
Perspectives in Diabetes

G Proteins and Modulation of Insulin Secretion

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Guanine nucleotide-binding proteins (G proteins) are critically important mediators of many signal-transduction systems. Several important sites regulating stimulus-secretion coupling and release of insulin from pancreatic β -cells are modulated by G proteins. G_s mediates increases in intracellular cAMP associated with hormone-induced stimulation of insulin secretion. G_i mediates decreases in intracellular cAMP caused by inhibitors of insulin secretion, e.g., epinephrine, somatostatin, prostaglandin E_2 , and galanin. G proteins also regulate ion channels, phospholipases, and distal sites in exocytosis. Cholera and pertussis toxins irreversibly ADP ribosylate G proteins and are important tools that can be used both to manipulate G-protein-dependent modulators of insulin secretion and detect and quantify G proteins by electrophoretic techniques. The stage is set to pursue these initial observations in greater depth and ascertain whether G-protein research will provide important new insights into normal and abnormal regulation of insulin secretion. *Diabetes* 40:1-6, 1991

Guanine nucleotide-binding proteins (G proteins) are essential to current concepts of stimulus-secretion coupling in the pancreatic islet. They link the receptors of many hormones to signal-transduction systems, e.g., adenylylcyclase, ion channels, phospholipases, and distal sites in exocytosis in the pancreatic islet (for review, see refs. 1 and 2). Hormones and autacoids that mediate their effects on islet cell function through G proteins include epinephrine, somatostatin, prostaglandin E_2 , and galanin. In this perspective, we present a brief history

of the development of G-protein research, describe mechanisms of action for G proteins and cholera and pertussis toxins, and speculate how answers to unresolved questions about G proteins might provide us with deeper insights into pancreatic islet physiology and pathophysiology.

HISTORY OF G PROTEINS

The first clue that G proteins would be important in mediating hormonal action was provided in the 1970s by reports that GTP was required for hormonal stimulation of intracellular cAMP production (3). That GTP interacted with an as yet unidentified plasma membrane protein was demonstrated by several groups (4). The studies largely responsible for the identification and characterization of this protein relied on the variant of S49 lymphoma cells, termed *cyc⁻*. The *cyc⁻* variant was so named because it could not be stimulated by the usual activators of adenylylcyclase (5). Reconstitution studies combining membrane proteins of *cyc⁻* and wild-type S49 cells revealed that plasma membranes of the normal wild-type cells contained a detergent-soluble protein that could correct the defect in the *cyc⁻* cell and thereby allow it to undergo hormonal stimulation of cAMP synthesis (6). This new protein, which had properties distinguishable from receptors and adenylylcyclase and was present in normal cells but missing in the *cyc⁻* cell, was initially termed the *G/F subunit*, later referred to as N_s , and now named G_s (G for guanine nucleotide binding, s for stimulatory). An important finding was that G_s was a substrate for ADP ribosylation by the bacterial toxin cholera toxin (7).

At roughly the same time, the first clues that another new protein had the opposite function, i.e., negative modulation of adenylylcyclase activity, were reported. Using pancreatic islets, Katada and Ui (8,9) discovered that pertussis toxin interfered with the mechanism whereby epinephrine inhibits insulin secretion. It was later learned that pertussis toxin specifically modulates the inhibition of adenylylcyclase (10) and catalyzes the ADP ribosylation of a membrane protein (11). The protein ADP ribosylated by pertussis toxin was also shown to be a G protein (12), and because it coupled hormone binding to inhibition of adenylylcyclase activity, it was

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termed G_i (i for inhibitory). Since then, several pertussis toxin substrates have been discovered (1,2). These include three subtypes of G_i (termed G_{i1} , G_{i2} , and G_{i3}) and two forms of G_o (o for other). In addition to inhibition of adenylylcyclase, pertussis toxin substrates appear to be involved in the regulation of K^+ and Ca^{2+} channels and phospholipases C and A_2 (13). There are nine separate genes that regulate the synthesis of 12 separate G proteins, with the probability that more will be uncovered in the future (1).

