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Review

Glucose and GTP-binding protein-coupled receptor cooperatively regulate transient receptor potential-channels to stimulate insulin secretion

Masafumi Kakei[1), 4)], Masashi Yoshida[1)], Katsuya Dezaki[2)], Kiyonori Ito[1)], Hodaka Yamada[1)], Shunsuke Funazaki[1)], Masanobu Kawakami[1), 3)], Hitoshi Sugawara[1)] and Toshihiko Yada[2)]

[1)] *Internal Medicine, Saitama Medical Center, Jichi Medical University, Saitama 330-8503, Japan*
[2)] *Integrative Physiology, Jichi Medical University, Shimotsuke 329-0498, Japan*
[3)] *Nerima-Hikarigaoka Hospital, Tokyo 179-0072, Japan*
[4)] *Saitama Citizens Medical Center, Saitama 331-0054, Japan*

Abstract. In pancreatic β-cells, glucose-induced closure of the ATP-sensitive K^+ (KATP) channel is an initial process triggering glucose-stimulated insulin secretion (GSIS). This KATP-channel dependent pathway has been believed to be a central mechanism for GSIS. However, since the resting membrane potential of cells is determined by the balance of the net result of current amplitudes in outward and inward directions, it must be taken into consideration that not only KATP channel inhibition but also inward current via the basal opening of non-selective cation channels (NSCCs) plays a crucial role in membrane potential regulation. The basal activity of NSCCs is essential to effectively evoke depolarization in concert with KATP channel closure that is dependent on glucose metabolism. The present study summarizes recent findings regarding the roles of NSCCs in GSIS and GTP-binding protein coupled receptor-(GPCR) operated potentiation of GSIS.

Key words: GPCR, Pancreatic β-cells, TRP channel, Insulin secretion

THE CONCEPT that glucose-stimulated insulin secretion (GSIS) in pancreatic β-cells is initiated by closure of the ATP-sensitive K^+ (KATP) channel as a result of an increase in the intracellular ATP/ADP ratio induced by glucose metabolism upon elevation of glucose concentration in the blood (Fig. 1; the triggering pathway), was proposed a long time ago [1-3]. Inhibition of the KATP channel is followed by membrane depolarization and activation of voltage-dependent Ca^{2+} channels (VDCCs) [4, 5]. Opening of the VDCCs brings about firings of action potential, cytosolic Ca^{2+} increase and initiation of GSIS [6, 7]. The triggering pathway is followed by time-dependent increase in insulin secretion that is potentiated by glucose exposure (the second phase, potentiating pathway or KATP-independent pathway) [3, 8] However, closure of the KATP channel alone is not sufficient to induce a shift in the membrane potential towards the threshold level and for the triggering pathway that can activate VDCCs, since membrane potential is theoretically determined by the overall balance of outward and inward currents. Modest background inward currents through opening of nonselective cation channels (NSCCs) are crucial for induction of membrane depolarization following KATP channel closure [9, 10]. This idea further suggests that regulation of a class of NSCCs may play an important role in producing effective depolarization of the membrane. Several types of NSCCs have been reported to be expressed in pancreatic β-cells, which, in terms of ion selectivity, are permeable to Na^+, K^+, and Ca^{2+}. In contrast to these ion channels, which have not been well studied, the classic-type ion channels; i.e., the voltage-dependent Na^+ channel, the voltage-dependent K^+ channel [11, 12], the voltage-dependent Ca^{2+} channel [13, 14] and the KATP channel have attracted a large amount of interest for a long time, because the first three types of these ion channels play an important role in membrane excitability and the KATP

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Correspondence to: Masafumi Kakei, Internal Medicine, Jichi Medical University, 1-847 Amanuma, Omiya, Saitama 330-8503, Japan. E-mail: mkakei@jichi.ac.jp

