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Regulation of postsynaptic plasticity genes' expression and topography by sustained dopamine perturbation and modulation by acute memantine: Relevance to schizophrenia

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ABSTRACT

A relevant role for dopamine–glutamate interaction has been reported in the pathophysiology and treatment of psychoses. Dopamine and glutamate may interact at multiple levels, including the glutamatergic postsynaptic density (PSD), an electron-dense thickening that has gained recent attention as a switchboard of dopamine–glutamate interactions and for its role in synaptic plasticity. Recently, glutamate-based strategies, such as memantine add-on to antipsychotics, have been proposed for refractory symptoms of schizophrenia, e.g. cognitive impairment. Both antipsychotics and memantine regulate PSD transcripts but sparse information is available on memantine's effects under dopamine perturbation. We tested gene expression changes of the Homer1 and PSD-95 PSD proteins in models of sustained dopamine perturbation, i.e. subchronic treatment by: a) GBR-12909, a dopamine receptor indirect agonist; b) haloperidol, a D2R antagonist; c) SCH-23390, a dopamine D1 receptor (D1R) antagonist; and d) SCH-23390 + haloperidol. On the last day of treatment, rats were acutely treated with vehicle or memantine. The *Homer1a* immediate-early gene was significantly induced by haloperidol and by haloperidol + SCH-23390. The gene was not induced by SCH-23390 per se or by GBR-12909. Expression of the constitutive genes *Homer1b/c* and *PSD-95* was less affected by these dopaminergic paradigms. Acute memantine administration significantly increased *Homer1a* expression by the dopaminergic compounds used herein. Both haloperidol and haloperidol + SCH-23390 shifted *Homer1a*/*Homer1b/c* ratio of expression toward *Homer1a*. This pattern was sharpened by acute memantine. Dopaminergic compounds and acute memantine also differentially affected topographic distribution of gene expression and coordinated expression of *Homer1a* among cortical–subcortical regions. These results indicate that dopaminergic perturbations may affect glutamatergic signaling in different directions. Memantine may help partially revert dopamine-mediated glutamatergic dysfunctions.

1. Introduction

A relevant role for dopamine–glutamate interaction has been suggested in the pathophysiology and treatment of schizophrenia (de Bartolomeis et al., 2005; Howes and Kapur, 2009; Javitt et al., 2012). Dopamine and glutamate may interact at multiple levels including the glutamatergic postsynaptic density (PSD), an electron-dense thickening constituted mainly by glutamate receptors, scaffolding and adaptor proteins, that has gained recent attention as a switchboard of dopamine and glutamate interactions and for its role in the regulation of synaptic plasticity (Bertaso et al., 2010; de Bartolomeis et al., 2013a; Iasevoli et al., 2013; Verpelli et al., 2012).

Among PSD scaffolding proteins, Homer1 and PSD-95, two interacting proteins, may be specifically relevant for psychosis pathophysiology and treatment. Homer1 belongs to the Homer family of proteins and is expressed in several brain regions, with high levels in cortex and striatum (Luo et al., 2012; Shiraishi-Yamaguchi and Furuichi, 2007).

Abbreviations: ACC, anterior cingulate cortex; AMPA, α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ANOVA, One-Way Analysis of Variance; CAb, core of the nucleus accumbens; CREB, Ca++ Responsive Element Binding Protein; D1R, dopamine D1 receptor; D2R, dopamine D2 receptor; DEPC, diethylpyrocarbonate; DL, dorsolateral caudate putamen; DM, dorsomedial caudate putamen; EDTA, ethylene-diamine-tetraacetic acid; GBR-12909, 1-[2-[Bis-(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine dihydrochloride; IC, insular cortex; LTD, long term depression; MAC, medial agranular cortex; MC, motor cortex; mGluR, metabotropic glutamate receptor; MSN, medium-sized spiny neurons; NMDA, N-methyl-D-aspartate; PBS, phosphate buffered saline; PSD, postsynaptic density; ROIs, Regions of Interest; SAb, shell of the nucleus accumbens; SCH-23390, (R)-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride; S.E.M., standard error mean; SS, somatosensory cortex; SSC, saline sodium citrate solution; VL, ventrolateral caudate putamen; VM, ventromedial caudate putamen.

