A role of PLC/PKC dependent pathway in GLP-1 stimulated insulin secretion

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Abstract

Glucagon-like peptide-1 (GLP-1) is an endogenous glucose-lowering hormone and GLP-1 receptor agonist are currently being used as antidiabetic drugs clinically. The canonical signalling pathway (including cAMP, Epac2, protein kinase A (PKA) and K_{ATP} channels) is almost universally accepted as the main mechanism of GLP-1-stimulated insulin secretion. This belief is based on *in vitro* studies that used nanomolar (1-100 nM) concentrations of GLP-1. Recently, it was found that the physiological concentrations (1-10 pM) of GLP-1 also stimulate insulin secretion from isolated islets, induce membrane depolarization and increase of intracellular [Ca^{2+}] in isolated β-cells/pancreatic islets. These responses were unaffected by PKA inhibitors, and occurred without detectable increases in intracellular cAMP and PKA activity. These PKA-independent actions of GLP-1 depend on protein kinase C (PKC), involve activation of the standard GLP-1 receptor (GLP1R) and culminate in activation of phospholipase C (PLC), leading to an elevation of diacylglycerol (DAG), increased L-type Ca^{2+} and TRPM4/ TRPM5 channel activities. Here we review these recent data and contrast them against the effects of nanomolar concentrations of GLP-1. The differential intracellular signalling activated by low and high concentrations of GLP-1 could provide a clue to explain how GLP-1 exerts different function in the central nervous system and peripheral organs.

Keyword

GLP-1, insulin secretion, PKA, PKC, K_{ATP} channel, TRP channels

Background

Glucagon-like peptide-1 (GLP-1), an endogenous glucose-lowering hormone, is released from L-cells in distal intestine and colon after meal. It stimulates insulin secretion in the pancreatic β-cells, and its effect is known to be glucose-dependent. The active form of glucagon-like peptide-1(7-36) (GLP-1) is broken down into its inactive form (GLP-1(9-36)) by dipeptidyl peptidase-4 (DPP-4) in the intestine [1], liver and lung [2]. The plasma concentration of GLP-1 is the highest in ileum (92 pM) [3] but falls to less than 10 pM in peripheral blood [4, 5]. Its physiological concentrations are not over 30 pM even after meal [6-8]. DPP-4 inhibitors, used widely as antidiabetic drugs, increase the peripheral blood concentration of GLP-1 by only a few picomolar, yet significantly enhances insulin secretion [9, 10]. In a clinical study, short-term intravenous infusion of picomolar GLP-1 achieved a full normalization of glucose concentrations in type-2 diabetic patients [11]. In contrast, high peripheral blood

concentrations of GLP-1 after subcutaneous injections caused several adverse effects, such as nausea [11].

Although picomolar concentrations of GLP-1 are physiologically relevant, most *in vitro* studies on pancreatic islets have been performed at nanomolar concentrations (1–100 nM) [12-15]. The use of such high concentrations was based on receptor binding assays or measurements of intracellular cAMP accumulation, which suggested an EC_{50} of 0.1-6.1 nM [16-19]. Because of this discrepancy, we asked the following questions: First, can the results obtained with nanomolar GLP-1 *in vitro* experiments reflect physiological functions *in vivo*? Secondly, is it correct to assume that picomolar concentrations of GLP-1 are insufficient to induce insulin secretion in experiments?

We have examined effective concentration of GLP-1 in MIN6 [20], freshly isolated pancreatic islets or dispersed β-cells of mice or humans [21], and found that 1-10 pM GLP-1 stimulate insulin secretion as strongly as ~10,000-fold higher concentrations. These results indicate that the mechanism of GLP-1 can and should be investigated at its physiologically relevant concentrations. Here, we review these recent observations and the underlying signalling pathways in pancreatic β-cells. We also summarize data obtained by applying nanomolar concentrations of GLP-1 on primary β-cells and briefly compare these with the mechanisms of picomolar levels of the hormone. For more exhaustive summaries of the signalling pathways activated by nanomolar GLP-1, readers are referred to previously published review articles [12, 14, 15].

cAMP/PKA dependent pathway

It is well established that nanomolar GLP-1 enhances insulin secretion via elevation of cAMP and subsequent activation protein kinase A (PKA). High cAMP also promotes insulin secretion via the cAMP-sensor Epac2 (exchange protein directly activated by cyclic adenosine monophosphate 2) [6, 12, 14, 15]. This is supported by the observation that whereas H89 (a PKA inhibitor) inhibited insulin secretion induced by 100 nM GLP-1 by half, a much stronger inhibition (80-90%) was seen when Epac2 was also inhibited [22]. Since PKA and Epac2 stimulate insulin secretion independently or in cooperation, the signalling pathways are complex (Figure 1). However, most studies agree that the cAMP/PKA-dependent pathways are critical for signalling of nanomolar GLP-1.

Unlike high concentration, picomolar GLP-1 induced no detectable increase in intracellular cAMP level [17, 19, 23], but nevertheless significantly stimulated insulin secretion [20, 21, 24]. In MIN6 cells, the stimulatory effect of low concentrations of

GLP-1 was completely resistant to the PKA inhibitor, KT5720 [20]. In primary mouse islets, PKA inhibitors, Rp-8-Br-cAMPS or myristoylated protein kinase inhibitor (myr-PKI), reduced insulin secretion but a PKA-resistant component persisted [21]. Using the same conditions, the PKA-inhibitor Rp-8-Br-cAMPS almost completely suppressed insulin secretion induced by nanomolar GLP-1. Physiological concentrations of GLP-1 (1 pM) evoked high-frequency action potentials in isolated β-cells both under control conditions and in the presence of Rp-8-Br-cAMPS or myr-PKI [21]. To summarize, these results suggest that picomolar concentrations of GLP-1 stimulate electrical activity and insulin secretion by cAMP/PKA-independent mechanisms. Additionally, it should be mentioned that picomolar GLP-1 increased cAMP in renal COS-7 cells transfected with human GLP-1 receptors [25], which suggests that it remains possible that low concentrations of GLP-1 also signal via cAMP in pancreatic β-cells.

