Effect of Peptides of the Insulin Superfamily on Glucose-6-Phosphate Dehydrogenase Activity in Skeletal Muscles of River Lamprey (*Lampetra fluviatilis*) during Prespawning Starvation

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Glucose-6-phosphate dehydrogenase activity in skeletal muscles of the lamprey (*Lampetra fluviatilis*) decreased during prespawning starvation (September-May). The observed changes were particularly pronounced in January. Insulin, insulin-like growth factor 1, and relaxin produce an *in vitro* stimulatory effect on the enzyme. Insulin was most potent in this respect. Inactivation of the enzyme was accompanied by a decrease in its sensitivity to the stimulatory effect of insulin and insulin-like growth factor 1.

Key Words: *glucose-6-phosphate dehydrogenase; insulin; insulin-like growth factor 1; relaxin; lamprey*

River lamprey (*Lampetra fluviatilis*) is a jawless fish, which belongs to the ancient class of vertebrate animals (Agnatha). The life cycle of this fish includes a long period of prespawning starvation (6-8 months). River lampreys migrate from the sea to the river in July-October. They live in the river and do not feed until May, which corresponds to the spawning season. Lampreys die after spawning. Skeletal muscles are the main place for storage of energy substrates to provide vital functions of this fish during the prespawning period. The weight of skeletal muscles is up to 70% of body weight. Vital activity of the lamprey during prespawning starvation requires adaptation of enzyme systems. Studying the hormonal mechanisms of enzyme regulation is an interesting problem.

Glucose-6-phosphate dehydrogenase (G-6-PDH, EC 1.1.1.49) is a key enzyme of the pentose phosphate pathway. This enzyme catalyzes the production of NADPH and ribose-6-phosphate, which serves as a nucleotide precursor in the cell. G-6-PDH couples the metabolism of carbohydrates and fats. Moreover, G-6-PDH is involved in the regulation of growth processes and antioxidant mechanisms [11,13]. Enzyme activity depends on nutrition. Tissue enzyme activity in the lower animals (fishes) and higher animals (mammals) decreases during starvation. Under these conditions, enzyme activity is regulated at the posttranscriptional level [6,12]. Insulin is one of the major hormonal regulators of G-6-PDH activity. Insulin increases activity of this enzyme. Little is known about the effect of insulin-like hormones, including insulin-like growth factor 1 (IGF-1) and relaxin, on enzyme activity. IGF-1 is a growth-stimulating factor, which regulates metabolic processes. Plasma hormone concentration correlates directly with feeding [10]. Relaxin and relaxin-like peptides are abundant in mammals. They were also revealed in invertebrate animals. Relaxin produces a tissue-specific effect on the reproductive system and affects a variety of other tissues, which attests to polyfunctional role of this hormone [9].

Here we studied activity of G-6-PDH and evaluated the regulatory effects of insulin, IGF-1, and

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relaxin on enzyme activity in skeletal muscles of lamprey during prespawning starvation (September, January, and May).

MATERIALS AND METHODS

Lampreys (*Lampetra fluviatilis*, 50-70 g body weight) were caught in the Neva River in September. They were maintained in an aerated-water aquarium at 1-8°C. The study was performed with skeletal muscles from 3 male and female specimens.

G-6-PDH activity was measured as described elsewhere [1]. Skeletal muscles of the lamprey were homogenized in a solution of 50 mM Tris-HCl (pH 8.0) and 0.15 M KCl (1:1). Hormones (10^{-11}-10^{-8} M) were *in vitro* added to the tissue homogenate and incubated at room temperature for 30 min under agitation. Tris-HCl (50 mM, pH 8.0) was added to control samples instead of hormones. G-6-PDH is a cytosolic protein. The supernatant fraction was isolated by centrifugation of the homogenate at 2400*g* for 10 min on ice. The reaction sample (2 ml) contained Tris-HCl (final concentration 50 mM, pH 8.0), $MgCl_2$ (final concentration 1 mM), NADP (final concentration 0.1 mM), glucose-6-phosphate (final concentration 0.2 mM), and supernatant (0.1-0.2 ml). These substrates were not added to the control sample. Enzyme activity was evaluated from the increase in optical density of the mixture during conversion of NADP into NADPH and expressed in μmol NADPH/mg/min. The measurements were performed on a Karl Zeiss spectrophotometer at 340 nm for 10 min. Protein concentration was measured by the method of Bradford using bovine γ-globulin as the standard.

