Pancreatic Hormones and Hormonal Regulation of Insulin Secretion

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SUMMARY

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Endocrine pancreas producing insulin, glucagon, somatostatin and pancreatic polypeptide is under the influence of different types of regulation; among them the regulatory role of enteropancreatic axis plays an important role. Incretin effect of glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide 1 (GLP-1) is significantly involved in the insulin secretion which is modulated by many other hormones. Diabetes mellitus, similarly to disturbances of other hormones, can cause impaired regulation of insulin and other pancreatic hormones.

Key words: pancreatic hormones, insulin secretion, incretin hormones.


BASIC TYPES OF HORMONES IN PANCREATIC CELLS

Endocrine islet cells produce large amounts of hormones (Fig. 1), the majority of them being physiologically anfunctional. Many of them are produced in endocrine cells of other organs, participate in hormonal as well as in paracrine regulations and may regulate secretion of intrinsic pancreatic hormones. This group includes gastrointestinal hormones, such as glucose-dependent insulinotropic peptide (GIP) or glucagon-like insulinotropic peptide-1 (GLP-1), participating in the “incretine effect” of insulin secretion. The four hormones produced in the basic four types of pancreatic cells are of predominant physiological significance.

Insulin

Insulin was discovered in 1921 and shortly afterwards was described as a protein, although its primary structure of it, like that of the first protein, was described in a detail by Sanger (winning the Nobel prize for his work) later on. (1). Insulin is a polypeptide hormone, composed of the A chain with 21 amino acids and the B chain with 30 amino acids. Both chains are held together by two disulphide bonds between the cystines in CysA7 and CysB7, resp. CysA20 and CysB19 positions. Another disulphide bond is internal, within the A chain itself (A6 a A11). Insulin structure is highly conserved among vertebrates, which is evidenced by its high degree of homology. Porcine insulin has a single amino acid variation from the human variety (alanine substituing threonine in position B30), while bovine insulin has three amino acid variations (alanine instead of threonine in positions A8 and B30 and valine instead of leucine in position A10). The total of six insulin molecules, producing a hexamer, binds to two zinc atoms. Three dimers, i.e. three pairs of insulin molecules, may be released from the structure. Spatial construction including polar and hydrophobic amino acids and relations between the individual amino acids within the chain, affect the molecule’s stability and insulin binding to a receptor. Proinsulin is converted to insulin by cleavage of the binding peptide (C-peptide), binding the A and B chains together. In contrast to insulin, the C-peptide structure is highly variable, considering amounts and types of its amino acids. Therefore it has been considered functionally insignificant in the process of biosynthesis, except for having an effect on the mutual configuration of both insulin chains. However, the C-peptide appears to exhibit certain endocrine functions, and some are mediated through stimulation of Na,K-ATPase (2, 3).

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Along with the normal insulin and proinsulin molecules, gene mutations with altered primary structures have also been described. (4). These include three types of varied insulin molecules PheB25Leu, PheB24Ser and ValA3Leu. Their biological activity is reduced, resulting in diabetes. Insulin gene mutations may impair release of insulin from the proinsulin molecule, which results in familiar hyperproinsulinemia. These variants include the HisB10Asp mutation. Proinsulin is less effective, has reduced clearance and accumulates in the circulation. The HisB10Asp insulin is released from proinsulin through proteolytic cleavage. Compared to other abnormal insulins, it exhibits increased affinity to the insulin receptor.

