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Role of prefrontal cortex in pharmacological models of schizophrenia and antipsychotic action

P. Celada[*,**]
A. Adell[*,**]
X. López-Gil[*,**]
L. Kargieman[*,**]
N. Santana[*,**]
A. Bortolozzi[*,**]
A. Castañé[*,**]
F. Artigas[*,**]

* Department of Neurochemistry and Neuropharmacology, Institut d'Investigacions Biomèdiques de Barcelona (CSIC), IDIBAPS Barcelona

** Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Ministry of Science and Innovation

SPAIN

ABSTRACT – NMDA receptor (NMDA-R) antagonists are extensively used as schizophrenia models due to their ability to evoke positive and negative symptoms as well as cognitive deficits similar to those of the illness. Likewise, 5-HT_{2A} receptor agonists display hallucinogen actions resembling psychotic symptoms. Overall, these drugs are useful models of schizophrenia for the screening of new antipsychotic drugs. However, the cellular and network elements involved in these actions are poorly known. Data obtained by several groups in recent years indicate that the prefrontal cortex (PFC) and anatomically related areas play a major role in these actions. This paper summarizes data obtained by the authors supporting that a) NMDA-R antagonists (phencyclidine –PCP–, dizocilpine –MK–801–) and 5-HT_{2A} agonists (DOI) alter the function of PFC in a similar fashion, and b) antipsychotic drugs exert their therapeutic action, at least in part, by normalizing hyperactivity states in PFC. While the actions of NMDA-R antagonists may involve blockade of these receptors in PFC and subcortical areas, that of antipsychotic drugs, in particular atypical drugs like clozapine, appear to be mediated essentially by a local action in PFC. These results help to better understand the neurobiological basis of the action of pharmacological models of schizophrenia and the mode of action of antipsychotic drugs.

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Introduction

The present report summarizes data obtained in recent years in one of the main research lines (antidepressants, antipsychotics, brain circuits) carried out by the "Systems Neuropharmacology" group of CIBERSAM.

Schizophrenia is associated with alterations in the anatomy and function of several cortical and subcortical areas. Among these, the prefrontal cortex (PFC) seems to play a key role in the pathophysiology of the illness[1,2]. Despite the obvious difficulty in modeling these alterations in experimental models of the illness, non-competitive *N*-methyl-D-aspartate (NMDA) receptor (antagonists such as the dissociative anaesthetics ketamine and phencyclidine (PCP) and MK-801 (dizocilpine), have been extensively used as pharmacological models of schizophrenia due to their ability to evoke positive and negative symptoms of schizophrenia as well as the cognitive deficits of the illness in humans. These agents elicit a potent behavioural syndrome as well as cognitive and sensory deficits in experimental animals that resemble human schizophrenia symptoms (see Geyer *et al*.[3]; Krystal *et al*.[4] for review). NMDA receptor antagonists also induce schizophrenia symptoms in healthy subjects and aggravate them in schizophrenic patients. Furthermore, the behavioural effects of NMDA receptor antagonists are sensitive to the treatment with antipsychotic drugs that alleviate psychotic symptoms in schizophrenic patients[4]. Also, serotonergic agents such as lysergic acid diathylamine and related compounds, which are agonists of 5-HT_{2A} receptors, can produce perceptual and psychic alterations[5]. DOI (1-[2,5-dimethoxy-4-iodophenyl-2-aminopropane]) is a partial 5-$HT_{2A/2C}$ agonist that evokes long-lasting alterations in consciousness and perception. DOI acts by over stimulating 5-HT_{2A} receptors, since its behavioral, neurochemical and electrophysiological effects are blocked by the selective 5-HT_{2A} receptor antagonist M100907.

To provide a deeper insight of the brain areas and neuronal types affected by NMDA-R antagonists and DOI, we have conducted a series of electrophysiological, histological and neurochemical studies to examine the cellular and population responses of PFC, paying also a special attention to the potential reversal of these actions by conventional and second generation (atypical) antipsychotic drugs.

