REVIEWS

THE MECHANISM OF AUTONOMIC NERVOUS SYSTEM IN THE REGULATION OF INSULIN SECRETION

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ABSTRACT

The regulation of insulin secretion is important for the maintenance of normal glucose homeostasis. There are at least four major pathways of the stimulation of insulin secretion that have been defined. The first major pathway is high concentration of glucose which causes inhibition of ATP-sensitive K^+ (K_{ATP}) channel and then depolarization of the plasma membrane. The second pathway is the K_{ATP} channel-independent pathway of glucose action or a distal effect. The third pathway is the activation of phospholipase C-β by pertussis toxin (PTX)-insensitive G protein, resulting in enhancement of the effect on stimulated secretion of agonists such as acetylcholine and cholecystokinin. The fourth pathway is the activation of adenyl cyclase by G_s, thereby activation of protein kinase A (PKA). The activation of PKA causes a small increase of $[Ca^{2+}]_i$ and a large increase of insulin secretion. The autonomic nervous system plays both positive and negative roles in the regulation of insulin secretion from pancreatic β-cells. Acetylcholine activates muscarinic M_3 receptor subtype, which is parasympathetic nervous system, then increases insulin secretion by combined effects of activation of phospholipase C-β and a distal effect independent of a rise of $[Ca^{2+}]_i$. For sympathetic nervous system, activation of $β_2$-adrenergic receptors coupled to G_s enhances insulin secretion, whereas activation of $α_2$-adrenergic receptors coupled to G_i/G_o inhibits insulin secretion. The mechanisms underlying $α_2$-adrenergic agonists inhibit insulin secretion are activation of K_{ATP} channel, inhibition of Ca^{2+}-channel, inhibition of adenylyl cyclase and others.

Key words : insulin, autonomic nervous system, regulation

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กลไกควบคุมการหลั่งฮอร์โมนอินซูลินโดยระบบประสาทอัตโนมัติ

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บทคัดย่อ

การควบคุมการหลั่งฮอร์โมนอินซูลิน มีความสำคัญยิ่งสำหรับการรักษาภาวะสมดุลย์ของน้ำตาลในร่างกาย กลไกสำคัญที่ควบคุมการหลั่งฮอร์โมนอินซูลินมีอย่างน้อย 4 กลไก คือ

1 ระดับความเข้มข้นของน้ำตาลในกระแสเลือดที่เพิ่มสูงขึ้น ทำให้ ATP-sensitive K^+ channel (K_{ATP}) ปิด ส่งผลให้เกิดการเปลี่ยนแปลงความต่างศักย์ เกิด depolarization ของผนังเซลล์ขึ้น

2 กลไกที่ไม่เกี่ยวข้องกับระดับน้ำตาลในกระแสเลือดและ K_{ATP} channel หรือที่เรียกว่า "distal effect"

3 กลไกที่เกี่ยวข้องกับการกระตุ้นเอนไซม์ phospholipase C-β โดยผ่านทาง pertussis toxin (PTX)-insensitive G protein มีผลทำให้เพิ่มฤทธิ์ของสาร agonist บางตัว เช่น acetylcholine และ cholecystokinin ในการกระตุ้นการหลั่งฮอร์โมนอินซูลิน

4 กลไกที่เกี่ยวข้องกับการกระตุ้น adenylyl cyclase โดย G_s ส่งผลกระตุ้น protein kinase A (PKA) ทำให้ระดับแคลเซียมภายในเซลล์เพิ่มสูงขึ้นเล็กน้อย และกระตุ้นการหลั่งฮอร์โมนอินซูลิน

