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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.

2.6. cAMP assay

Primary culture of PFC was prepared as described above and plated on 6-well plates in a final concentration of 10^6 neurons/well. After 2–3 weeks in vitro, neurons were washed twice and incubated for 10 min at 37 °C with 0.5 mM IBMX in PBS. Then cells were incubated in the presence of NMDA 100 μM, glycine 10 μM, SKF 81297 10 μM or forskolin 1 μM for the indicated time period at 37 °C and 5% CO2. Incubation media were removed and the reaction was stopped on ice with 0.5 ml of 0.1 N of HCl. cAMP was extracted and measured by radioimmunoassay (Perkin-Elmer Life Sciences, Boston, MA, U.S.A.) (Gbahou et al., 2006).

2.7. Measurement of intracellular Ca^{2+}

Cytosolic-free Ca^{2+} was monitored in neurons cultured on glass slides by quantitative ratio imaging of the fluorescent Ca^{2+} probe Fura-2

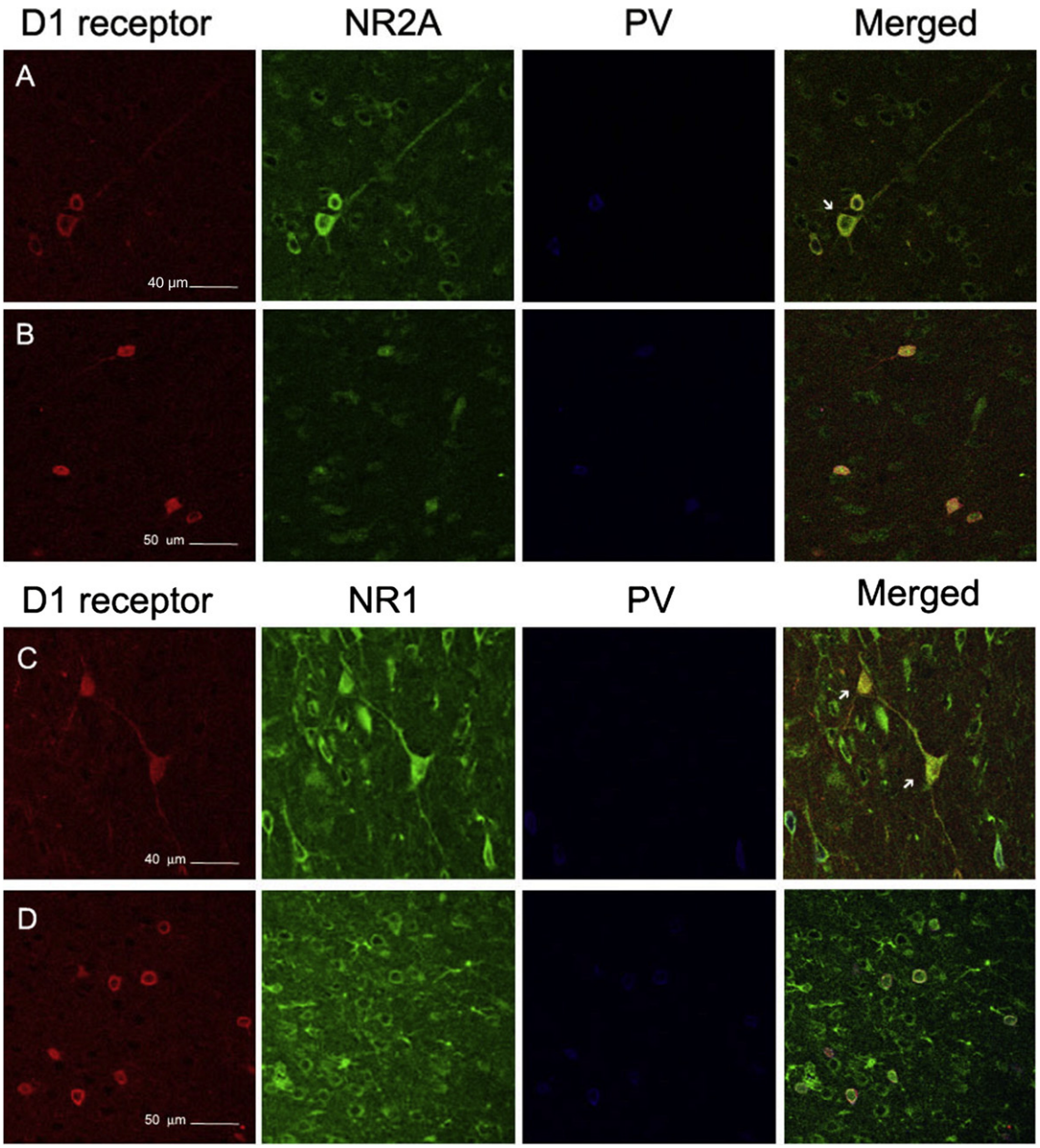

Figure 1 Confocal image of D1 receptor and NR1 and NR2A NMDA receptor subunits localization in rat PFC. Immunocytochemistry on coronal sections of PFC from adult Sprague-Dawley rats. Pyramidal neurons (white arrows) and parvalbumin-positive neurons (third column) in the medial PFC (mPFC) are co-labelled for both D1 receptor and NR2A (Fig. 1A and B, first and second columns respectively). Co-localization between D1 receptor and NR1 was also observed in both cell types (1C and D). Pyramidal neurons were identified based on morphological criteria including a pyramidal shaped cell body with dendrites emerging from both the apex and the base, a soma diameter $\geq$ 15 μm (Sun et al., 2005).

acetoxymethylester (Fura-2AM). Cells were loaded for 45 min with Fura-2AM (5 μM) in HEPES buffer. After loading, the glass slide was placed in a perfusion chamber where cells were exposed to test substances using a multichannel superfusion device. Fura-2AM fluorescence was monitored using a Hamamatsu monochromator coupled to a Nikon microscope. Cells were alternatively excited at 