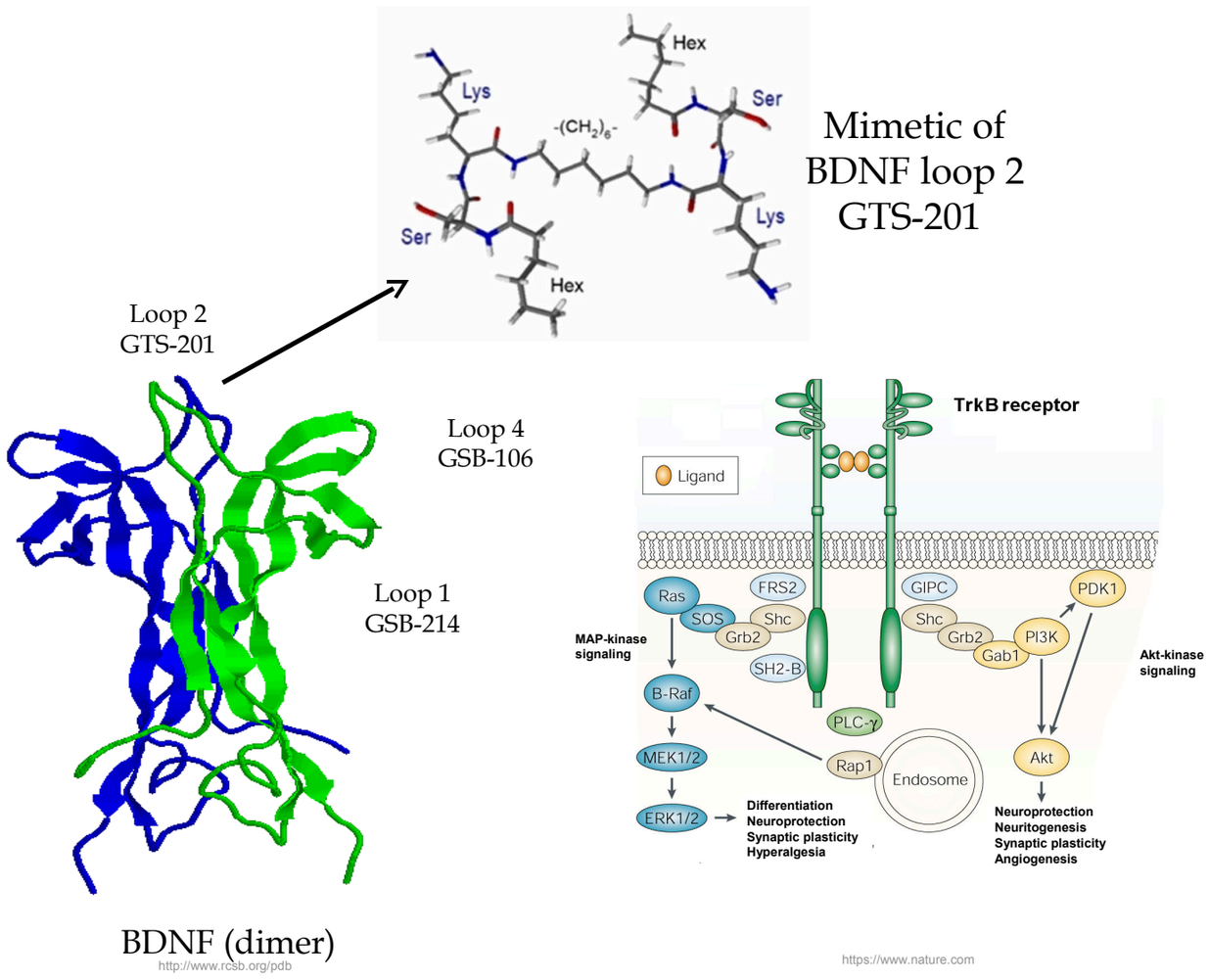
P.337

Low-molecular mimetic of BDNF loop 2 protected neurons against oxidative stress via the TrkB and MAPK/Erk activation

31ST ECNP CONGRESS 6-9 OCTOBER 2018 BARCELONA

Logvinov I.O., Antipova T.A., Tarasiuk A.V., Kruglov S.V., Gudasheva T.A., Seredenin S.B.
Zakusov Institute of Pharmacology, Moscow, Russia