Glucagon

Glucagon’s existence has already been envisaged by Banting and Best; however its structure was discovered in 1957. Its gene is located on chromosome 2. The molecule consists of 29 amino acids in a single chain which originates from the proglucagon molecule consisting of 160 amino acids through cleavage. Proglucagon originates in the A cells of the pancreas, as well as in the L cells of the ileus. The glucagon molecule contains the following: glicentin-related peptide (GRPP), glucagon, glucagon-like peptide 1 (GLP-1) and glucagon–like peptide 2 (GLP-2). The proglucagon molecule serves as a source of other peptides in the pancreas and in the intestine (Fig. 1). In the pancreas, proglucagon transforms to active glucagon, while glicentin-related pancreatic peptide (GRPP) and...
Glucagon is secreted by pancreatic A cells, which is affected by amino acids, mainly arginine and alanine, or by hypoglycemia. Already marginal or moderately lowered glycemia levels of about 3.4–3.7 mmol/l, stimulate glucagon release. Insulin inhibits glucagon secretion, which is evident from centrifugal blood perfusion of the islets. Catecholamines, cholecystokinin, glucose-dependent insulintropic peptide (GIP) and glucocorticoids facilitate secretion of the islets. Catecholamines, cholecystokinin, glucose-dependent insulintropic peptide (GIP) and glucocorticoids facilitate glucagon secretion, whereas somatostatin and high levels of free fatty acids have inhibitory effects. The circulatory half-time of glucagon is 3–6 minutes.

Glucagon enhances glucose production in the liver through stimulation of glycogenolysis as well as through gluconeogenesis. It binds to a hepatocyte receptor, which stimulates production of cyclic adenosinemonophosphate (cAMP) via G-protein. Activation of proteinkinase A then follows, which results in phosphorylation of proteins, primarily of enzymes participating in metabolic pathways. The mitogene – activated protein kinase (MAPK) pathway, as well as the phosphatidylinositol-3-kinase pathway are activated. The signaling cascade affects both gluconeogenetic pathways, i.e. glycogenolysis as well as gluconeogenesis. Lipolysis and ketogenesis is stimulated in a similar manner. The proportion of insulin and glucagon concentrations sustains cellular phosphorylating-dephosphorylating processes which activate or inhibit the regulating enzymes, thus affecting basic metabolic pathways.

**Somatostatin**

In 1973, somatostatin was identified in the hypothalamus, where it inhibited secretion of the growth hormone. Later on its activity in the digestive tract was discovered. (6). The somatostatin gene is located on chromosome 3, preprosomatostatin consists of 116 amino acids. The somatostatin molecule itself consists of 14 amino acids, forming a circle. Furthermore, about 5–10% of molecules containing 28 amino acids, participate in immune reactivity. It originates partially from the central and peripheral nervous system cells; however its main source are the D cells of the pancreas and GIT. It has several mechanisms, through which it exerts its activity: firstly, it is released in the circulation and, secondly, it has a typical paracrine activity. It exerts its modulatory, predominantly inhibitory effect on other gastrointestinal hormones, thus regulating a number of GIT functions. Binding to a specific receptor triggers the intracellular effects through pathways of the cyclic AMP production as well as of calcium ions transport.

In the pancreas, somatostatin exerts its modulatory activity both in the Langerhans islets and the exocrine part. The resulting effects depend on intrinsic interaction of the following three hormones: insulin, glucagon and somatostatin. While insulin secretion is inhibited by somatostatin in high, as well as in low glycemia levels, secretion of glucagon is inhibited only in low glucose levels. Therefore, interaction of the hormones within the islets is affected by other factors. Somatostatin-28 is up to ten times more effective in inhibiting insulin secretion than somatostatin-14.

Somatostatin inhibits secretion of exocrine pancreatic enzymes, while not affecting bicarbonate secretion. It inhibits gastrointestinal secretion and motility, both directly and through other hormones (e.g. it regulates secretion, therefore activity of cholecystokinin). It inhibits exocrine and endocrine secretion and motility of the stomach. Somatostatin inhibits gastric juice secretion through inhibition of histamine secretion, while gastrin secretion is regulated by somatostatin paracrinally. Also a gastric peptide, ghrelin, is influenced by somatostatin.

In the small intestine, somatostatin affects absorption of nutrients and intestinal motility. Somatostatin prolongs intestinal transit time, reduces electrolyte secretion into the lumen, and so it may have an effect on secretion diarrhea, while it has no significant effect on absorption of electrolytes. Somatostatin is a potent inhibitor of chloride secretion in the large intestine.

