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SHORT COMMUNICATION

Anti-ictogenic effects of the corticotropin-releasing factor-like peptide in the pentylenetetrazole model of seizure in brain slices

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Summary The present study provides evidence for anti-ictogenic activity of the mystixin 7 (MTX) mini-peptide in the pentylenetetrazole model of seizure. MTX was effective in inhibiting/suppressing ictal- and interictal-like activities over a long period of time (during 60—80 min). The peptide's anti-ictogenic effects were concentration- and time-dependent. An enzymatic treatment of MTX was accompanied by a decrease of the frequency pattern of epileptiform discharges, but their total blockade did not occurred. These findings indicate that the MTX mini-peptide has pronounced anti-ictogenic properties.

Introduction

Mystixin 7 mini-peptide (MTX) represents a novel class of synthetic corticotropin-releasing factor-like peptides (mystixins family); molecular formula: C51H84N12O9S; MW: 1.041 kDa; chemical structure: 4-anisoyl-arginyl-lysyl-leucyl-leucyl-thienyl-isoleucyl-leucinamide (http://www.sigmaaldrich.com/life-science.html. 1989). According to its amino acid structure it relates to mini-peptides (Gomazkov, 2012). In vivo studies have shown that MTX has anti-inflammatory effects on non-nervous cells (Thomas et al., 1993; Gjerde et al., 2000).

Recently, it was revealed that the MTX induced marked neurotropic effects in brain slices. Mini-peptide at concentrations of 10, 25, 50, 100 and 250 mg/mL inhibited the both AMPAR-and NMDAR-mediated postsynaptic processes in a dose-dependent manner. At concentrations of 10—50 mg/mL MTX depressed the $GABA_B$-ergic processes, while at high doses (100, 250 mg/mL) mini-peptide enhanced them. The action of each MTX concentration was recorded for 15 min to the lateral olfactory tract fibers (LOT) stimulation, which is the main afferent input to the neurons of the olfactory cortex. The detected effects of MTX were reversible, the both AMPARs and NMDARs dependent processes and inhibitory $GABA_B$-ergic processes were restored after washing (Mokrushin, 2011).

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Since the MTX inhibited AMPARs and, especially NMDA-dependent mechanisms, as well as upregulated of the inhibitory mechanisms, we hypothesized that MTX possesses the anti-ictogenic properties. The purpose of this study was to elucidate the role of the MTX mini-peptide in the pentylenetetrazole (PTZ) model of ictogenesis in olfactory cortex slices.

Materials and methods

Slice preparation and maintenance

The study was performed on male Wistar rats with the body weight of 180—200 g (vivarium of the Pavlov Institute of Physiology, RAS) in compliance with ethical standards of the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. The detailed description of the slices preparation and their incubation is provided in previous publications (Khama-Murad and Mokrushin, 2008; Khama-Murad et al., 2011; Mokrushin and Pavlinova, 2012). Briefly, tangential slices of the olfactory cortex were cut 450—500 μm thick and every slice was preincubated for 1 h in 1 mL of artificial cerebrospinal fluid (ACSF) with the following composition (mM): NaCl-124.0, KCl-5.0, $CaCl_2$-2.6, KH_2PO_4-1.24, $MgSO_4$-1.2; $NaCHO_3$-3.0, tris—HCL (pH 7.4)-23.0, glucose-10. ACSF was equilibrated with O_2 at 37 °C, pH 7.21—7.24. After preincubation, slices were one by one transferred into an interface recording chamber (Pavlov Institute Physiology, Russia) and continuously perfused with ACSF at 2.0 mL/min.

