BIOGERONTOLOGY

Effect of Tetrapeptide on Insulin Biosynthesis in Rats with Alloxan-Induced Diabetes

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Analysis of amino acid sequences of insulinotropic polypeptides revealed a common short fragment consisting of four amino acid residues. We synthesized $KEDW_a$ tetrapeptide, analog of this fragment protected from the effects of gastrointestinal proteinases. This tetrapeptide partially restored insulin synthesis in rats with alloxan-induced diabetes. The slope of the sugar curve in this case was similar to that in normal animals. Presumably, this tetrapeptide activates the preproinsulin gene promotor site via complementary interactions with the ggcagg and cctgcc nucleotide sequences of the leading strand of double-stranded DNA.

Key Words: *tetrapeptide; diabetes; preproinsulin; gene promotor; complementarity*

Numerous data on modulating effects of short peptides on various body systems, specifically, on the immune and neuroendocrine systems, on the hormonal system of the gastrointestinal tract, were obtained in recent years [1,2]. As a rule, these regulatory peptides (RP) emerge due to specific hydrolysis (processing) of longer peptides. This route rapidly provides RP in a needed place via hydrolysis of inert precursors [4]. Some short peptides are regarded as potential means for the treatment of diabetes mellitus. It is known that pancreatic β-cells produce and release insulin with hypoglycemic effect. Type I (insulin dependent) diabetes mellitus is characterized by insulin insufficiency or complete absence and permanently elevated glucose concentration in the blood.

The gastrointestinal peptide hormone system is a specific endocrine system, which largely independently coordinates the function of the stomach, pancreas, liver, and intestinum. Experimental studies showed that some endogenous peptide hormones activate the pancreas and stimulate the release of insulin (insulinotropic effect) [3,9,12]. For example, gastroinhibitory peptide (GIP) stimulates insulin release when glucose concentration is elevated. Incretin released by the duodenum and jejunum in response to the presence of glucose acts similarly.

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MATERIALS AND METHODS

We attempted to find a structure of a short peptide, which could act as an insulinotropic polypeptide mimetic. To this end we studied amino acid sequence of incretin [10]:

MVALKTCSLL LVLLFLAVGL GEKEEVEFRS HAKFAGPRPR GPRYAEGTFI[50] SDYSIAMDKI RQ QDFVNWLL AQKGK**KNDW**K HNLTQREARA LE LAGQSQRN[100] EEKEAQGSSL PKSLSDEDVL RDL LIQELLA WMADQAELCR LRSQ[144]

The polypeptide chain fragment in incretin composition corresponding to the GIP amino acid sequence is underlined, except arginine residue in position 61, which is substituted by histidine in GIP. Both insulinotropic hormones have a short **KNDW** fragment of 4 amino acid residues in their structure, which was not detected in other human regulatory peptides [7].

Regulatory peptide YG was isolated from *Lophius americanus* pancreatic islets: YPPKPETPGS NASP**E**

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DWASY QAAVRHYVNL ITRQRYG[37]. It contains an **EDW** chain fragment (a variant of **NDW** sequence) in which asparagine residue **N** is replaced by glutamic acid residue **E**.

Considering the possibility of using KNDW short peptide as a natural insulinotropic peptide mimetic, we should remember about its sensitivity to gastrointestinal proteinases.

Trypsin hydrolyzes the peptide bond between lysine (and arginine) and the next amino acid residue, and hence, **KN** bond can be easily hydrolyzed with trypsin because of high local density of positive charge (lysine and asparagine amide group). Replacement of asparagine residue with glutamic acid ensures local neutralization of the charge of lysine side group and protection of **KE** peptide bond from the action of trypsin. However, **WK** and **WA** peptide bond in natural polypeptides is hydrolyzed by chymotrypsin. Chymotrypsin specifically binds large hydrophobic side groups of phenylalanine, tyrosine, and tryptophan and disrupts their bond to the next amino acid residue, and hence, in the gastrointestinal tract **WK** and **WA** peptide bond is hydrolyzed by chymotrypsin. Tryptophan situated at the **KEDW** tetrapeptide terminal is not attacked by chymotrypsin. However, the tryptophan terminal carboxyl group should be amidated for protection from gastric juice elastase.