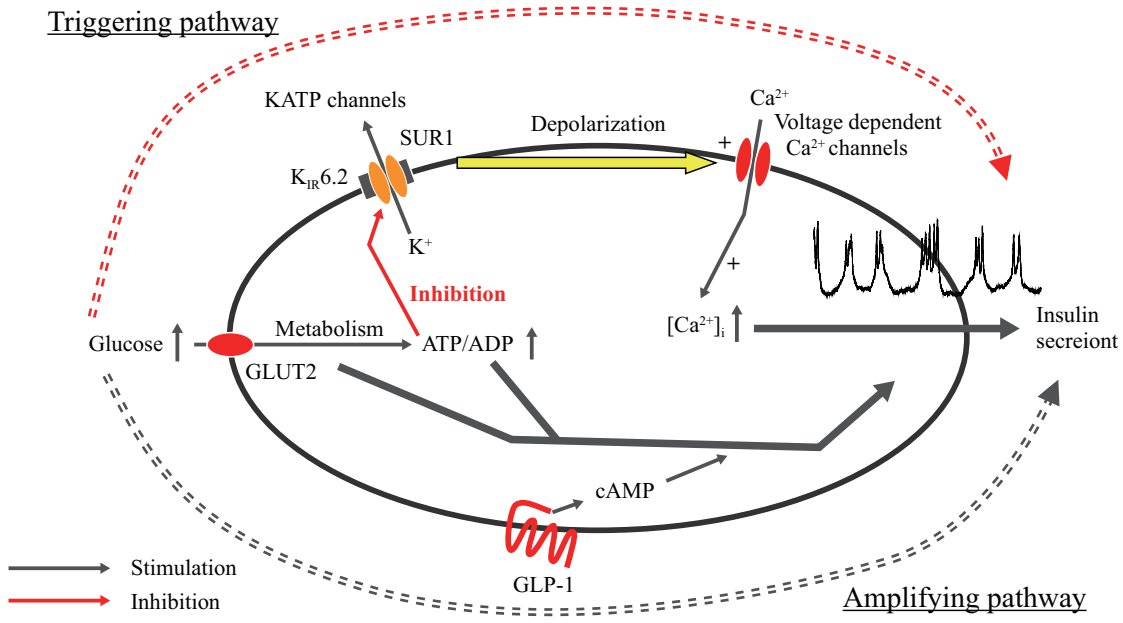

Fig. 1 Classic model of glucose-stimulated insulin secretion
An increase in the glucose concentration in the extracellular space induces metabolic and energy production processes with subsequent ATP/ADP elevation, KATP channel (composed of kir6.2 and SUR1 (sulfonyl-urea receptor 1) inhibition, membrane depolarization, VDCC activation and ultimately membrane excitement showing action potential firing. This glucose-induced initial event is called the first phase or triggering pathway (dashed red arrow) if it is followed by persistent and enhanced insulin secretion that is known as the second phase or potentiating pathway (dashed black arrow). The site of CLP-1/cAMP/PKA or EPAC2 signaling has been hypothetically proposed as locations to the KATP channel, VDCCs and the potentiating pathway [43, 47].

channel, a determinant of resting membrane potential [15]. However, in the present study we summarize recent findings regarding the physiological roles of NSCCs in GSIS of pancreatic β-cells and propose a new conceptual framework for regulation of the membrane potential in β-cells.

Membrane-potential regulation by background non-selective cation channels

The membrane potential of pancreatic β-cells is mostly determined by KATP channel activity. At low concentrations of extracellular glucose the resting membrane potential is approximately -70 mV, which is depolarized by 12 mV from the potassium equilibrium potential (E_K) that is calculated as -82 mV by assuming extra- and intracellular potassium concentrations of 5 mM and 130 mM, respectively (Fig 2A). This membrane potential shift is due to basal opening of NSCCs that is reversed in the current direction at 0 mV (broken line in Fig. 2A), because the NSCC current is permeable for Na^+, Ca^{2+} and K^+. Therefore the NSCC current is always inwardly-going in the physiological range of membrane potential. When the glucose concentration is increased, the resting membrane potential indicated by the red point at left in Fig. 2A moves to a more depolarized position (the red point at right) as a result of a decrease in the KATP-channel conductance (red line). If the β-cells were deficient in NSCCs, the resting membrane potential would be identical to E_K (Fig. 2B). In this situation, KATP-channel closure due to elevation of the glucose concentration does not produce any shift in the membrane potential. Thus, basal opening of NSCCs is a fundamental requirement for the membrane-potential shift towards the threshold level for activation of VDCCs. This means that membrane depolarization results from both closure of the KATP channel and basal opening of NSCCs. If the KATP channel activity is constant, i.e. at a low glucose concentration of 2.8 mM, and the NSCC current is increased, the membrane potential is depolarized over a small range (Fig. 2C). In contrast, at higher concentrations of glucose where the β-cell is not depolarized enough to evoke activation

