reconstructed the Sohma model using MATLAB/Simulink. To do this we have formulated an expression for the turnover rate of $Cl^-/2HCO_3^-$ exchange using network thermodynamics and we have estimated the constants from published experimental data. Preliminary data suggest that the 1 : 2 stoichiometry of SLC26A6 would favor HCO_3^- secretion at higher concentrations. J. Med. Invest. 56 Suppl. : 325-328, December, 2009

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MECHANISMS OF HCO_3^- SECRETION BY PANCREATIC DUCT

Pancreatic duct epithelium secretes a HCO_3^--rich isotonic fluid that is dependent on the activity of the cystic fibrosis transmembrane conductance regulator (CFTR) at the apical membrane. The HCO_3^- concentration of human and guinea-pig pancreatic juice reaches ~140 mM at maximal stimulation with secretin. Fig. 1 shows the current model for HCO_3^- transport by pancreatic duct cells. Accumulation of HCO_3^- across the basolateral membrane is mediated by Na^+-HCO_3^- cotransport and Na^+-H^+ exchange. In guinea-pig pancreatic duct, Na^+-HCO_3^- cotransport accounts for ~75% of HCO_3^- accumulation. HCO_3^- secretion across the apical membrane is mediated (i) by Cl^-/HCO_3^- exchange via an SLC26A6 anion transporter and (ii) by the HCO_3^- conductance of CFTR. The relative contribution of these two apical mechanisms varies depending on the anion composition of the luminal fluid (1). This model is based on measurements of intracellular pH (pH_i), Cl^- concentration ($[Cl^-]_i$), and membrane potential (P_d) in luminally-microperfused interlobular duct segments isolated from guinea-pig pancreas (2).

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Address correspondence and reprint requests to Makoto Yamaguchi, Human Nutrition, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan and Fax : +81-52-744-2183.

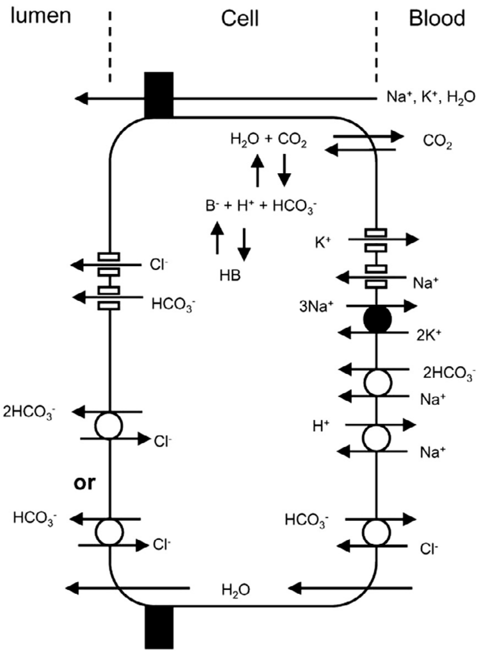

Fig. 1 Schematic representation of the ion transport systems in our mathematical model of electrolyte secretion by pancreatic duct epithelium. Alternative stoichiometries (1 : 1 and 1 : 2) are shown for Cl^-/HCO_3^- exchange at the apical membrane.

COMPUTER SIMULATION OF HCO_3^- SECRETION BY PANCREATIC DUCT

The transporters and channels in Fig. 1 are individually regulated by various factors and interact with each other physically and functionally via changes in pH_i, $[Cl^-]_i$ and P_d. A computer simulation of this model is required if we are to understand how such a complicated system secretes 140 mM HCO_3^- into an already HCO_3^--rich luminal fluid and achieves the observed fluid secretory rate.

Sohma, *et al.* (3, 4) have constructed a computer model of pancreatic duct epithelium using the FORTRAN programming language. In this model, the stoichiometry of the apical Cl^--HCO_3^- exchanger was assumed to be 1 : 1 and the HCO_3^-/Cl^- permeability ratio of CFTR was set at 0.2. When the luminal HCO_3^- concentration ($[HCO_3^-]_L$) was kept constant at 25 mM, the HCO_3^- concentration of the secreted fluid ($[HCO_3^-]_F$) was 145 mM and ~75% of the apical HCO_3^- secretion was mediated by Cl^-/HCO_3^- exchange (and the remaining 25% by CFTR). When $[HCO_3^-]_L$ was set to match the composition of the secreted fluid ($[HCO_3^-]_F = [HCO_3^-]_L$), as occurs *in vivo*, the model secreted only ~120 mM HCO_3^- and ~94% of apical HCO_3^- secretion was mediated by the HCO_3^- conductance of CFTR. To achieve 140 mM HCO_3^- secretion, additional assumptions were required to prevent Cl^- secretion and HCO_3^- absorption. These were : (i) a reduced apical Cl^- permeability and (ii) a reduced activity of the apical Cl^-/HCO_3^- exchanger when the apical membrane faces a high luminal HCO_3^- concentration. The first assumption was supported by experimental data on guinea-pig pancreatic duct cells (5) but the second one has not been verified.

