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TRANSPORT PHYSIOLOGY

High glucose inhibits HCO_3^- and fluid secretion in rat pancreatic ducts

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Abstract Cellular mechanisms underlying the impairment of pancreatic fluid and electrolyte secretion in diabetes were examined using interlobular ducts isolated from rat pancreas. Fluid secretion was assessed by monitoring changes in luminal volume. HCO_3^- uptake across the basolateral membrane was estimated from the recovery of intracellular pH following an 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 also severely impaired but could be largely reversed by incubation in normal-glucose solutions. Sodium-dependent glucose cotransporter 1 (SGLT1), glucose transporter (GLUT)1, GLUT2, and GLUT8 transcripts were detected by reverse transcriptase polymerase chain reaction in isolated ducts. Raising the luminal glucose concentration in microperfused ducts caused a depolarization of the membrane potential, consistent with the presence of SGLT1 at the apical membrane. Unstimulated ducts filled with high-glucose solutions lost luminal fluid by a phlorizin-sensitive mechanism, indicating that pancreatic ducts are capable of active glucose reabsorption from the lumen via SGLT1. In ducts exposed to high glucose concentrations, continuous glucose diffusion to the lumen and active reabsorption via SGLT1 would lead to elevation of intracellular Na^+ concentration and sustained depolarization of the apical membrane. These two factors would tend to inhibit the basolateral uptake and apical efflux of Cl^- and HCO_3^- and could therefore account for the impaired fluid and electrolyte secretion that is observed in diabetes.

Keywords Pancreatic duct cell · Glucose transport · HCO_3^- transport · Diabetes mellitus

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Introduction

Exocrine dysfunction is frequently found in patients with type I (insulin-dependent) and type II (noninsulin-dependent)

diabetes mellitus (DM) [8, 14]. Whereas the digestive enzymes in the pancreatic juice are synthesized and secreted by acinar cells, most of the HCO_3^--rich isotonic fluid is secreted by epithelial cells lining the pancreatic ducts, particularly the intercalated, intralobular and small interlobular ducts [1]. In DM patients, fluid and HCO_3^- secretion, as well as enzyme secretion, are reduced [6], suggesting that both acinar and ductal function are impaired.

It is generally accepted that acinar dysfunction in DM is due mainly to the absence of the trophic effects of insulin on acinar cells [22]. However, the mechanisms underlying the ductal dysfunction in DM (reduced fluid and HCO_3^- secretion) are unknown. In the present study, we have examined the effects of high glucose concentrations on fluid secretion and HCO_3^- transport in interlobular duct segments isolated from rat pancreas. We have found that either acute or chronic exposure of the ducts to high glucose potently inhibits fluid secretion. We have also demonstrated that the reduced secretion in ducts isolated from streptozotocin-induced diabetic rats can be reversed by normalizing the glucose concentration. To investigate the possible involvement of glucose transport in the glucose-induced impairment of ductal secretion, we have also examined the messenger RNA expression of glucose transporters in the ductal epithelium and we have assessed the capacity of isolated pancreatic ducts to mediate transepithelial glucose transport.

Methods

The study was approved by the Ethical Committee of Nagoya University on Animal Use for Experiment.

Animals and induction of diabetes

Male Wistar rats weighing 250–280 g were purchased from Japan SLC (Hamamatsu, Japan). When required, rats were rendered diabetic by a single intraperitoneal injection of streptozotocin at a dose of 82.5 mg/kg body weight and were used for experiments 7 days later. The serum glucose concentration on the day of the experiments was 27.7±2.7 mM (mean±SD, n=12) in the diabetic rats and 10.8±1.1 mM (n=8) in the control rats.

Isolation and culture of interlobular pancreatic ducts

Rats were anesthetized by inhalation of diethyl ether and euthanized. The pancreas was removed and interlobular ducts (diameter ~150 μm) were isolated by collagenase digestion and microdissection [24]. The interlobular duct segments were cultured at 37°C for 14–18 h in McCoy's 5A tissue culture medium supplemented with fetal calf serum, dexamethasone, and insulin. During the culture period, both ends of the duct segments sealed spontaneously, thus isolating the luminal space from the bathing medium. The glucose concentration in the culture medium was adjusted to 10.0 mM in the control experiments. To examine the effects of high glucose, either D-glucose (34.4 mM) or mannitol (34.4 mM, as an osmotic control) was added to the culture medium (Table 1).

Solutions

The standard HCO_3^--buffered solution contained (in mM): 115 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 D-glucose, and 25 $NaHCO_3$, and was equilibrated with 95% O_2–5% CO_2. The standard HEPES-buffered solution contained (in mM): 140 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, ten D-glucose, and ten 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and was equilibrated with 100% O_2. In solutions containing NH_4^+, the concentration of Na^+ was reduced to maintain osmolarity. All solutions were adjusted to pH 7.4 at 37°C. To examine the effects of high glucose, either D-glucose (34.4 mM) or mannitol (34.4 mM) was added to the solution (Table 1).

Measurement of fluid secretory rate

The fluid secretory rate into the closed luminal space was measured as described previously [24]. The ducts were superfused with HCO_3^--buffered solutions at 37°C on the stage of an inverted microscope, and bright-field images were obtained at 2-min intervals using a charged-couple device (CCD) camera. The initial values for the length (L_0), diameter ($2R_0$), and image area (A_0) of the duct lumen were measured in the first image using ARGUS-50 software (Hamamatsu Photonics, Hamamatsu, Japan). The initial volume (V_0) of the duct lumen was calculated, assuming cylindrical geometry, as $\pi R_0^2 L_0$. The values of L_0, R_0 and V_0 were 315±80 μm, 75±19 μm, and 6.0±2.6 nl, respectively (mean±SD, n=45). The luminal surface area of the epithelium was taken to be $2\pi R_0 L_0$. In subsequent images of the series, the luminal image area (A) was measured and expressed as relative area (A/A_0). Relative volume (V/V_0) was estimated from relative area assuming $V/V_0=(A/A_0)^{3/2}$. The rate of fluid secretion was calculated from the increment in the luminal volume and expressed as the secretory rate per unit area of ductal epithelium (nl $min^{-1} mm^{-2}$).

Measurement of intracellular pH

To examine the activity of the HCO_3^--uptake mechanisms at the basolateral membrane, intracellular pH (pH_i) was

Table 1 Glucose concentration of the culture media and experimental solution in each group

Group	Culture media	Experimental solutions
Isolated ducts from normal rats		
Control	10.0 mM	10.0 mM
High glucose—short term	10.0 mM	44.4 mM
High glucose—long term	44.4 mM	44.4 mM
Hyperosmotic	10.0 mM	10.0 mM
	+34.4 mM mannitol	+34.4 mM mannitol
Isolated ducts from diabetic rats		
Diabetic—high glucose	44.4 mM	44.4 mM
Diabetic—normal glucose	10.0 mM	10.0 mM

estimated by microfluorometry in sealed ducts using the pH-sensitive fluoroprobe 2′7′-bis (2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF). For the experiment shown in Fig. 2g, the sealed ends of the ducts were cut open and the lumen was microperfused [12] so that the bath and luminal solutions could be modified separately.