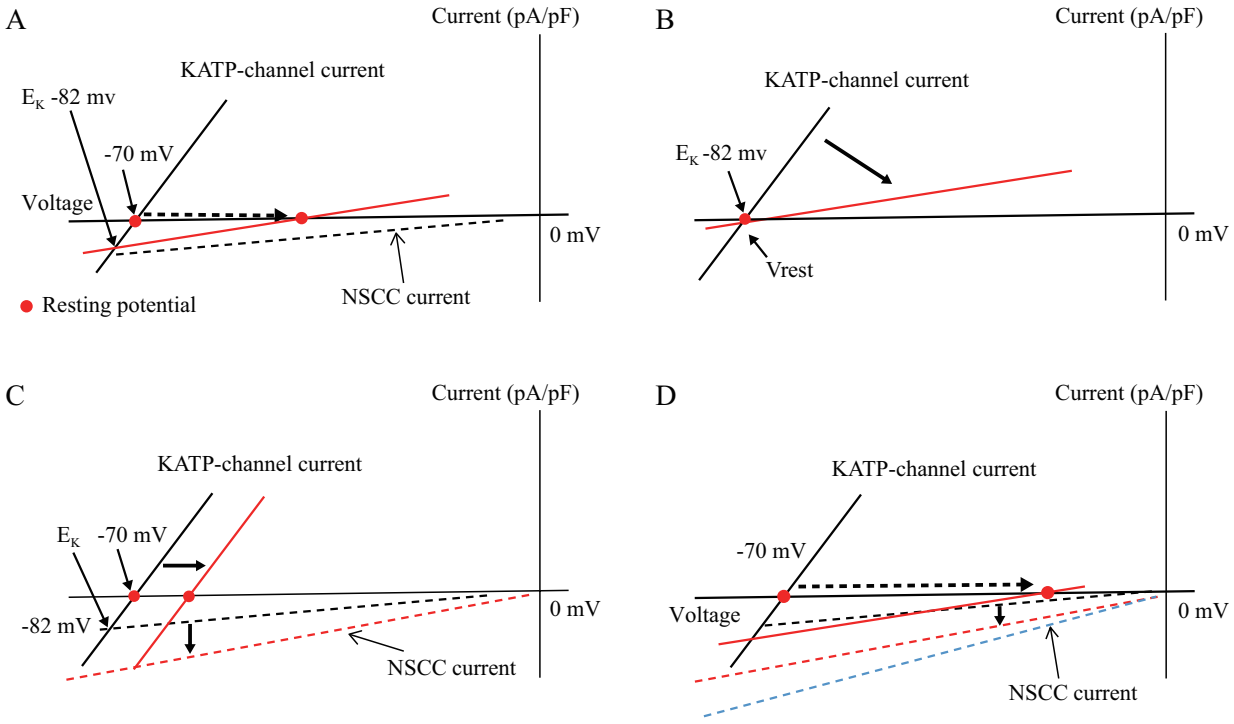

Fig. 2 Elements for determination of membrane potential
The membrane potential is determined by the balance between KATP and NSCC current amplitudes. **A**: Current-voltage (IV) relationship of the KATP channel and NSCC conductances. The resting membrane potential (-70 mV) is slightly shifted toward a positive potential from the potassium equilibrium potential (E_K, -82 mV) at a low glucose concentration (i.e.; 2.8 mM). The KATP channel conductance is shown as a solid black line crossing the zero current level at -70 mV (indicated by the red dot at left). When the KATP channel conductance is pharmacologically inhibited i.e. exposure to sulfonylurea (red line), the resting potential is moved to the red dot at right (broken arrowed line). The other broken line indicates NSCC conductance. This is the model that the β-cell is exposed to tolbutamide at low glucose concentration. **B**: When NSCCs are absent, the resting potential does not shift even when the KATP channel is inhibited (red line). The red point is unchanged. **C**: The resting potential shift with constant KATP-channel conductance is small but is clearly depolarized upon increasing the NSCC conductance (broken red line). The KATP channel conductance is shifted in parallel to the unbroken red line. This is the model that the β-cell is exposure to incretin hormone at low glucose concentration. **D**: The membrane conductance changes in GSIS and/or the incretin model. When the KATP channel conductance is inhibited by glucose (red line) and simultaneously the NSCC conductance is increased (broken red line), the resting membrane potential is more strongly depolarized (compare the right-side red point in A and D). Note that the slope conductances of the KATP channel before (solid black lines) and after inhibition of