GENERAL MECHANISM OF G-PROTEIN ACTION

All G proteins are heterotrimers composed of three subunits named α , β , and γ in order of decreasing molecular size (1,2). The β - and γ -subunits are exchangeable among various G proteins, but α -subunits are specific for individual G proteins. α -Subunits bind GDP and GTP. The favored theory explaining activation of G proteins involves a critical exchange of GTP for GDP on the α -subunit catalyzed by the interaction of the heterotrimeric G protein with the hormone-receptor complex (Fig. 1) (1). After association of the hormone-receptor complex with the heterotrimeric G protein and the GTP-GDP exchange, the β - and γ -subunits dissociate from the heterotrimeric complex. This allows the dissociated α -subunit-GTP complex to enter an activated state. It is this activated species that couples to effector systems, e.g., adenylylcyclase, to regulate effector-system function. The activated α -subunit possesses intrinsic GTPase activity that can hydrolyze the bound GTP to GDP, which terminates

activation of the α -subunit. Thus, closely integrated activation-autodeactivation of the G-protein α -subunit completes the cycle and allows the α -subunit-GDP complex to reassociate with β - and γ -subunits to reform the heterotrimer and return to the quiescent state.

MECHANISM OF ACTION OF PERTUSSIS AND CHOLERA TOXINS

Our understanding of signal transduction through G_s and G_i has been greatly enhanced by the use of cholera and pertussis toxins. The mechanisms of action of both of these toxins involve their covalent modification of G proteins through ADP ribosylation with NAD as substrate (14). ADP ribosylation of G-protein α -subunits by cholera toxin has been demonstrated for G_s (the G protein that activates adenylylcyclase) and G_i (or transducin, the major G protein in photoreceptors, which regulates a cGMP-specific phosphodiesterase; 13). The consequent incorporation of ADP-ribose into G_{sc} causes irreversible activation of G_s through inhibition of the intrinsic GTPase activity of the α -subunit. Similarly, ADP ribosylation of α -subunits by pertussis toxin has been demonstrated for G_i (which inactivates adenylylcyclase), G_i , G_K (which opens ligand-gated K^+ channels), G_o (function uncertain), G_{plc} (which activates phospholipase A_2), and G_{plc} (which activates phospholipase C) (13). Whether G_K , G_{plc} , and G_{plc} are totally distinct proteins or subtypes of G_i or G_o has not been resolved.

