Differential Effects of $M_1$ and 5-Hydroxytryptamine$_{1A}$ Receptors on Atypical Antipsychotic Drug-Induced Dopamine Efflux in the Medial Prefrontal Cortex

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ABSTRACT

Systemic administration of the $M_1$ receptor agonists $N$-desmethylclozapine (NDMC) and 4-[[3-[4-(butyl)piperidin-1-yl]-proplyl]-7-fluoro-4H-benzo[1,4]oxazin-3-one (AC260584) increase dopamine (DA) efflux in rat medial prefrontal cortex (mPFC). This increase is blocked by systemic administration of both telenzepine, a preferential $M_1$ receptor antagonist, and $N$-[2-[4-(2-methoxyphenyl)-1-piperazinyl][ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide (WAY-100635), a 5-hydroxytryptamine$_{1A}$ receptor antagonist. The present study sought to determine whether DA efflux in the mPFC induced by the atypical antipsychotic drugs clozapine, risperidone, and olanzapine is also mediated by $M_1$ receptor stimulation and, specifically, to determine whether these effects are mediated $M_1$ receptors in the mPFC through use of in vivo microdialysis in awake, freely moving Sprague-Dawley rats. Telenzepine (3 mg/kg) significantly attenuated clozapine- (20 mg/kg), olanzapine- (10 mg/kg), and risperidone- (1.0 mg/kg) induced increases in mPFC DA efflux. Local mPFC perfusion of NDMC, AC260584, clozapine, risperidone, or olanzapine (10–500 M), significantly increased DA efflux in the mPFC. Local mPFC perfusion of telenzepine (0.1 M) prevented increases in mPFC DA efflux induced by systemic administration of AC260584 (10 mg/kg), NDMC (20 mg/kg), and clozapine (10 mg/kg), but not by risperidone (1.0 mg/kg) or olanzapine (10 mg/kg). However, local mPFC perfusion of WAY-100635 (0.1 M) prevented mPFC DA efflux induced by clozapine, risperidone, and olanzapine, but not by AC260584 or NDMC. These results suggest that the AC260584-, NDMC-, and clozapine-induced DA efflux in the mPFC is mediated directly by mPFC $M_1$ receptors.

Atypical antipsychotic drugs (APDs), such as clozapine, risperidone, and olanzapine, when administered systemically, have been shown to enhance dopamine (DA) and acetylcholine (ACh) efflux in the medial prefrontal cortex (mPFC) with enhancement in the cortex at least equal to, but usually greater than, the enhancement produced in the nucleus accumbens (NAC) and striatum (Imperato and Angelucci, 1989; Moghaddam and Bunney, 1990; Shirazi-Southall et al., 2002). Meltzer and McGurk (1999) hypothesized that these effects contribute to improvements in cognitive function in schizophrenic patients treated with atypical APDs.

Although atypical APDs are more potent 5-HT$_{2A}$ receptor antagonists than $D_2$ receptor antagonists (Meltzer et al., 1989), stimulation of 5-HT$_{1A}$ receptors also may contribute to atypical APD-induced DA efflux in the PFC. For example, pretreatment with the 5-HT$_{1A}$ receptor antagonist WAY-100635 has been shown to attenuate PFC DA efflux induced by atypical APDs (Kuroki et al., 1999; Rollema et al., 2000). Furthermore, in a study by Diaz-Mataix et al. (2005), systemic administration of the 5-HT$_{1A}$ receptor agonist BAY-3702 increased DA efflux in the mPFC and ventral tegmental area (VTA). WAY-100635 reversed the effects of BAY-3702 in both regions. Moreover, this increase in DA efflux in the mPFC, but not the VTA, was blocked by frontocortical transection, suggesting that stimulation of 5-HT$_{1A}$ receptors in the mPFC is mediated by DA efflux in mPFC and VTA. Moreover, this study reported that the atypical APDs clozapine, olanzapine, and ziprasidone failed to elicit increased DA efflux in the mPFC after their systemic or local mPFC administration in 5-HT$_{1A}$ receptor knockout mice. Thus, stimulation of cortical 5-HT$_{1A}$ receptors by atypical APDs, through direct or indirect actions, may be a critical mechanism through which atypical APDs enhance cortical DA efflux. The evidence for the important role of 5-HT$_{1A}$

ABBREVIATIONS: APD, antipsychotic drugs; ACh, acetylcholine; DA, dopamine; 5-HT, 5-hydroxytryptamine, serotonin; mPFC, medial prefrontal cortex; NAC, nucleus accumbens; NDMC, N-desmethylclozapine; VTA, ventral tegmental area; WAY-100635, $N$-[2-[4-(2-methoxyphenyl)-1-piperazinyl][ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide; BAY $\times$ 3702, repinotan HCl.
receptors in schizophrenia and the action of antipsychotic drugs has been reviewed by Bantick et al. (2001).