the channel (solid red lines) in A and D are unchanged. Further increase in NSCC conductance upon GLP-1 exposure (broken blue line) induces more depolarization of the membrane independently of KATP channel inhibition. Notice that each slope conductance of the KATP channel (solid lines) and the NSCCs (broken lines) before (black lines) and after (red lines) the increase and decrease in the conductances is identical. E_K, potassium equilibrium potential.

of VDCCs, a small amount of NSCC-current increase would easily be able to depolarize the membrane to a threshold potential that could activate VDCCs, because the input resistance of the β-cell becomes higher during the process of KATP-channel inhibition under these conditions (Fig. 2D). This principle of membrane potential regulation leads us to a new concept of β-cell physiology; KATP channel and NSCC-operated and cooperative modulation of GSIS.

Non-selective cation channels in β-cells

The non-selective cation channels (transient receptor potential (TRP) channels) expressed in β-cells are listed in Table 1 [16-21]. Historically the concept that the TRP channel is a non-selective cation channel was first proposed by Montell *et al*. [22, 23]. The TRP channel is an ion channel that is permeable to Ca^{2+} [23]. The TRP channel includes 6 transmem-

Table 1 Non-selective cation channels expressed in β-cells

NSCCs		Ion selectivity (physiological roles, mediators)
TRPs	Trpv1,2,4	$Ca^{2+}>Na^{+}$ (sensor)
	Trpm2	$Ca^{2+}>Na^{+}$ [16] or $Ca^{2+}<Na^{+}$ [74, 75] (GLP-1, cyclic ADP-ribose, H_2O_2)
	Trpm4	$Ca^{2+}<<Na^{+}$ (Ca^{2+} store depletion, Ca^{2+})
	Trpm5	$Ca^{2+}<<Na^{+}$ (Ca^{2+} store depletion, Ca^{2+})
	Trpm7	mRNA in pancreas [20]
	Trpc1,3,4,6	Na^{+}, Ca^{2+}, K^{+} (Gq-PLC mediated)
5-HT (Htr3a)		Na^{+}, Ca^{2+}, K^{+} highly expressed during pregnancy

