## PROCEEDINGS

# Glucose transport in interlobular ducts isolated from rat pancreas

Sachiko Futakuchi[1], Hiroshi Ishiguro[2], Satoru Naruse[3], Shigeru BH Ko[1], Kotoyo Fujiki[4], Akiko Yamamoto[2], Miyuki Nakakuki[2], Ying Song[2], Martin C Steward[5], Takaharu Kondo[2], and Hidemi Goto[1]

*Departments of [1]Gastroenterology and [2]Human Nutrition, Nagoya University Graduate School of Medicine, Nagoya, Japan ; [3]Miyoshi Municipal Hospital, Aichi, Japan ; [4]Nagoya University of Arts and Sciences, Nagoya, Japan ; and [5]Faculty of Life Sciences, University of Manchester, Manchester, UK*

**Abstract : Pancreatic duct cells express $Na^+$-dependent glucose transporter, SGLT1 and $Na^+$-independent glucose transporters, GLUT1, GLUT2, and GLUT8. We examined transepithelial glucose transport by pancreatic duct. Interlobular ducts were isolated from rat pancreas. During overnight culture both ends of the duct segments sealed spontaneously. The lumen of the sealed duct was micropunctured and the luminal fluid was replaced by HEPES-buffered solution containing 10.0 mM or 44.4 mM glucose. The bath was perfused with HEPES-buffered solution at 37℃ containing 10.0 or 44.4 mM glucose. Transepithelial differences in osmolality were balanced with mannitol. Glucose transport across ductal epithelium was measured by monitoring changes in luminal volume. When the lumen was filled with 44.4 mM glucose, with either 10.0 or 44.4 mM glucose in the bath, the luminal volume decreased to 65~70% of the initial volume in 15 min. Luminally-injected phlorizin, an inhibitor of SGLT1, abolished the decrease in luminal volume. With 10.0 mM glucose in the lumen and 44.4 mM glucose in the bath, the luminal volume did not change significantly. Luminal application of phlorizin under identical condition led to an increase in luminal volume. The data suggest that both active and passive transport mechanisms of glucose are present in pancreatic ductal epithelium. J. Med. Invest. 56 Suppl. : 308-311, December, 2009**

***Keywords*** **:** *pancreatic duct cell, glucose transport, SGLT1*

## INTERACTION BETWEEN THE EXOCRINE AND ENDOCRINE PANCREAS

Exocrine pancreas is composed of acinar units and the duct system. Acinar cells secrete digestive enzymes and small amount of $Cl^-$-rich fluid, while ductal epithelium lining small (proximal) pancreatic ducts secretes large amount of $HCO_3^-$-rich isotonic fluid. Endocrine islets of Langerhans are dispersed throughout the exocrine tissue. A number of functional interrelations exist between exocrine and endocrine pancreas and most of them are mediated by the islet-acinar portal blood system that islet peptides directly regulate acinar cells (1). Exocrine dysfunction is frequently found in patients with type-I (insulin-dependent) and type-II (noninsulin-dependent) diabetes mellitus (DM). Fluid and $HCO_3^-$ secretion, as well as enzyme secretion, are reduced in DM (2), suggesting that both acinar and ductal functions are impaired. It is generally accepted that

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Address correspondence and reprint requests to Hiroshi Ishiguro, Research Center of Health, Physical Fitness, and Sports, Nagoya University, (Human Nutrition, Nagoya University Graduate School of Medicine), Furo-cho E5-2 (130), Chikusa-ku, Nagoya 464-8601, Japan and Fax : +81-52-789-3957.

acinar dysfunction is due mainly to the absence of the trophic effects of insulin on acinar cells (1). However, the mechanisms underlying the ductal dysfunction (reduced fluid and $HCO_3^-$ secretion) have been unknown.