Based on preliminary findings from our laboratory, stimulation of muscarinic M1 receptors may also be important for the ability of atypical APDs to increase both DA and ACh efflux in the mPFC. The putative atypical APD, N-desmethylclozapine (NDMC), an active metabolite of clozapine (Sur et al., 2003; Weiner et al., 2004), and the muscarinic receptor agonist AC260584 (Spalding et al., 2006), significantly increased DA efflux in the mPFC in our laboratory (Li et al., 2005, 2007). These effects were blocked by telenzepine, a preferential M1 receptor antagonist (Tanda et al., 2007) and WAY-100635 (Li et al., 2005, 2007). The increase in mPFC ACh efflux induced by NDMC or AC260584 was blocked by telenzepine but not by WAY-100635 (Li et al., 2005, 2007). These data suggest that M1 receptor stimulation contributes to the efflux of both DA and ACh by both NDMC and AC260584. Therefore, M1 receptors may represent a promising new target to treat cognitive dysfunction in schizophrenia and other neuropsychiatric disorders, including Alzheimer’s disease (Bymaster et al., 2002; Raedler et al., 2007).

It is well established that muscarinic receptors are important for the regulation of DAergic transmission. In situ hybridization studies have demonstrated the coexistence of DA and muscarinic receptors postsynaptic to DA terminals (Weiner et al., 1990). Cholinergic neurons of moderate to high density project from the mesopontine cholinergic nuclei (Ch5, Ch6) to DA cell bodies in the VTA (A10) and substantia nigra (A9) (Woolf and Butcher 1989; Bolam et al., 1991).

Electrophysiological studies have shown that muscarinic agonists injected near DA cell bodies stimulate the firing of A9 and A10 DA neurons (Gronier and Rasmussen, 1998). Moreover, the muscarinic receptor involved in this neuronal activation belongs to the M1 receptor subfamily (Gronier and Rasmussen, 1998). Thus, infusion of ACh, carbachol, and muscarine into the VTA increases DA cell firing which can be blocked by scopolamine (Lacey et al., 1990; Westerink et al., 1996). Furthermore, activation of muscarinic receptors in the mPFC or VTA has been shown to enhance DA efflux in the mPFC, VTA, and NAC (Gronier et al., 2000).

The atypical APDs clozapine and olanzapine have potent affinities to all muscarinic receptors, but risperidone does not (Schotte et al., 1996; Bymaster et al., 1999). Thus, it was of interest to determine whether M1 receptors are directly or indirectly involved in cortical DA efflux induced by these three APDs. The present study sought to determine whether mPFC DA efflux induced by clozapine, risperidone, and olanzapine is mediated by stimulation of M1 receptors, by assessing the effects of these drugs on mPFC DA efflux after systemic administration of telenzepine. In addition, we evaluated the effects of the atypical APDs clozapine, olanzapine, and risperidone and the muscarinic receptor agonists NDMC and AC260584 on mPFC DA efflux during local administration of these drugs into the mPFC by reverse microdialysis. Finally, the effect of local administration of telenzepine and WAY-100635 into the mPFC on mPFC DA efflux produced by systemic administration of clozapine, olanzapine, risperidone, NDMC, and AC260584 was also determined.

Materials and Methods

Animals. Male Sprague-Dawley albino rats (Zivic-Miller Laboratories, Zelienople, PA) weighing 250 to 350 g were housed two per cage and maintained in a controlled 12:12-h light/dark cycle and under constant temperature at 22°C, with free access to food and water. Animals used in this study were cared for in accordance with the guidelines of the Institutional Animal Care and Use Committee of Vanderbilt University. Principles of laboratory animal care (National Institutes of Health Publication 85-23, revised 1985) were followed.

