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Integrated regulation of AMPA glutamate receptor phosphorylation in the striatum by dopamine and acetylcholine

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SCH23390 (PubChem CID:5018)
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VU0152100 (PubChem CID:864492)
(-)-scopolamine hydrobromide (PubChem CID:517999)

ABSTRACT

Dopamine (DA) and acetylcholine (ACh) signals converge onto protein kinase A (PKA) in medium spiny neurons of the striatum to control cellular and synaptic activities of these neurons, although underlying molecular mechanisms are less clear. Here we measured phosphorylation of the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor (AMPAR) at a PKA site (S845) as an indicator of AMPAR responses in adult rat brains *in vivo* to explore how DA and ACh interact to modulate AMPARs. We found that subtype-selective activation of DA D1 receptors (D1Rs), D2 receptors (D2Rs), or muscarinic M4 receptors (M4Rs) induced specific patterns of GluA1 S845 responses in the striatum. These defined patterns support a local multitransmitter interaction model in which D2Rs inhibited an intrinsic inhibitory element mediated by M4Rs to enhance the D1R efficacy in modulating AMPARs. Consistent with this, selective enhancement of M4R activity by a positive allosteric modulator resumed the cholinergic inhibition of D1Rs. In addition, D1R and D2R coactivation recruited GluA1 and PKA preferentially to extrasynaptic sites. In sum, our *in vivo* data support an existence of a dynamic DA-ACh balance in the striatum which actively modulates GluA1 AMPAR phosphorylation and trafficking.

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1. Introduction

Dopamine (DA) D1 receptors (D1Rs) and D2 receptors (D2Rs) are the most abundant dopamine receptor subtypes in the striatum, a key structure in the basal ganglia and a focus of studies on various psychiatric disorders. These receptors are prominently segregated into two equally-populated subtypes of medium spiny neurons (MSNs) making up 90–95% of striatal neurons (Gerfen et al., 1990; Aubert et al., 2000; Bertran-Gonzalez et al., 2010). While D1Rs are primarily expressed in striatonigral output neurons giving rise to the direct pathway, D2Rs reside in striatopallidal output neurons triggering the polysynaptic indirect pathway (Onn et al., 2000). Both receptors are G protein-coupled receptors. Via distinct G proteins, D1Rs ($G_{\alpha s}$/G_{olf}-coupled) and D2Rs ($G_{\alpha i/o}$-coupled) respectively stimulate and inhibit adenylyl cyclase and the downstream cAMP formation and protein kinase A (PKA) activation (Neve et al., 2004). As a result, D1Rs are usually considered to enhance glutamatergic actions in striatonigral neurons, whereas D2Rs exert the opposite effects in striatopallidal neurons (Surmeier et al., 2007).

Acetylcholine (ACh) is another key transmitter in the striatum

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and is mainly provided by local large aspiny cholinergic interneurons. Despite few in number (only 1–2% of the total striatal cell population) (Bolam et al., 1984; Phelps et al., 1985), cholinergic interneurons widely influence surrounding MSNs via their extremely dense and branched axonal arbors. The intrinsic cholinergic transmission is traditionally viewed as a strategic drive balancing DA signaling and maintaining MSN homeostasis. Indeed, pharmacological blockade of muscarinic ACh receptors (mAChRs) caused hyperlocomotion and augmented DA-stimulated motor activities (Chou et al., 1992; Morelli et al., 1993; Wang and McGinty, 1996a) and gene expression in the striatum (Chou et al., 1992; Bernard et al., 1993; Morelli et al., 1993; Wang and McGinty, 1996a, 1996b; 1997). Thus, ACh through mAChRs acts as an inhibitory regulator of tonic and phasic DA signaling, although detailed molecular mechanisms, especially the mAChR subtype, underlying the ACh-DA interplay remain elusive.

The α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor (AMPAR) is critical for normal glutamatergic transmission and is linked to various mental illnesses (Dingledine et al., 1999). These receptors become functional upon homo- or hetero-tetrameric assembly of four subunits, GluA1-4 (formerly GluR1-4) (Greger et al., 2007). AMPARs are knowingly regulated by post-translational phosphorylation (Lu and Roche, 2012; Wang et al., 2014). Two well-characterized phosphorylation sites are serine 831 (S831) and serine 845 (S845) located in intracellular C-terminal tails of GluA1 subunits (Roche et al., 1996; Barria et al., 1997; Mammen et al., 1997; Serulle et al., 2007). The former is phosphorylated by protein kinase C and Ca^{2+}/calmodulin-dependent protein kinase II, while the latter is phosphorylated by PKA. As a labile, inducible and reversible process sensitive to changing synaptic input, phosphorylation of S831 and S845 controls synaptosomal delivery of GluA1 and physiological properties of GluA1-containing AMPARs (Lu and Roche, 2012; Wang et al., 2014).