ระบบประสาทอัตโนมัติมีผลทั้งในแง่กระตุ้นและยับยั้งการหลั่งฮอร์โมนอินซูลินจาก β-cells ของตับอ่อน Acetylcholine กระตุ้นตัวรับของระบบประสาทพาราซิมพาเธติกชนิด muscarinic (M_3) ส่งผลกระตุ้นการหลั่งฮอร์โมนอินซูลินได้โดยการกระตุ้นผ่านกลไก phospholipase C-β และ distal effect ซึ่งไม่เกี่ยวข้องกับการเพิ่มขึ้นของระดับแคลเซียมภายในเซลล์ สำหรับระบบประสาทซิมพาเธติก กลไกการกระตุ้นผ่านทาง β_2-adrenergic receptor เพิ่มการหลั่งฮอร์โมนอินซูลิน ในขณะที่กลไกการกระตุ้นผ่านทาง α_2-adrenergic receptor ยับยั้งการหลั่งฮอร์โมนอินซูลิน กลไกที่ α_2-adrenergic receptor ใช้ในการยับยั้งการหลั่งฮอร์โมนอินซูลิน คือ กระตุ้นการเปิด K_{ATP} channel ยับยั้ง Ca^{2+} channel ยับยั้งเอนไซม์ adenylyl cyclase และอื่นๆ

คำสำคัญ : อินซูลิน, ระบบประสาทอัตโนมัติ, การควบคุมการหลั่ง

1. Bl. glucose ↑ → K_{ATP} ปิด → depolarizat → ↑ insulin secret

2. ไม่เกี่ยวกับ bl glucose และ K_{ATP} ที่เรียกว่า distal ef.

3. กระตุ้น PLC-β ผ่าน PTX-insensitive Gpr → ↑ ฤทธิ์ของสาร agonist บางตัว ACh, cholecystokinin → ↑ insulin secret

4. กระตุ้น AC โดย G_s → ↑ PKA → ↑ Ca → ↑ insulin release

INTRODUCTION

The regulation of insulin secretion is important for the maintenance of normal glucose homeostasis. Insulin secretion from the β-cell is influenced by a variety of stimulatory, modulatory, and inhibitory influences [1-2]. The most important stimulator of insulin secretion is glucose. Also, many hormones, neurotransmitters and autacoids can stimulate or inhibit insulin secretion. Therefore, insulin secretion is thought to be regulated by the autonomic nervous system, nutrients, and peptide hormones [3-4]. Glucagon, glucagon-like peptide (GLP), acetylcholine and adrenergic input through the β-adrenergic receptor stimulate insulin secretion; whereas somatostatin, epinephrine/norepinephrine (acting predominantly through the α_2-adrenergic receptor in the islet), PGE_2, pancreastatin, peptide YY, calcitonin gene-related peptide and galanin reduce or completely block insulin secretion. The autonomic nervous system, therefore, has both positive and negative roles in the regulation of insulin secretion from β-cells. All of these agonists induce or reduce insulin secretion through mechanisms dependent on GTP binding proteins (G proteins). Various inhibitors use multiple mechanisms at the cellular level to block the secretion. The regulation of the inhibition of insulin secretion is very complex[5]. However, some of the inhibitory mechanisms simply reverse the stimulatory mechanisms. Therefore, we should understand the mechanisms of the stimulation of insulin secretion before understanding the mechanisms of the inhibition of insulin secretion. Although the details of stimulatory mechanisms have not been fully known yet, four major pathways of the stimulation of insulin secretion have been defined [6-7].

MAJOR PATHWAYS FOR THE STIMULATION OF INSULIN SECRETION

The first major pathway involves depolarization of the β-cell, resulting in increased Ca^{2+} influx via voltage dependent L-type Ca^{2+} channels (VDCC), increased intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), and increased rates of exocytosis. High concentration of glucose causes inhibition of ATP-sensitive K^+ (K_{ATP}) channel and then depolarization of the plasma membrane [8-11].

However, some agonists such as arginine may cause depolarization of the β-cell by the entry of the positively charged amino acid per se, stimulates Ca^{2+} influx through VDCC, increases $[Ca^{2+}]_i$ and thus triggers insulin release [12] without closing of K_{ATP} channels [4].