Whitcomb and Ermentrout constructed a simpler model using the authors' own program, XPPAUT (6). The model assumed (i) a small cell : lumen volume ratio (10 : 1), (ii) a constant inflow of Cl^--rich (acinar) fluid at the proximal end of the duct lumen, (iii) outflow of the mixture of acinar fluid and fluid secreted by duct cell at the distal end, and (iv) no Cl^-/HCO_3^- exchange activity in the basolateral membrane. The model achieved >140 mM HCO_3^- secretion which was not affected by the activity of the apical $1Cl^-/1HCO_3^-$ exchanger.

These computer simulations have significantly contributed to our understanding of HCO_3^- transport mechanisms in the pancreatic duct. However, considerable skill in computer programming is required for other researchers to explore the properties of these simulations and examine the effects of modifications to the model as more information becomes available. One of the aims of our study has therefore been to reconstruct the Sohma model (4) in a more user-friendly software environment, namely MATLAB (MathWorks, Natick, MA). This provides an interactive graphical environment for multidomain simulation (Simulink) in which block diagrams representing individual channels, transporters and solutes can be easily assembled into a time-varying system. Simulink models are also readily portable between users and relatively simple to modify.

An example of the need for this flexibility is the recent discovery that the apical Cl^-/HCO_3^- exchanger is probably a member of the SLC26 family of anion exchangers and that it may be electrogenic. SLC 26A3 and SLC26A6 have both been identified in pancreatic duct cells and are reported to mediate $2Cl^-/HCO_3^-$ and $Cl^-/2HCO_3^-$ exchange respectively when expressed in *Xenopus laevis* oocytes (7), although there remain some discrepancies between the data from different laboratories. Our second aim has therefore been to explore the effects of altering the stoichiometry of the apical anion exchanger on the behaviour of the model. In particular, we wish to determine whether the proposed 1 : 2 stoichiometry of SLC26A6 enhances the ability of the model to generate a HCO_3^--rich secretion.

EFFECTS OF ALTERED Cl^-/HCO_3^- EXCHANGER STOICHIOMETRY AT THE APICAL MEMBRANE

A simple calculation of the predicted equilibrium condition for apical Cl^-/HCO_3^- exchangers with various stoichiometries suggest that a $Cl^-/2HCO_3^-$ exchanger, such as SLC26A6, would be able to secrete HCO_3^- into a higher luminal HCO_3^- concentration than exchangers with 1 : 1 or 2 : 1 stoichiometries (1). The 1 : 2 exchanger would not be expected to reverse and reabsorb HCO_3^- until $[HCO_3^-]_L$ reaches ~136 mM. It might therefore not have to be suppressed in order to achieve a secreted HCO_3^- concentration of 140 mM. We have addressed this question using our reconstruction of the Sohma model in MATLAB/Simulink.

The first task was to formulate the turnover rate of a $Cl^-/2HCO_3^-$ exchanger from network thermodynamics (Fig. 2). This gave the following expression :

$$J_{slc26a6} = G_{slc26a6}\cdot\frac{\begin{array}{l}\{([Cl^-]_o/K_{Cl})\cdot([HCO_3^-]_i/K_{HCO3})^2\cdot\exp(F\cdot P_d/2\cdot R\cdot T)\}\\ -\{([Cl^-]_i/K_{Cl})\cdot([HCO_3^-]_o/K_{HCO3})^2\cdot\exp(-F\cdot P_d/2\cdot R\cdot T)\}\end{array}}{\begin{array}{l}\{([HCO_3^-]_o/K_{HCO3})^2\cdot\exp(-F\cdot P_d/2\cdot R\cdot T)+R_{k/l}([Cl^-]_o/K_{Cl})\}\\ \{1+([Cl^-]_i/K_{Cl})+([HCO_3^-]_i/K_{HCO3})^2\}\\ +\{([HCO_3^-]_i/K_{HCO3})^2\cdot\exp(F\cdot P_d/2\cdot R\cdot T)+R_{k/l}([Cl^-]_i/K_{Cl})\}\\ \{1+([Cl^-]_o/K_{Cl})+([HCO_3^-]_o/K_{HCO3})^2\}\end{array}}$$

where K_{Cl} and K_{HCO3} are the dissociation constants, $G_{slc26a6}$ is the permeability coefficient, and $R_{k/l}$ is the ratio of the velocity constants of the cross-membrane steps with and without the bound ions. R, T, and F have their usual meanings.

