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 (K_{ATP} channels) and opening of L-type voltage-dependent Ca^{2+} channels. The resultant elevation of cytosolic free Ca^{2+} triggers insulin exocytosis. This is termed the "K_{ATP}-dependent pathway" and is shared by sulfonylurea, which closes K_{ATP} channels. Glucose also stimulates insulin release independent of its action on K_{ATP} channels. This is referred to as the "K_{ATP}-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 Ca^{2+}-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 K_{ATP}-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 K_{ATP} 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-

Glucose

Incretin hormones (GLP-1, GIP)

GLUT

Gsa

AC

ATP

metabolism

cAMP

K_{ATP} channel

ATP production

K_{ATP} channel-independent pathway

Depolarization

Ca^{2+}

$\uparrow$ $[Ca^{2+}]_i$
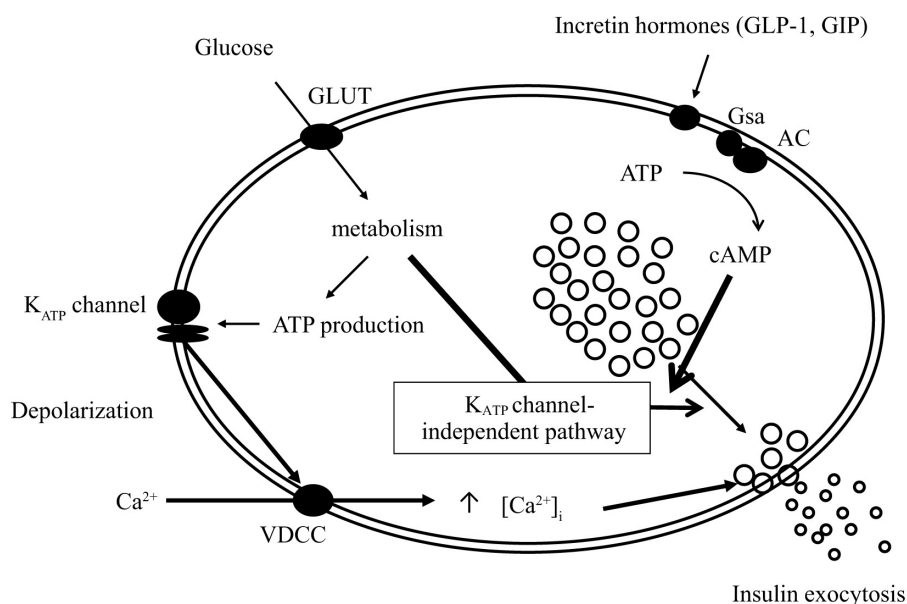
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Insulin exocytosis

Fig. 1 Stimulus-secretion coupling in pancreatic beta cells. Glucose stimulates insulin release *via* K_{ATP} channel-dependent and -independent pathways. The molecular basis for the latter pathways are largely unknown, and include several distinct mechanisms. Incretin hormones upregulate cAMP and enhance K_{ATP} channel-independent glucose action. GLP-1, glucagon-like peptide-1; GIP, glucose-dependent insulinotropic polypeptide; GLUT, glucose transporter; K_{ATP} channel, ATP-sensitive K^+ channel; $[Ca^{2+}]_i$, cytosolic free Ca^{2+} concentration.

ment of pancreatic beta cell function in the spectrum of prediabetic to diabetic range has been confirmed in obese and non-obese Caucasians [10, 11]. Defects in insulin secretion have a primary role in the evolution of T2D and are strongly enhanced by reduced insulin sensitivity [12].