Both toxins are important investigational tools for studies

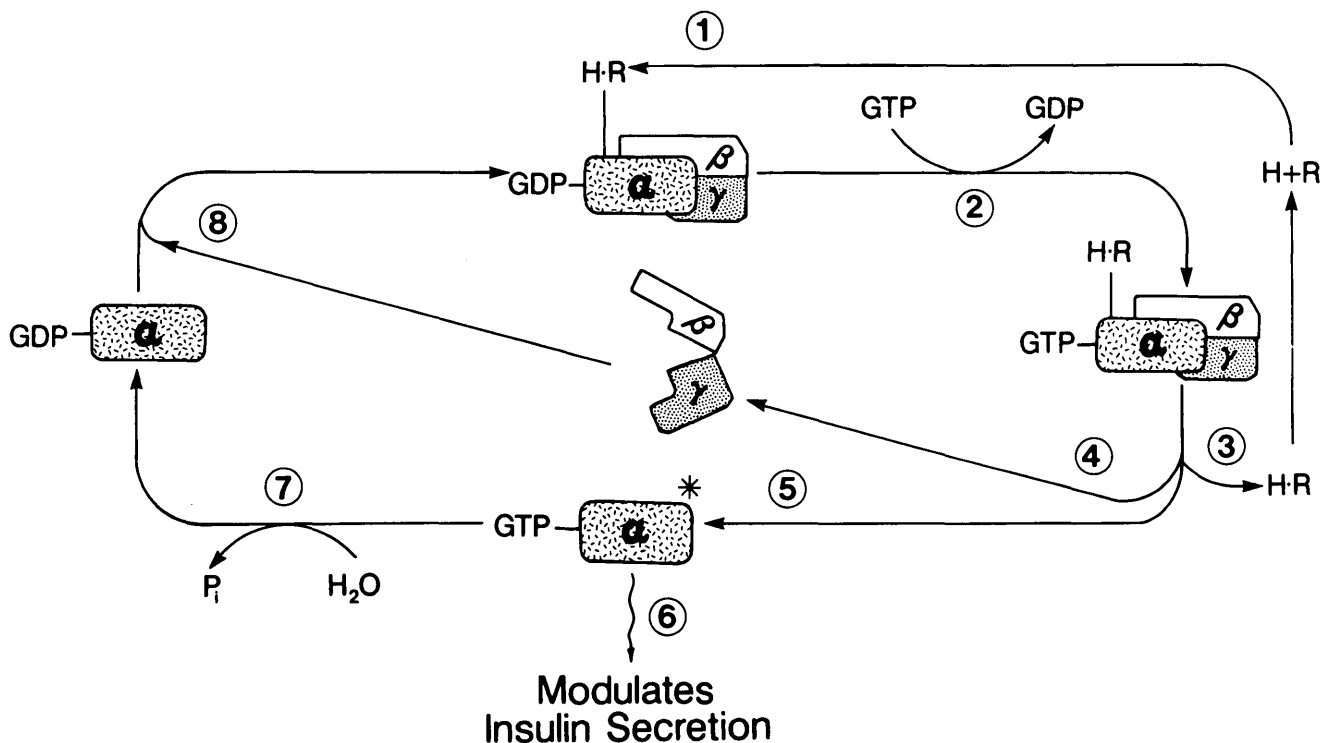


FIG. 1. Mechanism of action for G proteins. After formation of hormone-receptor complex (H-R; step 1), it associates with GDP-binding G protein (comprised of α -, β -, and γ -subunits). This union promotes exchange of GTP for GDP on α -subunit (step 2). This forms unstable complex that quickly dissociates into free H-R complex (step 3), free β - γ -subunits (step 4), and activated α -subunit binding GTP (step 5). This activated species modulates islet effector systems, e.g., adenylylcyclase, ion channels, phospholipases, and distal sites in exocytosis (step 6). Activated species is inactivated by intrinsic GTPase activity of α -subunit, which hydrolyzes bound GTP to GDP (step 7) and allows reassociation of α -GDP with β - γ -subunit (step 8). This completes cycle and returns G protein to its quiescent state. Cholera toxin irreversibly ADP ribosylates $G_{s\alpha}$ and $G_{i\alpha}$, blocks intrinsic GTPase activity, and consequently, prevents step 7. Pertussis toxin irreversibly ADP ribosylates G proteins such as $G_{i\alpha}$ and $G_{o\alpha}$ and prevents interaction of H-R with GDP-binding G protein.

of G-protein function and identification. With adenylyl cyclase as an example, cholera toxin can be used to amplify hormonal mechanisms that activate G_s and stimulate generation of cAMP. On the other hand, pertussis toxin can be used to dampen hormonal mechanisms that activate G_i and inhibit generation of cAMP. In this regard, it is important to understand that covalent modification of the α -subunits of G_s and G_i by cholera and pertussis toxins, respectively, have opposite functional effects on the G proteins. On the one hand, cholera toxin permanently activates G_{sa} , whereas on the other, pertussis toxin permanently inactivates G_{ia} . Consequently, depending on the type of cell, both cholera and pertussis toxins have the potential to increase levels of cAMP. Advantage has been taken of toxin-mediated ADP ribosylation to introduce ADP-ribose radiolabeled with ^{32}P into G proteins. This has allowed the creation of important G-protein detection and quantification procedures by electrophoretic techniques.

G PROTEINS IN PANCREATIC ISLETS

Abundant experimental data have accumulated in the past 25 yr that strongly support the concept that G proteins act as important modulators of pancreatic islet function. Early evidence for G_s as a regulator of insulin secretion can be found in reports that β -adrenergic agonists stimulate β -cell adenylyl cyclase, raise cAMP levels, and release insulin (15–18). Direct evidence for β -adrenergic binding sites in the islet was published by Cherksey et al. (19). Walseth et al. (20) reported the existence of two molecular forms of G_s in membrane preparations of HIT cells, a clonal cell line of transformed Syrian hamster pancreatic islet β -cells. Analysis with sodium dodecyl sulfate gels by examination with cholera toxin or by antisera specific for G_{sa} revealed 52,000- and 45,000-*M*, forms of G_{sa} . Interestingly, greater quantities of the 45,000-*M*, but not the 52,000-*M*, form were present with increasing passage of HIT cells. Evidence from assays of adenylyl cyclase and *cyc*⁻ cell reconstitution strongly suggested that the 45,000-*M*, form is more efficacious than the 52,000-*M*, form as a promoter of cAMP generation. Whether G_s plays a role in the physiological regulation of insulin secretion is a matter for future resolution. However, to the extent that cAMP augments glucose-induced insulin secretion, it would appear that G_s is an important modulatory protein for physiologically stimulated β -cell function.

