An antidiabetic effect of the new nerve growth factor (NGF) dipeptide mimetic GK-2 has been shown in rats with streptozotocin-induced diabetes. The peptide has a significant antihyperglycemic effect in the course of treatment: intraperitoneal injection at a dose of 0.5 mg/kg for two weeks before and four weeks after the toxin injection. GK-2 also completely prevents weight loss in rats caused by streptozotocin.

The prevalence of diabetes mellitus in the world is doubling every 10–15 years getting the character of a noninfectious epidemic. In Russia, 3,121,318 cases were registered on January 1, 2012, which is 2156 cases per 100,000 people [1]. Existing methods of therapy of diabetes mellitus are only symptomatic; therefore, searching for innovative tools that would not only hamper the development of the disease, but also eliminate its cause, is of great importance.

Nerve growth factor has a high therapeutic potential for the treatment of diabetes mellitus and its complications. It is known that NGF plays an important role in the differentiation and support of the functioning of beta cells of the pancreas [2]. Beta cells of the pancreas synthesize and secrete NGF, which stimulates the secretion of insulin through autocrine regulation; synthesis and secretion of NGF by beta cells are increased in response to an increasing concentration of extracellular glucose [3]. NGF and insulin secreted by beta cells are necessary for their survival. These properties of NGF (maintaining the viability and function of beta cells, as well as the ability to stimulate insulin secretion) determine its possible potential for the treatment of both type 1 and type 2 diabetes mellitus.

In the model of streptozotocin diabetes, it has been shown that NGF gene therapy prevents the development of diabetic neuropathy in mice [4], and, when administered in the form of eye drops, NGF prevents degeneration of the retina of diabetic rats [5].

Clinical trials of NGF in diabetic neuropathy patients were discontinued due to the development of significant side effects (pain at the injection site) and weak positive effects of the treatment [6]. The use of NGF in the clinic is limited because of a wide range of biological activity and poor pharmacokinetic properties.

In the Zakusov Institute of Pharmacology of the Russian Academy of Medical Sciences, the dimeric dipeptide mimetic GK-2 (hexamethylene diamide bis-(N-monosuccinyl glutamyl lysine)) was created on the basis of the β-bend of the NGF fourth loop [7]. It was shown that the peptide GK-2 had a high NGF-like neuroprotective activity at nanomolar concentrations in vitro [7] and was efficacious in in vivo models of Parkinsonism [8] and brain stroke [9].

The goal of this study was to investigate the effect of GK-2 on the blood glucose level in rats with experimental diabetes induced by intraperitoneal injection of streptozotocin. Streptozotocin diabetes is a common experimental model of diabetes, since streptozotocin injection allows to simulate both constantly evolving dysfunction of pancreatic beta cells, and impaired glucose tolerance, as well as the development of associated disorders [10].

NGF mimetic GK-2 was synthesized in the Zakusov Institute of Pharmacology, Russian Academy of Medical Sciences. Streptozotocin was obtained from Sigma (United States). A OneTouch Ultra blood glucose meter (United States) was used for measurements of the glucose level.

Experiments were performed on male Wistar rats weighing from 300 to 380 g obtained from the Stolbovaya Nursery of the Russian Academy of Medical Sciences. The animals were kept with free access to food and water and natural light regime. The work was performed according to the requirements of the European Community Council 86/609/EEC regulations on the use of animals for experimental research. The experimental animals were divided into three groups: (1) a passive control group (n = 12), (2) an active control group (n = 12), and (3) an active treatment group (n = 12), all receiving GK-2 treatment.
control group (streptozotocin diabetes, STZ) \((n = 3)\), and (3) an experimental group (streptozotocin + injection of GK-2, STZ+GK-2) \((n = 13)\).

Diabetes mellitus was simulated by intraperitoneal injection of streptozotocin (40 mg/kg). The passive control group, instead of streptozotocin, was injected with physiological saline. During two weeks before and four weeks after the streptozotocin injection, animals of the experimental group were injected daily with an aqueous solution of GK-2 (0.5 mg/kg, i.p.). The groups of active and passive control, instead of GK-2, were injected with distilled water. Immediately prior to streptozotocin injection and on days 3, 17, and 28 after injection, the groups of active and passive control, instead of GK-2, were injected with distilled water. Immediately prior to administration of streptozotocin, and on days 3, 17, and 28 after the injection of streptozotocin, blood from the tail vein was collected for glucose level determination. The mass of the rats was measured every three or four days.

In order to assess the statistical significance of the inter-group differences, the Kruskal–Wallis test was used, followed by comparison of the samples using the Mann–Whitney U test and Fisher’s exact test. The results were considered significant at \(p < 0.05\). The data were presented as medians and interquartile ranges of samples. The therapeutic effect of the dipeptide GK-2 was calculated using the formula

\[
\frac{b - a}{b - c} \cdot 100\%.
\]

where \(a\) is the value in the STZ+GK-2 group, \(b\) is the value in the STZ group, and \(c\) is the value in the control group.

During two weeks before streptozotocin injection, there were no differences between the experimental groups in the body weight or blood glucose level; i.e., chronic administration of GK-2 to healthy rats had no effect on these parameters. Streptozotocin induced reduction of the body weight gain and marked hyperglycemia in rats. The GK-2 dipeptide completely prevented the decrease in the body weight gain in rats (Fig. 1).

Hyperglycemia in rats with experimental diabetes was detected on the third day and was retained within four weeks after injection of streptozotocin.

On the third day after the administration of streptozotocin, GK-2 significantly reduced the blood glucose level with a therapeutic effect of 60%. On the 17th day after injection of streptozotocin, the GK-2 reduction of the blood glucose level was more pronounced: the therapeutic effect was 80%. On the 28th day after injection of streptozotocin, the antihyperglycemic effect of GK-2 was also 80% (Fig. 2).

It is known that NGF is involved in the pathogenesis of streptozotocin diabetes: intraperitoneal injection of streptozotocin in rats causes an increase in the