Three different modalities are available for treating insulin deficiency, which can be used as monotherapy or in various combinations. First, sulfonylurea (SU) and glinide stimulate insulin release by closure of ATP-sensitive K^+ channels (K_{ATP} channels). Second, dipeptidyl peptidase-4 (DPP4) inhibitor and glucagon-like peptide-1 (GLP-1) mimetic enhance insulin release by increasing cAMP levels in the beta cells. Finally, insulin injection can be used to directly supply insulin subcutaneously. SU stimulates insulin release from the pancreatic beta cells, thereby compensating for deficient insulin secretion and reduced plasma glucose levels. This class of antidiabetic agent has been used for over 50 years and there is a great deal of evidence for its efficacy in the treatment of T2D [13]. Glinide also stimulates insulin release via a similar mechanism with a rapid onset and short biological half-life, and so is especially suitable for controlling postprandial hyperglycemia. DPP4 inhibitor and incretin analogs, a new 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. Glucose-6-phosphate is subsequently metabolized by a cascade of reactions resulting in the production of pyruvate, which is transported into the mitochondria and subjected to further metabolism. ATP is produced as a result of mitochondrial metabolism. Increases in the cytosolic ATP concentration and/or the ATP/ADP ratio induce closure of K_{ATP} channels, a determinant of the membrane potential of pancreatic beta cells. Diminished K^+ 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 ($[Ca^{2+}]_i$) 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 diazoxide experiment [16, 17]. A stimulatory concentration of glucose does not cause elevation of $[Ca^{2+}]_i$ in the presence of diazoxide [18] or glibenclamide [15], because the K_{ATP} channels are kept open or closed, respectively. These were designated as 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 $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$ 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 acy-

lation [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 heterotrimeric 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]. Beta
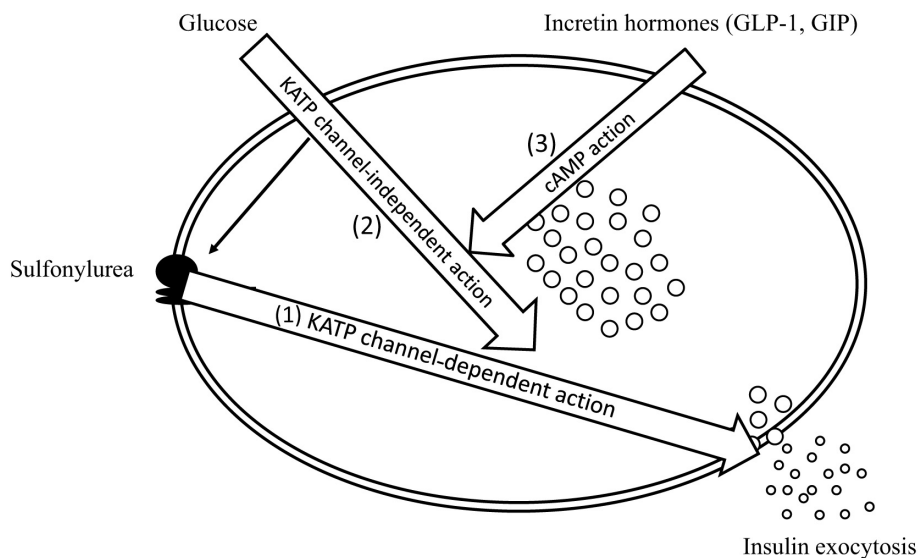
Fig. 2 Interactions among three major pathways leading to insulin exocytosis.

cell function has been considered profoundly impaired in an irreversible manner in patients with SU secondary failure. Additional administration of DPP4 inhibitor in such patients was expected to have little if any plasma glucose lowering effect. Unexpectedly, however, addition of DPP4 inhibitor to SU often resulted in significant improvement of hyperglycemia and even apparent hypoglycemia in some patients. Interestingly, addition of DPP4 inhibitor to SU did not produce as marked an effect in Caucasian patients. 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 Japanese T2D patients. As described above, SU and DPP4 inhibitor increase insulin release with distinct mechanisms in stimulus-secretion coupling. Fig. 2 illustrates three major pathways leading to insulin exocytosis. With SU "secondary failure," the beta cell is under tonic stimulation with excessive hyperglycemia and K_{ATP} channel closure, which is induced by a large amount of SU. It should be noted that SU simply binds to SUR1, a K_{ATP} channel subunit, leading to closure of Kir6.2, the counterpart of the channel; this process is not altered by hyperglycemia (Fig. 2 (1)). Thus, SU secondary failure is not associated with insufficient elevation of $[Ca^{2+}]_i$, but is associated with failure of glucose to enhance Ca^{2+}-stimulated insulin release in a K_{ATP} channel-independent manner (Fig. 2 (2)). In this situation, eleva-

