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RACK1 is involved in β-amyloid impairment of muscarinic regulation of GABAergic transmission

Wenhua Liu [a], Fei Dou [b], Jian Feng [a], Zhen Yan [a,*]

[a] *Department of Physiology and Biophysics, State University of New York at Buffalo, School of Medicine and Biomedical Sciences, Buffalo, NY 14214, USA*

[b] *Key Laboratory for Cell Proliferation and Regulation Biology Ministry of Education, Department of Cell Biology, College of Life Science, Beijing Normal University, Beijing 100875, China*

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Abstract

RACK1 (receptor for activated C-kinase 1), an anchoring protein that shuttles activated PKC to cellular membranes, plays an important role in PKC-mediated signal transduction pathways. A significant loss of RACK1 has been found in the brain of aging animals and Alzheimer's disease (AD) patients, which implicates the potential involvement of RACK1 in altered PKC activation associated with dementia. Our previous studies have demonstrated that GABAergic inhibition in prefrontal cortex, which is important for cognitive processes like "working memory", is regulated by muscarinic receptors via a PKC-dependent mechanism, and this effect is impaired by β-amyloid peptide (Aβ). In this study, we found that Aβ oligomers decreased RACK1 distribution in the membrane fraction of cortical neurons. Moreover, overexpression of RACK1 rescued the effect of muscarinic receptors on GABAergic transmission in Aβ-treated cortical cultures *in vitro* and Aβ-injected cortical neurons *in vivo*. These results suggest that the Aβ-induced loss of RACK1 distribution in the cell membrane may underlie the Aβ impairment of muscarinic regulation of PKC and GABAergic transmission. Thus, RACK1 provides a potential therapeutic target that can restore some of the impaired cellular processes by Aβ.

Keywords: Aβ; RACK1; PKC; Muscarinic acetylcholine receptors; IPSC; Sindbis virus; Stereotaxic injection

RACK1 is a member of the tryptophan-aspartate (WD) repeat family known for its propeller-like structure (Neer et al., 1994). Like many other WD domain proteins, RACK1 plays different roles upon binding to different partner proteins. RACK1 was first characterized as an intracellular receptor that binds activated PKC and is involved in activation-induced translocation of PKC to the membrane (Mochly-Rosen et al., 1991). RACK1 is present in the particulate fraction when binding activated PKC isozymes (Ron et al., 1994), bringing the signaling enzyme to the appropriate location, in close proximity with its substrate proteins (Jaken and Parker, 2000). In addition to PKC, RACK1 interacts with diverse proteins, including the small subunit of hetero-trimeric G protein Gβ (Dell et al., 2002), IP_3 receptors (Patterson et al., 2004), the neuronal transport protein Dynamin 1 (Rodriguez et al., 1999), $GABA_A$ receptors (Brandon et al., 1999), and NMDA receptors (Yaka et al., 2002). Thus, RACK1 has been implicated in multiple key neuronal functions, such as intracellular Ca^{2+} regulation, protein trafficking, synaptic transmission and plasticity (Sklan et al., 2006).

Changes in RACK1 levels have been found in a number of brain pathologies and during aging. For example, several reports show that RACK1 is decreased by ∼50% in membrane fractions of aging rat brains (Pascale et al., 1996; Battaini et al., 1997; McCahill et al., 2002). Reports on RACK1 changes in postmortem brains of AD patients are less consistent, with a reduction found in some studies (Battaini et al., 1999), but not others (Shimohama et al.,

* Corresponding author at: Department of Physiology and Biophysics, State University of New York at Buffalo, 124 Sherman Hall, 3435 Main St., Buffalo, NY 14214, USA. Tel.: +1 716 829 3058; fax: +1 716 829 2699.
E-mail address: zhenyan@buffalo.edu (Z. Yan).

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1998). Moreover, RACK1 levels are significantly decreased in the cortex of Down syndrome patients (Peyrl et al., 2002), all of who develop early onset AD. It suggests that loss of RACK1 may contribute to decreased PKC activity in the aging brain or AD.

The accumulation of β-amyloid (Aβ), a peptide generated from the amyloid precursor protein (APP), is one of the hallmarks of AD (Tanzi and Bertram, 2001; Selkoe and Schenk, 2003). Emerging evidence suggests that Aβ causes "synaptic failure" before the formation of senile plaques and the occurrence of neuron death (Selkoe, 2002). Our previous studies have found that Aβ impairs PKC-dependent regulation of synaptic functions by muscarinic acetylcholine receptors (mAChR) and metabotropic glutamate receptors (mGluR) in cortical neurons (Zhong et al., 2003; Tyszkiewicz and Yan, 2005). However, it is unclear about the mechanism underlying this action of Aβ. In this study, we provide evidence showing that the Aβ-induced impairment of PKC activation and synaptic regulation may be attributed to RACK1 deficit.