PLC/PKC dependent pathway

While the cAMP/PKA-dependent signalling has long been recognized as a main regulator of insulin secretion, protein kinase C (PKC) has been implicated with β-cell proliferation rather than insulin secretion in GLP-1 actions [12, 15, 26, 27]. To date, there are only a few studies that have examined the PKC-dependent signalling in response to nanomolar concentrations of GLP-1. The results have been quite variable. Whereas some studies indicate that phospholipase C (PLC) or PKC inhibitors do not affect insulin secretion induced by GLP-1 at all [12-15, 28] other studies led to a slightly different picture. For example, Suzuki et al. reported that PKC inhibitors, such as bisindolylmaleimide (BIM), reduced insulin secretion stimulated by 100 nM GLP-1 in INS-1 cells [29]. They also observed that forskolin-induced insulin secretion was suppressed by BIM. Since forskolin increases intracellular cAMP, they proposed that high concentrations of cAMP activate Epac2 and, in turn, stimulates PKC. Jacobo et al. [30] also showed that 50 nM GLP-1 stimulated insulin secretion in INS-1cells, an effect that was sensitive to BIM (~60% reduction) but resistant to the PLC inhibitor U73122.

Our own studies with freshly isolated mouse islets [21] demonstrated that the augmentation of insulin secretion by picomolar GLP-1 was almost entirely abolished by the PKC inhibitors BIM and calphostin C. Moreover, the stimulation of action potential firing by 1 pM GLP-1 was prevented by U73122. Direct evidence for the involvement PLC/PKC comes from our imaging of cytosolic/submembrane diacylglycerol (DAG), an activator of PKC. We found that 1 pM GLP-1increased DAG more strongly than a 10,000-fold higher concentration. In western blot analysis, protein kinase D1 (PKD1), a downstream product of PKC, was increased by 1 pM GLP-1, whereas phosphorylation

of PKA was not detected. The level of phosphorylation of PKD1 by 1 nM GLP-1 was lower than that produced by a 1000-fold lower concentration although this difference did not attain statistical significance. Collectively, these observations argue that PLC/PKC pathway plays a major role in the stimulation of insulin secretion produced by physiological/picomolar GLP-1 levels.

GLP-1 receptor and activation of G-proteins

It is well known from experiments using nanomolar concentrations of GLP-1 that the GLP-1 receptor interacts with the GTP-binding protein subunit Gαs, which activates adenylate cyclase (AC) with resultant stimulation of cAMP production [31]. Since picomolar GLP-1 activates PLC and PKC that were generally known as downstream substrates of Gαq, the possible involvement of an alternative GLP-1 receptor was suggested. To date, however, only one GLP-1 receptor has been identified (GLP-1R). In support of the involvement of GLP-1R, exendin9-39, a blocker of GLP-1R, completely inhibited the 1 pM GLP-1-induced insulin secretion [21]. Our own unpublished data (Ramracheya, Shigeto, Rorsman & Thorens) indicate that both pico- and nanomolar concentrations of GLP-1 are without stimulatory effect on insulin secretion in islets from GLP-1R knockout mice. Interestingly, several groups have reported that the GLP-1 receptor is capable of coupling to Gαq and Gαi in addition to Gαs [21, 32-35]. Collectively, these data suggest that both low and high concentrations of GLP-1 activate the same type of receptor, but differential downstream signalling pathways are activated. Gαq could be involved in the activation of PLC/PKC-dependent pathways by low concentrations of GLP-1. However, most of direct G-protein binding assays have been performed with nanomolar concentrations of GLP-1 [32-35], or the level of activity were too low to measure the activation of Gα subtypes in the picomolar range [21]. At present, there remains a possibility that GLP-1R activates PLC/PKC pathway in Gαq-independent way, and further technical improvement will allow us to elucidate this issue.

Calcium channels and Ca^{2+} dynamics

An elevation of $[Ca^{2+}]_i$ is essential for insulin secretion and application of GLP-1 produces a concentration-dependent increase in $[Ca^{2+}]_i$. However, previous studies based on the use of nanomolar concentration of GLP-1 produced conflicting results regarding the effect of nanomolar GLP-1 on Ca^{2+} dynamics. Some studies reported only subtle increases in $[Ca^{2+}]_i$ in response to GLP-1 [36-38], whereas other studies suggested more dramatic increases in $[Ca^{2+}]_i$, which disappeared when Ca^{2+}-free

extracellular solution or L-type Ca^{2+}-channel blockers were applied [38-41]. The latter data suggest that activation of L-type Ca^{2+}-channels mediate the increase in $[Ca^{2+}]_i$ evoked by GLP-1. However, from studies of this type it was not possible to discriminate between a direct effect of GLP-1 on the Ca^{2+} channels and a more indirect effect mediated by membrane depolarization due to K_{ATP} channel closure, and GLP-1 (10 nM) has been reported only to exert a marginal increasing effect on the amplitude of whole-cell Ca^{2+} current [42]. Nanomolar concentrations of GLP-1 have also been reported to promote calcium-induced calcium release by activation of ryanodine or inositol 1,4,5-trisphosphate (IP_3) receptors [12, 14, 15, 43]. The Ca^{2+} increase evoked by nanomolar GLP-1 in HEK 293 cells expressing cloned human GLP-1 receptor was totally suppressed by Rp-8-Br-cAMPS but unaffected by U73122 [44].