Surgery. Rats were anesthetized with the modified Equithesin mixture (810 mg of pentobarbital, 4.3 g of choral hydrate, 2.12 mg of MgSO4, 14 ml of ethanol, and 29 ml of propylene glycol were dissolved in saline and the final volume was 100 ml), and mounted in a stereotaxic frame (Stoelting, Wood Dale, IL). Stainless guide cannulae (21-gauge) with a dummy probe were placed and fixed by cranio-plastic cement (Plastic One, Roanoke, VA) onto the skull. Rats received probe implantation for the mPFC (coordinates: A +3.2, L +0.8, (10°C inclination), V −5.5 mm, relative to bregma). The incision bar level was 3.0 mm, according to Paxinos and Watson (1998).

Microdialysis Procedures. As reported previously (Li et al., 2003), rats were implanted, under slight anesthesia with isoflurane, with the probes 2 h before sample collection. The probes were perfused at a flow rate of 1.5 μl/min during the experiment. Then, the dialysate samples were collected every 15 min. Four baseline samples were collected every 15 min before local (reverse dialysis) or systemic drug administration and then successive dialysate samples were collected every 15 min. AC260584, NDMC, clozapine, risperidone, olanzapine, or haloperidol were perfused through the probe into the mPFC at concentrations of 10 to 500 μM. AC260584 (10 mg/kg s.c.), NDMC (20 mg/kg s.c.), clozapine (5 mg/kg s.c.), risperidone (1 mg/kg s.c.), olanzapine (10 mg/kg s.c.), or haloperidol (0.1 mg/kg s.c.) were also administered. In the systemic administration experiments with these compounds, WAY-100635 (0.1 μM; Sigma-Aldrich, St. Louis, MO), telenzepine (0.1 μM), or vehicle (i.e., Dulbecco’s phosphate-buffered saline solution, with Ca2+ added) was to be injected through the probe into the mPFC. Concentrictime 3-mm microdialysis probes were constructed in our laboratory as described in detail previously (Li et al., 2003).

DA Assay. We report here a new method for reverse microdialysis designed to prevent interference of DA detection and to increase the life of the analytical column. This modified system consists of an automated two-channel switch (channels A and B) installed in the outlet line that directs the outlet flow through one of two guard columns. Channel A routes the outlet flow through a guard column and then through an injection loop in an automatic injector. Channel B is not attached to the outlet flow, but rather, it is perfused continuously with a 20% acetylnitrite solution. Drugs are dissolved in Dulbecco’s phosphate-buffered solution (Sigma-Aldrich), with Ca2+ added, and perfused into the microdialysis probe, where it continues along the outlet line and passes through the guard column. In the guard column, drug passes through the column slower than DA does, which takes 15 min, and after DA passes through the guard column, the guard column is switched with the guard column on channel B. The drug remaining in the guard column is then washed out with the 20% acetylnitrite solution, and then after another 15 min, is exchanged with the guard column from column A. Thus, drugs are washed out of the system before entering the injection loop of the automatic injector.

As reported previously (Li et al., 2003), the concentration of DA in dialysate samples was determined by HPLC with electrochemical detection, and analyzed with a Millennium chromatogram manager (Waters, Milford, MA). The mobile phase consisted of 48 mM anhydrous citric acid and 24 mM sodium acetate trihydrate containing 0.5 mM EDTA-Na2, 2 mM dodecyl sulfate sodium salt, and 20% (v/v) acetonitrile, adjusted to pH 5.4 with NaOH, and was pumped (0.3
All results are given as mean ± S.E.M.

**Drugs.** NDNC, AC260584 (ACADIA Pharmaceutical Inc., San Diego, CA); clozapine (Novartis, Basel, Switzerland), olanzapine (Eli Lilly & Co., Indianapolis, IN), and risperidone (Janssen Pharmaceuticals, New Brunswick, NJ) were dissolved in 0.1 M H3PO4 and the pH was adjusted to approximately 7 with 0.1 N NaOH. WAY-100635 and telenzepine (Toronto Research Chemicals Inc., North York, ON, Canada) were dissolved in deionized water.

**Data Analysis.** The mean value of three consecutive stable samples before drug injection was set at 100% and considered the predrug basal level. Basal extracellular levels of DA were compared by one-way analysis of variance (ANOVA). The time-dependent effect of drugs on DA levels were analyzed by use of a two-way ANOVA with treatment group as fixed factor and time as the within-subject factor. ANOVA was followed by the least-squares significant difference post hoc pairwise comparison procedure (SPSS 15 and AMOS 7 Win; SPSS Inc., Chicago, IL). The level of significance was set at $p < 0.05$.

