Available online at www.sciencedirect.com
SCIENCE DIRECT®
Neuropharmacology 48 (2005) 949–955
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

ATP hydrolysis is required for the rapid regulation of AMPA receptors during basal synaptic transmission and long-term synaptic plasticity

Wonil Lim[a,1], John T.R. Isaac[a,b,*]

[a]*Department of Anatomy, MRC Centre for Synaptic Plasticity, University of Bristol, Bristol BS8 1TD, UK*
[b]*NINDS/NIH, 35 Convent Drive, Bethesda, MD 20892, USA*

Received 2 September 2004; received in revised form 16 December 2004; accepted 1 February 2005

Abstract

ATP hydrolysis is critical for many cellular processes; however, the acute requirement for ATP hydrolysis in synaptic transmission and plasticity in neurons is unknown. Here we studied the effects of postsynaptically applying the non-hydrolyzable ATP analogue adenosine 5′-[β,γ-methylene]triphosphate (AMP-PCP) into hippocampal CA1 pyramidal cells in hippocampal slices. The effects of this manipulation were investigated on basal transmission and on two forms of long-term synaptic plasticity, long-term potentiation (LTP) and long-term depression (LTD). AMP-PCP caused an increase in basal AMPA receptor (AMPAR)-mediated transmission, which occurred rapidly within minutes of infusing the drug. This effect was selective for AMPARs, since pharmacologically isolated NMDAR-mediated synaptic currents did not exhibit this run up. In two-pathway experiments infusion of AMP-PCP blocked the induction of both LTD and LTP. These findings show an acute and selective role for ATP hydrolysis in regulating AMPAR function both during basal transmission and long-term synaptic plasticity. Recent evidence indicates that AMPARs are selectively and acutely regulated by the ATPase *N*-ethylmaleimide-sensitive factor (NSF), which forms part of a multi-protein complex with AMPARs. Our data are consistent with the idea that such a mechanism that can acutely bi-directionally regulate AMPAR function at synapses and requires ATP hydrolysis is necessary for rapid activity-dependent changes in synaptic strength.

Keywords: AMPA receptor; *N*-Ethylmaleimide-sensitive factor; AMP-PCP; Long-term potentiation; Long-term depression

1. Introduction

Glutamate is the main excitatory transmitter in the mammalian CNS and its actions are mediated by three classes of ionotropic receptors: AMPA, kainate and NMDA receptors (Bettler and Mulle, 1995). Glutamatergic synapses undergo several forms of activity-dependent long-term synaptic plasticity (long-term potentiation, LTP; long-term depression, LTD) characterized by a change in the AMPA receptor (AMPAR)-mediated response. These forms of long-term synaptic plasticity are widely believed to be important mechanisms underlying learning and memory, development and certain pathological conditions (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999).

AMPARs are heteromeric assemblies composed of different combinations of four subunits, GluR1-4. The

* Corresponding author. Tel.: +1 301 451 7221; fax: +1 301 480 1789.
E-mail address: isaacj@ninds.nih.gov (J.T.R. Isaac).
[1] Present address: Department of Physiology, Gachon Medical School, 1198 Guwol-dong, Namdong-gu, Incheon 405-760, South Korea.

0028-3908/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2005.02.001

AMPAR subunits are known to undergo phosphorylation involving various kinases, and this can directly regulate AMPA receptor function and surface expression at synapses. There is a large body of evidence supporting a role for a number of kinases in the induction and expression of NMDA receptor-dependent LTP (Malenka and Nicoll, 1999; Soderling and Derkach, 2000; Song and Huganir, 2002). The targets of kinase activity during LTP have yet to be fully identified; however, there is good evidence that AMPAR phosphorylation is critical to the mechanism of LTP expression (Barria et al., 1997; Lee et al., 2000, 2003). In contrast, NMDA receptor-dependent LTD is associated with phosphatase activity (Mulkey et al., 1993, 1994; Morishita et al., 2001) and dephosphorylation of GluR1 AMPA receptor subunits is a key step in its expression mechanism (Kameyama et al., 1998; Lee et al., 1998, 2000). However, there is also evidence that kinase activity and phosphorylation of GluR2 also occurs during hippocampal LTD (Kim et al., 2001; Seidenman et al., 2003).