brane domains and cytoplasmic N- and C-termini. N-terminal Ankyrin repeats, a coiled coil domain, the TRP domain and the pore loop have been characterized as the channel. The TRP family is found in worms, flies, mice and humans. The family includes TRPC, TRPV, TRPM, TRPA and others [23]. These TRP family members are expressed in a variety of tissues [20, 24]. Many types of TRP subfamilies have been identified in pancreatic β-cells. Most of the channel family members are sensitive to temperature (heat), vision, taste and smell as well as touch [23]. Thus TRP channel expression in sensory neurons is critical for the fundamental function of these cells. These TRP channels are divided into two types of channel mediation; one is a channel regulation mechanism located downstream of stimulation of GTP-binding protein coupled receptor (GPCR) and the other is the direct regulation of the channel by a specific ligand binding to a site on the channel protein. TRPV1 was first described as the capsaicin receptor and heat-activated ion channel in the pain pathway [25]. TRPV1 is a non-selective cation channel that is permeable to Ca^{2+}, is activated at acid pH, and is inhibited by ruthenium red, which is a non-selective TRP channel blocker. This surprising and impactful report regarding TRPV1 was followed by a report of cold-sensitive TRPM8 by another group [26]. TRPV1 and TRPM8 are oppositely gated by heat and cold. Heat activates TRPV1 by shifting the voltage-dependent range for activation to physiological voltages and TRPM1 is similarly activated but by cold instead of heat. TRPM4 with a mutation is associated with progressive familial heart block type I (PFHBI) that exhibits autosomal dominant inheritance [27]. TRPM4 is a calcium-activated NSCC and may play a key role in the control of membrane potential and electrical activity of electrically excitable insulin secretory cells (INS1). The translocation of TRPM4 from a vesicular pool and its subsequent fusion with the plasma membrane via Ca^{2+}-dependent exocytosis may represent a key short- and midterm regulatory mechanism by which cells regulate electrical activity [28]. TRPM5 is also activated by Ca^{2+} and plays a central role in taste signaling of chemosensory cells [29]. Insulin downregulates the expression of TRPM5 in islets from leptin-deficient mice [30]. A recent report indicated that TRPM4/5 dependent stimulation of GSIS is mediated by a small dose of glucagon-like peptide (GLP-1) with a half-maximal effective concentration of 0.4 pM [31]. In addition the TRPC(1,3,4,6) family was identified in β-cells [32, 33]. For most of these TRPC channels, their physiological role and the mechanistic pathways regulating these channels in the GSIS process remain unclear.

The Htr3a channel is another type of NSCC, and this channel is sensitive to serotonin. The Htr3a activation by binding of serotonin to Htr3a receptor depolarizes the membrane potential and consequently stimulates the excitability of gestated β-cells in paracrine/autocrine mode. Serotonin production is pronounced in pregnant β-ells by activation of lactogen signaling and co-secreted with insulin and acts through Htr3a receptor to lower the β-cell threshold for glucose to GSIS thereby playing an essential role in the increased GSIS in pregnant animal [34]. Similarly co-secreted serotonin binds to Htr2b receptors of β-cells and this contributes to proliferation and increase in β-cell mass [35].

The important and more fully clarified TRP channel in pancreatic β-cells in terms of regulation of GSIS is TRPM2, which is described in the following paragraph.

Glucose-induced regulation of TRPM2-channel current and insulin secretion

It was reported that an increase in glucose concentration to 16.6 mM reversibly increases the NSCC current, and the current-voltage (I-V) relationship of the

NSCC current shows a reversal potential and slope conductance of -15.4 mV and 82.6 pS/pF, respectively [36]. Glucose at concentrations of 2.8, 5.6 and 16.6 mM dose-dependently increased the NSCC current. These effects of glucose on current increases in NSCC were not the result of glucose-induced KATP channel inhibition, because the same results were observed in the presence of the potassium channel blocker, tolbutamide. Similarly, NSCC-current increase was also evoked by increasing the glucose concentration to 16.6 mM in the absence of tolbutamide and further addition of tolbutamide did not influence the current level at the holding potential of -80 mV that is close to the potassium equilibrium potential (calculated as -82 mV). These findings suggested that the glucose effect on NSCC modulation was metabolism–dependent, and this notion was supported by the fact that the glucose-induced NSCC-current increase was not observed in the presence of the mitochondrial electron transport uncoupler, FCCP [36]. Thus, glucose metabolism evokes not only KATP channel inhibition as shown in Fig. 2D (broken black line), but it also increases the NSCC current (inward current) (Fig. 2D, broken red line). In fact, exposure to tolbutamide at 2.8 mM glucose depolarized the membrane only to -50.4 mV, whereas the membrane was further depolarized to -18.2 mV at 16.6 mM glucose [36]. Thus exposure of β-cells to tolbutamide alone at a low glucose concentration depolarizes the membrane to a lesser extent than that which occurs at a high glucose concentration (see and compare Fig. 2 A and D). We reported that this glucose-induced NSCC increase is due to opening of the TRPM2 channel, because this glucose effect was attenuated in β-cells from TRPM2-deficient mice [36]. These double effects of glucose on KATP-channel inhibition and NSCC (TRPM2) activation may synergistically and effectively depolarize the membrane to quickly trigger the first-phase of insulin secretion as illustrated in Fig. 2D. This mechanism may contribute to "priming" of the triggering pathway because glucose-induced TRPM2 activation is observed within a shorter time after exposure of the β-cells to glucose than glucose-induced KATP channel inhibition [36].