By contrast, picomolar concentrations of GLP-1 were found to consistently increase $[Ca^{2+}]_i$ [21, 28, 45] and to produce a small but statistically significant increases of the voltage-gated Ca^{2+} current in primary β-cells [21]. The increase in Ca^{2+} current was not seen in the presence of exendin9-39, PKC inhibitors (BIM and calphostin) and L-type Ca^{2+}-channel blocker (isradipine) but persisted in the presence of Rp-8-Br-cAMPS [21]. Taken together, these findings indicate that the L-type Ca^{2+}-channel is one of the targets of PKC and GLP-1. Additionally, activation of PLC will generate IP_3 in addition to DAG and it should therefore be considered whether GLP-1 also triggers Ca^{2+} release from intracellular stores (Fig. 2). Indeed, the capacity of GLP-1 to increase $[Ca^{2+}]_i$ persisted in hyperpolarized β-cells, i.e. when plasmalemmal Ca^{2+} entry through the voltage-gated Ca^{2+} channels was prevented. In summary, Ca^{2+} channel activation and intracellular Ca^{2+} dynamics are differentially regulated by GLP-1 in a concentration-dependent fashion and this may explain some of the earlier discrepant data.

K_{ATP} channels

ATP-sensitive K^+ (K_{ATP}) channels play a key role in controlling glucose-induced insulin secretion. They provide – via changes in the cytoplasmic ATP/ADP-ratio – a link between the metabolic state of the β-cell and electrical excitability. Importantly, some of K_{ATP} channels remain open at physiological glucose levels (≈5 mM) and this accounts for the maintenance of a negative membrane potential which is just subthreshold for the initiation of electrical activity and insulin secretion (~ -70 to -60 mV). Closure of these K_{ATP} channels leads to depolarization, which in turn triggers action potential firing and then enhances insulin secretion in β-cells [46-48]. Additionally, the activity of K_{ATP}

channels is reduced by cAMP [42]. This provides a potential mechanism explaining the capacity of GLP-1 to evoke electrical activity, $[Ca^{2+}]_i$ increases and insulin secretion.

In electrophysiology, a widely used experimental paradigm to examine the effect of GLP-1 on K_{ATP} channel activity is to measure the basal whole-cell conductance by application of small voltage pulses from the resting membrane potential (-70 mV) [42]. It has previously been reported that addition of 10 nM GLP-1 decreases the whole-cell conductance in primary β-cells by ~30% [24, 42]. This decrease was mediated via both PKA- [42, 49] and Epac2-dependent mechanisms [43]. However, it should be noted that this method assumes that only K_{ATP} channels are active under the resting conditions, which need not be the case. Thus, the ~30% decrease may underestimate the actual change of K_{ATP} conductance alone.

Surprisingly, and in contrast to the earlier studies using nanomolar concentrations, 1 pM GLP-1 did not decrease the resting membrane conductance [21]. To examine whether activation of another conductance could obscure the decrease in K_{ATP} channel activity, we applied GLP-1 in the presence of the K_{ATP} channel blocker tolbutamide. Any change in membrane conductance under these conditions must reflect changes in the activity of channels distinct from the K_{ATP} channel. Indeed, in the presence of tolbutamide, GLP-1 *increased* the remaining conductance by ~30% [21]. These data suggest that picomolar GLP-1 decreases K_{ATP} channel activity, but a simultaneous increase of K_{ATP}-independent conductance obscured the change. However, it is important to note that the two effects may co-operate to increase β-cell excitability and insulin secretion.

TRP channels

Using an electrophysiological technique, we could demonstrate that picomolar concentrations of GLP-1 remains capable of depolarizing even in the presence of tolbutamide [21]. In both human and mouse β-cells, tolbutamide resulted in membrane excitation which was suppressed by injecting a hyperpolarizing current. In such hyperpolarized β-cells, GLP-1 could induce K_{ATP}-independent depolarization.

Taken together with the data reviewed in the preceding section, these results suggest that physiological GLP-1 stimulates β-cell electrical activity by a combination of two effects: it inhibits the K_{ATP} channels and activates an inward depolarizing current. Clearly, this additional inward current is an important target of GLP-1's action but its identity was not known. The electrophysiological properties of this current implied that it is likely carried by Na^+ and/or Ca^{2+}, in line with earlier data [50, 51]. Since 1 pM GLP-1 could evoke membrane depolarization and action potential firing in

the presence of PKA inhibitors [21] and given that the effect of the hormone was abolished by PKC inhibitors, it appears that these channels may be regulated by PKC.

GLP-1 was found to remain capable of depolarizing β-cells in the presence of isradipine and tetrodotoxin, implying involvement of other cation-conducting channels than the voltage-gated L-type Ca^{2+} and Na^{+}-channels. The GLP-1 induced depolarization was fully reversed upon lowering of extracellular Na^{+} concentration [21], consistent with the proposal that GLP-1 activates a Na^{+}-permeable conductance [13, 50]. Thus, we propose that transient receptor potential (TRP) channels mediate the effects of GLP-1. Several TRP channels are expressed in pancreatic β-cells and influence insulin secretion. These include TRPM2, TRPM4 and TRPM5 [43].

