Are both protein kinase A- and protein kinase C-dependent pathways involved in glucagon-like peptide-1 action on pancreatic insulin secretion?

Glucagon-like peptide-1 (GLP-1) is an insulinotropic hormone classified as incretin. GLP-1 is secreted from L-cells, which are located in the lower gastrointestinal tract, by stimulation of several kinds of nutrition, such as carbohydrate. GLP-1 is transported to the pancreas probably through the bloodstream and nervous system, and not only regulates pancreatic hormones, but is also involved in differentiation, proliferation and regulation of β-cell mass. GLP-1 is inactivated by dipeptidyl peptidase-IV (DPP-IV) in a rapid manner. GLP-1 receptor agonists and DPP-IV inhibitors have been used as important antidiabetic drugs in clinical practice.

Administration of DPP-IV inhibitors increases GLP-1 concentration in the peripheral bloodstream by only a few picomolar levels, yet results in a significant increase in insulin release and reduction in serum glucose concentration. However, most previous in vitro experiments have been carried out at nanomolar GLP-1 concentrations. The use of this high concentration of GLP-1 was based on receptor binding assays and the measurement of intracellular cyclic adenosine monophosphate (cAMP; GLP-1 was applied to the MIN6 cell line). Furthermore, an increase of insulin secretion and calcium currents by 1 pmol/L GLP-1 was still significant in the presence of PKA inhibitor, 100 μmol/L 8-Br-RP-cAMPS, in primary cells. To sum up these observations, we conclude that the signaling pathway of picomolar GLP-1 is PKA-independent.

Pancreatic β-cells are equipped with a protein kinase C (PKC)-dependent pathway to modulate insulin secretion. For example, PKC is involved in insulin secretion stimulated by vasopressin. Recently, it became clearer that some fatty acids can enhance insulin secretion by binding G protein-coupled receptor 40 (GPR40), and PKC is also involved in the signal transduction. Despite that, the possibility that the PKC pathway is involved in insulin secretion by GLP-1 has been excluded by a study by Fridolf et al. They reported that the inhibitor of PKC did not affect insulin secretion stimulated by nanomolar GLP-1 in islets. Several reviews also suggested that the PKC pathway could be present in β-cells but its role in insulin secretion is minor.

The previous studies using nanomolar concentrations of GLP-1 have suggested that GLP-1 activates the protein kinase A (PKA) pathway by increasing cAMP through G protein-coupled receptor (GPCR; Figure 1). This has been regarded as the major signaling pathway for stimulating insulin secretion by GLP-1. Several studies have reported that the elevation of intracellular cAMP is not detectable or very small at 1 pmol/L, and always significant at 10 nmol/L GLP-1. In contrast, our study showed that 1 pmol/L GLP-1 induces significant insulin secretion as strongly as 10 nmol/L GLP-1 in MIN6 cells and isolated mouse islets, even in whole pancreas perfusion experiments. Furthermore, 1 pmol/L GLP-1 induced depolarization of membrane potential, and increased L-type calcium currents and membrane capacitance in isolated single β-cells. The discrepancy in the level of GLP-1 necessary for the elevation of cAMP and for insulin secretion implies that the insulin secretion by picomolar GLP-1 is mediated by the cAMP–PKA independent pathway. Consistently, we observed that PKA inhibitor, 100 nmol/L KT5720, had no effect on the time-course of the insulin secretion when 1 pmol/L GLP-1 was applied to the MIN6 cell line. Furthermore, an increase of insulin secretion and calcium currents by 1 pmol/L GLP-1 was still significant in the presence of PKA inhibitor, 100 μmol/L 8-Br-RP-cAMPS, in primary cells. To sum up these observations, we conclude that the signaling pathway of picomolar GLP-1 is PKA-independent.

