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Research report

Tetanically released zinc inhibits hippocampal mossy fiber calcium, zinc and synaptic responses

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Abstract

At the zinc-enriched mossy fiber synapses from hippocampal CA3 area, electrical or chemical stimulation evokes zinc release from glutamatergic synaptic vesicles that may cause different pre- or postsynaptic actions. Besides zinc that can be co-localized with glutamate and GABA, the mossy fibers contain a very high density of ATP-sensitive potassium channels that are activated by zinc. We have investigated the possibility that intensely released zinc inhibits presynaptic calcium changes and consequently zinc and glutamate release. The studies were made combining optical recording of fast presynaptic calcium and zinc signals, using the fluorescent indicators Fura-2 and *N*-(6-methoxy-8-quinolyl)-*para*-toluenesulfonamide, respectively, with measurements of field potentials. We have observed that strong tetanic stimulation caused posttetanic depressions of electrically induced presynaptic calcium and zinc signals and of synaptic responses, the depressions being blocked by zinc chelators. These results suggest that endogenously released zinc has an inhibitory role, mediated by presynaptic ATP-sensitive potassium channels and/or presynaptic calcium channels, that leads to the depression of zinc and glutamate release.

Theme: Excitable membranes and synaptic transmission
Topic: Presynaptic mechanisms

Keywords: Long-term potentiation (LTP); CA3 area; Fura-2; TSQ; TPEN; Posttetanic depressions

1. Introduction

The mossy fibers from hippocampal CA3 area contain one of the highest concentrations of chelatable zinc in the brain that is assumed to be co-released with glutamate [11,12]. Several studies, including recent ones using zinc indicators, have shown that zinc release depends on the frequency and intensity of stimulation and also that it is calcium-dependent and sensitive to tetrodotoxin [3,16,24,25,36,48]. Following release into the synaptic cleft and diffusion, hippocampal mossy fiber zinc may act on different pre- and postsynaptic mechanisms, be taken up by zinc transport systems or enter the postsynaptic neurons [12]. It has been shown that zinc modulates the action of multiple receptors and channels, enhancing AMPA and ATP-sensitive potassium (K_{ATP}) channel activity and inhibiting NMDA, $GABA_A$ and voltage-dependent channel responses [4,15,44]. Zinc may enter the postsynaptic region via calcium permeable AMPA/kainate and NMDA receptor channels or through voltage-dependent calcium channels [29,43], particularly following excessive zinc release [51].

The hippocampal mossy fibers have special features including a very high density of K_{ATP} channels [31,47,58] and co-localized GABA and zinc [38]. They also have characteristic forms of paired-pulse facilitation (PPF) and long-term potentiation (LTP), this form of LTP being NMDA-receptor independent and assumed to be expressed presynaptically [17,32]. It has been shown that released zinc has no effect on normal synaptic transmission or PPF [50,55]. Concerning the requirement of zinc for LTP induction, contradictory results have been obtained that

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are either in favor [25,28] or against [34,50,55] that requirement. Furthermore, exogenous zinc reduces PPF [22] and prevents the formation of both mossy fiber LTP and CA1 LTP [55].

Different lines of evidence suggest that, at the mossy fiber synapses, released zinc inhibits presynaptic calcium channels [55] and postsynaptic NMDA [50] and $GABA_A$ [38] receptors. The latter types of inhibition were not observed in studies made in zinc transporter 3 knockout mice [27], existing controversy about the existence of vesicular zinc in mocha mutant mice [21,45]. Recently, it has also been reported that released mossy fiber zinc activates presynaptic K_{ATP} channels [4]. It has been proposed that activation of these channels rapidly hyperpolarizes the cells causing reduced calcium influx and thereby a decrease in transmitter release, both in the case of glutamate [4] and GABA [41] release. It has also been shown that mossy fiber zinc inhibits NMDA-receptor-mediated responses at the recurrent mossy fiber synapses [30] and that zinc spillover causes a similar heterosynaptic inhibition in *stratum radiatum* [48].