340 and 380 nm wavelengths and the emissions were monitored at 510 nm. Both fluorescent images were digitized (8 video frames per digitized image, permitting the recording of one image per s). The camera dark noise was subtracted from the recorded crude image (camera and digitizing system were from Hamamatsu Photonics, Massy, France). Results were expressed as the ratio between fluorescence measured in single cells at 340 and 380 nm excitation wavelength (F_{340}/F_{380}). Cells were arbitrarily considered responsive to NMDA when F_{340}/F_{380} increased by more than 15% of the basal value.

3. Results

3.1. D1 and NMDA receptors co-localize in mPFC

Dopamine and glutamate innervations converge in deep layers of the mPFC. However, it is still not known whether D1 and NMDA receptors are in closed proximity in the rat mPFC. To address this question we first performed immunofluorescence followed by confocal microscopy analysis on PFC slices from adult rat. D1 receptor signal was found in the cell soma and in processes of pyramidal cells and parvalbumin positive cells (Fig. 1). NR1/NR2A expression was predominantly found in neuronal processes and somatic cell membrane of different cell types. D1 receptor co-localized with both NR2A (Fig. 1A and B) and NR1 subunits (Fig. 1C and D) in single pyramidal neurons (Fig. 1A and C, white arrows) and parvalbumin positive cells. Quantification analysis showed that 100% of the D1 receptor-positive neurons expressed NR2A and 65% were parvalbumin-positive neurons (n=27) (Fig. 1A and B, second and third columns respectively). On the other hand 90% of D1 receptor-positive neurons presented NR1 signal and 59% were parvalbumin-positive neurons (n=29) (Fig. 1C and D, second and third columns respectively). The majority of parvalbumin-interneurons presented D1 receptors (76% n=45) (Fig. 1, third column). In accordance to our observations it was recently shown that D1 receptors are expressed by a greater proportion on GABA rather than on pyramidal neurons in PFC (Santana et al.,in press).

