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Expression of 5-HT_{2A} receptors in prefrontal cortex pyramidal neurons projecting to nucleus accumbens. Potential relevance for atypical antipsychotic action

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ABSTRACT

The prefrontal cortex (PFC) is involved in higher brain functions altered in schizophrenia. Classical antipsychotic drugs modulate information processing in cortico-limbic circuits via dopamine D2 receptor blockade in nucleus accumbens (NAc) whereas atypical antipsychotic drugs preferentially target cortical serotonin (5-HT) receptors. The brain networks involved in the therapeutic action of atypical drugs are not fully understood. Previous work indicated that medial PFC (mPFC) pyramidal neurons projecting to ventral tegmental area express 5-HT_{2A} receptors suggesting that atypical antipsychotic drugs modulate dopaminergic activity distally, via 5-HT_{2A} receptor (5-HT_{2A}-R) blockade in PFC. Since the mPFC also projects heavily to NAc, we examined whether NAc-projecting pyramidal neurons also express 5-HT_{2A}-R. Using a combination of retrograde tracing experiments and *in situ* hybridization we report that a substantial proportion of mPFC-NAc pyramidal neurons in rat brain express 5-HT_{2A}-R mRNA in a layer- and area-specific manner (up to 68% in layer V of contralateral cingulate). The functional relevance of 5-HT_{2A}-R to modulate mPFC-NAc projections was examined in dual-probe microdialysis experiments. The application of the preferential 5-HT_{2A}-R agonist DOI into mPFC enhanced glutamate release locally (+66 ± 18%) and in NAc (+74 ± 12%) indicating that cortical 5-HT_{2A}-R activation augments glutamatergic transmission in NAc. Since NAc integrates glutamatergic and dopaminergic inputs, blockade of 5-HT_{2A}-R by atypical drugs may reduce cortical excitatory inputs onto GABAergic neurons of NAc, adding to dopamine D2 receptor blockade. Together with previous observations, the present results suggest that atypical antipsychotic drugs may control the activity of the mesolimbic pathway at cell body and terminal level.

1. Introduction

Schizophrenia is a severe psychiatric illness causing a large economic burden in developed societies (Kessler et al., 2005; Knapp et al., 2004; Smith, 2011). Alterations in several brain regions/networks have been reported in schizophrenia. In particular, the prefrontal cortex (PFC) is critically involved in many higher brain functions, including cognitive/executive functions and behavioral control, which are deeply altered in schizophrenic patients (Elvevag and Goldberg, 2000; Lewis and Lieberman, 2000; Weinberger et al., 2001). Autopsy and neuroimaging studies have revealed the existence of a reduced PFC volume, reduced layer thickness, reduced neuropil and tight packing of cortical neurons in the brains of schizophrenic patients (Harrison, 1999; Lewis and Lieberman, 2000; Selemon and Goldman-Rakic, 1999). A reduced energy metabolism in PFC has been related with negative symptoms (Andreasen et al., 1997; Potkin et al., 2002) whereas psychotic symptoms appear associated with hyperactivity of various cortical areas, including the PFC (Catafau et al., 1994; Dierks et al., 1999;

Abbreviations: CTB, cholera-toxin B; DA, dopamine; FG, Fluoro-Gold; mPFC, medial PFC; mRNA, messenger RNA; NAc, nucleus accumbens; PFC, prefrontal cortex; -R, receptor; VTA, ventral tegmental area.

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Shergill et al., 2000). Alterations in key neurotransmitters such as glutamate, GABA and dopamine (DA) have also been reported in PFC (Benes and Berretta, 2001; Lewis et al., 2005; Lewis and Lieberman, 2000). In particular, hyperactive subcortical (mesolimbic) and hypoactive mesocortical DA function have been reported (Abi-Dargham et al., 2002; Breier et al., 1997; Laruelle et al., 1996; Lewis and Lieberman, 2000).