Figure 1 | New concept of signaling pathway of Glucagon-like peptide-1 (GLP-1). Nanomolar GLP-1 bind to GLP-1 receptor, and then activate the cyclic adenosine monophosphate–protein kinase A (PKA) pathway and exchange protein directly activated by cyclic adenosine monophosphate 2 (Epac 2), which requires elevation of cyclic adenosine monophosphate (classical pathway). Picomolar GLP-1 activates protein kinase C (PKC) through the same GLP-1 receptor (high affinity site) and opens the L-type calcium channel (novel pathway; blue). AC, adenylyl cyclase; PLC, phospholipase C.
pathway is not involved in the signal transduction pathway of nanomolar GLP-1. In contrast, our recent study showed that the insulin secretion stimulated by 1 pmol/L GLP-1 was largely diminished by two PKC inhibitors, bisindolylmaleimide (100 nmol/L) and calphostin C (100 nmol/L), in primary mouse β-cells. Furthermore, 1 pmol/L GLP-1 increased the L-type Ca\(^{2+}\) current and exocytosis, and the PKC inhibitor abolished these effects\(^5\). These results showed that, different from nanomolar GLP-1, PKC participates in the action of picomolar GLP-1. In future, more experiments should be carried out to clarify the signal transduction cascade related to PKC.

The next question is how the signaling pathway can differ depending on the concentration of GLP-1. Simply, it could be argued that two different receptors might be involved in the actions of low and high concentrations of GLP-1. However, this answer is unlikely, because our preliminary experiments showed that the increase of insulin secretion by both picomolar and nanomolar GLP-1 were completely abolished in GLP-1 receptor-knockout mice\(^5\). Furthermore, exendin 9-39, an antagonist of a classical GLP-1 receptor, eliminated the stimulatory effect on insulin secretion of both low and high GLP-1 concentrations\(^6\). These results showed that low and high concentrations of GLP-1 act through the same receptor. Here, we imagine that GLP-1 could act on two distinct binding sites with different affinities in the same GLP-1 receptor, and each is involved in a different intracellular signaling pathway (Figure 1). The binding of GLP-1 to one site might inactivate the other by ligand-induced conformation change. This hypothesis could answer why the action of nanomolar GLP-1 stimulates the PKA pathway without activating the PKC pathway, and does not produce an additional increase of insulin secretion compared with the effect of picomolar GLP-1. Similar mechanisms have been reported for several family B GPCRs, such as β2-adrenoreceptors, in which conformational changes within the respective receptors initiate differential signaling\(^7\). The GLP-1 receptor is categorized as a family B GPCR, one of the most difficult proteins to analyze the structure and function of because of its instability in vitro. Therefore, further work is required, especially determination of the structure of the GLP-1 receptor, to test this hypothesis.

GLP-1 secreted from L-cells is transferred through the bloodstream, and the peripheral blood concentration of GLP-1 becomes a few picomolar when it reaches the pancreas. GLP-1 might also be transported to the pancreas through the nervous system. However, it is unlikely that a nanomolar concentration of GLP-1 acts in the pancreatic islets in the physiological condition. Our studies could answer how GLP-1 secreted from L-cells acts on pancreatic β-cells to stimulate insulin secretion\(^1\). How can a nanomolar concentration of GLP-1 be present around β-cells in vivo? For example, pancreatic α-cells can secrete a high local concentration of GLP-1 in islets. Ellingsgaard et al.\(^8\) have shown that GLP-1 was secreted from α-cells in the presence of high levels of interleukin-6 in humans. That is, although it might not happen under normal conditions, in the case of inflammation, the paracrine action of GLP-1 can stimulate insulin secretion. Presumably, high concentrations of GLP-1 could act as a supporting mechanism in the case of an emergency or dominantly regulating differentiation and proliferation. In conclusion, we propose a new idea that both PKC- and PKA-mediated pathways work in vivo to stimulate insulin secretion in pancreatic β-cells at picomolar and nanomolar concentrations of GLP-1, respectively.

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Makoto Shigeto\(^1,2\), Kohei Kaku\(^2,*\)

\(^1\)Oxford Center for Diabetes, Endocrinology and Metabolism, Churchill Hospital Site, Headington, Oxford, UK; \(^2\)Department of Diabetes, Endocrinology and Metabolism, Kawasaki Medical School, Kurashiki, Okayama, Japan

*E-mail: kka@med.kawasaki-m.ac.jp

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