Chapter

Redox Signaling is Essential for Insulin Secretion

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Abstract

In this review, we place redox signaling in pancreatic β-cells to the context with signaling pathways leading to insulin secretion, acting for example upon the action of incretins (GLP-1, GIP) and the metabotropic receptor GPR40. Besides a brief description of ion channel participation in depolarization/repolarization of the plasma membrane, we emphasize a prominent role of the elevated glucose level in pancreatic β-cells during glucose-stimulated insulin secretion (GSIS). We focus on our recent findings, which revealed that for GSIS, not only elevated ATP synthesis is required, but also fundamental redox signaling originating from the NADPH oxidase 4- (NOX4-) mediated H_2O_2 production. We hypothesized that the closing of the ATP-sensitive K^+ channel (K_{ATP}) is only possible when both ATP plus H_2O_2 are elevated in INS-1E cells. K_{ATP} alone or with synergic channels provides an element of logical sum, integrating both metabolic plus redox homeostasis. This is also valid for other secretagogues, such as branched chain ketoacids (BCKAs); and partly for fatty acids (FAs). Branched chain aminoacids, leucine, valine and isoleucine, after being converted to BCKAs are metabolized by a series of reactions resembling β-oxidation of FAs. This increases superoxide formation in mitochondria, including its portion elevated due to the function of electron transfer flavoprotein ubiquinone oxidoreductase (ETF:QOR). After superoxide conversion to H_2O_2 the oxidation of BCKAs provides the mitochondrial redox signaling extending up to the plasma membrane to induce its depolarization together with the elevated ATP. In contrast, experimental FA-stimulated insulin secretion in the presence of non-stimulating glucose concentrations is predominantly mediated by GPR40, for which intramitochondrial redox signaling activates phospholipase iPLA2γ, cleaving free FAs from mitochondrial membranes, which diffuse to the plasma membrane and largely amplify the GPR40 response. These events are concomitant to the insulin release due to the metabolic component. Hypothetically, redox signaling may proceed by simple H_2O_2 diffusion or via an SH-relay enabled by peroxiredoxins to target proteins. However, these aspects have yet to be elucidated.

Keywords: pancreatic β-cells, insulin secretion, redox signaling, NADPH oxidase 4, branched chain ketoacid oxidation, fatty acid β-oxidation, ATP-sensitive K^+ channel, GLP1, GPR40

1. Introduction

Recently, we revealed that physiological redox signaling is essential for the first phase of glucose-stimulated insulin secretion (GSIS) in pancreatic β-cells. Elevated glucose intake contributes to the increasing pentose phosphate pathway (PPP) supply of NADPH for NADPH oxidase 4 (NOX4), which directly produces H_2O_2. The burst of H_2O_2 then represents a redox signal, which fundamentally determines GSIS, while inducing a cooperative induction of plasma membrane depolarization together with ATP elevation (**Figure 1**) [1]. The latter originates from the increased ATP synthesis by oxidative phosphorylation (OXPHOS). Hypothetically, either a closure of the ATP-sensitive K^+ channel (K_{ATP}) is dependent on both H_2O_2 plus ATP; or H_2O_2 activates a synergic channel such as transient receptor potential

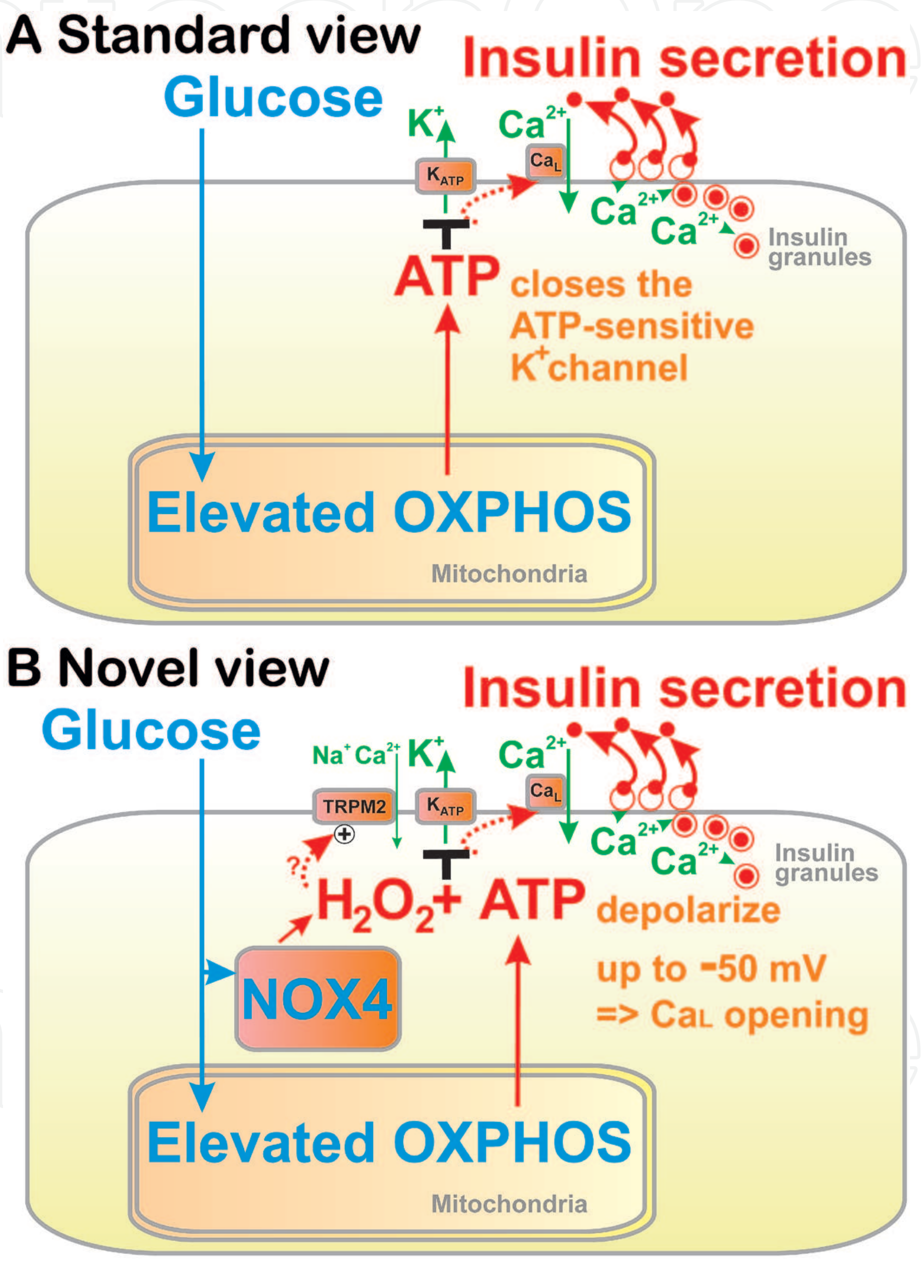

Figure 1.
(A) Traditional ("standard") view of the triggering mechanism of GSIS compared with (B) new paradigm in GSIS mechanism ("novel"), for which the redox signaling by NOX4-produced H_2O_2 is essentially required. Upon the glucose intake, PPP and redox shuttles supply cytosolic NADPH to increase NOX4 activity and thus elevate H_2O_2 which substantiates redox signaling. Either, the ATP-sensitive K^+ channel (K_{ATP}) is closed exclusively when both ATP plus H_2O_2 are elevated. Alternatively, H_2O_2 activates opening of TRPM2 or other nonspecific cation channels required for a depolarization shift to reach a threshold potential of −50 mV, at which the voltage-sensitive Ca^{2+} channels (Ca_L) become open, thus starting to fire the action potential. Resulting Ca^{2+} influx into the cell cytosol allows a complex process of exocytosis of the insulin granule vesicles (IGVs), beginning during the so-called 1st phase of GSIS by fusion of pre-attached IGVs with the plasma membrane and exposure of the IGV interior to the extracellular space (capillaries in vivo). Ca^{2+} also promotes the recruitment of distant IGVs towards the plasma membrane as well as ensures the late, so-called 2nd phase of GSIS, lasting about 1 hour in vivo.

melastin 2 (TRPM2) [2], required for sufficient depolarization. This principle of a logical summation of metabolic plus redox stimulation seems to be universal for other secretagogues (i.e. compounds stimulating insulin secretion), dependent on K_{ATP}. However, note that the redox signaling must be distinguished from the oxidative stress [3–6].