Hence, **KEDW$_a$** tetrapeptide, a synthetic insulinotropic hormone mimetic, protected from gastrointestinal proteinases, was designed and synthesized by the optimal scheme at Laboratory of Peptide Chemistry (Head: Cand. Chem. Sci. E. I. Grigor'ev) of St. Petersburg Institute of Bioregulation and Gerontology [11]. The biological activity of this tetrapeptide was tested on a model of alloxan diabetes in rats.

RESULTS

Experiment was carried out on 18 outbred male rats (375±35 g). After determination of blood insulin concentrations the animals were randomly divided into 2 groups: control and experimental (8 and 10 animals, respectively). All animals received a single intravenous injection of 1 ml alloxan (35 mg/kg). After 15 days diabetes developed; blood insulin concentration decreased 12-15-fold in comparison with the initial level (Table 1). Controls were then daily intraperitoneally injected with 0.3 ml 0.9% NaCl, experimental animals with KEDW$_a$ tetrapeptide (3 μg in 0.3 ml 0.9% NaCl solution) for 11 days.

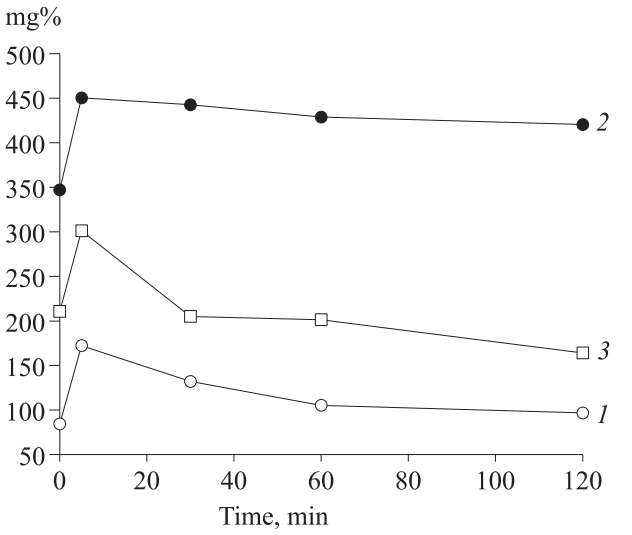

Fig. 1. Blood glucose concentration in rats after its intravenous injection. *1*) intact animals; *2*) rats with alloxan diabetes (control); *3*) rats with alloxan diabetes after treatment with KEDW$_a$ tetrapeptide.

After the end of tetrapeptide treatment blood insulin concentration increased in experimental animals, while in the blood of controls insulin was absent.

The dynamics of glucose assimilation was studied in all animals with alloxan diabetes 44 days after the end of tetrapeptide treatment. To this end the animals were intravenously injected with 1 ml 2% glucose and blood sugar was measured over 2 h. Intact rats with normal blood glucose level comprised control group 2. Two hours after glucose injection to intact rats its concentration decreased to virtually initial level (Fig. 1, *1*). Glucose injected to experimental animals receiving KEDW$_a$ peptide was rapidly assimilated and

TABLE 1. Effect of KEDW$_a$ Peptide on Blood Insulin Level in Rats with Alloxan Diabetes (*M*±*m*, μU/ml)

Group	Initial level	15 days after alloxan injection	Day after the end of tetrapeptide treatment				
			1	9	18	28	44
Control	24.3±2.1 (8)	2.0±0.7 (8)	0 (7)	0 (6)	0 (5)	0 (5)	0 (5)
Experimental (tetrapeptide)	23.8±2.8 (10)	1.5±0.4 (10)	3.6±0.7* (8)	3.2±0.5* (7)	4.3±0.5* (7)	4.1±0.6* (7)	3.9±1.1* (7)

Note. The number of animals is shown in parentheses. *p<001 compared to the control.

