INTRODUCTION

The history of the concept of the entero-insular axis in regulation of glucose levels started in 1931, when La Barre first used the term “incretin” to refer to a substance that is released by the intestinal mucosa in response to food intake and reduces the level of blood glucose. In 1964–1967, three scientific groups independently demonstrated that oral glucose administration caused a more pronounced influence on the insulin secretion than after intravenous administration: researchers attributed this difference to release the “incretin.” In 1971, J.C. Brown isolated an intestinal mucosa polypeptide inhibiting gastric acid secretion in dogs after exogenous administration and deduced its amino acid sequence; due to this physiological effect it was originally denominated as a gastric inhibitory polypeptide. Subsequently, however, J.C. Brown and colleagues recognized insulinotropic properties in this peptide and renamed it as glucose-dependent insulinotropic peptide (GIP). Somewhat later, in 1985, the second incretin, glucagon-like peptide-1 (GLP-1) was isolated [1].

1. INCRETINS

GIP, a peptide comprising 42 amino acids, is a product of post-translational processing of the precursor of 153 amino acids encoded by the gip gene (Fig. 1). Incretin is synthesized and secreted in response to nutrient stimulation of enteroendocrine cells (K cells) localized predominantly in duodenum and small intestine. The insulinotropic activity of GIP associated with the stimulation of a specific β-cell GIP receptor, coupled to the system of G-proteins activating adenylate cyclase; this results in increased levels of cAMP followed by the increase of intracellular calcium. Incretin is rapidly degraded by dipeptidyl peptidase 4 (DPP-4) [1, 2]. It should be noted that in diabetes mellitus type 2, plasma GIP concentration is either in normal or increased, but the insulinotropic effect is not sufficiently pronounced [2]. The mechanism underlying the reduced β-cell response to stimulation by GIP is not completely understood; studies by Lynn (2001) and Zhou (2007) suggest that hyperglycemia is accompanied by impaired regulation of GIP receptor expression [1, 2].

GLP-1, the second peptide exhibiting incretin activity, is the product of the proglucagon gene. It is mainly produced by enteroendocrine L-cells. Incretin exists in several forms; approximately 80% of the circulating biologically active human blood GLP-1 exist as GLP-1 (7–36) amide, while a smaller part exists as GLP-1 (7–37) (Fig. 1) [2]. Like GIP, GLP-1 exhibits the insulinotropic effect by binding to a specific β-cell GLP-1 receptor coupled to the system of Gs-proteins and additionally to Gq, Go, and Gi-proteins. GLP-1 receptor binding leads to an increase in intracellular calcium concentration and intracellular cAMP and activation of intracellular cascades, including protein kinases A and C, phosphoinositol-3-kinase, mitogen-
activated protein kinases, which phosphorylate proteins such as glucose transporter 2 (GLUT 2), sulfonylurea receptor 1, synaptosomal-associated protein (α-SNAP), etc. This blocks β-cell ATP-dependent potassium and voltage-dependent potassium channels and increases calcium levels, followed by membrane depolarization and insulin exocytosis [1].

Besides the insulinotropic action GLP-1 is involved in the regulation of the number of β-cells. Regulation of processes of regeneration and neogenesis of β-cells occurs via different ways; GLP-1 activates the system phosphoinositol-3-kinase (PI 3-K)/protein kinase B, which promotes translocation of the pancreatic duodenal homeobox-1 protein, (PDX-1) and further expression of PDX-1 via stimulation of phosphorylation of the transcription factor encoded by the foxo1 gene (forkhead box protein O1, Foxo 1) [3, 4]. In addition, GLP-1 binding to a specific receptor induces maturation of betacellulin; this involves membrane-bound metalloproteinases and transactivation of epidermal growth factor receptor followed by activation of PI 3-K [1]. GLP-1 also exhibits the stimulatory effect on β-cells proliferation via the transcription factor, a protein, which binds to the cAMP response element-binding protein (CREB). In the absence of cAMP, CREB interacts with the transcriptional co-activator (transducer of regulated CREB activity 2, TORC2) and the 14-3-3 protein and forms a complex with the serine/threonine protein kinase 2 (SIK2). The increase in the cAMP level induced by stimulation of GLP-1 receptor is accompanied by activation of protein kinase A, which inactivates SIK2 and causes dissociation of the complex CREB-TORC2-14-3-3 protein with release of 14-3-3 protein. The CREB-TORC2 complex is translocated to the nucleus, where it binds to the cAMP-responsive element and induces transcription of genes responsible for the survival of β-cells [5].

GLP-1 may inhibit apoptosis of β-cells induced by cytotoxic agents, including reactive oxygen species, high glucose concentrations, fatty acids, (e.g. palmitate), cytokines, tumor necrosis factor α, dexamethasone; this is associated with increased expression of gene encoding anti-apoptotic proteins (Bcl-2, Iap-2) [3–5].

Besides insulinotropic activity incretins are also characterized by other biological effects (Table 1) [2].

2. DIPEPTIDYL PEPTIDASE 4 AS A FACTOR REGULATING THE ACTIVITY OF INCRETINS

The therapeutic application of incretins for treatment of diabetes mellitus (DM) is hampered by their rapid inactivation. The biological activity of GLP-1 and GIP limited by dipeptidyl peptidase 4 (DPP-4), the enzyme involved in their degradation. The enzyme exists as membrane-bound and (small amounts of) free forms. In humans, DPP-4 is expressed on epithelial cells, capillary endothelium, lymphocytes, in the gastrointestinal tract, in the pancreatic exocrine acinar cells, kidney, thymus, lymph nodes, uterus, placenta, prostate, adrenals, parathyroid, sweat and breast glands, liver, spleen, lungs, and brain [6, 7]. The enzyme is a protein of 766 amino acids; it consists of two domains, including the N-terminal β-propeller and C-terminal α/β-hydrolase domain, which form a large cavity containing the active binding site. Although in the body DPP-4 may form dimers and tetramers, it exhibits catalytic activity only in one of these forms [7, 8].

Access to the active site of this enzyme is possible through the open center in the β-propeller or between the propeller and the hydrolase domain (Fig. 2). The active site of DPP-4 is formed by the following amino acid residues: the triad Ser 630, Asp 708 and His 740 in the hydrolase domain and Gly 205, Gly 206 in the...