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Pharmacological modulation of AMPA receptor phosphorylation by dopamine and muscarinic receptor agents in the rat medial prefrontal cortex

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Abstract

Two key transmitters in the medial prefrontal cortex (mPFC), dopamine and acetylcholine, are believed to interact with each other to modulate local glutamatergic transmission, although molecular mechanisms underlying their crosstalk are poorly understood. Here we investigated effects of pharmacological manipulations of dopamine and muscarinic receptors on phosphorylation of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the adult rat mPFC *in vivo*. We found that an agonist selective for $G_{\alpha s}$ -coupled dopamine D_1 receptors, SKF81297, increased AMPA receptor GluA1 subunit phosphorylation at a protein kinase A-sensitive site (S845), while SKF81297 had no effect on GluA1 phosphorylation at S831. An agonist for $G_{\alpha i/o}$ -coupled dopamine D_2 receptors, quinpirole, also increased S845 but not S831 phosphorylation. When coinjected, the two agonists induced an additive increase in S845 phosphorylation. The D_1 receptor antagonist SCH23390 blocked the SKF81297/quinpirole-stimulated S845 phosphorylation. The D_2 antagonist eticlopride also partially blocked S845 responses to SKF81297/quinpirole. VU0152100, a positive allosteric modulator selective for $G_{\alpha i/o}$ -coupled muscarinic M_4 receptors, reduced the S845 phosphorylation induced by SKF81297 and quinpirole injected alone or together. In contrast, coinjection of subthreshold doses of tropicamide, an M_4 antagonist, and SKF81297 facilitated S845 phosphorylation. Additionally, coadministered SKF81297 and quinpirole increased the abundance of mPFC GluA1 at extrasynaptic sites. These data reveal that both D_1 and D_2 receptors upregulate GluA1 phosphorylation in mPFC neurons probably via a direct and indirect mechanism, respectively. The indirect mechanism involves M_4 receptors which generally counteract the effect of dopamine on GluA1 phosphorylation.

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Keywords

D₁ receptor; D₂ receptor; M₄ receptor; S845; S831; protein kinase A; VU0152100; tropicamide

1. Introduction

The medial prefrontal cortex (mPFC) is a focus of studies in mental illness (Tzschentke, 2001). Increasing evidence links this area to the pathogenesis of a variety of common neuropsychiatric and neurological disorders (Bissonette et al., 2013; Morici et al., 2015). The mPFC is composed of infralimbic, prelimbic, and anterior cingulate cortex (from ventral to dorsal) (Kolb, 1984; McDonald, 1991). The rat mPFC is cytoarchitectonically characterized by a five-layered structure (layers I, II, III, V and VI; a discrete granule-cell layer 4 is lacking) hosting two main cell types: 1) pyramidal spiny output neurons (glutamatergic) in layers II, III, V and VI, and 2) smooth or sparsely spiny interneurons (GABAergic) in all layers (de Almeida et al., 2008; Puig and Gullledge, 2011). As one of major dopamine responsive regions in the limbic system, the entire mPFC is innervated by dopaminergic fibers primarily from the ventral tegmental area (A10 cell group) (Heidbreder and Groenewegen, 2003; de Almeida et al., 2008). Dopamine D₁ and D₂ receptors are two principal dopamine receptor subtypes in the mPFC (Tzschentke, 2001; Tritsch and Sabatini, 2012). Both G_{αs}-coupled D₁ receptors and G_{αi/o}-coupled D₂ receptors are present in certain percentages of pyramidal neurons and interneurons throughout layers (Bergson et al., 1995; reviewed in de Almeida et al., 2008; Tritsch and Sabatini, 2012; Dembrow and Johnston, 2014). D₂ receptors also noticeably reside on presynaptic terminals (Khan et al., 1998; Negyessy and Goldman-Rakic, 2005; Fitzgerald et al., 2012). It is evident that dopamine through both D₁ and D₂ receptors actively regulates mPFC neurons.