Recording techniques and data analysis

The extracellular field potentials (FPs) were evoked by stimulating the proximal part of the lateral olfactory tract (LOT) using platinum custom-made bipolar stimulating electrodes. Rectangular pulses (0.1 ms duration, 1.2—1.5 V intensity and frequency 0.003 Hz) were fed to the stimulating electrodes from the stimulator ESU-1 (Russia). FPs were detected using a glass microelectrode filled with 1 M NaCl with tip resistance 1—5 MΩ. The signals were recorded using an NTO-2 amplifier (Pavlov Institute Physiology, Russia) with a band-pass filter passing frequencies ranging from 0.016 kHz to 10 kHz, digitized at 20 kHz (MD-32 laboratory interface, Russia) and retained in a computer. Typical FPs in the piriform cortex evoked by orthodromic LOT stimulation consist of two main components: namely, presynaptic (AP LOT) and postsynaptic (AMPA, NMDA EPSP and IPSPslow). Amplitudes of the population AMPA and NMDA EPSPs were measured from the isoline to the peak level. The amplitudes of AMPA EPSP were measured over a 2—3 ms window centered at the peak of the response. Peak NMDA EPSP was measured as the average potential observed in a 7—8 ms window (Khama-Murad and Mokrushin, 2008; Khama-Murad et al., 2011).

The recording point was located in the piriform cortex of the olfactory cortex slice (Mokrushin, 1997; Khama-Murad et al., 2011). A silver reference electrode was in the chamber floor. The epileptiform activity of piriform cortex cells was analyzed offline using special home-made software. The changes of the average frequency of epileptiform discharges (EDs) were studied under the action of PTZ and subsequent exposure slices to MTX mini-peptide.

We analyzed total (interictal-like events and ictal-like discharges) epileptiform activity (Hoffman and Haberly, 1989; Panuccio et al., 2012) evoked by PTZ in slices of the olfactory cortex. For the analysis of interictal-like events and ictal-like discharges, caused by the PTZ in brain slices, in the program in the processing of the EDs was included clustering analysis similar as in Panuccio et al. (2012) studding. So, the processes longer than 2.5 s were considered as ictal activity, whereas all events with a duration of less than 2.5 s were considered as interictal processes. We used in the treatment program bin size equal to 5 ms and 2 ms for interictal and ictal events, respectively.

Drugs

The chemical compounds for preparation of ACSF were supplied by Roshimreaktiv (Russia), while 4B trypsin and PTZ were supplied by Sigma (USA). MTX was provided by the University of California, Berkeley (USA). PTZ and MTX were delivered to slices via bath perfusion. To achieve an enzymatic inactivation of the MTX, the ACSF containing the peptide at a concentration of 250 mg/mL was treated with immobilized trypsin in a sepharose 4B column loaded with activated CN—Br sepharose 4 using the appropriate method (Pavlov Institute Physiology, Russia). The solution containing MTX was passed through the column of 25 mL volume by portions 20 mL at 37 °C.

Data and statistical analysis

The statistical analyses of the changes in EDs frequencies were performed using Wilcoxon—Mann—Whitney nonparametric matched pairs signed-rank U test ($p \leq 0.01$). The data are presented as mean ± S.E.

Results

The measurement of pH of ACFS with MTX

The optimal range of pH for normal activity of brain slices is 7.2—7.4. Addition MTX in ACSF may evoke a modification of its pH and deteriorate the functioning of neurons in slice. By this reason pH of ACFS was measured (pH-meter pH-150, Izmeritel, Russia) twice viz., after preparation of MTX solution, and after perfusion of slices by ACSF with MTX. In both conditions temperature of medium was maintained at 37 °C. The pH of control ACFS (without MTX) was 7.24 ± 0.02 (n = 16), after adding MTX it was 7.27 ± 0.02 (n = 7) and after perfusion of slices by medium with MTX it was 7.31 ± 0.02 (n = 7). Data obtained indicate that MTX solution was slightly alkaline, but it remained within the optimal range of pH throughout experiment.

PTZ-induced epileptiform activity in slices

Experimental protocol was as follows. Initially PTZ was applied to the slices for 20 min in the concentration of 10 mg/mL to detect stable EDs. Then, the slices were