Methods

Animals. Adult male Wistar rats (250-300 g) (Iffa Credo; Lyon, France) were been used in most experiments. We also used 10-15 weeks old male homozygous 5-HT_{1A} receptor *knockouts* (5-HT_{1A} -/-, referred onwards as KO) and wild-type (5-HT_{1A} +/+, referred onwards as WT) mice of the same genetic background (C57BL/6). Animal procedures were performed according to the European Union regulations (O.J. of E.C. L358/1 18/12/1986) for the use of laboratory animals and were approved by the Institutional Animal Care and Use Committee.

Electrophysiological experiments. We examined the effect of psychotomimetic drugs such as the NMDA receptor antagonist phencyclicine (PCP), the preferential 5-HT_{2A} receptor agonist DOI (both with hallucinogen properties) and the antipsychotic drugs clozapine and haloperidol on the activity of PFC, assessed by a) single unit ex-

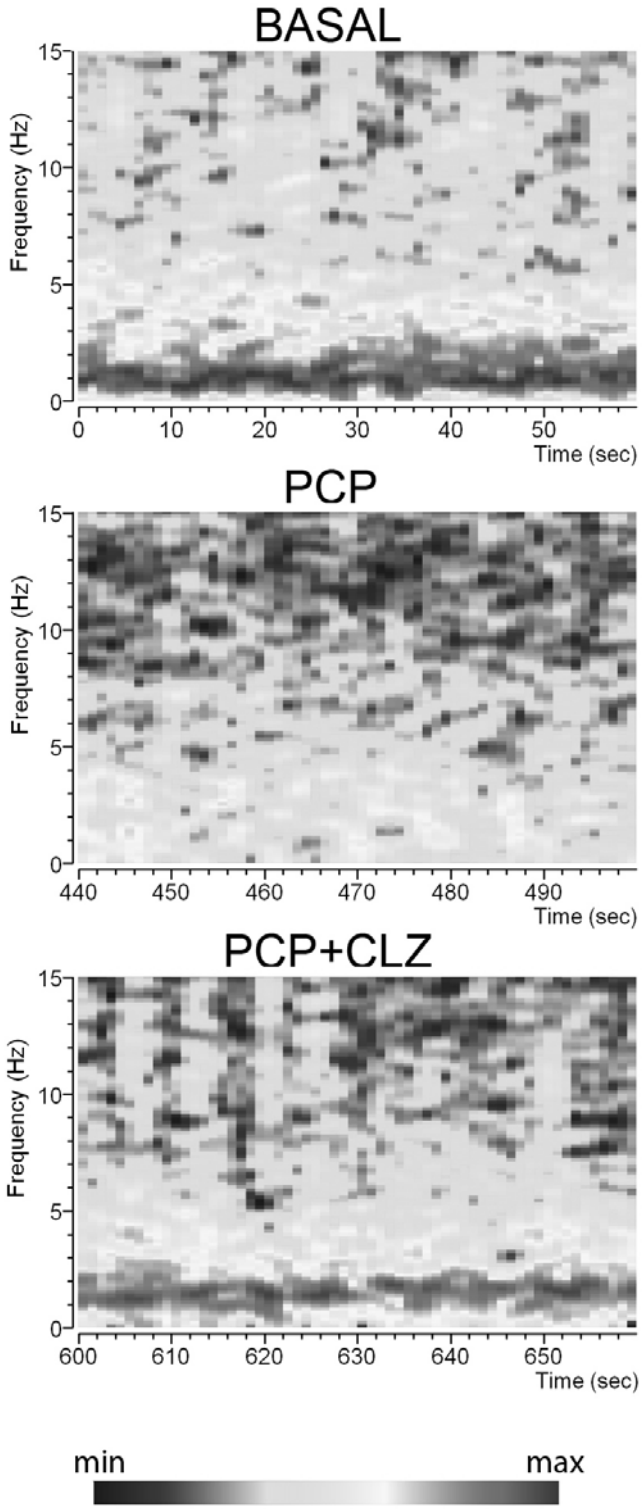

Figure 1. Representative spectrograms showing the effects of the administration of phencyclidine (PCP, 0.25 mg/kg i.v.) and clozapine (CLZ, 1 mg/kg i.v.) on low frequency oscillations recorded in mPFC. Note the marked reduction in the power spectrum induced by CPP (middle panel) and the reversal produced by CLZ. Abscissa is in s, ordinate is in Hz. The intensity of the power spectrum is color-coded (red = high intensity; blue = low intensitiy). Redrawn from data in Kargieman *et al.*[7]