Preparation of isolated acini

Rat pancreas was digested with collagenase and dispersed into individual acini by pipetting through polypropylene pipettes of decreasing diameter [18].

Reverse transcriptase-polymerase chain reaction

Messenger RNA expression of Na^+-dependent glucose transporters (SGLT) and Na^+-independent glucose transporters (GLUT) was examined in isolated pancreatic ducts by polymerase chain reaction (PCR) using the primers shown in Table 2. The PCR protocol was: 94°C, 30 s; 60°C, 30 s; 72°C, 30 s; 35 cycles. Primers were derived from published SGLT and GLUT sequences with GenBank accession numbers D16101 (SGLT1), U29881 (SGLT2), NM138827 (GLUT1), NM012789 (GLUT2), NM017102 (GLUT3), NM012751 (GLUT4), and NM053494 (GLUT8). Templates for positive controls were complementary DNAs (cDNAs) prepared from small intestine, kidney, lung, heart, and testis. β-actin specific primers (764 bp) were used for the positive controls.

Measurement of intracellular potential

The membrane potential was measured by impaling the basolateral membrane of microperfused ducts with glass microelectrodes. Pancreatic duct epithelium is a low-resistance epithelium [12, 19], and thus, the basolateral membrane potential is very similar in magnitude to the apical membrane potential.

Measurement of glucose transport across the ductal epithelium

The lumen of the sealed duct was micropunctured with a double-barreled micropipette [10], 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. The bath was perfused with HEPES-buffered solution containing normal or high glucose concentrations. Changes in the luminal volume were monitored using a CCD camera as described above. The initial values of the length, radius, and luminal volume of the ducts used for

Table 2 Primer pairs used to amplify glucose transporters

	Size (bp)	Sense	Antisense
SGLT1	499	5′-ATGGACAGTAGCACCTTGAGCC-3′	5′-TAGCCCCAGAGAAGATGTCTGC-3′
SGLT2	460	5′-CATTGTCTCAGGCTGGCACTGG-3′	5′-GGACACTGCCACAATGAACACC-3′
GLUT1	296	5′-GGAACTCCATGCTGATGATGAA-3′	5′-ACTGAGCAGTAGAGGCCACAAGTC-3′
GLUT2	314	5′-GGCTCAGCAGTTCTCTGGAATCAA-3′	5′-GACCTGGCCCAATCTCAAAGA-3′
GLUT3	434	5′-GTCAACCTGATTGCCATCCT-3′	5′-TCCTGGATCTCCTGGATCAC-3′
GLUT4	554	5′-GTGTGGTCAATACCGTCTTCACG-3′	5′-CCATTTTGCCCCTCAGTCATTC-3′
GLUT8	201	5′-TCATGGACAGAGCAGGGCG-3′	5′-ACATGCTGCCTACAGCCAG-3′

these experiments were 519±137 μm, 70±13 μm, and 8.6±4.9 nl, respectively (mean±SD, n=25).

Materials

Secretin was obtained from the Peptide Institute (Minoh, Japan); BCECF-AM was from Invitrogen (Carlsbad, CA, USA); streptozotocin, forskolin, phlorizin, and other standard laboratory chemicals were from Sigma (St. Louis, MO, USA).

Statistics

Tests for statistically significant differences were made with Student's t test. Data are presented as means ± standard error of the mean (SEM) unless indicated otherwise.

Results

Isolated interlobular pancreatic ducts secrete an isotonic, HCO_3^--rich fluid in response to secretin stimulation [21]. In this study, we examined the effects of high glucose concentrations on fluid secretion and HCO_3^- transport by isolated pancreatic ducts from normal and diabetic rats. The isolated ducts were kept in culture for 14–18 h, and then superfused on the stage of an inverted microscope for measurement of luminal volume and pH_i. The glucose concentrations of the culture media and experimental solutions (superfusate) for each experimental group are shown in Table 1. Mannitol was used as a control for the osmotic effects of glucose. We simulated normal and diabetic conditions by setting glucose concentrations at 10.0 mM and 44.4 mM, respectively.

Effects of high glucose on secretin-stimulated fluid secretion

In ducts isolated from normal rats and exposed to 10.0 mM glucose during the culture period, a small amount of spontaneous fluid secretion was observed when the ducts were subsequently superfused with a HCO_3^--buffered solution containing 10.0 mM glucose (Fig. 1a, *control* in Table 1). When 1 nM secretin was added to the bath, fluid secretory rate increased to 1.74±0.14 nl $min^{-1}mm^{-2}$ (n=8) in ~10 min, and was stable for over 20 min. When ducts cultured in 10.0 mM glucose were then superfused with a solution containing 44.4 mM glucose from 15 min prior to the measurement (Fig. 1b, *high glucose—short term* in Table 1), secretin-stimulated fluid secretion (1.06±0.07 nl $min^{-1}mm^{-2}$, n=8) was significantly (p<0.05) reduced compared with the normal-glucose control (Fig. 1a).

In ducts isolated from normal rats and exposed to 44.4 mM glucose during both the culture period and the experiment (Fig. 1c, *high glucose—long term* in Table 1), secretin-stimulated fluid secretion (1.02±0.15 nl min^{-1} mm^{-2}, n=8) was significantly (p<0.05) smaller than in the control (Fig. 1a), but not different from that in the *high glucose—short term* group (Fig. 1b). In ducts kept in solutions containing 10.0 mM glucose plus 34.4 mM mannitol during both the culture period and the experiment (Fig. 1d, *hyperosmotic* in Table 1), the fluid secretory rate under secretin stimulation (2.04±0.23 nl $min^{-1}mm^{-2}$, n=5) was not different from the control. These data (summarized in Fig. 1g) indicate that it is the high glucose concentration, not hyperosmolarity, that inhibits secretin-stimulated fluid secretion. Furthermore, it is clear that acute exposure to high glucose is sufficient to elicit the effect, which is therefore quite rapid in onset.

Secretin-stimulated fluid secretion in ducts isolated from diabetic rats

In ducts isolated from diabetic rats and exposed to 44.4 mM glucose during both the culture period and the experiment (Fig. 1e, *diabetic—high glucose* in Table 1), secretin-stimulated fluid secretion (0.70±0.07 nl $min^{-1}mm^{-2}$, n=8) was significantly (p<0.05) smaller than in the control (Fig. 1a). When the "diabetic" ducts were exposed to 10.0 mM glucose during both the culture period and the experiment (Fig. 1f, *diabetic—normal glucose* in Table 1), secretin-stimulated fluid secretion recovered significantly (p<0.05), reaching 1.35±0.17 nl $min^{-1}mm^{-2}$ (n=8). These data indicate that secretin-stimulated fluid secretion is severely impaired in the diabetic (high glucose) state but is substantially restored (by ~60%) following incubation of the ducts in normal glucose solution for 14–18 h (Fig. 1g).