3.2. Subcellular distribution of D1 and NMDA receptors

The expression of D1 and NMDA receptors in various subcellular compartments was investigated in rat PFC tissues (Fig. 2A). NR1 subunits were found in synaptosomal membranes (*LP1*, *lane 7*), and to a lesser extent in the light membrane (*P3*, *lane 5*) (Fig. 2A). As expected NR1 subunits were also present in the total homogenate (*H*, *lane 1*); cell soma, nuclei, and nuclei-associated membrane (*P1*, *lane 2*) and crude synaptosomal membrane (*P2*, *lane 3*). However, the NR1 subunit was not detectable in the cytosolic or soluble subcellular fractions (*S3 and LS2*, *lanes 6 and 9* respectively) or the synaptic vesicle-enriched (*LP2*, *lane 8*) fraction in accordance to previous studies (Dunah and Standaert, 2001). On the other hand, we examined the subcellular distribution of the D1 receptor (Fig. 2A). D1 receptor showed a similar subcellular distribution profile as NR1 subunit. However, D1 receptor was detected in the synaptic vesicle-enriched fraction (*LP2*, *lane 8*) while no signal was detected from NR1.

3.3. D1 receptors and NR1 subunits co-immunoprecipitate in mPFC

Previous studies in hippocampus and striatum show that D1 receptors and NR1 subunits directly interact through their intracellular domains. We then examined whether D1 and NR1 also formed a heterocomplex in the PFC by performing co-immunoprecipitation.

As shown in Fig. 2B, a 120-kDa band, which was detected by the anti-NR1 receptor antibody, was present in the proteins immunoprecipitated with the antibody raised against the D1 receptor (lane 1). As a positive control we used striatum (lane 2). Conversely, the anti-NR1 subunit receptor antibody immunoprecipitated a 60-kDa band corresponding to D1 receptor (Fig. 2C,

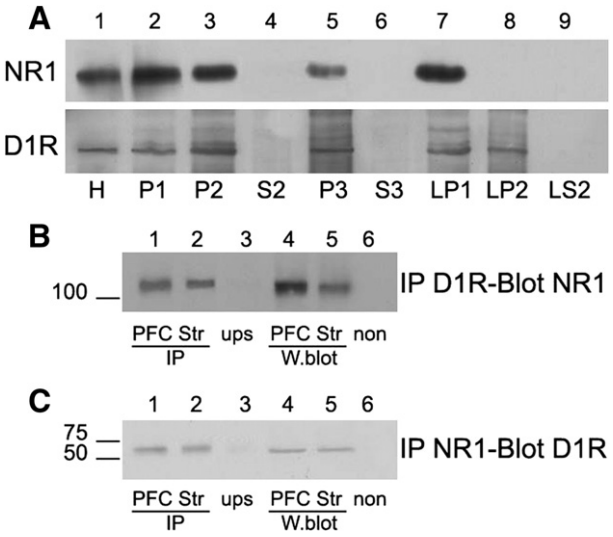

Figure 2 Co-localization and association of D1 and NMDA receptors in rat PFC. A. D1 receptor (D1R) and NR1 co-localized in different subcellular compartments in PFC. The PFC was homogenized immediately after dissection, separated into different biochemical fractions as described in Materials and methods, and resolved on SDS-polyacrylamide gels. Total protein (20 μg) from each fraction was loaded in each lane. The blots were probed with anti-D1R and anti-NR1 antibodies. *H*, Total homogenate; *P1*, nuclei and large debris; *P2*, crude synaptosomal fraction; *P3*, light membrane fraction; *LP1*, synaptosomal membrane fraction; *LP2*, synaptic vesicle-enriched fraction. *S2*, *S3*, and *LS2* are supernatants from *P2*, *P3*, and *LP2*, respectively. B. NR1 subunits and D1R co-immunoprecipitate in mPFC. Co-immunoprecipitation of NR1 subunit by the anti-D1R antibody (*lane 1 and 2*) but not by a non-specific IgG (reelin antibody, *lane3*). C. Co-immunoprecipitation of D1R by the anti-NR1 antibody (*lane 1 and 2*) but not by an irrelevant (*reelin*) antibody (*lane 3*) or by omission of a precipitating antibody (*lane 6*). 10 μg of whole homogenate from mPFC and striatum were loaded as positive controls (*lane 4 and 5*). The positions and sizes of molecular weight markers are indicated in kDa. Data are representative of three independent experiments.