To determine the values of the constants in this equation, we have constructed a model of the *Xenopus laevis* oocyte incorporating an SLC26A6 $Cl^-/2HCO_3^-$ exchanger and an intracellular pH buffering system. By fitting this model to the published experimental data for the effects of extracellular Cl^- substitution on pH_i, $[Cl^-]_i$, and P_d (7), values of K_{Cl} (40 mM), K_{HCO3} (40 mM), and $R_{k/l}$ (1.0) were determined.

We are now using our Simulink model of the pancreatic duct to examine the effects of this altered stoichiometry on HCO_3^- secretion when the duct lumen is perfused with 125 mM HCO_3^-, as it has been in many of our microperfusion experiments. Our preliminary results suggest that, under these conditions, a 1 : 2 exchanger such as SLC26A6 will

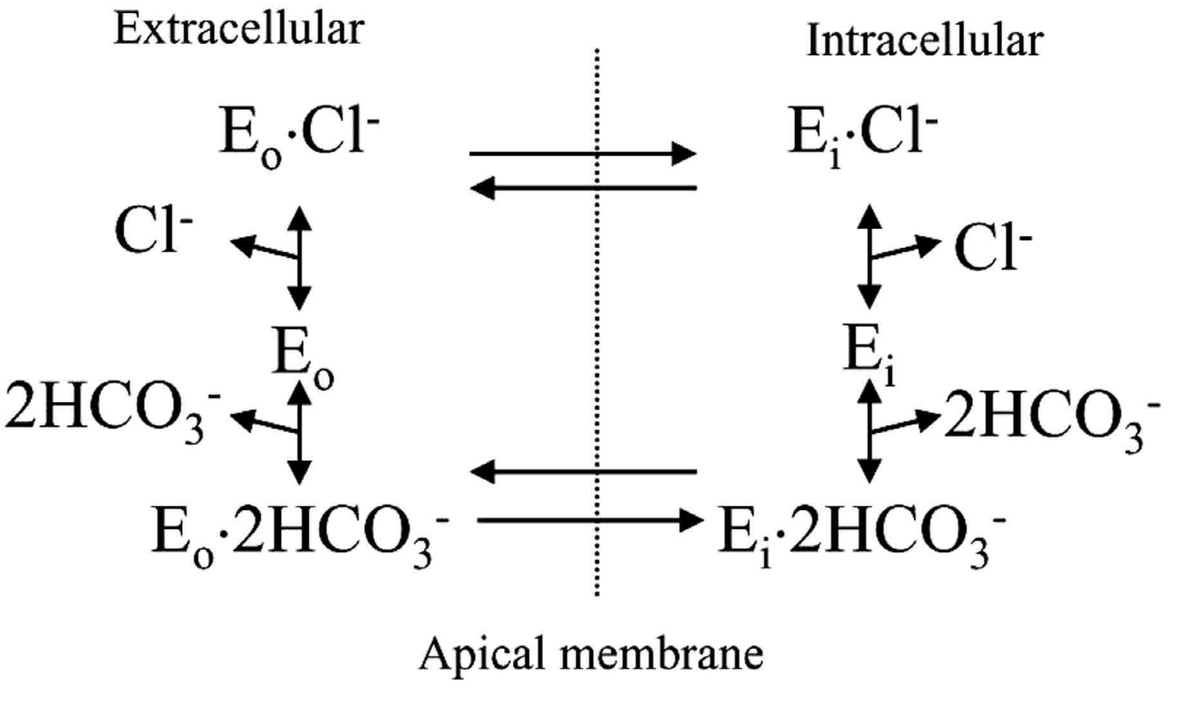

Fig. 2 Kinetic model for $Cl^-/2HCO_3^-$ exchange by SLC26A6. E_i and E_o represent the alternative conformations of the exchanger.

mediate apical HCO_3^- secretion whereas a 1 : 1 exchanger will mediate HCO_3^- absorption. This finding supports the idea that the 1 : 2 stoichiometry of SLC26A6 will facilitate the secretion of HCO_3^- at the higher concentrations observed in the guinea-pig and human pancreas.

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