EXPERIMENTAL BIOLOGY

Thyrotropin-Releasing Hormone Inhibits Long-Term Potentiation in Synaptic Systems of Rat Hippocampus

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 128, No. 12, pp. 690-693, December, 1999
Original article submitted May 27, 1999

The effects of thyrotropin-releasing hormone (TRH) on long-term potentiation of field responses in mossy fibers—CA3 and Shaffer collaterals — CA1 synaptic systems were studied on rat hippocampal slices. Incubation with micromolar concentrations of TRH inhibited the development of long-term potentiation in both synaptic systems. It is suggested that this phenomenon underlies the antiamnesic effect of TRH.

Key Words: *thyrotropin-releasing hormone; long-term potentiation; hippocampal slices; CA1; CA3*

Hypothalamic thyrotropin-releasing hormone (TRH, pGlu-His-ProNH$_2$) possesses central action and regulates a variety of brain functions [8]. One of its central effects is antiamnesic activity which manifests itself in electroconvulsive and other models of amnesia [5] associated with disturbed memory consolidation. The hippocampus plays a key role in memory consolidation [12] and contains a considerable number of TRH-immunoreactive nerve elements [9], therefore the antiamnesic effect of TRH can be attributed to the regulation of activity in the hippocampal neuronal networks.

Long-term potentiation (LTP), i.e. long-lasting enhancement of hippocampal pyramidal cell responsiveness after short-term high-frequency stimulation of excitatory synaptic input, is a manifestation of synaptic plasticity which constitutes the neurophysiological basis for learning [1]. This plastic phenomenon is sensitive to a number of drugs with antiamnesic activity [4,11].

The aim of this study was to assess the effect of TRH on the development of LTP in two synaptic systems of the hippocampus: mossy fibers (axons of dentate granular cells) — pyramidal cells of the CA3 region of the hippocampus and Shaffer collaterals (axon collaterals of CA3 pyramidal cells) — pyramidal cells of the CA1 hippocampal region.

MATERIALS AND METHODS

The study was carried out on hippocampal slices from 3-week-old male Wistar rats as described elsewhere [3]. Slices were perfused with a modified Ringer solution containing (in mM): 124 NaCl, 3 KCl, 2.5 $CaCl_2$, 2.5 $MgSO_4$, 1.25 Na_2HPO_4, 26 $NaHCO_3$, and 10 D-glucose and continuously saturated with carbogen (95% O_2+5% CO_2) at 29-30°C. The recording of electrical activity was started 1.5-2 h after slice preparation. CA1 or CA3 field responses to bipolar stimulation of the corresponding synaptic input (single square pulses, 0.1 msec) were recorded using glass microelectrodes filled with 1.5 M NaCl. Stimulus intensity was adjusted to evoke approximately a half-maximal population spike response (pop-spike). LTP was induced by short-term high-frequency stimulation (HFS; 30 or 100 pulses at 100 Hz) delivered through the same electrodes with the same stimulus intensity. Each slice received only one HFS during perfusion in the absence

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0007-4888/99/0012-1259$22.00

(control) or presence of various TRH concentrations. Single responses before and after HFS were tested at a frequency of 0.66 Hz (15 sec interstimulus interval).

TRH (Sigma) was dissolved in the perfusion medium immediately before the experiment and introduced into the bath 15 min before HFS by switching the perfusion system to the corresponding reservoir. Five min after HFS the perfusion system was switched back to the standard solution.

Changes in the responsiveness of pyramidal neurons were evaluated by relative changes in pop-spike amplitude with respect to the baseline (the mean value for 15-30 min before drug application).

The results are presented as means±standard errors. The data were analyzed statistically using Mann—Whitney *U* test.

RESULTS

LTP was induced by relatively weak (30 pulses) or standard (100 pulses) HFS. In the CA1 region, weak HFS caused only short-term potentiation lasting no more than 30 min, while standard HFS induced true LTP lasting over 60 min. In the CA3 region, weak HFS induced low-amplitude LTP in half of the studied slices.

