Molecular basis of potassium channels in pancreatic duct epithelial cells

Hayashi, M.; Novak, Ivana

Published in:
Channels (Austin)

DOI:
10.4161/chan.26100

Publication date:
2013

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Molecular basis of potassium channels in pancreatic duct epithelial cells

Mikio Hayashi** and Ivana Novak*

Department of Biology; University of Copenhagen; Copenhagen, Denmark;
**Current affiliation: Department of Physiology; Kansai Medical University; Hirakata, Japan

Keywords: cancer, EAG2, epithelia, HERG, pancreas, SK4, Slack, Slick, Slo1, TASK-2

Abbreviations: AKAP, A-kinase anchoring protein; BK, voltage- and Ca2+-dependent maxi-K+; BxPC3, human pancreas adenocarcinoma cell line; Capan-1, human pancreas adenocarcinoma cell line; CFPAC-1, human cystic fibrosis pancreatic adenocarcinoma cell line; CFTR, cystic fibrosis transmembrane conductance regulator; DC-EBIO, 5,6-dichloro-1-ethyl-1,3-dihydro-2H-benimidazole-2-one; DHS-I, dehydrosoyasaponin I; DIDS, 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid; E-4031, N-[(1R)-1-[2-(6-methylpyridin-2-yl)ethyl]piperidine-4-carbonyl]phenyl methanesulfonamide; EAG, ether-à-go-go gene; I-EBIO, 1-ethyl-2-benzimidazolinone; HERG, human ether-à-go-go related gene; HPAF, human pancreatic ductal adenocarcinoma cell line; ICA-105574, 3-nitro-N(4-phenoxypbenyl) benzamide; IK, intermediate-conductance Ca2+-activated K+; I_{IR}, short-circuit current; K_{ir} two-pore domain K+ channels; K_{ir} inward rectifier potassium channel; L-364,373, (3R)-1,3-dihydro-5-(2-fluorophenyl)-3-(1H-indol-3-ylmethyl)-1-methyl-2H-1,4-benzodiazepin-2-one; LY97241, N-ethyl-N-[4-(4-nitrophenyl)butyl] leptan-1-amine; NS11021, 1-(3,5-bis-trifluoromethyl-phenyl)-3-[(4-bromo-2-(1H-tetrazol-5-yl)-phenyl)-thio]urea; NS1608, N-(3-(trifluoromethyl)phenyl)-N'-(2-hydroxy-5-chlorophenyl)urea; PD-118057, [2-(4-[2-(3,4-dichloro-phenyl)-ethyl]-phenyl)-ethyl]-phenylamino)-benzoic acid; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; Ro-20-1724, 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone; Slack, sequence like a calcium-activated K+ channel; Slick, sequence like an intermediate conductance K+ channel; TALK, TWIK-related alkaline pH-activated K+ channel; TASK, TWIK-related acid-sensitive K+ channel; TRAM-34, 1-[(2-chlorophenyl)diphenylmethyl]-1H-pyrazole; TWIK, tandem of P-domains in a weak inward rectifying K+ channel; V_{1/2}, half-maximal voltage; XE991, 10,10-bis(4-pyridinylmethyl)-9(10H)-anthracenone

Potassium channels regulate excitability, epithelial ion transport, proliferation, and apoptosis. In pancreatic ducts, K+ channels hyperpolarize the membrane potential and provide the driving force for anion secretion. This review focuses on the molecular candidates of functional K+ channels in pancreatic duct cells, including KCNQ4 (K_{ir}), KCNMA1 (K_{ir}), KCNQ1 (K_{ir}), KCNH2 (K_{ir}), KCNH5 (K_{ir}), KCNH7 (K_{ir}), KCNT1 (K_{ir}), KCNT2 (K_{ir}), and KCNK5 (K_{ir}). We will give an overview of K+ channels with respect to their electrophysiological and pharmacological characteristics and regulation, which we know from other cell types, preferably in epithelia, and, where known, their identification and functions in pancreatic ducts and in adenocarcinoma cells. We conclude by pointing out some outstanding questions and future directions in pancreatic K+ channel research with respect to the physiology of secretion and pancreatic pathologies, including pancreatitis, cystic fibrosis, and cancer, in which the dysregulation or altered expression of K+ channels may be of importance.

Introduction

Potassium channels (K+ channels) are very important membrane proteins present in every cell. They determine the cell membrane potential and thereby regulate the excitability of neurons and myocytes and transport of ions and water in epithelia, such as the pancreas and salivary glands. Duct epithelial cells in the pancreas secrete a HCO3- rich pancreatic juice that neutralizes acid chyme in the duodenum. Secretin, acetylcholine, and ATP stimulate fluid secretion via signal transduction involving cAMP and Ca2+ signaling pathways. The generally accepted model for HCO3- secretion involves Cl––HCO3- exchangers (SLC26A3 and SLC26A6) that operate in parallel with cAMP-activated Cl– channels (CFTR) or Ca2+-activated Cl– channels (most likely TMEM16A) on the luminal membrane and Na+–coupled transporters such Na+-K+–Cl– co-transporter (NKCC1), Na+-HCO3- co-transporter (SLC4A4), and Na+-H+ exchanger (SLC9A1) and Na+-K+–pump on the basolateral membrane (Fig. 1).1,3 In addition, H+-K+–pumps are expressed on the luminal and basolateral membranes of pancreatic ducts.1,3 K+ channels are clearly important for setting the resting membrane potential and providing the driving force for anion exit and fluid secretion in a stimulated epithelium.1,3,5 K+ channels may also provide the transport partners for H+-K+–pumps.1,3 In addition, certain K+ channels could play an important role in pancreatic pathology, such as cystic fibrosis, pancreatitis, and pancreatic adenocarcinoma. Perhaps surprisingly, there are not so many K+ channels studies performed on pancreatic ducts.