Effects of high glucose on pH_i recovery from intracellular acid loading

In pancreatic duct cells, the recovery of pH_i from acid loading is mediated by basolateral Na^+-HCO_3^- cotransport and Na^+/H^+ exchange [21]. These two mechanisms also mediate HCO_3^- accumulation across the basolateral membrane during secretin-evoked HCO_3^- secretion [11]. To examine the effects of high glucose concentrations on the activity of these basolateral transporters, secretin-stimulated ducts were acid-loaded with a 2-min exposure to 20 mM NH_4Cl solution in the presence of HCO_3^--CO_2.

In ducts isolated from normal rats and exposed to 10.0 mM glucose during both the culture period and the experiment (Fig. 2a, *control* in Table 1), the initial rate of pH_i recovery under secretin (1 nM) stimulation was 0.093±

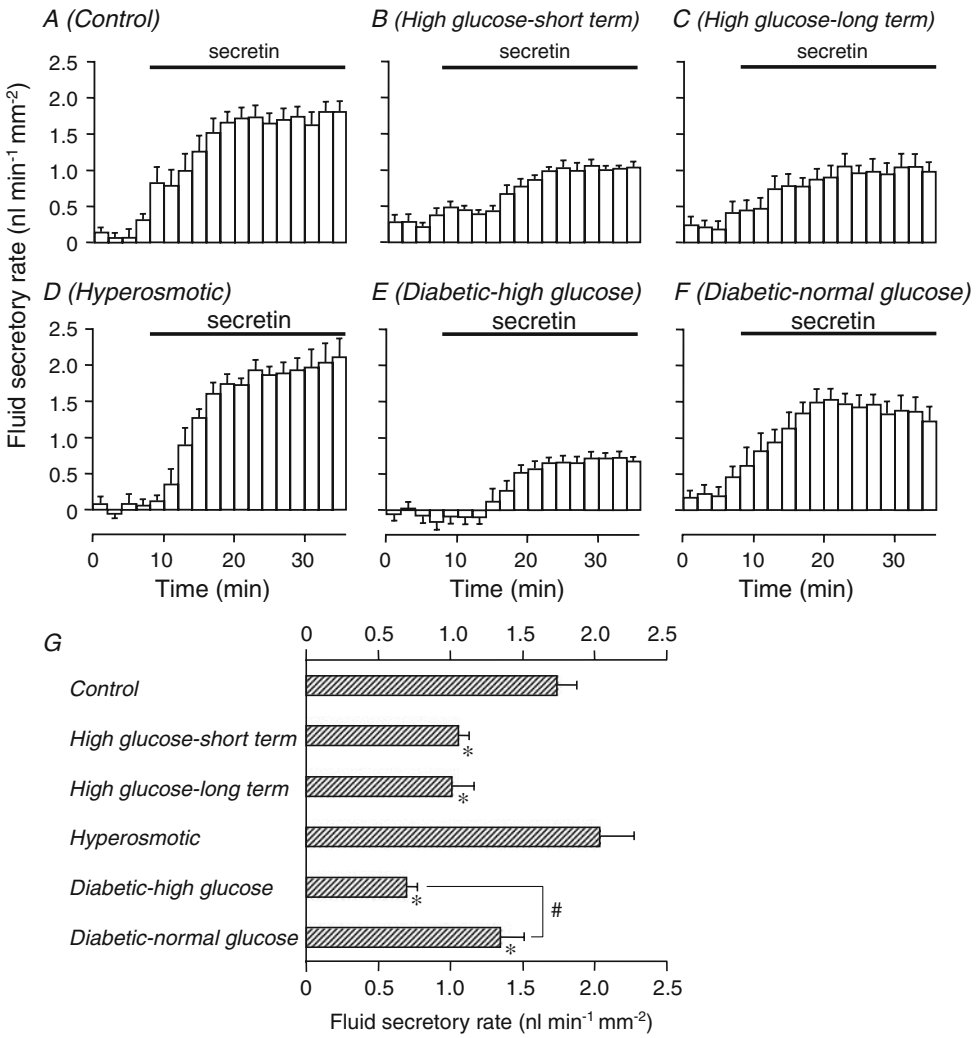

Fig. 1 Secretin-stimulated fluid secretion in interlobular pancreatic ducts isolated from normal and streptozotocin-induced diabetic rats. Isolated sealed ducts were superfused with HCO_3^--buffered solutions and secretin (1 nM) was added as indicated. Time courses of the fluid secretory rate are shown. Glucose concentrations of the culture media and experimental solutions in each group are shown in Table 1. **a**, **b**, **c**, and **d** Data from ducts isolated from normal rats. Ducts were maintained in 10.0 mM glucose throughout the culture period and experiment (**a**, Control), in 10.0 mM glucose during the culture period and then 44.4 mM glucose during the experiment (**b**, High glucose—short term), in 44.4 mM glucose throughout the culture period and experiment (**c**, High glucose—long term), or in 10.0 mM glucose plus 34.4 mM mannitol throughout the culture period and experiment (**d**, Hyperosmotic). **e** and **f** Data from ducts isolated from streptozotocin-induced diabetic rats. Ducts were maintained in 44.4 mM glucose (**e**, Diabetic—high glucose) or 10.0 mM-glucose (**f**, Diabetic—normal glucose) throughout the culture period and experiment. **g** Averaged fluid secretory rate during the last 10 min under secretin stimulation. Means±SEM of five to eight experiments; $^*p<0.05$ compared with control; $^\#$significant improvement in normal glucose ($p<0.05$)

0.011 pH unit min^{-1} ($n=6$). In ducts exposed to 44.4 mM glucose during the experiment only (Fig. 2b, *high glucose—short term* in Table 1) or exposed to 44.4 mM glucose during both the culture period and the experiment (Fig. 2c, *high glucose—long term* in Table 1), the rates of pH_i recovery were 0.069±0.007 ($n=6$) and 0.053±0.008 pH unit min^{-1} ($n=6$), respectively, both of which were significantly ($p<0.05$) slower than in the control (Fig. 2a). These data (summarized in Fig. 2f) suggest that basolateral HCO_3^- uptake is inhibited in the high-glucose condition.