TRH suppressed the induction of LTP in both synaptic systems after weak and standard HFS (Fig. 1,2). The inhibitory effect of TRH on weak HFS-induced potentiation showed no significant dose-dependence in the concentration range from 0.25 to 5 μM (Fig. 1, *b*). Using a broader concentration range (5 nM-10 μM) in the experiments with standard HFS we found U-shaped dose-dependence of the inhibitory effect of TRH, typical of drugs modulating learning and memory processes. The most efficient concentration was 0.5 μM. It not only completely blocked the induction of LTP, but also caused small, but long-lasting depression of responses (Fig. 2, *a*). Both higher and lower concentrations of TRH produced less pronounced inhibition of LTP (Fig. 2, *b*).

The results turned out to be surprising. Published data concerning the mechanisms of TRH action on hippocampal neurons show that TRH enhances excitatory responses mediated by glutamate receptors of the N-methyl-D-aspartate (NMDA) type and suppresses GABA-mediated inhibition [14,15]. Proceeding from this we could expect an enhancement, but not suppression of LTP induction. NMDA receptors are known to play a key role in the induction of LTP in the Shaffer collaterals-CA1 synaptic system [1], while disinhibition is an important prerequisite for synaptic plasticity [11]. According to current knowledge, Ca^{2+} entry through the channels coupled with NMDA receptors and voltage-gated calcium channels followed by the increase in intracellular Ca^{2+} concentration triggers the mechanisms of LTP [1]. Our data showed that despite no antagonism with NMDA receptors, TRH inhibited both NMDA-dependent (CA1) and NMDA-independent (CA3) LTP. This inhibitory effect can be attributed to suppression of Ca^{2+} entry through high-threshold voltage-gated Ca^{2+} channels [7] and attenu-