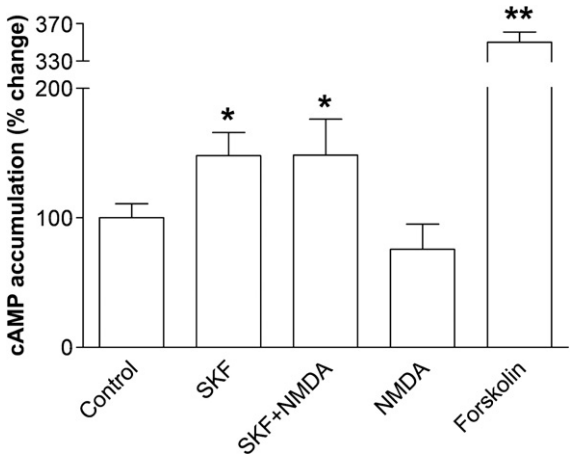

Figure 3 NMDA modulation of D1 receptor dependent-cAMP production in rat PFC primary culture. Bars indicate the % of cAMP accumulation ± SEM after different treatments. All neurons were exposed to IBMX 500 μM 10 min in PBS before any treatment. SKF (SKF81297, 10 μM, 10 min in PBS with Mg^{2+}). Pre-treatment with NMDA did not modify the cAMP response induced by SKF (NMDA 100 μM/glycine 10 μM, 30 s in PBS without Mg^{2+}). As positive control, neurons were incubated with forskolin (1 μm, 10 min). *p0.05; **p0.01 significantly different from control (Student's t test) from 5 independent experiments.

lane 1), indicating relevant complex formation between these two proteins in the rat mPFC *in vivo*. These bands did not appear when either an irrelevant antibody, such as that directed against reelin (lane 3) was used or the precipitating antibody was omitted (lane 6).

3.4. NMDA does not modulate the cAMP production induced by D1 receptor activation

The co-immunoprecipitation results demonstrated that D1 receptor and NR1 co-localize and associate in mPFC. Despite we cannot address whether this is a direct physical interaction between the intracellular domains, we, at least, can assume that both D1 and NMDA receptors are closed enough to modulate each others activity in the rat mPFC. We then examined the cell-physiological implications of D1–NMDA receptors interaction in the rat PFC.

To investigate whether the activation of NMDA receptors will modulate D1 receptor function, we tested the effects of NMDA receptor activation on D1 receptor-mediated cAMP accumulation in PFC primary cultures.

As expected, the selective D1-like receptor agonist SKF 81297 (10 μM for 10 min at 37 °C) stimulated cAMP accumulation significantly (Fig. 3, bar 2). When neurons were pretreated with 100 μM NMDA/10 μM glycine for 30 s, a protocol that showed to modulate the cAMP levels induced by D1 agonists in the striatum and hippocampus (Scott et al., 2002; Pei et al., 2004) the SKF-stimulated adenylate cyclase activity did not differ from neurons incubated with SKF 81297 alone (Fig. 3, bar 3). Treatment with NMDA alone did not modify significantly the cAMP (Fig. 3, bar 4). As expected, neurons incubated with forskolin (1 μM) for 10 min strongly increased the cAMP levels (Fig. 3, bar 5).