2. Two phases of GSIS

Two phases exist for GSIS in vivo [7–11]. They are also recognized in isolated pancreatic islets (PIs), but not in insulin-secreting β-cell lines. The consensus became that both K_{ATP}-dependent mechanism (also termed „triggering") and K_{ATP}-independent mechanisms contribute to both phases [12], while the K_{ATP}-independent mechanism still requires the elevation of cytosolic Ca^{2+} [13]. The 2nd phase in vivo was even considered to be independent of the extracellular glucose concentrations [14]. It depends more on the molecular mechanism of the increased sustained mobilization and priming of insulin granule vesicles (IGVs) [15].

The first rapid peak of insulin secretion is observed at 5–10 min after administration of a bolus of glucose in vivo or addition of glucose to the isolated PIs. The 1st phase involves the exocytosis of pre-docked juxtaposed IGVs, residing 100–200 nm from the plasma membrane prior to triggering [16, 17] and also possesses a contribution of deeper localized granules arriving within 50 ms, which were not initially pre-docked [18, 19]. The 2nd phase typically lasts over 1 hr. As a result, a predominant insulin amount is released in this phase. The 2nd phase results most likely from further delayed recruitment of IGVs belonging to the typically excessive reserve. The past hypothesis suggested a main reason for such a delay to involve the restricted passage through the filamentous actin (F-actin) cytoskeleton [20–22], but later a microfilament-independent movement of IGVs was reported [21, 23–25]. However, numerous cytoskeleton components play a more detailed role in the IGV exocytosis, not representing only a simple barrier. Generally, the IGV exocytosis relies on synaptogamin activation by Ca^{2+}, syntaxin, SNAP-25, and other target proteins of the SNARE family (SNAp REceptors, where SNAP is soluble NSF attachment proteins and NSF is a N-ethylmaleimide-sensitive fusion factor). They attract IGVs via the IGV-localized synaptobrevins (vesicle-associated membrane proteins), while forming a coiled-coil quarternaly structure [26]. The resulting SNARE core complex relocates the IGV and plasma membrane into proximity, thus facilitating establishment of so-called fusion stalk. Further zippering of coiled-coil structures allows fusion of larger part of the IGV membrane with the plasma membrane until a fusion pore is formed.

However, the recent explanation for the second phase is based on the fact that the two phases of insulin secretion exist when isolated pancreatic islets are studied, but do not exist for isolated primary pancreatic β-cells [27–29]. Hence, the role of inter-cellular contacts is emphasized for the 2nd phase. The inter-cellular contacts allow synchronization of the plasma membrane potential, while paracrine hormone secretion may also contribute to modification and termination of insulin release.

3. Mechanisms of the 1st phase of GSIS

GSIS has been consensually described to involve a so-called triggering mechanism accompanied by amplifying mechanism(s) [7, 12, 30–36]. The triggering is exclusively dependent on the K_{ATP} closure and attaining plasma membrane depolarization up to −50 mV. The latter is achieved in a synergy of K_{ATP} with other ion channels. The amplifying mechanisms are given by metabolism or stem from the action of incretins and other hormones. Also, mechanisms concerning other secretagogues, such as branched chain ketoacids (BCKAs) and fatty acids (FAs), were considered as merely amplifying. Nevertheless, we will show below the ambiguity of such a classification. The amplifying mechanisms originate from an incremental increase in Ca^{2+} elevations, not existing within the canonical "triggering" mechanism. Alternatively, they stem from facilitation via numerous proteins of the exocytotic machinery localized either on the IGV or plasma membranes. Therefore, some of these types of events might be Ca^{2+} independent and hence may also proceed at low glucose concentrations.

The traditional explanation of the triggering mechanism of GSIS relied exclusively on the ATP elevation (or elevation of the ATP/ADP ratio) in the cytosol of β-cells. Sole elevated ATP was considered to be sufficient for the K_{ATP} closing [30–33]. Any additional requirement for a parallel redox signaling was not considered, despite the findings that reactive oxygen species (ROS) have been implicated in insulin secretion. This concerned with at least ROS of mitochondrial origin [37], or resulting from mono-oleoyl-glycerol addition [38]. The blockage of PPP, that decreased insulin secretion, also shifted redox homeostasis [39]. An unspecified link of GSIS with the externally added H_2O_2 was reported, besides antioxidant effects at decreased glutathione by diethylmaleate [40]. Previously, also an unidentified isoform of NADPH oxidase was implicated in GSIS, since an antisense p47PHOX oligonucleotide [41] or an unspecific NOX inhibitors attenuated GSIS [38, 42, 43].

Recently, we have provided the evidence that the elevated OXPHOS is insufficient to initiate GSIS, despite the increased ATP levels and the elevated ATP/ADP ratio at the peri-plasma- membrane space [1]. We demonstrated that NOX4 is fundamentally required for GSIS [1]. In model rat pancreatic β-cells (INS-1E cells) with silenced NOX4 or in full NOX4 knockout (NOX4KO) mice and in mice with NOX4 knockout, specifically in pancreatic β-cells (NOX4βKO mice), the 1st phase of GSIS was largely blocked [1]. In both studied NOX4 KO mice strains and in their isolated PIs, the 1st phase of GSIS was abolished with NOX4 ablation, while in PIs, either overexpression of NOX4 (achieved at least in the peripheral spheroid layer of islets) or additions of H_2O_2 rescued this 1st phase. No effects were found in NOX2 KO mice, although NOX2 has been previously implicated to play an antagonistic role for redox homeostasis [44].

Moreover, using a patch-clamp of INS-1E cells, we demonstrated that the K_{ATP} closure is possible only when NOX4 is intact in INS-1E cells. After showing the well-known closure of K_{ATP} induced by high glucose concentration in cells transfected with scrambled siRNA, we observed no glucose-induced K_{ATP} closure in INS-1E cells silenced for NOX4 [1]. These experiments supported the model, in which K_{ATP} integrates metabolic and redox homeostasis and acts as a logical summation for which both elevated ATP plus elevated H_2O_2 exclusively lead to a triggering of GSIS (**Figure 1**). However, since without cation fluxes provided by nonspecific cation channels a threshold depolarization of −50 mV cannot be achieved, despite 100% K_{ATP} ensemble being closed [45, 46], we may also hypothesize that H_2O_2 alternatively or in parallel activates the TRPM2 channel [2], known to contain redox-sensitive Met residue [47].

Thus our results set a new paradigm for GSIS, since it had never been considered that the sole ATP increase is insufficient for GSIS and is insufficient particularly for the closing of K_{ATP}; as well as it had never been considered that any redox signaling might essentially participate in GSIS.