Experiments were performed with recombinant insulin and human IGF-1 (Sigma). Crystalline human relaxin 2 was kindly provided by Dr. Vide (University of Melbourne, Australia). Other reagents were purchased from Sigma.

The results are presented as the mean and standard error of the mean. The control and treatment groups (addition of hormones) were compared by paired Student's test. The differences were significant at $p<0.05$.

RESULTS

G-6-PDH activity in lamprey skeletal muscles during prespawning starvation was 11.5±0.2 μmol/NADPH/mg/min. Previous studies showed that tissue G-6-PDH activity in cold-blooded animals is higher than in warm-blooded animals [1]. Enzyme activity in lamprey is higher than in mammals and birds. For example, G-6-PDH activity in rat and chicken muscles [3] is 10-20% of enzyme activity in lamprey muscles in September. It can be hypothesized that the pentose phosphate pathway in cyclostomes plays an important role in the regulation of metabolism (similarly to other cold-blooded animals).

G-6-PDH activity in lamprey decreased significantly during natural starvation. Enzyme activity in January was one order of magnitude lower than at the beginning of the examined period (0.9±0.1 and 11.5±0.2 μmol NADPH/mg/min). Immediately before spawning (May), activity of G-6-PDH slightly increased and reached 4.0±0.1 μmol NADPH/mg/min (~35% of enzyme activity in September). It was mentioned above that enforced starvation is accompanied by a decrease in G-6-PDH activity in vertebrate animals. Enzyme activity in various tissues of the catfish (*Clarias batrachus*), including the skeletal muscles, decreases most significantly on day 35 of starvation (by 35-45%) [12].

We compared the effects of various peptides on G-6-PDH activity in lamprey. These peptides were shown to increase enzyme activity. Insulin was more potent than IGF-1 and relaxin in increasing G-6-PDH activity. Insulin and IGF-1 in a concentration of 10^{-11} M had the most pronounced effect on enzyme activity in September (Fig. 1, *a*). Relaxin had little effect on G-6-PDH. Enzyme activity was low in January. During this period, the greatest effect of hormones was observed at a much higher concentration (10^{-8} M; Fig. 1, *b*). G-6-PDH activity increased slightly in May. During this period, the greatest stimulatory effect of hormones on G-6-PDH was revealed at lower concentrations (as compared to that in January). The effects of insulin and IGF-1 were most significant at a concentration of 10^{-10} M. Relaxin in a concentration of 10^{-11} M had maximum effect on G-6-PDH (Fig. 1, *c*). Our results indicate that the decrease in G-6-PDH activity in lamprey is accompanied by a reduction of enzyme sensitivity to the stimulatory effect of insulin and IGF-1.

The question arises: what is the physiological role of variations in hormonal regulation with insulin and related hormones under conditions of natural starvation in the lamprey? The concentration of insulin and IGF-1 in the blood of vertebrate animals decreases during starvation. Published data show that blood insulin level and receptor binding of the hormone in skeletal muscles of lamprey decrease during the prespawning period [4]. IGF-1 receptors were found in various tissues of lamprey, including the skeletal muscles [4]. Our experiments showed that the stimulatory effect of insulin and IGF-1 on G-6-PDH became less pronounced in the prespawning period. It should be emphasized that both peptides cannot stimulate glycogen synthetase in skeletal muscles of lamprey during this period. Glycogen synthetase is a rate-limiting enzyme of glycogen synthesis [5]. Our findings and results of