Recovery of pH_i from intracellular acid loading in ducts isolated from diabetic rats

In ducts isolated from diabetic rats and exposed to 44.4 mM glucose during both the culture period and the experiment (Fig. 2d, *Diabetic—high glucose* in Table 1), the initial rate of pH_i recovery following acid loading under secretin stimulation was 0.043±0.005 pH unit min^{-1} ($n=6$), which was significantly ($p<0.05$) slower than in the control (Fig. 2a). When the diabetic ducts were exposed to

10.0 mM glucose during both the culture period and the experiment (Fig. 2e, *diabetic—normal glucose* in Table 1), the rate of pH_i recovery was 0.061±0.012 pH unit min^{-1} (n=6), which was still slower than in the control. These data suggest that basolateral HCO_3^- uptake is impaired in diabetic ducts (Fig. 2f).

Effects of high luminal glucose on the recovery from intracellular acid loading

To distinguish between the effects of high glucose concentrations applied to the apical and basolateral membranes, NH_4^+ pulses were applied to microperfused ducts in which the luminal glucose concentration was switched from 10.0 mM to 44.4 mM while the bath concentration remained at 10.0 mM (Fig. 2g). When luminal glucose was raised in secretin-stimulated ducts, basal pH_i dropped by ~0.05 unit. The initial rate of pH_i recovery with 10.0 mM luminal glucose was 0.107± 0.020 pH unit min^{-1} (n=4), whereas the pH_i recovery rate with 44.4 mM luminal glucose (0.072±0.025 pH unit min^{-1}) was significantly slower (p<0.05). This suggests that basolateral HCO_3^- uptake is inhibited by high glucose concentrations in the lumen.

Messenger RNA expression of SGLTs and GLUTs

Since the effects of high glucose concentrations on ductal secretion might be linked to glucose transport, we next examined the expression of messenger RNA (mRNA) for both Na^+-dependent and Na^+-independent glucose transporters in the pancreatic duct (Fig. 3). Amplified fragments from SGLT1, SGLT2, GLUT1, GLUT2, and GLUT4 transcripts were detected in the whole pancreas. Large amounts of SGLT1, GLUT1, GLUT2, and GLUT8 transcripts and smaller amounts of GLUT3 transcript were detected in isolated interlobular ducts. SGLT1 and GLUT1 transcripts were also detected in isolated acini. All of these transcripts were identical in size to those obtained with mRNA extracted from small

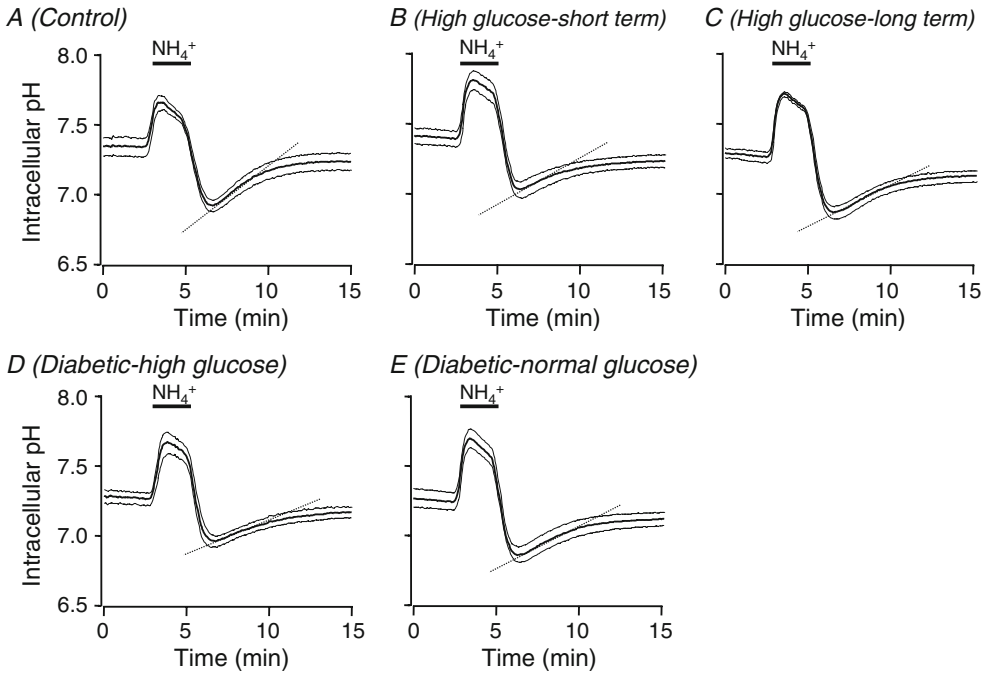

Fig. 2 Recovery of intracellular pH after acid loading in secretin-stimulated interlobular ducts isolated from normal and streptozotocin-induced diabetic rats. **a–e**: Isolated sealed ducts were superfused with HCO_3^--buffered solutions containing secretin (1 nM) and were acid-loaded by 2-min exposure to 20 mM NH_4Cl as indicated. Glucose concentrations of the culture media and experimental solutions in each group are shown in Table 1. **a**, **b**, and **c** Data from ducts isolated from normal rats. Ducts were maintained in 10.0 mM glucose throughout the culture period and experiment (**a**, Control), in 10.0 mM glucose during the culture period and then 44.4 mM glucose during the experiment (**b**, High glucose—short term), or in 44.4 mM glucose throughout the culture period and experiment (**c**, High glucose—long term). **d** and **e** Data from ducts isolated from streptozotocin-induced diabetic rats. Ducts were maintained in 44.4 mM glucose (**d**, Diabetic—high glucose) or 10.0 mM-glucose (**e**, Diabetic—normal glucose) condition throughout the culture period and experiment. **f** Initial rate of pH_i recovery after acid loading. Means±SEM of six experiments; $^*p$<0.05 compared with control. **g** The bath and lumen of isolated pancreatic ducts were perfused separately with HCO_3^--buffered solutions and the ducts were stimulated with secretin (1 nM). An NH_4^+ pulse (20 mM) was applied to the bath in the presence of normal (10 mM) and high (44.4 mM) luminal glucose concentrations. Representative of four experiments