In further work, we also demonstrated that the redox signaling upon GSIS is provided by elevations of cytosolic H_2O_2, whereas ROS in the mitochondrial matrix (both H_2O_2 and superoxide release) are diminished due to the enhanced operation of the redox shuttles upon GSIS [48]. One may expect that a portion of cytosolic NADPH as a substrate for NOX4 is provided by the glucose-6-phosphate dehydrogenase and also by 6-phosphogluconate dehydrogenase downstream within the PPP, whereas the second portion is generated due to the operation of redox shuttles. These shuttles become more active at higher glucose concentrations and increasingly produce NADPH. NADPH is particularly produced by isocitrate dehydrogenase 1 (IDH1) and malic enzyme 1 (ME1) in the cytosol upon operation of these redox shuttles [48].

In summary, we describe the revisited mechanism of the 1st phase of GSIS as follows. Elevated glucose metabolism and glycolysis allows an increased branching of the metabolic flux, particularly of glucose-6-phosphate G6P, toward PPP, which acts as a predominant source of NADPH. The essential role of PPP was emphasized elsewhere [49]. Amplification of the cytosolic NADPH is also provided by IDH1 and ME1 due to the elevated operation of the three redox shuttles. Since NOX4 was determined as the only NADPH oxidase producing H_2O_2 directly [50, 51], its reaction results in an increase of H_2O_2 release into the cell cytosol [1]. Finally, the elevated H_2O_2, together with concomitantly elevated ATP from the enhanced OXPHOS, is the only way for plasma membrane depolarization up to −50 mV [1]. This threshold subsequently induces Ca_L opening, followed by the Ca^{2+} influx into the cell cytosol, which in turn induces the exocytosis of insulin granule vesicles. The action potential spikes are then determined by the cycles of Ca_L opening, followed by the opening of voltage-dependent channels (K_V) in rodents [52] or calcium-dependent (K_{Ca}) K^+-channels in humans. Their action deactivates $Ca_{L,}$ which are, however, again activated in the next Ca_L-K_V cycle.

Pancreatic β-cells were undoubtedly adapted by phylogenesis to serve as a perfect glucose sensor. The glucose sensing is allowed by several key specific features. At first, specific isoforms of glucose transporters, GLUT2 in rodents and GLUT1 in humans, equilibrate the plasma glucose concentration with the glucose concentration in the cytosol of β-cells [53, 54]. Second, a specific isoform IV of hexokinase (also termed glucokinase) cannot be feed-back inhibited by its product glucose-6-phosphate. As a result, there is an efficient unidirectional flux towards the glycolysis [55, 56] and, most probably, this allows also branching into the PPP [49]. Originally, the PPP was accounted to utilize only 10% of glucose, due to presumably feed-back inhibition by glucose [57, 58]. However, metabolomics studies associated PPP intermediates with GSIS [59], confirming previous studies with various PPP inhibitors [59–62]. These results collectively demonstrated the important PPP contribution to GSIS. This contribution is also reflected by existing patients having a deficiency of glucose-6-phosphate dehydrogenase associated with the impaired 1st phase of GSIS [63].

The third aspect leading to the perfect glucose sensing lays in the virtual absence of lactate dehydrogenase in β-cells and inefficiency in pyruvate dehydrogenase kinases (PDK) [64]. PDKs would otherwise block pyruvate dehydrogenase (PDH). Thus the highly active PDH and other dehydrogenases, activated also by Ca^{2+} influx into the mitochondrial matrix [65], altogether enable that 100% of pyruvate and its equivalents (after pyruvate conversion by transaminases) is utilized by OXPHOS. A minor pyruvate flux ensures anaplerosis of oxaloacetate due to the reaction of pyruvate carboxylase [66]. Its reaction is also important also for the pyruvate/malate redox shuttle.

The fourth aspect reflects the in vivo inhibitory role of the mitochondrial ATPase inhibitory factor, IF1. IF1 adjusts a proper glucose concentration range for GSIS in rat pancreatic β-cells, INS-1E [67, 68]. This is suggested by the demonstration that IF1 silencing allows insulin secretion even at very low glucose approaching to zero in INS-1E cells [67]. In contrast, the IF1 overexpression inhibited GSIS in INS-1E cells [68]. This IF1 role awaits confirmation in vivo.

4. Plasma membrane events following K_{ATP} closure

Surprisingly, the plasma membrane of β-cells contains up to 60 channels of 16 ion channel families [69]. Moreover, ion channels are also located on the membrane of IGVs to facilitate fusion with the plasma membrane and insulin exocytosis. Resting plasma membrane potential (*V*p) is created predominantly by the activity of K^+-channels due to a higher concentration of K^+ inside the β-cell (~150 mM), exceeding that one established outside in capillaries or interstitial fluid (~5 mM). Experimentally, *V*p values are measured to be approximately of – 75 mV [70]. The K_{ATP} closure then induces depolarization [69, 71–73] and activation of Ca_L [74]. The action potential firing is the entity that activates Ca_L-K_V cycles (in rodents), however this firing is initiated by more channel types.

Surprisingly, the action potential firing is not induced until >90% of K_{ATP} channels are closed [75, 76]. As a result, only the closure of the remaining ~10% of the K_{ATP} population leads to depolarization [76]. In fact, the activity of the whole K_{ATP} population decreases exponentially with the increasing glucose concentration. Interestingly, 50% of the K_{ATP} population is already closed at 2–3 mM glucose, while *V*p remains steady. However, at about 7 mM glucose, 100% of the K_{ATP} ensemble is closed. This is being reflected by the completely vanished K_{ATP} current, which leads to action potential firing [69, 70]. This event is termed as a supra-threshold depolarization.

Thus, hyperpolarized interburst phases are induced, while a nearly permanent firing exists at high >25 mM glucose [70]. An intermediate depolarization at 10 mM glucose was reported for mouse β-cells, reversed upon withdrawal of Ca^{2+} and Na^+, supporting the participation of other channels, such as nonspecific cation channels, contributing to the depolarization (inward) flux [45]. Even an efflux of Cl^- was suggested to fulfil this role [77], including the opening of LRRC8/VRAC anion channels [78, 79]. The participation of TRPM4 and TRPM5 [80] providing inward currents of certain levels seem to be required for induction of sufficient membrane depolarization together with K_{ATP} closing [46]. This is because the measured resting *V*p of – 75 to −70 mV is already depolarized by a some extent from the equilibrium Vp^{equi} of −82 mV (5 mM vs. 130 mM [K^+]). The shift is probably due to the opening of nonspecific cation channels, since any of Na^+, Ca^{2+} and K^+ can penetrate them. The 100% K_{ATP} closing at higher glucose causes only an insufficient depolarization. Without nonspecific cation channels (or Cl^- channels), the established *V*p would only be equal to Vp^{equi}, so any shift to −50 mV required for Ca_L would not take place. Contribution by the basal opening of other synergic channels is therefore essential. Open synergic channels always induce the inward shift in *V*p, so to that depolarization given by 100% K_{ATP} closing reaches −50 mV. This allows opening of Ca_L and action potential firing. In summary, besides the heat-activated TRPV1 channel (capsaicin receptor), and TRPV2 or TRPV4, the H_2O_2-activated TRPM2 [2], or Ca^{2+}-activated TRPM4 and TRPM5 channels belong to the important group of possible synergic channels expressed in β-cells [46].

The same reasoning concerns with anion channels, particularly Cl^- channels. The active Cl^- transport is provided in β-cells by SLC12A, SLC4A, and SlC26A channels. These channels set the cytosolic Cl^- concentration above thermodynamic equilibrium. Besides $GABA_A$, $GABA_B$ and glycine receptor Cl^- channels considered to be part of the insulin secretion machinery, also volume-regulated anion channels (VRAC) were shown to be open at high glucose. VRACs are heteromers of the leucine-rich repeat containing 8 isoform A (LRRC8A) with other LLRC8 isoforms, forming anion channels [79]. Ablation of LRRC8 in mice led to delayed Ca^{2+} responses of β-cells to glucose and diminished GSIS in mice, demonstrating the modulatory role of LRRC8A/VRAC on membrane depolarization leading to Ca_L responses [78, 79].