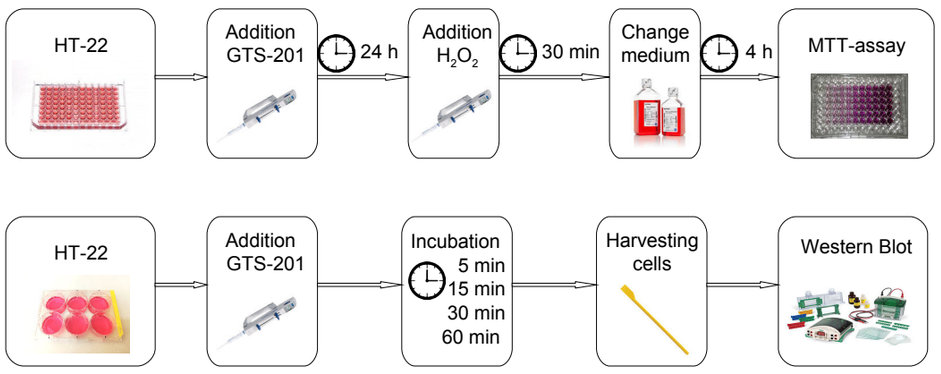


Purpose

Neurotrophins play an important role in the development of the nervous system, by influencing cell survival, differentiation and cell death. Brain derived neurotrophic factor (BDNF) belongs to the neurotrophin family. Desregulation of BDNF is involved in the pathogenesis of many neurodegenerative diseases.
It has been shown that BDNF rescues different types of neurons from ischemic, traumatic and toxic brain injury. The neuroprotective effect of BDNF are mediated through the activation of the TrkB neurotrophin receptors which causes to dimerization, autophosphorylation, and activation of two main signaling pathways phosphatidylinositol 3-kinase/AKT (PI3K/Akt) and mitogenactivated protein kinase/extracellular-signal-regulated kinases (MAPK/Erk) pathways. Unfortunately, the pharmacological application of BDNF is limited its rapid degradation in the organism, low ability to penetrate the blood-brain barrier, and undesirable side effects. Therefore, in order to develop neuroprotective drugs, it seems urgent to synthesize low-molecular mimetics of BDNF, which are capable of activating the TrkB receptor signaling pathways and have no disadvantages of full-length BDNF protein. In Zakusov Institute of Pharmacology was synthesized low-molecular dipeptide mimetic of BDNF – **GTS-201** (bis-(N-Hexanoyl-L-seril-L-lysine) hexamethylenediamide). GTS-201 was constructed on the basis of the β-turn of the second loop of BDNF. The objectives of this study were to explore neuroprotective properties of GTS-201 in model of oxidative stress in hippocampal neurons line HT-22 and the role of the two major signaling pathways activated via specific TrkB receptors in neuroprotective action of GTS-201.

Methods

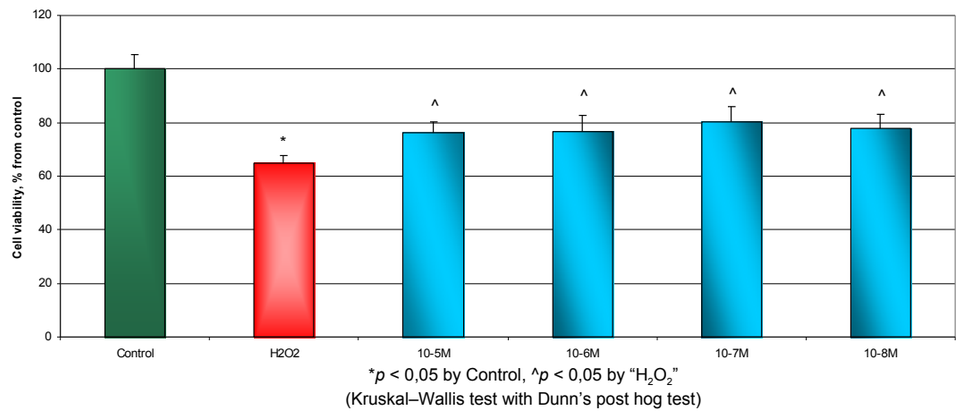
Experiments were performed on hippocampal neurons line HT-22. Cells were maintained at 37°C, 5% CO_2 in DMEM (Dulbecco's modified Eagle's medium) containing 5% FBS (fetal bovine serum). Oxidative stress was performed by addition H_2O_2 (1,5 mM). GTS-201 was added 24 hours before cell damage in range concentrations 10^{-5}-10^{-8}M. Cell viability was routinely assayed at 37°C, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The activation of TrkB, Erk, and Akt by GTS-201 was assessed by Western blot analysis using specific antibodies against the phosphorylated and non-phosphorylated forms of these kinases. Cells were collected 5, 15, 30 and 60 min after incubation with GTS-201. The Mann–Whitney U test and Kruskal–Wallis test with Dunn's post hog test were used. The data are presented as means ± standard deviation (SD). P-values <0.05 were considered significant.



