Effects of Delta Sleep-Inducing Peptide on Pre- and Postsynaptic Glutamate and Postsynaptic GABA Receptors in Neurons of the Cortex, Hippocampus, and Cerebellum in Rats

V. V. Grigor'ev, T. A. Ivanova, E. A. Kustova, L. N. Petrova, T. P. Serkova, and S. O. Bachurin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 8, pp. 149-151, August, 2006
Original article submitted September 14, 2005.

We studied the effect of delta sleep-inducing peptide on GABA receptors of hippocampal and cerebellar neurons in rats. It was shown that delta sleep-inducing peptide considerably and dose-dependently potentiates GABA-activated currents in these neurons and blocks NMDA-activated potentiation in cortical and hippocampal neurons. The peptide modulates activity of presynaptic NMDA receptors, which is seen from changes in $^{45}Ca^{2+}$ uptake into synaptosomes of the brain cortex after uptake stimulation with glutamate and NMDA.

Key Words: *delta sleep-inducing peptide; GABA receptors; NMDA receptors; $^{45}Ca^{2+}$ uptake into synaptosomes*

Delta sleep-inducing peptide (DSIP) was isolated from venous blood of animals under conditions of artificial sleep [9]. Injection of this peptide to other animals induced sleep [10]. Further experiments showed that injection of DSIP to experimental animals blocks N-methy-D-aspartate-induced convulsions [11]. DSIP blocked the increase in neuronal activity caused by microionophoretic application of glutamate [4]. Activation of GABA-ergic neurons in certain brain structures is a final phase of sleep onset, while other neurotransmitter systems (in particular, serotoninergic, cholinergic, and histaminergic systems) play an important role in the regulation of the sleep—wake cycle [5,8,12]. It is still unknown whether or not DSIP affects the GABA-ergic transmitter system in the CNS. In light of this, the study of the mechanisms underlying the effects of DSIP and other hormones and peptides on neuronal receptors is still a pressing problem.

Laboratory of Neurochemistry, Institute of Physiologically Active Substances, Russian Academy of Sciences, Chernogolovka

Here we studied the effects of DSIP on NMDA- and GABA-activated currents in cortical, hippocampal, and cerebellar neurons of rat brain and on $^{45}Ca^{2+}$ uptake into synaptosomes of rat brain cortex under conditions of glutamate and NMDA stimulation.

MATERIALS AND METHODS

Experiments were performed on cultured rat hippocampal neurons. Neurons were isolated from hippocampus of newborn rats (1-2 days) by trypsinization followed by pipetting. The cell suspension in culture medium (3 ml) was transferred into wells of a 6-well plate (Nunc) or Petri dishes with poly-L-lysine-coated coverslips. Cell concentration was 2.5×10^{-6}-5×10^{-6} cell/ml. The culture medium consisted of minimal Eagle medium and DME/F12 (1:1) supplemented with 10% FCS, 2 mM glutamine, 50 μg/ml gentamicin, 15 mM glucose, and 20 mM KCl, pH was brought to 7-7.4 with $NaHCO_3$. The plates were placed in a CO_2-incubator at 37°C

0007-4888/06/1422-0186

and 100% humidity. On day 2-3 of culturing, 10-20 μmol/ml cytosine-arabinoside was added. On days 6-7 in culture, 1 mg/ml glucose was added or the medium was replaced, depending on the subsequent experiment. Some experiments were carried out on freshly-isolated Purkinje neurons from the cerebellum of 12-17-day-old rats or cortical neurons obtained from 7-9-day-old rats (enzyme and mechanical treatment). Transmembrane currents induced by application of NMDA or GABA were recorded by the whole-cell patch-clamp method [7] using EPC-9 (HEKA) devise. The substances were applied by the method of rapid superfusion.

Synaptosomes were isolated from the cortex of newborn (9-day-old) Wistar rats by routine method proposed by Hajos [6]. For accumulation of radioactive label, P_2 fraction of synaptosomes was suspended in an incubation buffer A (132 mM NaCl, 5 mM KCl, 5 mM HEPES, pH 7.4, protein concentration 1.5-2.0 mg/ml). The uptake of $^{45}Ca^{2+}$ into synaptosomes was stimulated with glutamate (200 μM) or NMDA (200 μM NMDA+5 μM glycine). After 3-min incubation with NMDA receptor agonist at 37°C, the uptake was stopped by filtering through GF/B fiberglass filters (Whatman) and the filters were washed 3-fold with cold buffer B (145 mM KCl, 10 mM Tris, and 54 mM Trilon B, pH 7.4). Radioactivity was measured using a liquid scintillation β-counter. All experiments were performed in 4 repetitions in 3-4 independent experiments. $^{45}Ca^{2+}$ uptake into synaptosomes was determined by the difference in sample radioactivity after NMDA stimulation and without it and expressed in percents of control (100%).