All results are given as mean ± S.E.M.

**Results**

**Basal DA Levels in the Systemic and Local Administration Studies.** In the systemic administration experiments, mean basal cortical extracellular DA levels in the dialysates obtained from all rats used in these studies were 1.93 ± 0.11 (fmol/20 μl, mean ± S.E.M.; $n = 51$). There were no significant differences in basal extracellular DA levels among any of the groups in the systemic administration studies. In the local application experiments, the baseline samples were collected from the mPFC after a 2-h perfusion, and basal extracellular levels of DA remained stable before the drug perfusion. Basal extracellular DA levels in the dialysates obtained from all rats used in the local administration studies were 3.42 ± 0.11 (fmol/20 μl, mean ± S.E.M.; $n = 31$). There was no significant difference in basal extracellular DA levels between any of the groups in the local administration studies.

**Effect of Subcutaneous Administration of Telenzepine on DA Release in the mPFC Produced by Subcutaneous Administration of Clozapine, Risperidone, and Olanzapine.** Telenzepine, 3 mg/kg s.c., alone, did not alter the DA concentration in the mPFC in rats (Fig. 1). Pretreatment with telenzepine blocked the DA release produced by clozapine (Fig. 1A), risperidone (Fig. 1B), and olanzapine (Fig. 1C). Two-way ANOVA revealed significant effects of drug effect, time effect, and drug × time interaction for 1) clozapine treatment group: drug effect, $F_{9,25} = 20.246, p < 0.001$; time effect, $F_{9,25} = 22.004, p < 0.00001$, and time × drug interaction, $F_{27,44} = 21.306, p < 0.00001$; 2) risperidone treatment group: drug effect, $F_{3,25} = 16.479, p < 0.001$; time effect, $F_{9,25} = 17.010, p < 0.00001$, and time × group interaction, $F_{27,44} = 28.17, p < 0.00001$; and 3) olanzapine treatment group, drug effect $F_{3,24} = 15.163, p < 0.001$; time effect, $F_{9,24} = 19.921, p < 0.00001$, and time × group interaction, $F_{27,43} = 22.119, p < 0.00001$. Post hoc test indicated significant increases in DA efflux induced by clozapine ($p < 0.001$), risperidone ($p = 0.0045$), and olanzapine ($p = 0.0067$) compared with those of vehicle control groups. The effect of telenzepine to block cortical DA efflux induced by clozapine was incomplete whereas it completely blocked the DA efflux induced by risperidone and olanzapine (Fig. 1).

**Effect of Local Administration of AC260584, NDNC, Clozapine, Risperidone, and Olanzapine into the mPFC on DA Efflux in This Region.** At concentrations of 100 μM and 500 μM, local perfusion of AC260584 into the mPFC significantly increased DA efflux in the mPFC, compared with the baseline levels (repeated measures ANOVA followed by one-way ANOVA between two groups: $F_{1,6} = 9.236, p = 0.015$ for 100 μM and $F_{1,6} = 21.06, p < 0.0001$ for 500 μM). The effect was seen in all groups except for the vehicle group. The effect of pretreatment with telenzepine to block cortical DA efflux induced by clozapine was incomplete whereas it completely blocked the DA efflux induced by risperidone and olanzapine (Fig. 1).

![Dopamine (% of Baseline) vs Time (min)](image-url)
500 µM; Fig. 2A) and NDMC ($F_{1,10} = 13.374, p = 0.01$ for 100 µM and $F_{1,10} = 25.11, p < 0.0001$ for 500 µM; Fig. 2A). The effect of NDMC seemed to be greater than that of AC260584 at both concentrations (the comparison of net-AUC indicates a significant difference: $p < 0.01$; Fig. 2B). Perfusion of a low dose of either compound at a concentration of 10 µM, however, did not increase cortical DA efflux (data not shown). Unlike AC260582 and NDMC, clozapine ($F_{1,6} = 18.89, p = 0.0089$ for 10 µM and $F_{1,6} = 27.06, p < 0.0001$ for 100 µM; Fig. 2B), risperidone ($F_{1,6} = 17.89, p = 0.0092$ for 10 µM and $F_{1,6} = 25.22, p < 0.0001$ for 100 µM; Fig. 2), and olanzapine ($F_{1,8} = 10.222, p = 0.0756$ for 10 µM and $F_{1,8} = 11.568, p < 0.0001$ for 100 µM; Fig. 2C) all caused significant increases in cortical DA efflux after perfusion at high (100 µM) and low concentrations (10 µM) (Fig. 2D).

