## EXPANDED ABSTRACT

# Molecular characterization of Slc26a3 and Slc26a6 anion transporters in guinea pig pancreatic duct

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The majority of fluid and $HCO_3^-$ in pancreatic juice arises from the pancreatic duct cells. Secretin stimulates $HCO_3^-$ secretion *via* a mechanism that involves activation of the adenylate cyclase pathway, activation of both basolateral $K^+$ channels and the apical CFTR $Cl^-$ channel, and stimulation of an apical Slc26-mediated $Cl^-/HCO_3^-$ exchanger (1). The Slc26 anion exchangers, Slc26a3 and Slc26a6, have both been localized to the apical membrane of human pancreatic duct and mouse pancreatic duct although their functional roles in pancreatic $HCO_3^-$ secretion remain uncertain. The high rates of pancreatic bicarbonate secretion shared by guinea pig (gp) and human, in contrast to the low rates in mouse, prompted us to study the functional characteristics of gp Slc26 anion transporters. Furthermore, recent data from isolated guinea pig pancreatic duct experiments demonstrated that during stimulated $HCO_3^-$ secretion an apical Slc26a6-like $Cl^-/HCO_3^-$ exchanger is functionally coupled with CFTR (2). This study prompted us to clone cDNAs encoding Slc26a3 and Slc26a6 polypeptides from the gp pancreas and to compare their functional characteristics in *Xenopus* oocytes with those of their mouse and human orthologs (3-5), and with those of luminal $Cl^-/HCO_3^-$ exchange measured in isolated gp pancreatic interlobular ducts (2).

Whereas gp Slc26a6 mediated $Cl^-/Cl^-$ exchange and bidirectional exchange of $Cl^-$ for oxalate and $HCO_3^-$, gp Slc26a6 mediated only sulfate influx but not sulfate efflux in preliminary experiments. Gp Slc26a3 mediated robust $Cl^-/Cl^-$ exchange and minimal oxalate efflux in exchange for $Cl^-$, but did not mediate detectable influx of oxalate or sulfate. Measurement of intracellular pH by BCECF ratio fluorimetry in HEK cells transfected with Slc26a6 or in Slc26a3-expressing *Xenopus* oocytes during removal and restoration of $Cl^-_o$ in the presence of $HCO_3^-$ indicated that both gp Slc26a6 and Slc26a3 function as $Cl^-/HCO_3^-$ exchangers. These substrate specificity experiments reveal that whereas Slc26a3 functions predominantly as a $Cl^-/HCO_3^-$ exchanger, Slc26a6 can exchange a wider range of substrates, functioning as a $Cl^-/HCO_3^-$ exchanger or a $Cl^-$/anion exchanger. This finding allows for the possibility that Slc26a6 may have alternative roles in transepithelial solute transport in the pancreatic duct.

The apparent affinity of gp Slc26a6 for extracellular $Cl^-$ ($K_{1/2}$) was ~2 mM during $^{36}Cl^-_i/Cl^-_o$ exchange and ~30 mM during $^{14}$C-oxalate$_i$/$Cl^-_o$ exchange. These experiments reveal that binding and/or translocation rates of extracellular $Cl^-$ with respect to gp slc26a6 is/are dependent on the internal transanion substrate and show species-specificity (5). The high affinity for extracellular $Cl^-_o$ suggests that even when duct luminal $Cl^-$ is low, such as might occur with passage of pancreatic juice along the duct lumen, Slc26a6-mediated $Cl^-/HCO_3^-$ exchange would still be active. Whereas gp Slc26a6-mediated isotopic flux was moderately sensitive to DNDS (500 μM), niflumic acid (100 μM) and DIDS (500 μM), gp Slc26a3 was minimally sensitive to inhibition by DIDS and DNDS but strongly sensitive to niflumic acid. These pharmacological data, combined with

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those from isolated guinea pig interlobular ducts identifying a DIDS-sensitive $Cl^-/HCO_3^-$ exchanger that mediates the majority of forskolin-stimulated $HCO_3^-$ secretion during recovery from an intracellular alkaline load, suggest a dominant role for Slc 26a6 in guinea pig ductal $HCO_3^-$ secretion. In contrast, the role of Slc26a3-mediated $Cl^-/HCO_3^-$ exchange in pancreatic ductal secretion remains in question.

The ability of the pancreatic duct to secrete a high concentration of $HCO_3^-$ has been attributed, at least in part, to electrogenic apical $Cl^-/HCO_3^-$ exchange. However, the stochiometry of Slc26a6-and Slc26a3-mediated $Cl^-$/anion exchange in heterologous expression systems (5, 6) and in intestinal mucosa (7, 8) remains a point of controversy. Both gp Slc26a3 and Slc26a6-mediated electroneutral $Cl^-/HCO_3^-$ exchange in *Xenopus* oocytes, as judged by pH-sensitive microelectrode measurements and by two-electrode voltage clamp recording under the same experimental conditions. In contrast, measurements of membrane potential in isolated guinea pig interlobular ducts under conditions that activate apical $Cl^-/HCO_3^-$ exchange have suggested the presence of an electrogenic, DIDS-sensitive apical $Cl^-/HCO_3^-$ exchanger consistent with electrogenic Slc26a6 (2). These discrepant results suggest either that Slc26a6 requires an additional cofactor (such as CFTR) to mediate electrogenic anion exchange or, alternatively, that the DIDS-sensitive $Cl^-/HCO_3^-$ exchange measured in isolated ducts was not mediated by Slc26a6. Our current data reinforce the conclusion that functional differences are present among orthologous Slc26 anion exchangers of different species (3) and should be considered in modeling the role of Slc26 $Cl^-/HCO_3^-$ exchangers in pancreatic duct bicarbonate secretion.

In conclusion, data obtained to date with cloned guinea pig Slc26 anion exchangers continue to highlight species-specific differences in Slc26 anion transporter function. These differences may be important in formulating a model for the secretion of 140 mM $HCO_3^-$ by guinea pig pancreatic duct, and may suggest new insights into the molecular mechanism of human pancreatic bicarbonate secretion.

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