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A Novel Na^+-Dependent Transporter and NHE3 Mediate H^+ Efflux in the Luminal Membrane of the Pancreatic Duct: Regulation by cAMP

Min Goo Lee, Wooin Ahn, Joo Young Choi*, Shmuel Muallem*, Kyung Hwan Kim

Department of Pharmacology, Yonsei University College of Medicine, Seoul, Korea
Department of Physiology*, University of Texas Southwestern Medical Center, Dallas, U.S.A.

The pancreatic duct secretes fluid rich in Na^+ and HCO_3^- (1). HCO_3^- secretion across the luminal membrane (LM) requires H^+ secretion or HCO_3^- absorption across the basolateral membrane (BLM) to maintain constant cytosolic pH (pH_i) and replenish the secreted HCO_3^-. A Na^+/H^+ exchanger (NHE) in the BLM is assumed to help HCO_3^- secretion by controlling pH_i to prevent intracellular acidification during HCO_3^- secretion (2). Since the pancreatic duct is a HCO_3^- and Na^+ secreting tissue, NHEs are not expected to be present in the LM. However, using perfused ducts we and others reported a NHE activity in the LM of pancreatic ducts of different species (2, 3). The proteins mediating this activity, their regulation and physiological significance are unknown.

Key Words: *Na^+/H^+ Exchanger (NHE); Pancreas; Duct; Bicarbonate*

Address for correspondence: Professor Kyung Hwan Kim
Department of Pharmacology, Yonsei University College of Medicine, 134 Sinchon-dong, Seodaemoon-gu, Seoul 120-752, Korea, Tel: +82.2-361-5224, Fax: +82.2-313-1894, E-mail: hwan444@yumc.yonsei.ac.kr

In the present work, we characterized H^+ efflux mechanisms in pancreatic ducts from wild type (WT), NHE2-/- and NHE3-/- mice. RT-PCR analysis in combination with immunolocalization showed that the pancreatic duct expresses NHE1 in the basolateral membrane and NHE2 and NHE3 in the luminal membrane (not shown). NHE4 and NHE5 are not expressed in the pancreatic duct. Measurement of Na^+-dependent H^+ efflux in the microperfused duct demonstrated a basolateral activity inhibitable by 1.5 μM HOE 694, consistent with expression of NHE1, and a luminal activity inhibitable by 50 μM HOE 694, consistent with expression of NHE2 (Fig. 1).

However, disruption of the *NHE2* gene had no effect on luminal transport. By contrast, disruption of the *NHE3* gene reduced luminal Na^+-dependent H^+ efflux by about 45% (Fig. 2). Notably, the remaining luminal Na^+-dependent H^+ efflux in ducts from NHE3-/- mice was inhibited by 50 μM HOE 694. Hence, nearly 55% of luminal H^+ efflux in the pancreatic duct is mediated by

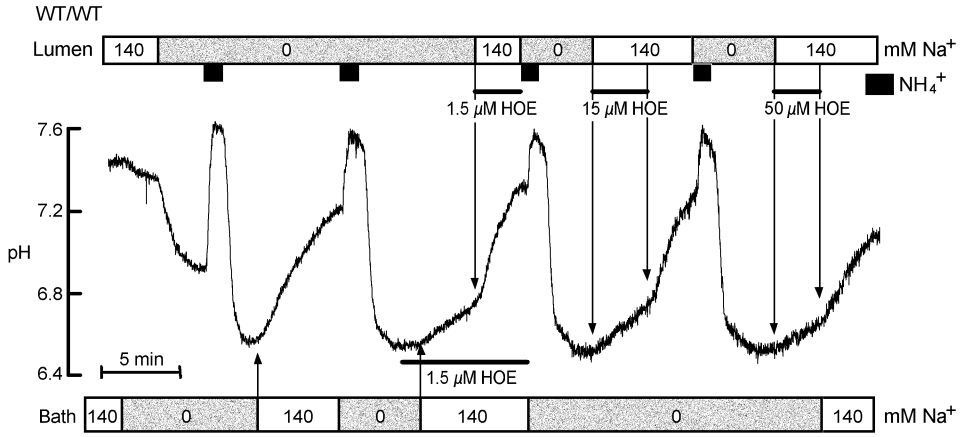

Fig. 1. Na^+-dependent H^+ efflux in the BLM and LM of the main pancreatic duct. BLM and LM NHE activity was measured in the main pancreatic duct. After NH_4^+-induced acidification, basolateral application of Na^+-containing medium cause a rapid increase in pH_i. BLM NHE activity is blocked by 1.5 μM HOE 694. By contrast, subsequent luminal application of a Na^+-containing medium with 1.5 μM HOE 694 shows a fast Na^+-dependent pH_i recovery. Inhibition of LM NHE activity require 50 μM HOE 694.