In addition to dopamine, acetylcholine acts as an important transmitter in the mPFC. Anatomically, the mPFC is innervated by diffuse cholinergic afferents primarily from basal forebrain cholinergic neurons (BFCN) (Henny and Jones, 2008; Bloem et al., 2014). Abundant muscarinic acetylcholine receptors are found in the mPFC. Notably, muscarinic M₄ receptors were seen on mPFC perikarya (Levey et al., 1991; Volpicelli and Levey, 2004). It is generally viewed that both dopamine and acetylcholine intimately interact with each other to maintain mPFC homeostasis, although molecular mechanisms underlying the dopamine-acetylcholine interplay are poorly understood.

The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor is enriched in dopamine-innervated regions, including the mPFC (Bernard et al., 1997; Kondo et al., 2000; Reimers et al., 2011). Most pyramidal neurons in mPFC express AMPA receptors (Pirrot et al., 1995). These receptors often function as homo- or heterotetrameric assembly of four subunits, GluA1-4 (formerly GluR1-4) (Greger et al., 2007) and are subjected to the regulation by a phosphorylation-dependent mechanism (Lu and Roche, 2012; Wang et al., 2014). For instance, GluA1 is phosphorylated in its intracellular C-terminal tails at serine 831 (S831) by protein kinase C and Ca²⁺/calmodulin-dependent protein kinase II and at serine 845 (S845) by protein kinase A (PKA) (Roche et al., 1996; Barria et al., 1997; Mammen et al., 1997; Serulle et al., 2007). Such phosphorylation is sensitive to changing

receptor and synaptic input and impacts expression and trafficking of GluA1 and physiology of GluA1-containing AMPA receptors (Lu and Roche, 2012; Wang et al., 2014).

In this study, we investigated the role of dopamine and acetylcholine in the regulation of GluA1 phosphorylation. We focused on the mPFC for this area has been less investigated compared to the striatum. We first investigated effects of a D₁ agonist and a D₂ agonist injected alone or coadministered on S845 and S831 phosphorylation. Effects of a D₁ antagonist and a D₂ antagonist were also analyzed. We then tested effects of a positive allosteric modulator (PAM) of M₄ receptors on basal GluA1 phosphorylation and effects of the PAM on the dopamine-regulated GluA1 phosphorylation. Finally, we examined effects of an M₄ antagonist on basal and dopamine-stimulated S845 phosphorylation.

2. Materials and Methods

2.1. Animals

We used Wistar rats in this study. These male animals weighing 210–300 g were purchased from Charles River (New York, NY). Animals were housed in a controlled facility with food and water available *ad libitum* (23°C). After 5–7 days of habituation, rats were used according to the US NIH Guide for the Care and Use of Laboratory Animals. The animal protocol was approved by the Institutional Animal Care and Use Committee.

2.2. Drug administration

Drugs were injected by an intraperitoneal (i.p.) injection at doses that were determined by previous studies (Wang and McGinty, 1995; Brady et al., 2008; Xue et al., 2017). Age-matched control rats were given an injection of physiological saline or vehicle. (±)-6-chloro-PB hydrobromide (SKF81297), (–)-quinpirole hydrochloride, R(+)-SCH23390 hydrochloride, S(–)-eticlopride hydrochloride and tropicamide were purchased from Sigma-Aldrich (St. Louis, MO). VU0152100 [3-amino-N-(4-methoxybenzyl)-4,6-dimethylthieno[2,3-b]pyridine carboxamide] was purchased from Axon Medchem (Reston, VA). All agents were freshly prepared at the day of experiments. VU0152100 was dissolved in 10% Tween 80 with the pH adjusted to approximately 7.0 using 1 N NaOH. Tropicamide was dissolved in a small volume of dimethyl sulfoxide and was then diluted in physiological saline. Other agents were dissolved in physiological saline.

2.3. Protein extraction

Rats were anesthetized after drug injection. After decapitation, rat brains were removed. Coronal slices were cut and the mPFC, including the anterior cingulate, prelimbic and infralimbic cortex (Heidbreder and Groenewegen, 2003), was dissected at 4°C. The dissected mPFC was homogenized in isotonic sucrose homogenization buffer (0.32 M sucrose, 10 mM HEPES, pH 7.4, 2 mM EDTA, and a protease/phosphatase inhibitor cocktail). After homogenates were centrifuged at 800 *g* (10 min), the supernatant was collected and centrifuged at 10,000 *g* (30 min). The pellet 2 (P2) was obtained. P2 membranes contain crude synaptosomal plasma membranes. To enrich synaptic and extrasynaptic membranes as described previously (Mao et al., 2013), the P2 sample was washed and resuspended in the isotonic sucrose homogenization buffer containing Triton