Upon the action potential firing thus metabolically driven *V*p oscillations occur due to the initial glucose rising [69, 70]. Cytosolic Ca^{2+} oscillations are superimposed from fast (2–60 s periods) and slow (up to several min) Ca^{2+} oscillations [81], stemming from *V*p oscillations and an interplay with Ca^{2+} efflux from the endoplasmic reticulum (ER) [82]. Collectively they lead to pulsatile insulin secretion. The ER involvement is given by the phospholipase C (PLC), responding to the glucose-stimulated Ca^{2+} influx. PLC produces inositol triphosphate (IP3), which opens the Ca^{2+} channel of IP3 receptor (IP3R) of ER; plus diacylglycerol (DAG). Importantly, DAG permits the opening of TRPM4 and TRPM5 via the protein kinase C (PKC) pathway. Another ER Ca^{2+} channel, the ryanodine receptor (RyR) may also participate, being activated by ATP, fructose, long-chain acyl-CoAs and cyclic adenosine 5′-diphosphate ribose [81]. Also, the role of other channels was demonstrated for permitting store-operated Ca^{2+} entry from ER, particularly of the ternary complex of TRPC1/Orai1/STIM1 [46, 83]. TRPC1 belongs to the transient receptor potential canonical (TRPC) family with a modest Ca^{2+} selectivity. TRPC1 interacts with Orai1 [84], and in such a functional complex, its channels are activated by STIM1, affecting the amplitude of Ca^{2+} oscillations, and correlating with GSIS.

As mentioned above, deactivation of Ca_L is ensured by the opening of voltage-dependent channels (K_V) in rodents [52] or calcium-dependent (K_{Ca}) K^+-channels in humans. Among the former, tetrameric K_V2.1 is the prevalent form in rodent β-cells. A delayed rectifier K^+-current is induced at positive *V*p down to −30 mV [85]. The opening of K_V2.1 channels repolarizes *V*p and thus closes Ca_L channels. Ablation of K_V2.1 thus reduces Kv currents by ~80% and prolongs the duration of the action potential, so more insulin is secreted. Mice with ablated K_V2.1 possess lower fasting glycemia but elevated insulin and reportedly improved GSIS [86]. In contrast, human β-cells use K_{Ca}1.1 channels (i.e. BK channels) for repolarization of *V*p [70]. Note also that down-regulation of K_V was observed after islet incubation with high glucose for 24 hr [87].

5. Possible redox regulations of K_{ATP} and other channels

The structure of K_{ATP} has been resolved and numerous mutagenesis studies of K_{ATP} have been conducted. Amino acid residues that are candidate redox targets are yet to be identified. The K_{ATP} channel is a hetero-octamer consisting of four external regulatory sulfonylurea receptor 1 (SUR1, a product of *Abcc8* gene) subunits and four pore-forming subunits of potassium inward rectifier, Kir6.2 (*Kcnj11* gene) [88, 89]. These Kir6.2 subunits cluster in the middle of ~18 nm size structure with a ~13 nm height [90]. The part exposed to the cytosol contains an ATP binding site, located about 2 nm below the membrane. A single ATP molecule was reported to close the channel, i.e. with the other three binding sites left unoccupied [91]. However, the ATP binding site overlaps with the binding site for phosphatidylinositol 4,5-bisphosphate (PIP_2), which stabilizes the open state. Palmitoylation of Cys166 of Kir6.2 was then reported to amplify the responsiveness to PIP_2 [92]. Upon the release of PIP_2 from the binding site, the open probability becomes decreased [90, 93, 94].

Diazoxide or cromakalim, as well as numerous other openers, set K_{ATP} pharmacologically in the open state even at a high ATP concentration [95]. In contrast, the artificial K_{ATP} closing by sulfonylurea derivatives, such as glibenclamide, takes place independently of ATP. Besides this sulfonylurea binding site, each of the four SUR1 subunits contains MgATP and MgADP binding sites. MgATP is hydrolyzed at the nucleotide binding fold 1 (NBF1) to MgADP. Resulting MgADP subsequently activates K_{ATP} at NBF2. This is indeed reflected by the ATP-sensitive increase in K^+ conductance and following lower excitability, accompanied by the lower sensitivity to ATP inhibition [91].

The phosphorylation of K_{ATP} reportedly sets the sensitivity of the K_{ATP} ensemble. The setting is such that transitions upon the glucose rise from 3 or 5 mM to 7 mM or > 10 mM result in the closing of the remaining 10% of the initially open channels by elevations between just the two ATP concentrations falling into the mM range. Any redox component in this was never indicated and should be studied. Nevertheless, phosphorylation mediated by the protein kinase A (PKA) was already reported to act in this unusual setting. Thus Thr224 [96] and Ser372 were reported to be the verified PKA phosphorylation sites. Their phosphorylation increases the open probability of K_{ATP} [97]. This might hypothetically provide closing mechanism acting at higher ATP concentration or even requiring H_2O_2. In a longer time scale, phosphorylation also increases the number of channels in the plasma membrane. Also, Thr224 was found to be phosphorylated by Ca^{2+} and calmodulin-dependent kinase II (CaMKII) while interacting with β_{IV}-spectrin [98]. In vivo, also autonomic innervations and paracrine stimulation ensure sufficient PKA-mediated phosphorylation of K_{ATP}.

Since the original discovery of the essential role of K_{ATP} in GSIS [99], only an indirect inhibition of K_{ATP} by H_2O_2 was observed in smooth muscle cells [100]. Nevertheless, other redox-sensitive targets have been identified in pancreatic β-cells. But we can exclude the possibility that the IGV exocytosis itself might be directly induced by H_2O_2, independently of Ca^{2+} [52], since the ability of exogenous H_2O_2 to induce insulin secretion in INS-1E cells was only partially blocked by NOX4-siRNA, but it was completely blocked by a Ca_L blocker nimodipine [1]. Consequently, albeit the used H_2O_2 doses exceeded 100 μM, they did not directly stimulate the K_{ATP}-independent exocytosis of insulin granules.

A second possibility would be that Ca_L channels themselves may be hypothetically co-activated by H_2O_2. Third, the plasma membrane depolarization might be redox sensitive, so that H_2O_2 could directly or indirectly inhibit repolarizing K^+-channels, such as K_V [101–103]. The fourth plausible redox link with GSIS would concern with the reported redox activation of TPRM2 depolarizing channels [2]. The latter is the most plausible, since it is related to a Ca^{2+}-induced [52, 104] or H_2O_2-induced exocytosis of insulin granules by the H_2O_2-activation of TPRM2 depolarizing channels [2, 105]. Note, our results excluded the Ca^{2+}-independent H_2O_2-induced exocytosis of IGVs at least in rat pancreatic β-cells [1]. Therefore, if the H_2O_2-activated TRPM2-dependent mechanism exists, it must provide the required synergy with K_{ATP}, to reach the −50 mV plasma membrane depolarization threshold. Note also, that TRPM2 was already implicated as a significant player in the GLP-1 potentiation of insulin secretion [106].

Finally, a competition for NADPH between NOX4 and a hypothetical NADPH-activated K^+-channel could exist. Nevertheless, using patch-clamped INS-1E cells in a whole cell mode, we demonstrated a closure of K_{ATP} by H_2O_2 produced by NOX4 at high glucose, since in cells silenced for NOX4, even ATP resulting from the metabolism of high glucose was unable to close the K_{ATP} channel [1].