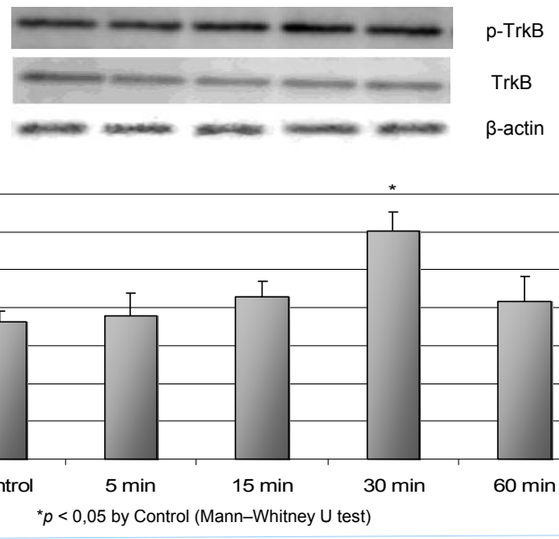
Results

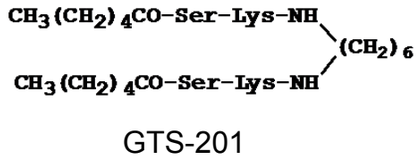
GTS-201 significantly ($p<0.05$) protected the cells from damage in the model of oxidative stress. Most effective was dipeptide concentration 10^{-7}M. We found that GTS-201 (10^{-7}M) activated TrkB receptors 30 min after its addition to the incubation medium (Control 4.6±0.38 RDU (Relative Density Units), GTS-201 7.6±0.61 RDU). In addition, the dipeptide mimetic of BDNF GTS-201 also increased the phosphorylation of Erk kinases 5 min after its addition to the incubation medium (Control 4.4±0.21 RDU, GTS-201 5.1±0.06 RDU). However, the activation of Akt kinases was not detected in any time intervals after the addition of GTS-201.

Neuroprotective effect of GTS-201 in model of oxidative stress. MTT assay.

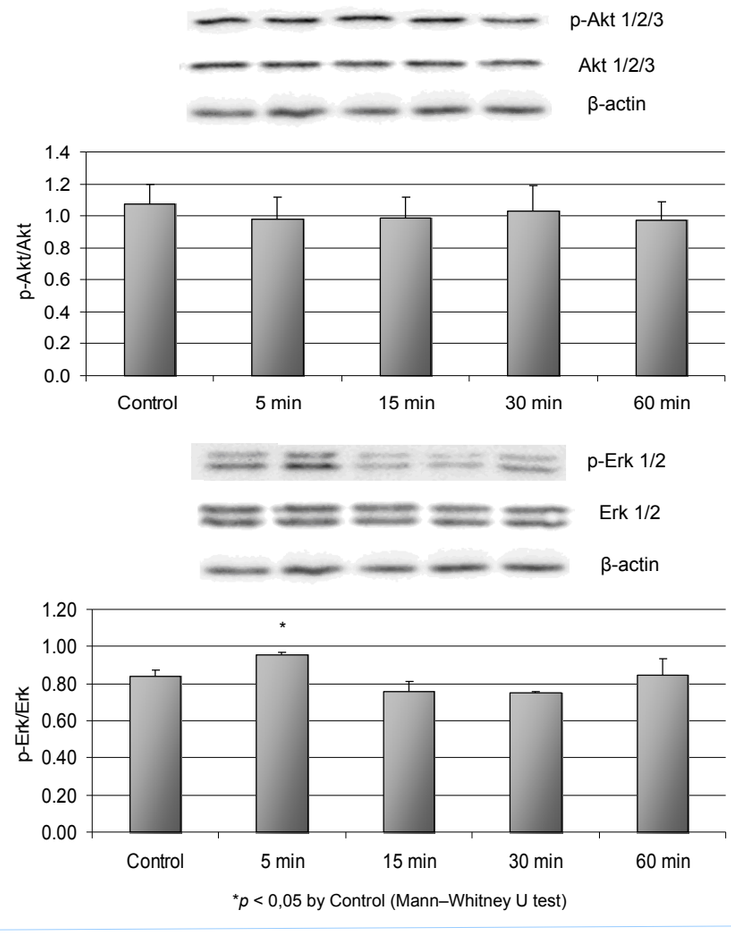


The ratio of p-TrkB to total TrkB in cultured HT-22 cells incubatted with GTS-201 (10-7M)
Western blot



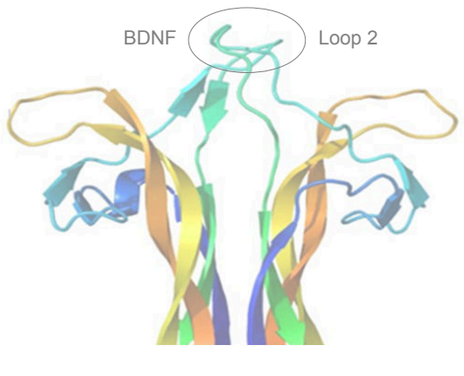


The ratio of p-Akt, p-Erk to total Akt, Erk in cultured HT-22 cells incubatted with GTS-201 (10-7M)
Western blot



Conclusions

Mimetic of BDNF loop 2 GTS-201 have neuroprotective properties and activates TrkB and MAPK/Erk signaling pathway, which are specific for the full-length protein, but does not activate the PI3K/Akt signaling pathway.



This work was supported by the Russian Science Foundation (projects number 14-15-00596)
Competing interests: The authors declare that they have no competing interests.

Contacts: logvinov@academpharm.ru