Effect of Local Administration of Telenzepine into mPFC on DA Efflux in This Region Induced by Subcutaneous Administration of AC260584, NDMC, Clozapine, Risperidone, and Olanzapine. Consistent with previous reports (Kuroki et al., 1999; Li et al., 2005, 2007), systemic administration of AC260584 (10 mg/kg s.c.), NDMC (20 mg/kg s.c.), clozapine (10 mg/kg s.c.), risperidone (1 mg/kg s.c.), and olanzapine (10 mg/kg s.c.) significantly increased DA efflux in the mPFC (Fig. 3). In previous reports, the samples were collected every 30 min for a 210-min period. In the present study, the samples were collected every 15 min for 60 min. After perfusion of telenzepine (0.1 µM) for 60 min in the mPFC, the increases in cortical DA efflux induced by administration of AC260584 (10 mg/kg s.c.) (a two-way ANOVA revealed a significant between groups effect; $F_{2,17} = 18.96, p < 0.001$; Fig. 3A), NDMC (20 mg/kg s.c.) ($F_{2,16} = 9.996, p = 0.03$; Fig. 3B), and clozapine (10 mg/kg s.c.) ($F_{2,17} = 13.144, p < 0.001$; Fig. 3C) were blocked completely. However, the increase in DA efflux induced by risperidone (1 mg/kg s.c.) ($F_{2,16} = 1.589, p = 0.194$; Fig. 3D) or olanzapine (10 mg/kg s.c.) ($F_{2,16} = 1.987, p = 0.098$; Fig. 3E) was not affected by telenzepine perfusion.

Effect of Local Perfusion of WAY-100635 into mPFC on DA Efflux in This Region Induced by Subcutaneous Administration of AC260584, NDMC, Clozapine, Risperidone, and Olanzapine. Figure 3 showed that perfusion of WAY-100635 (0.1 µM into the mPFC did not block mPFC DA efflux induced by AC260584 (10 mg/kg s.c.) ($F_{2,17} = 2.148, p = 0.785$; Fig. 3F) or NDMC (20 mg/kg s.c.) ($F_{2,16} = 13.144, p < 0.001$; Fig. 3C) were blocked completely. However, the increase in DA efflux induced by risperidone (1 mg/kg s.c.) ($F_{2,16} = 1.589, p = 0.194$; Fig. 3D) or olanzapine (10 mg/kg s.c.) ($F_{2,16} = 1.987, p = 0.098$; Fig. 3E) was not affected by telenzepine perfusion.

![Fig. 2. Local administration of muscarinic M₁ agonist NDMC (100 and 500 µM; n = 6) and AC260584 (100 and 500 µM; n = 4) (A) and atypical antipsychotic drugs clozapine (n = 4), risperidone (n = 4), and olanzapine (n = 5) (10 and 100 µM) (B) into the medial prefrontal cortex increased cortical dopamine release. In the local application experiments, the baseline samples were collected from the mPFC after a 2-h perfusion, and basal extracellular levels of DA remained stable before the drug perfusion. The net AUC data indicate that the extent of DA efflux produced by the high concentration of risperidone (100 µM) was not significantly different from that produced by the low concentration of risperidone (10 µM), whereas the other drugs produced greater DA increases at the 100 µM concentration than at the 10 µM concentration (C and D). ***, $p < 0.001$; **, $p < 0.01$ compared with the vehicle group. AUC, area under the curve; VEH, vehicle; CLOZ, clozapine; OLAN, olanzapine; RIS, risperidone.](image-url)
1.589, p = 0.144; Fig. 3G), but completely blocked mPFC DA efflux induced by clozapine (10 mg/kg s.c.) (F(3,22) = 17.111, p < 0.0001; Fig. 3H), risperidone (1 mg/kg s.c.) (F(3,22) = 14.26, p < 0.001; Fig. 3I) or olanzapine (10 mg/kg s.c.) (F(3,22) = 10.650, p = 0.007; Fig. 3J). The inhibitory effect of WAY-100635 on risperidone-induced DA efflux only lasted for 30 min after its perfusion, whereas it blocked the efflux of DA for the entire 60-min observation period in the case of clozapine or olanzapine.

