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Effects of Intranasal Administration of Epitalon on Neuron Activity in the Rat Neocortex

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This report discusses the properties of the synthetic tetrapeptide epitalon (Ala-Glu-Asp-Gly), synthesized on the basis of an epiphyseal peptide extract. Intranasal administration of epitalon was selected as a noninvasive means of applying the agent to the CNS by bypassing the blood-brain barrier. The aim of the present work was to assess the characteristics of the action of epitalon on the frequency of spontaneous neuron activity in the cerebral cortex of white rats. Studies were performed using male Wistar rats anesthetized with urethane (1 g/kg). Extracellular activity of cortical neurons was recorded with a glass microelectrode of resistance 1–2 MΩ. Recording of spontaneous neuron discharges for 10–15 min was followed by intranasal administration of epitalon solution and recording of neuron activity to 30 min after doses of 30 ng per animal. Significant activation of neuron activity was seen several minutes after dosage, with an increase (by factors of 2–2.5) in discharge frequency. In some experiments, the effect of epitalon was multiphasic. The first peak of increased neuron discharge frequency at 5–7 min was followed by peaks at 11–12 and 17–18 min. The increase in discharge frequency occurred because of an increase in the discharge frequency of neurons which were already active and the recruitment of previously silent neurons. At least the first peak of increased neuron activity following exposure to epitalon was found to be associated with the direct action of the peptide on cortical cells.

KEY WORDS: epitalon, neocortex, microelectrodes, intranasal administration, peptide.

Tissue-specific short peptides, collectively termed "cytomedins," have now been identified in the hypothalamus, epiphysis, thymus, neocortex, and many other parts of the brain. Cytomedins have been shown to be substances with high adaptogenic activity, oncostatics, anti-aging agents, and regeneration stimulators [5, 6, 14]. Interest in these agents as a new class of regulators of immune and neuroendocrine processes in the central nervous system continually increases.

Epithalamin is one of the first low-molecular weight peptide complexes obtained by acetic acid extraction from the epithalamic-epiphyseal region of the brain. It has marked oncostatic, anti-aging, antioxidant, and adaptogenic properties [1]. Studies in recent years have demonstrated that epiphyseal peptide preparations have active effects on CNS metabolism and neurological parameters in animals and humans [3]. Rats given epithalamin showed activation of melatonin production [2], which in turn led to normalization of circadian rhythms and increases in body resistance. Epithalamin replacement therapy completely eliminated memory and learning impairments in rats resulting from a deficiency in the epiphyseal production of peptide hormones associated with aging [7]. The use of epithalamin in elderly patients improved the performance of neuropsychological tests, with reversal of age-related changes in motor reaction times, improvements in visuomotor coordination, decreases in task performance times, and improvements in memory [3].

For clinical use, special interest is paid to the synthetic peptide preparation epitalon (Ala-Glu-Asp-Gly), con-

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structed on the basis of analysis of the amino acid composition of the epiphyseal extract epithalamin [14]. Like its precursor, it is a strong oncostatic, antioxidant, and anti-aging agent [9, 10, 12]. The physiological properties of epitalon are apparent at significantly lower doses than those of epithalamin [11].

Data on the protective actions of epiphyseal peptides on the CNS have mainly been obtained by intravenous administration of epitalon or epithalamin and by direct application of these substances to tissue cultures. However, penetration of peptides into the brain after intravenous administration is limited by the blood-brain barrier. Many investigators have shown that a number of substances, including metals, stains, viruses, and low-molecular weight peptides [15] penetrate the brain via the olfactory system, bypassing the blood-brain barrier [13]. Thus, from the point of view of the possible clinical application of cytomedins, there are good grounds for using intranasal administration as a noninvasive means of applying these agents to the CNS. We have previously observed that intranasal administration of epitalon led to a significant increase in c-Fos in the rat epiphysis, this being one of the triggers launching secretory processes in these cells [16]; it also led to a 2–3-fold increase in the frequency of spike activity in epiphyseal pinealocytes [8]. The physiological activity of the agent at the very low concentrations of 10^{-10}–10^{-14} M provides grounds for suggesting that the action of epitalon is based on binding with an unknown receptor.