## HIGH GLUCOSE INHIBITS $HCO_3^-$ AND FLUID SECRETION IN ISOLATED PANCREATIC DUCTS

We recently examined the cellular mechanisms underlying the impairment of pancreatic fluid and $HCO_3^-$ secretion in diabetes by using interlobular ducts isolated from rat pancreas (3). Interlobular ducts (diameter : ~150 μm) were isolated by microdissection and cultured overnight. During the culture both ends of the duct segments sealed spontaneously. Ducts were superfused with the standard $HCO_3^-$-buffered solution at 37°C. Fluid secretion was assessed by monitoring the luminal volume and $HCO_3^-$ accumulation across the basolateral membrane was estimated from the recovery of intracellular pH following an $NH_4^+$-induced acid load. Exposure to high glucose concentrations inhibited fluid secretion and reduced the rate of basolateral $HCO_3^-$ uptake in secretin-stimulated ducts isolated from normal rats. In ducts isolated from streptozotocin-treated diabetic rats, fluid secretion and basolateral $HCO_3^-$ uptake were severely impaired but largely reversed by incubation in normal-glucose solutions. The data suggest that the impaired fluid secretion and $HCO_3^-$ transport in diabetic ducts are largely reversible and attributed mainly to the inhibition of basolateral transporters by the high extracellular glucose concentration. Insulin depletion itself may not have any significant effects on ductal secretion. Correction to normoglycemia may improve pancreatic fluid secretion in DM patients.

## GLUCOSE TRANSPORT BY EPITHELIA

Epithelial tissues express $Na^+$-dependent glucose transporters (SGLT) and $Na^+$-independent glucose transporters (GLUT) in the plasma membrane. The small intestine absorbs luminal glucose by both active and passive pathways (4). The apparent $K_m$ for active glucose absorption in rat jejunum *in vivo* is ~27 mM, while the $K_m$ for passive component is ~56 mM. The active glucose absorption by enterocytes involves SGLT1 at the apical membrane and GLUT2 at the basolateral membrane.

Active re-absorption of luminal glucose has been also found in kidney proximal tubule, bile duct, and salivary gland. Takai *et al.* reported that active re-absorption mechanism of luminal glucose was present in rat submandibular gland but absent in sublingual gland (5). Glucose concentration of submandibular saliva evoked by chorda stimulation (0.05-0.50 mg/dl) was much lower than of plasma irrespective of the flow rate. Intraductal injection of phlorizin (an inhibitor of SGLT1) caused elevation of saliva glucose concentrations which show inverse relationship to the flow rate. The data also suggest the presence of passive glucose transport probably via the paracellular pathway.

## GLUCOSE TRANSPORT BY PANCREATIC DUCTAL EPITHELIUM

RT-PCR analysis of isolated interlobular ducts from rat pancreas revealed messenger RNA expression of SGLT1, GLUT1, GLUT2, and GLUT8 (3). Raising the luminal glucose concentration in microperfused ducts caused a depolarization of the membrane potential, which was consistent with the presence of SGLT1 at the apical membrane. By analogy with the intestine, we predict that the presence of SGLT1 in the apical membrane enables the ductal epithelium to reabsorb luminal glucose against a steep concentration gradient (Figure 1B). Indeed, it has been known that the concentration of glucose in human pancreatic juice (0.5~1 mM) is much lower than in plasma (6).

To examine transepithelial transport of glucose by pancreatic duct, the lumen of the sealed duct was micropunctured with a double-barreled micropipette (7) and the luminal fluid was replaced by HEPES-buffered solution containing 10.0 mM glucose (plus 34.4 mM mannitol) or 44.4 mM glucose (Figure 1A). The bath was perfused with HEPES-buffered solution ($HCO_3^-$-$CO_2$ was omitted to minimize the basal fluid secretion) containing normal or high glucose concentrations. Transepithelial differences in osmolality were balanced with mannitol. Because of the high water permeability of the ductal epithelium, glucose transport across ductal epithelium would be accompanied by water in isotonic proportions and would be detectable as changes in luminal volume. Figure 1C shows relative changes of the luminal volume during 15 min with normal or high glucose concentrations in bath and lumen