6. Receptor-mediated amplification of insulin secretion

G protein-coupled receptors activating heterotrimeric G proteins ensure pleiades of cell responses, mutually interrelated. G proteins typically regulate production of second messengers. Thus Gαs proteins increase generation of cyclic AMP (cAMP), whereas Gαi/o proteins decrease it [107–109]. The G proteins Gαq/11 initiate PLC-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate into diacylglycerol (DAG) and IP3 [110, 111]. Gα12/13 proteins promote protein RhoA for remodeling of the cytoskeleton [112]. Class of proteins termed β-arrestins initiates signaling via proximal MAP kinase, IκB, and Akt pathways [113]. The latter two G protein classes rather control long-term effects.

Let us emphasize downstream pathways that are important for acute effects in pancreatic β-cells, which predominantly lead to either modulation of the plasma membrane channels, typically Ca_L, K_{ATP} and K_V, so to ensure more intensive insulin secretion; or their action evokes stimulation of insulin secretion via ensuring the surplus Ca^{2+} influx to the cytosol from ER or mitochondria; or, else, their action targets proteins of the exocytotic machinery on the IGV or plasma membranes. The latter responses alter the kinetics of IGVs in docking, priming and fusion with the plasma membrane, so to facilitate exocytosis. Interestingly, these events could be independent of Ca_L and theoretically could take place at low glucose concentrations.

Activation of Gαs increases the activity of transmembrane adenylate cyclases (tmAC) producing cAMP from ATP [108, 109]. A number of phosphodiesterases (of 11 families) degrade cAMP (some also or exclusively cGMP). cAMP is a universal 2nd messenger having a specific function in amplifying of GSIS and insulin secretion stimulated with other secretagogues. Also, soluble adenylate cyclases (sACs) exist, notably in the mitochondrial matrix, while their reaction is potentiated by Ca^{2+} and bicarbonate. The major mediators of cAMP effects are cAMP-dependent PKA [114], including PKA tethered to the outer mitochondrial membrane [115, 116], and the parallel pathway of enhanced signaling via exchange proteins directly activated by cAMP 2 (EPAC2) [117–119].

In pancreatic β-cells, the PKA pathway is involved in signaling of incretin (GLP-1 and GIP) receptors [107, 120]. It exerts a minor contribution to signaling from metabotropic receptors, such as GPR40, which is sensing long chain fatty acids [111]. PKA typically amplifies the Ca^{2+}-dependent exocytosis of insulin granules. The core pathway involves PKA phosphorylation and hence activation of the Ca_L β2-subunit, in concert with K_{ATP} phosphorylation decreasing the ATP concentration range required for its closure (see above) [121]. In addition, PKA inhibits Kv channels, which otherwise terminate plasma membrane depolarization; hence this prolongs already more intensive Ca^{2+} influx via phosphorylated Ca_L and hence exocytosis of insulin granules [122].

Another PKA target is the exocytosis-modulating protein termed snapin, the phosphorylation of which allows its interaction with the other IGV proteins, which enhances the 1st GSIS phase [123]. Snapin participates in tethering of IGVs to the plasma membrane by coiled-coil interaction with a lipid-anchored protein SNAP-25 [124].

Altogether, the PKA pathway ensures about 50% of cAMP responses in β-cells [125], while the EPAC2 pathway ensures the remaining responses [117–119]. EPAC2 protein possesses a guanine nucleotide exchange activity, thus inducing the Ca^{2+}-induced Ca^{2+}release from ER via RyR [126] (questioned in [127]), occurring only at high glucose, since it requires the primary Ca_L opening [128], which also partially refills the ER Ca^{2+} stores. The EPAC2 pathway also affects the IGV proteins and thus facilitates the insulin exocytosis. For example, Rim2a protein is a target [129, 130], located on the inner plasma membrane surface and on IGVs, representing a scaffold for IGV exocytosis [131]. Rim2a interacts with Rab3A of IGVs and the resulting Rim2a-Rab3A complex facilitates docking of IGVs into the plasma membrane. This is followed by so-called priming, which is subsequently initiated by the Rim2a interaction with the Munc13–1 protein. Munc13–1 then opens syntaxin 1 from its closed conformation, thus allowing fusion with the plasma membrane. EPAC2 also interacts with NBD1 of SUR1, being released by cAMP [35]. Such locally released EPAC2 induces the release of Rim2 from the α1.2 Ca_L subunit.

The local Ca^{2+} influx within Ca_L ensures EPAC2 binding to Rim2, and subsequent interaction with another Ca^{2+} sensor termed Piccolo. The heterotrimeric complex then interacts with Rab3A and enables IGV exocytosis.

Interestingly, all necessary components of the PKA pathway were identified in the mitochondrial matrix, including sAC, PDE2A2 [132], and also PKA [133]. However, we may also speculate that some proteins can be phosphorylated by cytosolic PKA or by its fraction attached to OMM prior to their import to the mitochondrial matrix. There was also a consensus that cAMP cannot freely diffuse to the matrix [132]. Thus cAMP in the mitochondrial matrix may act as an independent pool [134, 135]. Its source is the matrix-located soluble adenylate cyclase sAC, which is activated by bicarbonate and Ca^{2+} [136, 137]. Since CO_2 is increasingly released when the Krebs cycle turnover increases upon GSIS, the matrix localized mtPKA can be activated in this way [138]. In any case, OXPHOS is facilitated in mitochondria of numerous tissues via phosphorylation of Complex I NDUFS4 subunit (facilitating its Hsp70-mediated import), Complex IV COXIV-1 subunit (preventing its inhibition by ATP) [139] as well as via IF1, enhancing ATP synthesis by disabling the inhibitory binding of phosphorylated IF1 dimers to the ATP synthase [140]. A link to redox homeostasis can be viewed in the observed release of the PKA catalytic subunits by the increased ROS [141, 142]. Thus mtPKA can act in parallel to the cytosolic PKA signaling initiated by GPR40 and GLPR or GIPR receptors. PKA targeting of at least IF1, and probably also of Complex I and Complex IV, should contribute to the amplification of insulin secretion by FAs or incretins.

The G protein Gαq/11 initiates signaling through the phospholipase C (PLC-)-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate into DAG and inositol triphosphate IP3 [110]. The main effector of DAG is protein kinase C (PKC), which is activated by DAG. One of the effectors of IP3 is the IP3 receptor (IP3R; subtypes IP3R1, IP3R2 and IP3R3), which is another important Ca^{2+} channel residing on ER membranes in β-cells [143]. Similarly to the EPAC2-RyR route of Ca^{2+} release from ER Ca^{2+}, the opening of this channel amplifies the primary Ca_L mediated Ca^{2+} signaling for insulin release. PKC contributes to the plasma membrane depolarization, while activating TRPM4 and TRPM5 [144]. Besides the canonical plasma membrane effects, PKC and downstream ERK1/2 signaling stimulates OXPHOS, hence mitochondrial ATP synthesis [145].

7. GSIS amplification by incretins GLP-1 and GIP

Glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP) have a prominent impact among other peptides belonging to incretins [107–109]. Oral glucose administration provides a higher insulin secretion response than when administered parenterally [146]. This surplus of potentiation of insulin secretion appears to be about equally ascribed to GLP-1 and GIP [147]. Indeed, diminished insulin secretion response to oral glucose was observed in GLP-1 knock out mice [148, 149] and was even more decreased in double knockout mice (GLP-1 plus GIP) [149].