**Pancreatic polypeptide**

Pancreatic polypeptide (PP) is secreted in PP cells located in the islets of the posterior part of the head of the pancreas (so-called processus uncinatus). PP consists of 36 amino acids. Its plasma concentration is increased under a combined diet, while intravenous administration of glucose or amino acids produces minor response. Incretine effect is expected (supposed to be GIP and GLP-1 conditioned). Physiological effects of the PP are unknown. In a number of active endocrine gastrointestinal tumors (mainly glucagonoma, VIPoma, gastrinoma and even insulinoma), increased plasma PP concentrations have been detected.

### INSULIN SYNTHESIS

Pancreatic B cells are a sole source of insulin in humans. It is synthesized here and released in the circulation. Both processes follow complicated regulations, which control physiological effects of the hormone in the body. Insulin is effective in sustaining metabo-
lic homeostasis with a direct control of glycemia. Any impairment of insulin synthesis and secretion is manifested as a pathology, resulting in hyperglycemia (diabetes mellitus or other glucose homeostasis impairments) or hypoglycemia (endogenous hyperinsulism in nesidiomas or hyperinsulinemic hypoglycemia in children, etc.). Physiological insulin synthesis is of major significance to the organism.

**Insulin synthesis regulation**

The B cell is the site of insulin synthesis and its storage in secretion granules in quantities sufficient for the current body requirements. Higher requirements induce increased insulin secretion and synthesis.

Human insulin gene is located on chromosome 11 between the thyrosinhydroxylase (TH) gene and the insulin-like growth factor 2 (IGF-2) gene. Insulin biosynthesis is initiated by the insulin promoter gene activation, mediated by several transcription factors (mainly Pdx1, neuroD and other factors) and the proinsulin release. Depending on ATP, Ca²⁺, glucose concentrations of 10–12 mmol/l (9). The biosynthesis is also increased in pregnancy and the secretion is stimulated at 4–6 mmol/l. The proinsulin biosynthesis reaches its maximum at glucose concentrations of 2–4 mmol/l, whereas secretion is stimulated at 4–6 mmol/l. The proinsulin biosynthesis is increased in pregnancy and its storage in secretion granules, coexists with the net-producing clathrin, develop within the Golgi complex. The granules maturate the following: loss of clathrin, progressive acidification (pH reaches 5.0–5.5) and conversion of proinsulin, which is pH-dependent. Proinsulin is cleaved by endopeptidases, type I splits the Arg₃₁ – Arg₃₂ bond, whereas type II splits the Lys₆₄ – Arg₆₅ bond. The single endopeptidase activity produces intermediate products, where the C-peptide remains linked to either the A chain (des-₃₁, ₃₂ proinsulin), or the B chain (des-₆₄, ₆₅ proinsulin). About 5–10 % of proinsulin and its intermediate products may be detected in the peripheral blood samples of healthy subjects under fasting condition. Insulin released in the granules linked to zink forms hexamers and crystalizes at low pH levels. Insulin crystals form the dense nucleus of the granules, which may be observed using electronmicroscopy.

Proinsulin biosynthesis is regulated by nutrients, hormones and neurotransmitters. Glucose stimulates synthesis at threshold concentrations of 2–4 mmol/l, whereas secretion is stimulated at 4–6 mmol/l. The proinsulin biosynthesis reaches its maximum at glucose concentrations of 10–12 mmol/l (9). The biosynthesis is also stimulated by other sugars and nutrients; if metabolized, however, their effect is less pronounced. The following hormones participate, primarily, in the insulin biosynthesis: the growth hormone, glucagon and glucagon-like peptide-1 (GLP-1). Catecholamines have inhibitory effects, while somatostatin has no effect on the synthesis. The proinsulin biosynthesis is increased in pregnancy and obesity, whereas starving and aging have inhibitory effects.

Insulin biosynthesis and its secretion are controlled by different factors (4). Biosynthesis is Ca²⁺ independent while it depends on Mg²⁺. Neither sulphonylurea derivates or diazoxide, or protein kinase C, have any effect on the synthesis. Long chain fatty acids have rather inhibitory effects.