RESULTS

DSIP in concentrations 10^{-14}-10^{-7} M increased GABA-induced responses. In cerebellar neurons, the maximum increase in ionic currents was observed after application of 10^{-14} M DSIP (250% from the control); further increase in DSIP concentration (Fig. 1) resulted in almost monotonous decrease in response potentiation to the control level (10^{-8} M). In hippocampal neurons, the dose-response curve had a complex shape with pronounced potentiation peaks corresponding to concentrations of 10^{-9} and 10^{-12} M (173% and 231%, respectively, Fig. 1).

DSIP in a concentration range 10^{-13}-10^{-7} M dose-dependently reduced NMDA-activated currents in cortical neurons (Fig. 2). Starting from DSIP dose of 10^{-13} M the response values were ~40% of the control. In hippocampal neurons, the effect of DSIP had a waveform pattern, the blocking effect was observed starting from the concentration of 10^{-12} M (Fig. 2).

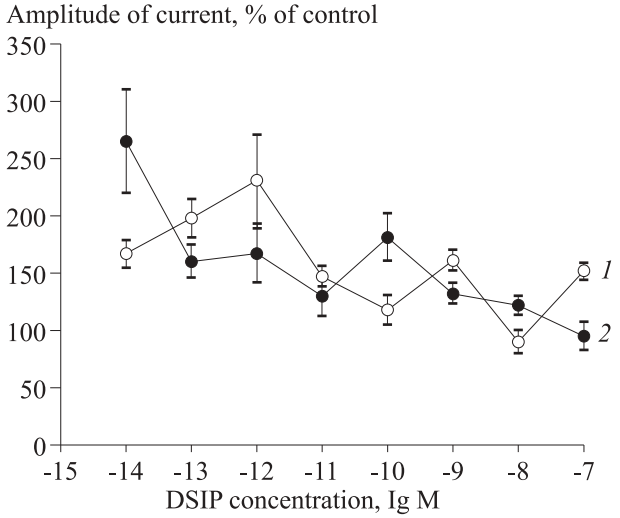

Fig. 1. Effect of DSIP on GABA-activated currents in rat hippocampal (*1*) and Purkinje neurons (*2*).

After $^{45}Ca^{2+}$ uptake stimulation with 200 μM glutamate, DSIP in concentrations of 10^{-10}, 10^{-8}, and 10^{-7} M had practically no effect on this process in synaptosomes from rat brain cortex. The inhibition of $^{45}Ca^{2+}$ uptake into synaptosomes was observed at DSIP concentrations of 10^{-9} M (27%). Application of 10^{-6} M DSIP increased $^{45}Ca^{2+}$ uptake into synaptosomes by 22% (Fig. 3). Thus, we showed that DSIP acts on postsynaptic glutamate receptors. For identification of the type of receptors affected by DSIP we stimulated $^{45}Ca^{2+}$ uptake with NMDA. It was found that DSIP inhibits NMDA-induced $^{45}Ca^{2+}$ uptake into synaptosomes in a concentration range of 10^{-12}-10^{-6} M. The maximum uptake inhibition (100%) was observed after ap-

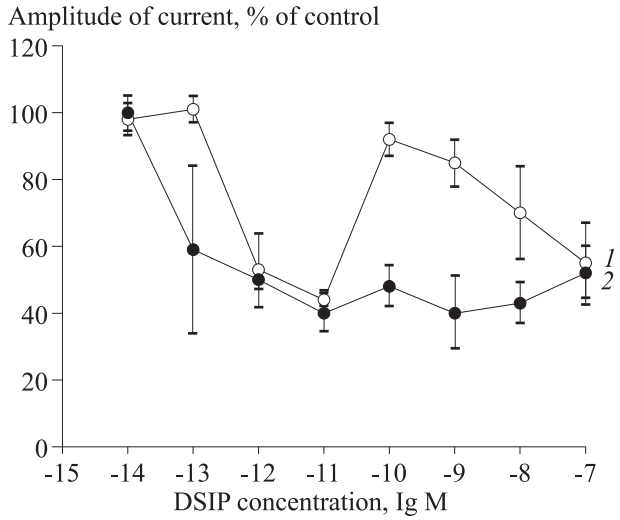

Fig. 2. Effect of DSIP on NMDA-activated currents in rat hippocampal (*1*) and cortical neurons (*2*).