TRPM2 channels as a GLP-1 mechanism potentiating GSIS

GLP-1 is an incretin hormone, which is secreted from L-cells at intestinal villus in response to nutrient in lower gut as glucose-dependent insulinotropic polypeptide (GIP) in K-cells from upper gut, stimulates insulin secretion after meal [37, 38]. There have been many efforts to determine the mechanistic pathway by which GLP-1 elicits a positive effect on insulin secretion and to identify the type of ion channels responsible or to clarify the mechanistic site involved in this fundamentally pivotal physiology [15, 39-46]. Rapid progress and findings regarding these open questions were made following the report that TRPM2-deficient mice showed a lack of effect of GLP-1 on insulin secretion [17, 36]. GLP-1 depolarizes the membrane potential at a low glucose concentrations and its mechanistic pathway is through cAMP and an exchange protein directly activated by cAMP 2 (EPAC2 [47, 48]), but not protein kinase A (PKA)-dependent [36]. Exendine-4, a GLP-1 analogue that is resistant to dipeptidyl peptidase 4 (DPP4) degradation, causes current activation of the TRPM2 that is a class of NSCCs when co-applied with a KATP channel inhibitor. This current increase was not observed in TRPM2-KO mice and was inhibited by ESI-09 (an EPAC inhibitor) and by 2-APB (a non-selective TRPM2 antagonist), but not by H89 (a PKA inhibitor). Furthermore, these currents (NSCC current) were activated by 8-CPT-2-O-Me-cAMP, an EPAC activator and the current activation was abolished in TRPM2-KO mice-cells. Thus, it was concluded that EPAC2 activates TRPM2 and that this activation is an important pathway underlying incretin-potentiated insulin secretion. TRPM2 is also activated by GIP [36] and pituitary adenylate cyclase-activating polypeptide **(**PACAP) (unpublished data). Interestingly these GLP-1 effects on insulin secretion associated with TRPM2 activation are mediated by GLP-1 concentrations between 10 pM and 1 nM. These concentrations are exactly within the physiological range of the change in GLP-1 concentration after meal [38].

EPAC2 serves as a major pathway for cAMP-mediated potentiation of GSIS [49]. Glucose-mediated NSCC current activation was attenuated in the presence of the EPAC2 inhibitor ESI-09 and in TRPM2-KO mice [36]. In TRPM2-KO mice, the GLP-1 effect on GSIS was completely attenuated [17]. The combined data suggested that GLP-1 mainly regulates membrane potential and potentiates insulin secretion via an EPAC2/TRPM2 pathway that may be a common pathway of both glucose and GLP-1 signals.

A receptor-operated TRP channel pathway may be the major regulatory mechanism of GPCR agonistic and antagonistic mediations in β-cells

