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Research report

Phosphodiesterase 4B (PDE4B) and cAMP-level regulation within different tissue fractions of rat hippocampal slices during long-term potentiation in vitro

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Abstract

Molecular events associated with mnemonic processes and neuronal plasticity are postulated to result in functional changes in synaptic structure. One possible site is the post-synaptic density, where activity-dependent changes modulate signal transduction cascades. In this report, we detail spatial–temporal changes for phosphodiesterase 4B (PDE4B) proteins and their substrate cAMP within three neuronal fractions during early and late long-term potentiation (LTP). The cAMP-dependent protein kinase A cascade – which can be regulated by distinct PDE4B activity – is required for mnemonic processes as well as mechanisms of neuronal plasticity, such as those during the maintenance or late-LTP. Fluorescence in situ hybridization studies (FISH) identified no translocation of PDE4B3 from the soma after late-LTP induction indicating a subtle, local control of PDE4B activity. Protein changes were detected within the PSD-enriched fraction. From these results, we conclude that either the changes in PDE4B are due to modulation of pre-existing mRNA, or that the protein is specifically translocated to activated synaptic structures. Furthermore, we report late changes in cAMP levels in the somato-dendritic fraction and discuss this result with the increased PDE4B1/3 doublet in the PSD-enriched fraction.

Theme: Excitable membranes and synaptic transmission
Topic: Long-term potentiation: physiology; Postsynaptic mechanisms

Keywords: Long-term potentiation; Hippocampus; Phosphodiesterase; cAMP; PDE4; Memory formation; Neuronal plasticity

1. Introduction

Molecular changes that are hypothesized to underlie long-term potentiation (LTP) – the most prominent cellular model for learning and memory formation – are assumed to occur within different neuronal loci. One possible site is a structure termed the post-synaptic density (PSD; [8,14]). Recent biochemical analyses have identified that a component of the PSD, the *N*-methyl-D-aspartate receptor (NMDAR; an excitatory glutamate receptor) forms a highly structured complex [35,57,58]. This is an interacting complex linked to signal transducer molecules, protein kinases A (PKA) and C (PKC) and cyto-architectural proteins, for example, *Arg3.1* [35,40,57,58]. Activity- and NMDAR-dependent responses during LTP induction evoke changes in second messenger levels. One subsequent outcome is the binding of these messengers to regulatory components of PKA and PKC and activating kinase-dependent cascades, whose effects include protein phosphorylation [1,28,53]. The initial

Abbreviations: ACD, Actinomycin D; CREB, cAMP-response-element-binding protein; EIA, Enzyme-immunoassay; HFS, High-frequency stimulation; LFS, Low-frequency stimulation; LTP, Long-term potentiation; NMDA, *N*-methyl-D-aspartate; PAGE, Poly-acrylamide gel electrophoresis; PBS, Phosphate buffered saline; PDE, Phosphodiesterase; PKA, cAMP-dependent protein kinase; PKC, Protein kinase C; STP, Short-term potentiation; FISH, Fluorescence in situ hybridization; Tet, Tetanization; TBST, Tris buffered saline Tween-20

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changes are involved in a transient protein-synthesis-independent phase of LTP (early-LTP) whereby the effects are postulated to (a) gate LTP induction, prime the synapse towards LTP [9,10] and, (b) polysomal de-repression, that is, local translation of mRNAs [38,48]. The prolonged maintenance of LTP (late-LTP), however, requires protein synthesis and for the prolonged maintenance also gene expression [17,20,22–29,39,46,47]. The activation of genes may take place already during earlier stages of LTP, however, the effect of the gene products is detectable only after their processing and cellular transport during later stages. The initial focus of our study was to analyze genes involved for late-LTP (beyond 4 h) and to assess how, or if, their products are related to the underlying processes of LTP [2]. Activation of protein kinase A (PKA) by increased levels of cAMP has been documented essential for late gene expression via activation of transcription factors [1,20,22–30,53,54]. cAMP-levels are potentiated by predominantly two mechanisms. The first is via Ca^{2+} entry through the activated NMDAR, which leads to activation of calmodulin, which then interacts with type I adenylate cyclase (AC-I) to raise cAMP levels, as described in juvenile animals [15,16]. The second mode, identified in adult animals, is via synergistic or reinforcing heterosynaptic inputs, for example, dopaminergic or aminergic neurones [5,27,28,50]. These inputs, unlike glutamatergic inputs, have receptors positively coupled to G-proteins, which – once activated – interact with type II adenylate cyclases (AC-II) to potentiate cAMP [43]. Interestingly, perturbations of cAMP homeostasis, either of proteins that potentiate/regulate cAMP, result in pleiotropic effects, which include memory deficits and metaplastic-associated phenomena [12,49,61]. Conversely, pharmacological inhibition of the type 4 phosphodiesterases (PDE4) gene products (which exclusively hydrolyse cAMP), results in enhanced LTP and improvements in learning tasks and amelioration of age or genetic deficits in rodents [4,6,13,37,45].

