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Effects of Typical and Atypical Antipsychotics and Receptor Selective Compounds on Acetylcholine Efflux in the Hippocampus of the Rat

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Some atypical antipsychotic drugs appear to improve cognitive function in schizophrenia and since acetylcholine (ACh) is of importance in cognition, we used in vivo microdialysis to examine the effects of antipsychotics administered acutely (SC or IP) at pharmacologically comparable doses on ACh outflow in the hippocampus of the rat. The atypical antipsychotics olanzapine and clozapine produced robust increases in ACh up to 1500% and 500%, respectively. The neuroleptics haloperidol, thioridazine, and chlorpromazine, as well as the atypical antipsychotics risperidone and ziprasidone produced modest increases in ACh by about 50–100%. Since most atypical antipsychotics affect a variety of monoaminergic receptors, we examined whether selective ligands for some of these receptors affect hippocampal ACh. Antagonists for the 5-HT_{2A} (MDL 100,907), the 5-HT_{2C} (SB 242,084), the 5-HT_6 (Ro 04-6790), the D_2 (raclopride) receptors, and the α_1-adrenoceptors (prazosin) modestly increased ACh by about 50%. The 5-HT_{1A} agonist R-(+)-8-OH-DPAT and the α_2-adrenoceptor antagonist yohimbine significantly increased ACh by about 100% and 50%, respectively. Thus, olanzapine and clozapine increased ACh to a greater extent than other tested antipsychotics, explaining perhaps their purported beneficial effect in cognitive function in schizophrenia. It appears that selective activity at each of the monoaminergic receptors studied is not the sole mechanism underlying the olanzapine and clozapine induced increases in hippocampal ACh.
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KEY WORDS: *Antipsychotic; Schizophrenia; Olanzapine; Clozapine; Microdialysis; Acetylcholine*

Impairments in cognitive processes, such as deficits in executive function, verbal memory and attention, are prevalent in most patients with schizophrenia (Breier 1999). Some atypical antipsychotic drugs appear to improve not only the positive symptoms, but also the negative symptoms and the cognitive deficits when compared with classical neuroleptics (Kinon and Lieberman 1996; Meltzer and McGurk 1999; Remington and Kapur 2000). For example, some atypical antipsychotic drugs have been shown to improve attention, motor and executive function in schizophrenic patients (Meltzer and McGurk 1999). It has been well documented that all clinically effective antipsychotic drugs block dopamine D2 receptors in the brain. Additionally, many preclini-

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cal and clinical studies have indicated that atypical antipsychotics, such as clozapine, olanzapine and risperidone, potently inhibit serotonin 5-HT_2 receptor function and affect a variety of other receptors in the brain (Meltzer et al. 1989; Leysen et al. 1992; Bymaster et al. 1996). In particular, clozapine and olanzapine show a complex pharmacological profile, being described as "multi-acting-receptor-targeted antipsychotic agents" (MARTAs) (Bymaster et al. 2000).

Acetylcholine has been shown to be an important neurotransmitter in motor function, attention and various aspects of cognition. Cholinergic synapses are prevalent in many areas of the brain, including the striatum, the prefrontal cortex and the hippocampus. Neuronal processes within the hippocampus, in particular, mediate declarative memory, and participate in cognitive mapping in both experimental animals and humans (Eichenbaum et al. 1999). It appears likely therefore that a dysfunction in cholinergic neurotransmission within the hippocampus may be responsible for the cognitive impairments often encountered in schizophrenia. In turn, the beneficial effects of some atypical antipsychotic drugs in the treatment of cognitive deficits in schizophrenia may be related to their selective action on cholinergic function in the hippocampus, as a consequence of their multireceptor pharmacological profile.

Earlier in vivo microdialysis experiments have revealed a selective action of clozapine on ACh outflow in limbocortical, dopaminergic regions of the brain, such as the prefrontal cortex, as compared with classical neuroleptics (Parada et al. 1997; Moore et al. 1999). In general, antipsychotic drugs appear to either increase or have no effect on ACh efflux within the striatum, a dopaminergic region of the brain related to motor function (Imperato et al. 1993; Ueda et al. 1995; DeBoer and Abercrombie 1996; Ikarashi et al. 1997; Parada et al. 1997; Moore et al. 1999). To this end, we sought to examine the effects the atypical antipsychotic drugs, olanzapine, clozapine, risperidone and ziprasidone, vis-à-vis the effects of the typical antipsychotic drugs, haloperidol, thioridazine and chlorpromazine, on ACh efflux in the hippocampus of the rat by means of in vivo microdialysis. In order to be able to differentiate between the effects of typical and atypical antipsychotics on ACh outflow, pharmacologically comparable doses of the tested antipsychotics were used, primarily based on ex vivo occupancy data for the D_2 and/or the 5-HT_2 receptors, i.e. doses that achieve considerable occupancy for these receptors (Schotte et al. 1996; Zhang and Bymaster 1999). We also examined the ACh responses to selective ligands for some monoaminergic receptors (D_2, 5-HT_{2A}, 5-HT_{2C}, 5-HT_6, 5-HT_{1A}, α_1- and α_2-adrenoceptors) that are targeted by the studied atypical antipsychotic drugs. Thus, we aimed at unraveling the receptor system(s) involved in the tentative differential effects of typical and atypical antipsychotic drugs on hippocampal ACh efflux.