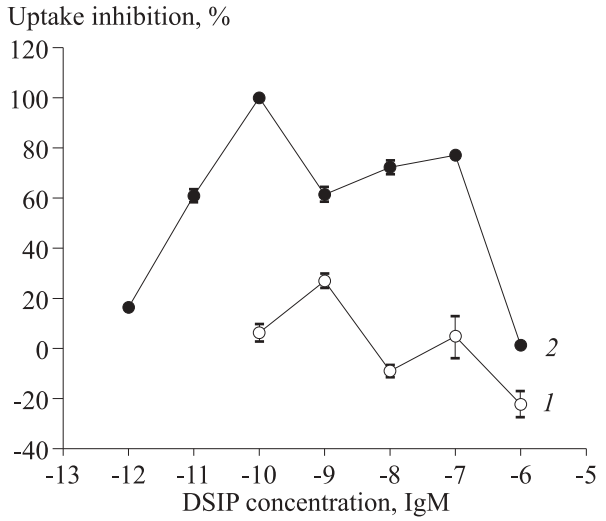

Fig. 3. Effect of DSIP on $^{45}Ca^{2+}$ uptake into synaptosomes from rat brain cortex. *1*) stimulation with 200 μM glutamate; *2*) stimulation with 200 μM NMDA+5 μM glycine.

plication of 10^{-10} M DSIP (Fig. 3). The curves illustrating the dependence of $^{45}Ca^{2+}$ uptake into synaptosomes on DSIP concentration under conditions of glutamate and NMDA stimulation had similar shape. Our findings suggest that DSIP affects presynaptic NMDA receptors.

Thus, in our experiments DSIP blocked the responses of NMDA receptors. This blockade was observed starting from very low concentration of the test peptide: 10^{-13} M in cortical neurons and 10^{-12} M in hippocampal neurons. However, further increase in DSIP concentration did not potentiated its blocking effect: maximum blockade in cortical and hippocampal neurons was observed after application of 10^{-12} and 10^{-11} M DSIP, respectively. Higher doses produces less pronounced inhibitory effects in hippocampal neurons (in cortical neurons all DSIP doses produced similar blockade).

We found that DSIP considerably potentiated the responses of GABA receptors in both cerebellar and hippocampal neurons. There were some differences in the effects of DSIP on GABA-activated currents in cerebellar and hippocampal neurons. The maximum potentiating effects in cerebellar neurons was produced by the lowest DSIP dose (10^{-14} M). Increasing the dose of the peptide was accompanied by a gradual decrease in its potentiating effect and its complete disappearance at DSIP doses of 10^{-8} and 10^{-7} M.

Our findings suggest that DSIP produces a hypnotic effects, which was doubted by some authorities [1-2]. Considerable potentiation of GABA receptors can be a factor promoting sleep onset. The fact that the time of DSIP life in the organism does not exceed several minutes cannot be a factor of its inefficiency.

Our experiments demonstrated also complex effect of DSIP on presynaptic NMDA receptors.

The study was supported by International Science and Technology Center (grant No. 2704).

REFERENCES

1. V. M. Koval'zon, *Zh. Evolut. Biokhim. Physiol.*, **30**, No. 2, 112-119 (1994).
2. V. M. Koval'zon, Yu. V. Rozhnov, V. N. Kalikhevich, and Z. A. Ardemasova, *Neurokhimiya*, **19**, No. 4, 288-292 (2002).
3. V. I. Fetisov, A. V. Kotov, P. B. Gordeev, *et al.*, *Dokl. Akad. Nauk*, **367**, No 6, 776-779 (1999).
4. P. E. Umryukhin, *Byull. Eksp. Biol. Med.*, **134**, No. 7, 9-11 (2002).
5. T. Gallopin, P. Fort, E. Eggermann, *et al., Nature*, **404**, 992-994 (2000).
6. F. Hajos, *Brain Res.*, **93**, No. 3, 485-489 (1975).
7. O. Hamill, E. Neher, B. Sakman, *et al.*, *Pflugers Arch.*, **391**, Nos. 1-2, 85-100 (1981).
8. W.B. Mendelson, *J. Clin. Psychyatry,* **62**, Suppl.10, 5-8 (2001).
9. M. Monnier, C.A. Schoenenberger, *Sleep.*, Eds. W. P. Koella, P. Levin. Basel (1976), pp. 257-263.
10. C. A. Schoenenberger, D. Schnelde-Helmert, *Trends Pharmacol. Sci.*, **4**, 307-318 (1983).
11. A. A. Shandra, L. S. Godlevskii, A. T. Brusentsov, *et al.*, *Neurosci. Behav. Physiol.*, **28**, No. 6, 694-697 (1998).
12. M. Steriade, R. W. McCarley, *Brainstem control of wakefulness and sleep*., N.Y. (1990).