The second major pathway is the K_{ATP} channel-independent pathway of glucose action or a distal effect. This pathway is so called a distal effect because glucose exerts its effect after the increase of $[Ca^{2+}]_i$ or at a step distal to the generation of secondary messengers, for example protein kinase C (PKC) and adenosine 3', 5'-cyclic monophosphate (cAMP)[13-15]. Although it has been widely assumed that the rise in $[Ca^{2+}]_i$ is required for glucose to exert an effect, it appears that this is not always true. Sharp et al. found that the K_{ATP} channel-independent action of glucose stimulates insulin secretion by acting at a late stage of stimulus-secretion coupling without any requiring increased $[Ca^{2+}]_i$ or its expression[16]. This Ca^{2+}-independent augmentation of secretion by glucose has been observed in Ca^{2+}-depleted pancreatic islets under Ca^{2+}- free conditions and is elicited by simultaneous activation of protein kinase A (PKA) and PKC. Little is known about this mechanism, except that it requires glucose metabolism. However, the underlying mechanism appears to be via the normal process of exocytosis because it is completely blocked by norepinephrine. Moreover, it is clear that this glucose-activated pathway is K_{ATP} channel-independent because neither sulfonylurea (K_{ATP} channel inhibitor) nor diazoxide (a K_{ATP} channel activator) affects the glucose-induced augmentation of insulin release. Therefore, it seems that there are two possibilities for the K_{ATP} channel-independent pathway. One is that glucose exerts its effect only in the presence of activated PKC or strongly in the presence of activated PKC and PKA. Hence, PKC and PKA would be permissive for the effect of glucose. Another possibility is that the simultaneous activation of PKC and PKA mimics the effect of elevated $[Ca^{2+}]_i$. If this is the case, this pathway should be the same mechanism underlying the K_{ATP} channel-independent effect of glucose that is thought to require an increase of $[Ca^{2+}]_i$. Regardless of its mechanism, this glucose-activated pathway exerts its effect at the distal site in stimulus-secretion coupling. In addition to this distal effect of glucose, other agonists have distal action to modulate insulin secretion, which

$ACh - M_3 - R \longrightarrow PLC - \beta \longrightarrow (+)$ insulin secret?

$\beta_2 - R \quad (+)$

$\alpha_2 - R \quad (-)$ [ลดการทำงานของ K_{ATP}, เพิ่ม Ca channel, inh AC

includes carbachol[17], epinephrine[18], and norepinephrine[19].

The third pathway is the activation of phospholipase C-β by pertussis toxin (PTX)-insensitive G proteins, which increases phosphoinositide (PI) turnover, thereby increases production of inositol trisphosphate (IP$_3$) and diacylglycerol (DAG)[4]. IP$_3$ mobilizes Ca^{2+} from the endoplasmic reticulum to increase [Ca^{2+}]$_i$[4] and DAG activates PKC, enhancing the effect on stimulated secretion of agonists such as acetylcholine and cholecystokinin.

The fourth pathway is the activation of adenyl cyclase by G$_s$, a cholera toxin-sensitive G protein, which causes a rise in cAMP, and then activates PKA. The activation of PKA causes a small increase of [Ca^{2+}]$_i$ and a large increase of insulin secretion. These four signaling pathways are shown in figure 1. There are several mechanisms for inhibition of stimulated insulin release as well. The proximal mechanisms, include activation of the K$_{ATP}$ channels and consequent repolarization of the membrane, direct inhibition of L-type Ca^{2+} channels, inhibition of PI hydrolysis and DAG production, and inhibition of adenylyl cyclase activity. The last pathway is the distal effect that would inhibit the final common pathway in stimulus-secretion coupling beyond the site of action of elevated [Ca^{2+}]$_i$ and beyond the generation of secondary messengers[4]. This mechanism is still not well understood. The inhibition of insulin release by norepinephrine, soma-tostatin, galanin and prostaglandins is blocked by treatment with PTX, suggesting that this effect is mediated by PTX-sensitive G protein-linked receptors.