MATERIALS AND METHODS

Animals and Surgery

Adult male Wistar rats (250–300 g; Harlan Sprague Dawley, Inc., Indianapolis, IN) were housed under 12-h light/dark cycle (lights on at 7:00 A.M.) in a temperature (23°C) controlled animal facility with food and water available ad libitum. Rats were anesthetized with a solution containing chloral hydrate (Sigma) and pentobarbital sodium (Bergen Brunswick) and were mounted in a stereotaxic apparatus (Stoelting) with the incisor bar 3 mm under the horizontal plane passing through the interaural line. Animals were implanted with a guide cannula (Bioanalytical Systems, Inc.) in the hippocampus (coordinates relative to bregma: AP = −5.2, ML = +5.0, DV = −3.8) according to the atlas of Paxinos and Watson (1986). While the animal was still anesthetized, a commercially available microdialysis probe (Bioanalytical Systems, Inc.), with an active membrane surface of 4.0 mm, was inserted through the guide cannula and secured in place. After surgery, rats were housed individually in plexiglass cages. Principles of laboratory animal care (*Guide for the Care and Use of Laboratory Animals*, National Academy Press 1996) were followed, and all protocols were approved by the Animal Care and Use Committee of Eli Lilly and Company.

Microdialysis Experiments and Biochemical Analysis

The day prior to microdialysis experiments, rats were transported from their home cage to the microdialysis testing room for habituation. All experiments were performed approximately 48 h postsurgery in awake, freely moving animals during the light period. Microdialysis experiments and the subsequent biochemical analysis were performed using an automated on-line injection system. On the day of the experiment, microdialysis probes were perfused with a modified Ringer's solution comprised of: 1.3 mM $CaCl_2$ (Sigma), 1.0 mM $MgCl_2$ (Sigma), 3.0 mM KCl (Sigma), 147.0 mM NaCl (Sigma), 1.0 mM $Na_2HPO_4 \times 7H_20$ (Malinckrodt), 0.2 mM $NaH_2PO_4 \times 2H_2O$ (Malinckrodt) and 0.3 μM neostigmine bromide (Sigma) (pH 7.2) at a rate of 2.4 μl/min set by a microinfusion pump (Scipro). The dialysate was loaded directly into a 100 μl sample loop of the injector (Valco Instruments Co.) and automatically injected into the analytical system (ESA, Inc.) every 15 min. The loading and injecting modes of the injector were computer driven (EZ Chrom software, Scientific Software). Dialysate concentrations of acetylcholine were determined by high-performance liquid chroma-

tography with electrochemical detection (HPLC-ED), with a 150 × 3mm ACH-3 column (ESA, Inc.) maintained at 35°C. The mobile phase was comprised of 100 mM di-Sodium hydrogen phosphate (Fluka), 2.0mM 1-octanesulfonic acid (Sigma), and 100 μl/2L of reagent MB (ESA, Inc.) (pH 8.0, adjusted with phosphoric acid) and was delivered by an HPLC pump (ESA, Inc.) at 0.4 ml/min. Pulse-dampeners (Alltech) were placed between the HPLC pump and injector. A coulometric detector was used for electrochemical detection (ESA Coulochem II) connected with a solid phase reactor for acetylcholine (ESA; ACH-SPR**)** and with an analytical cell with platinum target (ESA model 5041). This configuration allowed enzymatic conversion of acetylcholine in the solid phase reactor followed by electrochemical oxidation of hydrogen peroxide that was produced by the enzyme reactions. Chromatograms were simultaneously collected on a chart recorder (Kipp and Zonnen) and in a computer for further data analysis with the EZ Chrom software (Scientific Software). Under the present experimental conditions, inclusion of the sodium channel blocker tetrodotoxin (0.1μM, $n = 4$) in the perfusion solution or exclusion of Ca^{2+} (n = 4) from the perfusion solution decreased ACh outflow to <20% of baseline (data not shown), verifying the neuronal origin of the detected ACh.

