Regulatory Peptides 188 (2014) 1–4

Contents lists available at ScienceDirect

Regulatory Peptides

journal homepage: www.elsevier.com/locate/regpep

Rapid communication

Corticotropin-releasing factor-like peptide modifies the AMPA-, NMDA-dependent and $GABA_B$-ergic properties of synaptic transmissions in vitro

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ARTICLE INFO

Article history:
Received 3 April 2013
Received in revised form 10 November 2013
Accepted 12 November 2013
Available online 22 November 2013

Keywords:
Mystixin-7
AMPA-
NMDA EPSP
IPSPs
LTP/LTD

ABSTRACT

The aim of this study was to investigate the neurotrophic effects of the mystixin-7 mini-peptide (MTX, a synthetic corticotrophin-releasing-factor-like peptide-like peptide) using a slice-based system. The technique on-line monitoring of electrophysiological parameters (excitatory glutamatergic AMPAR-, NMDAR-dependent and inhibitory $GABA_B$-ergic postsynaptic mechanisms) in the olfactory cortex slices of the rat brain exposed to varied amounts of MTX was used. MTX in a dose-dependent manner inhibited both the AMPAR- and NMDAR-mediated postsynaptic processes. The peptide caused depression of inhibitory $GABA_B$-ergic processes only at low doses of MTX (10, 25, 50 mg/mL) while at higher doses (100, 250 mg/mL) it enhanced them. These effects of MTX were reversible. AMPA-dependent (but not NMDA-mediated mechanisms) and inhibitory processes were restored after washing.

Triple reperfusion of slices with MTX (100 mg/mL) accelerated the inhibitory processes and induced NMDAR desensitization. MTX evoked the long-term depression on θ burst stimulation of the slices. This study did not only lead to the conclusion that the functions of the MTX mini-peptide is not limited to anti-inflammatory effects, but also is included modifications of excitatory glutamatergic AMPAR-, NMDAR-dependent and inhibitory $GABA_B$-ergic postsynaptic mechanisms.

1. Introduction

Recently, it was demonstrated that identifying and testing mini-peptides with desired properties is a very promising approach for neuropharmacology. Research of mini-peptides is aimed at the discovery of agents with therapeutic potential based on their effects on different receptors in the central nervous system. The development of mini-peptides may allow us to create medications with improved efficacy based on their high diffusion through the intercellular space, impact on specific targets, and persistent effects due to their ability to retain properties for a long-time as compared to protein macromolecules [1].

The mystixin-7 mini-peptide (MTX) was investigated in this study. MTX represents a novel class of synthetic CRF-like peptides (mystixins family) with molecular formula: C51H84N12O9S and MW 1.041 kDa. MTX has the following amino acid structure: 4-anisoyl-arginyl-lysyl-leucyl-leucyl-thienyl-isoleucyl-leucinamide [2].

In vivo studies have shown that MTX has anti-inflammatory effects on non-nervous cells [3–7]. Based on the pleiotropic activity of peptides it has been hypothesized that MTX may affect the glutamatergic and GABAergic synaptic transmissions. To study the unknown properties of MTX, olfactory cortex slices have been employed in the present study. Slices of the olfactory cortex provide a convenient experimental object. These slices are less traumatic as compared to hippocampal slices due to the intact pial surface. The cutting line is located on the inside, allowing one to keep the normal function of cellular structures for the analysis of incoming sensory input. The morphological structures of the slices are easily defined under slight magnifying. It allows one to consistently and reliably localize the stimulating and recording electrodes at selected points for the extracellular potential recording. At registration the electrical activity as the separate exciting pre- and postsynaptic NMDA and AMPA processes as well as inhibitory $GABA_B$ergic mechanisms are reliably identified [8]. The objectives of the present research were to study the effects of MTX on presynaptic propagation of excitation in the lateral olfactory fibers (LOT), by recording the compound action potential (AP LOT) and analyze the changes of the postsynaptic processes by measuring of the excitatory glutamatergic postsynaptic potentials (AMPA- and NMDA EPSP) and inhibitory $GABA_B$-ergic slow inhibitory postsynaptic potentials (IPSPs). We also intended to study the reversibility of MTX effects. For studying desensitization of the NMDA-dependent mechanisms were tested for the effect of repeated MTX applications. The effects of MTX on the LTP have also been studied as a model for a non-associative form of learning.