3.5. D1 receptor activation potentiates iCa^{2+} increase induced by NMDA

To study the physiological relevance of D1–NMDA receptors interaction we measured variations of intracellular calcium concentration in PFC primary cultures by ratiometric imaging of Fura-2AM loaded.

Bath application of NMDA (20 μM, 30 s) alone evoked an immediate rise in $[Ca^{2+}]_i$ in 48 out of 60 neurons studied (Fig. 4A). After NMDA washed-out, $[Ca^{2+}]_i$ rapidly returned to the baseline levels. Re-application of NMDA evoked a similar $[Ca^{2+}]_i$ response (Fig. 4A). Only neurons that responded to NMDA were used for further studies. Bath application of D1 agonist, SKF 38393 (50 μM, 2 min) alone had no effect on $[Ca^{2+}]_i$ (n=48). In contrast application of NMDA and SKF 38393 for 30 s, resulted in a higher increase in $[Ca^{2+}]_i$ in 52% of the NMDA responsive cells compared with NMDA alone (n=25 $p<0.001$) (Fig. 4A). The average increase was 14% over the NMDA treatment (Fig. 4B, bar NMDA vs bar NMDA+SKF). Fig. 4A inset illustrates cortical cells showing little or no effect of SKF 38393 on NMDA-Ca^{2+} signal whereas the arrow shows a pyramidal-shaped neuron with significantly enhanced NMDA-Ca^{2+} signal after SKF 38393 exposure.

The D1 antagonist SCH 23390 (10 μM) did not alter the NMDA-Ca^{2+} response (data not shown) but abolished the SKF-38393 potentiating effect (n=7 $p<0.01$) (Fig. 4B), confirming the involvement of D1 receptor on this effect.

It is well known that D1 receptor couples to Gs proteins activating the adenylate cyclase (AC)-cAMP-PKA signaling pathway. Here, we tested whether the D1 receptor-potentiation was mediated by PKA. The treatment with the PKA antagonist H-89 alone (1 μM, 10 min) did not affect the NMDA response (data not shown). However, pretreatment with H-89 followed by incubation with NMDA and SKF 38393 (20 μM, 10 min and 50 μM respectively, 30 s) abolished the enhancing effect induced by D1 receptor agonist on the NMDA-$[Ca^{2+}]_i$ response (n=7 $p<0.01$) (Fig. 4B).

4. Discussion

This is the first study on D1 and NMDA receptors localization, co-localization and functional interaction in rat PFC in vivo and in vitro using neurons in primary cultures. We demonstrate that D1 receptors expressed in pyramidal-shaped neurons and in parvalbumin-containing interneurons in the rat medial PFC co-localize with the essential NMDA subunit, NR1, and the NR2A subunit in both cell types. NR1 and D1 receptor physically and functionally interact since they are found in membrane fractions in which they co-immunoprecipitate and D1 agonist enhances the NMDA-induced increase in cytosolic calcium concentration in cultured prefrontal cortical neurons. The D1-mediated potentiation of the NMDA-Ca^{2+} response is dependent on PKA signaling. Together these findings provide further evidence for a synergistic action of D1 and NMDA receptors in PFC and emphasize a mechanism involving Ca^{2+} in a PKA-dependent manner.

The localization of D1 receptor that we found in pyramidal cells and interneurons in rat PFC, is comparable to what was seen in monkey PFC (Smiley et al., 1994; Muly et al., 1998; Goldman-Rakic et al., 2000). Studies in rodents have reported a $D1_A$-like dopamine receptor staining that was primarily found in larger-sized neurons throughout the neocortex (Ariano and