On completion of the microdialysis experiments, the animals were sacrificed and their brains were removed and stored in a 10% formalin solution (Fisher). Each brain was sliced at 50 μM on a cryostat (Leica), stained (Cresyl Violet), and examined microscopically to confirm probe placement.

Drug Treatments

The effects of several typical and atypical antipsychotic drugs on ACh efflux in the hippocampus were examined: Haloperidol (1 mg/kg, SC)-Research Biochemicals, Inc. Natick, MA (RBI), thioridazine (10 mg/kg, SC)-Sigma, chlorpromazine (10 mg/kg, SC)-RBI, as well as olanzapine (1 and 3 mg/kg, SC)-Lilly Research Laboratories (LRL), clozapine (5 and 10 mg/kg, SC)-RBI, risperidone (1 and 2 mg/kg, SC)-RBI, and ziprasidone (3 mg/kg, SC and IP)-LRL were dissolved in a 5.5% glucose solution with 10 μl of acetic acid. Ziprasidone was administered both IP and SC to minimize possible differences in the pharmacokinetics of the drugs tested, due to the fact that this solution was prepared as a fine suspension. In addition, we examined the effects of selective serotonergic, noradrenergic and dopaminergic receptor compounds on ACh efflux in the hippocampus: MDL 100,907 (1 mg/kg, SC)-LRL, prazosin (1 mg/kg, SC)-RBI and yohimbine (5 mg/kg, SC)-RBI were dissolved in a 5.5% glucose solution with 10 μl of acetic acid. Ro 04-6790 (10mg/kg, SC)-RBI was dissolved in a 10% 2-hydroxypropyl-β-cyclodextrin solution (RBI). SB 242,084 (3mg/kg, SC)-LRL was dissolved in a 5% 2-hydroxypropyl-β-cyclodextrin solution (RBI)/10% cremophor EL (Sigma) solution with 10 μl of acetic acid. Both R(+)-8-OH-DPAT (0.05 mg/kg)-RBI and raclopride (1mg/kg, SC)-RBI were dissolved in saline. The selection of the doses of antipsychotic drugs and selective receptor compounds was based on previous ex vivo, in vivo or other pharmacological studies on receptor occupancy of relevant receptors, e.g. D_2, 5-HT_2, muscarinic (Schotte et al. 1996; Zhang and Bymaster 1999; see Discussion). Control animals received injections with the appropriate drug vehicle. The volume of vehicle or drug solution injected for SB 242,084 was 2ml/kg. The volume of vehicle or drug solution injected was 1 ml/kg for all other drugs.

Data Analysis

The corresponding ACh peaks in both the standard solution and the dialysate samples were analyzed using the EZ Chrom Elite software (Scientific Software) program. Microdialysis data were expressed as percent changes (mean ± S.E.M.) of baseline that was defined as the average absolute value (in pmol/sample) of the four samples before drug injection. Effects of the drug and control treatments were statistically evaluated by 2-way (treatment × time) analysis of variance (ANOVA) followed by the Bonferroni test for between groups multiple comparisons (Figures 1, 2, 3, 5, 6). Data were also presented as the average mean (± S.E.M.) percent changes over a 2-h period post-injection and analyzed with a 1-way ANOVA followed by the Newman-Keuls test (Figures 4, 7). In all statistical measures, a *p*-value less than .05 was considered significant. Data were statistically evaluated by the Graph Pad Prism program.

RESULTS

Basal Concentrations of Acetylcholine in Microdialysates from the Hippocampus of the Rat

Under the present experimental conditions, the basal ACh concentrations in all animals tested ranged from 0.05 to 2.5 pmol/sample, whereas the average basal values in the groups formed was approximately 0.86 pmol/sample. Between group comparisons of the ACh basal values did not reveal any statistically significant differences.

Effects of Systemic Administration of the Typical Antipsychotic Drugs Haloperidol, Thioridazine, and Chlorpromazine on Extracellular Concentrations of Acetylcholine from the Hippocampus of the Rat

Vehicle injections did not significantly affect extracellular concentrations of ACh in the hippocampus (Figure 1). Haloperidol (1mg/kg, SC) and thioridazine (10mg/