tion of cellular cAMP by DPP4 inhibitor may restore the K_{ATP} channel-independent enhancement of Ca^{2+}-stimulated insulin release (Fig. 2 (3)). We constructed this hypothesis based on circumstantial evidence, as mentioned above, and further studies are required for verification. Recently, Epac2 was proposed as a common molecular target of SU and cAMP [40, 41]. However, as SU-evoked insulin secretion is completely dependent on elevation of $[Ca^{2+}]_i$, Epac2 is unlikely 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

References

1. Saad MF, Knowler WC, Pettitt DJ, Nelson RG, Charles MA, Bennett PH (1991) A two-step model for development of non-insulin-dependent diabetes. *Am J Med* 90(2): 229-235.
2. Kadowaki T, Miyake Y, Hagura R, Akanuma Y, Kajinuma H, Kuzuya N, Takaku F, Kosaka K (1984) Risk factors for worsening to diabetes in subjects with impaired glucose tolerance. *Diabetologia* 26(1): 44-49.
3. Weyer C, Bogardus C, Mott DM, Pratley RE (1999) The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J Clin Invest* 104(6): 787-794.
4. Taniguchi A, Nakai Y, Fukushima M, Kawamura H, Imura H, Nagata I, Tokuyama K (1992)Pathogenic factors responsible for glucose intolerance in patients with NIDDM. *Diabetes* 41(12): 1540-1546.
5. Sato Y, Komatsu M, Katakura M, Ohfusa H, Yamada S, Yamauchi K,Hiramatsu K, Ichikawa K, Aizawa T, Hashizume K (2002) Diminution of early insulin response to glucose in subjects with normal but minimally elevated fasting plasma glucose. Evidence for early beta-cell dysfunction. *Diabet Med* 19(7): 566-571.
6. Mitrakou A, Kelley D, Mokan M, Veneman T, Pangburn T, Reilly J, Gerich J (1992) Role of reduced suppression of glucose production and diminished early insulin release in impaired glucose tolerance. *N Engl J Med* 326(1): 22-29.
7. Ferrannini E, Gastaldelli A, Miyazaki Y, Matsuda M, Pettiti M, Natali A, Mari A, DeFronzo RA (2003) Predominant role of reduced beta-cell sensitivity to glucose over insulin resistance in impaired glucose tolerance. *Diabetologia* 46(9): 1211-1219.
8. Goldfine AB, Bouche C, Parker RA, Kim C, Kerivan A, Soeldner JS, Martin BC, Warram JH, Kahn CR (2003) Insulin resistance is a poor predictor of type 2 diabetes in individuals with no family history of disease. *Proc Natl Acad Sci U S A* 100(5): 2724-2729.
9. Weyer C, Tataranni PA, Bogardus C, Pratley RE (2001) Insulin resistance and insulin secretory dysfunction are independent predictors of worsening of glucose tolerance during each stage of type 2 diabetes development. *Diabetes Care* 24(1): 89-94.
10. Defronzo RA. Banting Lecture (2009) From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes* 58(4): 773-795.
11. Tabak AG, Jokela M, Akbaraly TN, Brunner EJ, Kivimaki M, Witte DR (2009) Trajectories of glycaemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: an analysis from the Whitehall II study. *Lancet* 373(9682): 2215-2221.
12. Katakura M, Komatsu M, Sato Y, Hashizume K, Aizawa T (2004) Primacy of beta-cell dysfunction in the development of hyperglycemia: a study in the Japanese general population. *Metabolism* 53(7): 949-953.
13. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA (2008) 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med* 359(15): 1577-1589.
14. Aizawa T, Sato Y, Komatsu M (2002) Importance of nonionic signals for glucose-induced biphasic insulin secretion *Diabetes* 51 Suppl 1: S96-98.
15. Best L, Yates AP, Tomlinson S (1992) Stimulation of insulin secretion by glucose in the absence of diminished potassium (86Rb$^+$) permeability. *Biochem Pharmacol* 43(11): 2483-2485.

