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Interaction of dopamine D1 with NMDA NR1 receptors in rat prefrontal cortex

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Abstract

Despite the tremendous importance of D1 and NMDA receptors to cognition (working memory, executive functions) and synaptic plasticity in the prefrontal cortex (PFC), little is known about the molecular mechanisms underlying D1–NMDA receptors interactions in this brain area. Here, we show that D1 receptors and the NMDA receptor co-localize in single pyramidal neurons and interneurons in adult rat PFC. NR1 and NR2A expression are found in different neuronal types. Conversely, D1 receptors are predominantly localized in pyramidal-like cells and parvalbumin positive cells. NR1 co-immunoprecipitates with D1 receptor in adult medial PFC. In prefrontal primary cultures, NMDA does not affect the D1 receptor dependent-cAMP production. In contrast, activation of D1 receptor potentiates the NMDA mediated increase in cytosolic Ca^{2+}, an effect that was blocked by a PKA inhibitor. We conclude that D1 receptor potentiates the NMDA-Ca^{2+} signal by a PKA-dependent mechanism.

1. Introduction

The PFC is a critical component of the cortical network that is essential for higher cognitive functions related to attention, working memory and future planning. Dopamine which is elevated in the PFC during performance of working memory tasks acts mainly through the D1/D5 but not the D2 receptors to modulate glutamate neural activity (Goldman-Rakic, 1998). Glutamate and dopamine fibres converge on pyramidal cells in deep layers of the PFC. Ultrastructural analysis in monkey PFC shows D1 receptors in distal dendrites and spines of pyramidal cells and interneurons in close proximity to glutamatergic terminals (Goldman-Rakic et al., 2000; Paspalas and Goldman-Rakic, 2005). Nevertheless, the cellular and subcellular associations between D1 and NMDA receptor systems in the rat PFC are still unknown.

Using electrophysiology on in vivo and in vitro preparations, we and others have clearly shown that dopamine via

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the D1 receptor strongly modulates glutamatergic activity in deep layers of the prelimbic region of the rat PFC and produces an increase in NMDA receptor-mediated LTP through a post-synaptic mechanism (Gurden et al., 1999, 2000; Seamans et al., 2001; Wang and O'Donnell, 2001) yet little is known about the mechanisms that underlie these effects. Studies using whole cell patch clamp on PFC slices and in vivo approaches indicate that D1 receptor increases NMDA current through a postsynaptic signaling cascade involving Ca^{2+}, PKA, PKC and CaMKII (Jay et al., 1998; Gurden et al., 2000; Wang and O'Donnell, 2001; Gonzalez-Islas and Hablitz, 2003).

More recently, it has been shown in the striatum and hippocampus that D1 receptor directly interacts with the NR1 subunit of the NMDA receptor to form a hetero-complex. This conformation represents a novel type of regulation since D1 receptors modulate NMDA receptor-gated current and NMDA regulates D1-mediated cAMP accumulation through direct protein–protein interaction (Lee et al., 2002; Fiorentini et al., 2003; Pei et al., 2004).

In the present study we examined for the first time the crosstalk between D1 and NMDA receptors in rat PFC at both anatomical and functional levels. The results show that D1 and NMDA receptors are located in the cytoplasm and synaptic membranes of both pyramidal cells and interneurons in which activation of D1 receptors enhances NMDA receptor-mediated Ca^{2+} responses.

2. Materials and methods

2.1. Prefrontal cortex cultures

Frontal cerebral cortices were isolated from E18 Sprague-Dawley rats and the meninges removed in sterile Ca^{2+}/Mg^{2+} free PBS solution containing 0.6% glucose. The tissue was minced in 5 ml Ca^{2+}/Mg^{2+} free PBS containing 0.003% deoxyribonuclease I (Dnase; Calbiochem Corp.). Cells were dissociated by trituration with a syringe. The cells were counted, viability estimated by trypan blue exclusion, and plated onto poly-D-ornithine (0.15–1.5 μg/ml; Sigma)-coated coverslips. Cells were grown in Neurobasal medium (Invitrogen) supplemented with 2% B-27 (GIBCO), 0.5 mM glutamine, and penicillin/streptomycin at 37 °C in an atmosphere of 5% CO_2 and the medium was replaced every 4 days. Cultures were used for experiments between weeks 2 and 3.