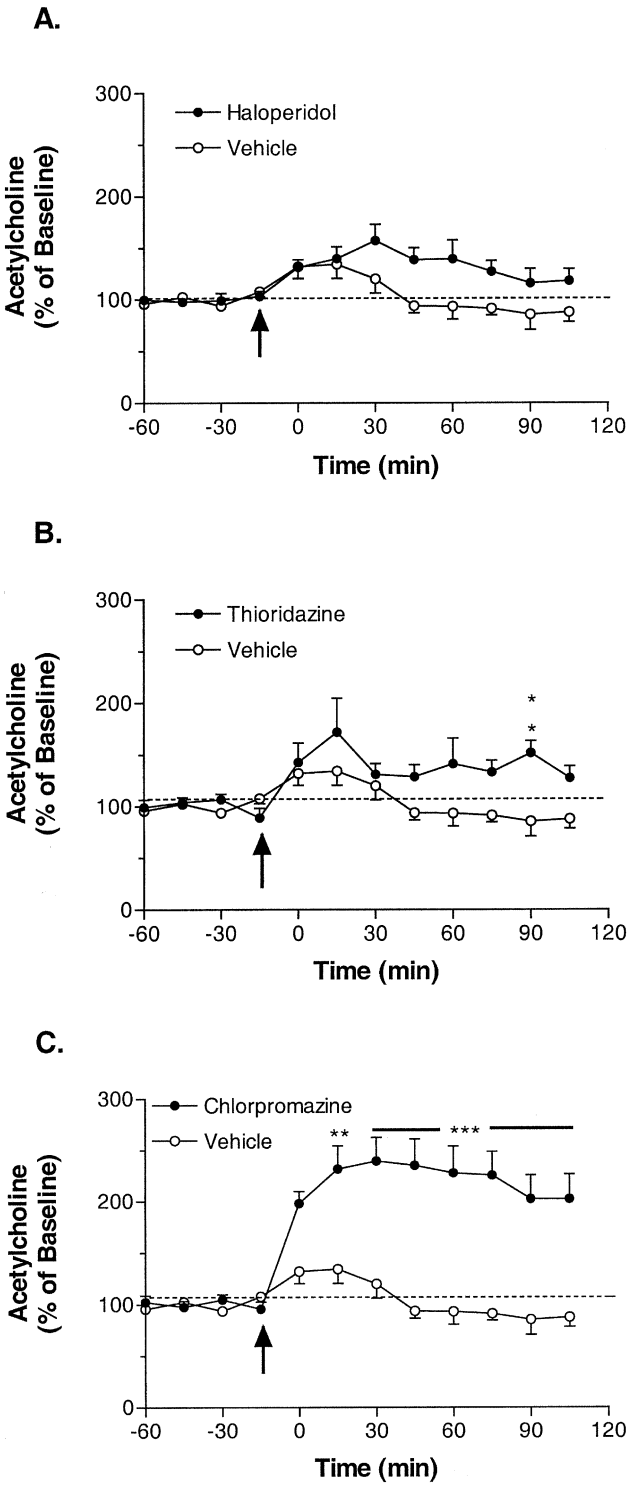

Figure 1. Panel A: Effects of haloperidol (1mg/kg, SC, n = 6) or vehicle (SC, n = 7) on dialysate concentrations of acetylcholine from the hippocampus. Panel B: Effects of thioridazine (10mg/kg, SC, n = 7) or vehicle (SC, n = 7) on dialysate concentrations of acetylcholine from the hippocampus. Panel C: Effects of chlorpromazine (10mg/kg, SC, n = 7) or vehicle (SC, n = 7) on dialysate concentrations of acetylcholine from the hippocampus. Data are presented as mean ± S.E.M. percent changes of baseline values. ** $p < .01$, ***$p < .001$ compared with vehicle; arrows indicate last baseline sample.

kg, SC) produced about a 50% increase in ACh release (see Figure 1, Panels A and B). Compared with vehicle, the effect of haloperidol did not attain statistical significance, whereas the effect of thioridazine reached statistical significance ($p < 0.01$) at the 90min postinjection interval. Chlorpromazine (10 mg/kg, SC) produced over 100% increase in ACh release, which was statistically significant within the 15–105-min postinjection interval as compared with the control group ($p < .01$–$.001$, see Figure 1, Panel C).

Effects of Systemic Administration of the Atypical Antipsychotic Drugs Olanzapine and Clozapine on Extracellular Concentrations of Acetylcholine from the Hippocampus of the Rat

Vehicle injections did not significantly affect extracellular concentrations of ACh in the hippocampus (Figure