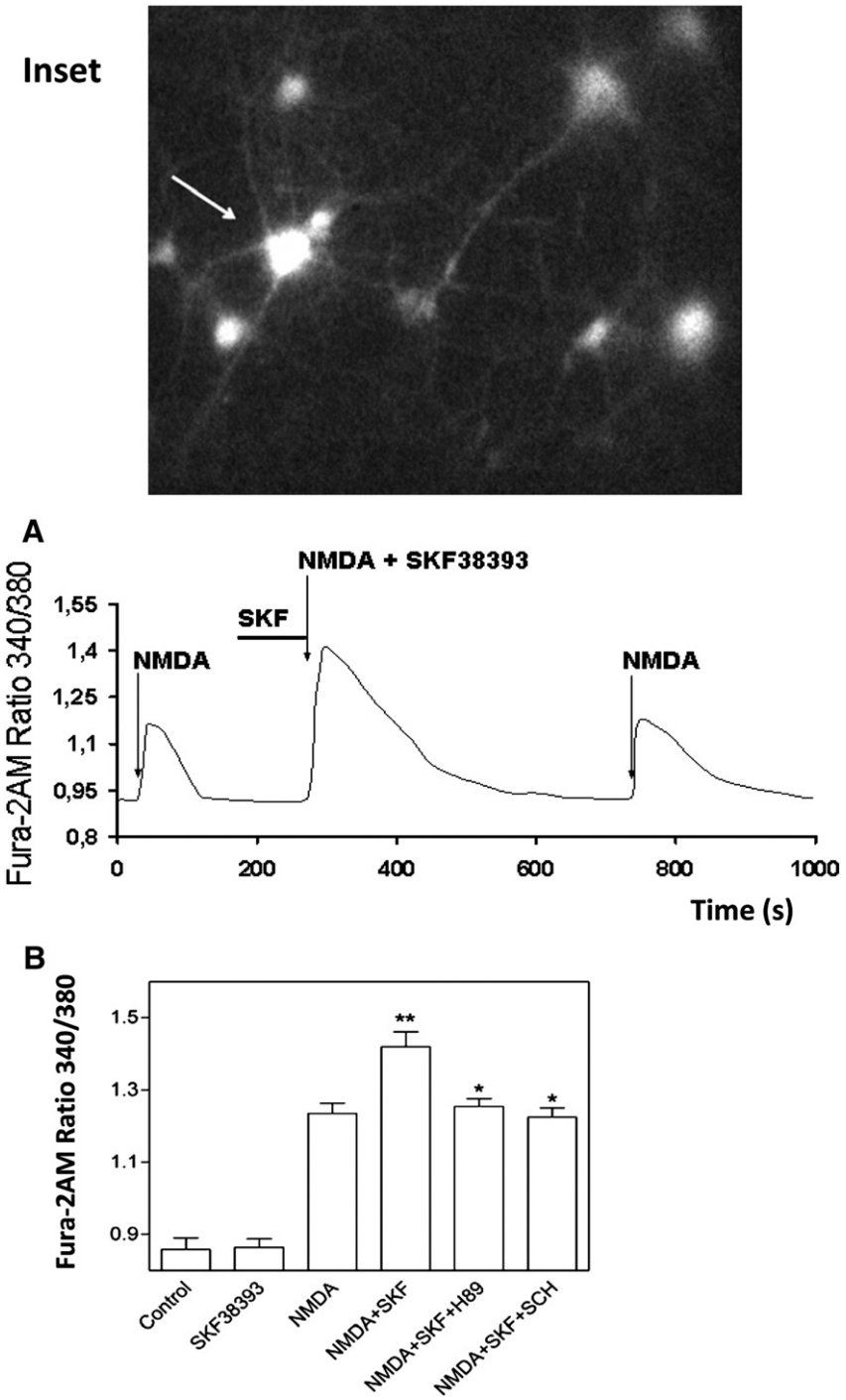

Figure 4 D1 receptor modulation of NMDA-dependent Ca^{2+} response in rat PFC primary culture. Average of 3 representative cells. In these cultured PFC cells, application of NMDA (20 μM, 30 s) reproducibly induced a transient rise in internal Ca^{2+}. Application of SKF 38393 (SKF, 50 μM, 30 s) increased the internal Ca^{2+} induced by NMDA (20 μM, 30 s). Inset shows the effects of SKF 38393 on NMDA-Ca^{2+} signal. B. Bar diagrams showing the average of Fura 2AM ratio 340/380. In the presence of the PKA antagonist H-89 (1 μM, 10 min) or the D1 receptor antagonist SCH 23390 (SCH, 10 μM, 10 min), SKF was unable to potentiate the NMDA-Ca^{2+} response. *p0.05; **p0.01 (Student's t test) from at least 5 independent experiments.

Sibley, 1994). In addition, a higher expression of D1 mRNA was measured in cortical output PFC neurons and a subpopulation of interneurons containing parvalbumin (Gaspar et al., 1995; Le Moine and Gaspar, 1998). A robust expression of the NR1 and NR2A and/or NR2B subunits in dendritic shafts and spines with a minor localization in axons has been reported in monkey and human PFC and a similar distribution pattern has been described in the neocortex of rats (Petralia et al., 1994; Huntley et al., 1997; Conti et al., 1999). In interneurons, the role of D1 receptor has received major attention but little is known about the role of NMDA receptors. In monkeys, NR2A and NR1 NMDA subunits were seen in dendrites of interneurons but few data are available in

rats. Spines of pyramidal neurons and parvalbumin dendrites are known to be targets of dopamine and glutamatergic terminals (Carr and Sesack, 2000). The present study shows that in rat PFC, individual neurons and interneurons express D1, NR1 and/or NR2A mainly on the neuronal cell body and dendritic shaft.

The colocalization of D1 dopamine receptor and NMDA receptor subunits led us to suspect physical and functional interactions in pyramidal cells similarly to what was already reported in striatal medium spiny neurons or in hippocampal prefrontal cortical neurons. However, the consequences of NMDA and D1 receptors interactions are diverse and controversial. Several mechanisms for interactions between D1 and NMDA receptors have been described, some of which facilitate and some of which inhibit responses to receptor activation (Lee et al., 2002; Fiorentini et al., 2003). Studies performed in the striatum and in the hippocampus indicate that NMDA receptor activity is regulated by D1 dopamine receptor activation (Cepeda and Levine, 1998; Yang, 2000; Mockett et al., 2007). In pyramidal cells of rat PFC, in vitro physiological studies have demonstrated that the amplitude of NMDA-mediated response was increased by the D1 receptor agonist SKF38393 and this facilitation was dependent upon PKA and intracellular