Early electrophysiological studies using microelectrodes and patch-clamp methods indicated that pancreatic ducts expressed voltage- and Ca2+-activated K+ channels, consistent with maxi-K+ channels (BK channels), intermediate-conductance Ca2+-activated
K⁺ channels (IK channels), and pH/HCO₃⁻ sensitive K⁺ channels. Recent studies focusing on molecular candidates have shown that pancreatic ducts express the following channels that could be candidates for above functional channels: Kᵥ 3.1 channels coded by the KCNMA1 and KCNB1 genes (α– and β-subunits of the BK channel); the Kᵥ 3.1 protein is mediated by the KCNN4 gene (IK channel); the KCNK5 gene (Kᵥ 5.1); and they also express: KCNQ1 (Kᵥ 7.1, KVLUQT1), KCNH2 (Kᵥ 11.1, HERG), KCNH5 (Kᵥ 10.2, EAG2), KCNT1 (Kᵥ 4.1, Slack), and KCNT2 (Kᵥ 4.2, Slick), the functions of which remain unclear in duct cells.

It is not known whether many of these candidates are functional in pancreatic ducts or what is their localization and regulation. Therefore, their physiological and possibly pathophysiological functions have not to be confirmed. The aim of this review is to provide an overview of the above mentioned K⁺ channels with respect to their electrophysiological and pharmacological characteristics and functions, as we know from other cell types, preferably in epithelia, and, where known, their identification and functions in pancreatic ducts is given (Table 1). We also address some outstanding questions and future directions in pancreatic K⁺ channel research.

**Table 1**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Tissue</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCNMA1</td>
<td>Pancreatic ducts</td>
<td>Strong</td>
</tr>
<tr>
<td>KCNB1</td>
<td>Pancreatic ducts</td>
<td>Strong</td>
</tr>
<tr>
<td>KCNN4</td>
<td>Pancreatic ducts</td>
<td>Strong</td>
</tr>
<tr>
<td>KCNK5</td>
<td>Pancreatic ducts</td>
<td>Strong</td>
</tr>
<tr>
<td>KCNQ1</td>
<td>Pancreatic ducts</td>
<td>Strong</td>
</tr>
<tr>
<td>KCNH2</td>
<td>Pancreatic ducts</td>
<td>Strong</td>
</tr>
<tr>
<td>KCNH5</td>
<td>Pancreatic ducts</td>
<td>Strong</td>
</tr>
<tr>
<td>KCNT1</td>
<td>Pancreatic ducts</td>
<td>Strong</td>
</tr>
<tr>
<td>KCNT2</td>
<td>Pancreatic ducts</td>
<td>Strong</td>
</tr>
</tbody>
</table>

**Figure 1.** Model of ion transport in a pancreatic duct cell. Intracellular HCO₃⁻ is derived from CO₂ through the action of carbonic anhydrase (CA) and from HCO₃⁻ uptake via the Na⁺–HCO₃⁻ cotransporter. H⁺ is extruded at the basolateral membrane by the Na⁺–H⁺ exchanger and H⁺–K⁺ pump. HCO₃⁻ efflux across the luminal membrane is mediated by Cl⁻–HCO₃⁻ exchangers and/or Cl⁻ channels, and the H⁺–K⁺ pump may provide a buffering/protection zone for the alkali-secreting epithelium. K⁺ channels provide an exit pathway for K⁺ and play a vital role in maintaining the membrane potential, which is a crucial component of the driving force for anion secretion.

**Tissue expression**

*KCNN4* coding for the Kᵥ 3.1 protein was cloned from the human ductal cells. Functional expression of the *KCNN4* gene has been demonstrated in colonic crypts, salivary acini, and pancreatic ducts. Immunoreactivity of the Kᵥ 3.1 protein has also been reported in the esophagus, stomach, small intestine, proximal colonic crypts, salivary glands, luminal membrane of lacrimal gland duct cells, and intercalated and intralobular ducts of the pancreas. Interestingly, Kᵥ 3.1 channel immunoreactivity was shown to be localized in both the basolateral and luminal membranes in pancreatic ducts and monolayer of Capan-1, a human pancreas adenocarcinoma cell line, though its expression appeared to be stronger in the luminal membrane. Consistent with this finding, the short-circuit current (Iₛ) of the Capan-1 cell monolayer was enhanced by the Kᵥ 3.1 channel activator DC-EBIO in luminal or basolateral bathing solution. Kᵥ 3.1 could potentially be an important candidate for luminal K⁺ channels in pancreatic ducts. Importantly, equivalent circuit analysis revealed that luminal K⁺ conductance contributed to a minimum of 10% of the total K⁺ conductance in pancreatic ducts. Moreover, stimulation of the rat pancreas with secretin caused a marked increase in K⁺ concentrations in the pancreatic juice, which was equal to twice that in the plasma, indicating that K⁺ was secreted. K⁺ efflux was also shown to be mediated via mucosal Kᵥ 3.1 channels in other epithelia, such as the distal colon, and provided, in part, the driving force for agonist-induced anion secretion. Another example is salivary acini, in which both Kᵥ 1.1 and Kᵥ 3.1 were shown to be expressed on the apical membrane and contribute to optimal secretion. Furthermore, H⁺–K⁺–pumps were reported to be expressed on the luminal membranes of pancreatic ducts and their function, such as contributing to local epithelial protection, appeared to depend on the operation of K⁺ channels.