PARASYMPATHETIC REGULATION OF INSULIN SECRETION

Islets of Langerhans are innervated by parasympathetic and sympathetic nervous systems. It is clear that autonomic activity has an important modulating effect on the insulin secretion. Stimulation of vagal nerve fibers or the administration of acetylcholine enhances insulin secretion from pancreatic β-cells. These effects are abolished by atropine, suggesting that they are mediated by muscarinic receptors. In addition, the slight but significant impairment of glucose-induced insulin secretion has been observed after vagotomy in the fasted rat [20]. The sight, smell and taste of food can stimulate insulin secretion through activation of parasympa-

thetic and inhibition of sympathetic nervous system [21].

The subtypes of muscarinic receptor in pancreatic islets is M$_3$ [22]. Activation of muscarinic receptors couples to G$_{q/11}$, a PTX-insensitive G protein, which stimulates phospholipase C-β. The activation of PKC may potentiate the stimulation of insulin secretion by sensitizing the secretory apparatus to Ca^{2+}. It is clear that muscarinic receptor activation stimulates insulin secretion by the combined effects of activated phospholipase C-β to increase DAG and to mobilize Ca^{2+}. Since inhibition of PKC reduces or abolishes muscarinic effect on insulin secretion in islet β-cells [23,24] and in clonal hamster β-cell line (HIT) [25]. The elevation of [Ca^{2+}]$_i$ and activation of PKC act in synergy to bring about the full physiological response of β-cells to muscarinic activation. However, Sharp et al. found that activation of muscarinic receptors of an insulin secreting clonal β-cell line (RINm5F) stimulated insulin secretion by the mechanism that was independent of the rise in [Ca^{2+}]$_i$ and independent of the activation of PKC. This suggested that the stimulation of insulin secretion by muscarinic activation in these cells is also exerted at the distal stage in stimulus-secretion coupling, a stage that is independent of a rise of [Ca^{2+}]$_i$ [17].