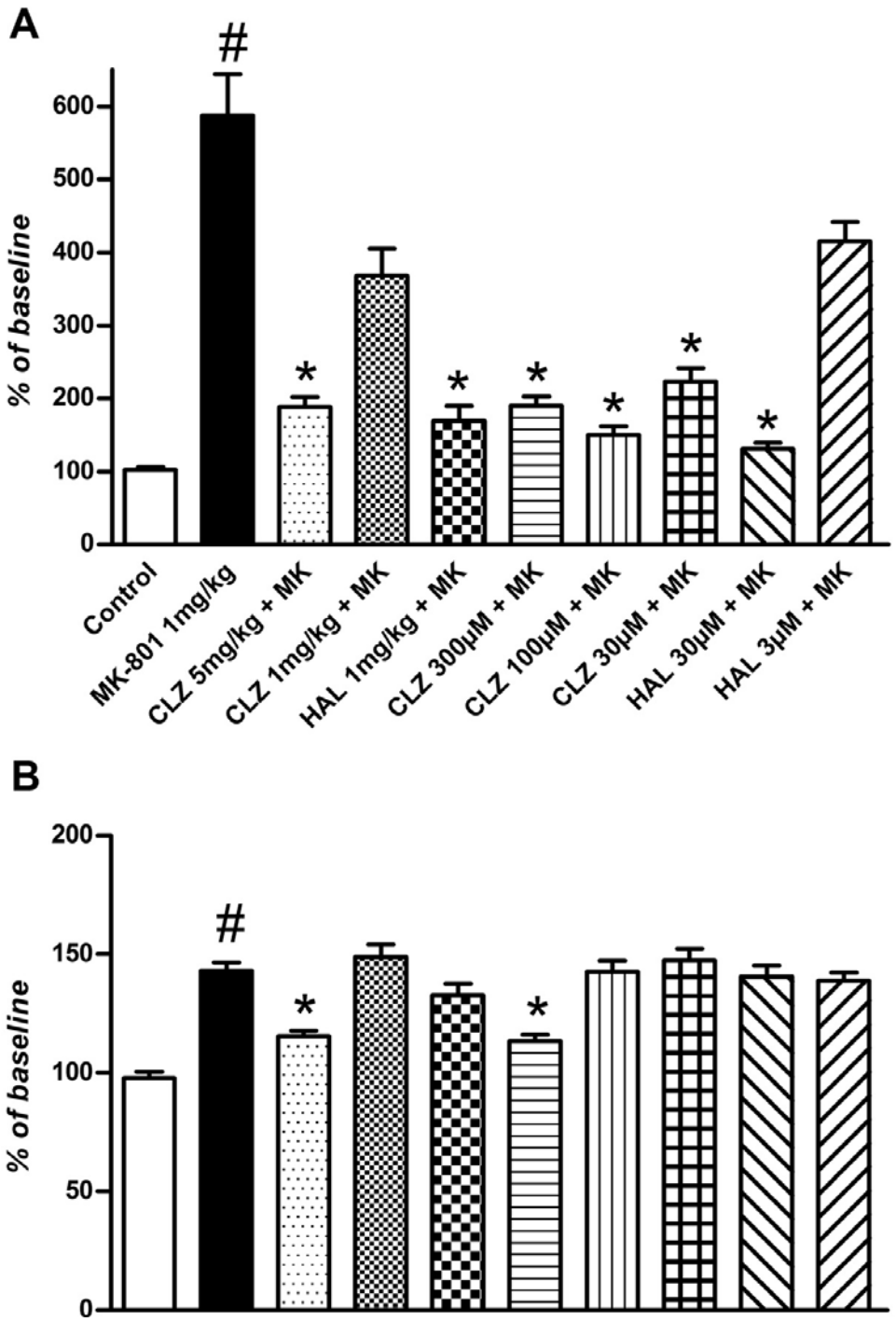

Figure 2. Bargraph showing the effect of MK-801 (MK) alone or in combination with the antipsychotic drugs clozapine (CLZ) or haloperidol (HAL) on the glutamate and serotonin output in rat PFC. Antipsychotic drugs were given systemically (CLZ, 1 and 5 mg(kg; HAL 1 mg/kg) or locally, by reverse dialysis at the stated concentrations. Redrawn from data in López-Gil *et al*.[10]