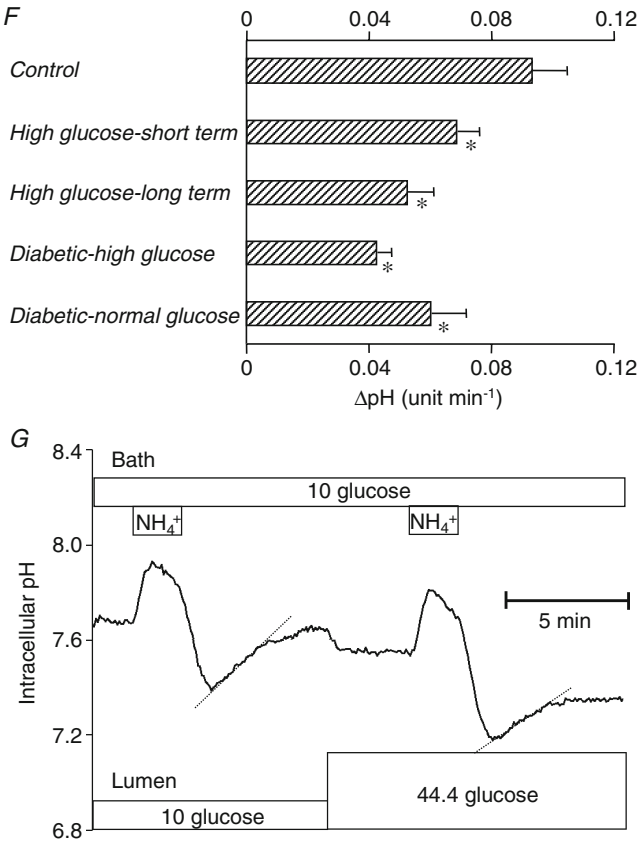

Fig. 2 (continued)

intestine (SGLT1 and GLUT2), kidney (SGLT2, GLUT1, GLUT2, GLUT3, and GLUT8), lung (GLUT1 and GLUT2), heart (GLUT4) and testis (GLUT8).

Effects of high glucose concentrations on membrane potential

Since the Na^+-dependent glucose transporter SGLT1 is electrogenic, we attempted to determine its location in the pancreatic duct cells by examining the effects of high luminal or basolateral glucose on membrane potential (V_m) in microperfused ducts (Fig. 4a). Application of 1 μM forskolin, an activator of adenylate cyclase, caused an initial depolarization as previously observed. When the glucose concentration in the lumen was then increased from 10.0 to 44.4 mM, by replacing 34.4 mM mannitol with glucose, V_m depolarized further by 8.2±1.2 mV ($p<0.05$, $n=3$). In contrast, raising the glucose concentration in the bath (from 10.0 to 44.4 mM) caused a significant hyperpolarization of 15.0±1.5 mV ($p<0.05$, $n=3$). Because increased SGLT1 activity normally leads to depolarization, these data suggest that SGLT1 is localized to the apical membrane. The hyperpolarization evoked by raising the bath glucose concentration is probably due to the activation of K^+ channels by cell swelling as a result of passive glucose entry across the basolateral membrane.

To confirm that the depolarization evoked by raising the luminal glucose concentration was due to Na^+ entry via SGLT1, we used phlorizin, a specific inhibitor of SGLT1 (Fig. 4b). Application of phlorizin (0.5 mM) to the lumen initially hyperpolarized the cells by 5.7±0.8 mV ($p<0.05$, $n=3$) in the presence of 10 mM luminal glucose. The subsequent depolarization, caused by raising the luminal glucose concentration to 44.4 mM, was reduced by 57% in

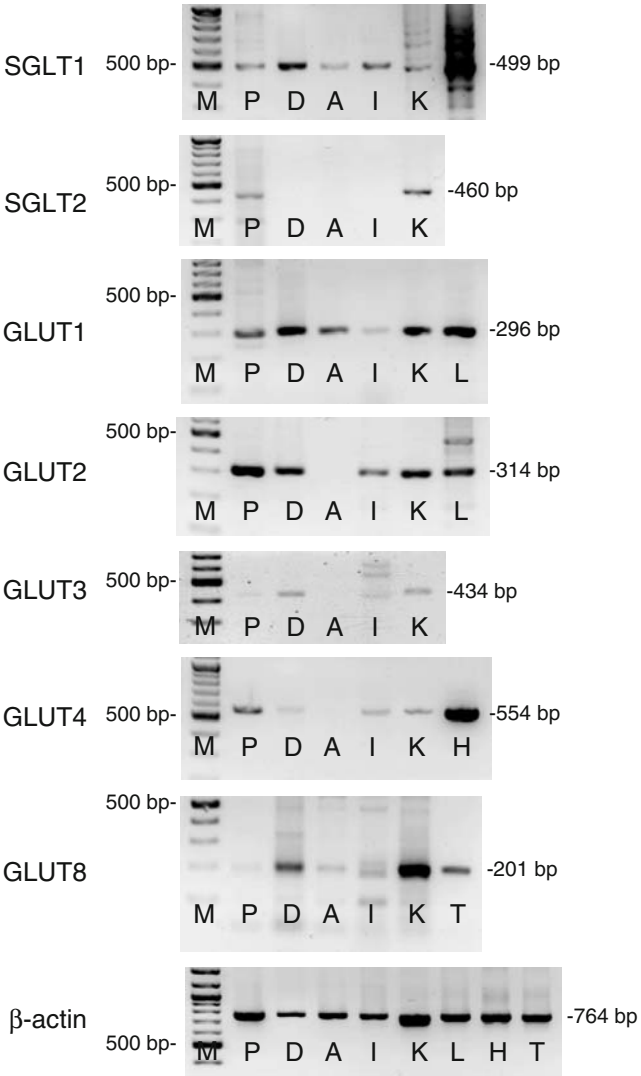

Fig. 3 RT-PCR analysis of expression of Na^+-glucose cotransporter (SGLT) and glucose transporter (GLUT) transcripts. Messenger RNA was extracted from whole pancreas (*P*), isolated interlobular pancreatic ducts (*D*), isolated pancreatic acini (*A*), and small intestine (*I*) and reverse transcribed. PCR was performed using each cDNA as template and with SGLT- and GLUT-specific sense and antisense primers (Table 2). cDNAs from kidney (*K*), lung (*L*), heart (*H*), and testis (*T*) were used as positive controls, and β-actin was used as a reference. 100-bp DNA ladder (*M*)

