MOLECULAR REGULATION OF HORMONE SECRETION, GROWTH AND APOPTOSIS OF GLP-1-PRODUCING CELLS

AKADEMISK AVHANDLING som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Aulan, plan 6, Södersjukhuset.

Fredagen den 18 januari, 2013, kl 09.00

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Stockholm 2013
ABSTRACT

Type 2 diabetes (T2D) spreads like an epidemic in today’s society, and there is a great need for new and improved treatments. T2D is characterized by hyperglycemia, resulting from impaired insulin production and insulin resistance in peripheral tissues. Incretin hormones, such as glucagon-like peptide-1 (GLP-1), secreted from L-cells dispersed along the intestinal tract, potentiate meal-stimulated insulin secretion in a glucose-dependent manner. Defective GLP-1 secretion has been indicated in T2D and administration of GLP-1 to T2D patients restores glucose-induced insulin secretion and normalizes glycemia, making stable analogs of GLP-1 among the best available treatments for T2D today. However, enhancing endogenous GLP-1 productionsecretion by direct stimulation of GLP-1 secretion/promotion of growth and viability of L-cells may be a novel and more physiological option in incretin-based diabetes therapy. The aim of this work was to determine the effect of diabetic conditions and anti-diabetic agents on GLP-1-producing cells, in order to unravel some of the mechanisms regulating growth, survival and function of this cell type.

Studies I-III were performed in vitro using the murine GLUTag cell line as a model. In study I, direct effects of metformin on apoptosis, and function of GLP-1-secreting cells were determined. Simulated diabetic hyperlipidemia resulted in increased caspase-3 activity and DNA fragmentation, indicating lipoapoptosis. Metformin treatment significantly decreased this lipoapoptosis in conjunction with increased phosphorylation of AMPK. In addition, metformin treatment stimulated GLP-1 secretion.

In study II, we determined molecular mechanisms mediating lipotoxicity and metformin-induced lipoprotection in GLP-1-secreting cells. Diabetic hyperlipidemia was simulated in this cell system by addition of the fatty acid palmitate. Palmitate increased ROS production in GLP-1-secreting cells, and the lipotoxic effects of palmitate were abolished in the presence of the antioxidant Trolox. Further, palmitate phosphorylated p38 MAPK and inhibition of this enzyme significantly reduced lipoapoptosis. Pre-incubation with metformin further increased palmitate-induced ROS production, while significantly reducing the expression of p38 MAPK.

Study III focused on direct effects of insulin and exendin-4/GLP-1 on lipoapoptosis and function of GLP-1-secreting cells. The GLP-1R was found to be expressed in the GLUTag cells, and diabetic lipotoxicity was partially inhibited by pre-incubation with insulin or the stable GLP-1 analog exendin-4. The lipoprotective effect of exendin-4 was GLP-1R-dependent, while independent of PKA activity. In addition, both insulin and exendin-4 significantly stimulated acute and long term GLP-1 secretion in the presence of glucose. In study IV, we investigated if a high fat diet (HFD) reduces the number of enteroendocrine GLP-1-secreting L-cells in C57/Bl6 mice. We also determined the effects of a HFD on GLP-1 plasma levels and possible effects on these parameters by metformin treatment. A HFD rapidly induced a diabetic phenotype with increased HbA1c levels, as well as fasting plasma insulin levels in conjunction with reduced oral glucose tolerance – indicating the manifestation of insulin resistance. A 14 day oral administration of metformin reduced HbA1c, fasting insulin and prandial FFA levels. The number of L-cells was significantly reduced after 12 weeks on a HFD, while -- in contrast -- there was a clear trend toward increased prandial plasma GLP-1 levels despite reduced food intake in HFD-fed mice.

These findings may be of pathogenic significance not only in understanding mechanisms of the impaired incretin response characterizing T2D patients, but may also be harnessed to therapeutic advantage in efforts to enhance endogenous GLP-1 production. Such an approach has hitherto received little attention but may be superior to contemporary incretin-based antidiabetic therapy, which does not faithfully mimic physiologic GLP-1 release in for instance terms of secretory pattern (e.g. pulsatility) and actions on topographically adjacent hormone receptors (e.g. in the portal vein).