The mechanisms of action of epitalon on the CNS after intranasal administration, particularly on the excitability of neurons in central brain structures, have received virtually no study. The aim of the present work was to address these effects. The actual task was to characterize the dynamics of the actions of intranasal administration of epitalon on the electrical activity of neocortical neurons.

METHODS

Experiments were performed on 17 male Wistar rats weighing 180–200 g. Procedures were undertaken in the second half of the light part of the day, from 15:00 to 18:00. Animals were anesthetized with i.p. urethane (1 g/kg), after which bilateral craniotomy was performed from 0 to 3 mm frontal to the bregmal suture and to 2 mm lateral to the sagittal suture. Rats were placed in a stereotaxic apparatus and a glass microelectrode filled with saturated sodium citrate solution (resistance 1–2 MΩ) was placed in the appropriate part of the neocortex. The indifferent electrode was a silver chloride plate placed in the scalped area. Extracellular cortical neuron activity was recorded starting at the microelectrode insertion depth of 700 μm. The signal from the microelectrode was passed to an ML319 amplifier and was then digitized at a sampling frequency of 30 kHz using an MD93 analog-to-digital converter for subsequent analysis of measures of multineuron activity using our Bioactivity Recorder 4.0 program.

Neurons were identified in multineuron activity on the basis of the shapes of individual action potentials (amplitude, duration of depolarization, duration of repolarization, presence or absence of spike trains). Cluster analysis (the KlustaKwik algorithm) was used to identify 2–8 categories of spikes, of which one category always consisted of low-amplitude noise, the others corresponding to the "voices" of 1–7 neurons.

Intranasal administration of epitalon was performed bilaterally, with recording of neuron activity for 10–30 min before and after administration. Threshold doses of epitalon were determined in the first four experiments by giving 2 μl of solution containing 1, 5, 10, 20, and 50 ng per rat, with recording of subsequent neuron activity in the neocortex to 20 min after dosage (38 neurons were studied). In the following 14 experiments, intranasal administration was performed using the resulting threshold dose. In most experiments, repeat doses were given after intervals of 40–60 min, with recording of observed effects. The actions of epitalon (experimental) and physiological saline (control) were observed for 30–40 neurons in each animal. A total of 322 experimental neurons and 55 control neurons were identified.

In three experiments, epitalon was added to the solution used for filling the microelectrodes (sodium citrate) to a final concentration of 10^{-11} M. The microelectrode was attached to a hermetic holder attached to a plunger. Neuron activity was recorded before and after microinjection of epitalon into the intercellular space (0.5 μl, assessed by displacement of the fluid meniscus in the microelectrode). A total of 60 neurons were identified.

For statistical analysis, absolute values of the neuron discharge frequency in experiments (after administration) were expressed as percentages of the baseline activity (before administration). Transformed neuron discharge frequencies during time intervals prior to epitalon infusions and at 1–3 min, 5–7 min, and 9–12 min showed normal distributions, such that Student's test could be used to evaluate differences between neuron discharge frequencies during these time periods.

RESULTS AND DISCUSSION

Studies of the effects of intranasal administration of epitalon in the acute physiological experiment showed that the spontaneous neuron activity frequency recorded in the parietal and frontal areas of the neocortex showed significant changes. The results of four experiments showed that the threshold dose for intranasal application of epitalon was 20 ng per animal. Depending on the level of anesthesia and the depth of microelectrode insertion, the absolute neuron discharge frequencies in different experiments varied signifi-