The results were also analyzed with the three conditions together for each drug (vehicle group, drug treatment alone group, telenzepine plus drug group, and WAY-100635 plus drug group). 1) The effect of telenzepine and WAY-100635 on DA release produced by AC260584 (Fig. 3, A and F). Two-way ANOVA revealed significant effects of drug (F(3,22) = 17.111, p < 0.0001), time (F(9,99) = 20.008, p < 0.0001), and time × drug interaction (F(27,58) = 26.040, p < 0.0001). Post hoc analysis revealed a significant difference between groups of AC260584 and telenzepine plus AC260584 (p = 0.0011) and nonsignificant difference between groups of AC260584 and WAY-100635 plus AC260584 (p = 0.145). 2) The effect of telenzepine and WAY-100635 on DA release produced by NDMC (Fig. 3, B and G). Two-way ANOVA revealed a significant effect of drug (F(3,22) = 15.50, p < 0.001), time (F(9,99) = 10.389, p < 0.001), and time × drug interaction (F(27,58) = 17.79, p < 0.001). Post hoc analysis revealed a significant difference between groups of NDMC and telenzepine plus NDMC (p = 0.0187), and nonsignificant difference between groups of NDMC and WAY-100635 plus NDMC (p = 0.178). 3) The effect of telenzepine and WAY-100635 on DA release produced by clozapine (Fig. 3, C and H). Two-way ANOVA revealed a significant effect of drug (F(3,22) = 28.19, p < 0.0001), time (F(9,99) = 13.33, p < 0.0001), and time × drug interaction (F(27,58) = 15.004, p < 0.0001). Post hoc analysis revealed significant differences between groups of clozapine and telenzepine plus clozapine (p < 0.0001), and between groups of clozapine and WAY-100635 plus clozapine (p < 0.0001). 4) The effect of telenzepine and WAY-100635 on DA release produced by risperidone (Fig. 3, D and I). Two-way ANOVA revealed a significant effect of drug (F(3,22) = 8.881, p = 0.0208), time (F(9,99) = 9.74, p = 0.0094), and time × drug interaction (F(27,57) = 10.22, p = 0.027). Post hoc analysis revealed a nonsignificant difference between the groups of risperidone and telenzepine plus risperidone (p = 0.199), and a significant difference between the groups of risperidone and WAY-100635 plus risperidone (p = 0.0095). 5) The effect of telenzepine and WAY-100635 on DA release produced by olanzapine (Fig. 3, E and J). Two-way ANOVA revealed a significant effect of drug (F(3,22) = 22.04, p < 0.001), time (F(9,21) = 19.2, p < 0.001), and time × drug interaction (F(27,57) = 12.229, p < 0.001). Post hoc analysis revealed a nonsignificant difference between the groups of olanzapine and telenzepine plus olanzapine (p = 0.111), and a significant difference between the groups of olanzapine and WAY-100635 plus olanzapine (p < 0.0001).

**Discussion**

The main findings of the present study are: 1) systemic administration of the preferential M1 receptor antagonist telenzepine blocked cortical DA efflux induced by systemic administration of the atypical antipsychotic drugs clozapine, olanzapine, and risperidone; 2) local administration of AC260584, NDMC, clozapine, risperidone, and olanzapine into the mPFC increased mPFC DA efflux; 3) local administration of telenzepine into the mPFC blocked DA efflux in the mPFC induced by systemic administration of AC260584, NDMC, and clozapine, but not risperidone or olanzapine; and 4) local administration of WAY-100635 into the mPFC blocked DA efflux in the mPFC by systemic administration of clozapine, risperidone, and olanzapine, but not AC260584 or NDMC. These results suggest that 1) AC260584 and NDMC increase cortical DA efflux through the direct stimulation of M1, but not 5-HT1A receptors in the mPFC; 2) both cortical M1 and 5-HT1A receptors contribute to clozapine-induced DA release; and 3) the 5-HT1A receptors, but not M1 receptors in the mPFC are directly involved in cortical DA efflux induced by risperidone and olanzapine.