**HORMONAL REGULATION OF INSULIN SECRETION**

Insulin secretion is controlled by autocrine, paracrine, neurocrine and endocrine factors, which modulate the final effect of glucose in both directions- stimulating or inhibiting it. Effects observed in vitro are not always applicable to effects within the whole organism, particularly when autocrine and paracrine control is concerned. Hormones regulate the B cell and the insulin secretion via receptors, then activating “secondary messengers” systems. These include cAMP, inositolphosphate and diacylglycerol. cAMP is generated from ATP by adenylylase, regulates protein kinase A to stimulate insulin secretion either via phosphorylation and calcium channel activation, or via amplification- potentiation of calcium ions effect on exocytosis. GIP, GLP-1 and glucagon use this mechanism. Acetylcholine operating on muscarine receptors has an effect on membrane phospholipase C, degrading phosphoinositides. Diacylglycerol, linked to the cellular membrane is released and it activates proteinkinase C, which amplifies calcium ions – mediated secretion (10). Furthermore, a by product – inositoltriphosphate – is formed. It binds to receptors of the endoplasmatic reticular membrane and pumps Ca²⁺ from the ER lumen into the cytoplasm. (11). This way controls the activation signal, i.e. the amount of the intracellular calcium. However, acetylcholine exhibits a more complex activity within the B cell. Its activity results in increased flow of natrium ions, which have a direct effect on the calcium channel. (12). Also cholecystokinin, a neurotransmitter, works via the diacylglycerole pathway. Both incretins, i.e. GIP and GLP-1, stimulate glucose-mediated insulin secretion. (13). Besides the incretin effect, activity of both hormones, particularly of GLP-1, is so complex, including its effects on food intake, on CNS functions and on other organs, that it may have other, indirect, effects on insulin secretion, as well. (14).

**Glucose-dependent insulinotrophic peptide (GIP)**

This substance was originally described in 1969 and its effect was studied with respect to inhibition of gastric secretion. Recent studies have shown that, under physiological conditions, this hormone, produced mainly in duodenal K cells, stimulates postprandial secretion of insulin, as well as fat deposition. It was its effect on insulin secretion via a substance secreted in the proximal intestine, which resulted in its original labelling as “incretin”, the existence of which was expected much earlier than the GIP was discovered. Insulinotropic activity of GIP was then confirmed in several experimental models. It was proved that dependence of its effect on the glucose level is essential, as it prevented undesirable hypoglycemia caused by inadequate insulin stimulation on a low-saccharide diet. Based on both the above effects, it is evident that the GIP activity overlaps to a great degree with the insulin activity (e.g. their lipogenetic effect), which may become significant in dysfunctions of insulin synthesis or secretion. In type 1 diabetes mellitus, reduced serum GIP concentrations were detected, while in type 2 diabetes mellitus, the concentrations were elevated or normal. Studies of GIP receptors (GIPR) have shown that in type 2 diabetes mellitus, the receptors are desensitized as a result of elevated GIP concentrations, or their expression is low.(15). Furthermore, reduced GIP half-time and impaired food – induced stimulation of GIP secretion have been demonstrated. (15). The pathophysiology of these varia-
GLP-1 secretion from the intestinal mucosal cells is regulated directly by nutrients contained in the chyme, while mixed diet has a strong stimulatory effect. The released GLP-1 stimulates insulin secretion from pancreatic B cells and secretion of somatostatin from D cells, while glucagon secretion from A cells is inhibited. The effect is mediated by blood circulation within the islets of Langerhans from their centre to the periphery and is modulated by effects of increased insulin secretion on A cells, while GLP-1 has a stimulatory effect on isolated A cells. Furthermore, GLP-1 stimulates transcription of the insulin gene and its biosynthesis. Insulinotropic activity of GLP-1 is glucose-dependent, because glucose is required to exert its effects on B cells. GLP-1 activity results in inhibition of glucose production in the liver and in its increased clearance in adipose and muscle tissue, mediated by insulin. The effects are not induced by GLP-1 itself in the peripheral tissues, but result from effects on pancreatic hormones. GLP-1 is of substantial significance for insulin secretion, as it has been proved that absence of GLP-1 receptors in mice results in diabetes mellitus.

