Experimental Models of Diabetes Mellitus Types 1 and 2 in Rats: Regulation of Activity of Glycogen Synthase by Peptides of the Insulin Superfamily and by Epidermal Growth Factor in Skeletal Muscles

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Abstract—The regulatory effect of peptides of the insulin superfamily—insulin, insulin-like growth factor (IGF-1), and relaxin, as well as of epidermal growth factor (EGF) on activity of glycogen synthase (GS) in rat skeletal muscles was studied in normal state and in experimental diabetes mellitus types 1 and 2 (DM1, DM2). Normally, the peptides stimulated GS activity to the maximum at a concentration of $10^{-8}$ M in vitro. The efficiency ranking of the peptide action was as follows: insulin > IGF-1 > relaxin. In DM1 the basal GS activity did not change, while the effect of insulin in vitro decreased more sharply on the 30th day of diabetes as compared to IGF-1 and relaxin, i.e. the efficiency ranking was as follows: IGF-1 = relaxin > insulin. Administration of insulin in vivo did not recover the sensitivity of the enzyme to the action of the hormone in DM1. In DM2, GS activity (both in total and in the active form) decreased while the stimulatory effect of the peptides and EGF on the enzyme was absent. Insulin administered in vivo did not lead to the recovery of the enzyme activity. We conclude that it is insulin resistance pronounced in DM2 that mostly affects the basal GS activity as well as the enzyme regulation by peptides of insulin type and EGF in rat skeletal muscles, while insulin deficiency in DM1 is of lesser importance.

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Key words: glycogen synthase, insulin, insulin-like growth factor-1, relaxin, epidermal growth factor, diabetes mellitus, skeletal muscles, rat.

INTRODUCTION

Peptides of the insulin superfamily, i.e. insulin, insulin-like growth factor-1 (IGF-1), and relaxin, share common evolutionary origin, are structurally and functionally similar, and have several metabolic effects [1, 2]. The main effects of insulin include amplification of glucose uptake, stimulation of glycogen synthesis, and hypoglycemia. Insulin deficiency leads to hyperglycemia—a primary diagnostic sign of insulin-dependent diabetes mellitus, or the type 1 diabetes (DM1). Type 2 diabetes (DM2), also referred to as insulin-independent, and is marked by reduced ability of peripheral target tissues, especially muscles, to respond to normal concentrations of circulating insulin. The primary and most crucial diagnostic sign of DM2 is insulin resistance [3]. IGF-1 mainly exerts growth-stimulating effects by controlling proliferation and differentiation processes and alongside with these processes plays critical role in anabolism. Relaxin is involved in regulation of
reproductive and a number of metabolic processes [1].

Glycogen synthase (GS, EC 2.4.1.11) is an enzyme limiting the synthesis rate of glycogen. GS catalyzes transfer of glucose residue from UDP-glucose to glycogen molecule. Rat skeletal muscles contain GS of the skeletal muscle type, which differs from that found in liver. Activity of the enzyme is regulated by many hormones, predominantly via two main mechanisms: phosphorylation/dephosphorylation and allosterically by glucose 6-phosphate (G6P). Insulin stimulates glycogen synthesis by dephosphorylating and thus activating GS as the enzyme is converted from its inactive and G6P-dependent form GS-D to the active and G6P-independent GS-I [4, 5]. The effects of IGF-1 and relaxin on GS activity remain almost unstudied. In addition to IGF-1, some other growth factors stimulate GS activity and glycogen synthesis in skeletal muscles of rats and humans, e.g., epidermal growth factor (EGF). The use of experimental diabetes mellitus models proved to be fruitful for understanding etiology and pathogenesis of this disease. Streptozotocin (ST) administered to adult rats induces DM1, whereas its injection to newborn rats leads to development of the main symptoms of DM2 as observed in humans [6]. Disturbed glycogen synthesis in skeletal muscles is one of the essential changes in carbohydrate metabolism in diabetes. Patients with DM2 have been found to have an insulin-independent increase of GS phosphorylation [3]. The goal of this study was to evaluate disturbances of hormonal regulation of GS activity under conditions of insulin deficiency and insulin resistance. Our particular tasks were as follows: to determine GS activity in rat skeletal muscles in normal state, in DM1, and in DM2; to compare the effects of peptides of insulin nature and of EGF on activity of the enzyme in control and diabetic animals.

MATERIALS AND METHODS

The experiments were carried out on male Wi- star rats. Used in the study was the muscle tissue (m. gastronomies) by taking into account that skeletal muscles are the primary organ-target for insulin and related hormones.

DM1 was produced by intraperitoneal injection of ST (65 mg/kg) dissolved in acidified (pH 4.5) physiological 0.9% saline directly before the experiment. Control animals were injected with 0.9% saline. Hyperglycemia (20–26 mmol/l), persistent glucosuria (28–56 mmol/l), and polyuria developed in rats 24 h after the ST injection. DM2 (neonatal) was induced by intraperitoneal injection of ST (85 mg/kg) to the 2-day-old rat pups. Control newborn rats were administered with 0.9% saline. DM2 symptoms developed after 2.5–3 months and were revealed by using the glucose tolerance test. Selected for these experiments were the rats with high blood sugar (up to 7–9 mmol/l), in which the glycemia level 2 h after the glucose load did not return to the initial values. During in vivo experiments, insulin was administered intraperitoneally at the dose of 90 ng/g body weight for 30 minutes. Used in the experiments were 5 groups, each composed of 10 animals: control, DM1 at the 7th and 30th day, and DM2 at the 80th and 180th day.

Study of GS was performed in the soluble muscle protein fraction (supernatant) isolated after the 10-min centrifugation of homogenate at 1800 g and 4°C [4]. The GS activity was measured by using the previously described spectrophotometric method [8]. Both the total GS activity (GS-t) in the presence of G6P and the active GS-I form in the absence of G6P were determined. The GS activity was expressed in micromol NAD/mg protein/min or as the ratio of GS-I to the total GS activity (I/t), which indicates the active state of the enzyme.

Protein determination was performed by Brad- ford’s method with use of bovine γ-globulin for construction of calibration curve.

The following chemicals were used in this work: Tris-HCl, G6P, uridine diphosphate glucose, gly- cogen, cysteine, NADH, phosphoenolpyruvate (sodium salt), pyruvate kinase and lactate dehydrogenase (Sigma, USA), EGF (Sigma, USA), recombinant human insulin and IGF-1 (Sigma, USA). Crystalline human relaxin-2 was kindly provided by Dr. Wade (University of Melbourne, Australia). The glycemia level was measured by using the One Touch Ultra kit (LifeScan, Belgium). All results are presented as the mean ± the standard error. Differences between the control and experimental values were estimated by using.