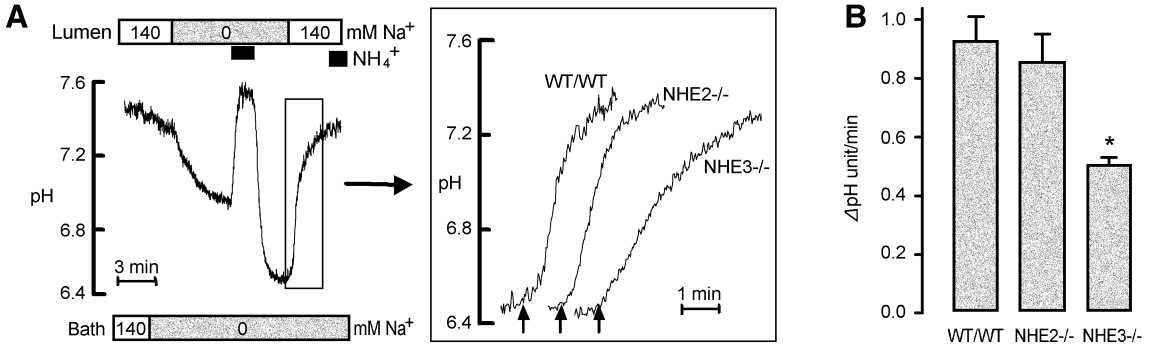

Fig. 2. Luminal Na^+-dependent H^+ efflux in pancreatic ducts of WT, NHE2-/- and NHE3-/- mice. Luminal Na^+-dependent H^+ efflux was measured in ducts acidified to the same level using the indicated protocol. Panel **A** shows examples of individual traces from each mouse line. Panel **B** shows the summary of 5, 4 and 6 experiments performed with ducts from WT, NHE2-/- and NHE3-/- animals, respectively.

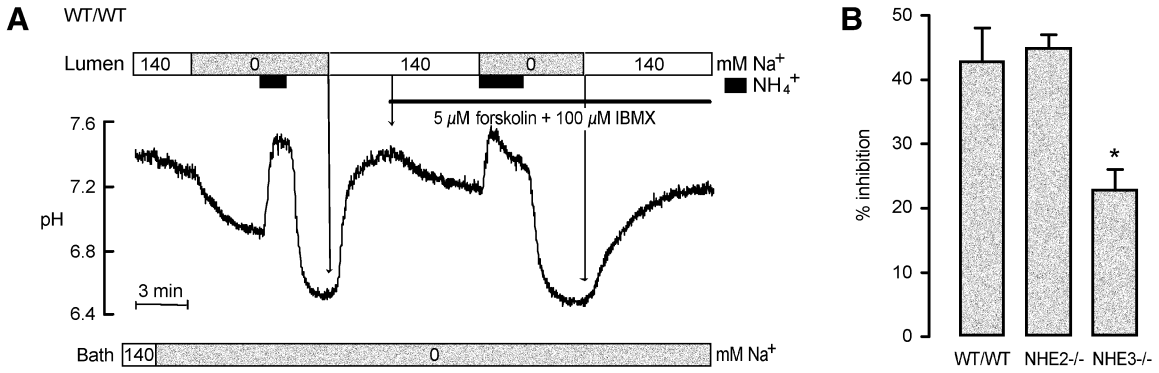

Fig. 3. Regulation of luminal NHE activities by Forskolin. After the control period ducts were treated with a Forskolin cocktail (5 μM forskolin and 100 μM IBMX) for 5 min. **(A)** A representative trace using the pancreatic duct of a WT mouse. **(B)** Summary of the multiple experiments. Forskolin treatment reduces the luminal NHE activity by 43±5% (n=5) in WT, 45±2% (n=6) in NHE2-/-, and 23±6% (n=6) in NHE3-/- mice. *$p<0.05$: difference from WT.

a novel, HOE 694 (or amiloride)-sensitive, Na^+-dependent mechanism. H^+ transport by the two luminal mechanisms is inhibited by cAMP stimulation, albeit to a different extent (Fig. 3).

In summary, we believe that the present work highlights an under appreciated but important function of the pancreatic duct, that is the control of pancreatic secretion and the composition of the pancreatic juice under basal conditions. It appears that luminal NHE3 and a novel Na^+-dependent, amiloride-sensitive H^+ efflux mechanisms reabsorb Na^+, HCO_3^- and fluid, probably to produce an acidic, Cl^--rich pancreatic juice under resting conditions. Upon cell stimulation by agonists that act through cAMP, these mechanisms are inhibited to allow production of a HCO_3^--rich pancreatic juice.

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