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Effects of clozapine and 2,5-dimethoxy-4-methylamphetamine [DOM] on 5-HT_{2A} receptor expression in discrete brain areas

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Abstract

Activation of 5-HT_{2A} receptors has been shown to be an essential component of the discriminative stimulus effects of indoleamine and phenethylamine hallucinogens. The objective of the present study was to determine the neuroanatomical location of the 5HT_{2A} receptors which may be responsible for the stimulus effects of the phenethylamine hallucinogen [−]2,5-dimethoxy-4-methylamphetamine (DOM). It was hypothesized that brain areas containing altered 5-HT_{2A} receptor expression in the context of a similar alteration in DOM-induced stimulus control might be important in mediating the stimulus effects of DOM. Fisher 344 rats were treated with either clozapine (25 mg/kg/day) or DOM (2 mg/kg/day) for 7 days, and the consequences of these drug treatment regimens on DOM-induced stimulus control and on 5-HT_{2A} receptor expression in several brain areas were determined. Chronic administration of clozapine was associated with a widespread decrease in levels of 5-$HT_{2A/2C}$ receptors. Conversely, treatment with DOM had varied effects including a neuroanatomically selective decrease in 5-$HT_{2A/2C}$ receptor levels that was restricted to the olfactory nucleus. Both chronic treatment with DOM and clozapine decreased the stimulus effects of DOM. The present findings suggest a role for the olfactory nucleus in producing the stimulus effects of DOM.

Keywords: 2,5-dimethoxy-4-methylamphetamine (DOM); Clozapine; 5-HT_{2A} receptor; Receptor regulation; Quantitative autoradiography; Drug-induced stimulus control

1. Introduction

Administration of indoleamine (e.g., LSD, psilocybin) and phenethylamine (e.g., DOM, mescaline) hallucinogens exert profound behavioral effects such as distortion of perception, disrupted awareness of time, as well as alterations in mood, affective state and cognition. Many of these same effects are observed in individuals with schizophrenia. For example, schizophrenic patients exhibit positive symptoms such as delusions and hallucinations as well as negative symptoms such as impaired cognition and flattening of affect (Andreasen and Black, 2001; Vollenweider and Geyer, 2001). Furthermore, psilocybin, an indoleamine hallucinogen, produces a psychosis-like syndrome in humans that resembles the first manifestations of schizophrenia (Vollenweider et al., 1998).

In the case of both schizophrenia and the effects of indole and phenethylamine hallucinogens, the 5-HT_{2A} subfamily of serotonin receptors appears to play a critical role (Dean, 2003; Winter et al., 1999). Thus, several studies have reported a lower density of 5-HT_{2A} receptors in schizophrenic patients (Dean et al., 1999; see Dean, 2003). Further, atypical antipsychotics share the common feature of being high-affinity antagonists at the 5-HT_{2A} receptor (Meltzer et al., 2003; Roth et al., 2004), and allelic variations in the 5-HT_{2A} receptor gene influences the clinical response to these compounds (Ellingrod et al., 2002; Lane et al., 2002). Similarly, affinity at 5-HT_2 receptors for a series of phenethylamines correlates both with the potency of these compounds as hallucinogens

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(Titeler et al., 1988; Sadzot et al., 1989) as well as with their potency to substitute for DOM as a discriminative stimuli (Glennon et al., 1984). Using antagonist correlation analysis, Fiorella et al. (1995a,b) concluded that the stimulus effects of LSD involved activation of 5-HT_{2A} receptors rather than 5-HT_{2C} receptors. Similarly, M100907, a selective 5HT_{2A} receptor antagonist, but not the 5HT_{2C} receptor antagonist SB 200,646 blocked the discriminative stimulus effects of phenethylamine hallucinogens (Schreiber et al., 1994; Eckler et al., 2004).