The GLP-1 mediated cAMP/EPAC2/TRPM2 pathway is inhibited by ghrelin, an acylated 28-amino acid peptide that is produced in the stomach and that has been found to be the endogenous ligand for a GPCR, the growth hormone (GH) secretagogue-receptor (GHSR) [50]. Ghrelin inhibits GSIS from the perfused pancreas, isolated islets and β-cell lines [51-55]. Ghrelin and GHSR are located in the pancreatic islets [20, 56]. We have found that the insulinostatic actions of ghrelin are mediated via the pertussis toxin (PTX)-sensitive G-protein Gαi2 in β-cells, which leads to attenuation of cAMP and $[Ca^{2+}]_i$ signaling in β-cells and insulin release from islets by glucose stimulation [57, 58]. This unique signal coupling to the cAMP level in the cell antagonizes the glucose stimulation of insulin secretion that activates EPAC2/TRPM2 as has been described above and potentiates the triggering pathway in GSIS [36, 59]. This hypothesis can be confirmed because ghrelin antagonizes GSIS by counteracting cAMP production by glucose [60] (see Fig. 3, P-3 and P-4). NSCC-current increase due to 8.3 mM glucose (TRPM2-current increase) was attenuated in the presence of 10 nM ghrelin. This antagonistic effect of ghrelin was not observed in β-cells from growth hormone secretagogue-receptor (GHSR) deficient mice. A glucose-induced increase in NSCC could not be observed in TRPM2-deficient mice [36, 59]. Both cAMP production and $[Ca^{2+}]_i$ increase induced by glucose were suppressed by ghrelin in β-cells of GHSR-null/Ins-Cre mice in which GHSR was re-expressed in islet cells [59]. Presumably some level of cAMP *per se* in the cytosol is essential to maintain normal GSIS. The fact that ghrelin inhibits GSIS via cAMP production also suggests that intrinsically produced cAMP is critical for normal GSIS process. Similarly, a membrane permeable cAMP analogue also effectively maintains GSIS during a normal response [61].

Both glucose metabolism and GLP-1 receptor stimulation increased the activity of TRPM2 channels via the cAMP-EPAC pathway but not via the PKA path-

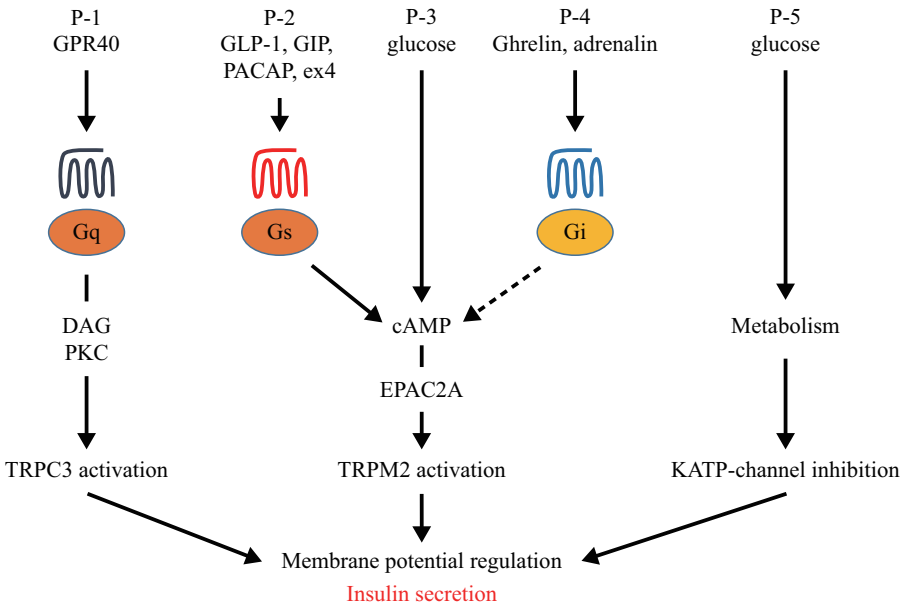

Fig. 3 TRP-dependent model for modulation of GSIS
The P-1 (pathway 1) shows the GPR40 pathway that activates TRPC3. The P-2 (pathway 2) shows the incretin hormone-dependent cAMP/EPAC2 pathway that activates TRPM2. The novel glucose-stimulated pathway is indicated by P-3 (pathway 3). Its antagonistic pathway that is utilized by ghrelin or adrenalin is indicated by P-4 (pathway 4) and this pathway may inhibit P-3 at the cAMP production site. The P-3 pathway synergistically depolarizes the membrane in concert with KATP channel inhibition that is the classical pathway of metabolism-dependent KATP-channel inhibition (P-5).

way [36]. As increases in glucose concentration reportedly induce oscillations of cAMP in the cytoplasmic space in β-cells and these oscillations are preceded and potentiated by elevation of $[Ca^{2+}]_i$ [62], the activation of the cAMP/EPAC/TRPM2 channel by glucose metabolism may further facilitate glucose-induced depolarization that depends on KATP channel closure (Fig. 3, P-3 and P-5).