1. Materials and methods

1.1. Aβ oligomer preparation

Oligomeric Aβ was prepared as what was previously described (Dahlgren et al., 2002; Gu et al., 2009). Briefly, $A\beta_{42}$ peptide (Sigma) was dissolved in hexafluoroisopropanol (HFIP) to 1 mM. HFIP was then removed under vacuum. The remaining peptide was then dissolved in DMSO to 5 mM and diluted in PBS to 100 μM. The oligermeric Aβ was formed by incubating at 4 °C for 24 h.

1.2. Primary neuronal culture

All experiments were performed with the approval of the State University of Buffalo Animal Care Committee. Rat cortical cultures were prepared as previously described (Wang et al., 2003). Brief, frontal cortex was dissected from embryonic day 18 embryos, and cells were dissociated using trypsin and triturated through a Pasteur pipette. Neurons were plated on poly-L-lysine coated coverslips in Dulbecco's modified Eagle's medium with 10% fetal calf serum at a density of 1×10^5 cells/cm^2. When neurons attached to the coverslip within 24 h, the medium was changed to neurobasal medium with B27 supplement (Invitrogen, Carlsbad, CA). Neurons were maintained for 2–3 weeks.

1.3. Whole-cell recordings

Standard voltage-clamp techniques (Liu et al., 2006) were used for whole-cell recordings of spontaneous IPSC in cultured neurons. The external solution contained (mM): 127 NaCl, 5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, 12 glucose, pH 7.3–7.4, 300–305 mOsm. The internal solution contained (in mM): 100 CsCl, 30 *N*-methyl-D-glucamine, 10 HEPES, 1 $MgCl_2$, 4 NaCl, 5 EGTA, 0.1 QX314, 12 phosphocreatine, 2 MgATP, 0.2 Na_3GTP, 0.1 leupeptin, pH 7.2–7.3, 265–270 mOsm. The AMPA/KA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM) and NMDA receptor antagonist D-aminophosphonovalerate (APV, 25 μM) were present in the external solution throughout the recording. Cells were held at −70 mV.

Whole-cell voltage-clamp techniques were used for recordings of slices from young adult (3–4 weeks postnatal) Sprague–Dawley rats (Zhong et al., 2003). The brain slice (300 μm) was submerged in oxygenated artificial cerebrospinal fluid (ACSF) containing CNQX (10 μM) and APV (25 μM). The internal solution was the same as that used for culture recordings. Cells were visualized with a water-immersion lens and illuminated with near-IR light. Tight seals (2–10 GΩ) from visualized pyramidal neurons were obtained by applying negative pressure. The membrane was disrupted with additional suction and the whole-cell configuration was obtained. The access resistances ranged from 13 to 18 MΩ and were compensated 50–70%. The recording of spontaneous IPSC were performed on neurons (held at −70 mV) using a Multiclamp 700 A amplifier (Axon Instruments).

1.4. Subcellular fractionation of proteins and Western blot analysis

Cultured cortical neurons (DIV 11–16) were treated with Aβ oligomer (1 μM) for 48–72 h. After treatment, cultured neurons were homogenized on ice with the lysis buffer (0.3 M sucrose, 0.15 M NaCl, 20 mM Tris–HCl (pH 7.4), 2 mM EDTA, 0.3 mM PMSF, and 10 μg/ml leupeptin). Homogenates were centrifuged at $1000 \times g$ for 10 min at 4 °C, and supernatant fractions were collected for ultracentrifugation. Cytosol and membrane fractions were separated by ultracentrifugation at $100{,}000 \times g$ for 90 min at 4 °C. The supernatant constituted the cytosol fraction, and the pellet was resuspended and homogenized in the above lysis buffer with 0.2% Triton X-100 added. This resuspended fraction represented the membrane fraction.

Samples were boiled in 2× SDS loading buffer for 5 min, loaded at 30 μg of cytosol fractions and membrane fractions, separated on 10% SDS-polyacrylamide gel and transferred to nitrocellulose membranes. The blots were incubated with primary antibodies, including RACK1 (Santa Cruz), PKC (Santa Cruz), or actin (Santa Cruz). For detecting activated PKC, a phospho-PKC (pan) antibody (Cell Signal) that recognizes PKCα, βI, βII, ε, η, and δ isoforms only when phosphorylated at a carboxy-terminal residue homologous to Ser660 of PKCβII was used in the Western blot analysis. The Aβ antibody (Chemicon, 6E10, 1:500) was used for detecting oligomeric Aβ. After incubation with secondary antibodies conjugated with horseradish peroxidase