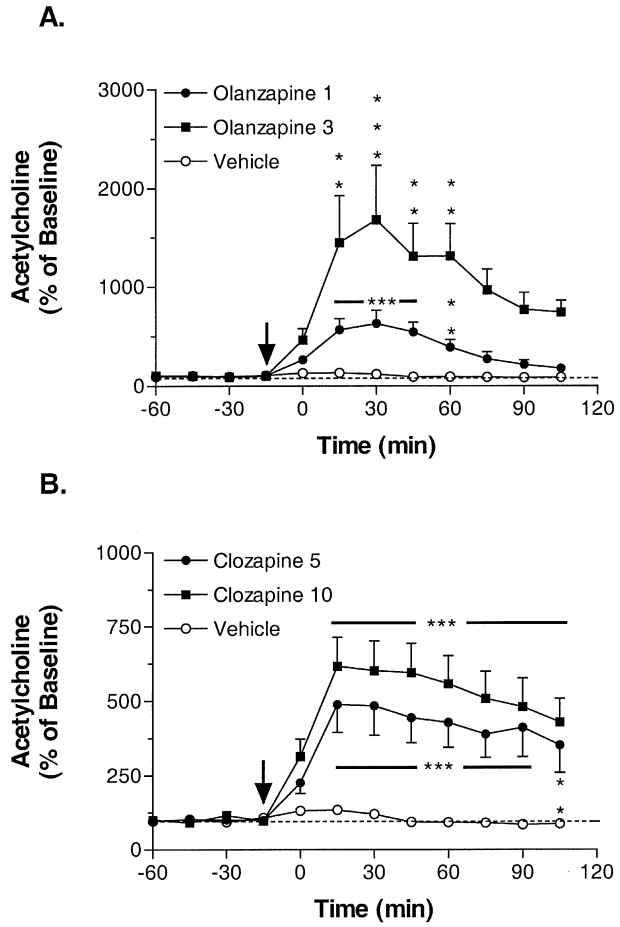

Figure 2. Panel A: Effects of olanzapine (1mg/kg, SC, n = 7 and 3mg/kg, SC, n = 7) or vehicle (SC, n = 7) on dialysate concentrations of acetylcholine from the hippocampus. Panel B: Effects of clozapine (5mg/kg, SC, n = 5 and 10mg/kg, SC, n = 5) or vehicle (SC, n = 7) on dialysate concentrations of acetylcholine from the hippocampus. Data are presented as mean ± S.E.M. percent changes of baseline values. ** $p < .01$, ***$p < .001$ compared with vehicle; arrows indicate last baseline sample.