In addition to these effects, experimental studies have shown GLP-1 to induce expression of the transcription factor IDX-1 in progenitor cells of pancreatic ducts, which implicates their transformation to B cells. Experiments performed with diabetic mice have shown that the transcription factor IDX-1, transferred using adenoviral vectors, can transform a hepatocyte subpopulation into B cells producing insulin, which resulted in recovery from the diabetic symptoms. Neogenetic activity of GLP-1 plays a substantial role in differentiation, therefore also in reconstruction of islet B cells, which are known to have a life span of 30–40 days. Then they undergo a programmed death (apoptosis). GLP-1 agonist, so called exendin-4, has a similar effect. Both substances applied into the AR42J ductal cell culture resulted in their transformation into cells producing glucagon to start with, then they concomitantly produced insulin, and in the next stage they produced insulin and pancreatic polypeptide. This report brings essential information on a potential development of hormones in the embryonic pancreas. Transcription factors are crucial for a respective pancreatic gene expression. Factor PDX1 activates the insulin and somatostatin gene, and inhibits the glucagon gene. Therefore its inactivation results in increased number of glucagon producing cells and, consequently, in increased glucagon production.

However, GLP-1 exhibits a number of other effects. It inhibits gastric motility and secretion and, as a consequence, it delays gastric emptying, increases satiety, which finally results in reduction of food intake. However, specific mechanisms of this regulation remain unknown. Pancreatic and extrapancreatic effects of GLP-1 have been recently summarized in a general report. Clinically, GLP-1 have become of a primary interest amongst
diabetologists. While GLP-1 secretion is normal in type 1 diabetes, reduced GLP-1 secretion has been detected in type 2 diabetes, which may be responsible for impaired insulin secretion in this type of the disorder, (33). Administration of GLP-1 in type 2 diabetes patients adjusted insulin secretion as a result of the improved B cell response to glucose. (34). Experiments have shown that administration of the GLP-1 agonist, exendin-4, prevented the onset of type 2 diabetes mellitus following partial pancreatectomy in rats, while control animal subjects, „treated“ with physiological solution, developed the disorder (35). Treatment with exendin-4 normalized postprandial glycemia. Therefore GLP-1 appears to be a perfect substance to use in the type 2 diabetes mellitus treatment. Its continual administration does not cause hypoglycemia, because its effect is glucose-dependent. Administration of GLP-1 to patients with the type 2 diabetes mellitus resulted in nearly normal glycemic values, whereas its discontinuation caused repeated elevations of glycemia (36). However, a very short half-time of GLP-1 (4–5 minutes), caused by its renal excretion and, in particular, by its fast DPPIV – mediated breakdown (5). Therefore current studies also concentrate on development of this enzyme’s inhibitors, which might improve the GLP-1 effect on insulin secretion. (37). Another pathway concentrates on development of the GLP-1 receptor agonists. Activation of the GLP-1 receptors appears to be one of the most promising steps towards the type 2 diabetes mellitus treatment. When GLP-1 is compared with GIP, it is evident that GLP-1 is a much more potent substance for treatment of the type 2 diabetes mellitus than GIP. Direct stimulatory effect of GIP on glucagon secretion, which then reduces its total effect, may contribute or the GLP-1 effect (38). Therefore, GLP-1 appears to be more suitable for the type 2 diabetes mellitus treatment than GIP.

Furthermore, in obese subjects, impaired GLP-1 secretion, to which leptin resistance is expected to contribute, has been detected (39). Leptin stimulates GLP-1 secretion from L cells, reduced lep-
tin activity is associated with impaired secretion of GLP-1. These pathophysiological correlations could explain alterations of insulin secretion in the metabolic syndrome, mainly in combination with the type 2 diabetes mellitus.