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Homer1 links group I mGluRs (mGluR1 and mGluR5) to downstream targets, such as Transient Receptor Potential channels (Smani et al., 2014), inositol triphosphate receptors (Tu et al., 1998), and ryanodine receptors (Feng et al., 2008). Constitutively expressed Homer1b/c multimers have been shown to mediate ligand-dependent signaling, while the shorter splice variant Homer1a, coded by an immediate-early gene that is induced by neuronal activation, acts as a dominant negative to the constitutive isoforms causing relevant changes in the downstream signaling (Luo et al., 2012; Shiraishi-Yamaguchi and Furuichi, 2007).

Homer1a gene expression has been found to be induced by glutamatergic and dopaminergic stimuli, including: activation of N-Methyl-D-Aspartate (NMDA) and possibly non-NMDA glutamatergic receptors (Iasevoli et al., 2007; Sato et al., 2001); activation of D1Rs (Yamada et al., 2007); acute indirect agonism at dopaminergic receptors (G.C. Zhang et al., 2007; Ghasemzadeh et al., 2009; Hashimoto et al., 2007); and acute and chronic blockade of D2Rs by antipsychotics (de Bartolomeis et al., 2013b; Iasevoli et al., 2009, 2010a,b, 2011; Tomasetti et al., 2011). Mechanistically, Homer1a has been demonstrated to play a role in homeostatic synaptic downscaling of postsynaptic, mostly medium-sized spiny neurons (MSN) (Hu et al., 2010; Siddoway et al., 2014). Therefore, *Homer1a* gene induction likely represents a marker of network activity of postsynaptic neurons.

PSD-95 interacts with NMDA receptor, D1R, and type 2A serotonin receptors (Xia et al., 2003; Zhang et al., 2009), modulating their trafficking and association with intracellular signaling cascades. Notably, PSD-95 negatively modulates D1R/NMDA receptor heteromeric association (Zhang et al., 2009) and inhibits D1R-mediated signaling (J. Zhang et al., 2007).

Despite the efficacy of antipsychotic therapy, still approximately 30% of schizophrenia patients respond poorly or do not respond to current available antipsychotic treatment (Elkis, 2007), and glutamate-based interventions have been proposed in the last decade to alleviate residual and negative symptoms of schizophrenia (de Bartolomeis et al., 2012; Papanastasiou et al., 2013). Among glutamate-based approaches, the modulation of NMDA receptor is an important one, considering the accumulating evidence both at the preclinical (de Bartolomeis et al., 2014) and clinical (Chang et al., 2014) level on the role of NMDA receptors in aberrant dopamine–glutamate interaction. Moreover NMDA receptors are highly represented at the PSD.

Memantine is a moderate affinity, partial uncompetitive trapping blocker of NMDA receptor channels (Kotermanski et al., 2009; Parsons et al., 1999), licensed for the treatment of Alzheimer's disease. The compound has been recently proposed as add-on to treat residual symptoms of schizophrenia, however the results are controversial (Sani et al., 2012). It has been suggested that memantine action may be more similar to that of endogenous Mg^{++} than other voltage-dependent antagonists such as ketamine and use-dependent antagonists, such as MK-801 (Parsons et al., 1999). A potential explanation for these differences is the faster blocking/unblocking kinetics and greater voltage dependency of memantine as compared with other NMDA receptor antagonists (Parsons and Gilling, 2007). Indeed, in preclinical studies, memantine has been found to improve cognitive functioning (Nagakura et al., 2013; Zajaczkowski et al., 1997) and to reduce or prevent glutamate-mediated excitotoxic damage (Thellung et al., 2013; Yeganeh et al., 2013). These features suggest that memantine may balance NMDA-mediated glutamate signaling, preventing both hyper- and hypo-activation of this system (Parsons et al., 2007).

