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Expression of noradrenergic α1, serotoninergic 5HT2a and dopaminergic D2 receptors on neurons activated by typical and atypical antipsychotic drugs

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Abstract

Antipsychotic agents produce activation of a subset of largely dynorphinergic/GABAergic neurons in the shell of nucleus accumbens (AcbShB), central amygdaloid nucleus (CeA) and midline thalamic central medial nucleus (CM) in rats. It is not known why these particular neurons respond to antipsychotic drugs. The present study tested the hypothesis that activated neurons bear subtypes of monoamine receptors to which antipsychotic drug are known to bind, including dopaminergic D2, serotoninergic 5HT2a and noradrenergic α1 receptors. Rats were treated with the typical antipsychotic haloperidol or the atypical antipsychotic clozapine. Double immunofluorescence labeling was performed with antibodies directed against (1) the expression of Fos proteins, indicating drug-induced cell activation, and (2) each of the monoamine receptor proteins noted. All three receptors examined were expressed in haloperidol- and clozapine-activated neurons in AcbSh. Furthermore, noradrenergic α1 receptors were extensively expressed in activated neurons in CeA and CM, as well. The results suggest that bearing monoamine receptors with high binding affinity for typical and/or atypical antipsychotic drugs might be a key feature of neurons which respond to these drugs. In AcbSh, activated neurons appeared to bear each receptor and, therefore, it is possible that not only the individual but also the combined effect of antipsychotic drugs at multiple receptors may explain why they directly activate certain cells and not others. Also, bearing noradrenergic α1 receptor neurons was a shared feature of all activated cells in each location tested, suggesting inhibition of noradrenergic α1 receptors may contribute to antipsychotic drug action at these sites.

Keywords: Antipsychotic drugs; Central amygdaloid nucleus; Fos; Midline thalamic central medial nuclei; Monoamine receptors; Shell of nucleus accumbens

1. Introduction

Antipsychotic drugs differ in side effects and, by degree, in their potency in treating positive and negative symptoms of psychosis. However, they all share substantial efficacy in ameliorating the core symptoms of psychotic disorders. In animal studies, immunohistochemical detection of Fos-like proteins has been used as a reliable marker to identify cells responding to antipsychotic drugs or other stimuli (Deutch et al., 1992; Robertson and Fibiger, 1992; Nguyen et al., 1992; Robertson et al., 1994; Deutch et al., 1995; Wan et al., 1995; Cohen and Wan, 1998; Cohen et al., 2003). In some regions of brain, the effects of antipsychotic drugs are quite different. We and others have reported that the typical antipsychotic drug haloperidol induces greater Fos protein expression than clozapine does in the dorsal lateral striatum, while the atypical antipsychotic clozapine induces a higher degree of Fos expression compared to haloperidol in the prefrontal cortex (Deutch et al., 1992; Nguyen et al., 1992; Robertson and Fibiger, 1992; Robertson et al., 1994; Wan et al., 1995). Of great interest, a broad range of typical and atypical antipsychotic agents induce Fos protein expression in cells located in nucleus accumbens shell (AcbSh), central amygdala (CeA) and thalamic central medial nucleus (CM) (Deutch et al., 1992; Wan et al., 1995; Sebens et al., 1995; Cohen and Wan, 1998; Cohen et al., 1998 Suzuki et al., 1998; Morelli et al., 1999;

Abbreviations: AcbSh, shell of nucleus accumbens; CeA; central amygdaloid nucleus; CM, thalamic central medial nucleus; DAD2, dopaminergic D2; NEα1, noradrenergic alpha 1.

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Cohen and Yurgelum-Todd, 2001; Cohen et al., 2003). These findings suggest that cells in AcbSh, CeA and CM are targets of both typical and atypical antipsychotic drugs, and that these cells may be involved in mediating shared effects of these drugs, possibly including their therapeutic effects.