AMPARs are enriched in DA-innervated forebrain regions, including the striatum (Bernard et al., 1997; Kondo et al., 2000; Reimers et al., 2011). In the striatum, GluA1-containg AMPARs exist in all MSNs (Bernard et al., 1997). These AMPARs are believed to be tightly regulated by DA given that D1R agonists readily elevated GluA1 phosphorylation usually at S845 in striatal neurons (Price et al., 1999; Snyder et al., 2000; Chao et al., 2002; Swayze et al., 2004). However, how the D1R-D2R interaction and DA-ACh interplay regulate GluA1 phosphorylation is unclear. Here we investigated the cooperative role of D1Rs and D2Rs in regulating GluA1 AMPAR phosphorylation in the rat striatum and evaluated the detailed contribution of the DA-ACh balance to controlling AMPAR trafficking *in vivo*.

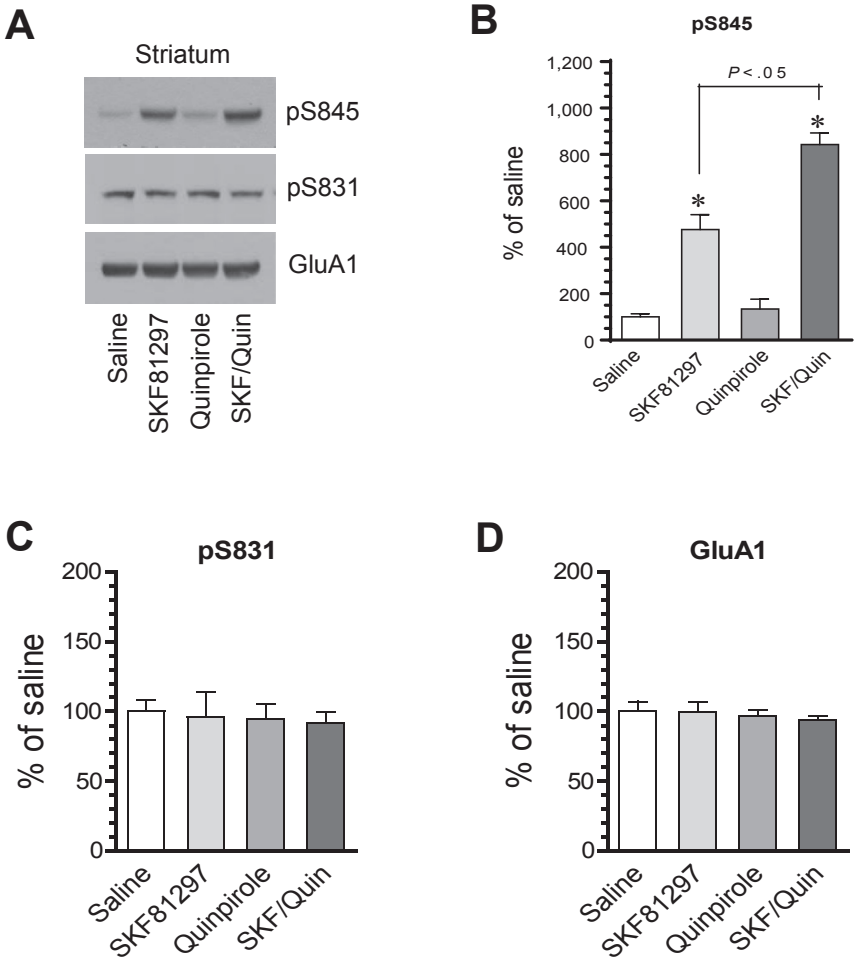

Fig. 1. Effects of the D1R and D2R agonists on basal GluA1 phosphorylation in the rat striatum. (A) Representative immunoblots illustrating effects of SKF81297 and quinpirole on GluA1 phosphorylation in the striatum. **(B–D)** Quantification of effects of SKF81297 and quinpirole on GluA1 phosphorylation at S845 (B) and S831 (C) and total GluA1 expression (D). Note that SKF81297 increased S845 phosphorylation, while quinpirole did not. Co-administration of the two agonists induced a greater increase in pS845 levels than that induced by the D1R agonist alone. Rats were given an i.p. injection of SKF81297 (SKF, 3 mg/kg), quinpirole (Quin, 3 mg/kg) or both drugs and were sacrificed 20 min after drug injection for immunoblot analysis. Data are presented as means ± SEM (n = 4 per group). *$P < 0.05$ versus saline.