TRPM2 is a non-selective cation-conducting channel, and it is activated by heat, ADP-ribose and PKA in β-cells [52, 53]. Based on experiments with *Trpm2*$^{-/-}$ mice TRPM2 was suggested to mediate the PKA-dependent stimulatory effect of nanomolar concentrations of GLP-1 on insulin secretion [54]. The involvement of TRPM2 in the signalling of picomolar GLP-1 has not been investigated yet.

TRPM4 and TRPM5 are Ca^{2+}-sensitive cation-conducting channels that are also regulated by PKC and ATP. TRPM4 and TRPM5 are structurally and functionally related to each other, sharing ~40 percent amino acid identity [55]. TRPM4 is influenced by PKC [55-57] and has been suggested to control membrane potential and electrical activity in insulin secretion stimulated by glucose and arginine-vasopressin [58, 59]. We found that the effects of picomolar GLP-1 on insulin secretion and membrane depolarization were strongly suppressed in *Trpm4*$^{-/-}$ mice and *Trpm5*$^{-/-}$ mice [21]. Thus, we concluded that picomolar concentrations of GLP-1 influence β-cell membrane potential by a combination of activation of a background Na^{+} current through TRPM4 and TRPM5 channels and reduction of K_{ATP} channel activity. The both effects are mediated by PKC- and PKA-pathways, respectively.

Conclusion

Here, we highlight some aspects of GLP-1's action on the insulin secretion that are of immediate relevance to the understanding of its physiological and pharmacological regulation.

First, the action of GLP-1 requires the presence of physiological glucose concentrations [60]. Under low glucose conditions, the β-cell membrane potential is extremely hyperpolarized due to massive opening of K_{ATP} channels. In this condition, the GLP-1-induced depolarization will not be enough to exceed the threshold of membrane excitation for insulin secretion. When the glucose concentration approaches

the threshold for initiation of glucose-induced electrical activity/insulin secretion, the small changes in membrane conductance produced by GLP-1 will have a dramatic stimulatory effect on β-cell electrical activity that translates into enhanced insulin secretion. Therefore, small variations of the metabolic state of the β-cells, especially near the normal glucose level, will have significant effects on the responses to GLP-1, which may account for why different groups have reported discrepant findings concerning GLP-1 effects.

Second, both GLP-1 and glucose share the same signalling mechanisms (PKA or PKC-dependent pathways) to control the β-cell activity [61, 62]. Since the both pathways are already activated to some degree by glucose, it is difficult to determine whether responses to pharmacological kinase inhibitors result from the reduction of GLP-1-induced signalling processes or simply from inhibiting substrate activities that already exist.

Third, there are several types of endocrine cells in the pancreatic islets that communicate via paracrine mechanisms. Thus, an effect on insulin secretion might be mediated by a direct effect on the β-cell, via changes in the release of other hormones or a combination of both, making the experimental data difficult to interpret.

Fourth, results obtained *in vitro* experiments do not necessarily reflect the behavior of *in vivo* and caution is warranted when interpreting the results.

Finally, the concept that different concentrations of one agonist signal by distinct intracellular second messenger systems is not without precedent. Glucagon is processed from proglucagon, like GLP-1, and stimulates hepatic glucose output. The conventional wisdom is that glucagon receptors activate cAMP/PKA pathways, but Rodgers demonstrated that physiological concentrations of glucagon (<100 pM) induce glycolysis without increasing cAMP in hepatic cells and it is rather mediated through PLC and IP_3 [63]. In addition, the concentration-dependent action of GLP-1 has also been observed in cultured human primary adipose cells: picomolar GLP-1 promoted lipogenesis without elevating intracellular cAMP, whereas nanomolar GLP-1 induces lipolysis by increasing cAMP [64]. Ghrelin, a hormone regulating energy homeostasis, stimulates insulin secretion at 1 pM concentration but inhibits at 10 nM in insulin-secreting cells [65, 66]. These examples show that concentration-dependent regulation of GLP-1 is not extraordinary in signalling processes. However, further investigations are needed to clarify molecular mechanisms by which different concentrations of GLP-1 can activate different signalling pathways via the same receptor. The receptor may have two distinct binding sites with different affinities, or a conformational change may switch the binding affinity. Once GLP-1 receptor has been crystallized [67, 68], it will

allow more direct assessment of this tantalizing possibility.

These observations raise the question as to why GLP-1 employs two different mechanisms depending on its concentration. One possible reason is that GLP-1, by analogy to ghrelin, has dual roles: stimulation of insulin secretion in pancreatic β-cells, and function as a neuropeptide in brain. The capacity to function as a neuropeptide was suggested by GLP-1 synthesis in preproglucagon-expressing neurons in the brain [69]. It is conceivable that nanomolar concentrations may occur in the narrow synaptic clefts and we speculate that GLP-1 relies on the PKA-dependent pathway in its capacity as neuropeptide, whereas its endocrine/hormonal effects are mediated by PKC-dependent pathway in response to picomolar concentrations. It is important to separate the function of GLP-1 as a neuropeptide and as a circulating hormone [70, 71], and employing distinct intracellular signalling pathways provides a possible and elegant solution. This might explain some of side effects of GLP-1 analogues in the clinic [11]. Liraglutide, a GLP-1 analogue that can cross the blood brain barrier, causes headache, at nanomolar peripheral blood concentrations [72]. On the other hand, DPP-4 inhibitor increases only a few picomolar of GLP-1 [10] and is not associated with these undesired side effects. This can also provide an explanation why only GLP-1 analogues reduce appetite and weight of obese patients.

We acknowledge that many of the details of GLP-1 actions in the islets or the brain are not fully understood and more work is certainly needed. The data that will emanate from such studies may ultimately help to fully define and exploit GLP-1's therapeutic potential.