In previous studies, we have shown that the muscarinic M1 receptor agonists NDMC (Sur et al., 2003; Weiner et al., 2004; Li et al., 2005) and AC260584 (Spalding et al., 2006; Li et al., 2007) given systemically increased DA efflux in rat mPFC and hippocampus and that the effect of these drugs on DA efflux was inhibited by pretreatment with telenzepine (Li et al., 2005, 2007), a preferential muscarinic M1 receptor antagonist (Eltze
Fig. 3. Local administration of preferential muscarinic M₁ antagonist telenzepine (0.1 μM) blocked medial prefrontal cortex dopamine release produced by systemic administration of M₁ receptor agonists AC260584 (20 mg/kg s.c.; n = 6) and NDMC (10 mg/kg s.c.; n = 5) and atypical antipsychotic drugs clozapine (10 mg/kg s.c.; n = 6), but not risperidone (1.0 mg/kg s.c.; n = 5) or olanzapine (10 mg/kg s.c.; n = 5) (A–E). Local administration of 5-HT₁₆ receptor antagonist WAY-100635 (0.1 μM) blocked medial prefrontal cortex dopamine release produced by systemic administration of atypical antipsychotic drugs clozapine (10 mg/kg s.c.; n = 6), risperidone (1.0 mg/kg s.c.; n = 6), and olanzapine (10 mg/kg s.c.; n = 5), but not M₁ receptor agonists AC260584 (20 mg/kg s.c.; n = 6) or NDMC (10 mg/kg s.c.; n = 7) (F–J).
et al., 1985; Doods et al., 1987; Tanda et al., 2007), suggesting that these actions are mediated by telenzepine-targeted receptors, such as M₁ subtype receptors, which are present predominantly in rat cortex. In the present study, we examined the role of M₁ receptors in the action of atypical APDs after either systemic administration or mPFC local administration. The results showed that both subcutaneous and local administration of AC260584 or NDMC into the mPFC induced significant increases in mPFC DA efflux. Either systemic administration or local mPFC perfusion of telenzepine was able to block the increased DA efflux by these M₁ receptor agonists administered either systemically or locally. These results are consistent with our hypothesis that telenzepine-prefering M₁ receptors in rat cortex mediate the effect of the systemic administration of atypical antipsychotic drugs to stimulate cortical DA efflux (Li et al., 2005, 2007).

Risperidone, which does not have an appreciable affinity for M₁ receptors (Schotte et al., 1996), and olanzapine, which has a high affinity for M₂ receptors (Bymaster et al., 1999), have been reported to enhance cortical DA efflux after systemic administration (Kuroki et al., 1999; Ichikawa et al., 2001). In the present study, local administration of telenzepine into the mPFC was unable to alter mPFC DA efflux induced by either olanzapine and risperidone, thus indicating that M₁ receptors in mPFC are involved in the DA release induced by them. However, the complete inhibition of DA efflux induced by the two drugs after WAY-100635 perfusion in the mPFC suggest that 5-HT₁₅ receptors in mPFC are required for olanzapine and risperidone to increase mPFC DA efflux. Consistent with this hypothesis, it has been shown that the mPFC contains an abundance of 5-HT₁₅ receptor-expressing cells (Pompeiano et al., 1992; Amargó-Bosch et al., 2004), and 5-HT₁₅ receptors may be located in the axon hillock of cortical pyramidal neurons (Czyszak et al., 2003; Cruz et al., 2004). Based on the present findings, we conclude that the 5-HT₁₅ receptors important for clozapine-, risperidone-, and olanzapine-induced DA efflux are located in the mPFC, which is consistent with previous suggestions from this laboratory and others (Relloma et al., 1997; Ichikawa et al., 2001). However, Rollema et al. (1997) found that WAY-100635 blocked ziprasidone- and clozapine-, but not olanzapine-induced DA efflux in the cortex, although there was a trend toward a statistically significant attenuation of DA efflux. In addition, Assié et al. (2005) found that WAY-100635 failed to block DA efflux induced by clozapine, risperidone, ziprasidone, and olanzapine. The discrepancy is probably due to the dose of WAY-100635 and the route of drug administration. In the two studies above, very low doses of WAY-100635, 0.1 and 0.16 mg/kg, respectively, were administered. The 5-HT₁₅ receptors important for the DA efflux induced by systemic administration of AC260584 and NDMC seem to be those located in the VTA (Li et al., unpublished data) or dorsal raphe nucleus. Our observation that systemic telenzepine inhibited increased DA release in mPFC by systemic administration of olanzapine or risperidone could indicate an indirect pathway involving noncortical M₁ receptors for the two drugs. Muscarinic receptors also directly affect midbrain DA neurons, which may influence mPFC DA efflux. Cholinergic neurons of moderate to high density project from the mesopontine cholinergic nuclei (Ch₅, Ch₆) to the DA cell bodies in the VTA (A10) and substantia nigra (A9) (Woolf and Butcher, 1989; Bolam et al., 1991), and in situ hybridization studies have demonstrated the coexistence of postsynaptic muscarinic receptors on VTA DA neurons (Weiner et al., 1990). Electrophysiological studies also have shown that muscarinic agonists injected near DA cell bodies in the A9 and A10 regions increase the firing rate of DA neurons (Gronier and Rasmussen, 1998). Thus, muscarinic receptor antagonists acting in the VTA may, in turn, limit atypical APD-induced increases in DA efflux from mesocortical DA neurons.