calcium (Tseng and O'Donnell, 2004). To better characterize the potentiating mechanism in PFC, we first demonstrated that the NR1 NMDA subunit and D1 receptors are both present in fractions containing cellular membranes but not in cytosolic fractions originating from brain slices of adult rat. Such NR1 and D1 co-expression was also found in the basolateral amygdala in typical glutamatergic terminals arising from projecting prefrontal neurons (Pickel et al., 2006). As observed in hippoccampal and striatal neuronal membranes (Lee et al., 2002; Fiorentini et al., 2003; Pei et al., 2004) we found that D1 receptors and the NMDA receptor subunit, NR1 reciprocally co-immunoprecipitate in rat mPFC homogenates suggesting that the two receptors are closed enough to affect their activity. Looking for the functional significance of this interaction, we demonstrated that activation of D1 receptor enhances the NMDA-induced increase in cytosolic calcium concentration. It is known that D1 receptors are preferentially couple to Gs proteins and stimulate adenylate cyclase activity and protein kinase A-dependent (PKA) pathways. In fact, the potentiating effect of dopamine could be prevented by the PKA inhibitor H89. In contrast to what was reported in hippocampal and striatal neurons (Scott et al., 2002; Pei et al., 2004), we found that NMDA did not affect the cAMP production induced by D1 receptor activation in cultured PFC neurons. Physiological studies have also shown that D1 receptor activation enhances the amplitude of NMDA-mediated EPSCs in both the neocortex and striatum (Chen and Yang, 2002; Flores-Hernandez et al., 2002; Gonzalez-Islas and Hablitz, 2003). This synergism which occurs at the postsynaptic level appears to be mediated through both a PKA and Ca^{2+}-dependent mechanisms (Wang and O'Donnell, 2001; Tseng and O'Donnell, 2003, 2004). D1 receptors and activation of the cAMP-PKA pathway could directly phosphorylate NMDA receptor subunits and through this mechanism modulates the synaptic strength (Snyder et al., 1998). D1 receptor could also facilitate the opening of L-type Ca^{2+} channels through a PKA dependent process (Cepeda and Levine, 1998; Snyder et al., 1998). However, L-type Ca^{2+} channels do not seem to contribute to the enhancement of NMDA calcium current (Liu et al., 2004). In the striatum, D1 receptor activation was shown to increase NMDA receptor availability in plasma membrane (Dunah and Standaert, 2001) that in turn could result in an increase of the intracellular Ca^{2+} levels. Thus, the modulation of receptor trafficking at postsynaptic sites could be one of the mechanisms by which D1 receptor potentiates the NMDA response. Although there is no evidence linking D1 receptors and trafficking of NMDA receptors in neocortical neurons, it is possible that a similar mechanism also takes place in the PFC.

