

# New concept of the glucagon-like peptide-1 signaling pathway on pancreatic insulin secretion

Glucagon-like peptide-1 (GLP-1), a member of the incretin family, is a hormone released from L cells in the distal intestine/colon after a meal. GLP-1 analogs or its receptor agonists are widely used as antidiabetic drugs, which stimulate glucose-dependent insulin secretion. The active form of GLP-1 (7–36) is quickly degraded to an inactive form (9–36) by dipeptidyl peptidase-4 (DPP-4), mainly in the intestine. Thereby, a steady-state physiological concentration of GLP-1 is <10 pmol/L in the peripheral blood, and not >30 pmol/L even after a meal<sup>1</sup>. In fact, DPP-4 inhibitors increase plasma concentrations of GLP-1 only up to ≤20 pmol/L in diabetes patients, yet significantly enhance insulin secretion from the pancreas.

In contrast, many studies investigating the effects of GLP-1 on pancreatic islet function have been carried out at nanomolar levels of GLP-1, which are at least 100-fold higher than those seen physiologically. Historically, such high concentrations of GLP-1 have been used customarily in *in vitro* studies for the cellular mechanism of this hormone action. Actually, an EC<sub>50</sub> of a few nmol/L was suggested in the receptor-binding assay and intracellular cyclic adenosine monophosphate (cAMP) accumulation. High concentrations (≥0.1 nmol/L) of GLP-1 induce the receptor coupling to the guanosine 5'-triphosphate-binding protein G $\alpha$ s, which activates adenylyl cyclase and is followed by activation of the cAMP-dependent protein kinase A (PKA) pathway. The intracellular GLP-1 signaling pathway, which involves cAMP, exchange protein directly activated by cAMP (Epac2), PKA and mediated channels, has been widely accepted to explain a mechanism of GLP-1-stimulated insulin secretion<sup>2</sup>.

As physiological levels (pmol/L) of GLP-1 are much lower than those used in the studies (nmol/L) for the aforementioned GLP-1 signaling pathway, the following question is raised: whether a cAMP–PKA-dependent pathway shown at high concentrations of GLP-1 is universal as an action mechanism of this hormone or not. Our previous *in vitro* study might provide a clue to deal with the question<sup>3</sup>. The study supported a possible effect of low concentrations (pmol/L) of GLP-1, which was seen in the case of DPP-4 inhibitor use, on insulin secretion. In that study, a high concentration (10 nmol/L) of GLP-1 increased insulin secretion from MIN6 cells with a significant increase in intracellular cAMP accumulation. In this condition, TK5720, a selective PKA inhibitor, suppressed insulin secretion. In contrast, a low concentration (1 pmol/L) of GLP-1 also stimulated insulin secretion without a

significant accumulation of intracellular cAMP. Insulin secretion was not affected by a PKA inhibitor, but was inhibited by calcium channel blockers, such as verapamil and dantrolene, and an intracellular Ca<sup>2+</sup> chelating agent, 1,2-Bis(2-amino-phenoxy)-ethane-N,N,N',N'-tetraacetic acid. These data showed that picomolar GLP-1-induced insulin secretion is dependent of intracellular calcium concentration, but independent of the cAMP–PKA pathway. In response to these results, we speculated on a new possibility for a unique mechanism of picomolar GLP-1 action different from that shown at high nanomolar concentrations<sup>4</sup>.

The recent study reported by Shigeto *et al.*<sup>5</sup> has made the possibility real. First of all, the study examined the dose-dependent properties of GLP-1 effects on glucose-stimulated insulin secretion (GSIS) in mouse and human islets. It was again confirmed that GLP-1 between 0.1 pmol/L and 10 pmol/L enhanced GSIS dose-dependently with EC<sub>50</sub> of ~0.4 pmol/L in isolated mouse islets. A potency of 1 pmol/L GLP-1 was almost the same as that of high concentrations (~10 nmol/L). In experiments to assess electrical activity in  $\beta$ -cells, the membrane potential generated in dispersed  $\beta$ -cells exposed to physiological concentration (6 mmol/L) of glucose was observed in just 10% of the total number of cells, but the addition of 1 pmol/L GLP-1 significantly and reversely initiated or enhanced action potential firing in almost whole cells. The potency of 1 pmol/L GLP-1 on the electrical activity in  $\beta$ -cells was almost equal to that evoked by a higher concentration (10 nmol/L) of this hormone.

