Glucose-incretin interaction revisited

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Abstract. Pancreatic beta cell dysfunction is pivotal to the development of diabetes, and restoration of insulin action is of primary importance. Here, we present a review of the mechanism of insulin secretion by pancreatic beta cells and discuss the mutual interaction of signaling pathways in stimulus-secretion coupling to better understand the scientific basis of pharmacological treatment for insulin secretion deficiency. Glucose stimulates insulin secretion via membrane depolarization by closure of ATP-sensitive K⁺ channels (KATP channels) and opening of L-type voltage-dependent Ca²⁺ channels. The resultant elevation of cytosolic free Ca²⁺ triggers insulin exocytosis. This is termed the “KATP-dependent pathway” and is shared by sulfonylurea, which closes KATP channels. Glucose also stimulates insulin release independent of its action on KATP channels. This is referred to as the “KATP-independent pathway,” the molecular basis of which remains elusive. In the pancreatic beta cell, incretin hormones increase cAMP level, which enhances glucose-stimulated insulin release by protein kinase A-dependent and -independent mechanisms. Importantly, cAMP does not directly augment Ca²⁺-stimulated insulin release per se. The stimulatory level of ambient glucose is an absolute requirement for incretin to enhance insulin release. Therefore, incretin/cAMP enhances KATP-independent insulinotropic action of glucose. The robust glucose-lowering effect of DPP4 inhibitor add-on in diabetic patients with sulfonylurea secondary failure is intriguing. With the clinical availability of DPP4 inhibitor and GLP-1 mimetics, the importance of the interactions between cAMP signaling and KATP channel-independent actions of glucose is reappraised.

Key words: Glucose, Insulin secretion, ATP-sensitive K⁺ channel, Incretin, cAMP

INSULIN is the only hormone that lowers plasma glucose concentration. Therefore, insufficient insulin activity leads to chronic elevation of plasma glucose and diabetes mellitus, and restoration of insulin activity is vital for normalization of metabolism in diabetes. Here, we present a review of the pathophysiology of insulin deficiency in type 2 diabetes (T2D), stimulus-secretion coupling in the pancreatic beta cells, and the interaction of glucose and cAMP signal to provide a comprehensive view regarding the basis of pharmacological treatment of patients with T2D.

1. Evolution of T2D

Both insulin deficiency and insulin resistance play pivotal roles in the development of T2D. Early in 1991, the “two-step theory” was proposed for the evolution of T2D [1]. That is, a sedentary lifestyle initially causes obesity and reduced insulin sensitivity. As a compensation, insulin secretion by the pancreatic beta cell increases, which is often insufficient to restore normoglycemia. The result is non-diabetic hyperglycemia associated with hyperinsulinemia. Then, as the beta cell function deteriorates, T2D develops with impaired insulin secretion. However, the importance of impaired insulin secretion as a risk factor for future T2D in the non-diabetic population had long been recognized prior to this hypothesis [2]. The two-step theory was corrected in 1999 even in Pima Indians [3]. In Asian populations, insulin resistance is less prominent than in Caucasians with a similar incidence of T2D in both groups. This strongly suggests the importance of insulin deficiency in the pathogenesis of T2D. In fact, not only in Japanese but also in other ethnic groups, glucose-induced insulin secretion is clearly diminished in subjects in whom plasma glucose concentration is slightly elevated but remains within the normal range [4-9]. More recently, profound and progressive impair-
class of antidiabetic agents, are attracting a great deal of attention because they increase insulin secretion via mechanisms distinct from those of SU and glinide. They enhance insulin secretion in a glucose-dependent manner, therefore avoiding hypoglycemia caused by inappropriate insulin secretion.

2. Mechanisms of insulin secretion from the pancreatic beta cells

Fig. 1 shows the mechanisms of insulin release stimulated by glucose, which is the most potent insulin secretagog. Glucose enters the pancreatic beta cells via glucose transporters. In the cytosol, glucose is phosphorylated by glucokinase to glucose-6-phosphate, which is the rate-limiting step for glucose metabolism in beta cells. Increases in the cytosolic ATP concentration and/or the ATP/ADP ratio induce closure of K\textsubscript{ATP} channels, a determinant of the membrane potential of pancreatic beta cells. Diminished K\textsuperscript{+} outflow due to
closure of $K_{ATP}$ channels causes membrane depolarization and opening of voltage-dependent $Ca^{2+}$ channels, which is followed by $Ca^{2+}$ influx. Finally, elevation of cytosolic free $Ca^{2+}$ concentrations ([Ca$^{2+}$]) beneath the plasma membrane immediately promotes fusion of the beta granule and the plasma membrane. The pairing of the membrane-soluble $N$-ethylmaleimide-sensitive factor (t-SNARE) with the vesicle membrane SNARE (v-SNARE) occurs as in the nerve endings upon excitation. Studies of the detailed molecular mechanism by which $Ca^{2+}$ stimulates insulin exocytosis are currently in progress.