References

1. Hansen L, Deacon CF, Orskov C, Holst JJ (1999) Glucagon-like peptide-1-(7-36)amide is transformed to glucagon-like peptide-1-(9-36)amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. Endocrinology 140: 5356-5363. DOI 10.1210/endo.140.11.7143

2. Deacon CF, Pridal L, Klarskov L, Olesen M, Holst JJ (1996) Glucagon-like peptide 1 undergoes differential tissue-specific metabolism in the anesthetized pig. Am J Physiol 271: E458-464

3. Mortensen K, Christensen LL, Holst JJ, Orskov C (2003) GLP-1 and GIP are colocalized in a subset of endocrine cells in the small intestine. Regul Pept 114: 189-196

4. Seino Y, Ogata H, Maekawa R, Izumoto T, Iida A, Harada N, Miki T, Seino S, Inagaki N, Tsunekawa S, et al. (2015) Fructose induces glucose-dependent insulinotropic polypeptide, glucagon-like peptide-1 and insulin secretion: Role of adenosine triphosphate-sensitive K(+) channels. J Diabetes Investig 6: 522-526. DOI 10.1111/jdi.12356

5. Velasquez-Mieyer PA, Cowan PA, Umpierrez GE, Lustig RH, Cashion AK, Burghen GA (2003) Racial differences in glucagon-like peptide-1 (GLP-1) concentrations and insulin dynamics during oral glucose tolerance test in obese subjects. Int J Obes Relat Metab Disord 27: 1359-1364. DOI 10.1038/sj.ijo.0802415

6. Bloomgarden ZT (2004) Gut-derived incretin hormones and new therapeutic approaches. Diabetes Care 27: 2554-2559

7. Mojsov S, Weir GC, Habener JF (1987) Insulinotropin: glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. J Clin Invest 79: 616-619. DOI 10.1172/JCI112855

8. Toft-Nielsen MB, Damholt MB, Madsbad S, Hilsted LM, Hughes TE, Michelsen BK, Holst JJ (2001) Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. J Clin Endocrinol Metab 86: 3717-3723. DOI 10.1210/jcem.86.8.7750

9. Ahren B (2007) DPP-4 inhibitors. Best Pract Res Clin Endocrinol Metab 21: 517-533. DOI 10.1016/j.beem.2007.07.005

10. Balas B, Baig MR, Watson C, Dunning BE, Ligueros-Saylan M, Wang Y, He YL, Darland C, Holst JJ, Deacon CF, et al. (2007) The dipeptidyl peptidase IV inhibitor vildagliptin suppresses endogenous glucose production and enhances islet function after single-dose administration in type 2 diabetic patients. J Clin Endocrinol Metab 92: 1249-1255. DOI 10.1210/jc.2006-1882

11. Nauck MA, Baranov O, Ritzel RA, Meier JJ (2013) Do current incretin mimetics exploit the full therapeutic potential inherent in GLP-1 receptor stimulation? Diabetologia 56: 1878-1883. DOI 10.1007/s00125-013-2953-6

12. Doyle ME, Egan JM (2007) Mechanisms of action of glucagon-like peptide 1 in the pancreas. Pharmacol Ther 113: 546-593. DOI 10.1016/j.pharmthera.2006.11.007

13. Fridolf T, Ahren B (1991) GLP-1(7-36) amide stimulates insulin secretion in rat islets: studies on the mode of action. Diabetes Res 16: 185-191

14. Gromada J, Brock B, Schmitz O, Rorsman P (2004) Glucagon-like peptide-1: regulation of insulin secretion and therapeutic potential. Basic Clin Pharmacol Toxicol 95: 252-262. DOI 10.1111/j.1742-7843.2004.t01-1-pto950502.x

15. MacDonald PE, El-Kholy W, Riedel MJ, Salapatek AM, Light PE, Wheeler MB (2002) The multiple actions of GLP-1 on the process of glucose-stimulated insulin secretion. Diabetes 51 Suppl 3: S434-442

16. Coopman K, Huang Y, Johnston N, Bradley SJ, Wilkinson GF, Willars GB (2010) Comparative effects of the endogenous agonist glucagon-like peptide-1 (GLP-1)-(7-36) amide and the small-molecule ago-allosteric agent "compound 2" at the GLP-1 receptor. J Pharmacol Exp Ther 334: 795-808. DOI 10.1124/jpet.110.166009

17. Gefel D, Hendrick GK, Mojsov S, Habener J, Weir GC (1990) Glucagon-like peptide-I analogs: effects on insulin secretion and adenosine 3',5'-monophosphate formation. Endocrinology 126: 2164-2168. DOI 10.1210/endo-126-4-2164

18. Goke R, Fehmann HC, Linn T, Schmidt H, Krause M, Eng J, Goke B (1993) Exendin-4 is a high potency agonist and truncated exendin-(9-39)-amide an antagonist at the glucagon-like peptide 1-(7-36)-amide receptor of insulin-secreting beta-cells. J Biol Chem 268: 19650-19655

19. Green BD, Gault VA, Flatt PR, Harriott P, Greer B, O'Harte FP (2004) Comparative effects of GLP-1 and GIP on cAMP production, insulin secretion, and in vivo antidiabetic actions following substitution of Ala8/Ala2 with 2-aminobutyric acid. Arch Biochem Biophys 428: 136-143. DOI 10.1016/j.abb.2004.05.005

20. Shigeto M, Katsura M, Matsuda M, Ohkuma S, Kaku K (2008) Low, but physiological, concentration of GLP-1 stimulates insulin secretion independent of the cAMP-dependent protein kinase pathway. J Pharmacol Sci 108: 274-279

