Karolinska Institutet

Institutionen för molekylär medicin och kirurgi

Studies on factors modulating glucose homeostasis in healthy and diabetic rats

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Rehabsalen S2:01 Norrbacka, Karolinska Universitetssjukhuset Solna

Fredagen den 21 oktober 2011, kl 09.00

av

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Stockholm 2011

ABSTRACT

Glucose is the most common substrate for energy metabolism. Despite the varying demands for glucose, the body needs to regulate its internal environment and maintain a constant and stable condition. Glucose homeostasis requires harmonized interaction between several tissues, achieving equilibrium between glucose output and uptake. In this thesis, we aimed to investigate factors modulating glucose homeostasis in a rat model of type 2 diabetes, the Goto-Kakizaki (GK) rat. In addition, we investigated sex differences in hepatic carbohydrate and lipid metabolism in healthy rats.

In **Paper I,** three-week but not three-day treatment with a Southeast Asian herb, *Gynostemma pentaphyllum* (GP), significantly reduced plasma glucose (PG) levels in GK rats. An intra-peritoneal glucose tolerance test (IPGTT) was significantly improved in GP-treated compared to placebo-treated group. In the GP treated rats, the glucose response in an intra-peritoneal pyruvate tolerance test was significantly lower, indicating decreased gluconeogenesis, and hepatic glucose output (HGO) was reduced. GP-treatment significantly reduced hepatic glycogen content, but not glycogen synthase activity. The study provides evidence that the GP extract exerted anti-diabetic effect in GK rats, reducing PG levels and HGO, suggesting that GP improves the hepatic insulin sensitivity by suppressing gluconeogenesis.

In **Paper II,** shikonin, a naphthoquinone derived from the Chinese plant *Lithospermum erythrorhizon*, increased glucose uptake in L6 myotubes, but did not phosphorylate Akt. Furthermore we found no evidence for the involvement of AMP-activated protein kinase (AMPK) in shikonin-induced glucose uptake. Shikonin increased the intracellular levels of calcium in these cells and stimulated the translocation of GLUT4 from intracellular vesicles to the cell surface in L6 myotubes. In GK rats treated with shikonin once daily for 4 days, PG levels were significantly decreased. In an insulin sensitivity test, the absolute PG levels were significantly lower in the shikonin-treated rats. These findings suggest that shikonin increases glucose uptake in muscle cells via an insulin-independent pathway dependent on calcium.

In **Paper III,** GK and control Wistar rats were injected daily for up to 4 weeks with either a non-hematopoietic erythropoietin analog ARA290 or with placebo. PG levels in GK but not Wistar rats were significantly lower in ARA290-treated rats compared to placebo-treated rats. After 2 and 4 weeks, the IPGTT was significantly improved in ARA290 treated GK rats. In insulin and pyruvate tolerance tests, glucose responses were similar in ARA290 and placebo groups. In isolated GK rat islets, glucose-stimulated insulin release was two-fold higher and islet intracellular calcium concentrations in response to several secretagogues were significantly higher in ARA290-treated rats than in placebo-treated GK rats. These findings indicate that treatment with ARA290 significantly improved glucose tolerance in diabetic GK rats, most likely due to improvement of insulin release.

In **Paper IV,** sex differences in hepatic carbohydrate and lipid metabolism were characterized in healthy rats. No sex-differences were observed regarding hepatic triglyceride content, fatty acid oxidation rates or insulin sensitivity. Male rats had higher ratios of insulin to glucagon levels, increased hepatic glycogen content, a lower degree of AMPK phosphorylation, a higher rate of glucose production and higher expression levels of gluconeogenic genes, as compared to female rats. A sex-dependent response to mild starvation was observed, with males being more sensitive. In conclusion, sex-differences reflect a higher capacity of the healthy male rat liver to respond to increased energy demands.

Key words: glucose homeostasis, type 2 diabetes, GK rats, L6 myotubes, hepatic glucose output, insulin sensitivity, sex differences.

ISBN 978-91-7457-483-8