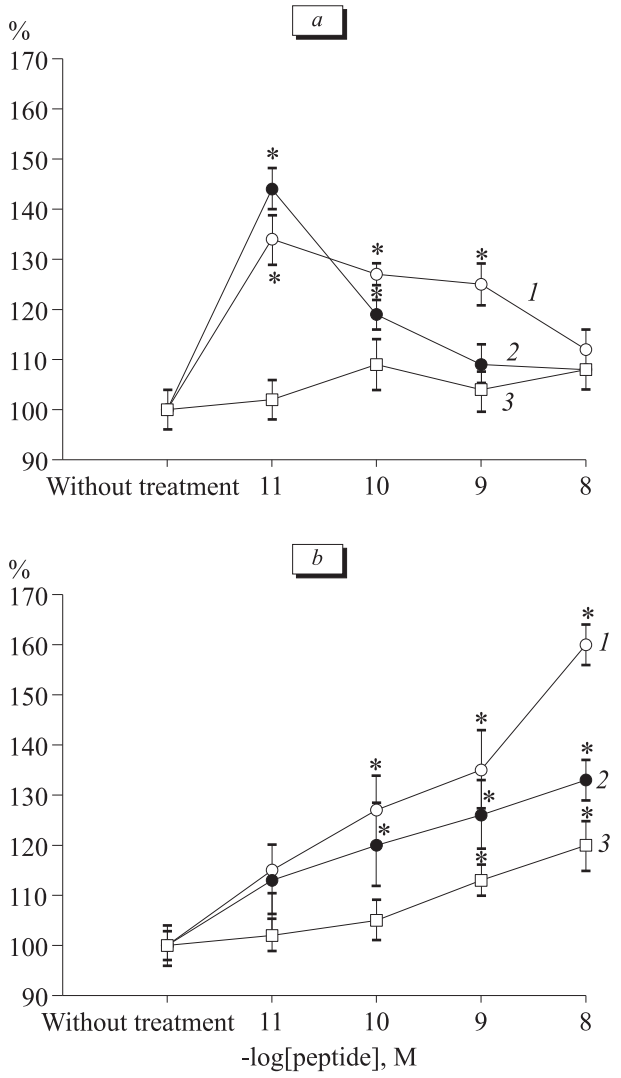

Fig. 1. Effects of insulin, IGF-1, and relaxin on G-6-PDH activity in lamprey skeletal muscles during the prespawning period. (*a*) September; 100%, enzyme activity 11.5±0.2 μmol NADPH/mg/min. (*b*) January; 100%, enzyme activity 0.9±0.1 μmol NADPH/mg/min. (*c*) May; 100%, enzyme activity 4.0±0.1 μmol NADPH/mg/min. Insulin (*1*), IGF-1 (*2*), and relaxin (*3*). *$p<0.05$ compared to enzyme activity without treatment.

previous experiments show that the role of insulin and IGF-1 in the regulation of carbohydrate metabolism in lampreys decreases during starvation.

Much attention was paid to the effect of relaxin on mammalian reproduction [9]. The concentration of endogenous relaxin is associated with the reproductive cycle in the lower animals. For example, plasma relaxin level in male hammerheads (*Sphyrna tiburo*) increases during late gametogenesis and copulation [8]. The functional role of relaxin in cyclostomes remains unknown. The significant increase in the sensitivity of G-6-PDH to relaxin immediately before spawning indicates that the role of this hormone in lamprey skeletal muscles is associated with reproductive function.

There are several mechanisms for the regulation of G-6-PDH activity by insulin peptides. First, insulin increases enzyme activity in some tissues (*e.g.*, in skeletal muscles) due to stimulation of the expression of enzyme mRNA [7]. And second, growth factors (epidermal growth factor and platelet growth factor) affect the intracellular localization of G-6-PDH in renal tissue. The enzyme bound to intracellular structures is translocated into the cytosol. These changes are accompanied by the increase in enzyme activity [11]. Enzyme translocation depends on tyrosine phosphorylation of receptors and activity of phosphatidylinositol-3-kinase. However, our previous studies showed that the increase in enzyme activity due to the intracellular redistribution does not play a role in smooth muscles of the mollusk (*Anodonta cygnea*) and skeletal muscles of chick embryos and chickens [1,3]. The stimulatory effect of relaxin on G-6-PDH was observed in tissues of mammals and invertebrate animals [2]. The mechanism for this effect remains unknown. Further studies are required to evaluate the molecular mechanism of the stimulatory effect of insulin, IGF-1, and relaxin on G-6-PDH.

We conclude that insulin peptides produce a stimulatory effect on G-6-PDH in lamprey skeletal muscles during the prespawning period. These data extend the knowledge on adaptive mechanisms maintaining

cellular homeostasis in anadromous migrants during starvation for several months.

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