X-100 (0.5%, v/v) for 20 min with gentle rotation. After we centrifuged samples for 20 min (32,000 *g*), the supernatant was yielded, which was enriched with Triton X-100-soluble extrasynaptic membranes, while the pellet was enriched with Triton X-100-insoluble synaptic membranes. P2, synaptic, and extrasynaptic membranes were solubilized in the sucrose homogenization buffer containing 0.5% Triton X-100, 1% sodium dodecyl sulfate (SDS), and a protease/phosphatase inhibitor cocktail with gentle rotation (1 h, 4°C). Protein concentrations were determined. Samples were stored at 80°C until use.

2.4. Western blot

Western blot was performed as described previously (Xue et al., 2017). In brief, proteins were separated on SDS NuPAGE Novex 4–12% gels (Invitrogen, Carlsbad, CA) and transferred to polyvinylidene fluoride membranes (Millipore, Bedford, MA). Membranes were blocked and incubated with a primary antibody overnight at 4°C. These antibodies included rabbit antibodies against phospho-S831 (pS831, PhosphoSolutions, Aurora, CO), phospho-S845 (pS845, PhosphoSolutions), GluA1 (Millipore), or tubulin (Millipore). After the primary antibody incubation, membranes were incubated in a solution containing a horseradish peroxidase-linked secondary antibody. An enhanced chemiluminescence reagent (GE Healthcare Life Sciences, Piscataway, NJ) was used to visualize immunoblots on membranes. In all cases, a single blot was probed once with a phospho-specific antibody (either an anti-pS845 or an anti-pS831 antibody). Optical density of immunoblots was measured using NIH gel analysis software. The amount of pS845 and pS831 signals was normalized to total GluA1 proteins.

2.5. Statistics

The data (means $\pm$ S.E.M.) were statistically analyzed using GraphPad Prism 6 software (GraphPad Software, San Diego, CA) with a one-way analysis of variance (ANOVA) followed by a Tukey's multiple comparison *post hoc* test for comparing multiple groups or a two-tailed unpaired Student's *t*-test, as appropriate. A statistically significant level was $P < 0.05$.

3. Results

3.1. Both D₁ and D₂ agonists enhance GluA1 S845 phosphorylation

We started with a study testing the effect of a D₁ or a D₂ receptor subtype-selective agonist on GluA1 phosphorylation in the mPFC. Rats were given a single i.p. dose of the D₁ agonist SKF81297 (3 mg/kg) or the D₂ agonist quinpirole (3 mg/kg). They were then killed 20 min after drug injection. Changes in GluA1 phosphorylation at S845 and S831 in the mPFC were monitored using immunoblots with phospho- and site-specific antibodies. We found that SKF81297 induced an increase in pS845 levels over saline control (Fig. 1A and 1B). Interestingly, like SKF81297, quinpirole enhanced pS845 signals. We also examined the effect of coadministration of the two agonists. The two agonists caused an additive increase in pS845 levels. In contrast to pS845, the two agonists injected either alone or together had a minimal impact on pS831 levels (Fig. 1A and 1C). A total amount of GluA1 proteins remained unchanged following all drug treatments (Fig. 1A). These data demonstrate a positive linkage of SKF81297-sensitive D₁ receptors to GluA1 in S845 phosphorylation in

mPFC neurons *in vivo*. Notably, activation of D₂ receptors also enhanced S845 phosphorylation. Since D₂ receptors inhibit the cAMP-PKA pathway, the D₂ agonist-stimulated GluA1 phosphorylation at the PKA site (S845) is thought to be mediated via an indirect mechanism (see below).