In the presence of 6 mmol/L glucose, the induction of [Ca<sup>2+</sup>]<sub>i</sub> oscillations was observed in just 5% of the cells, which was similar to the fraction of cells shown in the effect on electrical activity. However, 1 pmol/L GLP-1 again significantly increased the recruitment of cells, in which a spontaneous Ca<sup>2+</sup> current was induced. Exocytosis of insulin granules evoked by the membrane depolarization was also enhanced by the addition of 1 pmol/L GLP-1. As these effects of 1 pmol/L GLP-1 were inhibited by the GLP-1 receptor antagonist, exendin (9–39), it was strongly suggested that the stimulatory effect of physiologically low levels of GLP-1 on GSIS would be mediated by the GLP-1 receptors that are currently recognized.

Shigeto *et al.*<sup>5</sup> further showed that 1 pmol/L GLP-1 potentiated the integrated Ca<sup>2+</sup> current in mouse  $\beta$ -cells. This effect was thought to be mediated by the activation of L-type Ca<sup>2+</sup> channels, because isradipine, an inhibitor of these channels, significantly reduced the Ca<sup>2+</sup> current induced by 1 pmol/L

GLP-1. GSIS potentiated by a low concentration of GLP-1 was also inhibited by isradipine. These results were confirmed in human  $\beta$ -cells. In the experiment using the membrane permeable PKA inhibitor, myristoylated PKI or 8-Br-Rp-cAMP, the stimulatory effect of 1 pmol/L GLP-1 on GSIS was significantly inhibited, indicating the possibility that the stimulatory effects of picomolar GLP-1 on insulin secretion are mediated by a PKA-independent mechanism. In contrast, 1 pmol/L GLP-1 stimulated cAMP accumulation in renal COS7 cells transfected with human GLP-1 receptor, suggesting that the cAMP–PKA-dependent mechanism also remained to a certain degree.

From the above process, a PKA-independent/PKC-dependent mechanism was found to be involved in the stimulatory effects of picomolar GLP-1 on insulin secretion. Then, it has been suggested that GLP-1 receptor binding activates not only  $G\alpha_s$ , but also  $G\alpha_i$  and  $G\alpha_q$ , which are linked with phospholipase C (PLC) and protein kinase C (PKC) activation. The imaging of cytosolic/submembrane diacylglycerol (DAG), a PKC activator, strongly supported an involvement of PLC/PKC, which played a significant role in picomolar levels of GLP-1-stimulated insulin secretion. Furthermore, a physiological (pmol/L) GLP-1-induced adenosine triphosphate-sensitive-independent depolarization was closely related with an activation of a  $Na^+$ -permeable conductance, using an electrophysiological technique<sup>5</sup>.

Suggested  $Na^+$ -permeable conductance was activated by a low level of GLP-1, and transient receptor potential (TRP) channels were assumed to mediate GLP-1 effects on insulin secretion. TRP cation channel subfamily M member 4 (TRPM4) and TRPM5 were chosen from several TRPMs expressed in pancreatic  $\beta$ -cells. These two TRPM, which are  $Ca^{2+}$ -sensitive cation-conducting channels and share approximately 40% amino acid identity, are functionally similar to each other. TRPM4 is known to be involved in the PKC pathway. Interestingly, the picomolar GLP-1-induced membrane depolarization was significantly suppressed in *Trpm4*<sup>-/-</sup> or *Trpm5*<sup>-/-</sup> mice<sup>5</sup>.