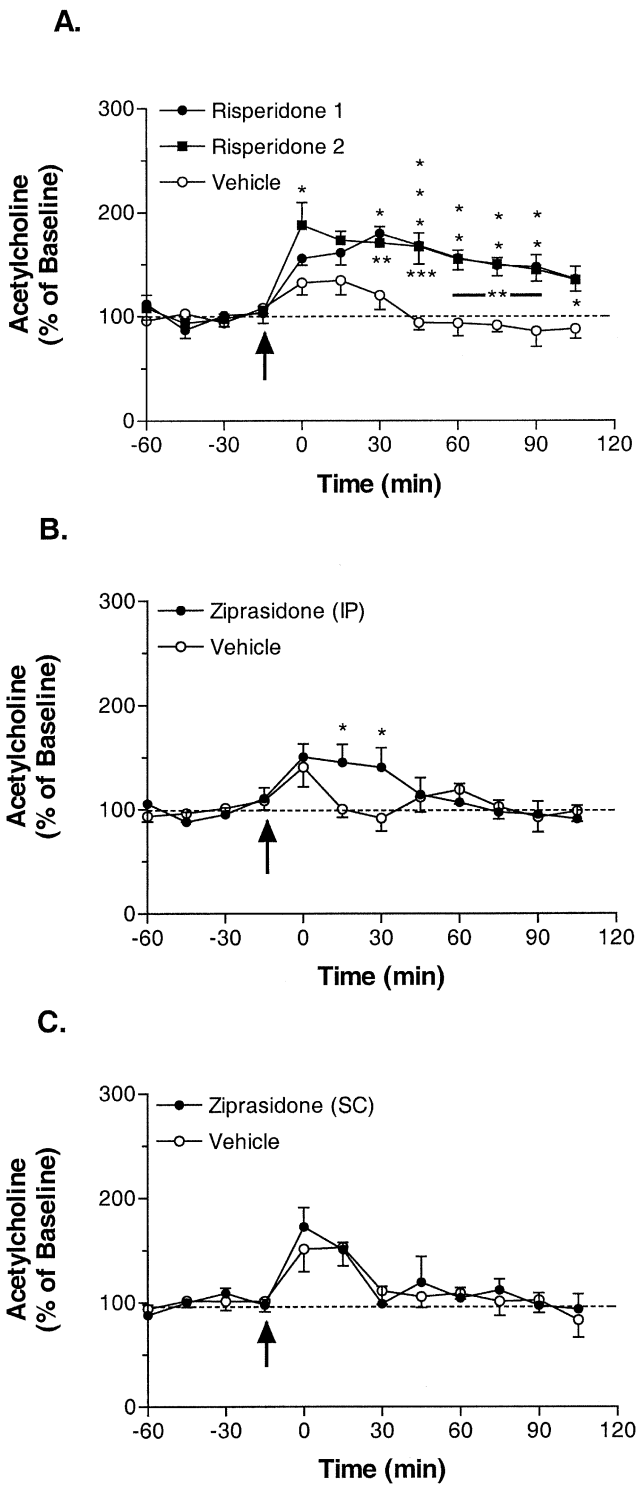

Figure 3. Panel A: Effects of risperidone (1mg/kg, SC, n = 5 and 2mg/kg, SC, n = 5) or vehicle (SC, n = 7) on dialysate concentrations of acetylcholine from the hippocampus. Panel B: Effects of ziprasidone (3mg/kg, IP, n = 6) or vehicle (IP, n = 6) on dialysate concentrations of acetylcholine from the hippocampus. Panel C: Effects of ziprasidone (3mg/kg, SC, n = 5) or vehicle (SC, n = 5) on dialysate concentrations of acetylcholine from the hippocampus. Data are presented as mean ± S.E.M. percent changes of baseline values. *$p < .05$, ** $p < .01$, ***$p < .001$ compared with vehicle; arrows indicate last baseline sample.

2). Olanzapine (1 and 3mg/kg, SC) produced a marked, dose dependent increase in ACh release, up to 1500% of baseline (see Figure 2, Panel A). Post-hoc analysis showed that the 1 and 3mg/kg dose of olanzapine significantly increased ACh concentrations within the 15–60-min postinjection interval as compared with the vehicle control group ($p < .01$–$.001$, see Figure 2, Panel A). Clozapine (5 and 10 mg/kg, SC) also increased ACh release by 500% from basal values (see Figure 2, Panel B). Post-hoc analysis showed that the 5 and 10 mg/kg concentration of clozapine significantly increased ACh concentrations within the 15–105-min postinjection interval as compared with the vehicle control ($p < .01$–$.001$, see Figure 2, Panel B).

Effects of Systemic Administration of the Atypical Antipsychotic Drugs Risperidone and Ziprasidone on Extracellular Concentrations of Acetylcholine from the Hippocampus of the Rat

Vehicle injections did not significantly affect extracellular concentrations of ACh in the hippocampus. Risperidone (1 and 2mg/kg, SC) increased ACh outflow by about 75% (see Figure 3, Panel A). These effects of risperidone reached statistical significance during various time points postinjection when compared with the vehicle control ($p < .05$–$.001$, see Figure 3, Panel A). Ziprasidone (3mg/kg) also increased ACh outflow by about 50% when administered either SC or IP (see Figure 3, Panels B and C). However, this effect reached statistical significance only with the IP administration at the 15–30-min postinjection interval compared with the vehicle control group ($p < .05$, Figure 3, Panel C).

Overall Effects of Typical and Atypical Antipsychotic Drugs on Extracellular Concentrations of Acetylcholine from the Hippocampus of the Rat

The effects of typical and atypical antipsychotic drugs on ACh concentrations over a 2-h postinjection period are presented in Figure 4. Statistical evaluation of all data depicted in this figure revealed a statistically significant treatment effect ($F_{12,91} = 34.68$, $p < .001$). Post-hoc analysis indicated that only olanzapine and clozapine significantly increased the mean overall ACh release compared with the respective vehicle control groups ($p <$ .01–.001, see Figure 4).

Effects of Systemic Administration of Selective Receptor Compounds on Extracellular Concentrations of Acetylcholine from the Hippocampus of the Rat

To determine the underlying mechanism of action of atypical antipsychotic drugs, we examined the effects of