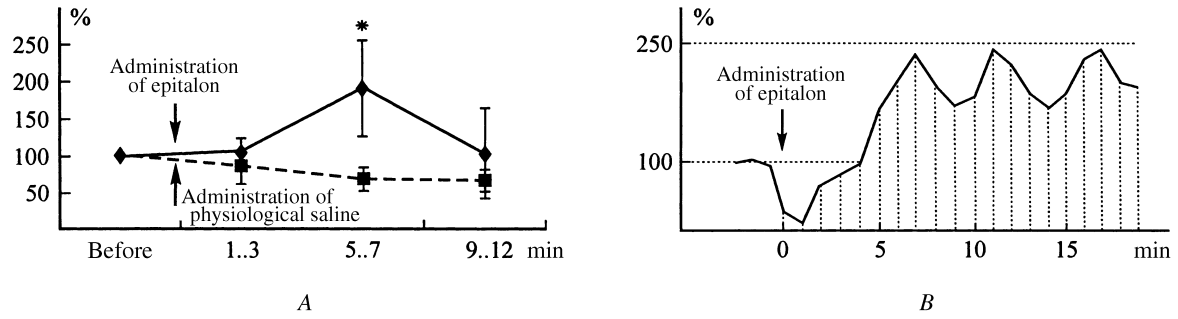

Fig. 1. Dynamics of changes in the spontaneous activity frequency of cortical neurons after intranasal administration of epitalon; the arrow shows the time of administration. *A*) Mean neuron discharge frequency on administration of epitalon and physiological solution; *significant differences ($p < 0.05$); *B*) example of changes in the discharge frequency of a single neuron. The horizontal axes show time, min; the vertical axes show neuron discharge frequency, % of baseline.

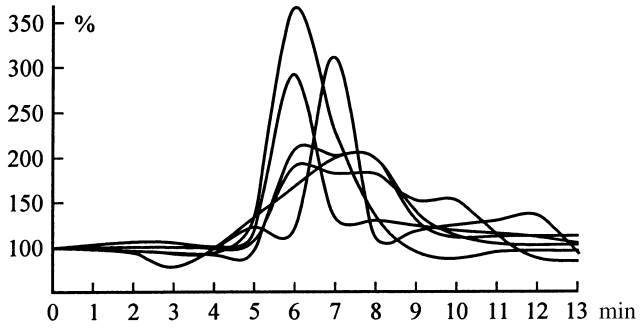

Fig. 2. Superimposition of plots showing changes in neuron discharge frequency from the moment of epitalon administration. The horizontal axis shows time from the moment of epitalon administration, min; the vertical axis shows neuron discharge frequency, % of baseline.

cantly, though the general tendency to an increase in frequency after administration of epitalon persisted. Figure 1, *A* illustrates this tendency using the results obtained from averaged data obtained in 12 experiments, which showed a significant increase in the neuron discharge frequency 5–7 min after administration of epitalon as compared with baseline activity before treatment. Control administration of physiological saline produced no significant changes in this measure. Significant decreases in the mean spike frequency 9–12 min after application of both epitalon and control administration of physiological saline appeared to be associated with the harmful effects of prolonged microelectrode recording on the surrounding cortical tissue.

Discharge frequencies in different experiments reached a peak with a small time shift. Figure 2 shows superimposition of plots of changes in the discharge frequencies of six individual neurons recorded in different experiments.

In four experiments, the responses of cortical neurons to intranasal administration of epitalon solution consisted of several phases. Figure 1, *B* shows an example of the dynamics of changes in discharge frequency in one such experiment. During the first three minutes after administration, the frequency did not increase and could even decrease. We regard this as a nonspecific reaction to stimulation of the olfactory epithelium, as it was induced equally effectively by intranasal physiological saline. Significant increases in neuron spike activity occurred 5–7 min after administration of epitalon in most experiments. As shown in Fig. 1, neuron discharge frequency reached a level of 200–250% of baseline after intranasal administration of epitalon. The increase in cortical neuron activity was short-lived, and decayed by 8–9 min after administration of epitalon; a further three phases of increased neuron discharge frequency were sometimes observed. The first peak of increased frequency at 5–7 min was followed by peaks at 11–12 and 17–18 min (Fig. 1, *B*). Unfortunately, the nature of this multiphasic reaction remains unclear. The appearance of two, three, or more peaks of increased neuron activity may be associated with effects mediated by other parts of the CNS.

