
PHYSIOLOGY

Delta Sleep-Inducing Peptide Blocks Excitatory Effects of Glutamate on Rat Brain Neurons

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 7, pp. 9-11, July, 2002
Original article submitted March 13, 2002

Microiontophoresis of delta sleep-inducing peptide primarily activated neurons in the dorsal hippocampus, anteroventral thalamic nucleus, lateral hypothalamus, and sensorimotor cortex. Microiontophoretic administration of glutamate markedly enhanced neuronal activity, while preliminary microiontophoresis of delta sleep-inducing peptide blocked the excitatory effect of glutamate on neurons in these brain structures.

Key Words: *delta sleep-inducing peptide; glutamate; microiontophoresis*

Published data show that delta sleep-inducing peptide (DSIP) possesses pronounced antistress activity [2, 3,14]. Radioimmunoassay revealed DSIP in various brain regions, including the thalamus, hypothalamus, hippocampus, and other limbic structures [1,6,13]. Under conditions of emotional stress intraperitoneal injection of DSIP inhibits overexpression of immediate early gene *c-fos* in the hypothalamus and limbic structures [4,15]. The mechanisms underlying the inhibitory effect of DSIP on expression of immediate early genes during emotional stress remain unclear.

Emotional stress potentiates the influence of excitatory neurotransmitters in the central nervous system, which contributes to glutamatergic excitotoxicity [9,10]. Glutamate binds to NMDA receptors on brain neurons and acts as a major excitatory neurotransmitter in the central nervous system [8,11]. The excitatory neurotransmitters activating NMDA receptors stimulate expression of immediate early genes in brain structures [5,7].

Here we studied whether DSIP modulates the excitatory effect of glutamate on individual neurons in various brain structures.

MATERIALS AND METHODS

Experiments were performed on 18 male Wistar rats weighing 280-320 g. The animals were maintained at room temperature and had free access to food and water. The rats were scalped 1 day before the experiment under ether anesthesia. During the experiment these animals were narcotized with urethane (1 g/kg intraperitoneally), body temperature was maintained at 37-38°C with a rubber heater. Three-channel glass microelectrodes were stereotactically implanted into rat brain. One channel contained 3 M NaCl, and the others were filled with substances for microiontophoresis. The electrodes were tracked through the sensorimotor cortex, dorsal hippocampus, anteroventral thalamic nucleus, and lateral hypothalamus. We recorded pulse activity of neurons in these brain structures. The indifferent electrode was implanted into the nasal cavity.

Extracellular neuronal activity was recorded. After recording stable baseline activity, DSIP (Serva, 20 nA, holding current -5 nA) and glutamate (-20 nA, holding current 5 nA) were applied microiontophoretically. The concentrations of DSIP and glutamate were 100 and 50 µg/ml, respectively.

In the control series, microelectrode channels were filled with the solvent. In this series we observed no

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effects typical of microiontophoretic application of DSIP and glutamate. These data indicate that changes in neuronal activity were not associated with the effect of electrical current.

The animals were decapitated, and localization of the microelectrode tips was verified on rat brain slices [12]. Frequency histograms were constructed using a special software calculating the number of spikes over time interval. MS Excel and Statistica 6.0 were also used.

RESULTS

The effect of microiontophoretic application of DSIP was studied on 85 neurons in various brain structures (Fig. 1). DSIP activated 52% neurons in the studied brain structures. The number of neurons activated by DSIP was maximum in the dorsal hippocampus and anteroventral thalamic nucleus. Inhibition of neuronal activity after microiontophoretic application of DSIP was observed primarily in the sensorimotor cortex. Typical response of thalamic neurons to DSIP was reproduced after several consecutive microiontophoretic applications of the peptide (Fig. 2, *a*).

Changes in pulse activity produced by microiontophoretic application of glutamate were studied on 30 neurons (Fig. 1). Glutamate increased pulse activity in 67% brain neurons. The number of neurons reacting to microiontophoretic application of glutamate was

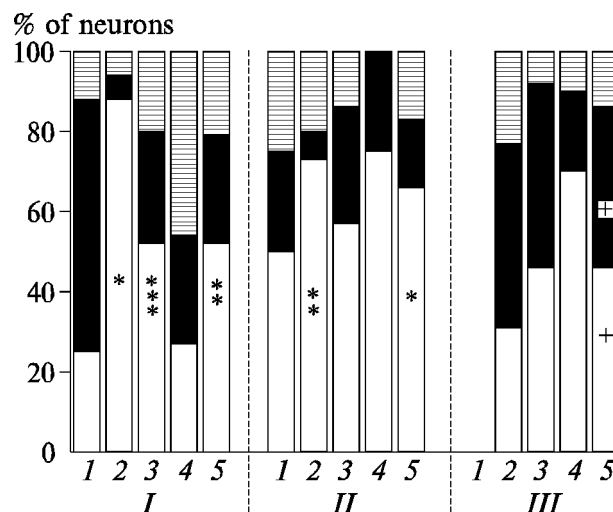


Fig. 1. Response of neurons in the sensorimotor cortex (1), dorsal hippocampus (2), anteroventral thalamic nucleus (3), and lateral hypothalamus (4) to microiontophoretic administration of delta sleep-inducing peptide (I), glutamate (II), and glutamate after microiontophoretic application of delta sleep-inducing peptide (III). All structures (5). Light bar: activation; dark bar: inhibition; shaded bar: no response. * $p < 0.001$, ** $p < 0.01$, and *** $p < 0.05$ compared to inhibition and absence of the response. * $p < 0.05$ compared to glutamate. The combined action (III) of delta sleep-inducing peptide and glutamate was studied only on 1 neuron of the sensorimotor cortex (inhibition).