3.2. Effects of D₁ and D₂ antagonists on GluA1 phosphorylation

To further characterize the role of D₁ and D₂ receptors, we investigated effects of D₁ and D₂ antagonists on S845 phosphorylation in the mPFC. We gave rats an injection of a D₁ antagonist SCH23390 (0.1–0.5 mg/kg) or a D₂ antagonist eticlopride (0.5 mg/kg) alone or 15 min prior to coadministration of SKF81297 and quinpirole at 3 mg/kg. We then killed rats 20 min after the final drug injection to detect changes in S845 and S831 phosphorylation. SCH23390 almost completely blocked the response of S845 phosphorylation to coadministered SKF81297/quinpirole (Fig. 2A and 2B). Noticeably, eticlopride induced a partial blockade of the S845 phosphorylation induced by SKF81297/quinpirole, while in the rats pretreated with eticlopride, the two agonists still elevated pS845 to a level significantly higher than that seen in the rats treated with eticlopride + saline ($P < 0.05$). S831 phosphorylation was not altered in response to all drug treatments (Fig. 2A and 2C). These results indicate that D₁ receptors largely mediate the S845 phosphorylation stimulated by SKF81297 and quinpirole. In addition, D₂ receptors partially mediate the S845 phosphorylation response to SKF81297 and quinpirole, probably via an indirect and muscarinic receptor-mediated mechanism (see below).

3.3. Effects of a muscarinic receptor subtype-selective agent on basal GluA1 phosphorylation

M₄ receptors are negatively coupled to adenylyl cyclase via G_{ai/o} proteins (Wess, 1996). Activation of M₄ receptors leads to inhibition of the cAMP-PKA pathway. We next investigated whether the M₄-mediated cholinergic transmission is involved in the modulation of GluA1 in its phosphorylation. To this end, we chose a PAM VU0152100 which is selective for M₄ receptors and is able to enhance M₄ receptor activity in the brain after a systemic administration *in vivo* (Brady et al., 2008). In the first attempt with this PAM, we tested its effects on basal GluA1 phosphorylation. We found that VU0152100 after an i.p. injection at 6 mg/kg had no effect on basal pS845 levels in the mPFC (Fig. 3A and 3B). At a higher dose (60 mg/kg), VU0152100 showed a tendency to reduce pS845 levels, although it did not reach a statistically significant level. VU0152100 did not alter S831 phosphorylation (Fig. 3A and 3C). Thus, enhancement of M₄ receptor activity by a subtype-selective PAM has no significant impact on constitutive GluA1 phosphorylation in mPFC neurons under normal conditions.

3.4. The M₄ PAM reduces the dopamine-stimulated GluA1 S845 phosphorylation

To determine the influence of the M₄ PAM over the dopamine-stimulated S845 phosphorylation, we investigated the effect of VU0152100 on the SKF81297-stimulated GluA1 phosphorylation in the rat mPFC. As expected, SKF81297 alone (3 mg/kg, 20 min) induced an increase in pS845 levels. Pretreatment with VU0152100 (60 mg/kg, 20 min prior to SKF81297) significantly reduced this increase (Fig. 4A and 4B). The pS831 level was not

altered by VU0152100, SKF81297 or both (Fig. 4A and 4C). Apparently, the M₄ PAM possesses the ability to reduce the D₁ agonist-stimulated S845 phosphorylation.

Coactivation of D₁ and D₂ receptors induced a greater increase in S845 phosphorylation than that induced by activation of D₁ or D₂ receptors alone (Fig. 1A and 1B). Regarding the D₂-mediated component in this event, we hypothesized that D₂ receptors stimulate S845 phosphorylation via an indirect mechanism involving the M₄-mediated cholinergic transmission. Specifically, activation of inhibitory D₂ receptors reduces local acetylcholine release in the mPFC. This lowers the inhibitory M₄ receptor tone and thus enhances the responsivity of the cAMP-PKA system to D₁ receptor stimulation, resulting in a greater increase in PKA-mediated S845 phosphorylation. This hypothesis is supported by our observations that enhancing M₄ receptor activity by the M₄ PAM resumed the cholinergic inhibition and thereby reduced the S845 phosphorylation induced by D₁ and D₂ agonists. As shown in Fig. 4D and 4E, SKF81297 and quinpirole (3 mg/kg, 20 min) produced a significantly less increase in pS845 signals in the rats pretreated with VU0152100 (60 mg/kg) than the rats pretreated with vehicle.

As aforementioned, quinpirole alone increased S845 phosphorylation in the mPFC (Fig. 1A and 1B). This might also occur as a result of the D₂ agonist-induced reduction of the acetylcholine/M₄-mediated inhibitory system. If this is the case, the M₄ PAM that enhances M₄ receptor activity might suppress S845 phosphorylation in response to a D₂ agonist. Indeed, administration of VU0152100 reduced although not prevented the quinpirole-induced S845 phosphorylation (Fig. 5A and 5B). This supports the role of the M₄-mediated cholinergic transmission in mediating the D₂ agonist-stimulated S845 phosphorylation.