tracellular responses and/or b) local filed potentials in the chloral hydrate anesthetized rats. These variables permit to examine cellular and population responses, respectively, to drug administration. A full account of the procedures used can be found in Kargieman *et al*.[6,7] and Celada *et al*.[8]

Histological experiments. The effects of PCP and clozapina on PFC function were also examined by using the expression of the immediate early gene *c-fos* as a marker of neuronal activity. This was conducted using double *in situ* hybridization, labeling *c-fos* mRNA with radioactive oligonucleotides and the cellular phenotype (glutmatergic or GABAergic neurons) with non-radioactive oligonucleotides directed respectively towards the vesicular glutamate transporter 1 (vGLuT1) pr the GABA-synthesizing enzyme $GAD_{65/67}$ (glutamate acid decarboxylase). See Kargieman *et al*.[6,7] for details.

Microdialsyis experiments. These experiments were aimed at examining the effect of NMDA receptor antagonists and/or antipsychotic drugs on the *in vivo* release of neurotransmitters in PFC: serotonin (5-HT), dopamine (DA) and glutamate, as an index of the activity of these neuronal groups in response to drug administration. A full description of microdialysis procedures can be found in Amargós-Bosch *et al*.[9], López-Gil *et al*.[10,11]

Data analysis. The effects of drugs on the different variables used in the different studies (neurotransmitter concentrations, neuronal discharge rate, power of cortical oscillations, neuronal numbers, etc) have been assessed by one- or two-way ANOVA for independent or repeated measures, as appropriate. Student's *t*-tests have also been used. Data are expressed as means ± SEM. Statistical significance has been set at the 95% level (two-tailed).

Results

Effects of PCP on neuronal activity in PFC. Reversal by antipsychotic drugs

PCP induces a marked disruption of the activity of the PFC in the rat, increasing and decreasing the activity of 45% and 33% of the pyramidal neurons recorded, respectively (22% of the neurons were unaffected)[6,12]. Concurrently, PCP markedly reduced cortical synchrony in the delta frequency range (0.3-4 Hz) as assessed by recording local field potentials. The subsequent administration of the antipsychotic drugs haloperidol and clozapine reversed PCP effects on pyramidal cell firing and cortical synchronization[6]

Histological studies showed that PCP increased *c-fos* expression in PFC pyramidal neurons, an effect prevented by the administration of clozapine. PCP also enhanced *c-fos* expression in the centromedial and mediodorsal (but not reticular) nuclei of the thalamus, suggesting the participation of enhanced thalamocortical excitatory inputs[6,7].

Effects of DOI on neuronal activity in PFC. Reversal by antipsychotic drugs

Similarly to PCP, DOI markedly disrupts cellular and network activity in the rat PFC. DOI altered pyramidal discharge in mPFC (39% excited, 27% inhibited, 34% unaffected; n = 51)[12]. In all instances, DOI concurrently reduced low frequency oscillations (0.3-4 Hz; power spectrum: 0.25 ± 0.02 and 0.14 ± 0.01 μV^2 in basal conditions and after 50-300 μg/kg i.v. DOI, respectively; n = 51). Moreover, DOI disrupted the tempo-

ral association between active phase of local field potentials (LFP) and pyramidal discharge[8]. Both effects were reversed by M100907 (5-HT_{2A} receptor antagonist) and were not attenuated by thalamic lesions, supporting an intracortical origin of the effects of DOI.

As also observed for PCP, the alteration of low frequency oscillations induced by DOI was significantly reversed by the antipsychotic drugs haloperidol (0.1-0.2 mg/kg i.v.) and clozapine (1 mg/kg i.v.)[8].