In all experiments, the overall discharge frequency stabilized or returned to the control level within 30 min.

Thus, intranasal administration of epitalon was followed by an interval of 5–6 min before the appearance of cortical neuron reactions. We suggest that epitalon enters the cerebral circulation and that the delay observed here is associated with the time taken for transport of the peptide to the areas of the cortex under study. Our previous histological studies demonstrated the penetration of fluorescence-labeled epitalon into the brain via the blood flow and its binding with individual neurons [4]. In addition, intranasally administered epitalon also reaches many other parts of the CNS, arriving at some in a little more than 5 min and at others in a rather shorter period of time [15]. In this case, the physiological effect of epitalon could be mediated by other parts of the CNS rather than being associated with the direct action of the tetrapeptide on cortical neurons.

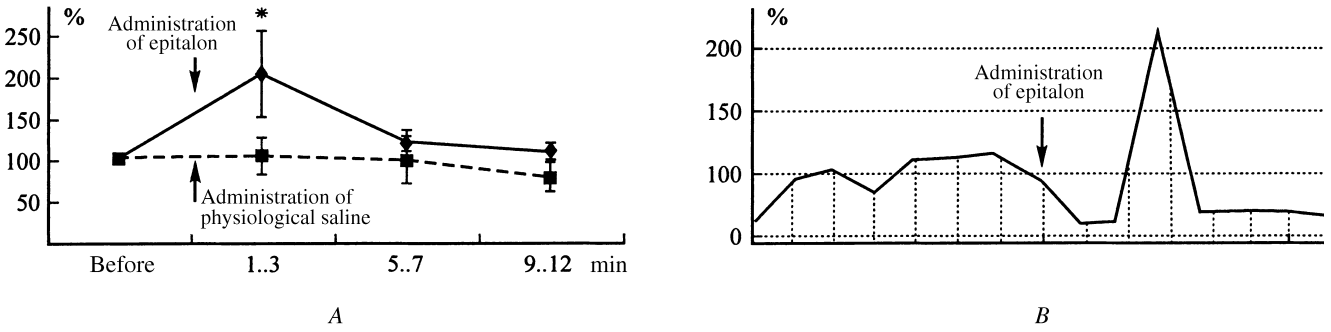

Fig. 3. Dynamics of changes in the spontaneous discharge frequency of cortical neurons on direct application of epitalon solution; the arrow shows the time of administration. The abscissa shows time, with markers every 10 sec. For further details see caption to Fig. 1.