SYMPATHETIC REGULATION OF INSULIN SECRETION

The sympathetic nervous system plays both positive and negative roles in the regulation of insulin secretion. Both α- and β-adrenergic receptors have been demonstrated in pancreatic islets. Activation of β_2-adrenergic receptors enhances insulin secretion, whereas activation of α_2-adrenergic receptors inhibits insulin secretion. For β_2-adrenergic receptors, activation of these receptors couples to G$_s$, a cholera toxin-sensitive G protein, stimulates adenylyl cyclase, thus causing an increase in cAMP and activation of PKA. The activated PKA causes a small augmentation of [Ca^{2+}]$_i$ and a large increase of insulin secretion [4]. Epinephrine and norepinephrine have important influences on metabolic processes. They decrease the uptake of glucose by peripheral tissues because of the effect on insulin secretion. Stimulation of the sympathetic nervous system attenuates insulin secretion from β-cells, unless α_2-adrenergic receptors in the pancreas are blocked. Also,α_2-adrenergic receptor antagonists alone on both
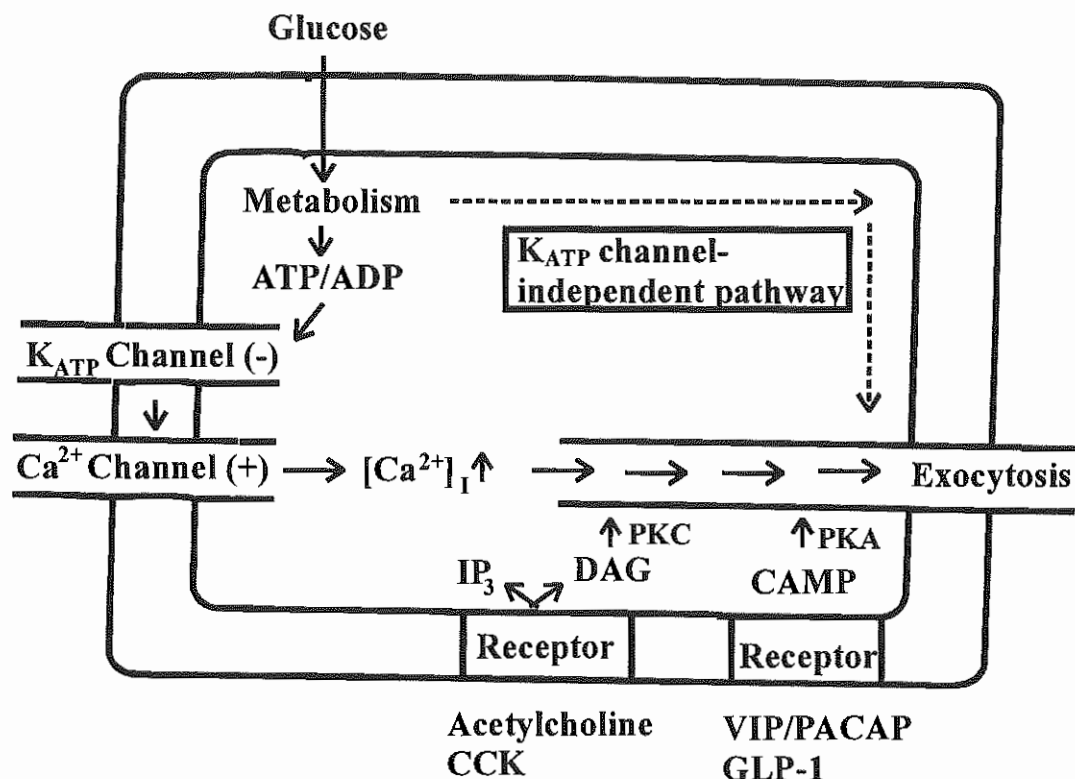
Figure 1. Four major pathways involved in stimulation of insulin release. Shown is K_{ATP} channel-dependent pathway in which increased blood glucose concentrations and consequent increased β-cell metabolism result in a change in intracellular ATP to ADP ratio.   This is thought to be a contributory factor in closure of ATP-dependent K^+ channels, depolarization of β-cell membrane, and increased L-type channel activity.   Increased channel activity and increased Ca^{2+} influx result in increased intracellular Ca^{2+} and stimulated insulin release. Also shown is important K_{ATP} channel-independent pathway that augments Ca^{2+}-stimulated insulin release of K_{ATP} channel-dependent pathway. Major potentiation of release results from hormonal and peptidergic activation of receptors positively linked to adenylyl cyclase, for example, vasoactive intestinal peptide (VIP), pituitary adenylyl cyclase-activating peptide (PACAP), and glucagon-like peptide 1 (GLP-1). Occupation of these receptors by the hormones results in activation of the enzyme, increased cAMP levels, and potentiation of release by 2 mechanisms. These are activation of protein kinase A (PKA) and phosphorylation of the L-type Ca^{2+} channel to increase Ca^{2+} entry and phosphorylation at an as yet unknown distal site in stimulus-secretion coupling to enhance stimulated release. A second potentiating mechanism is due to activation of receptors linked to phospholipase C, increased phosphoinositide hydrolysis, and increased production of inositol triphosphate (IP_3) and diacylglycerol (DAG). As is the case for adenylyl cyclase stimulation, the 2 products of phospholipase C activity result in increased $[Ca^{2+}]_i$ and potentiation of insulin release by protein kinase C (PKC) activation and phosphorylation of another as yet unknown distal site in stimulus secretion coupling (Modified from Sharp GWG, 1996).

α-and β-adrenoceptors, but the increase insulin secretion. Catecholamines act predominant effect seen with epinephrine and norepinephrine on insulin secretion is inhibition. This effect has been found both in perfused pancreas and in isolated islets of Langerhans, suggesting that circulating levels of epinephrine or norepinephrine from sympathetic nerve terminals play an important role in the physiological regulation of insulin secretion[19]. These agonists act on α_2-adrenergic receptor in β-cells, coupled to G_i/G_o, PTX-sensitive G proteins, leading to inhibition of insulin secretion via many mechanisms that are described as follows [5].