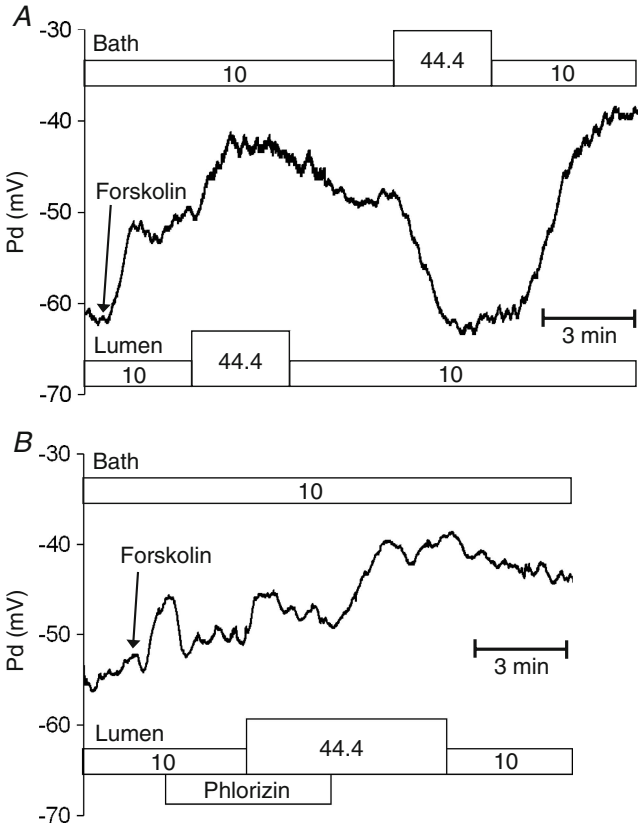

Fig. 4 Effects of high luminal or bath glucose concentrations on membrane potential in interlobular pancreatic ducts isolated from normal rats. Isolated pancreatic ducts were microperfused and bathed with HCO_3^--buffered solutions and stimulated with forskolin (1 μM). Membrane potential was measured with respect to the bath using conventional intracellular microelectrodes. **a** The glucose concentration of either the luminal or bath perfusate was increased from 10.0 to 44.4 mM, as indicated, by replacement of mannitol. Representative of 3 experiments. **b** The glucose concentration of the luminal perfusate was raised from 10.0 to 44.4 mM in the presence of phlorizin (0.5 mM). Representative of three experiments

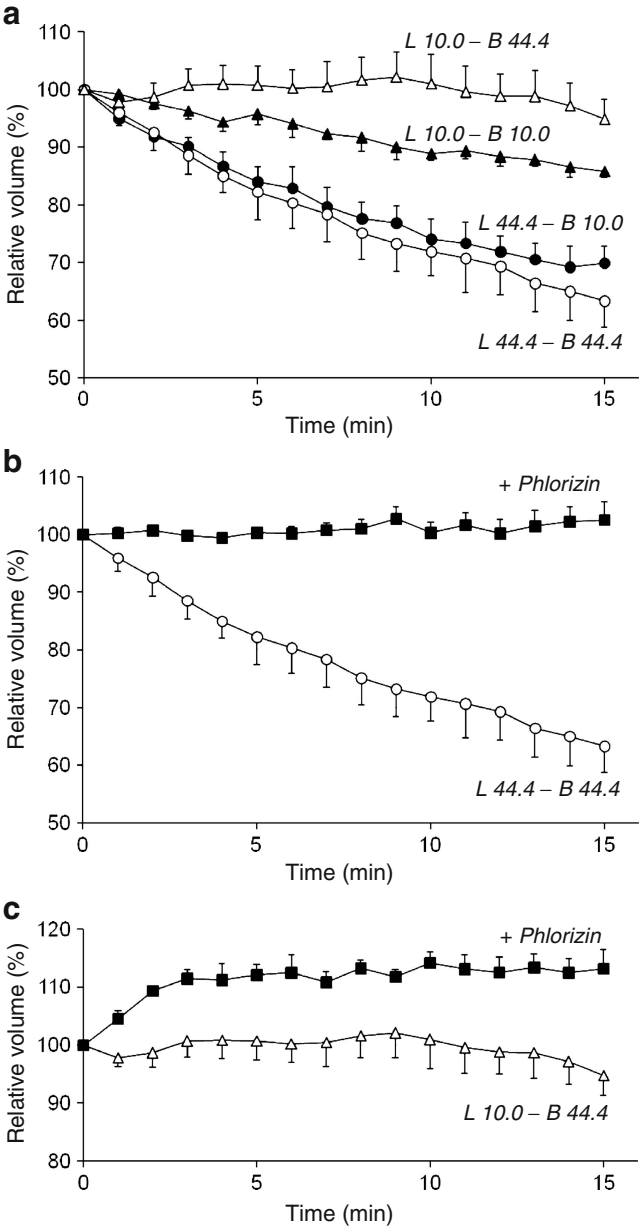

Fig. 5 Reabsorption of luminal glucose in interlobular pancreatic ducts isolated from normal rats. The lumen of the sealed ducts was micropunctured and the luminal fluid was replaced with HEPES-buffered solutions containing 10.0 mM glucose (+ 34.4 mM mannitol) or 44.4 mM glucose. The bath was also perfused with HEPES-buffered solution containing 10.0 mM glucose (+ 34.4 mM mannitol) or 44.4 mM glucose. **a**, **b**, and **c** Time courses of changes in luminal volume. *L10.0-B44.4*: 10.0 mM glucose in the lumen and 44.4 mM glucose in the bath. *L10.0-B10.0*: 10.0 mM glucose in both lumen and bath. *L44.4-B10.0*: 44.4 mM glucose in the lumen and 10.0 mM glucose in the bath. *L44.4-B44.4*: 44.4 mM glucose in both lumen and bath. **b** and **c** 0.5 mM phlorizin was injected into the lumen as indicated (+Phlorizin). Means±SEM of four to five experiments. **d** Images of a duct at the beginning and end of a representative experiment (*L44.4-B44.4*). The sealed duct was immobilized with a holding pipette (*top left*) and an oil droplet was injected into the lumen to avoid leakage of the luminal solution. In this example, the luminal volume had decreased by 32.9% after 15 min

the presence of luminal phlorizin ($p<0.05$, $n=3$) but was restored upon its withdrawal.

Glucose transport across the ductal epithelium

The presence of SGLT1 at the apical membrane and passive glucose permeability at the basolateral membrane suggests that the ductal epithelium has the capacity to reabsorb glucose from the lumen. To examine this possibility, the luminal fluid in sealed isolated ducts was replaced by micropuncture with solutions containing either 44.4 mM glucose or 10.0 mM glucose and the subsequent changes in the luminal volume were monitored (Fig. 5). In these experiments the ducts were unstimulated and HCO_3^--CO_2 was omitted to minimize the basal fluid secretion. Trans-epithelial differences in osmolality were balanced with mannitol. Because of the high water permeability of the

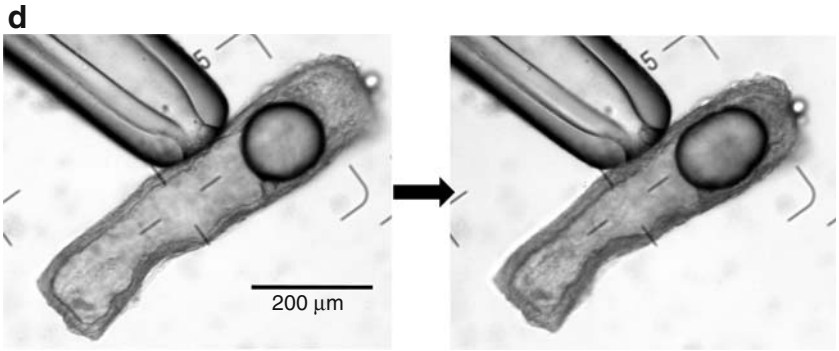

Fig. 5 (continued)

ductal epithelium, glucose reabsorption from the duct lumen to the bath would be accompanied by water in isotonic proportions and would be detectable as a decrease in luminal volume.