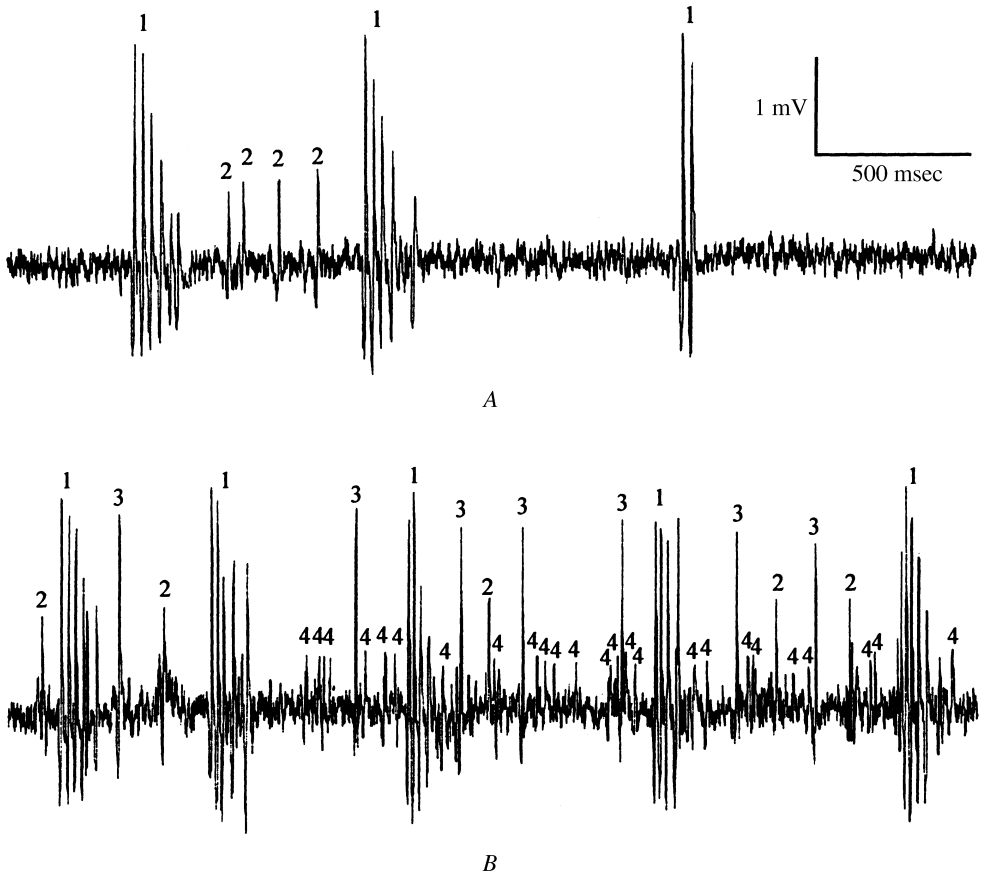

Fig. 4. Example of a multicellular trace of neuron discharges before (*A*) and 5 min after (*B*) intranasal epitalon. Numbers show discharges from individual identified neurons.

This possibility was investigated by performing an additional four experiments with direct microinjection of epitalon (10^{-11} M) into the intercellular medium from the tip of a microelectrode. When epitalon acted on neurons adjacent to the electrode tip (Fig. 3, *A*, *B*), the increase in neuron activity (an increase by 400 ± 211%) was seen within 10–20 sec from microinjection and was similar to that seen after intranasal administration, but without the 5-min delay. Thus, we can suggest that epitalon acts directly on neocortical cells, and at least the first peak increase in neu-

ron discharge frequency after intranasal administration is associated with the direct action of epitalon on the cortex and is not mediated by other systems.

The increase in total discharge frequency itself consisted of at least two components. Firstly, the discharge frequency of already active cells increased; secondly, previously silent neurons were recruited. An example of this process can be seen in Fig. 4, which presents an example trace of neuron activity in neocortical cells before (*A*) and 8 min after (*B*) intranasal epitalon. Neurons 1 and 2 responded to the experimental treatment with increases in neuron discharge frequency, while neurons 3 and 4 (previously silent) showed activity only after exposure to epitalon. In addition, our experiments recorded a population of neurons for which no relationship between discharge frequency and the actions of epitalon could be demonstrated. The greatest statistical weighting in changes in total discharge activity in response to epitalon was associated with cells with a single type of discharges. There was no significant increase in the number of spikes in trains after as compared with before administration of epitalon.

We believe that the increase in spontaneous neuron activity frequencies in the rat neocortex induced by epitalon may be associated with the decrease in the excitability threshold of cortical neurons and weakening of inhibition in the cortex. Despite the fact that the mechanism by which this effect is mediated requires further study, our data may be of interest in relation to the clinical application of epitalon for the treatment of impairments of CNS activity.

CONCLUSIONS

Intranasal administration of epitalon solution was followed 5–7 min later by a transient (2–3 min) increase in neuron discharge frequency in the rat neocortex.

Intranasal administration of epitalon induced several waves of increased neuron discharge frequency with periods of 3–4 min, this effect lasting no more than 30 min from the time of treatment.

The overall increase in neuron spike activity results from an increase in the discharge frequency of active neurons as well as recruitment of previously silent neurons.

At least the first peak of increased neuron spike activity after treatment with epitalon is associated with the direct action of the peptide on cortical cells.

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