Clozapine has high affinity for many G-protein-coupled receptors, including 5-HT₁₅ and M₄ receptors (Bolden et al., 1992; Schotte et al., 1996). It has been shown to be an M₁ antagonist (Bolden et al., 1992; Zorn et al., 1994; Weiner et al., 2004), whereas its major metabolite, NDMC, is an M₁ muscarinic receptor partial agonist, in addition to having other appreciable receptor affinities (Sur et al., 2003; Weiner et al., 2004). Clozapine can also produce a selective increase in DA efflux in rat prefrontal cortex that is, in large part, mediated via activation of 5-HT₁₅ receptors (Rollema et al., 1997; Kuroki et al., 1999). The results reported here showed that systemically or locally administered clozapine increased mPFC DA efflux, which was inhibited by either systemically or locally administered telenzepine and WAY-100635. These observations indicate that clozapine enhances DA efflux through stimulation of both mPFC 5-HT₁₅ and muscarinic, probably M₁, receptors. Local administration of WAY-100635, however, failed to attenuate NDMC-induced increases in mPFC DA efflux, even though NDMC has been reported to be a 5-HT₁₅ partial agonist, as is clozapine (Kuoppamäki et al., 1993; Weiner et al., 2004). The role of 5-HT₁₅ receptors in mPFC DA efflux induced by NDMC or AC260584 is apparent when either drug is given systemically because WAY-100635 is able to attenuate mPFC DA efflux following this route of administration (Li et al., 2005, 2007). The basis for the effect of NDMC and AC260584 to increase 5-HT₁₅-mediated mPFC DA efflux requires further study, but, based on these present findings, mPFC 5-HT₁₅ receptors seem to mediate clozapine-, but not NDMC-induced mPFC DA efflux. Together, the present findings, along with receptor binding data, suggest that the effects of clozapine on mPFC neurotransmission are the results of the combined action of both clozapine and its metabolite NDMC. A large component of the effect produced by these two compounds may result from M₁ receptor stimulation by NDMC. Indeed, pretreatment with risperidone significantly potentiated mPFC DA efflux induced by AC260584 (Li et al., 2007). Thus, the inability of 5-HT₁₅ receptor blockade to attenuate NDMC- and AC260584-induced mPFC DA efflux in the present study demonstrates an important difference between these compounds, and clozapine, olanzapine, and risperidone.

In summary, the present findings further support the importance of muscarinic receptors and 5-HT₁₅ receptors for atypical APD effects. The present results suggest that M₁ receptors in the mPFC contribute to the AC260584-, NDMC-, and clozapine-induced DA efflux in the mPFC, whereas M₁ receptors in the mPFC do not seem to be involved in mPFC DA efflux induced by risperidone and olanzapine, despite attenuation of these effects by a systemically administered M₁ receptor antagonist. These findings suggest that M₁ receptors in other regions may also be directly or indirectly involved in mPFC DA efflux produced by risperidone or olanzapine. Moreover, a refinement of the ligands used for this research may further explain these different findings, in particular, because neither NDMC nor telenzepine are highly selective for M₁ receptors. Nor, for that matter, is the 5-HT₁₅ receptor antagonist WAY-100635, which is a common ligand.
used for blocking these receptors. For example, WAY-100635 recently has been shown to be a high-affinity D₄ receptor agonist (Chemel et al., 2006). Currently, however, these findings illuminate a potentially important role for M₁ and 5-HT₁A receptors in mediating atypical APD effects, and could become important pharmacological targets for new atypical APDs.

References


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Differential Effects of M₁ and 5-HT₁A Receptors
