Effect of Tetrapeptide on Insulin Biosynthesis in Rats with Alloxan-Induced Diabetes
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Analysis of amino acid sequences of insulinotrophic polypeptides revealed a common short fragment consisting of four amino acid residues. We synthesized KEDW, 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 promoter site via complementary interactions with the ggcgg and cctgcc nucleotide sequences of the leading strand of double-stranded DNA.

Key Words: tetrapeptide; diabetes; preproinsulin; gene promoter; 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.

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]:

\[
\text{MVALKTCSLL LVLFLAVGL GEKEEEVEFRS HAKFAGPRPR GPRYAEGETEI\text{SDYSIAMDKLRO}}
\]
\[\text{QDFVNNLLL AQKGGKKNWDW HNLTQREARA LE LAGQSQRN}^{109} \text{EEKEAQGSSL PKSLSDEVL RDL LIQELLA WMADQCAELCR LRSQ}^{144}\]

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 NASF

DWASY QAAVRHYVNL ITRQRYG. 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, KEDWa 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 KEDWa tetrapeptide (3 μg in 0.3 ml 0.9% NaCl solution) for 11 days.

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, I). Glucose injected to experimental animals receiving KEDWa peptide was rapidly assimilated and

![Fig. 1. Blood glucose concentration in rats after its intravenous injection. I) intact animals; 2) rats with alloxan diabetes (control); 3) rats with alloxan diabetes after treatment with KEDWa tetrapeptide.](image)

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial level (μU/ml)</th>
<th>15 days after alloxan injection</th>
<th>Day after the end of tetrapeptide treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>24.3±2.1 (8)</td>
<td>2.0±0.7 (8)</td>
<td>0</td>
</tr>
<tr>
<td>Experimental</td>
<td>23.8±2.8 (10)</td>
<td>1.5±0.4 (10)</td>
<td>3.6±0.7* (8)</td>
</tr>
</tbody>
</table>

Note. The number of animals is shown in parentheses. *p<0.001 compared to the control.
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 KEDW₆ tetrapeptide in the DNA major groove [5,8]. This sequence consists of 6 b. p. gccagg and cctgcc in the preproinsulin gene DNA leading chain [13], the promoter 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 gccagg nucleotide sequence of DNA double spiral, is presented (Fig. 2).

Hence, the designed tetrapeptide KEDW₆ 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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REFERENCES