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Research Report

Regulation of group I metabotropic glutamate receptor expression in the rat striatum and prefrontal cortex in response to amphetamine *in vivo*

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ABSTRACT

G protein-coupled metabotropic glutamate receptors (mGluRs) are expressed in widespread regions of the mammalian brain and are involved in the regulation of a variety of neuronal and synaptic activities. Group I mGluRs (mGluR1 and mGluR5 subtypes) are expressed in striatal medium spiny output neurons and are believed to play an important role in the modulation of cellular responses to dopamine stimulation with psychostimulants. In this study, we investigated the effect of a single dose of the psychostimulant amphetamine on mGluR1/5 protein expression in the rat forebrain *in vivo*. We found that acute systemic injection of amphetamine at a behaviorally active dose (5 mg/kg) was able to reduce mGluR5 protein levels in a confined biochemical fraction of synaptosomal plasma membranes enriched from the striatum. In contrast to the striatum, amphetamine increased mGluR5 protein levels in the medial prefrontal cortex. These changes in mGluR5 expression in both the striatum and the medial prefrontal cortex were transient and reversible. In addition, protein levels of mGluR1 in the enriched synaptosomal fraction from both the striatum and the medial prefrontal cortex remained stable in response to acute amphetamine. Similarly, Homer1b/c proteins, which are prominent anchoring proteins of mGluR1/5 and are highly expressed in the striatum and the medial prefrontal cortex, showed no change in their protein abundance in striatal and cortical synaptosomes after amphetamine administration. These data demonstrate differential sensitivity of mGluR1 and mGluR5 expression to amphetamine. Acute amphetamine injection is able to alter mGluR5 protein levels at synaptic sites in a subtype- and region-specific manner.

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1. Introduction

Metabotropic glutamate receptors (mGluRs) belong to G protein-coupled receptors. These receptors are densely expressed in mammalian brains and are involved in the modulation of a variety of neuronal and synaptic activities. Based on sequence homology, pharmacological profiles, and signaling mechanisms, eight subtypes of mGluRs so far cloned are organized into three functional groups (Conn and Pin, 1997). Group I mGluRs (mGluR1 and 5 subtypes) are positively coupled to membrane-bound phospholipase Cβ1 (PLCβ1) through Gαq proteins. Activation of group I mGluRs increases phosphoinositol hydrolysis, resulting in intracellular Ca^{2+} release and protein kinase C activation (Conn and Pin, 1997). Both group II (mGluR2 and 3 subtypes) and group III (mGluR4, 6, 7, and 8 subtypes) receptors are negatively coupled to adenylyl cyclase through Gαi/o proteins. Activation of these receptors reduces cAMP formation and inhibits protein kinase A (Conn and Pin, 1997). The linkages of mGluRs to those diverse intracellular signaling pathways enable mGluRs to dynamically modulate their activities in response to changing excitatory synaptic inputs.

The striatum represents a basal ganglia site highly innervated with glutamatergic afferents from widespread regions of the forebrain (McGeer et al., 1977; McGeorge and Faull, 1988). Similarly, glutamate receptors, including group I mGluRs, are densely expressed in striatal medium spiny neurons. A low to moderate level of mGluR1 and a high level of mGluR5 mRNAs are expressed in the vast majority of either striatonigral or striatopallidal projection neurons (Testa et al., 1994, 1995; Kerner et al., 1997). Immunostaining of the two receptor proteins is also found in neurons throughout the striatum (Tallaksen-Greene et al., 1998; Testa et al., 1998). Both mGluR1 and 5 are primarily postsynaptic on dendrites and spines and concentrated in an annulus surrounding the edge of the postsynaptic density as opposed to ionotropic glutamate receptors that are located centrally in the synapse (Takumi et al., 1999; Paquet and Smith, 2003; Mitrano and Smith, 2007). These characteristic subsynaptic distributions of mGluR1/5 imply a distinct role of these receptors in the modulation of excitatory synapses in the striatal region.