However, TRPM2 is modulated by many other metabolites or specific activators including adenosine diphosphoribose (ADPR) or hydrogen-peroxide, H_2O_2. H_2O_2 over-production is closely related to β-cell death, possibly because H_2O_2 opens a number of TRPM2 channels and excess entry of Ca^{2+} through the channels may result in Ca^{2+} overload and apoptosis [63]. The hypothesis that cyclic ADP-ribose (cADPR) produced from NAD^+ by ADP-ribosyl cyclase (CD38) mobilizes Ca^{2+} from intracellular stores and stimulates insulin secretion in the GSIS process was proposed a long time ago. In support of this possibility TRPM2 channels are expressed in lysosomal Ca^{2+} stores and also in the plasma membrane [63]. It may be required to revisit this previously postulated hypothesis [64]. This cADPR hypothesis may hold true and, if so, cADPR may be considered as a mediator of GSIS because cADPR is a potent activator of TRPM2 channels [16, 65].

GPR40 signaling is mediated by TRPC3

We further propose a novel regulation of GPCR-mediated TRP-channel activity and insulin secretion (Fig 3, P-1). GPR40 is a Gq-coupled protein receptor and unsaturated medium- or long-chain free fatty acids are the ligands of this receptor [66]. Stimulation of GPR40 activates phospholipase C (PLC) that hydrolyzes phosphatidylinositol 4,5- bisphosphate (PIP_2), resulting in the production of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). Increased IP_3 binds to the IP_3 receptor of the endoplasmic reticulum (ER) and mobilizes Ca^{2+} to increase $[Ca^{2+}]_i$ release from the ER [67-72]. DAG promotes F-actin remodeling and potentiates GSIS via protein kinase D1 [73]. A GPR40-specific agonist (fasiglifam) potentiated GSIS at glucose concentrations that were close to the threshold but insufficient to elicit membrane excitement followed by firing of action potential and insulin secretion [59]. The NSCC current activated by fasiglifam was abolished by the presence of the TRPC3 (transient receptor potential canonical) channel blocker, pyrazole-3. Inhibitors of PLC or protein kinase C (PKC) inhibited the increases in GSIS and the NSCC current induced by GPR40. Stimulation of the GPR40–PLC/PKC–TRPC3 channel pathway is suggested to function as GPR40 agonist-induced GSIS. TRPC3 activation potentiates the depolarization of the plasma membrane in rat or mouse pancreatic β-cells.

In summary, Fig 3 shows our concluding hypothesis in which glucose and GPCR ligands induce initiation and potentiation of GSIS via regulation of the membrane potential. As has been established for a long time, glucose metabolism closes the KATP channel and depolarizes the membrane to trigger GSIS; however, as shown in the figure, another glucose pathway that uses a different mechanism opens the TRMP2 channel by utilizing cAMP/EPAC2 molecules (Fig. 3, P-3). The latter pathway facilitates membrane depolarization together with glucose/metabolism/KATP channel closure (P-5). Thus, glucose-induced membrane depolarization results not only from closure of the KATP channel but also from opening of TRPM2 channels. Incretin hormones activate the cAMP/EPAC2/TRPM2 pathway to further potentiate GSIS. Receptor-operated TRPM2 opening is attained in a shorter time than glucose metabolism-induced KATP channel closure. These effects of incretin action on this pathway that lead to modulation of GSIS may contribute to “the priming” of β-cells for triggering of GSIS by glucose, although further confirmation is required. Ghrelin or adrenalin antagonizes these glucose/cAMP/TRPM2 pathways (P-4 vs. P-3), and presumably antagonizes the incretin/cAMP/TRPM2 pathway as well (P-2). However the latter effect has not yet been confirmed. We also propose here that another GPCR ligand for GPR40 activates the TRPC3 channel via the Gq/PLC/DAG (PKC) pathway (P-1). Further molecular mechanisms linking PKC and TRPC3 remain to be elucidated.

Acknowledgments

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