K_{ATP} CHANNEL ACTIVATION

One α_2-adrenergic mechanism is to decrease the β-cell membrane potential. The α $_2$-adrenergic agonists activate the K_{ATP} channel, hyperporalize the membrane, and thus inhibit the insulin secretion and also inhibit the action of all secretagogues that act to depolarize the β-cell via the inhibition of this channel[4]. However, the effect of these inhibitors via this mechanism is frequently not of great magnitude and is often transient, and fails to completely reverse the depolarizing effect of glucose[26,27]. Therefore, the significance of α_2-adrenergic receptor mediated membrane repolarization in β-cells for the inhibition of insulin secretion is still unclear.

CA^{2+} CHANNEL INHIBITION

The second action of α_2-adrenergic agonists is the direct inhibition of voltage-dependent L-type Ca^{2+} channels. α_2-adrenergic agonists also inhibit the effect of all secretagogues that act to stimulate Ca^{2+} influx through VDCC by closing K_{ATP} channels.

INHIBITION OF ADENYLYL CYCLASE

The third mechanism of norepinephrine/epinephrine is to inhibit the activity of adenylyl cyclase, thereby reducing cAMP levels in the pancreatic β-cells[28,29], consequently inhibiting insulin secretion. The action of these inhibitors also decreases the effect of other secretagogues, for example glucagon and glucose, that stimulate insulin secretion via activation of adenylyl cyclase[30]. Inhibition of exocytosis in electrically permeabilized islets bathed with high concentration of Ca^{2+}, norepinephrine inhibited

Ca^{2+}-induced insulin secretion with similar efficacy to that of the inhibition of glucose-induced insulin secretion from intact islets[18,19]. This inhibition of Ca^{2+}-induced insulin secretion from permeabilized islets was blocked by the α_2-adrenergic blocker, yohimbine, but was not reversed by cyclic AMP (cAMP). Moreover, Ca^{2+}-independent stimulation of insulin secretion by glucose, mediated via distal sites, was totally blocked by norepinephrine. It is likely that the inhibitory action of the α_2-adrenergic agonist occurs late in stimulus-secretion coupling, for example, after the elevation of $[Ca^{2+}]_i$ or after the generation of secondary messengers and is referred to as the distal or late effect. This distal inhibitory action is mediated via PTX-sensitive G proteins, G_i/G_o.

Because of little information about the nature of the distal inhibitory effect, little is known about the mechanism involved in the late stages of stimulus-secretion coupling and exocytosis. However, by using the information gained in yeast and neuronal system as the basis for studies on the pancreatic exocytosis, it is now possible to see an outline of the distal steps as followings.