Amphetamine (AMPH) is a prototypic dopamine psychostimulant that stimulates dopamine release from the dopaminergic nerve terminals in the striatum (Hernandez et al., 1987; Butcher et al., 1988). In addition to the stimulating effect on dopamine, AMPH enhances extracellular glutamate levels in the striatum (Reid et al., 1997; Gray et al., 1999; Del Arco et al., 1998, 1999). Enhanced glutamate in concert with dopamine thereby increases output of the basal ganglia and induces a transient increase in peripheral motor activity. In response to altered synaptic levels of dopamine and glutamate, postsynaptic glutamate receptors such as group I mGluRs in medium spiny neurons may undergo dynamic alterations in expression. However, to date, the sensitivity of mGluR1/5 expression to AMPH exposure has not been thoroughly investigated.

In this study, we investigated the regulation of mGluR1 and 5 protein expression following AMPH administration. Alterations in mGluR1/5 protein abundance in the striatum and the medial prefrontal cortex (mPFC) were assessed in rats following a single intraperitoneal (i.p.) injection of the drug at a behaviorally active dose. In addition, key scaffold proteins for mGluR1/5, Homer1b/c, were detected in parallel with mGluR1/5 for its sensitivity to AMPH.

2. Results

2.1. *Effects of AMPH on mGluR5 expression*

To explore possible changes in mGluR5 expression in striatal and cortical neurons following AMPH administration, we subjected rats to a single dose of AMPH (5 mg/kg, i.p.). The rats were then sacrificed 1 h after AMPH injection for Western blot assessments of changes in mGluR5 protein levels in the striatum and mPFC. The selection of 1 h was based on preliminary studies. For Western blot analysis, we purified synaptic proteins by solubilizing membrane-bound proteins from synaptic plasma membrane preparations (Fig. 1A). In this form of protein extracts, functional mGluR5 was notably displayed as monomers (~130 kDa) and dimers (~250 kDa) at low and high levels (Fig. 1B), respectively, as described previously (Kuwajima et al., 2007). Following an acute injection of AMPH, basal mGluR5 protein levels in the striatum (both monomers and dimers) were markedly reduced as compared to those in the saline-treated animals (Fig. 1C). As shown in a quantitative graph in Fig. 1C, protein levels of mGluR5 monomers and dimers in AMPH-treated rats were reduced to 50.7±5.0% of saline ($p<0.05$) and 57.0±5.7% of saline ($p<0.05$), respectively. There was no change in striatal actin protein levels in AMPH-treated rats relative to saline-treated rats (Fig. 1C). These results demonstrate that synaptic mGluR5 (both monomers and dimers) is sensitive to AMPH. A single dose of AMPH reduces the synaptic level of the receptor in the striatum.

The mPFC is also enriched with mGluR5 and is implicated in processing drug effects (Berke and Hyman, 2000; Wise, 2002). We therefore investigated whether mGluR5 expression in the mPFC is subject to the modulation by AMPH. Interestingly, in contrast to the reduction of mGluR5 in the striatum, the mGluR5 protein level in the mPFC was elevated in rats treated with AMPH relative to rats treated with saline. As shown in Fig. 1D, mGluR5 dimers were elevated to 188.8±5.8% of saline ($p<0.05$), even though mGluR5 monomers remained unchanged 99.1±29.3% of saline, $p>0.05$). Thus, mGluR5 in the mPFC, primarily in the form of dimers, is sensitive to AMPH, and can be enhanced in its expression in response to AMPH.

2.2. *Effects of AMPH on mGluR1 expression*

Using the same protein samples, protein levels of mGluR1 in AMPH- and saline-treated rats were assessed using Western blots. Like mGluR5, functional mGluR1 receptors exist in monomer and dimer forms in a synaptic protein-enriched fraction from the striatum (Kuwajima et al., 2007). In gel electrophoresis, mGluR1 proteins migrated into a very light monomer band (~130 kDa) and a predominant dimer band (~250 kDa) (Fig. 2A). Due to the dominant nature of dimers, we focused our quantification analysis of mGluR1 protein expression on the form of dimers. In rats treated with a single dose of