3.5. The M₄ antagonist augments the SKF-stimulated GluA1 S845 phosphorylation

An M₄ receptor antagonist tropicamide was used to further clarify the role of M₄ receptors in the regulation of S845 responses to the D₁ agonist. Tropicamide is a systemically active agent (Lazareno et al., 1990) and has been recently demonstrated to dose-dependently (2.5 and 10 mg/kg, i.p.) alleviated motor deficits after dopamine lesions in wild type but not M₄ receptor knockout mice *in vivo* (Ztous et al., 2016). In this study, we found that tropicamide (10 mg/kg, i.p.; 15 min) produced an increase in pS845 levels in the mPFC (Fig. 6A and 6B), while the antagonist did not affect pS831 levels (Fig. 6C). Tropicamide at a lower dose (2 mg/kg, i.p.) did not significantly alter S845 phosphorylation (Fig. 6D and 6E). Neither did SKF81297 alone at a low dose of 0.5 mg/kg (i.p.). Remarkably, coadministration of tropicamide and SKF81297 at these subthreshold doses induced a significant increase in pS845 levels. No significant change in pS831 levels was seen after coadministration of two drugs (Fig. 6F). These data support the notion that M₄ receptors inhibit basal and D₁ agonist-stimulated GluA1 S845 phosphorylation in mPFC neurons.

3.6. Coinjected D₁ and D₂ agonists elevate GluA1 expression at extrasynaptic sites

Phosphorylation of GluA1 at S845 may regulate trafficking of GluA1, thereby controlling the number of the receptor in discrete subsynaptic compartments (Lu and Roche, 2012; Wang et al., 2014). In this study, we thus investigated whether costimulation of D₁ and D₂ receptors that has been shown to induce a robust S845 phosphorylation in the mPFC (see

above) leads to a change in the abundance of GluA1 proteins in distinct synaptic and/or extrasynaptic locations. Coadministration of SKF81297 and quinpirole (3 mg/kg, i.p.; 90 min) induced a moderate increase in S845-phosphorylated protein levels in the protein pool enriched from synaptic membranes (Fig. 7A). However, GluA1 expression was not altered in this protein pool. Noticeably, the abundance of GluA1 was elevated to a significant level in the protein pool enriched from extrasynaptic membranes (Fig. 7B). The amount of S845-phosphorylated proteins was also increased to a magnitude higher than that seen in the synaptic location. No significant changes in the amount of S831-phosphorylated proteins were seen in either the synaptic or extrasynaptic location. These results indicate that costimulation of D₁ and D₂ receptors primarily recruits S845-phosphorylated GluA1 and total GluA1 proteins to the extrasynaptic compartment of mPFC neurons.

4. Discussion

We investigated the dopaminergic and cholinergic regulation of AMPA receptor phosphorylation. We focused on the mPFC in this study. We found that a systemic injection of a D₁ agonist SKF81297 or a D₂ agonist quinpirole induced an increase in GluA1 S845 phosphorylation, while an M₄ PAM VU0152100 did not. SKF81297 and quinpirole after coadministration produced a greater increase in S845 phosphorylation. Pretreatment with VU0152100 reduced the S845 phosphorylation induced by SKF81297 and quinpirole injected alone or together. In contrast, pretreatment with tropicamide augmented the SKF81297-stimulated S845 phosphorylation. These data demonstrate a characteristic pattern of S845 phosphorylation in response to pharmacological manipulations of dopamine receptors and muscarinic receptors and jointly support a dynamic dopamine-acetylcholine balance controlling GluA1 phosphorylation in mPFC neurons *in vivo*.