In addition, glucose shows insulinotropic actions independent of its effects on $K_{ATP}$ channels [14]. The existence of this glucose action was demonstrated by the strong stimulation of insulin release by glucose even when $K_{ATP}$ channels are pharmacologically clamped either open or closed [15-17]. That is, glucose stimulates insulin release in the presence of diazoxide or glibenclamide, which open and close $K_{ATP}$ channels, respectively. Insulin release in response to a depolarizing concentration of $K^+$ is augmented in the case of $K_{ATP}$ channel-independent glucose actions. The $K_{ATP}$ channel-independent insulinotropic action of glucose is concentration-dependent with the curve shifted slightly to the left compared to glucose-stimulated insulin release under regular conditions [19]. This suggests that the metabolic threshold is lower for $K_{ATP}$ channel-independent vs. $K_{ATP}$ channel-dependent actions of glucose. Importantly, elevation of [Ca$^{2+}$] is not mandatory for the $K_{ATP}$-independent glucose action event to trigger insulin exocytosis, at least in the experimental setting, because the physiological range of glucose elicits robust insulin release without any elevation of [Ca$^{2+}$] under stringent Ca$^{2+}$-free conditions given an activator of protein kinase C (PKC), and an activator of adenylyl cyclase is present [20, 21]. Time-dependent potentiation of beta cells by glucose, which primes the beta cell secretory machinery for subsequent stimulation, occurs in a $K_{ATP}$-independent and Ca$^{2+}$-independent manner [22]. The molecular basis for the $K_{ATP}$ channel-independent action of glucose, which encompasses Ca$^{2+}$-independent action, is unclear. ATP [23], GTP [24, 25], malonyl-CoA [26-28], protein acylation [29, 30], and NADPH [31] have been proposed as candidate mediators of $K_{ATP}$ channel-independent glucose action. The recently identified glucoreceptors in beta cells may also be involved [32].

The incretins are a group of hormones secreted in the intestine upon meal intake, and have long been recognized as physiological enhancers of insulin release [33]. Glucose-dependent insulinotropic polypeptide (GIP), which is secreted from K cells in the upper small intestine, and GLP-1, which is secreted from L cells in the lower small intestine and large intestine, both stimulate insulin release. Incretins bind to specific heterotrimetric membrane receptors in beta cells, resulting in activation of adenylyl cyclase and increased cellular cAMP levels. Enhancement of insulin release by cAMP is shown schematically in Fig. 1. cAMP enhances insulin release in protein kinase A (PKA)-dependent and -independent manners [34-36]. More than 10 years ago, we found that cAMP enhances glucose-stimulated insulin release but not Ca$^{2+}$-stimulated insulin release per se if ambient glucose concentration is substimulatory [37]. Forskolin, an activator of adenylyl cyclase, and GLP-1 fail to augment insulin release elicited by a depolarizing concentration of K$^+$ in the absence of glucose [37]. As suggested by this finding, cAMP strongly augments the $K_{ATP}$ channel-independent, Ca$^{2+}$-independent, insulinotropic actions of glucose (Fig. 1) [37, 38]. The interactions of PKC, a mediator of acetylcholine-induced insulin release, and Ca$^{2+}$ signals are different in that PKC activators robustly augment Ca$^{2+}$-stimulated insulin release even in the absence of glucose, suggesting a direct interaction. Taken together, these observations refute the simplistic view that Ca$^{2+}$ elevation triggers insulin exocytosis and that cAMP has a direct enhancing effect.

3. Glucose-incretin interaction

In view of stimulus-secretion coupling of the pancreatic beta cells, the recent clinical observation of a combinatorial effect of DPP4 inhibitor and SU in patients with T2D is intriguing. Addition of DPP4 inhibitor not only effectively improves glucose control in a significant number of diabetic patients with poor glycemic control with maximum SU dose, but also induced a substantial number of severe hypoglycemic episodes. The effectiveness of all antidiabetic agents decreases over time. For SUs, this phenomenon is referred to as “secondary failure” [39].
The synergism between SU and DPP4 inhibitor, i.e., Ca^{2+} and cAMP signals, in the presence of excessive hyperglycemia is notable, because hypoglycemia was practically absent with DPP4 inhibitor monotherapy in Caucasian patients. The synergism between SU and DPP4 inhibitor, i.e., Ca^{2+} and cAMP signals, is likely to be involved in the synergism between SU and cAMP under physiological conditions.

4. Perspectives

Physiological insulin secretion in vivo is coordinately regulated by a number of stimulatory, modulatory, and inhibitory factors. Among them, glucose is the most important regulator of insulin secretion. Plasma glucose concentration changes gradually and never fluctuates abruptly under physiological conditions in contrast to the changes seen under experimental conditions. An important feature of the regulation of insulin secretion is the interaction between glucose and non-glucose fuels, such as amino acids and fatty acids, incretin hormones, and neurotransmitters, all of which show gradual increases and decreases in concentration. In insulin release experiments, the extracellular Ca^{2+} concentration is usually set to between 2 and 2.5 mM, which is necessary to reliably demonstrate
glucose-stimulated insulin secretion in vitro. However, this is almost double the physiological plasma concentration of Ca^{2+}. Therefore, caution is required in interpreting and extrapolating these in vitro results into humans. The physiological relevance of various mechanisms leading to insulin exocytosis may be overlooked or exaggerated.

Stimulus-secretion coupling in pancreatic beta cells has not been fully elucidated. In particular, the mechanism of glucose-stimulated insulin release is only partially understood. The K_{ATP} channel-dependent pathway has been relatively well characterized, while K_{ATP} channel-independent pathways are unclear. Clinical application of DPP4 inhibitor and GLP-1 mimetics reinforced the importance of K_{ATP} channel-independent glucose action. To implement safe and effective pharmacological treatment for insulin deficiency and to further explore novel targets of antidiabetic agents in pancreatic beta cells, it will be essential to determine the molecular basis of K_{ATP} channel-independent glucose action.

Acknowledgments

We thank Dr. Toru Aizawa for helpful discussion. This work was supported by a Grant-in-Aid for Mitsuhisa Komatsu from the Ministry of Education, Culture, Sports, Science and Technology, Japan (No. 21591128).

References

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