Numerous ATPases are expressed in neurons that regulate many key cellular functions. Of particular interest in synaptic function is the ATPase *N*-ethylmaleimide-sensitive factor (NSF), which is an essential component of various membrane fusion events including the exocytosis of synaptic vesicles, the activity of which is required in the disassembly of the SNARE complex (Sollner and Rothman, 1994; Jahn et al., 2003). NSF has also been identified as a postsynaptic protein that interacts with the GluR2 AMPAR subunit (Nishimune et al., 1998; Osten et al., 1998; Song et al., 1998). This interaction has been shown to rapidly regulate AMPA receptor function at synapses (Nishimune et al., 1998; Song et al., 1998; Luscher et al., 1999; Luthi et al., 1999; Noel et al., 1999; Lee et al., 2002) and plays a role in hippocampal LTD (Luscher et al., 1999; Luthi et al., 1999; Lee et al., 2002). In addition NSF, in an ATP-dependent manner, has been shown to disassemble a SNARE-like complex containing GluR2, SNAPs and PICK1, a PDZ protein that interacts at a distinct extreme C-terminal site on GluR2 (Hanley et al., 2002). This NSF-dependent mechanism has been proposed as a key step in the rapid activity dependent trafficking of AMPA receptors at synapses.

Taken together, these studies indicate that the NSF-GluR2 interaction as well as kinase activity phosphorylating synaptic proteins including AMPARs are required in the rapid regulation of AMPARs at synapses. However, it is unclear at what step in these processes ATP hydrolysis is required and whether there is an acute requirement for this in the regulation of AMPAR function during LTP and LTD. To investigate this we have used the non-hydrolyzable ATP analogue, adenosine 5′-[β,γ-methylene]triphosphate (AMP-PCP; Blum et al., 1977; Pang and Briggs, 1977). Infusion of AMP-PCP inside hippocampal CA1 neurons caused a rapid increase in the amplitude of AMPA receptor-mediated excitatory postsynaptic currents (EPSCs) but had no effect on NMDA receptor-mediated synaptic responses. Furthermore, AMP-PCP blocked both LTP and LTD. These data indicate an important role for ATP hydrolysis in the rapid regulation of AMPA receptor function both during basal transmission and bidirectional long-term synaptic plasticity. One possible mechanism for this is the ATPase activity of NSF in complex with AMPARs at synapses.

2. Methods

2.1. Slice preparation

Hippocampal slices were prepared from 12 to 16 day-old Wistar rats. Rats were anesthetized and decapitated and the brain was placed in ice-cold extracellular solution. Hippocampal slices (400 μm thick) were prepared on a vibratome and were allowed to recover for at least 1 hr at room temperature. They were then transferred to a recording chamber and continuously superfused with extracellular solution saturated with 95% O_2 and 5% CO_2. The extracellular solution contained (in mM): 119.0 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 1.0 $NaHPO_4$, 26.2 $NaHCO_3$, 2.5 $CaCl_2$, 11.0 glucose, 0.05 picrotoxin (pH 7.4).

2.2. Electrophysiology

Whole-cell voltage clamp recordings were made from CA1 pyramidal cells using electrodes with a resistance of 3–5 MΩ when filled with the intracellular solution. The intracellular solution contained (in mM): 130.0 Cs methanesulfonate, 8.0 NaCl, 10.0 HEPES, 0.5 EGTA, 4.0 MgATP, 0.3 NaGTP, and 5.0 QX-314 (pH 7.2 with CsOH; 285 mOsm). ATP was replaced by 2 mM AMP-PCP where indicated. In all experiments, the effects of AMP-PCP were compared to interleaved experiments using control intracellular solution. EPSCs were recorded at a holding potential of −70 mV and evoked by electrical stimulation of Schaffer collateral–commissural axons at a frequency of 0.1 Hz using a bipolar stimulating electrode placed in the striatum radiatum. In most cells, two pathways were stimulated alternately. In experiments studying NMDA receptor-mediated EPSCs, 0.1 mM extracellular Mg^{2+} was used, 2 μM NBQX was included in the bath and cells were held at −70 mV. LTP was induced with a pairing protocol of 100 stimuli at 1 Hz with a holding potential of −10 mV applied to one pathway. LTD was induced using a protocol of 300 stimuli at 1 Hz paired with a holding potential of −40 mV applied to one pathway (Daw et al., 2000). For all long-term plasticity experiments,