D₁ receptors are positively linked to GluA1 S845 phosphorylation in mPFC neurons. This was demonstrated by the finding that the D₁ agonist SKF81297 increased S845 phosphorylation, which was blocked by the D₁ antagonist SCH23390. These results are consistent with a previous report in which the D₁ agonist SKF38393 stimulated S845 phosphorylation in rat cultured prefrontal cortical neurons (Swayze et al., 2004). Thus, mPFC neurons, like striatal neurons (Price et al., 1999; Snyder et al., 2000; Chao et al., 2002), contain a D₁-dependent signaling pathway linking D₁ receptors to S845 phosphorylation. The D₁ receptor is a known G protein-coupled receptor. Via G_{αs} proteins, D₁ receptors activate adenylyl cyclase to trigger cAMP formation and PKA activation (Neve et al., 2004). Thus, D₁ receptors are assumed to stimulate their conventional cAMP-PKA pathway to enhance GluA1 phosphorylation at the PKA-catalyzed site (S845).

Interestingly, the D₂ agonist quinpirole also increased S845 phosphorylation in the mPFC. The D₂ receptor is a G_{αi/o} protein-coupled receptor which inhibits the adenylyl cyclase and associated cAMP-PKA pathway (Neve et al., 2004). Thus, D₂ receptors are reasoned to elevate the PKA-dependent S845 phosphorylation through an indirect mechanism (see below). Of note, the D₂ agents induced almost opposite changes in S845 phosphorylation in the striatum compared to the mPFC. In the striatum, while quinpirole had little effect on S845 phosphorylation, eticlopride substantially increased it (Xue et al., 2017). It has been well demonstrated that D₂ receptors are segregated into one phenotype of striatal projection

neurons, striatopallidal neurons (Gerfen et al., 1990; Aubert et al., 2000; Bertran-Gonzalez et al., 2010). Thus, the eticlopride-induced increase in S845 phosphorylation was thought to occur in D₂ receptor-bearing striatopallidal neurons as a result of direct blockade of the inhibitory D₂ receptor tone (Xue et al., 2017).

An important finding in this study is the role of M₄ receptors in suppressing the dopamine-stimulated GluA1 S845 phosphorylation in the mPFC. We found that the M₄ receptor selective PAM VU0152100 reduced all responses of S845 phosphorylation to SKF81297 and quinpirole injected alone or together. The dose of VU0152100 (60 mg/kg, i.p.) used in this study is deemed to be M₄ receptor selective because VU0152100 at 56.6 mg/kg (i.p.) blocked motor responses to the dopamine receptor indirect agonist amphetamine in rats and wild type mice but not in M₄ receptor knockout mice (Brady et al., 2008; Byun et al., 2014). Thus, enhancement of M₄ receptor activity leads to inhibition of phosphorylation of GluA1 at a PKA site in response to D₁ or D₂ receptor signals. In support of the notion that M₄ receptors inhibit the D₁-mediated S845 phosphorylation, we found that the M₄ receptor blockade by tropicamide led to a higher efficiency of the D₁ agonist in stimulating S845 phosphorylation. M₄ receptors are G_{ai/o}-coupled receptors which upon activation inhibit adenylyl cyclase and the cAMP-PKA pathway (Wess, 1996). Thus, M₄ and D₁ receptors if they are co-expressed in mPFC neurons can antagonistically converge onto the cAMP-PKA pathway, thereby forming a dynamic dopamine-acetylcholine balance controlling AMPA receptor activity via S845 phosphorylation.

While the D₁ agonist appears to directly stimulate S845 phosphorylation in mPFC neurons, the D₂ agonist may regulate S845 phosphorylation via an indirect mechanism. A possible scenario is that the D₂ agonist inhibits, but not eliminates, acetylcholine release in the local mPFC (Brooks et al., 2007), thereby reducing activity of postsynaptic M₄ receptors on pyramidal neurons. This reduces the M₄-mediated inhibition of the cAMP-PKA system and therefore increases PKA activity and PKA-dependent phosphorylation of GluA1. In support of this scenario, enhancement of M₄ receptor activity by an exogenously administered M₄ PAM reduced the elevation of S845 phosphorylation induced by the D₂ agonist. Similarly, the M₄ PAM partially blocked the S845 phosphorylation induced by coactivation of D₁ and D₂ receptors. However, it should be pointed out that the mPFC is a highly heterogeneous area in its anatomical and functional aspects (Tritsch and Sabatini, 2012). Future studies need to elucidate detailed responses of S845 phosphorylation to dopamine and muscarinic receptor agents in various subpopulations of pyramidal neurons or interneurons at defined subsets of synapses.

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Figure 1.