The rationale for this study stemmed from multiple considerations. Antipsychotics and memantine have been shown to modulate transcripts of PSD genes (de Bartolomeis et al., 2013c). Dopamine perturbation is involved both in schizophrenia pathophysiology and treatment, since all available antipsychotics share an antagonist action at D2Rs, although with different degrees (Ginovart and Kapur, 2012). Sparse information is available on the effect of NMDA receptor modulation by memantine in models of sustained dopamine perturbation, as occurs in human pathology and therapy. Based on these considerations, herein we explored the effects of acute memantine administration in paradigms of sustained dopamine perturbation, i.e. indirect dopamine agonism (by GBR-12909), D2R antagonism (by haloperidol), D1R antagonism (SCH-23390), and D1R/D2R simultaneous blockade, on *Homer1a*, *Homer1b/c*, and *PSD-95* transcripts by molecular imaging of their quantitative expression and topographic distribution in cortical and sub-cortical brain regions.

The rationale for choosing these specific paradigms of dopamine perturbation resided in their translational potential to mimic distinct molecular aspects of psychosis. GBR-12909 is a blocker of the dopamine transporter and an indirect agonist at dopamine receptors, providing a condition of sustained hyper-dopaminergia, which has considered to occur in psychosis, mostly in striatal sites (Howes and Kapur, 2009). SCH-23390 is a D1R selective antagonist, mimicking a condition of D1R-mediated hypo-dopaminergia that has been hypothesized to occur in psychosis (Remington et al., 2011), mostly in cortical sites. Haloperidol is a D2R selective antagonist, since blockade of D2Rs is considered the necessary mechanism of antipsychotic action (Seeman, 2006). Finally, haloperidol + SCH-23390 provided a condition of sustained hypo-dopaminergia, as in some antipsychotic-related conditions.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (mean weight 250 g) were obtained from Charles-River Labs. (Lecco, Italy). The animals were housed and let to adapt to human handling in a temperature and humidity controlled colony room with 12/12 h light–dark cycle (lights on from 6:00 a.m. to 6:00 p.m.) with ad libitum access to laboratory chow and water. All procedures were conducted in accordance with the NIH Guide for Care and Use of Laboratory Animals (NIH Publication No. 85–23, revised 1996) and to the EU Directive 2010/63/EU for animal experiments, and were approved by local Animal Care and Use Committee. All efforts were made to minimize animal number and suffering.

2.2. Drug treatment and group composition

Memantine hydrochloride (gently supplied by H. Lundbeck A/S, Copenhagen, Denmark), SCH-23390 (R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride) (Tocris Cookson, Bristol, UK), GBR-12909 (1-{2-[bis-(4-fluorophenyl)-methoxy]ethyl}-4-(3-phenylpropyl)piperazine) (Sigma-Aldrich, Milano, Italy), were all supplied as a powder and dissolved in saline solution (NaCl 0.9%). Haloperidol was provided as an injectable solution (Lusofarmaco, Milano, Italy) and then diluted at the experimental dosages. All solutions were adjusted to physiological pH value and injected i.p. at a final volume of 1 ml/kg.

Rats were randomly assigned to one of the following treatment groups (n = 14 for each treatment group): vehicle (NaCl 0.9%, VEH); SCH-23390 1 mg/kg (SCH); haloperidol 0.8 mg/kg (HAL); haloperidol 0.8 mg/kg + SCH-23390 1 mg/kg (HAL + SCH); GBR-12909 15 mg/kg (GBR). All drugs were given at behaviorally active doses, known to elicit gene expression (Ambesi-Impiombato et al., 2007; Iasevoli et al., 2009). The above-listed drugs were administered once a day for seven consecutive days. All animals received the drugs in one injection a day. Care was taken to carry out injections at the same hour.

On the last injection day, subjects in each group were split in two subgroups to receive an additional injection containing either vehicle or memantine 5 mg/kg (de Bartolomeis et al., 2013c). This adjunctive injection was administered 30 min after the injection of the corresponding dopaminergic compound. Therefore, all treatment groups were subdivided in half animals receiving acute vehicle plus the dopaminergic compound (thereafter referred to as: VEH/VEH; SCH/VEH; HAL/VEH; HAL + SCH/VEH; and GBR/VEH) and half animals receiving acute memantine plus