Abbreviations: MTX, mystixin-7; ACSF, artificial cerebrospinal fluid; LOT, lateral olfactory tract; FP, field potential; EPSP, excitatory postsynaptic potential; NMDA, N-methyl-D-aspartate; AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic; IPSPs, postsynaptic potential; LTP/LTD, long-term potentiation/depression; TBS, θ burst stimulation.

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0167-0115/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.regpep.2013.11.001

2. Materials and methods

2.1. Preparation of slices

Studies were performed on male Wistar rats with a body weight of 100–150 g (vivarium of the Pavlov Institute of Physiology, RAS). The method of slice preparation and incubation was described in detail previously [9,10]. Briefly, slices of olfactory cortex were cut 450–500 μm thick, and every slice was preincubated for 1 h in 1 mL of artificial cerebrospinal fluid (ACSF) at 37 °C, and pH 7.21–7.24. The composition of ACSF was as follows (mM): NaCl — 124.0, KCl — 5.0, $CaCl_2$ — 2.6, KH_2PO_4 — 1.24, $NaCHO_3$ — 3.0, Tris–HCl (pH 7.4) — 23.0, and glucose — 10.0. ACSF was equilibrated with O_2. After preincubation, slice by slice was transferred into the interface recording chamber.

2.2. Electrical stimulation and recording techniques

Field potentials (FPs) were evoked using platinum custom-made bipolar stimulating electrodes positioned onto the proximal part of the LOT. Stimulation was applied as the rectangular pulses (duration — 0.1 ms, intensity — 1.2–1.5 V, frequency — 0.003 Hz) using the stimulator ESU-1 (Russia). Potentiating trains for the induction of LTP consisted of 10 sets of four pulses at 100 Hz delivered at 200 msec intervals to LOT (θ burst stimulation — TBS).

The FPs were recorded using a glass microelectrode filled with 1 M NaCl with a tip resistance of 1–5 MΩ. Signals were registered with an NTO-2 amplifier (Russia), digitized by an analog-to-digital converter MD-32 (Russia) and stored in a computer. The recording point was located in the piriform cortex of the olfactory cortex slice [8,10]. A silver reference electrode was located in the chamber floor. Typical FPs in the piriform cortex evoked by orthodromic stimulation of the LOT anterior part consist of two main components: namely, presynaptic (AP LOT) and postsynaptic (AMPA, NMDA EPSP and IPSPs). The components of FPs, their characteristics, pharmacological identification and methods of measuring their amplitudes were described in detail earlier [9,10].

2.3. Drugs

Chemical compounds for the preparation of ACSF were supplied by Chimreactiv company (Russia), L-glutamate was received from Sigma (USA). MTX was provided by the University of California, Berkeley (USA). MTX was dissolved in ACSF immediately before testing. The prepared solution was filtered and kept in the thermostat at 37 °C until use.

2.4. Statistical analyses

The statistical analyses of the changes in amplitudes of separate FP components were performed using the nonparametrical Wilcoxon–Mann–Witney matched pairs signed-rank test ($P \leq 0.01$). The data are presented as mean ± S.E.

3. Results and discussion

3.1. Effects of MTX on FP amplitudes modification in brain slices

Synaptic responses in the piriform cortex were registered during the perfusion of slices with ACSF (15 min) so as to determine the initial peak amplitudes of separate FP components (expressed as control). Then synaptic responses in the slice were recorded following addition of MTX at different doses in the bathing medium. The FP traces were registered at the end of the 15 min of MTX action.

As shown in Fig. 1A and B, MTX in concentrations of 10 mg/mL and 100 mg/mL reduced both AMPA and NMDA EPSP amplitudes in a dose-dependent manner. At these concentrations of MTX slight AP LOT amplitude changes were seen. The amplitude of the inhibitory postsynaptic component FPs–IPSPs decreased with MTX at 10 mg/mL. On the contrary, it remained equal to the control value with MTX at 100 mg/mL (Fig. 1A and B).