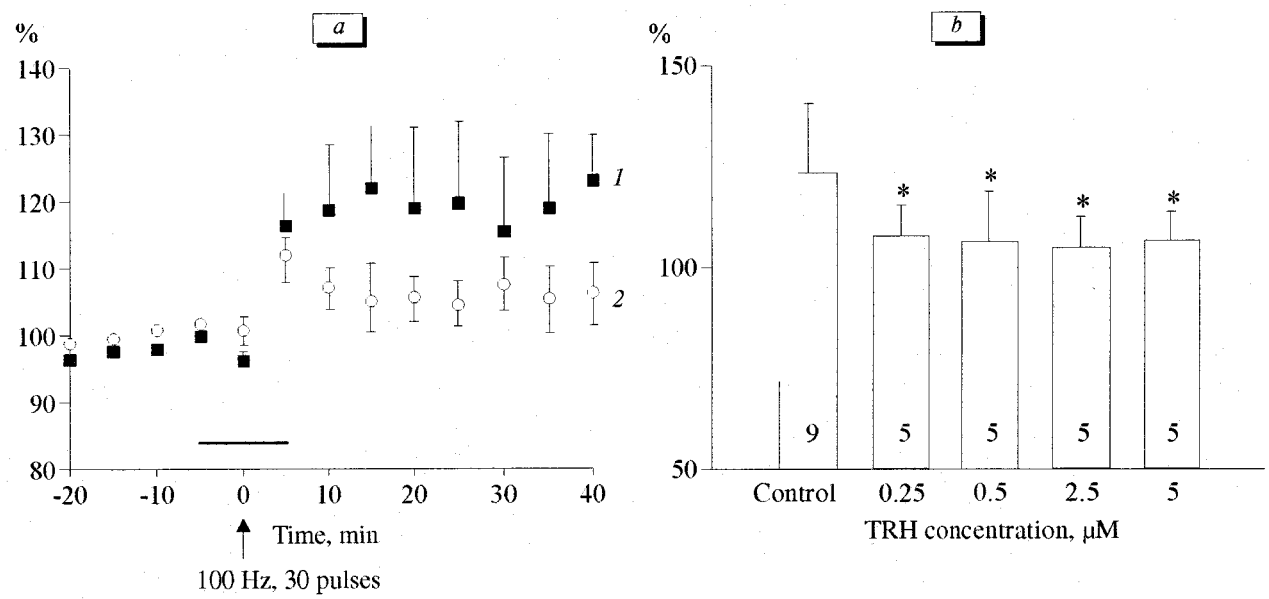

Fig. 1. TRH-induced inhibition of mossy fiber LTP in the hippocampus. Here and in Fig. 2: *a*, posttetanic dynamics of responsiveness in the control (*1*) and after 20-min incubation with 0.5 μM TRH (*2*). Horizontal line marks the period of application. Ordinate: relative changes in the mean amplitude of pop-spike, percent of baseline. *b*, effects of various concentrations of TRH on LTP magnitude. The number of experiments in each series is indicated by numbers within the bars. *$p<0.002$ in comparison with the control. Each bar represents the mean responsiveness over 0-40 min (in Fig. 2 over 30-60 min) after high-frequency stimulation (percentage of the baseline).

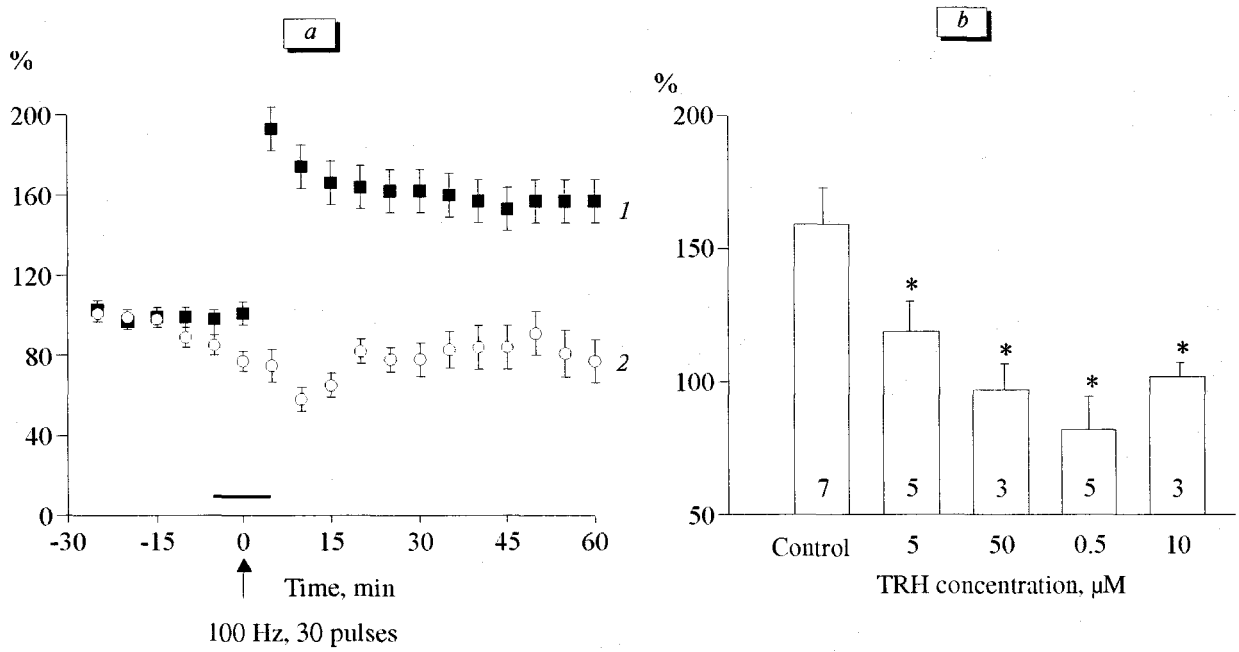

Fig. 2. TRH-induced inhibition of LTP in Shaffer collateral-CA1 system of the hippocampus.

ation of glutamate-induced accumulation of intracellular Ca^{2+} [6]. The more pronounced inhibition of LTP induction under conditions of relatively strong HFS and the appearance of moderate depression of responsiveness imply that this mechanism can underlie the antiamnesic effect of TRH in electroshock-induced amnesia. Electroconvulsive stimulation results in an LTP-like increase in hippocampal responsiveness [13] and "saturates" the excitatory connections, thus impairing learning [2]. TRH intensely released in the hippocampus during seizure activity [10] can significantly reduce the level of potentiation, thus preventing amnesia.

The study was supported by the Russian Foundation for Basic Reseach (grants No. 98-04-48311 and 96-05-97764).

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