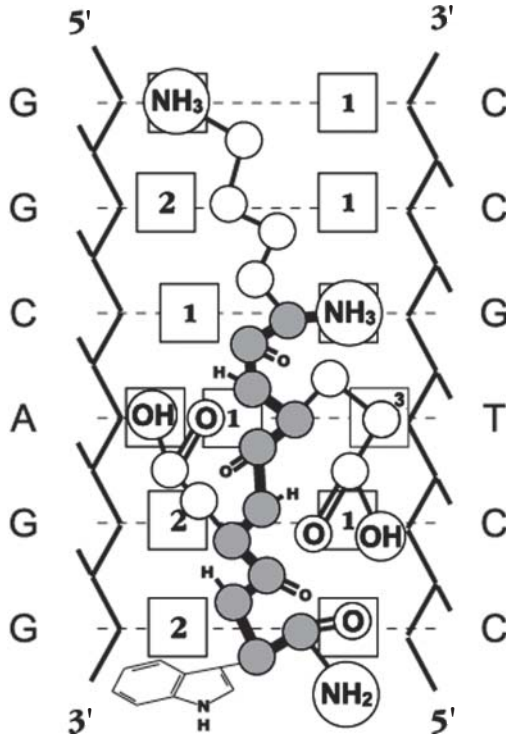

Fig. 2. Complementary binding of $KEDW_a$ tetrapeptide with nucleotide bases in the DNA double strand major groove. 1) adenine and cytosine amino groups; 2) adenine and guanine ^7N atoms; 3) thymine methyl group. Interrupted lines: planes of nucleotide pairs. Dark circles: main peptide chain backbone; light circles: atoms of side groups of amino acid residues. Tryptophan aromatic group is arranged parallel to the guanine plane (perpendicular to the figure plane), as it is oriented by the stacking effect.

after 2 h its concentration was lower than before injection (Fig. 1, *2*). The rate of glucose assimilation in rats with experimental diabetes treated with the peptide was comparable to that in intact animals. In the control group initially elevated blood glucose concentration increased after injection of glucose and then decreased very slowly, not reaching the initial level (Fig. 1, *3*).

The mechanism of experimental diabetes mellitus is as follows. By its structure alloxan is close to natural pyrimidine bases uracyl and thymin, therefore it can compete with natural nucleotide bases, incorporate (by the intercalation mechanism) into RNA and DNA structures, and inhibit protein (including insulin) synthesis. A possible mechanism of induction of insulin biosynthesis by $KEDW_a$ peptide seems to be caused by interaction of this tetrapeptide with the preproinsulin gene promotor site. Tetrapeptide binding to the major groove of DNA double strand in the promotor site of the gene can presumably lead to competitive subsiding of alloxan by the tryptophan residue hydrophobic side group. Preproinsulin is a product of this gene translation. Signal peptide of this molecule facilitates membrane transport of insulin precursor and is cleaved after transfer. The resultant proinsulin molecule contains peptide chains A and B connected by site C. After enzymatic removal of this site disulfide bonds specific of natural insulin form between chains A and B.

Using a previously developed method for evaluation of complementary interactions of short peptides with nucleotide sequences of DNA double strand, we determined nucleotide sequence capable of complementary binding the $KEDW_a$ tetrapeptide in the DNA major groove [5,8]. This sequence consists of 6 b. p. ggcagg and **cctgcc** in the preproinsulin gene DNA leading chain [13], the promotor site of which (2423 b. p.) contains 14 such sites, which gives sufficient grounds for experimental verification of the hypothesis. The projection of the tetrapeptide side groups, reacting with functional groups of ggcagg nucleotide sequence of DNA double spiral, is presented (Fig. 2).

Hence, the designed tetrapeptide $KEDW_a$ can initiate gene transcription and insulin synthesis in rats with alloxan diabetes. This will enable the development of new drugs for the treatment of patients with diabetes mellitus.

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