1. After activation of β-cells, the granules translocate to the plasma membrane.

2. After translocation, the granules must dock.

3. The granules are then primed.

4. The granule membrane fuses with the plasma membrane and exocytosis.

After the fourth step, the endocytosis must follow exocytosis to complete the cycle and allow exocytosis to occur again for lengthy periods. Nevertheless, there is the evidence supporting that exocytosis in the β-cell is analogous to that in the yeast and in the neuronal synapse, and that will be consistent with synaptosome-associated protein (SNAP) receptor (SNARE) hypothesis[31-33]. The complex of vesicle-associated membrane protein (VAMP; also called synaptobrevin), syntaxin, and SNAP-25 (synaptosome-associated protein of relative molecular weight 25,000) is the receptor for SNAP, the soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein. The translocation step appears to involve low-molecular weight G proteins of the Rab family. Docking involves formation of a core complex including syntaxin and SNAP-25 from the plasma membrane, the target (t)-SNARE, and a VAMP/synaptobrevin, the vesicle (v)-SNARE. Synaptotagmin on the granule is a Ca^{2+} sensor related in priming-fusion process. Finally, the
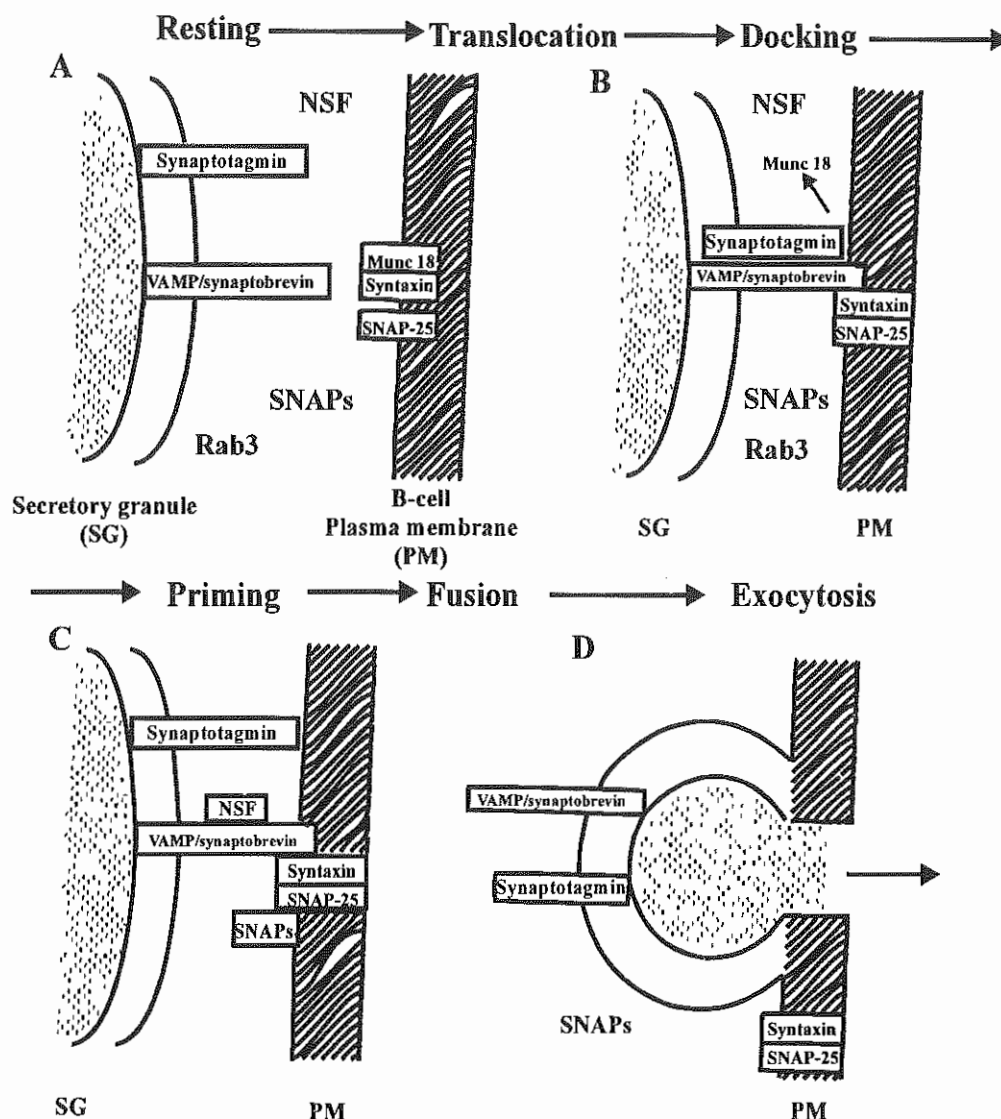
Figure 2. Scheme for distal steps in stimulus-secretion coupling and exocytosis in pancreatic β-cell. Shown are some of the components necessary for exocytosis and events that are thought to occur during final stages of stimulus secretion coupling and exocytosis. In basal or resting state (A, left) a secretory granule (SG) is shown in apposition to β-cell plasma membrane (PM). Some of the components necessary for exocytosis, which have been identified in the β-cell, are shown. After translocation to the PM, a process that may involve the Rab3 GTP-binding proteins, vesicle-associated membrane protein (VAMP)/synaptobrevin (the v-SNARE) in the granule membrane can bind to syntaxin and synaptosome-associated protein of relative mol. Wt. 25,000 (SNAP-25; the 2 making up the t-SNARE) in the plasma membrane (B). As the mammalian homologue of the *Caenorhabditis elegans unc*-18 gene (Munc 18) inhibits the binding of the v- and t-SNARE, binding or docking requires the prior dissociation of Munc 18. Synaptotagmin association with the complex is also thought to be inhibitory, and dissociation of synaptotagmin from the complex is necessary so the system can be "primed". Subsequently, *N*-ethylmaleimide-sensitive factor (NSF) and soluble NSF attachment protein (SNAP) complete the formation of the fusion complex (C), which leads finally to exocytosis (D). (Modified from Sharp GWG, 1996).

