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PHARMACOLOGY AND TOXICOLOGY

Antidiabetic Properties of Low-Molecular-Weight BDNF Mimetics Depend on the Type of Activation of Post-Receptor Signaling Pathways

R. U. Ostrovskaya, S. S. Yagubova, T. A. Gudasheva, and S. B. Seredenin

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Reduced proliferation and enhanced apoptosis of β cells in diabetes mellitus are associated with a deficiency of brain-derived neurotrophic factor (BDNF). Low-molecular weight compounds similar to different BDNF loops were synthesized at the V. V. Zakusov Research Institute of Pharmacology. They produce a potentiating effect on TrkB phosphorylation, but differently activate post-receptor signaling pathways. We compared their effects on the severity of streptozotocin-induced diabetes mellitus in C57Bl/6 mice. The antidiabetic effect (estimated from the degree of hyperglycemia and dynamics of body weight) was typical of GSB-214 compound that selectively activates PI3K/Akt. This activity was not revealed in GTS-201, selective activator of MAPK/Erk. GSB-106 compound activating both signaling pathways exhibited weak antidiabetic activity. Our results indicate that the antidiabetic effect is mainly related to activation of the PI3K/Akt signaling pathway.

Key Words: *diabetes; C57Bl/6 mice; streptozotocin; low-molecular-weight BDNF mimetics*

Previous experiments showed that brain-derived neurotrophic factor (BDNF) is synthesized and released by pancreatic β cells and produces the insulinotropic effect [15]. The decrease of endogenous BDNF in diabetic patients is followed by suppression of proliferation and increase in β cell apoptosis. A correlation was found between the decrease in plasma BDNF level and degree of insulin resistance [12]. Intracerebral infusion of BDNF is accompanied by the decrease in food consumption and blood glucose concentration [7]. Little is known about the effects of systemic treatment with BDNF. Subcutaneous injection of BDNF in a dose of 20 mg/kg for 8 weeks was shown to decrease the concentration of glucose in db/db mice with genetically determined obesity and diabetes mellitus [14]. Other experiments showed that subcutaneous injection of BDNF in a dose of 20 mg/kg potentiates the hypoglycemic effect of insulin in C57Bl/6Ncrj mice with streptozotocin-induced diabetes [13]. These data indicate that BDNF holds much promise for the therapy of type 2 diabetes mellitus. However, native molecule of neurotrophin cannot be used for therapy due to inappropriate pharmacokinetic properties and pleiotropic activity, which may cause a variety of side effects.

BDNF interacts with TrkB receptors, which results in their dimerization and autophosphorylation. This process is followed by activation of the following related signaling pathways: MAPK/Erk, which includes mitogen-activated protein kinases (MAPK) and extracellular signal-regulated kinases (Erk); and PI3L/Akt, which includes phosphatidylinositol 3-kinase and Akt proteinase [7].

V. V. Zakusov Research Institute of Pharmacology, Moscow, Russia. ***Address for correspondence:*** rita.ostrovskaya@gmail.com. R.U. Ostrovskaya

Previous studies at the V. V. Zakusov Research Institute of Pharmacology allowed us to hypothesize that some loops of neurotrophins can activate various pathways for signal transduction [2]. Low-molecular-weight dimeric dipeptide mimetics for different BDNF loops were synthesized to confirm this hypothesis [3]. It was shown that these compounds cause phosphorylation of TrkB and selectively activate the signaling pathways [1,4]. Moreover, some compounds stimulate neurogenesis in the hippocampus of mice [9].

This work was designed to compare the effects of dimeric dipeptide mimetics of BDNF, which differently modulate post-receptor processes, on the diabetogenic activity of streptozotocin.

MATERIALS AND METHODS

Experiments were performed on C57Bl/6 mice weighing 23-28 g and obtained from the Stolbovaya nursery. The animals were maintained in a vivarium under standard conditions and had free access to food (except for 16 h prior to administration of streptozotocin) and water. The study was conducted in accordance with the Directive 86/609/EEC on the protection of animals (Council of the European Communities). The experimental design was approved by the Biomedical Ethics Committee of the V. V. Zakusov Research Institute of Pharmacology.

The diabetogenic toxin streptozotocin (STZ, Sigma) in a dose of 100 mg/kg was dissolved in citrate buffer (pH 4.5) and injected intraperitoneally to animals after 16-h starvation to induce the development of type 2 diabetes. This dose was selected as the most effective for C57Bl/6 mice [10].

We studied the effects of GSB-214 (hexamethylenediamine bis-(N-monosuccinyl-L-methionyl-L-serine), GTS-201 (hexamethylenediamine bis-(N-hexanoyl-L-seryl-L-lysine), and GSB-106 (hexamethylenediamine bis-(N-monosuccinyl-L-seryl-L-lysine). These compounds are dimeric dipeptide mimetics of loops 1, 2, and 4 of BDNF, which activate various signaling pathways. They were synthesized at the Department for Chemistry of Medicinal Products as described previously [3].