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 steadily decreased, reaching 69.9±2.9% (n=4) of the initial volume after 15 min (closed circles, L44.4-B10.0 in Fig. 5a). The presence of high (44.4 mM) glucose in the bath did not affect the rate of volume decrease (open circles, L44.4-B44.4) indicating that the absorptive flux was not due to passive glucose efflux from the lumen. Figure 5d shows images of a duct at the beginning and end of a representative experiment.

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 (Fig. 5b, n=4). 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 more slowly, reaching 85.8±1.1% (n=4) of the initial volume after 15 min (closed triangles, L10.0-B10.0, Fig. 5a). With 10.0 mM glucose in the lumen and 44.4 mM glucose in the bath, the luminal volume did not change significantly (open triangles, L10.0-B44.4, Fig. 5a and c), suggesting that this bath-to-lumen glucose gradient abolished the net transepithelial flux of solute. In support of this interpretation, inhibition of the apical SGLT1 with phlorizin, under otherwise identical conditions, led to an increase in luminal volume, reaching 113.2±3.3% (n=4) of the initial volume after 15 min (Fig. 5c). This was most 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.

Discussion

Fluid secretion by pancreatic duct epithelium is driven by the active vectorial transport of Cl^- and HCO_3^- to the duct lumen [1]. Accumulation of Cl^- and HCO_3^- across the basolateral membrane is mediated mainly by Na^+-K^+-$2Cl^-$ cotransport [7] and by Na^+-HCO_3^- cotransport [11], respectively, while Cl^- and HCO_3^- secretion across the apical membrane depends on the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel [21]. Na^+,K^+-ATPase maintains the transmembrane Na^+ gradient, which provides the driving force for basolateral Cl^- and HCO_3^- uptake.

In this study we have attempted to simulate normal and diabetic conditions in the experiments using isolated pancreatic ducts. The choice of an appropriate glucose concentration for the diabetic condition is not straightforward. It is known that serum glucose concentration of rats is higher than that of human. The serum glucose concentration of normal rats was ~11 mM (~195 mg/dl) in our present study and ~200 mg/dl in a previous study [20]. The serum glucose concentration of streptozotocin-induced diabetic rats was ~28 mM (~500 mg/dl) in our study and ~33 mM (~600 mg/dl) in the previous study. In a study using the Capan-1 pancreatic duct cell-line, concentration-dependent changes in the activity of aldose reductase were observed over a wide range of glucose concentrations (5.5-110 mM), i.e. one half to ten times normal [4]. In the present study we chose 44.4 mM (800 mg/dl), i.e. four times normal, as representative of a moderately severe diabetic condition.

Inhibition of fluid secretion and basolateral HCO_3^- uptake by high glucose

The present study demonstrates that exposure of isolated rat pancreatic ducts to high glucose concentrations inhibits secretin-stimulated fluid secretion and reduces the baso-

lateral uptake of HCO_3^-. In diabetic ducts (isolated from streptozotocin-treated rats, a model for type-I DM), secretin-stimulated fluid secretion and HCO_3^- uptake were similarly impaired when the ducts were maintained at high glucose concentrations. Although the diabetic ducts were briefly exposed to insulin in the culture medium prior to the experiments, the remarkable recovery of fluid secretion, by as much as ~60% following incubation in normal-glucose solutions, indicates that insulin depletion itself does not have any significant long-term effect on secretion. These results suggest, therefore, that the impaired fluid secretion and HCO_3^- transport observed in diabetic ducts are largely reversible and may be attributed mainly to the inhibition of basolateral transporters by the high extracellular glucose concentration.

Pancreatic duct reabsorbs luminal glucose via Na^+-dependent and Na^+-independent glucose transporters

The impairment of fluid secretion by high glucose concentrations could be attributed either to the adverse effects of high intracellular glucose on cellular metabolism or to interactions between glucose transport and the driving forces for electrolyte secretion. The first of these hypotheses is supported by a report that high glucose activates polyol metabolism in Capan-1 pancreatic duct cells, which results in a decrease in Na^+, K^+-ATPase activity [4]. However, the second hypothesis is more likely to explain the present data which show that quite short periods of exposure to high glucose have marked inhibitory effects on HCO_3^- transport and fluid secretion (Figs. 1 and 2) i.e. the effect is quite rapid in onset.

Several lines of evidence point to the likelihood that pancreatic duct cells actively transport glucose out of the ductal lumen. First, we have shown that duct cells express Na^+-dependent (SGLT1) and Na^+-independent (GLUT1, GLUT2, GLUT3, and GLUT8) glucose transporters, at least at the mRNA level (Fig. 3). Second, our measurements of glucose-induced changes in membrane potential suggest that there is electrogenic uptake of glucose, indicative of SGLT1 activity, at the apical membrane (Fig. 4). Third, we have observed fluid reabsorption in unstimulated ducts filled with high glucose solutions (Fig. 5a), which was abolished by injection of phlorizin into the duct lumen (Fig. 5b) suggesting that the fluid movement was coupled to glucose transport via SGLT1. Fourth, in the presence of a bath-to-lumen glucose gradient, we could observe passive glucose transport into the lumen when glucose reabsorption via SGLT1 was blocked with luminal phlorizin (Fig. 5c).

Our functional data (Figs. 2, 4, and 5) suggest that SGLT1 is present and active at the apical membrane of pancreatic duct cells. A recent study has used an improved antibody to localize SGLT1 in various rat organs [2]. Although the pancreas was not examined, the apical membranes of small ducts in the submandibular gland and small bile ducts in the liver showed positive staining for SGLT1.