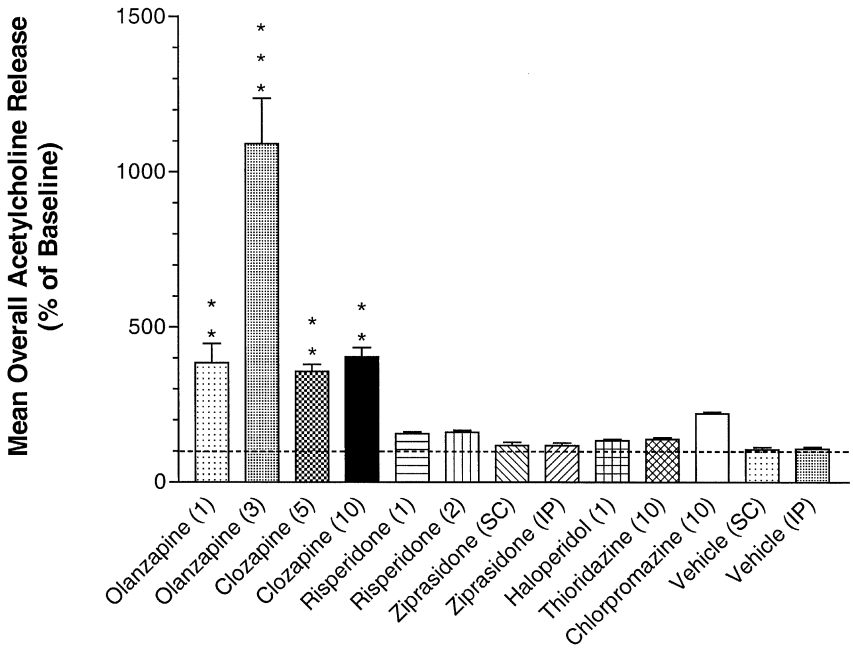

Figure 4. Effects of typical and atypical antipsychotic drugs and their respective vehicle on acetylcholine outflow in the hippocampus. Each bar represents the mean ± S.E.M. percent change of baseline values over a 2-h postinjection interval. Data were analyzed with a 1-way analysis of variance, followed by the Newman-Keuls test. $^{**}p < .01$, $^{***}p < .001$ compared with the respective vehicle.

selective compounds for receptors that atypical antipsychotic drugs show relatively high affinity for (Figures 5 and 6). The selective 5-HT_{2A} receptor antagonist MDL 100,907 (1 mg/kg, SC) increased ACh outflow by about 50%; this effect, however, did not attain statistical significance compared with the vehicle treated group (see Figure 5, Panel A). Similarly, the selective 5-HT_{2C} receptor antagonist SB 242,084 (3 mg/kg, SC) increased ACh outflow by about 60%, an effect that did not reach statistical significance compared with the control group (see Figure 5, Panel B). The selective 5-HT_6 receptor antagonist Ro 04-6790 (10 mg/kg, SC) produced a modest (50%) increase in ACh outflow; this effect was not statistically different from the action of vehicle (see Figure 5, Panel C). The selective 5-HT_{1A} agonist R(+)-8-OH-DPAT (0.05 mg/kg, SC) increased ACh release by about 100% compared with vehicle controls. Post-hoc analysis showed that R(+)-8-OH-DPAT significantly increased ACh concentrations within the 0–60-min postinjection interval as compared with the vehicle control ($p < .05$–.01, see Figure 5, Panel D). The selective D_2 receptor antagonist raclopride (1mg/kg, SC) increased ACh concentrations by about 50%; this effect reached significance at the 30min postinjection interval ($p < .05$, see Figure 6, Panel A). The selective α_1-adrenergic antagonist prazosin (1mg/kg, SC) increased ACh outflow by about 50%, an effect that was not statistically different from the actions of vehicle (see Figure 6, Panel B). On the other hand, the α_2-adrenergic antagonist yohimbine (5mg/kg, SC) induced an about 50% increase in ACh efflux that lasted the entire postinjection interval, attaining statistical significance at several time points when compared with vehicle control ($p < .01$, Figure 6, Panel C).

Overall Effects of Selective Receptor Compounds on Extracellular Concentrations of Acetylcholine from the Hippocampus of the Rat

The effects of selective receptor compounds on ACh concentrations over a 2-h postinjection period are presented in Figure 7. Statistical evaluation of all data depicted in this figure revealed a statistically significant treatment effect ($F_{10,77} = 5.23$, $p < .001$). Post-hoc analysis indicated that only the selective α_2-adrenergic antagonist yohimbine and the selective 5-HT_{1A} agonist R(+)-8-OH-DPAT significantly increased the mean overall ACh release compared with the respective vehicle control groups ($p < .05$, see Figure 7).

DISCUSSION

The major finding of the present study is that olanzapine and clozapine increase ACh outflow in the hippocampus of the rat to a greater extent than other antipsychotic drugs tested, either typical (haloperidol, chlorpromazine, thioridazine) or atypical (risperidone, ziprasidone). In addition, selective action at only one of the monoaminergic receptor sites affected by antipsychotic drugs, e.g. D_2, 5-HT_{2A}, 5-HT_{2C}, 5-HT_6, 5-HT_{1A}, α_1- and α_2-adrenoceptors, does not appear to exclusively account for the marked increases in hippocampal ACh efflux observed with both olanzapine and clozapine. The increased release of ACh in the hippocampus could contribute to the improvement of cognitive functions observed with some atypical antipsychotic drug use, particularly with olanzapine, in schizophrenia (Purdon et al. 2000; Cuesta et al. 2001). In clinical studies, both