More extensive data have been reported supporting the concept that G_i is a physiologically important β -cell regulatory protein. The first reports that epinephrine inhibited glucose-induced insulin secretion appeared >25 yr ago (21,22). The ability of epinephrine to inhibit insulin secretion was shown to be dependent on α -adrenergic activity (23), and direct evidence for α -adrenergic receptors in the islet has been reported (19). In the classic studies of Takahiko and Ui (24) and Katada and Ui (25), pertussis toxin prevented the inhibitory effects of epinephrine on insulin release. They reported that pretreatment of rats with a pertussis vaccine, whose active component was initially termed *islet-activating protein* and now referred to as pertussis toxin, could prevent the inhibitory effects of epinephrine on glucose-induced insulin secretion in the rats. These investigators also demonstrated that pertussis toxin treatment of pancreatic islets prevented the ability of epinephrine to lower islet cAMP

level (9). In the course of their studies, pertussis toxin was shown to ADP ribosylate a substrate in pancreatic islet cell membranes, which provided direct evidence for the existence of G_i (11,12). More recently, Robertson et al. (26) reported a pertussis toxin substrate in membranes from HIT cells. Further work with this cell line in our laboratory indicates that there may be as many as five pertussis toxin substrates in HIT cell membranes, which appear on Western analysis to represent three forms of G_i and two forms of G_o .

In addition to epinephrine, three other well-established inhibitors of insulin secretion—somatostatin, prostaglandin E_2 , and galanin—have been shown to be dependent on the activity of β -cell G_i . In their initial reports, Katada and Ui (9) demonstrated that pertussis toxin pretreatment of animals could prevent the inhibitory effects of somatostatin on cAMP levels and insulin secretion in isolated rat islets. More recently, Seaquist et al. (27) completed extensive studies demonstrating that preincubation of HIT cells before perfusion with somatostatin prevents the inhibitory effects of this peptide on both first and second phases of glucose-induced insulin secretion (Fig. 2). This was accompanied by a decrease in the ability of somatostatin to lower cAMP levels in HIT cells. Interestingly, the work of Seaquist et al. indicated that pertussis toxin completely prevented the effects of somatostatin but only partially prevented the effects of epinephrine on insulin secretion (Fig. 2). This led us to conclude that all inhibitory effects of somatostatin on β -cell function are mediated by pertussis toxin substrates, whereas only a portion of the inhibitory effects of epinephrine are dependent on these substrates. This may indicate that epinephrine, unlike somatostatin, has the ability to inhibit insulin secretion through at least two separate mechanisms, i.e., one that is dependent on and another that is independent of pertussis toxin-sensitive G proteins.

Inhibition of insulin secretion by prostaglandin E_2 (PGE_2) was demonstrated in vitro (28) and in vivo (29) in 1973, an effect that was independent of α -adrenergic activity (29). In more recent studies, we provided direct evidence for a PGE_2 binding site on HIT cell plasma membranes (26). Occupancy of this receptor was associated with decreased cAMP levels, and pertussis toxin partially reversed PGE_2 inhibition of insulin secretion in static incubations. In subsequent studies, Seaquist et al. (30) demonstrated that pertussis toxin reverses the inhibitory effects of PGE_2 on both first- and second-phase glucose-induced insulin secretion from perfused HIT cells.

Galanin is a 29-amino acid peptide that was reported in the mid-1980s as an inhibitor of insulin secretion (31). Binding sites for galanin have been identified in a hamster pancreatic β -cell tumor (32) and RINm5F cells (33), work that we confirmed with membranes from HIT cells. Amiranoff et al. (34,35) examined the mechanism of galanin and demonstrated in the β -cell line RINm5F that galanin inhibits cAMP production while simultaneously inhibiting insulin release. They observed that adenylyl cyclase in membranes from these cells was inhibited by galanin and that pretreatment of the cells with pertussis toxin completely abolished the inhibitory effect of galanin on insulin release, cAMP production, and adenylyl cyclase activity. They also demonstrated that pertussis toxin ADP ribosylated a protein in the cell membranes that was consistent in size with G_i .