OTHER HORMONAL EFFECTS

Secretion of insulin is further significantly controlled by the growth hormone, which potentiates its growth, insulin biosynthesis and its secretion, via somatogenic and lactogenic B cell receptors (40). The growth hormone deficiency presents with glucose-induced impaired insulin secretion. Ghrelin (discovered in 1999), released from gastric and intestinal endocrine cells, a stimulator of the growth hormone secretion, regulates insulin secretion via its receptors (GHS-R – growth hormone secretagogue receptors) located also on B cells. It inhibits the glucose-stimulated insulin secretion (41). Glucocorticoids stimulate secretion of insulin indirectly through stimulation of insulin resistance and through its effects on gluconeogenesis. However, their effect on B cells is inhibitory and results from impaired effect of calcium ions on exocytosis (42). Vitamin D also has a positive effect on insulin secretion (43) through 1,25-dihydroxycholecalciferol receptors detected in B cells (44). Female gonadal steroids have insulinotrophic effects and increase insulin secretion by modification of calcium entry into cells. (45).

Impaired insulin secretion is mediated both by reduced calcium entry into the B cell, and by reduced efficacy of calcium ions. The mechanism depends on opposite changes to those described during activation reduced cAMP, opening of ATP-sensitive potassium channels are the principal mechanisms, however, other, distally

located processes with a direct effect on exocytosis, e.g. GTP – binding proteins, are expected to participate as well. Cate-
cholamines, released during exercise and stress, have a negative physiological effect on insulin secretion, then galanin and somato-
statin, released from the digestive tract following a high-fat meal (somatostatin-28), whereas somatostatin-14 , originating from pan-
creatic D cells has a less pronounced effect. (46). Also leptin inhibits insulin secretion, probably through activation of phospho-
diesterase, decreasing cAMP and opening ATP-sensitive potassium channel. Apart from regulation of secretion, leptin inhibits insulin biosynthesis (47). Existence of adipo-insuline axis is expected (48), and its role is supposed to be impaired in leptin resistance.

Thyroid hormones reduce insulin content of B cells, probably through inhibition of proinsulin mRNA (49) which results in delayed inhibition of insulin secretion following a glucose stimulus. Thyroxin reduces insulin in pancreas and glucose stimulated insulin secretion via facilitated B cell apoptosis (50). Other hormones with inhibitory activity on insulin secretion include IGF-1, pancreastatin released from B cells – a substance with autocrine activity, some opioids, calcitonin gene-related peptide (CGRP) and islets amyloid polypeptide (IAPP) (1a). However, under physiological conditions, their effect is minor.

Islets of Langerhans are supplied by adrenergic and cholinergic autonomic nerves. Adrenergic stimulation inhibits insulin secretion, while parasympathetic stimulation mediated by the vagus nerve, has stimulatory activity. Also a number of neurotransmitters and hormonal substances participate in this regulation. (51). Although autonomic nervous system activity has been demonstrated in studies, its in vivo regulatory role has not been fully explained. Howev-

er, two clinical observations document its physiological signifi-
cance. Firstly, the role of the insulin secretion cephalic phase, controlled by the vagus nerve, affects postprandial regulation of glycemia and, furthermore, reduced glucose tolerance has been documented following vagotomy. (52). Secondly, following pancreatic transplantation, impaired posprandial insulin secretory response has been recorded (53). Functional autonomic nervous system, in conjunction with hormones, participates in in vivo physiological regulation of the insulin secretion.

CONCLUSION

Secretion of pancreatic hormones is controlled by many regula-
tory actions, which result from interactions of pancreatic endocrine cells (endocrine and paracrine effects), as well as from modular actions of the gastrointestinal hormones (with incretine effects) and other hormones. Diabetes mellitus is not only a dysfunction of the insulin synthesis and secretion, but also a cause of dysregulation of incretine effects of the gastrointestinal hormones. This may affect their modulatory effects on the endocrine pancreas.

Abbreviations

cAMP – cyclic adenosinmonophosphate
CGRP – calcitonin gene-related peptide
DPPIV – dipeptidylpeptidase IV
GHS-R – growth hormone secretagogue receptors
GIP – glucose-dependent insulintropic peptide
GLP-2 – glucagon-like peptide 2
GLP-1 – glucagon-like peptide-1
GRPP – glicentin-related peptide
IGF-1 – insulin-like growth factor 1
IGF-2 – insulin-like growth factor 2
MAPK – mitogene-activated protein kinase
PP – pancreatic polypeptide
TH – thyroxinhydroxylase
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