Effects of NMDA-R antagonists on neurotransmitter release in PFC. Reversal by antipsychotic drugs

The systemic, but not local (in PFC), administration of the NMDA-R antagonists PCP, ketamine and MK-801 increased the *in vivo* 5-HT release in PFC[9,10]. Further investigations with MK-801 indicated that it also produced a large increase in the efflux of glutamate[10], possibly as a neurochemical correlate of the increase in pyramidal cell activity seen with NMDA-R[6,13].

Interestingly, whereas the local application of MK-801 could not increase glutamate efflux, both the local (in PFC) and systemic administration of clozapine were able to reverse the increased glutamate efflux induced by systemic MK-801 administration[10], suggesting an intracortical action of clozapine. Further studies examining the likely receptors affected by clozapine suggest interactions with 5-HT_{2A}, 5-HT_{1A} and $_{-1}$-adrenoceptors present in PFC pyramidal cells[11]. However, despite 5-HT_{1A}eceptors in PFC appear necessary for the atypical antipsychotic-induced increase in cortical (PFC) dopamine release[14], they play a minor role in the actions of MK-801 to modulate dopamine release[15].

Discussion

Despite the widespread use of NMDA receptor antagonists as pharmacological models of schizophrenia, their neurobiological basis of action is still poorly known. Neuroimaging studies indicate that a sub-anesthetic dose of ketamine increases the activity of the prefrontal cortex (PFC) in human volunteers[16]. In experimental animals, NMDA receptor antagonists such as MK-801 or PCP increase neuronal activity[6, 13,17]. Recent observations also indicate that NMDA receptor antagonists and 5-HT_{2A} receptor agonists produce a marked loss of slow oscillations in PFC[6,8] reflecting a disruption of the function of cortical networks, which possibly reflects the psychotomimetic properties of these compounds. This effect is accompanied by a marked expression of the immediate early gene *c-fos* in pyramidal (but not GABAergic) neurons. The differential effect of PCP in pyramidal and GABAergic neurons is consistent with a preferential blockade of NMDA receptors in GABAergic neurons[18], subsequently leading to pyramidal cell disinhibition. However, since thalamic neurons also expressed *c-fos,* it cannot be discarded that PCP can also act in subcortical areas, this leading to an activation of thalamocortical inputs.

The increased PFC activity observed in electrophysiological experiments is also paralleled by an increased neurotransmitter release in PFC[9-11,15,19,20]. This likely reflects the activation of local and extended neuronal networks, including the activation of PFC descending afferents to the monoaminergic midbrain nuclei (raphe nuclei and ventral tegmental area) which contain the cell bodies of serotonergic and dopamienrgic neurons, respectively.

Interestingly, the above effects produced by NMDA receptor antagonists (and –when

examined– by 5-HT_{2A} receptor agonists), such as increased pyramidal neuron activity, loss of cortical synchrony, increased *c-fos* expression and increased neurotransmitter release, are antagonized or reversed by classical (haloperidol) and atypical (clozapine) antipsychotic drugs. This suggests that the above alterations in PFC function are intimately related to schizophrenia. One interesting observation is that both the local and systemic administration of antipsychotic drugs were able to antagonize the drug-induced PFC abnormalities, supporting that antipsychotric drugs normalize cortical function by a local action in PFC, yet some differences exist between haloperidol and clozapine when antagonizing MK-801 effects on serotonin and glutamate release. This may reflect a distinct interaction of classical and atypical drugs with monoamine receptors in PFC which is possibly related to the distinct activity of both drugs on negatrive/cognitive symptoms. Overall, the above observations suggest that the normalization of PFC function by antipsychotic drugs is related to their therapeutic activity in schizophrenia.

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Corresponding author:
Francesc Artigas, PhD
Dept. of Neurochemistry and Neuropharmacology
IIBB-CSIC (IDIBAPS)
Rosselló, 161, 6th floor
08036 Barcelona
Spain
Phone: +3493-363 8315
Fax: +3493-363 8301
E-mail: fapnqi@iibb.csic.es