Although 5-HT_{2A} receptors play a prominent role in mediating the stimulus effects of hallucinogenic drugs, the anatomical location of the relevant receptors is unclear. 5-HT_{2A} receptors are distributed throughout the brain, but are found in high density in various cortical regions (especially the frontal cortex), caudate nucleus, nucleus accumbens, olfactory nucleus, and claustrum (Pazos et al., 1985; Appel et al., 1990; López-Giménez et al., 1997). The objective of the present study was to identify the neuroanatomical location of the 5-HT_{2A} receptors that may be responsible for mediating the stimulus effects of DOM, a 5-HT_{2A} receptor agonist with hallucinogenic activity, in the rat. It is hypothesized that brain areas containing altered expression of 5-HT_{2A} receptors in the context of a similar alteration in DOM-induced stimulus control might be important in mediating the discriminative stimulus effects of DOM. Thus in the present study, behavioral results using drug-induced stimulus control after chronic treatment with clozapine or DOM were correlated with changes in 5-HT_{2A} receptor expression in discrete brain areas as measured by quantitative autoradiography. The choice of clozapine and DOM was based on reports that: (i) chronic administration of clozapine, an atypical antipsychotic that blocks several neurotransmitter receptors including 5-HT_{2A} receptors, but not haloperidol, a typical antipsychotic without significant affinity at the 5-HT_{2A} receptor, reduces DOM-induced stimulus control (Doat et al., 2002); (ii) repeated treatment with DOM decreases 5-HT_2 receptor-mediated head-twitch (Leysen et al., 1989); and (iii) chronic exposure to DOM or clozapine alters the density of 5-HT_2 receptors (Leysen et al., 1989; Burnet et al., 1996; Kuoppamäki et al., 1995). In addition, as a clinical relevant drug, any insight obtained on clozapine might be beneficial in elucidating its mechanism of action.

2. Methods

2.1. Subjects

Male Fisher 344 rats obtained from Harlan Sprague–Dawley Inc. (Indianapolis, IN) were used in all experiments. Animals were housed in pairs under a 12 h light dark cycle beginning at 6:00 a.m. and allowed free access to water. All training and testing took place during the light cycle. For rats used in behavioral studies caloric intake was controlled to maintain a mean body weight of 250 g. Subjects were fed following experimental sessions. Caloric control has been shown to lengthen the life span and decrease the incidence of a variety of pathologies in Fischer 344 rats (Keenan et al., 1994). Animals used in these studies were maintained in accordance with the 'Guide for Care and Use of Laboratory Animals' of the Institute of Laboratory Animals Resources, National Research Council and the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) were followed. All procedures using animals were approved by the Institutional Animal Care and Use Committee at the University of Buffalo.

2.2. Drugs

The negative isomer of DOM, [−]-DOM, was employed in all experiments and is referred to as DOM throughout the manuscript. DOM was provided by the National Institute on Drug Abuse. Clozapine was purchased from RBI (Natick, MA). DOM was dissolved in water and injected in a volume of 1.0 ml/kg body weight. Clozapine was dissolved in a minimal volume of 8.5% lactic acid, diluted to the appropriate concentration with 0.9% NaCl, and injected in a volume of 2 ml/kg. All injections were intraperitoneal.

2.3. Drug treatments

In the behavioral studies, after stimulus control was established, animals were treated with either vehicle (saline), clozapine (25 mg/kg/day), or DOM (2 mg/kg/day) for seven days. Animals were tested with the training dose 30 h after the last injection in order to minimize the effects of any residual drug on the stimulus properties of DOM.

During treatments, DOM or saline discrimination training was suspended to prevent additional learning. Previously we demonstrated that there is no significant difference in DOM-appropriate responding to the training dose of DOM (0.3 mg/kg) before and after a 21-day suspension of training (Doat et al., 2002). Furthermore, in all experiments the percent DOM-appropriate responding after drug treatment was compared to the percent DOM-appropriate responding after treatment with vehicle. In the autoradiography studies, untrained animals were treated with vehicle, 2 mg/kg/day DOM, or 25 mg/kg/day clozapine for seven days and sacrificed 30 h after the last injection.

3. Drug-induced stimulus control

3.1. Apparatus

Animal test chambers (Coulbourn Instruments Model E10-10) housed in larger lightproof, sound-insulated boxes were used for all experiments. Each box had a house light and exhaust fan. Chambers contained two levers mounted