In this work, we have investigated the effect of endogenously released zinc on presynaptic calcium changes. For this purpose, we have applied intense stimulation to the mossy fiber pathway, consisting of different groups of tetani, and investigated their effect on presynaptic calcium signals induced by single stimuli. Calcium influx contributes to these signals via P/Q- and N-type voltage-dependent calcium channels that mediate fast synaptic transmission and also via R-type calcium channels that may evoke release following high-frequency stimulation [9,10,37]. Involvement of calcium release from intracellular stores in presynaptic calcium changes has been detected but only during brief high-frequency trains [23,26]. Since both zinc and glutamate release are calcium-dependent, we have also examined the effect of intense stimulation on single presynaptic zinc signals, characterized earlier [36], and on field potentials. The optical transients were obtained using the permeant form of the calcium indicator Fura-2 [14] and the fluorescent permeant zinc probe *N*-(6-methoxy-8-quinolyl)-*para*-toluenesulfonamide (TSQ) [39] that forms fluorescent complexes with mossy fiber vesicular zinc [11]. The involvement of endogenous zinc on the posttetanic signals was investigated using the permeant and impermeant zinc chelators N,N,N′,N′-tetrakis (2-pyridylmethyl)ethylenediamine (TPEN) [2] and ethylenediaminetetraacetic acid (Ca-EDTA), respectively. We had previously observed, in agreement with other studies, that the application of two tetani (100 Hz, 1 s) induced mossy fiber LTP [36]. In order to look for synaptic zinc actions independent of LTP-inducing events, which might mask those actions, we have applied different groups of tetani after LTP was established.

Most of the present findings have been reported in abstract form [33,35].

2. Experimental procedures

2.1. Electrophysiology

Wistar rats (4–6 weeks old) were anesthetized with ethyl ether and decapitated. Transverse slices (400 μm thick) were prepared from the hippocampus and transferred to the experimental chamber where they were perfused (1.5–2 ml/min) with oxygenated artificial cerebrospinal fluid (ACSF) (30–32 °C), containing (in mM): NaCl 124; KCl 3.5; $NaHCO_3$ 24.0; NaH_2PO_4 1.25; $MgCl_2$ 2.0; $CaCl_2$ 2.0 and glucose 10.0. The mossy fibers were stimulated using stainless steel bipolar electrodes, with single current pulses (0.2–0.5 mA; 100 μs; 0.016 Hz), equal to 40% of the saturation value. Extracellular field potentials were recorded in the CA3 pyramidal layer using glass microelectrodes (1–10 MΩ), containing a 2 M NaCl solution. Mossy fiber LTP was elicited with two 100-pulse trains (at 100 Hz/1s) with a 30-s interval and monitored with pulses given at baseline stimulus strength.

Electrically induced presynaptic zinc and calcium changes were detected in the *stratum lucidum* using a silicon photodiode and an optical setup for transfluorescence. The signal from the photodiode passed through an I/V converter with 1 GΩ feedback resistance and through an AC-coupled amplifier with a low cutoff frequency (1 Hz). For zinc measurements, the slices were incubated (60–90 min, 35–39 °C) in the normal medium containing 30 μM TSQ. The permeant zinc chelator TPEN (20 μM) or the impermeant chelator Ca-EDTA (2.5 mM) was applied in the bath for 1 h prior to the recording period. The group II metabotropic glutamate receptors (mGluRs) agonist (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV) (DCG-IV) was added (1 μM) to the perfusion medium at the end of the experiments. Zinc measurements were performed with a tungsten/halogen lamp (12V, 100 W) and 400 nm excitation light, selected using a linear variable interference filter (400–700 nm; Schott) with slit widths of 0.6 mm. Emission light was selected by a 500 nm long-band pass filter.

The calcium studies were performed using Fura-2/AM, the permeant form of the fluorescent calcium indicator Fura-2, pressure-injected in the mossy fiber tract more than 500 μm away from the recording site at least 1 h before starting the measurements. The excitation light, from a xenon lamp (75 W), was selected using a 380 nm narrow band interference filter (10 nm bandwidth) and the emission light using a 500 nm long-band pass filter. Although Fura-2 is normally used as a calcium indicator, it has a much higher affinity for zinc than for calcium and forms complexes with these ions that have similar spectral shifts [14] but different isosbestic points [49]. In these studies, the Fura-2 signals were measured using 380 nm excitation light that has approximately the isosbestic wavelength of the Fura-2/zinc complex [49].

The zinc and calcium signals were also observed in the presence of bath-applied 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (10 μM) and D(-)-2-amino-5-phosphonopentanoic acid (D-APV) (50 μM), antagonists of α-amino-3-