Glucose absorption in the small intestine involves SGLT1 at the apical membrane and GLUT2 at the basolateral membrane. The apparent K_m for active glucose absorption in rat jejunum in vivo is ~27 mM [13] while the K_m for glucose of SGLT1 expressed in Xenopus oocytes is ~0.5 mM [23]. By analogy with the intestine, we would predict that the presence of SGLT1 in the apical membrane would enable the ductal

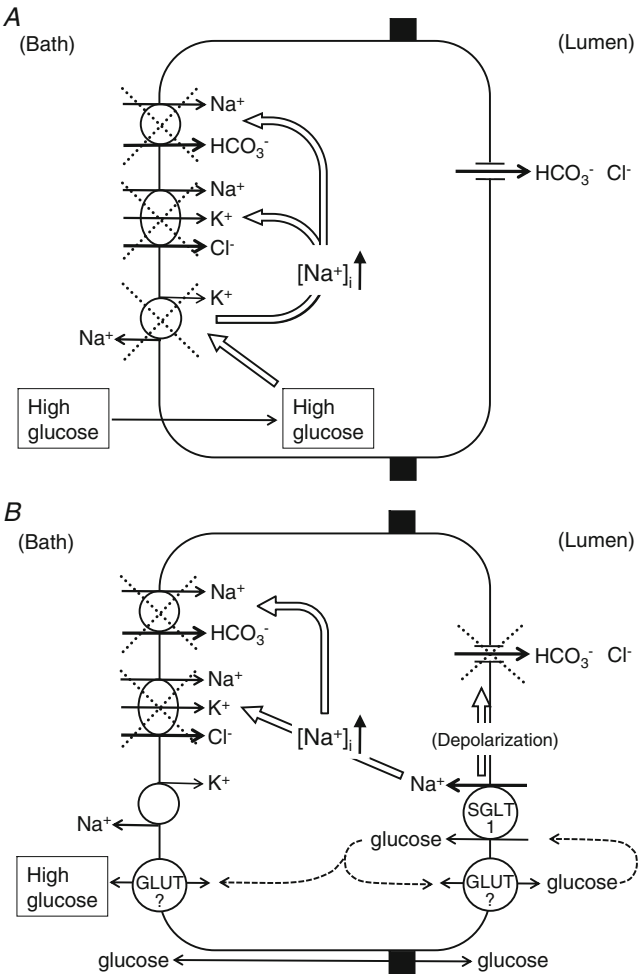

Fig. 6 Hypothetical models for glucose-induced inhibition of fluid and electrolyte secretion by pancreatic duct cells **a** Existing model. High glucose inhibits Na^+, K^+-ATPase. The resultant elevation of $[Na^+]_i$ attenuates the Na^+ gradient across the basolateral membrane, which reduces Cl^- and HCO_3^- uptake via Na^+ -coupled transporters at the basolateral membrane such as the Na^+-HCO_3^- cotransporter. **b** New model. Active absorption of luminal Na^+ and glucose via SGLT1 increases $[Na^+]_i$ and depolarizes the apical membrane. $[Na^+]_i$ elevation reduces Cl^- and HCO_3^- uptake as in the existing model (**a**). The apical depolarization reduces the driving force for Cl^- and HCO_3^- secretion via CFTR at the apical membrane. When the sealed duct is exposed to high glucose concentrations in the bath, the passive glucose flux from bath to lumen and reabsorption of luminal glucose via SGLT1 cause recycling of glucose across the apical membrane

epithelium to reabsorb luminal glucose against a steep concentration gradient. Indeed, it has been known for some time that the concentration of glucose in human pancreatic juice (0.5 ~ 1 mM) is much lower than in plasma [3].

The presence of a significant passive permeability to glucose, via apical and basolateral GLUTs or via the paracellular pathway, would lead to continuous SGLT1 activity when the ducts are placed in a high-glucose solution. Under these conditions, and presumably also in the untreated diabetic condition, a steady state would be established in which glucose diffuses passively into the ductal lumen down its concentration gradient and is then actively transported back out of the lumen via SGLT1. Passive glucose transport has previously been demonstrated in both the intestine [13] and bile duct [17].

Possible mechanisms for the glucose-induced impairment of fluid secretion

Figure 6 shows two possible explanations for how fluid secretion could be inhibited by exposure to high glucose. Previous studies have demonstrated inhibition of Na^+, K^+-ATPase by high intracellular glucose in pancreatic duct cells [4, 9]. It is thought that the resultant elevation of intracellular Na^+ concentration ($[Na^+]_i$) attenuates the Na^+ gradient across the basolateral membrane, which consequently reduces the uptake of Cl^- and HCO_3^- via Na^+-coupled transporters, such as the Na^+-HCO_3^- cotransporter, at the basolateral membrane (Fig. 6a). This in turn leads to a reduction in Cl^-, HCO_3^- and fluid secretion.

Our data suggest a modification to this model (Fig. 6b). We propose that the active reabsorption of luminal glucose via SGLT1 has two effects. First, the influx of Na^+ via SGLT1 raises $[Na^+]_i$, thereby reducing the driving force for basolateral uptake of Cl^- and HCO_3^-. Second, and perhaps more importantly, the electrogenicity of SGLT1 depolarizes the apical membrane (Fig. 4). This will markedly reduce the driving force for Cl^- and HCO_3^- secretion via the CFTR channel [12] and lead to a corresponding reduction in the fluid secretory rate.

We propose that the inhibitory effect of glucose on secretion is sustained, even in sealed ducts where glucose does not have direct access to the lumen, because glucose in the bath can enter the lumen by passive transport via basolateral and apical GLUTs or via the paracellular pathway. In effect, the continuous "recycling" of glucose leads to continuous SGLT1 activity and therefore sustained inhibition of secretion.

Clinical implications of glucose-induced inhibition of fluid secretion

A reduction in the HCO_3^- concentration and volume output of pancreatic juice is frequently observed in patients with DM, both type I and type II. Okabayashi *et al.* reported that pancreatic juice secretion in response to secretin was also significantly reduced in streptozotocin-treated diabetic rats (20). The present study suggests that the dysfunction of pancreatic duct cells in DM is probably due mainly to the high extracellular glucose concentration rather than insulin depletion. More importantly, our data also suggest that the ductal dysfunction is reversible and that correction to normoglycemia will improve fluid and electrolyte secretion. These findings are consistent with previous clinical studies. For example, hyperglycemia-clamping was found to reduce the pancreatico-biliary output of volume, HCO_3^-, and amylase in healthy subjects [15]. In addition, the severity of the exocrine pancreatic dysfunction in DM is not related to the duration or severity of the condition [5, 16].

In summary, we have demonstrated a novel interaction between the exocrine and endocrine pancreas whereby fluid secretion and HCO_3^- transport by the pancreatic duct epithelium are inhibited by the high extracellular glucose concentrations associated with DM. Acute exposure to high glucose inhibits secretin-stimulated fluid secretion in interlobular ducts isolated from rat pancreas. This is most likely attributable to the inhibition of Na^+-coupled transporters at the basolateral membrane and of Cl^- and HCO_3^- secretion via CFTR at the apical membrane. Secretion is also severely impaired in isolated ducts from diabetic rats, but can be largely restored when the ducts are exposed to normal extracellular glucose concentrations. We have also found that pancreatic duct cells express SGLT1 and GLUTs which mediate the active reabsorption of luminal glucose. This process appears to be responsible for the impairment of secretion caused by high glucose concentrations. We propose that $[Na^+]_i$ elevation and apical membrane depolarization, resulting from the increased activity of apical SGLT1, inhibit the basolateral uptake and apical efflux of Cl^- and HCO_3^- thus leading to the observed inhibition of fluid and electrolyte secretion.

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