D₁ and D₂ agonists increase GluA1 S845 phosphorylation in the rat mPFC. (A) Representative immunoblots illustrating effects of SKF81297 and quinpirole on GluA1 phosphorylation at S845 and S831. (B and C) Quantifications of effects of SKF81297 and quinpirole on S845 (B) and S831 (C) phosphorylation. Note that both SKF81297 and quinpirole alone increased S845 but not S831 phosphorylation, while coadministration of both agonists induced a significantly greater increase in S845 phosphorylation than either agonist alone. Rats were given an injection of SKF81297 (SKF, 3 mg/kg, i.p.), quinpirole (Quin, 3 mg/kg, i.p.) or both drugs and were killed 20 min after drug injection for immunoblot analysis. Data are presented as means $\pm$ S.E.M. (n = 4 per group) and analyzed using one-way ANOVA: pS845/GluA1: $F(3,12) = 120.9$, $P < 0.001$; pS831/GluA1: $F(3,12) = 0.44$, $P = 0.73$. * $P < 0.05$ versus saline. + $P < 0.05$ versus SKF81297 or quinpirole alone.

Figure 2.

Pharmacological blockade of D₁ or D₂ receptors alters SKF81297/quinpirole-stimulated GluA1 S845 phosphorylation in the rat mPFC. (A) Representative immunoblots illustrating effects of the D₁ antagonist SCH23390 and the D₂ antagonist eticlopride on basal and SKF81297/quinpirole-stimulated GluA1 phosphorylation. (B and C) Quantifications of effects of SCH23390 and eticlopride on basal and SKF81297/quinpirole-stimulated GluA1 phosphorylation at S845 (B) and S831 (C). Note that SCH23390 blocked the S845 phosphorylation induced by SKF81297 and quinpirole, while eticlopride partially blocked the SKF81297/quinpirole-stimulated S845 phosphorylation. Rats were given an i.p. injection of SCH23390 (SCH, 0.1–0.5 mg/kg) or eticlopride (Eti, 0.5 mg/kg) 15 min prior to saline (sal) or SKF81297 (SKF)/quinpirole (Quin) at 3 mg/kg and were killed 20 min after the final drug injection for immunoblot analysis. Data are presented as means $\pm$ S.E.M. (n = 4 per group) and analyzed using one-way ANOVA: pS845/GluA1: $F(5,18) = 93.8$, $P < 0.001$; pS831/GluA1: $F(5,18) = 0.40$, $P = 0.84$. * $P < 0.05$ versus saline + saline. + $P < 0.05$ versus saline + SKF81297/quinpirole.

Figure 3.

Effects of the M_4 PAM on basal GluA1 phosphorylation in the rat mPFC. (A) Representative immunoblots illustrating effects of the M_4 PAM VU0152100 on S845 phosphorylation. (B and C) Quantifications of effects of VU0152100 on S845 (B) and S831 (C) phosphorylation. Rats were given an i.p. injection of vehicle (Veh) or VU0152100 (VU) at 6 or 60 mg/kg and were killed 20 min after drug injection for immunoblot analysis. Data are presented as means $\pm$ S.E.M. ($n = 4$ per group) and analyzed using one-way ANOVA: pS845/GluA1: $F(2,9) = 1.09$, $P = 0.38$; pS831/GluA1: $F(2,9) = 0.01$, $P = 0.99$.

Figure 4.

Effects of VU0152100 on the dopamine receptor agonist-stimulated GluA1 phosphorylation in the rat mPFC. (A) Representative immunoblots illustrating effects of VU0152100 on the efficacy of SKF81297 in modulating GluA1 phosphorylation. (B and C) Quantification of effects of VU0152100 on the efficacy of SKF81297 in modulating GluA1 phosphorylation at S845 (B) and S831 (C). (D) Representative immunoblots illustrating effects of VU0152100 on GluA1 phosphorylation responses to SKF81297/quinpirole. (E and F) Quantification of effects of VU0152100 on S845 (E) and S831 (F) phosphorylation responses to SKF81297/quinpirole. Note that VU0152100 significantly reduced the SKF81297- and SKF81297/quinpirole-stimulated S845 phosphorylation. Rats were given an i.p. injection of vehicle (Veh) or VU0152100 (VU, 60 mg/kg) 20 min prior to saline (sal), SKF81297 (SKF, 3 mg/kg), or SKF81297/quinpirole (SKF/Quin, 3 mg/kg) and were killed 20 min after the final drug injection for immunoblot analysis. Data are presented as means $\pm$ S.E.M. ($n = 4-5$ per group) and analyzed using one-way ANOVA: pS845/GluA1 (panel B): $F(2,11) = 23.0$, $P < 0.001$; pS831/GluA1 (panel C): $F(2,11) = 0.04$, $P = 0.96$; pS845/GluA1 (panel E): $F(2,9) = 44.8$, $P < 0.001$; pS831/GluA1 (panel F): $F(2,9) = 0.30$, $P = 0.75$. * $P < 0.05$ versus vehicle + saline. + $P < 0.05$ versus vehicle + SKF81297 (B) or vehicle + SKF81297/quinpirole (E).