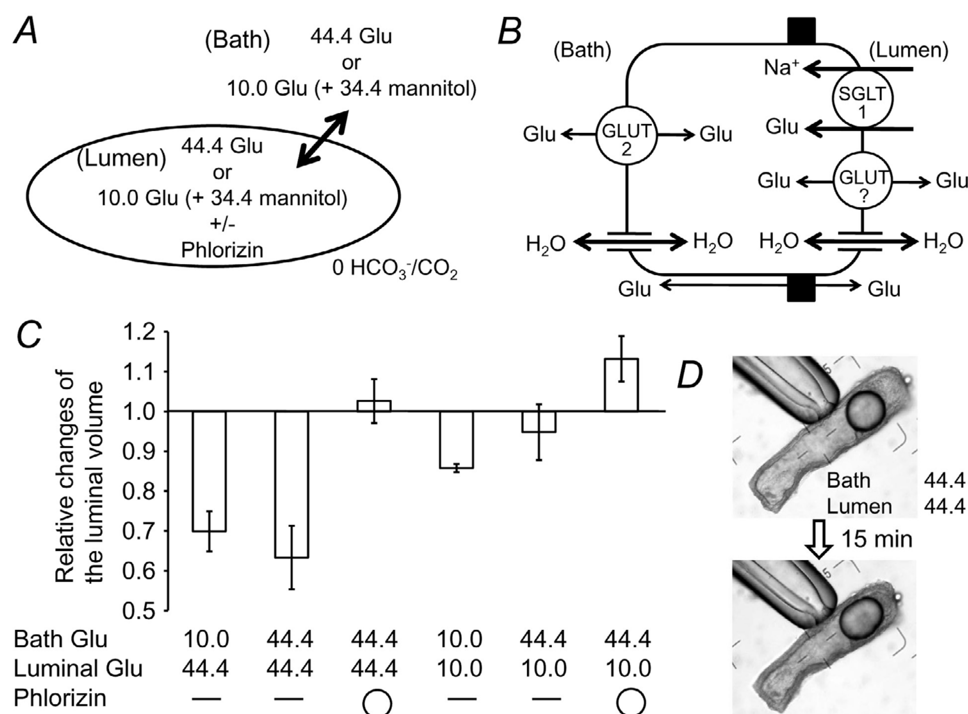


Figure 1. Absorption of luminal glucose in interlobular ducts isolated from rat pancreas.
*A* : Schema of the protocol. The lumen of the sealed ducts was micropunctured and the luminal fluid was replaced with HEPES-buffered solutions containing 10.0 mM glucose (+34.4 mM mannitol) or 44.4 mM glucose +/- phlorizin (0.5 mM). The bath was also perfused with HEPES-buffered solution containing 10.0 mM glucose (+34.4 mM mannitol) or 44.4 mM glucose. *B* : A hypothetical model of glucose transport by pancreatic duct. *C* : Relative changes of the luminal volume in 15 min with normal or high glucose concentrations in bath and lumen with or without luminal phlorizin. Means ± SD of 4-5 experiments. *D* : Images of a duct at the beginning and end of a representative experiment (Bath glucose : 44.4, Luminal glucose 44.4). The sealed duct was immobilized with a holding pipette and an oil droplet was injected into the lumen to avoid leakage of the luminal solution. In this example, the luminal volume had decreased to 67.1% in 15 min.

with or without luminal phlorizin.

When the lumen was filled with the 44.4 mM glucose solution in the presence of 10.0 mM glucose (plus 34.4 mM mannitol) in the bath, the luminal volume decreased to ~70% of the initial volume in 15 min. The presence of high (44.4 mM) glucose in the bath did not affect the rate of volume decrease, indicating that the absorptive flux was not due to passive glucose efflux from the lumen. Figure 1D shows images of a duct at the beginning and end of a representative experiment in this condition. When phlorizin (0.5 mM) was included in the luminal solution, together with the 44.4 mM glucose, the decrease in luminal volume was abolished. This suggests that SGLT1-mediated glucose transport across the apical membrane is involved in the reabsorption of fluid under these conditions. When the lumen was filled with the 10.0 mM glucose solution, with 10.0 mM glucose present in the bath, the luminal volume decreased to ~80% in 15 min. With 10.0 mM glucose in the lumen and 44.4 mM glucose in the bath, the luminal volume did not change significantly. Inhibition of the apical SGLT1 with phlorizin under identical condition led to an increase in luminal volume. This was likely due to a passive flux of glucose from bath to lumen that was previously balanced by the SGLT1-mediated transport of glucose from lumen to bath.

Our data suggest that both active and passive transport mechanisms of glucose are present in pancreatic ductal epithelium, We propose that glucose transport by pancreatic ductal epithelium explains the inhibitory effects of extracellular high glucose on fluid secretion and $HCO_3^-$ transport in isolated pancreatic ducts. Transepithelial glucose transport accompanies water and thus would affect fluid secretion. Moreover, active absorption of glucose induces apical influx of $Na^+$ and membrane depolarization both of which would affect $HCO_3^-$ and $Cl^-$ transport. Elevation of intracellular $Na^+$ concentration will reduce the driving force for basolateral uptake of $Cl^-$ and $HCO_3^-$ *via* $Na^+$-$HCO_3^-$ cotransport and $Na^+$-$K^+$-$2Cl^-$ cotransport. Membrane depolarization will reduce the driving force for $Cl^-$ and $HCO_3^-$ secretion via cystic fibrosis transmembrane conductance regulator (CFTR) anion channel.

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