Experiments were performed on mice (n=62) divided into 5 groups. Group 1 animals (passive control, n=10) received daily intraperitoneal injections of distilled water (DW) for 31 days. Group 2 animals (active control, n=13) received a single injection of STZ after intraperitoneal treatment with DW for 14 days. Administration of DW was continued for the next 16 days. The animals of treatment groups 3, 4, and 5 received GSB-106, GSB-214, and GTS-201, respectively, in the prophylactic-and-therapeutic regimen (before and after injection of STZ). These compounds in a daily dose of 0.5 mg/kg were injected intraperitoneally for 14 days. STZ was administered 30 min after the last treatment with test compound on day 15. The compounds were repeatedly injected for the next 16 days.

Each treatment group consisted of 13 animals. The solutions of the test compounds were prepared daily. DW served as a solvent.

Blood samples were obtained from the caudal vein. Blood glucose level was measured with a One Touch Ultra glucometer before injection of STZ and on days 5, 12, 20, 30, and 60 after treatment. The animals were weighted at 3-4-day intervals.

The results were analyzed with Statistica 8.0 software. The statistical significance of between-group differences in study parameters was estimated by nonparametric Mann—Whitney U test. The differences were significant at $p<0.05$. The data are presented as the means and standard errors of the means ($M\pm SEM$).

The dynamic effect of test compounds was evaluated from the relative antihyperglycemic activity (Ag) as follows:

$$Ag=glSTZ-(glSTZ+compound)/glSTZ-glDW\times 100\%,$$

where glSTZ is blood glucose level in group 2 (active control); glSTZ+compound is blood glucose level in treatment groups 3, 4, and 5; and glDW is blood glucose level in group 1 (passive control).

RESULTS

Preventive treatment with the test compounds for 14 days had no effect on blood glucose level in mice (in comparison with the passive control group). Injection of diabetogenic toxin STZ in a dose of 100 mg/kg was followed by hyperglycemia. Blood glucose concentration in group 2 animals (active control) increased to 17-24 mmol/liter (Fig. 1) and remained unchanged for 60 days of the study.

Injection of GSB-214 attenuated the degree of hyperglycemia in mice. The effect of this compound was observed starting from the 5th day after STZ administration. Blood glucose level in these animals was similar to that in specimens of the passive control group. The antihyperglycemic effect of test compound was revealed not only during therapy, but also for a long period after drug withdrawal. Antihyperglycemic activity of GSB-214 remained high even on day 44 of the study.

The antihyperglycemic effect of GSB-106 was observed only on day 12 after treatment with STZ (day 27 of therapy) and persisted for a short period (day 20 after injection of STZ).

GTS-201 had little antihyperglycemic effect in mice. Blood glucose level in mice of this group on

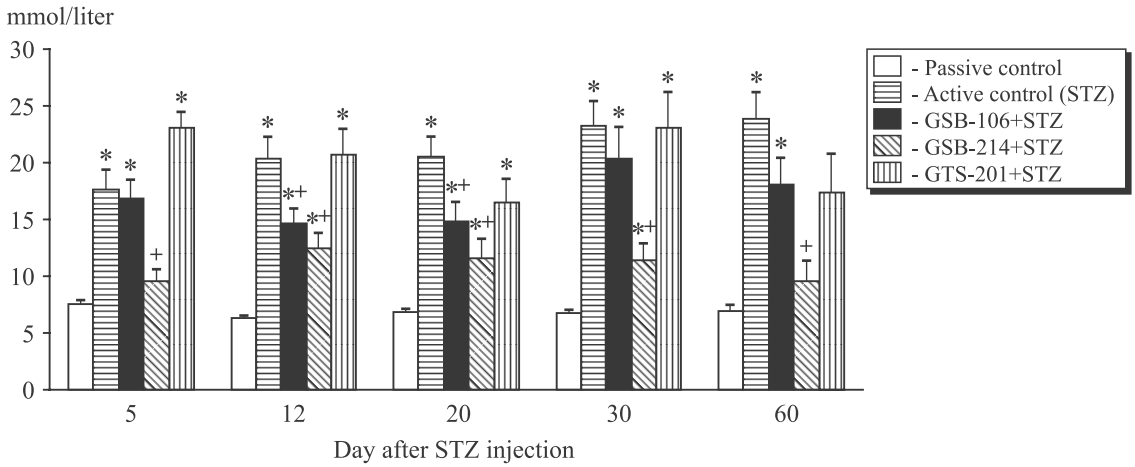

Fig. 1. Blood glucose level in mice after STZ injection. Here and in Fig. 2: $p<0.05$ in comparison with *passive control group (DW), +active control group (STZ).

days 5 and 12 after injection of STZ was higher than in group 2 animals (active control; Fig. 1).

The dynamics of the relative antihyperglycemic activity for test compounds is compared in Table 1.