completed core complex associates with NSF, SNAP, and perhaps other players to promote fusion and exocytosis.

The above information and figure 2 provide the document for illustrating the potential sites for the distal inhibitory effect. For instance, Munc 18 inhibits the binding of syntax and SNAP-25, so that the inhibitor may act to prevent the dissociation of Munc 18 at the docking stage. In addition, synaptotagmin is bound to VAMP/-synaptobrevin and inhibits its action on the SNAP-25 and syntaxin complex. Therefore, any inhibitor that acts to prevent the dissociation of synaptotagmin from this complex would inhibit exocytosis. However, there are many potential targets for inhibitor's action at this stage, so that further studies in this area are warranted [4].

OTHER POSSIBLE MECHANISMS

Norepinephrine and epinephrine may inhibit insulin secretion from the pancreatic β-cells via other possible mechanisms, such as inhibition of glucose metabolism, activation of sulfonylurea-insensitive low-conductance K^+ channels, inhibition of fatty acid metabolism, elevation of guanosine 3',5'-cyclic mono-phosphate (cGMP) and increase in F-actin content[4]. Actin microfilaments inhibit the access of secretory granules to the plasma membrane before exocytosis. Norepinephrine increases F-actin contents, thereby causing the exocytosis more difficult to occur.

In addition, epinephrine may have an inhibitory action on insulin synthesis. Zhang et al found that epinephrine not only decreased insulin secretion but also decreased levels of insulin mRNA and intracellular insulin content and this effect was prevented by PTX [34]. Also, another study found that epinephrine inhibited HIT-T15 cell expressions of a reporter gene

(gene whose products are easily detected) in a concentration-dependent manner that paralleled the inhibition of insulin secretion when they were transfected with the reporter gene driven by the human insulin gene 5'-regulatory sequence (promoters). This finding suggested that epinephrine may inhibit insulin secretion by decreasing the rate of insulin gene transcription [35]. However the mechanisms by which epinephrine inhibits gene transcription are not clear. Therefore, further studies are needed to clarify all the above statements [1]. The regulation of insulin secretion is of most importance in the maintenance of normal glucose homeostasis. Although glucose is the major stimulator of insulin secretion, autonomic nervous system can modulate the effect of glucose-induced insulin secretion from pancreatic β-cell. Moreover, the direct stimulation of parasympathetic and sympathetic nerve fibers can enhance and diminish insulin secretion, respectively. Under a stress, serum catecholamine concentrations are elevated or the sympathetic nervous system is activated, thereby decrease insulin secretion. Also, elevated catecholamine levels enhance glycogenolysis, thus increasing blood glucose that is the primary fuel for the brain and other critical organs. It is the mechanism underlying the fighting reflex of body under stress or pressure condition. This would be beneficial to the body system, if it occurs in short term or in emergency condition. In long term stress condition, however, it may contributes to the impairment of insulin secretion or glucose intolerance in type II diabetes mellitus or stress-induced glucose intolerance in non-diabetic persons and animals. Therefore, the understanding of the regulation of insulin secretion by autonomic nervous system is invaluable for both physiological and pathological views.

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