**Channel properties**

Patch-clamp studies using *Xenopus* oocytes and mammalian expression systems established the basic electrophysiological and pharmacological properties of Kᵥ 3.1 channels. Single-channel openings were observed at both positive and negative membrane potentials, and this gating showed no significant voltage-dependency. The single-channel current–voltage relationship showed weak inward rectification with conductance of 30–54 pS in heterologous expression systems. Interestingly, intermediate-conductance K⁺ channels exhibited a conductance of 80 pS in rat pancreatic duct cells. One explanation for this discrepancy is that unidentified auxiliary proteins for Kᵥ 3.1 channels or additional *KCNN4* genes may exist in rodent cells. Regarding pharmacology, Kᵥ 3.1 currents were inhibited by charybdotoxin, clotrimazole, TRAM-34, and maurotoxin with Kₐ values of 2–28 nM, 25–150 nM, 20 nM, and 1 nM, respectively. Kᵥ 3.1 currents were also activated by 1-EBIO and DC-EBIO with Kₐ values of 15–84 μM and 0.8 μM, respectively.

**Regulation**

Regarding regulation, it is well established that Kᵥ 3.1 channels are activated by the Ca²⁺/calmodulin signaling pathway. For example, heterologously expressed Kᵥ 3.1 channels were previously shown to be activated by submicromolar free Ca²⁺ concentrations with EC₅₀ values of 0.1–0.3 μM. There is also strong evidence to suggest that the Ca²⁺ sensitivity of Kᵥ 3.1 channels is mediated by calmodulin and calmodulin...
In addition, ATP/UTP was shown to regulate $K_{Ca}$ channels via purinergic receptors in pancreatic cell lines and rat pancreatic duct cells. Both P2Y$_2$ and P2Y$_4$ receptors upregulated $K_{Ca}$ activity in the *Xenopus* oocyte expression system. Importantly, luminal ATP/UTP, most likely delivered by secretory acini, was reported to stimulate ductal secretion. The physiological role of $K_{Ca}$ channels in pancreatic secretion could be also investigated with respect to secretin, which acts predominantly via the cAMP/cAMP-dependent protein kinase (PKA) signaling pathway, however, until this becomes available, we need to resort to studies on other cell types. A membrane-associated PKA has been proposed to activate $K_{Ca}$ channels

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Conductance ($pS$)</th>
<th>Blockers ($K_i$)</th>
<th>Activators ($K_d$)</th>
<th>Regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCNN4</td>
<td>$K_{Ca}3.1$</td>
<td>30–54$^{15,28,29}$</td>
<td>charrybdotoxin (2–28 nM)$^{15,28,29}$, clotrimazole (25–150 nM)$^{15,28,31}$, TRAM-34 (20 nM)$^{30}$, maurotoxin (1 nM)$^{32}$</td>
<td>1-EBIO (15–84 μM)$^{28,29,31,33}$, DC-EBIO (0.8 μM)$^{33}$</td>
<td>$Ca^{2+}$, calmodulin$^{18,28,34}$, PKA$^{19,42,43}$, extracellular UTP$^{11}$ cell swelling$^{7,48}$</td>
</tr>
<tr>
<td>KCMA1</td>
<td>$K_{Ca}1.1$</td>
<td>100–270$^{30,56}$</td>
<td>tetraethylammonium (0.14 mM)$^{30}$, charrybdotoxin (1–31 nM)$^{57,58,62}$, iberiotoxin (1–9 nM)$^{58,61,62}$, paxiline (2–9 nM)$^{58,60}$</td>
<td>NS1608 (2 μM)$^{52}$, NS11021 (0.4 μM)$^{53}$</td>
<td>membrane potential$^{7,50,56,64}$, $Ca^{2+}$, PKA$^{7,56}$, extracellular UTP$^{11}$</td>
</tr>
<tr>
<td>KCNQ1</td>
<td>$K_{Ca}7.1$</td>
<td>0.7–4$^{60,81}$</td>
<td>chromanol 293B (10–41 μM)$^{68,82,86,87}$, azimilide (77 μM)$^{86}$, XE991 (0.8 μM)$^{70}$</td>
<td>L-364,373$^{90}$</td>
<td>membrane potential$^{78,81}$ cAMP$^{97}$, cytosolic pH$^{83}$</td>
</tr>
<tr>
<td>KCNQ1/KCNE1</td>
<td>$K_{Ca}7.1/minK$</td>
<td>4.5–16$^{60,81}$</td>
<td>chromanol 293B (3–10 μM)$^{58,62}$, azimilide (5.6 μM)$^{66}$, XE991 (11 μM)$^{68}$, Mefloquine (0.9 μM)$^{89}$</td>
<td>DIDS$^{96}$, mefenamic acid$^{96}$</td>
<td>membrane potential$^{78,81}$ cAMP$^{93,92}$, cytosolic pH$^{93}$</td>
</tr>
<tr>
<td>KCNH2</td>
<td>$K_{Ca}11.1$</td>
<td>10–13$^{102,104}$</td>
<td>E-4031 (7–1250 nM)$^{104,105,106,111}$, BeKm-1 (3–12 nM)$^{106,110}$, ergotoxin (4.5–17 nM)$^{107,109}$, LY97241 (2.2–19 nM)$^{111,112}$</td>
<td>mallotoxin (0.5 μM)$^{114}$, PD-118057 (3.1 μM)$^{115}$, ICA-105574 (0.5 μM)$^{108}$</td>
<td>membrane potential$^{116}$ PKA$^{110,120}$</td>
</tr>
<tr>
<td>KCNH5</td>
<td>$K_{Ca}10.2$</td>
<td></td>
<td></td>
<td></td>
<td>membrane potential$^{97}$ PKC$^{107}$</td>
</tr>
<tr>
<td>KCNT1</td>
<td>$K_{Ca}4.1$</td>
<td>180$^{122}$</td>
<td>bepridil (1 μM)$^{125}$, quinidine (90 μM)$^{25}$</td>
<td>bithionol (0.8 μM)$^{125}$, niclosamide (2.9 μM)$^{126}$, loxapine (4.4 μM)$^{126}$, niflumic acid (2.7 mM)$^{127}$</td>
<td>membrane potential$^{123,122}$ $Ca^{2+}$, $Na^{+}$, $Cl^-$, PKC$^{130}$</td>
</tr>
<tr>
<td>KCNT2</td>
<td>$K_{Ca}4.2$</td>
<td>140$^{122}$</td>
<td>quinidine$^{122}$, isoflurane$^{128}$</td>
<td>meclofenamic acid (80 μM)$^{127}$, flufanemic acid (1.1–4 mM)$^{127,129}$, niflumic acid (2.1 mM)$^{127,129}$</td>
<td>membrane potential$^{122}$ $Na^{+}$, $Cl^-$, intracellular ATP$^{122}$ PKC$^{130}$</td>
</tr>
<tr>
<td>KCNK5</td>
<td>$K_{Ca}5.1$</td>
<td>50–78$^{133,136,137}$</td>
<td>quinine (22 μM)$^{131}$, clofilium (25 μM)$^{138}$, bupivacaine (26 μM)$^{139}$, ropivacaine (95 μM)$^{139}$</td>
<td>halothane, isoflurane, chloroform$^{40}$</td>
<td>extracellular pH$^{133,138,140,141}$ PKC$^{40}$ osmolality$^{38}$</td>
</tr>
</tbody>
</table>
in human erythrocytes, the T84 human colonic crypt cell line, and rat submandibular acinar cells.\textsuperscript{39,42,43} Interestingly, the PKA consensus phosphorylation site at serine 334 in K\textsubscript{Ca}\textsubscript{3.1} channels was not involved in PKA-dependent activation.\textsuperscript{45} In contrast to these studies, heterologously expressed K\textsubscript{Ca}\textsubscript{3.1} channels were not affected by PKA activators and/or inhibitors,\textsuperscript{29,30} or were inhibited by the catalytic subunit of PKA.\textsuperscript{55} Given these contradictory results, it is tempting to speculate that K\textsubscript{Ca}\textsubscript{3.1} channels may be activated via the phosphorylation of a closely associated protein, the expression of which is tissue-specific. One candidate for this protein is A-kinase anchoring protein (AKAP), which is able to scaffold PKA and components of cAMP signaling pathways, including G protein-coupled receptors and ion channels.\textsuperscript{66}