Taken together, the present results suggest that the presence of D1 and NMDA receptors in the same compartments is an input-specific and/or target-specific dopaminergic modulation of glutamate transmission whereas the presence of both D1 and NMDA receptors in different cell types (i.e. glutamatergic and GABAergic neurons) may indicate a tuned regulation of synaptic input. It is well known that the actions of dopamine on mPFC pyramidal neurons depend on the timing and strength of synaptic inputs as well as on the membrane potential range at which medial PFC neurons are operating (Au-Young et al., 1999). A recent elegant in vivo study (Tseng et al., 2006) showed that VTA stimulation increased fast spiking parvalbumin-interneurons (FSI) firing with a temporal course matching the inhibition of pyramidal neurons.

It has been proposed that dopamine supports working memory and other cognitive functions by increasing the detection of strong relevant signals and reducing irrelevant activity (O'Donnell, 2003). Thus, the presence of D1 and NMDA receptors in both pyramidal cells and parvalbumin-interneurons may contribute to modulate the balance between pyramidal neurons and FSI firing in PFC to filter weak or irrelevant stimuli. The studies present herein could also explain one of the mechanisms by which dopamine and D1 receptor enhance the NMDA receptor-dependent LTP in rat mPFC (Gurden et al., 1999, 2000). D1 receptor could contribute to the increases of Ca^{2+} entry into the postsynaptic neuron through NMDA receptors and promote phosphorylation of different proteins known to support LTP long hours or even days.

In conclusion, the functional crosstalk between D1 and NMDA receptors may provide important new insights into the neuropathological mechanisms underlying neuropsychiatric illnesses such as schizophrenia, depression, Parkinson's disease and healthy aging. There is now evidence that NMDA receptor antagonists potentiate the therapeutic effects of L-DOPA in Parkinson's disease patients (Missale et al., 2006) and that simultaneous stimulation of both D1 and NMDA receptors may be involved in the acute action of the atypical antipsychotic clozapine (Chen and Yang, 2002). Future therapeutic approaches should focus on simultaneous modulation of D1 and NMDA receptors. In this context, the results from the present work could contribute to the development of new and more efficient therapeutic strategies in neuropsychiatry disorders involving dopamine and glutamate imbalance.

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Contributors

M.S.K. and T.M.J. designed the research; M.S.K performed all biochemical experiments. M.S.K. and J.P. performed calcium experiments and analyzed data; M.S.K. and T.M.J. wrote the manuscript. M.O.K. provided inputs and suggestions that improved the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest.

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