We previously reported the isolation of a cAMP-specific PDE4 within single, acutely isolated rat hippocampal slices in vitro. The transcript and the protein of PDE4B3 were specifically modulated during different LTP-phases in area CA1 with consequences on cAMP levels [2]. This contrasts with the area dentata where PDE4B changes were dependent upon mRNA derepression and translation within a specific cellular compartment [3]. Furthermore, several reports have detailed that PDE4B proteins are compartmentalized within two cell fractions, where they tightly regulate cAMP-levels [33,36]. To investigate further, as to whether PDE4B3 is translocated within the cell as described for other gene products, like ARC [55,56], we have investigated transcript location by fluorescence in situ hybridization (FISH) and protein and cAMP-level changes within three different cell fractions of the hippocampal slices after LTP induction in area-CA1.

2. Materials and methods

2.1. Electrophysiological experiments

In the studies detailed here, 7-week-old male Wistar rats of the outbred strain MOL: WIST (SHOE) were used. The animals were maintained in accordance with Institutional, Federal and German Government guidelines on animal care, with the minimum number used for statistical purposes. Rats were sacrificed, the brain rapidly removed and the right hippocampus dissected out, from which four 400-μm-thick transverse hippocampal slices from the dorsal–ventral area were prepared. Slices were maintained in an artificial cerebro-spinal fluid (ACSF) perfused/carbogenated in an interface chamber (for more details, see [2,26,50]). Two monopolar stainless steel electrodes (for electrical stimulation) were placed opposite one another in the stratum radiatum, for orthodromic signal generation of two separate synaptic inputs to one neuronal populations (Fig. 1A). Two further electrodes were placed in the stratum radiatum or pyramidale of area CA1 for field excitatory post-synaptic potential (fEPSP) and population spike (PS) recordings, respectively. The electrophysiological protocols for early- (1 tetanus (Tet): 1× 100 Hz train, 1 s) and late-LTP (3 Tet: three 1× 100 Hz trains, 1 s at 10-min intervals) induction and low-frequency stimulation (LFS) were as described previously [2,23]. In the studies described, a single slice was always used for the electrophysiological study, which was then used for the subsequent molecular or biochemical analyses, that is, in the reported data, the number *n* represents the number of electrophysiologically treated and biochemically analyzed single slices from n animals.

2.2. Molecular and biochemical studies

For fluorescence in situ hybridization (FISH) studies, all solutions and materials were treated and prepared with diethyl-pyrocarbonate (DEPC) treated sterile water. After 3 Tet-LTP hippocampal slices were immediately submerged for 1 h in ice cold 3% paraformaldehyde (PFA)–0.1 M phosphate-buffered saline (PBS) pH 7.4 solution. The slices were then transferred to 30% sucrose in PBS for 2 h, mounted onto a cryostat section block, covered with Tissue-Tek (Sigma) and frozen at −20 °C. Cryosections (8 μm thick) were then prepared from these slices. Slice sections were then incubated, free floating, in hybridization buffer: 2× SSC; 50 mM Tris–HCl; 0.5 mM EDTA; 2× Denhardt's reagent; 50% v/v formamide; 1 mg/ml poly-A; 2 mg/ml yeast tRNA (Sigma) pH 7.5. Sections were initially pre-hybridized at 50 °C for 3 h, then incubated with buffer containing denatured cRNA *PDE4B3* or *Arg3.1* (*PDE4B3* probe preparation with fluoroscein green-UTP; detailed in [2]). *Arg3.1* probe (a kind gift from Dr. D Kuhl; Hamburg University) was labeled with Texas red-UTP as detailed in [40], (chromaphore-UTPs from Molecular Probes Inc., Eugene Oregon; USA) and hybridized with gentle shaking