In addition to transepithelial transport, K\textsubscript{Ca}\textsubscript{3.1} channels were also shown to be stimulated by cell swelling, which triggered regulatory volume decreases.\textsuperscript{7,48} Notably, KCNN4 mRNA levels were upregulated in primary pancreatic tumors, and the growth of ductal adenocarcinoma cell lines in vitro was inhibited by blockers of K\textsubscript{Ca}\textsubscript{3.1} channels, which indicated that these were correlated with the proliferation of pancreatic cancer.\textsuperscript{49}

\textbf{KCNMA1 (K\textsubscript{Ca}\textsubscript{1.1}, Slo1, α-subunit of BK) and KCNBMB (β-subunits)}

\textbf{Tissue expression}

The KCNMA1 coding K\textsubscript{Ca}\textsubscript{1.1} (Slo1) protein was cloned from brain and skeletal muscle.\textsuperscript{50} Functional expression of the KCNMA1 gene has been demonstrated in the colon,\textsuperscript{31} salivary acini,\textsuperscript{17} pancreatic acini,\textsuperscript{52} and pancreatic ducts.\textsuperscript{31,53} The K\textsubscript{Ca}\textsubscript{1.1} protein is located in the luminal membrane of colonic epithelia,\textsuperscript{31,54} salivary acini and ducts,\textsuperscript{27,55} and pancreatic ducts.\textsuperscript{33} It is noteworthy that there was no labeling of the basolateral membrane of guinea-pig pancreatic duct cells, although the first recordings of maxi-K\textsuperscript{+} currents were made on the basolateral membrane of rat pancreatic ducts.\textsuperscript{7} Venglovecz et al.\textsuperscript{53} proposed that luminal K\textsubscript{Ca}\textsubscript{1.1} channels, which are activated by bile acids in the lumens, regulate HCO\textsubscript{3}~ secretion in pancreatic ducts. Nevertheless, experiments on K\textsubscript{Ca}\textsubscript{1.1} regulation have also indicated that some channels may be confined to the basolateral membrane (see below). Luminal K\textsubscript{Ca}\textsubscript{1.1} channels in the distal colon were shown to be responsible for resting and stimulated Ca\textsupscript{2+}-activated K\textsuperscript{+} secretion.\textsuperscript{51}

\textbf{Channel properties}

K\textsubscript{Ca} channels have the largest single-channel conductance of all K\textsuperscript{+} selective channels: 100–270 pS in symmetrical 150 mM KCl.\textsuperscript{50,56} Maxi-K\textsuperscript{+} currents in isolated rat pancreatic duct cells had a conductance of 170–180 pS.\textsuperscript{7,14} Regarding pharmacology, the α-subunit of K\textsubscript{Ca}\textsubscript{1.1} was inhibited by tetraethylammonium, charybdotoxin, iberiotoxin, and paullin with K\textsubscript{0} values of 0.14 mM, 1–31 nM, 1–9 nM, and 2–9 nM, respectively.\textsuperscript{50,57,62} The α-subunit of K\textsubscript{Ca}\textsubscript{1.1} was also activated by NS1608 and NS11021 with K\textsubscript{v} values of 2 μM and 0.4 μM, respectively.\textsuperscript{50,63} Interestingly, dehydrosoyasaponin I (DHS-I) activated the α-subunit of K\textsubscript{Ca}\textsubscript{1.1} only if co-expressed with the β1-subunit, an auxiliary protein for K\textsubscript{Ca}\textsubscript{1.1} channels.\textsuperscript{77}