Classical antipsychotic drugs are thought to ameliorate psychotic symptoms by blocking an excessive activation of DA D2 receptors (D2-R) located in ventral striatum (nucleus accumbens – NAc – and adjacent structures). This therapeutic action is associated to severe motor side effects due to D2-R blockade in dorsal striatum. Likewise, D2-R blockade induces negative symptoms in healthy individuals, possibly by dampening cortical DA transmission (Artaloytia et al., 2006). In contrast, atypical antipsychotic drugs – and particularly clozapine, the gold standard in antipsychotic treatments (Leucht et al., 2009) – produce much lesser D2-R occupancy and preferentially target serotonin (5-HT) receptors such as 5-HT_{2A}, 5-HT_{2C} and 5-HT_{1A} receptors of which 5-HT_{2A}-R appear to play a key role (Arnt and Skarsfeldt, 1998; Artigas, 2010; Bymaster et al., 1996; Chou et al., 2003). These 5-HT receptors (in particular 5-HT_{2A}-R) are mainly cortical and are densely expressed in various subfields of the rat PFC, particularly in its medial aspect (Pompeiano et al., 1992, 1994). Several studies have established their presence in a large proportion of PFC pyramidal and GABAergic neurons in rodent and human brains (Amargós-Bosch et al., 2004; de Almeida and Mengod, 2007, 2008; Santana et al., 2004). The physiological release of 5-HT excites and inhibits the activity of pyramidal neurons in mPFC via 5-HT_{2A}-R and 5-HT_{1A}-R, respectively (Amargós-Bosch et al., 2004; Puig et al., 2005). Likewise, the systemic administration of the preferential 5-HT_{2A} agonist DOI also evokes an overall increase of pyramidal neuron activity in mPFC (Puig et al., 2003).

The brain networks involved in the therapeutic action of atypical drugs are not fully understood. In particular, the pathways by which dopamine D2 receptor blockade (mainly subcortical) and 5-HT_{2A} receptor blockade (mainly cortical) modulate brain function to evoke clinical antipsychotic effects are unclear. Previous microdialysis studies have indicated an important role of 5-HT_{A1}-R and 5-HT_{2A}-R in the control of DA release in cortical and subcortical regions, which contributes to atypical antipsychotic action (Bortolozzi et al., 2010; Díaz-Mataix et al., 2005; Ichikawa and Meltzer, 1991, 1995, 2000; Ichikawa et al., 1995; Ichikawa et al., 2001; Kuroki et al., 1999; Li et al., 2005; Yamamoto et al., 1994; see for review see Meltzer and Huang, 2008). However, the fact that systemic drug administration was used in most of these studies hampers the precise identification of the neuronal pathways involved.

Anatomical and functional studies indicate the existence of a 5-HT_{2A}-R-mediated control of DA neurons in the ventral tegmental area (VTA). On the one hand, PFC pyramidal neurons project densely to the VTA (Gabbott et al., 2005; Sesack et al., 1989) making synapses onto dopaminergic and GABAergic neurons (Carr and Sesack, 2000; Sesack and Pickel, 1992). These glutamatergic inputs are important to switch discharge of DA neurons from tonic to phasic (burst) discharge (Gariano and Groves, 1988; Murase et al., 1993; Tong et al., 1996a). On the other hand, a large percentage of mPFC pyramidal neurons projecting to midbrain structures, including the VTA and the dorsal raphe nucleus, express 5-HT_{2A}-R (Vázquez-Borsetti et al., 2009). Consistent with these anatomical observations, the local stimulation of 5-HT_{2A}-R in mPFC enhanced DA burst firing and DA release in rodent brain (Bortolozzi et al., 2005, 2010). These observations indicate that PFC 5-HT_{2A}-R distally control VTA DA neuronal activity and raise the possibility that atypical antipsychotic drugs may attenuate an excessive dopaminergic activity in schizophrenia by antagonizing excitatory 5-HT_{2A}-R in pyramidal neurons projecting to VTA. This pharmacological effect would help atypical antipsychotic drugs to reduce dopaminergic function without the massive blockade of striatal DA D2-R produced by classical antipsychotic drugs (Kapur et al., 1999).

The ventral striatum is a brain structure with two main sources of innervation, descending excitatory inputs from prefrontal cortex using glutamate as neurotransmitter, and ascending dopaminergic inputs from the VTA (mesolimbic pathway). Hence, the mPFC projects heavily to NAc (Gabbott et al., 2005) where descending glutamatergic terminals synapse on spiny neurons also receiving dopaminergic inputs (Sesack and Pickel, 1992). These observations suggest that 5-HT_{2A}-R in mPFC pyramidal neurons might also be involved in the control of NAc neurons, and therefore in the modulation of signals along basal ganglia circuits. We tested this hypothesis using a combination of tract-tracing techniques and *in situ* hybridization to examine whether mPFC pyramidal neurons projecting to NAc express 5-HT_{2A}-R. The functional relevance of 5-HT_{2A}-R in the activity of descending mPFC-NAc afferents has been assessed using dual-probe microdialysis.