Body weight loss (or delay in body weight gain) is an important criterion for the diabetogenic effect of STZ. Body weight in mice of the passive control group progressively increased, which reached 15% by the end of observations (in comparison with the baseline level). Body weight of untreated diabetic mice was shown to decrease initially, but slowly increased in the follow-up period (7% gain by the end of observations). Body weight gain in the group of diabetic mice receiving GSB-214 was 16%. Therefore, body weight in these animals was completely recovered to the passive control level. Body weight in mice receiving GSB-106 and GTS-201 was increased by 11 and 5%, respectively, compared to the level observed before treatment with STZ (Fig. 2).

Our results indicate that GSB-214 exhibits a high antidiabetic activity. The effect of this compound persists even after therapy withdrawal. It should be emphasized that subcutaneous treatment with the native BDNF molecule in a dose of 20 mg/kg produces the antihyperglycemic effect in mice with STZ-induced diabetes [13]. GSB-214, a mimetic of DBNF loop 1, was effective in a 40-fold lower dose. The efficiency of GSB-106 was lower than that of GSB-214 compound. GTS-201 compound did not attenuate, but even potentiated the hyperglycemic effect of STZ at the beginning of observations.

Phosphorylation of TrkB and Akt and Erk kinases was studied on hippocampal HT-22 neurons by the Western blot analysis with monoclonal antibodies. The dipeptide mimetic of BDNF loop 1, GSB-214, activated only one signaling pathway (PI3K/Akt) [4,8]. The dipeptide mimetic of BDNF loop 4, GSB-106, was shown to produce an activating effect on the Trk-

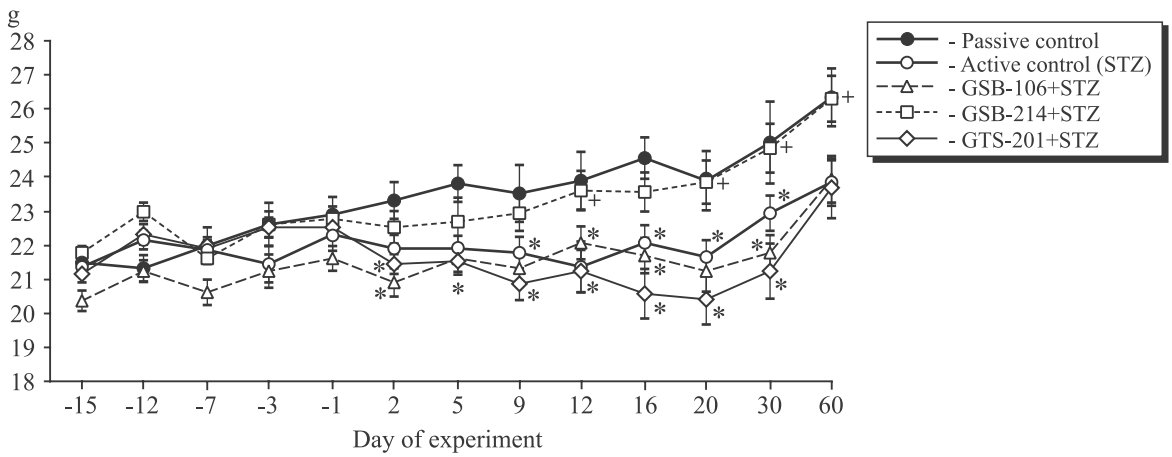

Fig. 2. Dynamics of body weight in mice. Day "0": STZ injection.

TABLE 1. Criterion for Antihyperglycemic Activity (Ag, %) of BDNF Mimetics

Group	Period of study				
	day 5 after STZ (day 20 of therapy)	day 12 after STZ (day 27 of| therapy)	day 20 after STZ (day 4 after therapy withdrawal)	day 30 after STZ (day 14 after therapy withdrawal)	day 60 after STZ (day 44 after therapy withdrawal)
3 (GSB-106)	7.65	40.59	41.76	17.74	34.26
4 (GSB-214)	80.02	55.91	65.20	72.15	84.49
5 (GSB-201)	-54.07	-2.64	29.67	1.51	38.28

B receptor and its signaling pathways (MAPK/Erk and PI3K/Akt) [8]. The dipeptide mimetic of BDNF loop 2, GTS-1, activated only the MAPK/Erk pathway [4].

Our previous studies on C57Bl/6 mice [5] and rats [6] revealed an antihyperglycemic effect of GK-2, a mimetic of NGF loop 4 that activates only one of the TrkA-related signaling pathways (PI3K/Akt) [1]. Published data show that this pathway is mainly involved in the neuroprotective effects of neurotrophins [11].

We conclude that the antihyperglycemic activity is typical of GSB-214 (mimetic of BDNF loop 1) and GK-2 (mimetic of NGF loop 4) [5], which selectively activate the same signaling pathway of PI3K/Akt. The antihyperglycemic effect is not produced by GTS-201 (mimetic of BDNF loop 2) that selectively activates the MAPK/Erk pathway. Therefore, the antidiabetic effect of BDNF is mainly related to activation of the PI3K/Akt signaling pathway. This pathway provides the epigenetic effects, which are related to the survival of pancreatic β cells [7].

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