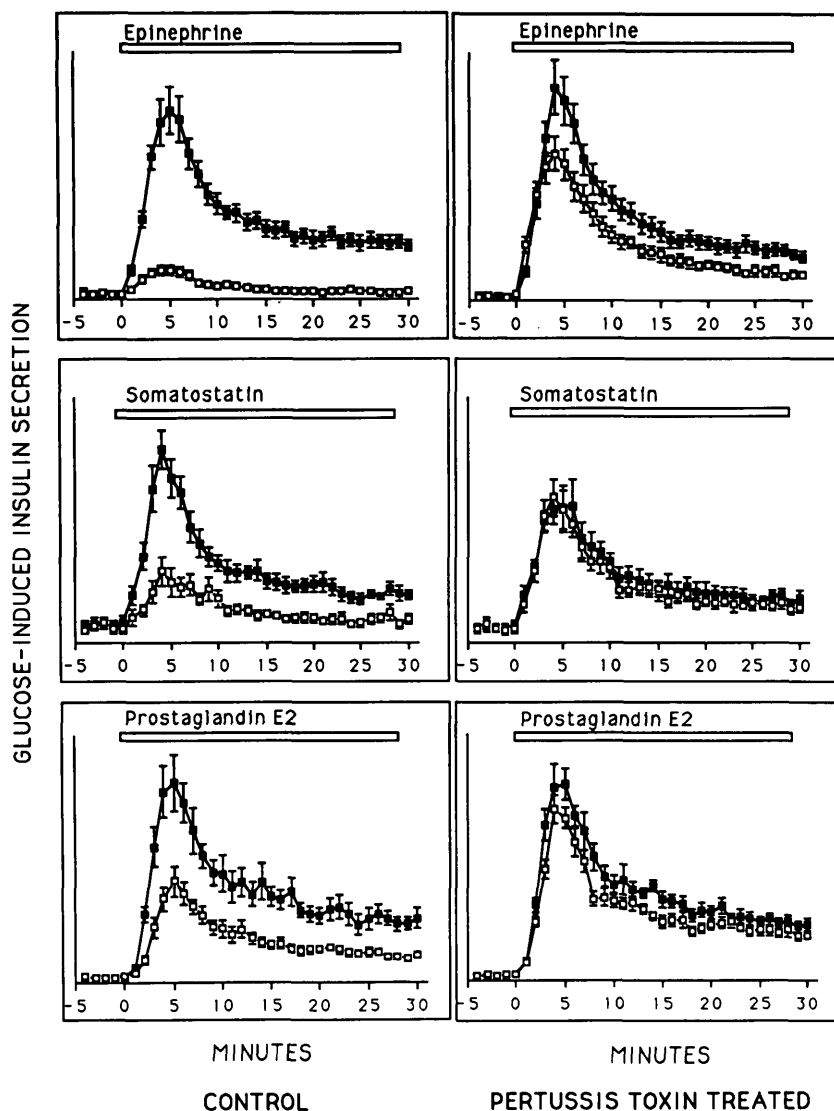


FIG. 2. Effect of pertussis toxin pretreatment on inhibitory effects of epinephrine, somatostatin, and prostaglandin E_2 on glucose-induced insulin secretion. Perfusion of HIT cells with 11.1 mM glucose and 0.1 mM 3-isobutyl-1-methylxanthine for 0–30 min elicits biphasic pattern of insulin secretion. Epinephrine (10 μ M, $n = 10$), somatostatin (0.1 μ M, $n = 6$), and prostaglandin E_2 (1 μ M, $n = 10$) all inhibit phasic glucose-induced insulin release (left panels). Pretreatment of HIT cells with 10 ng/ml pertussis toxin for 18 h prevents effects of these inhibitors (right panels). Effect of pertussis toxin on epinephrine and prostaglandin E_2 is partial, indicating that these agents modulate insulin secretion through mechanism that is only partially dependent on pertussis toxin-sensitive G proteins. Pertussis toxin completely prevents inhibitory effects of somatostatin, demonstrating that pertussis toxin substrates mediate all of inhibitory effects of this hormone on insulin secretion. ■, Control insulin secretion; □, inhibited insulin secretion.