2. Materials and methods

2.1. Surgery and tissue preparation

A total of 5 male albino Wistar rats (Iffa Credo, Lyon, France) weighting 250–275 g were used. Animals were kept in a controlled environment (12 h light–dark cycle and 22 ± 2 °C room temperature) with food and water provided *ad libitum*. Animal care followed the European Union regulations (O.J. of E.C. L358/118/12/1986) and protocols used were approved by the Ethical Committee for Animal Research of the University of Barcelona and the "Departament de Medi Ambient i Habitatge" from the Catalan Government (Generalitat de Catalunya).

Animals were deeply anesthetized with sodium pentobarbital (60 mg/kg ip), and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). Glass capillary tubes were heated and pulled with a Narishige PE-2 pipette puller (Narishige Sci. Inst., Tokyo, Japan). Tips were broken to 30 μm diameter under microscopic control. Such micropipettes were filled with a solution of cholera-toxin B subunit (CTB, 2% in distilled water; List Biological Laboratories, Campbell, CA, USA) or Fluoro-Gold (FG, 2% in cacodylate buffer, pH 3.8; Fluorochrome LLC, Denver, CO, USA). Retrotracers were injected into the nucleus accumbens (NAc) at stereotaxic coordinates 1.7 mm AP, −-1.6 mm L, −6.8 mm DV from bregma, according to the rat brain atlas of Paxinos and Watson (1998). The injections were done by microiontophoresis using a Midgard Precision current source device (Stoelting, Wood Dale, IL, USA) using a 5 μA positive-pulsed direct current for a total of 5 min (7 s on/off for 10 min). After surgery animals were housed individually to prevent any risk of injury from other animals. Fifteen days after surgery rats were deeply anesthetized and perfused transcardially with 120 mL of calcium-free Tyrode's solution (6.8 g/L NaCl; 0.4 g/L KCl; 0.32 g/L $MgCl_2 \cdot 6H_2O$; 0.10 g/L $MgSO_4 \cdot 7H_2O$; 0.17 g/L $NaH_2PO_4 \cdot H_2O$; 2.2 g/L $NaHCO_3$; 1.1 g/L glucose) containing 0.1% heparin at room temperature, followed by 60 mL of fixative (4% paraformaldehyde in 0.1 M phosphate buffer, pH 6.9) at room temperature and 300 mL of cold (4 °C) fixative. The brains were quickly removed, immersed in fixative for 90 min, kept in 10% sucrose in 0.1 M phosphate buffer for 2 days at 4 °C, and finally frozen in isopentane at −30 °C.

Fourteen μm thick slices were cut using a microtome-cryostat (Microm HM560, Walldorf, Germany) and thaw-mounted onto microscope glass-slides pretreated with HistoGrip (Invitrogen, Frederick, MD, USA) and kept at −20 °C until used. The correct placement of tracer injection in the NAc was verified by direct observation of FG fluorescence at the microscope or by immunohistochemical labeling of CTB (see procedure described below). Fig. 1 illustrates the precise location of microinjections performed in the different animals used.

2.2. Histochemistry

Tissue sections were hybridized with oligodeoxyribonucleotide probes complementary to 5-HT_{2A} receptor mRNA. We used 3 oligonucleotide probes complementary to bases 669–716, 1923–1970, and 1482–1529 (GenBank accession no. X13971.1) (Pritchett et al., 1988). The specificity of the hybridization signal obtained with these probes has been previously established (Pompeiano et al., 1994) using the following control procedures: 1) the thermal stability of the hybrids obtained was checked for every probe, 2) for every oligonucleotide probe, the hybridization signal was completely blocked by competition of the labeled probe in the presence of 50-fold excess of the same unlabeled oligonucleotide, and 3) the 3 probes used independently demonstrated identical distributions of hybridization signal at regional and cellular levels. All oligonucleotides were synthesized and HPLC purified by Isogen Bioscience BV (Maarsden, The Netherlands). Each probe (2 pmol) was individually labeled at the 3′-end with [^{33}P]α-dATP (>2500 Ci/mmol; Perkin–Elmer, Boston, MA, USA)