2.2. Animals and section preparations

Sprague-Dawley rats weighting 250–300 g were used in this study. All experimental procedures were carried out according to the French (87-848, Ministère de l'Agriculture et de la Forêt) and the European Economic Community (86-6091, EEC) guidelines for care of laboratory animals. Animals were maintained on a 12:12 h light/dark cycle, with food and tap water available ad libitum, until the time of the experiment. The animals were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and perfused transcardially with 1 l of cold 4% paraformaldehyde (PFH) in PBS, pH 7.4. The brains were removed immediately and left in 4% PFH over night. The day after brains were placed in PBS containing 30% sucrose 48 h at 4 °C. After that, the forebrains were serially sectioned with the vibratome and 40-μm-thick coronal sections (Bregma +4.2–2.2 mm) were collected for the following immunohistochemistry and data analysis.

2.3. Immunocytochemistry and confocal microscopy

Sections were incubated in PBS containing 0.1% Tween 20 and 10% normal goat serum (NGS) for 1 h at room temperature. The samples were then incubated with rat anti-D1 receptor (Sigma, 1:300 dilution), rabbit anti-NR1 (Chemicon, USA; 1:300 dilution) or rabbit anti-NR2A (Upstate; 1:300 dilution) and mouse anti-parvalbumin (Sigma, 1:500 dilution) in PBS containing 2% goat serum and 0.1% Tween 20 over night at 4 °C. Antibodies against NR1 and D1 receptor were utilized by other authors (Paspalas and Goldman-Rakic, 2005; Pickel et al., 2006), and their specificity was also confirmed by Western blot (Free et al., 2007). Subsequently, samples were rinsed in PBS for 30 min and then incubated with goat anti-rabbit 488, goat anti-rat 546 and goat anti-mouse 647 (Immunochim, all 1:200) for 2 h at room temperature. Finally, after washing, samples were mounted on glass slides and examined with a Leica TCS SP inverted confocal scanning laser microscope. As control, primary antibody was omitted in some sections and sections were then processed with the same procedures as described above. In those control sections the fluorescence staining intensity obtained was used as a marker to identify the positive staining since a low staining intensity similar to background was not regarded as positive immunoreactivity.

2.4. Immunoprecipitation studies

Sprague-Dawley rats were killed by decapitation and medial PFC and striatum was rapidly dissected out and thawed on ice. D1 and NR1 subunit receptors were immunoprecipitated as described (Trivedi et al., 2004). Briefly, the tissue slices were homogenized in immunoprecipitation (IP) buffer containing (in mM) 150 NaCl, 50 Tris–HCl, 1 EDTA, 1 orthovanadate, 1 phenylmethylsulfonyl fluoride (PMSF) and 1% NP-40 and protease inhibitor cocktail, pH 7.4. The homogenate was centrifuged at 5000 ×*g* for 10 min. The supernatant (1.0 mg protein/ml) was incubated with 10 μg of D1 receptor antibody (Sigma) over night to allow the formation of antigen and antibody complex. The samples were then incubated with protein G agarose for 2 h at 4 °C. The D1 receptor/NR1-antibody-protein G complex attached to agarose beads was settled down and was washed once with IP buffer and finally with 50 mM Tris · HCl, pH 8.0. All the steps of IP were carried out at 4 °C. Finally, the D1 receptor/NR1-antibody-protein G complex was dissociated with 2× Laemmeli buffer at 85 °C for 10 min. The samples were vortexed and centrifuged at room temperature, and the supernatant was used for electrophoresis.

2.5. Subcellular fractionation studies

Biochemical fractionation was performed as described previously (Dunah and Standaert, 2001). Dounce homogenates (H) of the pellets in ice-cold TEVP buffer [containing (in mM) 10 Tris–HCl, pH 7.4, 5 NaF, 1 Na_3VO_4, 1 EDTA, and 1 EGTA] containing 320 mM sucrose was centrifuged at 1000 ×*g* to remove nuclei and large debris (P1). The supernatant (S1) was centrifuged at 10,000 ×*g* to obtain a crude synaptosomal fraction (P2) and subsequently was lysed hypo-osmotically and centrifuged at 25,000 ×*g* to pellet a synaptosomal membrane fraction (LP1). Then the resulting supernatant (LS1) was centrifuged at 165,000 ×*g* to obtain a synaptic vesicle-enriched fraction (LP2). Concurrently, the supernatant (S2) above the crude synaptosomal fraction (P2) was centrifuged at 165,000 ×*g* to obtain a cytosolic fraction (S3) and a light membrane enriched fraction (P3). After each centrifugation the resulting pellet was rinsed briefly with ice-cold TEVP buffer before subsequent fractionations to avoid possible crossover contamination.