21. Shigeto M, Ramracheya R, Tarasov AI, Cha CY, Chibalina MV, Hastoy B, Philippaert K, Reinbothe T, Rorsman N, Salehi A, et al. (2015) GLP-1 stimulates insulin secretion by PKC-dependent TRPM4 and TRPM5 activation. J Clin Invest 125: 4714-4728. DOI 10.1172/JCI81975

22. Kashima Y, Miki T, Shibasaki T, Ozaki N, Miyazaki M, Yano H, Seino S (2001) Critical role of cAMP-GEFII--Rim2 complex in incretin-potentiated insulin secretion. J Biol Chem 276: 46046-46053. DOI 10.1074/jbc.M108378200

23. Moens K, Heimberg H, Flamez D, Huypens P, Quartier E, Ling Z, Pipeleers D, Gremlich S, Thorens B, Schuit F (1996) Expression and functional activity of glucagon, glucagon-like peptide I, and glucose-dependent insulinotropic peptide receptors in rat pancreatic islet cells. Diabetes 45: 257-261

24. Gromada J, Bokvist K, Ding WG, Holst JJ, Nielsen JH, Rorsman P (1998) Glucagon-like peptide 1 (7-36) amide stimulates exocytosis in human pancreatic beta-cells by both proximal and distal regulatory

steps in stimulus-secretion coupling. Diabetes 47: 57-65

25. van Eyll B, Lankat-Buttgereit B, Bode HP, Goke R, Goke B (1994) Signal transduction of the GLP-1-receptor cloned from a human insulinoma. FEBS Lett 348: 7-13

26. Buteau J, Foisy S, Rhodes CJ, Carpenter L, Biden TJ, Prentki M (2001) Protein kinase Czeta activation mediates glucagon-like peptide-1-induced pancreatic beta-cell proliferation. Diabetes 50: 2237-2243

27. Bernal-Mizrachi E, Kulkarni RN, Scott DK, Mauvais-Jarvis F, Stewart AF, Garcia-Ocana A (2014) Human beta-cell proliferation and intracellular signaling part 2: still driving in the dark without a road map. Diabetes 63: 819-831. DOI 10.2337/db13-1146

28. Gromada J, Dissing S, Bokvist K, Renstrom E, Frokjaer-Jensen J, Wulff BS, Rorsman P (1995) Glucagon-like peptide I increases cytoplasmic calcium in insulin-secreting beta TC3-cells by enhancement of intracellular calcium mobilization. Diabetes 44: 767-774

29. Suzuki Y, Zhang H, Saito N, Kojima I, Urano T, Mogami H (2006) Glucagon-like peptide 1 activates protein kinase C through Ca2+-dependent activation of phospholipase C in insulin-secreting cells. J Biol Chem 281: 28499-28507. DOI 10.1074/jbc.M604291200

30. Jacobo SM, Guerra ML, Hockerman GH (2009) Cav1.2 and Cav1.3 are differentially coupled to glucagon-like peptide-1 potentiation of glucose-stimulated insulin secretion in the pancreatic beta-cell line INS-1. J Pharmacol Exp Ther 331: 724-732. DOI 10.1124/jpet.109.158519

31. Drucker DJ, Philippe J, Mojsov S, Chick WL, Habener JF (1987) Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. Proc Natl Acad Sci U S A 84: 3434-3438

32. Bavec A, Hallbrink M, Langel U, Zorko M (2003) Different role of intracellular loops of glucagon-like peptide-1 receptor in G-protein coupling. Regul Pept 111: 137-144

33. Hallbrink M, Holmqvist T, Olsson M, Ostenson CG, Efendic S, Langel U (2001) Different domains in the third intracellular loop of the GLP-1 receptor are responsible for Galpha(s) and Galpha(i)/Galpha(o) activation. Biochim Biophys Acta 1546: 79-86

34. Montrose-Rafizadeh C, Avdonin P, Garant MJ, Rodgers BD, Kole S, Yang H, Levine MA, Schwindinger W, Bernier M (1999) Pancreatic glucagon-like peptide-1 receptor couples to multiple G proteins and activates mitogen-activated protein kinase pathways in Chinese hamster ovary cells. Endocrinology 140: 1132-1140. DOI 10.1210/endo.140.3.6550

35. Thompson A, Kanamarlapudi V (2015) Agonist-induced internalisation of the glucagon-like peptide-1 receptor is mediated by the Galphaq pathway. Biochem Pharmacol 93: 72-84. DOI 10.1016/j.bcp.2014.10.015

36. Cane MC, Parrington J, Rorsman P, Galione A, Rutter GA (2016) The two pore channel TPC2 is dispensable in pancreatic beta-cells for normal Ca(2+) dynamics and insulin secretion. Cell Calcium 59: 32-40. DOI 10.1016/j.ceca.2015.12.004

37. Hodson DJ, Tarasov AI, Gimeno Brias S, Mitchell RK, Johnston NR, Haghollahi S, Cane MC, Bugliani M, Marchetti P, Bosco D, et al. (2014) Incretin-modulated beta cell energetics in intact islets of Langerhans. Mol Endocrinol 28: 860-871. DOI 10.1210/me.2014-1038

38. Tsuboi T, da Silva Xavier G, Holz GG, Jouaville LS, Thomas AP, Rutter GA (2003) Glucagon-like peptide-1 mobilizes intracellular Ca2+ and stimulates mitochondrial ATP synthesis in pancreatic MIN6 beta-cells. Biochem J 369: 287-299. DOI 10.1042/BJ20021288