16. Gembal M, Gilon P, Henquin JC (1992) Evidence that glucose can control insulin release independently from its action on ATP-sensitive K$^+$ channels in mouse B cells. *J Clin Invest* 89(4): 1288-1295.

17. Sato Y, Aizawa T, Komatsu M, Okada N, Yamada T (1992) Dual functional role of membrane depolarization/Ca^{2+} influx in rat pancreatic B-cell. *Diabetes* 41(4): 438-443.

18. Gembal M, Detimary P, Gilon P, Gao ZY, Henquin JC (1993) Mechanisms by which glucose can control insulin release independently from its action on adenosine triphosphate-sensitive K$^+$ channels in mouse B cells. *J Clin Invest* 91(3): 871-880.

19. Asanuma N, Aizawa T, Sato Y, Schermerhorn T, Komatsu M, Sharp GW, Hashizume K (1997) Two signaling pathways, from the upper glycolytic flux and from the mitochondria, converge to potentiate insulin release. *Endocrinology* 138(2): 751-755.

20. Komatsu M, Schermerhorn T, Aizawa T, Sharp GW (1995) Glucose stimulation of insulin release in the absence of extracellular Ca^{2+} and in the absence of any increase in intracellular Ca^{2+} in rat pancreatic islets. *Proc Natl Acad Sci U S A* 92(23): 10728-10732.

21. Komatsu M, Schermerhorn T, Noda M, Straub SG, Aizawa T, Sharp GW (1997) Augmentation of insulin release by glucose in the absence of extracellular Ca^{2+}: new insights into stimulus-secretion coupling. *Diabetes* 46(12): 1928-1938.

22. Yamada S, Komatsu M, Aizawa T, Sato Y, Yajima H, Yada T, Hashiguchi S, Yamauchi K, Hashizume K (2002) Time-dependent potentiation of the beta-cell is a Ca^{2+}-independent phenomenon. *J Endocrinol* 172(2): 345-354.

23. Sato Y, Henquin JC (1998) The K$^+$-ATP channel-independent pathway of regulation of insulin secretion by glucose: in search of the underlying mechanism. *Diabetes* 47(11): 1713-1721.

24. Komatsu M, Noda M, Sharp GW (1998) Nutrient augmentation of Ca^{2+}-dependent and Ca^{2+}-independent pathways in stimulus-coupling to insulin secretion can be distinguished by their guanosine triphosphate requirements: studies on rat pancreatic islets. *Endocrinology* 139(3): 1172-1183.

25. Metz SA, Meredith M, Rabaglia ME, Kowluru A (1993) Small elevations of glucose concentration redirect and amplify the synthesis of guanosine 5'-triphosphate in rat islets. *J Clin Invest* 92(2): 872-882.

26. Komatsu M, Sharp GW (1998) Palmitate and myristate selectively mimic the effect of glucose in augmenting insulin release in the absence of extracellular Ca^{2+}. *Diabetes* 47(3): 352-357.

27. Komatsu M, Yajima H, Yamada S, Kaneko T, Sato Y, Yamauchi K, Hashizume K, Aizawa T (1999) Augmentation of Ca^{2+}-stimulated insulin release by glucose and long-chain fatty acids in rat pancreatic

islets: free fatty acids mimic ATP-sensitive K$^+$ channel-independent insulinotropic action of glucose. *Diabetes* 48(8): 1543-1549.