maximum in the dorsal hippocampus, anteroventral thalamic nucleus, and lateral hypothalamus. Figure 2, *b* shows a typical response of neurons in the dorsal

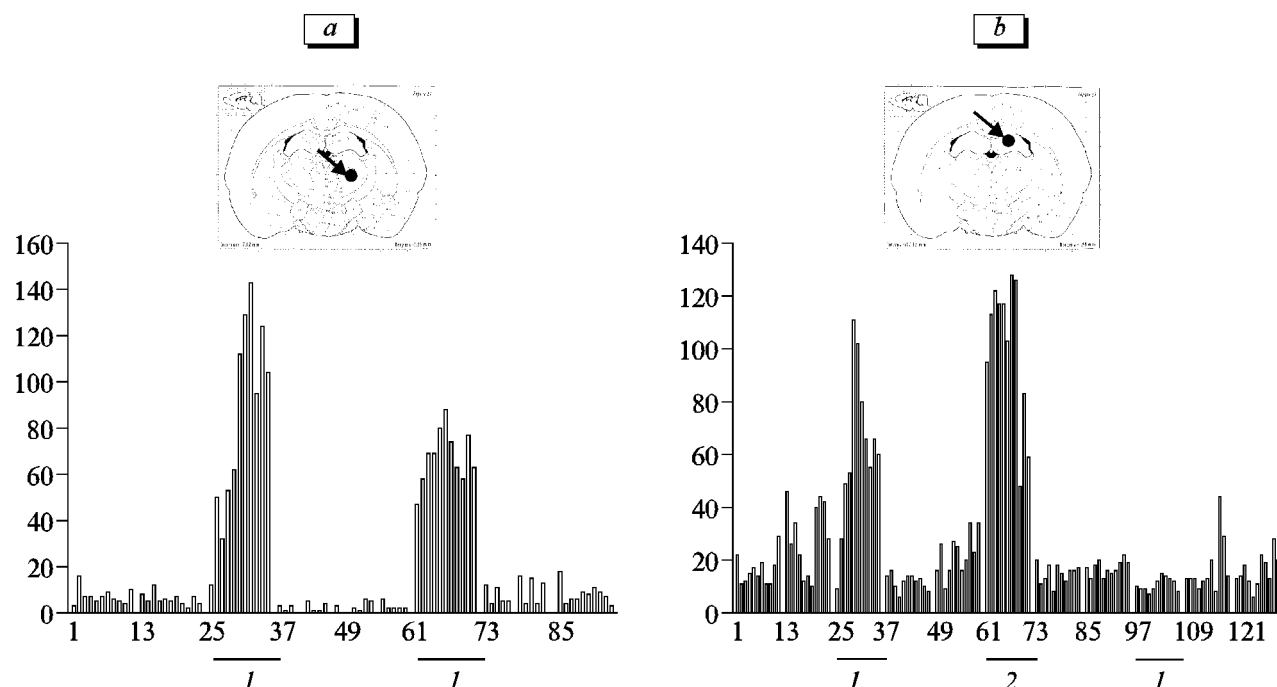


Fig. 2. Response of neurons in the anteroventral thalamic nucleus (*a*) and dorsal hippocampus (*b*) to microiontophoretic application of delta sleep-inducing peptide (DSIP, *a*) and glutamate after microiontophoresis of DSIP (*b*). Localization of microelectrode is shown in the upper scheme. Abscissa: 2.7-sec intervals. Ordinate: number of spikes over 2.7 sec. Lines below the chart shows application of DSIP (1) and glutamate (2).

hippocampus to microiontophoretic administration of glutamate.

The effects of glutamate administered microiontophoretically after microiontophoretic application of DSIP were studied on 37 neurons (Fig. 1). DSIP abolished the effect of glutamate in 14% neurons. In 41% neurons glutamate not stimulated, but even inhibited pulse activity. DSIP most markedly blocked the excitatory effect of glutamate on neurons of the dorsal hippocampus and anteroventral thalamic nucleus. The typical response of neurons in the dorsal hippocampus to microiontophoretic administration of glutamate after microiontophoretic application of DSIP is shown in Fig. 2, *b*.

Thus, microiontophoretic administration of DSIP primarily excites neurons in various brain structures (particularly, in the dorsal hippocampus and thalamic area). This reaction was typically reproduced after several consecutive applications of the peptide. The number of neurons activated with glutamate markedly decreased after preliminary microiontophoretic application of DSIP. In the dorsal hippocampus microiontophoresis of glutamate against the background of DSIP inhibited pulse activity of most neurons. Our previous experiments showed that intraperitoneal injection of DSIP stimulates expression of the immediate early gene *c-fos* in the paraventricular hypothalamus, septum, and amygdala [4,15]. Our findings indicate that DSIP blocks the excitatory effect of glu-

tamate on neurons of the dorsal hippocampus, sensorimotor cortex, lateral hypothalamus, and anteroventral thalamic nucleus.

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