Incretin-cAMP signaling amplifies GSIS by both PKA-dependent and EPAC2A-dependent pathways. As described above, the EPAC2 pathway is partially dependent on the Ca_L opening, and the PKA pathway enables synergy among actions of K_{ATP}, Ca_L, and Kv channels, leading again to a more effective Ca_L opening. This knowledge complies with the traditional view, considering that the incretin signaling does not stimulate insulin release in the low glucose conditions [150, 151]. The GLP1R-cAMP-EPAC2-TRPM2 pathway was suggested to be one of the major routes [106].

GLP-1 is secreted by enteroendocrine L-cells, residing predominantly in the distal ileum and colon. Secretion is initiated by postprandial stimuli, i.e. by glucose, fatty acids, or lipids, as well as proteins [152, 153]. Only 10 to 15% of active GLP-1 likely reaches the pancreas via the circulation [154]. Thus concentrations of biologically active GLP-1 in human plasma at fasting account for about 2 pmol/l and maximum10 pmol/l postprandially [155], peaking 30 to 60 min after a carbohydrate or protein intake and 120 min after ingestion of lipids [156]. The most efficient truncated variants are GLP-$1_{(7–37)}$ and variant GLP-$1_{(7–36amide)}$ [152]. The latter is ~80% abundant in humans [157]. Note that full peptide GLP-$1_{(1–37)}$ is much less efficient in GSIS potentiation [150, 151]. Moreover, paracrine GLP-1 signaling acts among the different types of PI cells [150], similarly to the paracrine and endocrine secretion of other hormones. On the systemic level, central control by the brain and nervous system, including GLP-1 secretion in the *nucleus tractus solitarii* of the brainstem [152], further provides an indispensable top level of regulation for the insulin secretion. GLP-1 effects related to β-cell proliferation or apoptosis are beyond the scope of this review.

GLP-1 from the bloodstream acts through its receptor (GLP1R) residing in the plasma membrane of pancreatic β-cells [158]. GLP1R activation stimulates Gαs and Gαq/11 and recruits β-arrestin, depending on biased agonism relative to different agonists, such as exendin-4 and oxyntomodulin [159, 160]. As a scaffold protein, β-arrestin facilitates signaling via Gαs to cAMP but also to CREB [160], extracellular regulated kinase ERK1/2 [161], and insulin receptor substrate 2 (IRS-2), the effects promoting β-cell growth, differentiation, and maintenance [160]. The stimulation of Gαs leads via enhanced cAMP to the initiation of PKA [162] and EPAC2A pathways [163]. Continuous cAMP production and partial potentiation of GSIS was found even for the internalized GLP1R [164].

The PKA pathway provides a surplus intracellular Ca^{2+} above that of the net GSIS without any receptor stimulation. This is ensured by phosphorylation-induced closing among the population of K_{ATP}, stimulation of Ca_L opening, and closing of Kv channels [165]. The latter prolongs Ca^{2+} stimulation of IGV exocytosis and hence may also potentiate the 2nd phase of GSIS. In parallel, PKA engages snapin interaction with IGVs, reportedly potentiating the 1st GSIS phase [123, 124]. Simultaneously, the EPAC2 pathway promotes Ca^{2+}-induced RyR-mediated Ca^{2+} release from ER, which must be, however, initiated by the ongoing Ca_L opening [163]. The EPAC2 pathway also facilitates docking and priming of IGVs by promoting Rab3A interaction with Rim2a [131] and hypothetically interaction of EPAC2-Rim2-Picollo trimers with Rab3A, enabling IGV exocytosis [152]. Stimulation of GLP1R biased downstream via stimulation of Gαq/11 also contributes by a surplus to intracellular Ca^{2+}, while inducing the IP3R-mediated Ca^{2+} release from ER.

When GLP1 effects were simulated and IGV kinetics was monitored using total internal reflection fluorescence microscopy, cAMP and 8-Br-cAMP were found to increase the frequency of fusion events, i.e. IGV fusion with the plasma membrane in both phases of GSIS [25]. EPAC2A was found to interacts also with a small G protein Rap1, affecting its conformation so to release the catalytic region, which subsequently binds and thus activates another G protein Rap113. In EPAC2A knockout mice, most of the potentiation of the 1st GSIS phase vanished [25]. Thus speculatively, the 2nd phase amplification can be due to the PKA pathway.

8. Mechanism of insulin secretion stimulated by branched-chain keto-acids

Postprandial response by insulin secretion is also given by substances other than glucose. These substances, which induce the secretion of insulin, are termed secretagogues in general. One important type of secretagogues is branched-chain keto-acids (BCKAs), metabolites of branched-chain amino acids (BCAAs) (**Figure 2**). We found that the alternative to the NOX4-mediated redox signaling exists for some other insulin secretagogues, particularly for BCKAs [1]. For the redox signaling in this case, the mitochondrial redox signaling replaced that one originating from NOX4. Thus we demonstrated that H_2O_2 signaling originating from mitochondria is essentially required for insulin secretion stimulated by BCAAs metabolized onto BCKAs, such as 2-ketoisocaproate (KIC; also termed 2-oxoisocaproate, OIC; leucine metabolite), 2-ketoisovalerate (KIV; valine metabolite) and 2-ketomethylvalerate (KMV; isoleucine metabolite) [166, 167]. This mechanism was evidenced by the effects of mitochondrial-matrix-targeted antioxidant SkQ1. We observed that SkQ1 did not affect GSIS in INS-1E cells, but completely inhibited insulin secretion stimulated by KIC [1]. Thus the NOX4 source of H_2O_2 cannot be efficiently inhibited by SkQ1 located within the inner phospholipid leaflet of the inner mitochondrial membrane, whereas the redox signaling originating from mitochondrion must be blocked.

Metabolism of BCKAs begins in the mitochondrial matrix by the reaction of the BCKA dehydrogenase complex (BCKDH), since there is no branched-chain amino acid aminotransferase (BCAT) in the cell cytosol [168]. BCKDH forms isovaleryl-CoA, isobutyryl-Co and methyl-isobutyryl-CoA from KIC, KIV and KMV, respectively. This is followed by a series of reactions resembling β-oxidation of fatty acids. This series, as well as FA β-oxidation, elevates formation of superoxide in the mitochondrial matrix by several ways. The major way is due to the reoxidation of the BCKDH co-factor $FADH_2$ by the electron-transfer flavoprotein (ETF), the one electron carrier. Two electrons from the two ETF molecules are accepted by the electron-transfer flavoprotein: ubiquinone oxidoreductase (ETF:QOR) [169].