\textbf{Regulation}

Significant diversity has been reported in the functional characteristics of K\textsubscript{Ca}\textsubscript{1.1} channels. It is well established that K\textsubscript{Ca}\textsubscript{1.1} channels are activated by membrane depolarization alone, intracellular Ca\textsuperscript{2+} alone, or synergistically by depolarization and Ca\textsuperscript{2+}.\textsuperscript{7,56,57} The single-channel open probability of K\textsubscript{Ca}\textsubscript{1.1} channels markedly increased when the cytoplasmic face of a patch membrane was exposed to 10 μM Ca\textsuperscript{2+} and voltage was changed over a range of −60 to +80 mV. Under these conditions, the half-maximal voltage (V\textsubscript{1/2}) was +23 mV in 10 μM Ca\textsuperscript{2+};\textsuperscript{50} however, these were unphysiological conditions for pancreatic ducts. Importantly, maxi-K\textsuperscript{+} channels on pancreatic duct cells were activated by much lower Ca\textsuperscript{2+} concentrations. For example, maxi-K\textsuperscript{+} channels exposed to 3 μM Ca\textsuperscript{2+} reached V\textsubscript{1/2} at −4 mV.\textsuperscript{77} This difference indicated that the β-subunit exists in pancreatic duct cells. Maxi-K\textsuperscript{+} channels on Xenopus oocytes that heterologously expressed both the α – and β1-subunits of K\textsubscript{Ca}\textsubscript{1.1} proteins were about 10-fold more sensitive to activation by voltage and Ca\textsuperscript{2+} concentration than channels composed of the α-subunit alone.\textsuperscript{57} Indeed, KCNMB1 coding the β1 subunit was detected in isolated pancreatic ducts.\textsuperscript{11}

Interestingly, UTP was shown to inhibit K\textsubscript{Ca}\textsubscript{1.1} channels via the P2Y\textsubscript{1} receptor,\textsuperscript{11} and appeared to lead to a decrease in secretion. The basolateral application of ATP/UTP inhibited K\textsuperscript{+} conductance in rat duct cells and secretion in guinea-pig ducts and human duct cell monolayers.\textsuperscript{35,39,40} These results collectively indicated that P2Y\textsubscript{1} receptors on the basolateral membrane appeared to downregulate secretion via K\textsubscript{Ca}\textsubscript{1.1} channels in the ductal system.

Regarding the cAMP/PKA signaling pathway, cAMP-dependent phosphorylation can also activate maxi-K\textsuperscript{+} channels on pancreatic duct cells.\textsuperscript{77} The functional response of K\textsubscript{Ca}\textsubscript{1.1} channels to PKA phosphorylation depends on the splice-variant of the α-subunit. For example, PKA was shown to activate the ZERO splice variant, whereas PKA inhibited the STREX variant. PKA activation of the ZERO variant requires a conserved C-terminal PKA site.\textsuperscript{57} Indeed, the ZERO splice variant has been shown to conduct adrenaline-induced K\textsuperscript{+} secretion in the distal colon.\textsuperscript{66}

\textbf{KCNQ1 (K\textsubscript{7.1}, KVLQT1) and KCNE1 (minK)}

\textbf{Tissue expression}

The KCNQ1 coding K\textsubscript{7.1} protein was cloned from the heart.\textsuperscript{67} Functional expression of the KCNQ1 gene has also been demonstrated in the kidney, stomach, small intestine, colon,\textsuperscript{68–71} pancreatic acini,\textsuperscript{69,72,73} and pancreatic ducts.\textsuperscript{13} Immunoreactivity of the K\textsubscript{7.1} channel protein was reported in the parietal cells of the stomach, in the basolateral membrane of small intestinal and colonic crypt cells,\textsuperscript{69,74,75} and in acinar and duct cells of the pancreas.\textsuperscript{13,69} K\textsubscript{7.1} resides in the tubulovesicular and canalicular membranes of gastric parietal cells together with H\textsuperscript{+}–K\textsuperscript{+} pumps and participates in gastric acid secretion.\textsuperscript{71,74,75} K\textsubscript{7.1} was localized in the luminal membrane of pancreatic duct cells,\textsuperscript{13} and may be involved in cell volume regulation during purinergic stimulation in epithelial transport,\textsuperscript{76,77} and/or may potentially be associated with H\textsuperscript{+}–K\textsuperscript{+} pumps expressed by pancreatic ducts.\textsuperscript{4}
The K\textsubscript{7.1} protein can assemble with the KCNE family of regulatory β-subunits to fulfill various physiological functions. For example, minK coded by the KCNE1 gene has been shown to modify K\textsubscript{7.1} activity by increasing unitary conductance, slowing activation, causing a right shift in the voltage dependence of activation, and modulating pharmacology.\textsuperscript{92-98} It is worth noting that the acidification of cytosolic pH increased K\textsubscript{7.1}–minK, but decreased K\textsubscript{7.1} currents, whereas alkalization decreased K\textsubscript{7.1}–minK, but increased K\textsubscript{7.1} currents.\textsuperscript{93} Indeed, the whole pancreas expresses KCNE1 and KCNE2 genes.\textsuperscript{48,49} The K\textsubscript{7.1} current was shown to be strongly diminished and membrane targeting of the K\textsubscript{7.1} protein was impaired in acinar cells in K\textsubscript{CNE1} knockout mice.\textsuperscript{69} The expression and function of KCNE in duct cells has not yet been investigated.

