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Differential redistribution of native AMPA receptor complexes following LTD induction in acute hippocampal slices

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Abstract

AMPAR trafficking is crucial for the expression of certain forms of synaptic plasticity. Here, using surface biotinylation of hippocampal slices and subsequent synaptosome isolation we assessed AMPAR surface expression in synaptosomes following NMDA-evoked long-term depression (NMDA-LTD). Surface levels of GluR1, GluR2 and GluR3 in synaptosomes were markedly reduced 90 min after NMDA-LTD induction. Consistent with endocytosis and degradation, whole-cell surface and total expression levels of GluR2 and GluR3 were also reduced. In contrast, whole-cell surface levels of GluR1 were unaltered at 90 min suggesting that AMPARs with different subunit composition are redistributed to different non-synaptic compartments following LTD induction in acute hippocampal slices.

Keywords: AMPA receptor; NMDA receptor; Long-term depression; Synaptosome; Hippocampal slice; Dendritic spine; AMPAR surface expression; Synapse

1. Introduction

α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) are responsible for most fast excitatory neurotransmission in the mammalian central nervous system and their regulation directly controls synaptic efficacy (Palmer et al., 2005). There are four different AMPAR subunits, GluR1–4, which assemble as tetramers (Greger et al., 2003). The subunit composition of AMPARs is critical in determining the functional and trafficking properties of resulting channels (Malinow and Malenka, 2002). GluR2-containing AMPARs have low Ca^{2+} permeability and consequently display outwardly rectifying current–voltage (I–V) relationships, whereas GluR2-lacking AMPARs have high Ca^{2+} permeability and are inwardly rectifying (Hollmann et al., 1991; Burnashev et al., 1992). In hippocampal neurons AMPARs comprise mainly GluR2 (Ozawa and Lino, 1993) with either GluR1 (GluR1/2) or GluR3 (GluR2/3), although there is evidence for GluR1 homomers and GluR2-lacking heteromers (Wenthold et al., 1996). Based on experiments using recombinant subunits it has been proposed that GluR1/2 complexes are driven into synapses during hippocampal long-term potentiation (LTP), whereas GluR2/3 complexes are continuously inserted into synapses regardless of activity (Shi et al., 2001). More recently, it has been reported that native GluR2-lacking AMPARs are transiently incorporated into synapses during hippocampal LTP, which are later replaced by GluR2-containing receptors (Plant et al., 2006). The rules regulating synaptic AMPAR trafficking during the inverse process of long-term depression are, however, less well defined.

Low frequency stimulation (LFS) is typically used to induce LTD in acute hippocampal slices (Dudek and Bear, 1993) although prolonged synaptic depression can also be evoked by bath application of NMDA (3 min, 20 μM; (Lee et al., 1998)). By mimicking NMDA-LTD in dispersed neuronal culture, several studies have provided valuable insight into the mechanisms of NMDA-induced AMPAR trafficking

Abbreviations: AMPAR, α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors; LTD, long-term depression; NMDA, *N*-methyl-D-aspartate; I–V, current–voltage; LTP, long-term potentiation; ACSF, artificial cerebral spinal fluid; *f*EPSP, field excitatory post-synaptic potential.

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(Beattie et al., 2000; Ehlers, 2000; Ashby et al., 2004; Lee et al., 2004). As yet, it is unclear how these processes correlate with LTD in slices that, from electrophysiological studies, are considered a more physiologically relevant preparation than dispersed cell cultures.

Using acute hippocampal slices, we have assessed the effects of NMDA-LTD induction on the surface localisation of native GluR1, GluR2 and GluR3. We show that all three subunits are removed from the surface of synaptosomes following NMDA-LTD induction. Interestingly, whole-cell surface and total levels of GluR1 are unaltered under these conditions. In contrast, whole-cell surface and total levels of GluR2 and GluR3 are reduced suggesting that AMPARs with different subunit composition are redistributed to different non-synaptic sites during LTD.

2. Methods

2.1. Electrophysiology

Hippocampal slices (400 μm) were prepared from P21 to 23 male Wistar rats in ice-cold artificial cerebrospinal fluid (ACSF; composition in mM: 124 NaCl; 3 KCl; 26 $NaHCO_3$; 1.25 NaH_2PO_4; 2 $CaCl_2$; 1 $MgSO_4$; 10 D-glucose; saturated with 95% O_2 and 5% CO_2). After removing the CA3 region, slices were transferred to a submersion storage chamber where they were maintained in ACSF for 1 h at room temperature. Field excitatory post-synaptic potentials (*f*EPSPs) were measured at 28 °C using glass recording electrodes filled with ACSF. *f*EPSPs were evoked in the CA1 region by stimulating Schaffer collaterals with 0.2 ms pulses delivered using two bipolar stimulating electrodes. Baseline responses were collected by stimulating once every 15 s using a stimulating intensity of 20 μA. The stimulating intensity was then reduced (5–15 μA) so that the *f*EPSP peak amplitude was 70% of the value obtained using a 20 μA stimulating intensity. LTD was evoked by transient NMDA application (5 min; 20 μM in ACSF; Tocris). *f*EPSPs were recorded for at least 3 h after NMDA treatment. Analysis was performed using the LTP221p Reanalysis computer programme (Anderson and Collingridge, 2001). *f*EPSP slope and fibre volley peak amplitude values were then imported into Sigmaplot where they were normalised against the initial 40 min baseline. Data recorded 10 min before drug treatment and 90 min after drug treatment were compared using paired Student's *t*-tests.