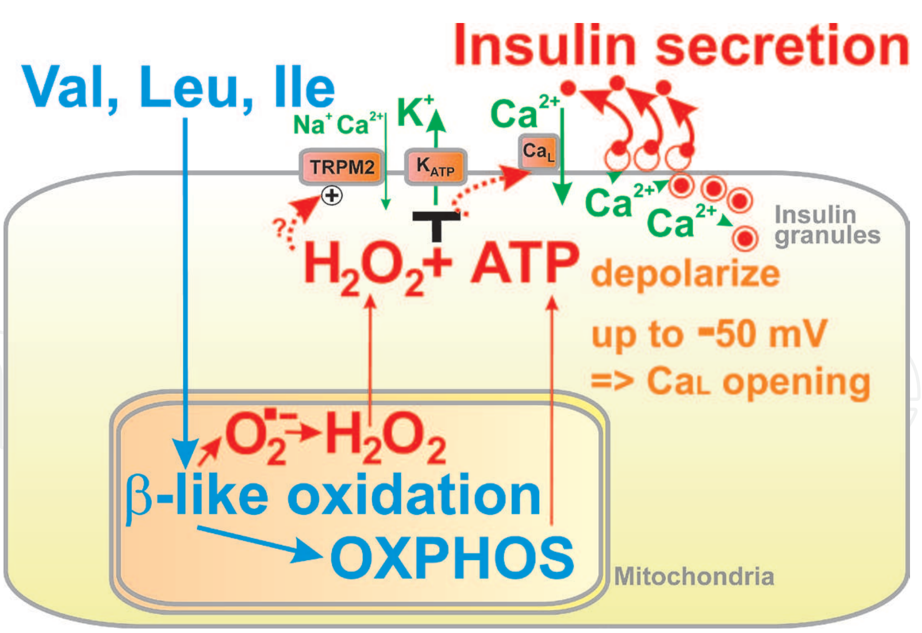

Figure 2.
The mechanism of branched chain keto acid-stimulated insulin secretion involves redox signaling of mitochondrial origin. 2-ketoisocaproate (KIC), 2-ketomethylvalerate (KMV) and 2-ketoisovalerate (KIV) resulting from leucine, isoleucine, and valine, respectively, due to the branched chain aminotransferase reaction in mitochondria (BCAT2), are metabolized by the branched chain ketoacid dehydrogenase (BCKDH) in mitochondria. A series of reactions, BCKA oxidation leads to the electron transfer from the co-factor $FADH_2$ of BCKDH, via ETF towards the ETF:QOR reaction reducing Q to QH_2. This effectively retards the competing reaction of the Complex I of the mitochondrial respiratory chain, leading to the superoxide formation. In the mitochondrial matrix, superoxide is transformed to H_2O_2 by MnSOD, whereas by CuZnSOD in the intermembrane space and cytosol. The elevated mitochondrial/cytosolic H_2O_2 substitutes the redox signal of NOX4 origin. Consequently, such redox signaling, together with elevated ATP, allows the sufficient depolarization of the plasma membrane.

ETF:QOR reaction is coupled to ubiquinone (Q) oxidation to ubiquinol (QH_2). This effectively competes with the Complex I reaction of the respiratory chain, also providing Q oxidation to QH_2 driven by NADH. The electron transfer within the Complex I is thus effectively retarded and this results in a higher superoxide formation. Superoxide is then most probably increasingly formed at the I_Q site (i.e. at the proximity of the Q-binding site), similarly as due to the reverse electron transfer.

Alternatively, the Complex I electron transfer is retarded upon the acetyl-CoA entry (propionyl-CoA entry for KIV; through methylmalonyl and Succinyl-CoA) into the Krebs cycle. Also, acetoacetate influences redox homeostasis, as one of the final products of leucine metabolism. After superoxide conversion to the elevated H_2O_2 in the mitochondrial matrix, the H_2O_2 is elevated in the cytosol and thus represents the mitochondrial retrograde redox signaling. Its target could be again K_{ATP} (or K_{ATP} and TRPM2) which would depolarize the plasma membrane due to this redox signaling ongoing in parallel with the elevated ATP due to concomitantly enhanced OXPHOS.

The BCAA oxidation involves the following sequence of reactions: isovaleryl-CoA dehydrogenase (IVD), methylcrotonyl-CoA carboxylase (MCC), methylglutoconyl-CoA hydratase (MGCoAH) and 3-hydroxy-3-methylglutaryl-CoA lyase (HMGCoAL). The end-products are acetyl-CoA and acetoacetate. Similarly, as for pyruvate metabolism via PDH, the common end-product acetyl-CoA drives the Krebs cycle. This may also increase mitochondrial superoxide formation. Acetyl-CoA is linked to the above acyl-CoA dehydrogenase reaction by the reaction of the ETF:QOR, using ubiquinone (CoQ or Q) to oxidize it to ubiquinol QH. Also, the ETF:QOR itself may produce superoxide.

In summary, independently of the molecular mechanism, BCAA metabolism leads to the increased mitochondrial superoxide formation. After conversion to H_2O_2 by the matrix MnSOD and the intermembrane space CuZnSOD, the ongoing H_2O_2 efflux from mitochondria can be regarded as redox signaling. We have clearly demonstrated that the absence of such redox signaling, for example, in the presence of the mitochondrial matrix-targeted antioxidants SkQ1 leads to a blockage of insulin secretion, which is otherwise stimulated with BCKAs [1]. Likewise, the silencing of BCKDH led to the inhibition of insulin secretion stimulated with BCKAs.

9. Mechanism of fatty acid-stimulated insulin secretion

Fatty acids (FAs) appear in pancreatic islet capillaries either bound to albumin or being part of postprandial chylomicrons resulting from dietary fat lipids. FA pool of lipoproteins can be also considered. The dietary fat lipids are rich in triglycerides, which are cleaved locally in pancreatic islet capillaries by lipoprotein lipase secreted by β-cells. Resulting 2-monoacylglycerol (2MAG) and long chain FAs [170–173] stimulate each own two receptors GPR119 [157] and GPR40/FFA1, respectively. Therefore, fatty acid-stimulated insulin secretion (FASIS) could be defined as the net insulin secretion induced at the low glucose concentration, which itself does not stimulate insulin secretion. It is still controversial, whether such a net FASIS exists, since some previous reports observed that glucose should always be present for fatty acid to induce insulin secretion response. In contrast, the other reports described FASIS at 3 mM glucose, but not at zero glucose. Physiologically, postprandial responses should be due to all secretagogues resulting from major saccharide, fat and protein components. FASIS may dominate late responses upon feeding by fatty meal or an experimental high-fat diet [174].

Theoretically, FASIS must concern with the two components (**Figure 3**). The first one should depend on metabolism and the second one should rely on the stimulation