**Channel properties**

K\textsubscript{7.1} channels have very small conductance. Noise analysis revealed estimated single-channel conductances of 0.7–4 pS\textsuperscript{80,81} Small conductance K\textsuperscript{+} channels had 1 pS and were inhibited by chromanol 293B, a K\textsubscript{7.1} blocker, in the basolateral membrane of rat pancreatic acinar cells.\textsuperscript{73} Chromanol 293B inhibited α-subunit of K\textsubscript{7.1} with K\textsubscript{v} values of 10–41 μM in Xenopus oocytes and mammalian expression systems.\textsuperscript{68,82,86,87} Importantly, KCNE β-subunits increase the sensitivity of K\textsubscript{7.1} to chromanol 293B. K\textsubscript{v} values for K\textsubscript{7.1}/KCNE1, K\textsubscript{7.1}/KCNE2 and K\textsubscript{7.1}/KCNE3 were 3–10 μM, 0.4 μM, and 3–4 μM, respectively.\textsuperscript{68,70,82,86,87} Voltage-gated K\textsuperscript{+} currents in pancreatic acinar cells were shown to be inhibited by chromanol 293B with a K\textsubscript{v} value of 3 μM.\textsuperscript{72} This result supports voltage-gated K\textsuperscript{+} channels being composed of K\textsubscript{7.1} and KCNE1 β-subunit in acinar cells. Azimilide inhibited K\textsubscript{7.1} and K\textsubscript{7.1}/KCNE1 in the same manner as chromanol 293B with K\textsubscript{v} values of 77 μM and 5.6 μM, respectively.\textsuperscript{86} In contrast, XE991 inhibited K\textsubscript{7.1} and K\textsubscript{7.1}/KCNE1 with K\textsubscript{v} values of 0.8 μM and 11 μM, respectively.\textsuperscript{88} Mefloquine inhibited K\textsubscript{7.1}/KCNE1 with a K\textsubscript{v} value of 0.9 μM.\textsuperscript{89} DIDS and mefenamic acid activated K\textsubscript{7.1}/KCNE1, but not K\textsubscript{7.1}.\textsuperscript{80,86} On the other hand, L-364,373 activated K\textsubscript{7.1}, but did not affect K\textsubscript{7.1}/KCNE1.\textsuperscript{90}

**Regulation**

Regarding regulation, voltage-gated K\textsubscript{7.1} channels are known to be regulated by the cAMP signaling pathway.\textsuperscript{91} In addition, AKAPs are required for cAMP regulation of recombinant K\textsubscript{7.1} channels in mammalian cell lines.\textsuperscript{92} Interestingly, a K\textsuperscript{+} current was elicited by cAMP stimulation in CFTR-transfected, but not untransfected CFPAC-1 cells derived from a cystic fibrosis patient with deletion in Phe-508 in CFTR.\textsuperscript{93} AKAPs also mediate PKA compartmentalization with CFTR,\textsuperscript{94} therefore, these findings imply that functional CFTR regulates the K\textsubscript{7.1} channel, presumably in the luminal membrane of pancreatic duct cells.

**KCNH2 (K\textsubscript{11.1}, HERG) and KCNH5 (K\textsubscript{10.2}, EAG2)**

**Tissue expression**

The KCNH2 coding K\textsubscript{11.1} (HERG) protein was isolated from the hippocampal cDNA library.\textsuperscript{95} Functional expression of the KCNH2 gene has been demonstrated in colon carcinoma cells.\textsuperscript{96} Immunoreactivity of the K\textsubscript{11.1} protein was also reported in colon carcinoma cells\textsuperscript{96} and the luminal membrane of pancreatic duct cells.\textsuperscript{13} The KCNH5 coding K\textsubscript{10.2} (EAG2) protein was identified in the thalamus and was expressed in the brain, testes, skeletal muscle, heart, placenta, lung, liver, and at low levels in the kidney and whole pancreas.\textsuperscript{97,98} Notably, K\textsubscript{10.2} was shown to promote medulloblastoma tumor progression by regulating cell volume dynamics.\textsuperscript{99} KCNH2 and KCNH5 are clearly expressed in rodent and human pancreatic duct cells.\textsuperscript{13} However, the physiological or potentially pathophysiological role of K\textsubscript{11.1} and K\textsubscript{10.2} channels remains unclear. The related K\textsubscript{10.1} (KCNH1) channel has been shown to be upregulated in several cancers including pancreatic cancer, based on studies of human pancreatic adenocarcinoma cell lines.\textsuperscript{100,101}

**Channel properties**

K\textsubscript{11.1} channels have small conductance of 10–13 pS.\textsuperscript{92-101} Regarding pharmacology, K\textsubscript{11.1} was inhibited by E-4031, BeKm-1, and ergotxin with K\textsubscript{v} values of 7–1250 nM, 3–12 nM, and 4.5–17 nM, respectively.\textsuperscript{104-111} K\textsubscript{11.1} channels formed with KCNE2 were about 2-fold more sensitive to E-4031.\textsuperscript{104} LY97241 was shown to inhibit K\textsubscript{10.2} and K\textsubscript{11.1} currents with K\textsubscript{v} values of 1.5 μM and 2.2–19 nM, respectively.\textsuperscript{111,113} K\textsubscript{11.1} currents were also activated by mallowtoxin, PD-118057, and ICA-105574 with K\textsubscript{v} values of 0.5 μM, 3.1 μM, and 0.5 μM, respectively.\textsuperscript{108,114,115}

**Regulation**

K\textsubscript{11.1} currents were activated at voltages more positive than −50 mV and V\textsubscript{1/2} was ~15–11.1 mV,\textsuperscript{116} whereas K\textsubscript{10.2} currents were activated at around ~100 mV and V\textsubscript{1/2} was ~35.5 mV.\textsuperscript{97} However, a 14–3–3 protein was associated with K\textsubscript{11.1} in a phosphorylation-dependent manner at specific PKA sites and shifted V\textsubscript{1/2} in a hyperpolarizing direction by ~11.1 mV.\textsuperscript{117} K\textsubscript{11.1} may exist in a macromolecular signaling complex that includes 14–3-3 proteins and possibly AKAPs.\textsuperscript{118} Importantly, the K\textsubscript{11.1} protein can also assemble with KCNE1 or KCNE2 regulatory β-subunits.\textsuperscript{103,104} Regarding inhibition, phorbol 12-myristate 13-acetate (PMA), an activator of protein kinase C (PKC), produced a potent dose-dependent block of K\textsubscript{10.2} or K\textsubscript{11.1} currents.\textsuperscript{97,119} In addition, K\textsubscript{11.1} currents were reduced by the cAMP-specific phosphodiesterase inhibitor Ro-20–1724 or the adenylate cyclase inhibitor forskolin, which were shown to result in increased cAMP levels and PKA stimulation.\textsuperscript{120}