We have recently identified the types of cells responding to antipsychotic drugs in these sites by double immunofluorescence labeling with antibodies directed against Fos proteins and a series of specific cell markers. We found that haloperidol- and clozapine-activated cells in AcbSh, CeA and CM are (1) neurons, rather than glia; (2) inhibitory GABAergic neurons, but not acetylcholinergic neurons; and (3) dynorphin-containing, but not M-enkephalin-containing GABAergic neurons (Ma et al., 2003). These findings suggest that dynorphinergic/GABAergic neurons may participate in mediating the effects of antipsychotic drugs. In addition, in those experiments, we have observed that only some dynorphinergic/GABAergic neurons are activated by antipsychotic drugs. In the present study, we begin to ask what determines that these particular neurons respond to typical and atypical antipsychotic drugs. Since both classes of antipsychotic drugs have preferred binding affinity for several types of monoamine receptors (Meltzer et al., 1989; Arnt and Skarsfeidt, 1998; Brooks et al., 1999; Roth et al., 2004), the authors examined whether these types of receptors are expressed in antipsychotic drug-responsive neurons and might, thereby, provide a mechanistic basis for the actions of these drugs. Among the monoamine receptors to which antipsychotic drugs bind, dopaminergic D2 (DAD2), noradrenergic α1 (NEα1) and serotoninergic 5HT2a receptors have been well studied, and there is in vitro and in vivo evidence that both drugs have substantial affinity for each receptor (Table 1). Thus, while haloperidol has highest affinity for dopamine D2 receptors, both in vitro and ex vivo, it may be only three-fold less potent at NEα1 receptors and 10- to 20-fold less potent at 5HT2a receptors. Clozapine has high affinity at NEα1 and 5HT2a receptors and much lower affinity at dopamine D2 receptors. Table 1 presents representative findings in rats, but human postmortem studies, as well, show that haloperidol has roughly equal affinity for dopamine D2 and NEα1 receptors (Richelson and Nelson, 1984) or slightly higher affinity for NEα1 than dopamine D2 receptors (Richelson, 1984), while clozapine has about a 10-fold greater affinity for NEα1 versus dopamine D2 receptors. Haloperidol probably begins to occupy other receptors when it occupies more than 25% of dopamine receptors (Remington and Kapur, 1999) and, in clinical use, doses of haloperidol are higher, often considerably higher, than those needed to occupy dopamine receptors and produce the extrapyramidal side effects typical of dopamine blockade (Baldessarini et al., 1984, 1988; Vuckovic et al., 1990). Thus, it is possible that actions of monoamine receptors in addition to dopamine D2 receptors are relevant to the actions of haloperidol just as they are thought to be relevant to the actions of clozapine.

There are specific antibodies available for dopamine D2, NEα1 and 5HT2a receptors, and to address this question, we have examined the expression profiles of dopamine D2, 5HT2a and NEα1 receptors in haloperidol- and clozapine-activated neurons in AcbSh, CeA and CM in rat brain.

Table 1
Affinities of haloperidol and clozapine for dopaminergic D2 (DAD2), noradrenergic α1 (NEα1) and serotoninergic 5HT2a receptors in the rat

	DAD2	NEα1	5HT2α
In vitro affinities (K, nM)[a]			
Haloperidol	4.4	14	45
Clozapine	380	17	29
Ex vivo receptor occupancy (ED50, mg/kg)[b]			
Haloperidol	0.13	0.42	2.6
Clozapine	9.0	0.58	1.3

[a] Peroutka and Synder (1980).
[b] Schotte et al. (1993).

2. Methods

2.1. Animal

Sprague Dawley rats (Charles River, body weight 300–350 g) were maintained in groups of three to four per cage with food and water ad libitum in a reversed light room with a 12-h on/12-h off light schedule. Rats were frequently handled in the days before the study to reduce the effects of stress associated with drug treatment. Animals were treated in accordance with the provisions of the "Guide for the Care and Use of Laboratory Animals" of the US Department of Health and Human Services, and all procedures were approved by the McLean Hospital Institutional Animal Care and Use Committee.

2.2. Drug administration

Rats received an intraperitoneal (i.p.) injection of 1 mg/kg haloperidol, 20 mg/kg clozapine or vehicle (2% lactic acid). There were five rats in each treatment group. Drug-treated rats were studied concurrently with vehicle-treated control animals. The doses of haloperidol and clozapine chosen were consistent with those previously used in pharmacological studies in rat (Robertson and Fibiger, 1992; Robertson et al., 1994; Wan et al., 1995; Cohen et al., 2003; Ma et al., 2003).

2.3. Immunocytochemical techniques

Two hours after the i.p. injection, rats were deeply anaesthetized with sodium pentobarbital (60 mg/kg) and transcardially perfused with 200 ml of 0.01 M phosphate-buffered saline (PBS) followed by 200 ml of freshly prepared 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4. Brains were post-fixed in 4% paraformaldehyde overnight and subsequently cryoprotected with 20% sucrose in 0.01 M PBS for 24 h at 4 °C. Serial 40 μm coronal sections were then cut from each brain on a frozen microtome. Double immunofluorescence labeling was performed at room temperature unless otherwise indicated. The sections were washed three times for 5 min, then incubated with 0.3% Triton-X-100 (PBST)