Thus, abundant evidence exists indicating the presence of both G_s and G_i in pancreatic β -cells and that these G proteins positively and negatively, respectively, modulate adenylyl cyclase activity. Although the contribution of G_s to physiological insulin secretion is uncertain, four separate physiological inhibitors of glucose-induced insulin secretion have been shown to be at least partially dependent on the activity of pertussis toxin-sensitive G protein. In addition to G_s and G_i , evidence has also accumulated suggesting the presence of other G proteins in the islet.

FUTURE DIRECTIONS

It is generally accepted that glucose-induced insulin secretion involves closure of K^+ channels, depolarization of the β -cell, opening of Ca^{2+} channels, influx of Ca^{2+} , and activation of exocytosis. Ullrich and Wollheim (36) demonstrated in isolated islets that 8-bromo-cAMP failed to reverse the inhibitory effects on insulin secretion of epinephrine. This led Ullrich and Wollheim (37) and Metz (38) to suggest the presence of G proteins at a more distal site, perhaps in the exocytotic apparatus, which may mediate some of the inhibitory effects of epinephrine. In contrast, we have recently

observed partial prevention by 8-bromo-cAMP of epinephrine and somatostatin effects on insulin secretion from HIT cells, which suggests that there are proximal sites of action involving G_i for both somatostatin and epinephrine. There may be other sites for both hormones that are distal to the K^+ and Ca^{2+} channels. This is supported by our unpublished experiments showing noncompetitive inhibition by epinephrine and somatostatin for glipizide-induced insulin release, indicating a site of action beyond the K^+ channel. Additional unpublished experiments revealed that both inhibitors were additive to inhibitory effects of nickel chloride, indicating that there is a site of action for both inhibitors beyond the Ca^{2+} channel. Nonetheless, because pertussis toxin completely prevents the inhibitory effects of somatostatin on phasic glucose-induced insulin release from HIT cells, it can be concluded that all of the suggested sites of action for the inhibitory effects of somatostatin involve pertussis toxin substrates and, presumably, G proteins of one variety or another. On the other hand, epinephrine appears to be partially mediated by pertussis toxin substrates and partially independent of these substrates, because pertussis toxin incompletely prevents the inhibitory effects of epinephrine on β -cell function.

A potential candidate for the non-pertussis toxin-sensitive portion of the inhibitory effects of epinephrine might be G_z (1,2).

The stage is now set to intensively investigate the regulatory role of G proteins in pancreatic islet function. Diverse results from different laboratories have raised many unresolved issues about G-protein regulation of hormone- and substrate-induced stimulation and inhibition of insulin secretion. In addition, our knowledge about whether synthesis of pancreatic islet G proteins themselves is regulated by stimulators of hormonal secretion and/or hormones synthesized by the islet is embryonic. It seems possible that both stimulators and products of secretion might themselves exert regulatory and counterregulatory influences, respectively, over the synthesis of G proteins. Potential regulatory steps might involve cellular accumulation of G-protein mRNA by altering gene transcription, translation, or degradation of mRNA.

Additionally, it seems probable that knowledge of the mechanisms whereby toxins affect G-protein activity will lead to the design of new pharmacological agents for therapeutic manipulation of islet function. For example, many stress states are known to lead to pathophysiological consequences by virtue of the associated inhibition of insulin secretion, which could be mediated through G_i . Amelioration of abnormal stress states by drugs that finely titrate ADP ribosylation of G_i might prove helpful in clinical management.

Finally, examples of G proteins playing pivotal roles in intrinsic defects in several diseases have been uncovered. Erythrocytes from patients with pseudohypoparathyroidism have been demonstrated to have reduced levels of G_{sa} (39). This α -subunit deficiency is considered to be a likely explanation for the tissue resistance to parathyroid hormone action that results in the clinical characteristics of this syndrome. More recently, absence of intrinsic GTPase activity of G_{sa} in some patients with acromegaly has been suggested to be responsible for the unbridled release of growth hormone and consequent clinical abnormalities found in these patients (40). Because G proteins clearly play a dominant role in the regulation of pancreatic islet β -cell function, it seems reasonable to wonder whether abnormalities of G proteins, whether in the regulation of their synthesis, degradation, or activity, might play a pathogenetic role in patients with the abnormal insulin secretion characteristic of non-insulin-dependent diabetes mellitus.

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