39. Delmeire D, Flamez D, Hinke SA, Cali JJ, Pipeleers D, Schuit F (2003) Type VIII adenylyl cyclase in rat beta cells: coincidence signal detector/generator for glucose and GLP-1. Diabetologia 46: 1383-1393. DOI 10.1007/s00125-003-1203-8

40. Gromada J, Dissing S, Rorsman P (1996) Desensitization of glucagon-like peptide 1 receptors in insulin-secreting beta TC3 cells: role of PKA-independent mechanisms. Br J Pharmacol 118: 769-775

41. Holz GG, Leech CA, Heller RS, Castonguay M, Habener JF (1999) cAMP-dependent mobilization of intracellular Ca2+ stores by activation of ryanodine receptors in pancreatic beta-cells. A Ca2+ signaling system stimulated by the insulinotropic hormone glucagon-like peptide-1-(7-37). J Biol Chem 274: 14147-14156

42. Gromada J, Ding WG, Barg S, Renstrom E, Rorsman P (1997) Multisite regulation of insulin secretion by cAMP-increasing agonists: evidence that glucagon-like peptide 1 and glucagon act via distinct receptors. Pflugers Arch 434: 515-524. DOI 10.1007/s004240050431

43. Leech CA, Dzhura I, Chepurny OG, Kang G, Schwede F, Genieser HG, Holz GG (2011) Molecular physiology of glucagon-like peptide-1 insulin secretagogue action in pancreatic beta cells. Prog Biophys Mol Biol 107: 236-247. DOI 10.1016/j.pbiomolbio.2011.07.005

44. Gromada J, Rorsman P, Dissing S, Wulff BS (1995) Stimulation of cloned human glucagon-like peptide 1 receptor expressed in HEK 293 cells induces cAMP-dependent activation of calcium-induced calcium release. FEBS Lett 373: 182-186

45. Cullinan CA, Brady EJ, Saperstein R, Leibowitz MD (1994) Glucose-dependent alterations of intracellular free calcium by glucagon-like peptide-1(7-36amide) in individual ob/ob mouse beta-cells. Cell Calcium 15: 391-400

46. Ashcroft FM, Harrison DE, Ashcroft SJ (1984) Glucose induces closure of single potassium channels in isolated rat pancreatic beta-cells. Nature 312: 446-448

47. Ashcroft FM, Rorsman P (2013) K(ATP) channels and islet hormone secretion: new insights and controversies. Nat Rev Endocrinol 9: 660-669. DOI 10.1038/nrendo.2013.166

48. McTaggart JS, Clark RH, Ashcroft FM (2010) The role of the KATP channel in glucose homeostasis in health and disease: more than meets the islet. J Physiol 588: 3201-3209. DOI 10.1113/jphysiol.2010.191767

49. Light PE, Manning Fox JE, Riedel MJ, Wheeler MB (2002) Glucagon-like peptide-1 inhibits pancreatic ATP-sensitive potassium channels via a protein kinase A- and ADP-dependent mechanism. Mol

Endocrinol 16: 2135-2144. DOI 10.1210/me.2002-0084

50. Kato M, Ma HT, Tatemoto K (1996) GLP-1 depolarizes the rat pancreatic beta cell in a Na(+)-dependent manner. Regul Pept 62: 23-27

51. Miura Y, Matsui H (2003) Glucagon-like peptide-1 induces a cAMP-dependent increase of [Na+]i associated with insulin secretion in pancreatic beta-cells. Am J Physiol Endocrinol Metab 285: E1001-1009. DOI 10.1152/ajpendo.00005.2003

52. Togashi K, Hara Y, Tominaga T, Higashi T, Konishi Y, Mori Y, Tominaga M (2006) TRPM2 activation by cyclic ADP-ribose at body temperature is involved in insulin secretion. EMBO J 25: 1804-1815. DOI 10.1038/sj.emboj.7601083

53. Lange I, Yamamoto S, Partida-Sanchez S, Mori Y, Fleig A, Penner R (2009) TRPM2 functions as a lysosomal Ca2+-release channel in beta cells. Sci Signal 2: ra23. DOI 10.1126/scisignal.2000278

54. Uchida K, Dezaki K, Damdindorj B, Inada H, Shiuchi T, Mori Y, Yada T, Minokoshi Y, Tominaga M (2011) Lack of TRPM2 impaired insulin secretion and glucose metabolisms in mice. Diabetes 60: 119-126. DOI 10.2337/db10-0276

55. Nilius B, Prenen J, Tang J, Wang C, Owsianik G, Janssens A, Voets T, Zhu MX (2005) Regulation of the Ca2+ sensitivity of the nonselective cation channel TRPM4. J Biol Chem 280: 6423-6433. DOI 10.1074/jbc.M411089200

56. Earley S, Straub SV, Brayden JE (2007) Protein kinase C regulates vascular myogenic tone through activation of TRPM4. Am J Physiol Heart Circ Physiol 292: H2613-2622. DOI 10.1152/ajpheart.01286.2006

57. Guinamard R, Demion M, Launay P (2010) Physiological roles of the TRPM4 channel extracted from background currents. Physiology (Bethesda) 25: 155-164. DOI 10.1152/physiol.00004.2010

58. Cheng H, Beck A, Launay P, Gross SA, Stokes AJ, Kinet JP, Fleig A, Penner R (2007) TRPM4 controls insulin secretion in pancreatic beta-cells. Cell Calcium 41: 51-61. DOI 10.1016/j.ceca.2006.04.032

59. Marigo V, Courville K, Hsu WH, Feng JM, Cheng H (2009) TRPM4 impacts on Ca2+ signals during agonist-induced insulin secretion in pancreatic beta-cells. Mol Cell Endocrinol 299: 194-203. DOI 10.1016/j.mce.2008.11.011