A line of evidence draws the conclusion that a physiologically low level (pmol/L) of GLP-1, which is enough to stimulate insulin secretion from  $\beta$ -cells, affects cell membrane potential by collaboration of  $Na^+$  current activation through TRPM4 and TRPM5 channels involved in the PKC-dependent pathway and PKA-dependent reduction of adenosine triphosphate-sensitive channel activity. Thus, it is clearly shown that the effects of picomolar GLP-1 on insulin secretion are mediated by the PKC-dependent pathway, in addition to a PKA-dependent mechanism as a common pathway of GLP-1 action.

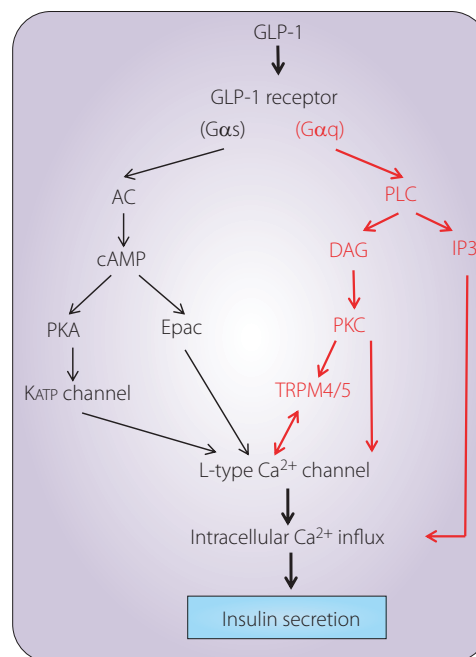
Here, I advocate a new concept of the intracellular GLP-1 signaling pathway on insulin secretion (Figure 1). Low (~30 pmol/L) concentrations of GLP-1 stimulate insulin secretion from pancreatic  $\beta$ -cells in the presence of physiological levels of glucose accompanied by membrane depolarization and an increase in the intracellular  $[Ca^{2+}]_i$  via L-type  $Ca^{2+}$  channel. Unlike nanomolar levels of GLP-1, this response is mediated in a considerable part by the PKA-independent pathway,

including induction of  $G\alpha_q$  activity followed by PLC and PKC activation. Furthermore, TRPM4 and TRPM5 channels play a role in the PKC-induced  $Ca^{2+}$  current. This pathway is a unique action mechanism of GLP-1 at physiologically low concentrations.

The reason why an intracellular mechanism of GLP-1 action is dependent of its concentration is not easy to explain clearly. GLP-1 is known to work in many ways as a hormone in the pancreas and as neuropeptides in the brain. Although the actual concentrations of GLP-1 in each organ are not always definite, it is speculated that GLP-1 functions at suitable concentrations according to the purpose in its target organs.

The concept that some receptor ligands act through distinct signaling pathways is well known; for example, epinephrine binding to its  $\alpha$ -receptor activates not only  $G\alpha_s$ , but also  $G\alpha_i$  according to the role.

Furthermore, it has not been excluded that GLP-1 stimulates insulin secretion neurally; that is, through activation of vagovagal reflexes, suggesting its concentration is higher than that seen in peripheral blood. However, a series of events clearly showed that picomolar levels of GLP-1 are physiologically relevant and sufficient to stimulate insulin secretion, even if the contribution of the PLC–PKC pathway in picomolar GLP-1 action might be different in each animal type. Each different intracellular signaling pathway observed at high and low concentrations of GLP-1



**Figure 1** | A new concept for intracellular signaling pathway of glucagon-like peptide-1 (GLP-1)-stimulated insulin secretion. A common pathway of GLP-1 in any concentration is drawn in black and a unique pathway of GLP-1 at physiologically low levels (pmol/L) is in red. AC, adenylyl cyclase; DAG, diacylglycerol; IP3, inositol triphosphate; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; TRPM, transient receptor potential cation channel subfamily M member.

presents a hint to understanding a multifunctional property of this hormone in various organs.

#### DISCLOSURE

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