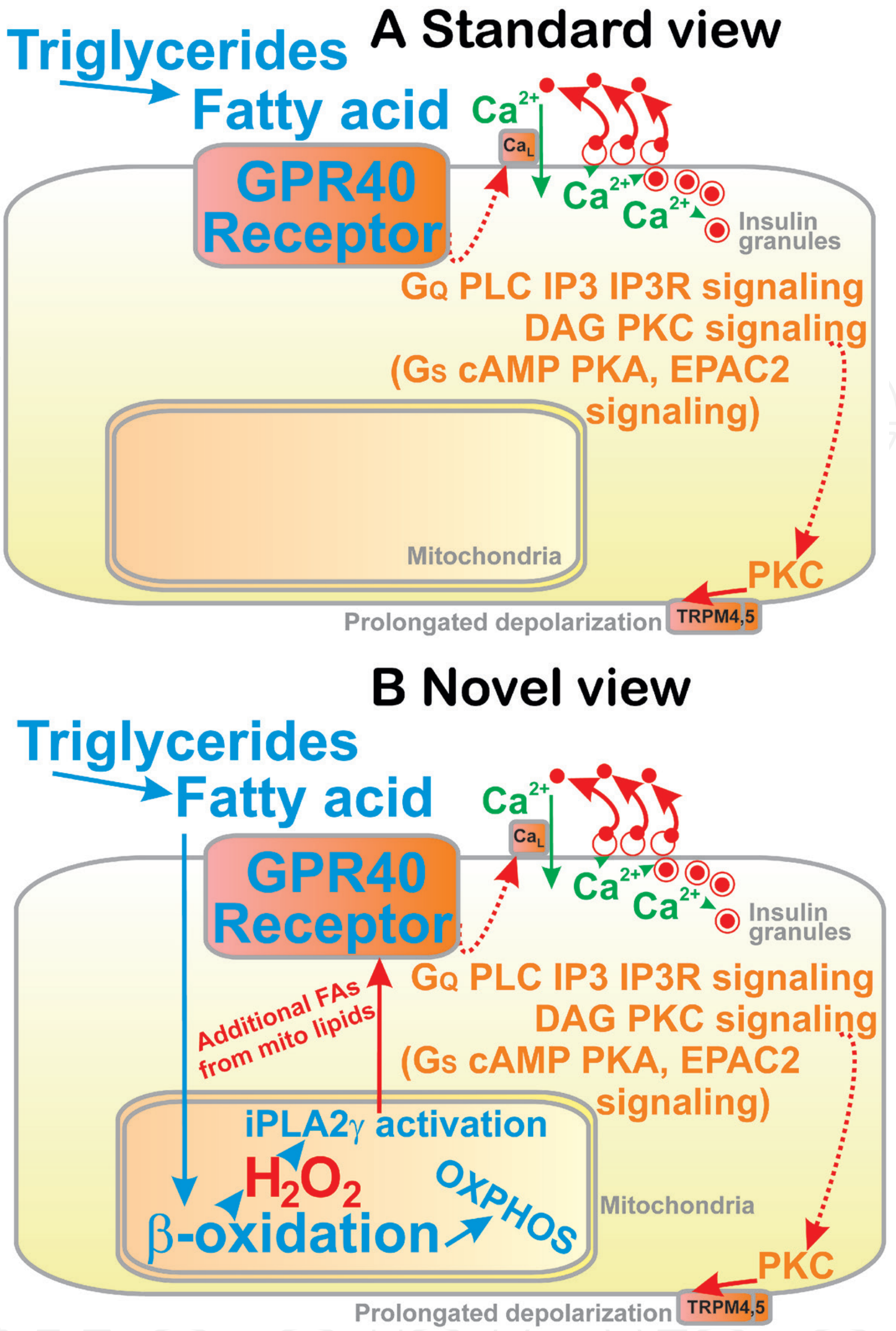

Figure 3.
(A) Traditional ("standard") view of FASIS compared with (B) FASIS with GPR40 receptor supplied by the mitochondrial fatty acids mechanism ("novel view"). The mechanism of FASIS at low glucose appears to be predominantly mediated by GPR40. Its ligands are free long-chain FAs. An excessive supply of GPR40 ligands and hence a substantial amplification of the GPR40 downstream response is given by the redox-activated mitochondrial phospholipase iPLA2γ/PNPLA8. The phospholipase iPLA2γ is directly activated by the elevated H_2O_2 in the mitochondrial matrix and cleaves both saturated and unsaturated FAs from the phospholipids of mitochondrial membranes. The cleaved free FAs diffuse up to the plasma membrane, where they activate GPR40.

of the metabotropic receptor GPR40. In vivo, FASIS-GPR40 axis is paralleled by a portion of insulin secretion stimulated via another metabotropic receptor, GPR119, to which monoacylglycerol (MAG) binds as the second major component of triglycerides. The metabolic component undoubtedly involves fatty acid β-oxidation, providing both ATP from the elevated OXPHOS and H_2O_2 from the enhanced superoxide formation by the respiratory chain and ETF:QOR, similarly as for BCKAs [175]. This component is thus directly dependent on K_{ATP}, since it leads to its closure and to the canonical downstream events identical to those during GSIS.

The receptor component of FASIS may be at least partly K_{ATP}-independent and even Ca_L-independent, hence may partly proceed independently of high glucose. In other words, FASIS at low glucose is theoretically possible. The major pathway downstream of GPR40 relies on Gαq/11, which induces PLC-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate into DAG and IP3 [110, 111]. The latter would amplify the primary Ca_L-mediated Ca^{2+} signaling for the insulin release by mediating Ca^{2+} release from ER via the Ca^{2+} channel of the IP3 receptor [143]. However, this would happen provided that some basal Ca_L would be initiated by the metabolic component, i.e. due to partial fatty acid metabolism by β-oxidation followed by H_2O_2 plus ATP elevations. Also, PKC could be activated downstream of GPR40 as being the main effector of DAG. The PKC pathway could increase the extent of plasma membrane depolarization since it activates TRPM4 and TRPM5 [144]. Moreover, this may act at low glucose, again providing that the initial triggering is ensured by the metabolic component, and so that certain basal H_2O_2 plus ATP elevations exist, leading to the K_{ATP} closure. Also, another route downstream of GPR40 would involve the Gαq/11-PLC-TRPC-induced Ca^{2+} efflux from ER [176]. As mentioned above, the TRPC1-Orai1-STIM1 complex was demonstrated to act during GSIS, while contributing to Ca^{2+} oscillations [46, 83, 84]. The action of such a complex has yet to be studied during experimental FASIS as well as its dependence on Ca_L.

Also, biased (promiscuous) pathways of GPR40, i.e., those involving $G\alpha_S$-cAMP initiation of information signaling may exist and contribute to a certain extent to FASIS. Both downstream pathways of GPR40-$G\alpha_S$-cAMP stimulation, i.e. the PKA and EPAC2 pathway, could target components of IGV interactions with the plasma membrane, hence being independent of Ca_L. These speculations await experimental evidence.

Our in vitro and in vivo experiments with mice (unpublished) demonstrated that approximately 2/3 of the GPR40 response (amplitude of insulin secretion) is given by the amplifying mechanism due to the mitochondrial phospholipase iPLA2γ/PNPLA8 [175]. This phospholipase cleaves both saturated and unsaturated FAs from the phospholipids of mitochondrial membranes. The cleaved free FAs subsequently diffuse up to the plasma membrane, where they activate GPR40. Moreover, the phospholipase iPLA2γ is directly activated by the elevated H_2O_2 in the mitochondrial matrix. The reader may remain that this is just the FA β-oxidation, which via the increased superoxide formation, due to the function of ETF:QOR and respiratory chain, produces H_2O_2, while the concomitant OXPHOS provides elevated ATP. As a result, the sufficient plasma membrane depolarization is enabled. The proof of co-existence of the GPR40 receptor component and metabolic component of FASIS is suggested by the experiments when FASIS in iPLA2γ knockout mice or in its isolated islets yielded only ~30% insulin secretion peak in the 1st phase when compared to wt mice (Holendová B., Jabůrek M, et al., unpublished). Incidentally, a similar portion remains when GW1100 antagonist of GPR40 was applied. These results show that in parallel with the GPR40 pathway, a 1/3 portion of FASIS still results from FA β-oxidation, having a similar mechanism as described for ketoacids. The abolished FASIS in the iPLA2γ knockout mice then supports the existence of such an acute mechanism in vivo, when GPR40 is supplied with mitochondrial fatty acids.

10. Redox relay as a hypothetical carrier for redox signaling

It has been established that the content of glutathione (GSH) is rather low in pancreatic β-cells [177–180], in contrast to the content of thioredoxins and glutaredoxins [181, 182], peroxiredoxins and other proteins capable of redox relay. Therefore, these proteins are able to conduct and spread the redox signals [183, 184]. From this point of view, the pancreatic β-cell appears to be a well-integrated redox system.

Redox signal spreading may be accomplished either by the direct diffusion of H_2O_2 or may be facilitated by the specialized proteins. Redox signals can be traced experimentally as instantly oxidatively modified cysteine residues, which are spread via different sets of proteins in different tissues. However, one may consider their majority as passive targets. For the case of NOX4 residing in the proximity of K_{ATP}, undoubtedly, the direct diffusion of H_2O_2 would be sufficient. Nevertheless for more distant NOX4 molecules, this would be difficult. Also, for mitochondrial redox signaling towards targets residing in the plasma membrane, a distance over 500 nm must be overcome. Redox signal across such high distances could be conducted through the action of thiol-based proteins capable of redox relay to the target, such as peroxiredoxins (regenerated via thioredoxins and glutaredoxins). The relay would provide a common redox signal transfer. It is yet to be established whether a redox relay exists via an array of peroxiredoxin oligomers.

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