28. Prentki M, Vischer S, Glennon MC, Regazzi R, Deeney JT, Corkey BE (1992) Malonyl-CoA and long chain acyl-CoA esters as metabolic coupling factors in nutrient-induced insulin secretion. *J Biol Chem* 267(9): 5802-5810.

29. Yajima H, Komatsu M, Yamada S, Straub SG, Kaneko T, Sato Y, Yamauchi K, Hashizume K, Sharp GW, Aizawa T (2000) Cerulenin, an inhibitor of protein acylation, selectively attenuates nutrient stimulation of insulin release: a study in rat pancreatic islets. *Diabetes* 49(5): 712-717.

30. Yamada S, Komatsu M, Sato Y, Yamauchi K, Aizawa T, Kojima I (2003) Nutrient modulation of palmitoylated 24-kilodalton protein in rat pancreatic islets. *Endocrinology* 144(12): 5232-5241.

31. Huypens P, Pillai R, Sheinin T, Schaefer S, Huang M, Odegaard ML, Ronnebaum SM, Wettig SD, Joseph JW (2011) The dicarboxylate carrier plays a role in mitochondrial malate transport and in the regulation of glucose-stimulated insulin secretion from rat pancreatic beta cells. *Diabetologia* 54(1): 135-145.

32. Nakagawa Y, Nagasawa M, Yamada S, Hara A, Mogami H, Nikolaev VO, Lohse MJ, Shigemura N, Ninomia Y, Kojima I (2009) Sweet taste receptor expressed in pancreatic beta-cells activates the calcium and cyclic AMP signaling systems and stimulates insulin secretion. *PLoS One* 4(4): e5106.

33. Drucker DJ (2006) The biology of incretin hormones. *Cell Metab* 3: 153-165.

34. Ozaki N, Shibasaki T, Kashima Y, Miki T, Takahashi K, Ueno H, Sunaga Y, Yano H, Matsuura Y, Iwanaga T, Takai Y, Seino S (2000) cAMP-GEFII is a direct target of cAMP in regulated exocytosis. *Nat Cell Biol* 2(11): 805-811.

35. Seino S, Shibasaki T (2005) PKA-dependent and PKA-independent pathways for cAMP-regulated exocytosis. *Physiol Rev* 85(4): 1303-1342.

36. Seino S, Takahashi H, Fujimoto W, Shibasaki T (2009) Roles of cAMP signalling in insulin granule exocytosis. *Diabetes Obes Metab* 11: 180-188.

37. Yajima H, Komatsu M, Schermerhorn T, Aizawa T, Kaneko T, Nagai M, Sharp GW, Hashizume K (1999) cAMP enhances insulin secretion by an action on the ATP-sensitive K$^+$ channel-independent pathway of glucose signaling in rat pancreatic islets. *Diabetes* 48(5): 1006-1012.

38. Yamada S, Komatsu M, Sato Y, Yamauchi K, Kojima I, Aizawa T, Hashizume K (2002) Time-dependent stimulation of insulin exocytosis by 3',5'-cyclic adenosine monophosphate in the rat islet beta-cell. *Endocrinology* 143(11): 4203-4209.

39. Matthews DR, Cull CA, Stratton IM, Holman RR,

Turner RC (1998) UKPDS 26: Sulphonylurea failure in non-insulin-dependent diabetic patients over six years. UK Prospective Diabetes Study (UKPDS) Group. *Diabet Med* 15(4): 297-303.

40. Zhang CL, Katoh M, Shibasaki T, Minami K, Sunaga Y, Takahashi H, Yokoi N, Iwasaki M, Miki T, Seino S (2009) The cAMP sensor Epac2 is a direct target of antidiabetic sulfonylurea drugs. *Science* 325(5940): 607-610.

41. Herbst KJ, Coltharp C, Amzel LM, Zhang J (2011) Direct activation of Epac by sulfonylurea is isoform selective. *Chem Biol* 18(2): 243-251.