Figure 5.

Effects of the M₄ PAM on quinpirole-stimulated GluA1 S845 phosphorylation in the rat mPFC. (A and B) Representative immunoblots (A) and quantifications (B) illustrating effects of VU0152100 on the efficacy of quinpirole in stimulating S845 phosphorylation. Rats were given an i.p. injection of vehicle (Veh) or VU0152100 (VU, 60 mg/kg) 20 min prior to saline (sal) or quinpirole (Quin, 3 mg/kg) and were killed 20 min after the final drug injection for immunoblot analysis. Data are presented as means $\pm$ S.E.M. ($n = 4$ per group) and analyzed using one-way ANOVA: $F(2,9) = 19.9$, $P < 0.001$. * $P < 0.05$ versus vehicle + saline. + $P < 0.05$ versus vehicle + quinpirole.

Figure 6.

Effects of tropicamide on basal and SKF81297-stimulated GluA1 phosphorylation in the rat mPFC. (A) Representative immunoblots illustrating effects of tropicamide on basal phosphorylation of GluA1. (B and C) Quantifications of effects of tropicamide on GluA1 phosphorylation at S845 (B) and S831 (C). Note that tropicamide significantly elevated S845 phosphorylation. Rats were given an i.p. injection of vehicle (Veh) or tropicamide (Tro, 10 mg/kg) and were killed 15 min after drug injection for immunoblot analysis. (D) Representative immunoblots illustrating effects of coadministration of tropicamide and SKF81297 on GluA1 phosphorylation. (E and F) Quantifications of effects of coadministration of tropicamide and SKF81297 on GluA1 phosphorylation at S845 (E) and S831 (F). Note that coadministration of tropicamide and SKF81297 at low doses induced an increase in S845 phosphorylation (D and E). Rats were given an i.p. injection of vehicle (Veh) or tropicamide (Tro, 2 mg/kg) 15 min prior to saline (sal) or SKF81297 (SKF, 0.5 mg/kg) and were killed 20 min after the final drug injection for immunoblot analysis. Data are presented as means $\pm$ S.E.M. ($n = 4-5$ per group) and analyzed using Student's *t* test (B and C) or one-way ANOVA (E and F): pS845/GluA1: $F(3,12) = 4.5$, $P = 0.02$; pS831/GluA1: $F(3,12) = 0.20$, $P = 0.90$. * $P < 0.05$ versus vehicle (B) or vehicle + saline (E).

Figure 7.

Effects of D₁ and D₂ agonists on GluA1 phosphorylation and expression in synaptic and extrasynaptic fractions of rat mPFC proteins. (A) Effects of SKF81297/quinpirole on the amount of pS845, pS831, and GluA1 proteins in the synaptic fraction. (B) Effects of SKF81297/quinpirole on the amount of pS845, pS831, and GluA1 proteins in the extrasynaptic fraction. Representative immunoblots are shown left to the quantified data. Note that coadministration of SKF81297 and quinpirole elevated pS845 protein levels in the synaptic pool and pS845 and GluA1 protein levels in the extrasynaptic pool. Rats were given an injection of saline or coadministration of SKF81297 (SKF) and quinpirole (Quin) at 3 mg/kg (i.p.) and were killed 90 min after drug administration for enriching synaptic and extrasynaptic membranes. Data that were normalized to tubulin are presented as means $\pm$ S.E.M. (n = 5 per group) and analyzed using Student's *t* test. **P* < 0.05 versus saline.