**KCNT1 (K\textsubscript{4.1}, Slo2.2, Slack) and KCNT2 (K\textsubscript{cα4.2}, Slo2.1, Slick)**

**Tissue expression**

KCNT1 (K\textsubscript{4.1}, Slo2.2, or Slack), which encodes for the Na⁺-activated K⁺ channel, was isolated from the brain cDNA library.\textsuperscript{121} KCNT1 and KCNT2 (K\textsubscript{cα4.2}, Slo2.1, or Slick) are expressed in the heart, kidney and testis, as well as in the brain.\textsuperscript{121-123} The functional expression of K\textsubscript{cα4.1} has been demonstrated in the basolateral membrane of the thick ascending limbs of Henle's loop.\textsuperscript{124} Pancreatic duct cells also expressed KCNT1 and KCNT2.\textsuperscript{125} Interestingly, the expression pattern of KCNT1 and KCNT2 was different between Capan-1 cells expressing functional CFTR channels and CFPAC-1 cells derived from a cystic fibrosis patient with a mutation in CFTR. Capan-1 cells express KCNT1, but not KCNT2, while CFPAC-1 cells express KCNT2,
but not KCNT1. This discrepancy indicates that the expression of KCNT1 and KCNT2 channels is in some way associated with the expression of functional CFTR. However, the function of these K+ channels in pancreatic duct cells remains to be investigated.

**Channel properties**

KCa.4.1 and KCa.4.2 channels have large conductances of 180 pS and 140 pS in symmetrical 130 mM KCl. In the basolateral membrane of the thick ascending limbs of Henle’s loop, Na+-activated K+ channels had a conductance of 140–180 pS. Regarding pharmacology, KCa.4.1 was inhibited by bepridil and quinidine with K values of 1 μM and 90 μM, respectively. KCa.4.1 was activated by bithionol, niclosamide, loxapine, and niflumic acid with K values of 0.8 μM, 2.9 μM, 4.4 μM, and 2.7 mM, respectively. KCa.4.2 was inhibited by 1 mM quinidine and isoflurane, and was activated by meclofenamic acid, flufenamic acid, and niflumic acid with K values of 80 μM, 1.1–1.4 mM, and 2.1 mM, respectively.

**Regulation**

KCa.4.1 was shown to be unusually inhibited by intracellular Ca2+ at 1 μM. However, KCa.4.1 may co-assemble with KCa.1.1 subunits to generate Ca2+-activated K+ channels. KCa.4.1 and KCa.4.2 channels were reported to be activated by intracellular Na+ and K+ values of 41 mM and 89 mM in the presence of 30 mM internal Cl−, respectively. These channels were also activated by intracellular Cl− or synergistically by Na+ and Cl−. Intracellular ATP inhibited KCa.4.2 directly, via the presence of a consensus ATP binding motif. A similar ATP binding motif has not been demonstrated in the KCa.4.1 sequences. Interestingly, the PKC activator PMA increased KCa.4.1 currents, but inhibited KCa.4.2 currents.

**KCNKS (KCa.5.1, TASK-2)**

**Tissue expression**

Two-pore domain K+ channels (KCa) generate background K+ currents over the whole membrane potential range. The pH-sensitive KCa subunits (TALK-1, TALK-2 and TASK-2) were shown to be expressed in pancreatic acini. An electrophysiological study indicated that TASK-2 was expressed in HPAF, a human pancreatic ductal adenocarcinoma cell line. KCNK5 coding TASK-2 (KCa.5.1) was isolated from the brain cDNA library. KCNK5 is expressed in the kidney, liver, stomach, small intestine, colon, and pancreatic acini. The functional expression of KCa.5.1 has been demonstrated in kidney proximal convoluted tubule cells, which could be involved in volume regulation and HCO3− transport. Clofilium-sensitive K+ conductance, possibly KCa.5.1, was located in the luminal membrane of the monolayer of HPAF. pH-sensitive K+ channels on the luminal membrane of pancreatic duct cells may be physiologically relevant in terms of maintaining the electrical driving force for electrogenic HCO3− secretion and providing an exit pathway for K+ secretion.

**Channel properties**

KCa.5.1 channels have an intermediate conductance of 50–78 pS. Regarding pharmacology, KCa.5.1 was inhibited by quinine, clofilium, bupivacaine, and ropivacaine with K values of 22 μM, 25 μM, 26 μM, and 95 μM, respectively. KCa.5.1 was activated by halothane, isoflurane, and chloroform, which are volatile anesthetics.