2.2. Slice treatment for biochemical analysis

To compare multiple NMDA- and ACSF (control)-treated slices simultaneously we used a two-chamber perfusion system. Before treating the slices the cortex was removed leaving the CA1/CA2 region. Four to seven slices per group (groups of seven slices were necessary for synaptosome isolation) were equilibrated at room temperature for 1 h before being placed into the chambers of the two-chamber perfusion system (maintained at 28 °C). Following ACSF perfusion for 40 min, one chamber was perfused with ACSF plus 20 μM NMDA for 5 min, while the other chamber was perfused with ACSF alone. Both sets of slices were then perfused with ACSF for a further 15–90 min prior to biochemical analysis.

2.3. Slice biotinylation and homogenisation

Slices were washed once with ice-cold ACSF (5 min) and then incubated with Sulfo-NHS-SS-Biotin (Pierce; 0.5 mg/ml in ACSF) for 30 min on ice. Excess biotin was removed by two brief washes with 50 mM NH_4Cl (in ACSF) and two ACSF washes. Slices were then homogenised in 1 ml of homogenisation buffer (320 mM sucrose; 10 mM Tris; pH 7.4) and centrifuged at 1000 *g* for 5 min to remove nuclear material and cell debris.

2.4. Isolation of hippocampal synaptosomes

Post-nuclear supernatants were layered onto discontinuous step gradients consisting of 20, 10, 6 and 2% percoll (diluted in homogenisation buffer). Purified synaptosomes were collected from the 20–10% percoll interface following 5 min of centrifugation at 35,000 *g* (Nakamura et al., 1993; Grilli et al., 2004). Synaptosomes were then resuspended in 500 μl of lysis buffer (150 mM NaCl, 20 mM HEPES, 2 mM EDTA, 1% Triton, 0.1% SDS, pH 7.4), sonicated and placed on a head-over-head shaker for 2 h. Samples were cleared at 16,000 *g* for 20 min and the protein concentration of the resulting supernatant was determined using a BCA kit (Pierce). Protein matched samples were then used for streptavidin pull downs.

2.5. Isolation of post-synaptic density

Synaptosomes were pelleted by centrifugation (16,000 *g*; 5 min; 4 °C) and resuspended in 300 μl 0.32 M sucrose and 0.1 mM $CaCl_2$. The synaptosomes were then diluted 1:10 in ice-cold 0.1 mM $CaCl_2$ and mixed with an equal volume of 2× solubilisation buffer (2% Triton X-100, 40 mM Tris, pH 6.0). Following 30 min incubation at 4 °C the insoluble material (synaptic junctions) was pelleted by centrifugation (40,000 *g*, 30 min, 4 °C). The pellet was resuspended in 10 volumes of 1× solubilisation buffer (1% Triton X-100, 20 mM Tris, pH 8.0) and incubated for 30 min at 4 °C. The suspension was then centrifuged at 40,000 g for 30 min at 4 °C. The pellet contained the insoluble post-synaptic density (Phillips et al., 2001).

2.6. Isolation of hippocampal membranes

Post-nuclear supernatants were centrifuged at 100,000 *g* for 1 h and supernatants were discarded. The hippocampal cell membranes were resuspended in 1 ml of lysis buffer, sonicated and then treated as above.

2.7. Streptavidin pull down

Streptavidin beads (40 μl; Sigma) were washed three times with lysis buffer. Lysed biotinylated samples were added to the beads (50 μg total protein) and mixed on a head-over-head shaker for 4 h. Beads were then centrifuged at 800 *g* and the supernatants removed. Beads were subsequently washed three times with lysis buffer and biotinylated proteins were eluted from the beads using 2× SDS-PAGE loading buffer (containing β-mercaptoethanol) at 90 °C for 5 min. The beads were then vortexed for 10 s and centrifuged at 16,000 *g*. Supernatants were removed and stored at −20 °C until further use.

2.8. Quantitative immunoblotting

Proteins were resolved by SDS-PAGE and immunoblotting was performed using a rabbit polyclonal antibody to GluR1 (Upstate; 0.6 μg/ml) and mouse monoclonal antibodies to GluR2 (Chemicon; 1 μg/ml), GluR3 (Chemicon; 2 μg/ml), β-actin (Sigma 0.5 μg/ml), β-tubulin (Sigma; 0.5 μg/ml) and *N*-Cadherin (BD Transduction Laboratories; 1 μg/ml). Quantitative densitometric analysis was performed using NIH Image J.

2.9. Calculation of AMPAR subunit surface expression

Increasing amounts (3.13, 6.25, 12.5, 25 and 50 μg) of total protein were resolved alongside the streptavidin bead-bound and un-bound fractions. Following Western blot analysis, optical density values were obtained for each of the bands representing the input and bound fractions. By plotting these values the percentage surface expression of each subunit was determined by normalising the bound optical band density value to the input band density values. To quantify NMDAR-evoked changes in subunit expression (surface and total) the optical density value of the band representing the NMDA-treated slices was divided by the optical density value of the band representing the ACSF-treated slices. Pooled ACSF band density values were