60. Goke R, Wagner B, Fehmann HC, Goke B (1993) Glucose-dependency of the insulin stimulatory effect of glucagon-like peptide-1 (7-36) amide on the rat pancreas. Res Exp Med (Berl) 193: 97-103

61. Nesher R, Anteby E, Yedovizky M, Warwar N, Kaiser N, Cerasi E (2002) Beta-cell protein kinases and the dynamics of the insulin response to glucose. Diabetes 51 Suppl 1: S68-73

62. Shibasaki T, Takahashi T, Takahashi H, Seino S (2014) Cooperation between cAMP signalling and sulfonylurea in insulin secretion. Diabetes Obes Metab 16 Suppl 1: 118-125. DOI 10.1111/dom.12343

63. Rodgers RL (2012) Glucagon and cyclic AMP: time to turn the page? Curr Diabetes Rev 8: 362-381

64. Villanueva-Penacarrillo ML, Marquez L, Gonzalez N, Diaz-Miguel M, Valverde I (2001) Effect

of GLP-1 on lipid metabolism in human adipocytes. Horm Metab Res 33: 73-77

65. Date Y, Nakazato M, Hashiguchi S, Dezaki K, Mondal MS, Hosoda H, Kojima M, Kangawa K, Arima T, Matsuo H, et al. (2002) Ghrelin is present in pancreatic alpha-cells of humans and rats and stimulates insulin secretion. Diabetes 51: 124-129

66. Dezaki K, Hosoda H, Kakei M, Hashiguchi S, Watanabe M, Kangawa K, Yada T (2004) Endogenous ghrelin in pancreatic islets restricts insulin release by attenuating Ca2+ signaling in beta-cells: implication in the glycemic control in rodents. Diabetes 53: 3142-3151

67. Donnelly D (2012) The structure and function of the glucagon-like peptide-1 receptor and its ligands. Br J Pharmacol 166: 27-41. DOI 10.1111/j.1476-5381.2011.01687.x

68. West GM, Willard FS, Sloop KW, Showalter AD, Pascal BD, Griffin PR (2014) Glucagon-like peptide-1 receptor ligand interactions: structural cross talk between ligands and the extracellular domain. PLoS One 9: e105683. DOI 10.1371/journal.pone.0105683

69. Vrang N, Larsen PJ (2010) Preproglucagon derived peptides GLP-1, GLP-2 and oxyntomodulin in the CNS: role of peripherally secreted and centrally produced peptides. Prog Neurobiol 92: 442-462. DOI 10.1016/j.pneurobio.2010.07.003

70. Barrera JG, Sandoval DA, D'Alessio DA, Seeley RJ (2011) GLP-1 and energy balance: an integrated model of short-term and long-term control. Nat Rev Endocrinol 7: 507-516. DOI 10.1038/nrendo.2011.77

71. Trapp S, Richards JE (2013) The gut hormone glucagon-like peptide-1 produced in brain: is this physiologically relevant? Curr Opin Pharmacol 13: 964-969. DOI 10.1016/j.coph.2013.09.006

72. Juhl CB, Hollingdal M, Sturis J, Jakobsen G, Agerso H, Veldhuis J, Porksen N, Schmitz O (2002) Bedtime administration of NN2211, a long-acting GLP-1 derivative, substantially reduces fasting and postprandial glycemia in type 2 diabetes. Diabetes 51: 424-429

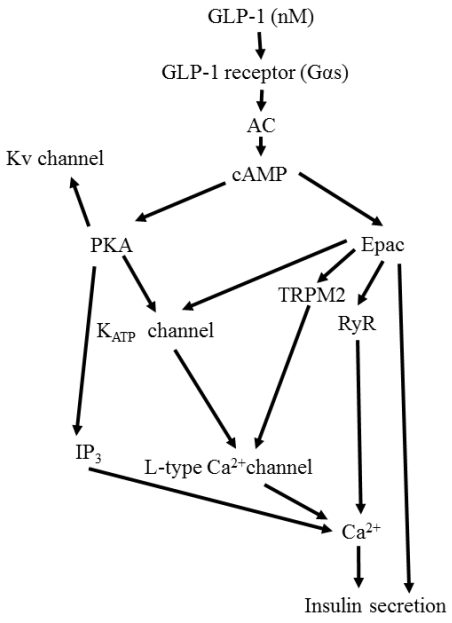

Figure 1

The conventional cAMP/Epac2/PKA pathway for insulin secretion triggered by nanomolar GLP-1.

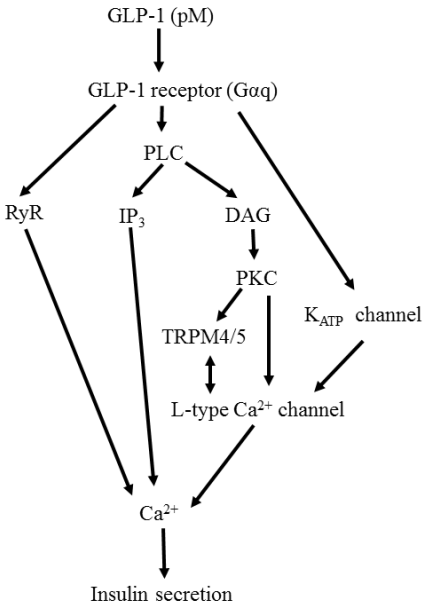

Figure 2

The PKC-dependent pathway for insulin secretion stimulated by physiological concentration of GLP-1.