**Potassium Channels in Pancreatic Cancer**

Ion channels have been associated with the malignant phenotype of cancer cells, as well as contributing to virtually all basic cellular processes, including crucial roles in maintaining tissue homeostasis such as proliferation, differentiation, and apoptosis. Several potassium channels have been suggested as the hallmarks of cancer, including pancreatic duct adenocarcinoma. For example, KCa.3.1 channels have been correlated with the proliferation of pancreatic cancer. In addition, the expression of G protein-activated inward rectifier potassium channel 1 (KCa.3.1) was markedly higher in pancreatic adenocarcinomas than in a normal pancreas, whereas KCa.1.3 expression was decreased in pancreatic adenocarcinomas. Downregulation in the expression of KCa.1.3 has been associated with metastatic tumors. KCa.1.5 was also shown to be highly expressed in pancreatic adenocarcinomas. Furthermore, a specific monoclonal antibody that inhibits the function of KCa.10.1 (EAG1) reduced tumor growth of BxPC3, a human pancreas adenocarcinoma cell line, which implicates this channel in cancer progression. Altered pH homeostasis is known to be one of the key hallmarks of cancer. Thus, pH-sensitive KCa channels may also play a role in pancreatic adenocarcinoma. The human duct adenocarcinoma cell line, HPAF, cells, were reported to express KCa.5.1 channels. However, its contribution to cancer progression is still unknown. Although further studies on K+ channels in pancreatic cancer must be performed, some candidates, such as KCa.10.1, already have the potential to be diagnostic tools and therapeutic targets.

**Concluding Remarks**

This review described the current status on the molecular basis for a number of K+ channels found in pancreatic ducts. Electrophysiological studies on ducts and duct cells using microelectrode, patch-clamp, and Ussing chamber methods showed how some of these K+ channels contribute to physiological processes in ductal secretion by providing the driving forces for anion transport and as partial accompanying partners in...
secretion. Future studies are needed to verify the localization of K+ channels to a polarized ductal epithelium and affirm their participation in cell proliferation and apoptosis. The pancreas and especially the ductal epithelium are involved in a number of diseases including cystic fibrosis and pancreatitis. Some target therapies should include K+ channel openers to maintain or upregulate pancreatic secretion. Our knowledge regarding the role of K+ channels in duct cell homeostasis remains relatively sparse. Because some K+ channels are being regarded as the hallmark of cancer progression and emerging studies on pancreatic adenocarcinoma foreshadow similar trends, more knowledge is required in this area before specific K+ channel openers or inhibitors can be used in the treatment of pancreatic diseases.

Disclosure of Potential Conflicts of Interest No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by the Pancreas Research Foundation of Japan, JSPS KAKENHI Grant Number 24790226, the Lundbeck Foundation, and Danish Council for Independent Research Natural Sciences (10–085217).

Neylon CB, D’Souza T, Reinhart PH. ATP storage and uptake by pancreatic duct epithelial cells. Pflügers Arch 2000; 440:809-16; http://dx.doi.org/10.1007/s00424-000-0364-6

Neylon CB, D’Souza T, Reinhart PH. Protein kinase A inhibits intermediate conductance Ca²⁺-activated K⁺ channels expressed in Xenopus oocytes. Pflügers Arch 2004; 448:613-20; http://dx.doi.org/10.1007/s00424-004-1302-5


Hoffmann EK, Lambert IH, Pedersen SF. Physiology of cell volume regulation in vertebrates. Physiol Rev 2009; 89:153-277; PMID:1926278; http://dx.doi.org/10.1152/physrev.90003.2007


McManus OB. Blockage of intermediate-conductance K⁺ channels. J Biol Chem 1999; 274:3178-84; PMID:10542200; http://dx.doi.org/10.1074/jbc.74.3.31784


Krug HH, Schrøder RL, Jensen BS, Strøbæk D, Olesen SP. Calcium-activated potassium channels of the SLO family. J Biol Chem 1993; 268:9743-52; PMID:8528244; http://dx.doi.org/10.1038/35003200


Meera P, Wallner M, Toro L. A neuronal β subunit (KCNMB4) makes the large conductance, voltage – and Ca²⁺-activated K⁺ channel resistant to charybdotoxin and ibotenic acid. Proc Natl Acad Sci U S A 2000; 97:5562-7; PMID:10792958; http://dx.doi.org/10.1073/pnas.100118597

Bentzen BH, Nardi A, Callies K, Madsen LS, Olesen SP, Grunnet M. The small molecule NS11021 is a potent and specific activator of Ca²⁺-activated big-conductance K⁺ channels. Mol Pharmacol 2007; 72:1033-44; PMID:17636045; http://dx.doi.org/10.1124/mol.107.038331

Magleby KL. Gating mechanism of BK (Slo) channels: so near, yet so far. J Gen Physiol 2003; 121:81-96; PMID:12566537; http://dx.doi.org/10.1085/jgp.20028721


www.landesbioscience.com


Sesti F, Goldstein SA. Single-channel characteristics of wild-type I_{Ks} channels and channels formed with two MinK mutants that cause long QT syndrome. J Gen Physiol 1998; 112:651-63; PMID:9834139; http://dx.doi.org/10.1085/jgp.112.6.65.

Yuan H, Sigworth FJ. Single-channel properties of I_{Ks} potassium channels. J Gen Physiol 1998; 112:665-78; PMID:9834139; http://dx.doi.org/10.1085/jgp.112.6.65.


Sesti F, Goldstein SA. Single-channel characteristics of wild-type I_{Ks} channels and channels formed with two MinK mutants that cause long QT syndrome. J Gen Physiol 1998; 112:651-63; PMID:9834139; http://dx.doi.org/10.1085/jgp.112.6.65.


Bhattacharjee A, Joiner WJ, Wu M, Yang Y, Sigworth FJ, Kaczmarek LK. Slick (Slo2.1), a rapidly-gating sodium-activated potassium channel inhibited by ATP. J Neurosci 2003; 23:11681-91; PMID:14684870


Yang B, Gribkoff VK, Pan J, Damagno Y, Dworetszy SI, Boissard CG, Bhattacharjee A, Yan Y, Sigworth FJ, Kaczmarek LK. Pharmacological activation and inhibition of Slack (Slo2.1) channels. Neuropharmacology 2006; 51:896-906; PMID:16687260; http://dx.doi.org/10.1016/j.neuropharm.2006.06.003