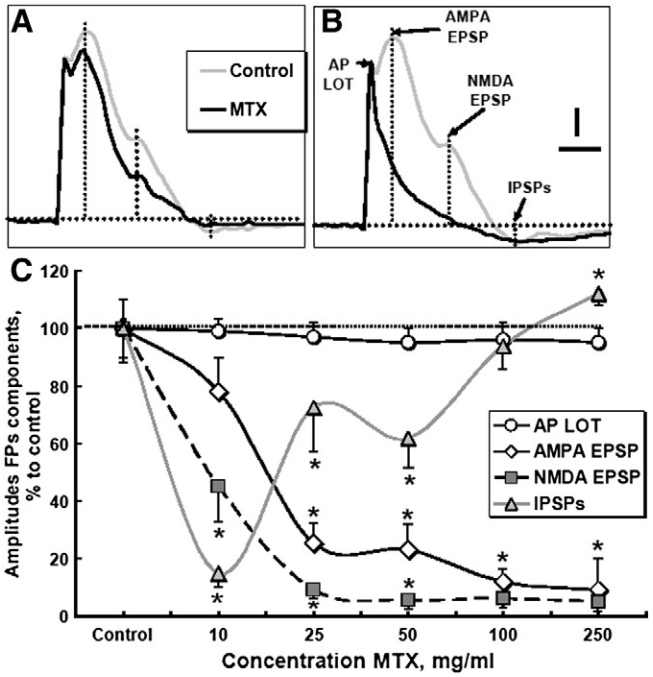

Fig. 1. Dose–response relationship for changes in the amplitudes of separate FP components under the action of various concentrations of MTX. A, B. Traces of FPs under the action of MTX at concentrations of 10 mg/mL (A) and 100 mg/mL (B). Calibrations: 0.1 mV; 3 msec. C. Decrease in the activity of FP components postsynaptic ionotropic glutamatergic mechanisms (AMPA and NMDA EPSP) with increasing MTX concentrations, and a simultaneous increase the activity of inhibitory processes ($GABA_B$-ergic inhibitory postsynaptic processes). X-axis is irregular. Asterisks — the data are significantly different from control values for every curve ($P \leq 0.05$, $n = 7$ for every point, U-criterion, Wilcoxon–Mann–Whitney test).

3.2. Dose- and time-dependent relationships

With increasing concentrations of MTX in the bathing medium the AMPA- and NMDA-dependent processes decreased exponentially and reached the plateau at 25 mg/mL for NMDAR and 100 mg/mL for AMPAR (Fig. 1C). These results indicate that MTX attenuates the efficacy of the AMPA- and NMDA-dependent synaptic transmission in the piriform cortex in a dose-dependent manner.

The application of MTX on slices induced a small depressive effect on the amplitude of the AP LOT with the peptide doses of 50, 100 and 250 mg/mL in the bathing medium. These findings indicate that MTX did not significant influences the process of conduction excitation along the fibers of LOT (Fig. 1C).

The maximum depression of the inhibitory $GABA_B$-ergic mechanisms occurred when MTX was applied at a concentration of 10 mg/mL ($U = 9$, $n = 8$ — control; $n = 17$ — MTX, $P \leq 0.05$). Increasing the concentration of MTX in the bathing medium led to gradual recovery of the IPSPs amplitude, and at 250 mg/mL The latter was higher than the control level ($P \leq 0.05$) (Fig. 1C).

There were no changes in the amplitudes of separate FP components at MTX concentrations of 1.0, 0.5, and 0.1 mg/mL in the bathing medium (the data are not shown in Fig. 1C).

The dose–response relationships depend on the exposure time slices mini-peptide.

Data shown in the Table 1 indicate that the latent time of MTX effects depend on its concentration in a washing medium.

So, at MTX concentrations of 10 and 25 mg/mL the latent period of MTX effects for AMPARs, NMDAR and $GABA_BR$ was the greatest. The