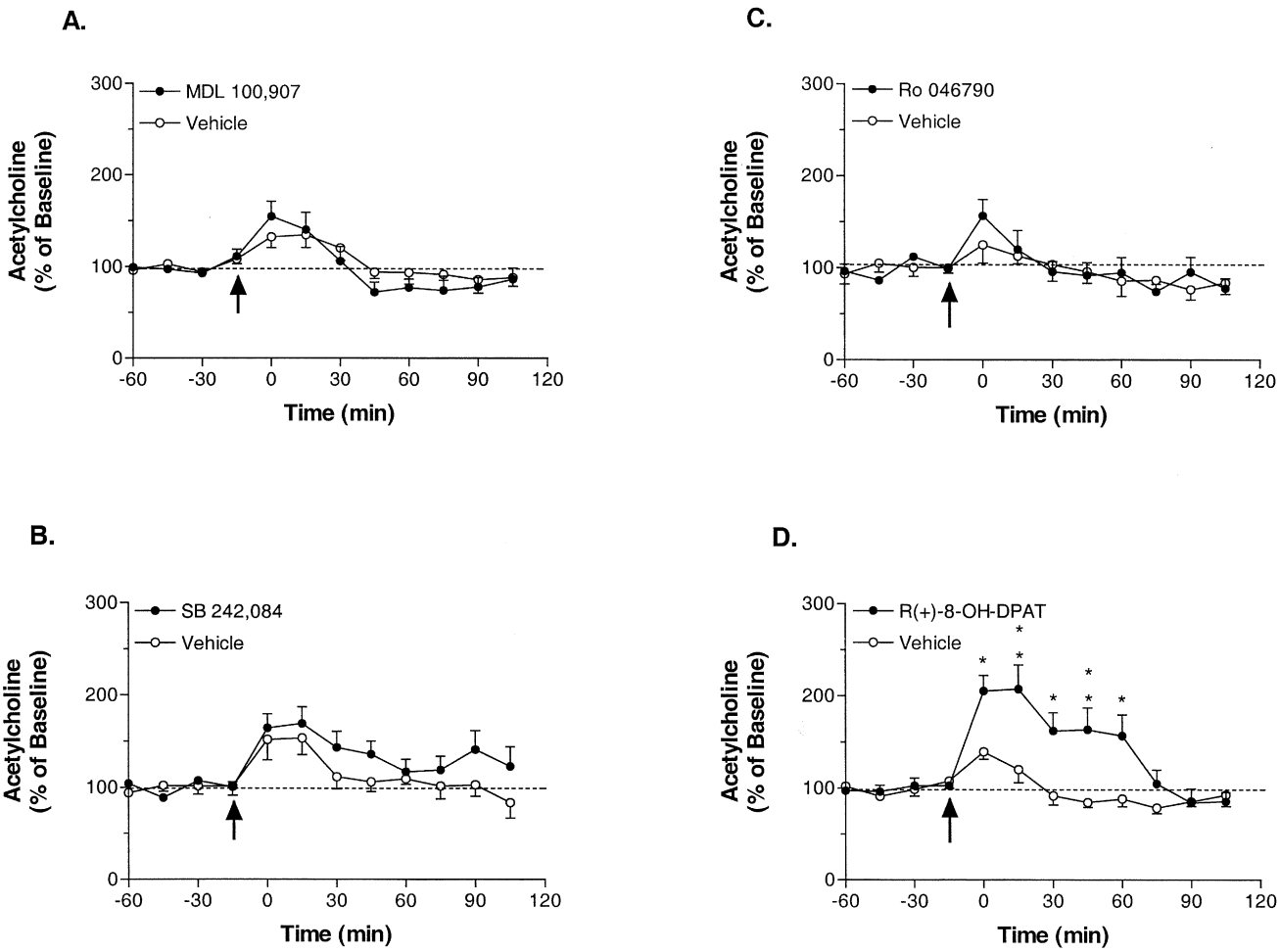

Figure 5. Panel A: Effects of MDL 100, 907 (1mg/kg, SC, n = 7) or vehicle (SC, n = 7) on dialysate concentrations of acetylcholine from the hippocampus. Panel B: Effects of SB 242,084 (3mg/kg, SC, n = 6) or vehicle (SC, n = 5) on dialysate concentrations of acetylcholine from the hippocampus. Panel C: Effects of Ro 04-6790 (10mg/kg, SC, n = 7) or vehicle (SC, n = 6) on dialysate concentrations of acetylcholine from the hippocampus. Panel D: Effects of R(+)-8-OH-DPAT (0.05mg/kg, SC, n = 6) or vehicle (SC, n = 6) on dialysate concentrations of acetylcholine from the hippocampus. Data are presented as mean ± S.E.M. percent changes of baseline values. *$p < .05$, ** $p < .01$, ***$p < .001$ compared with vehicle; arrows indicate last baseline sample.

olanzapine and clozapine, and to some extent risperidone, have been found to be beneficial in improving procedural learning tasks; these cognitive improvements have not been apparent with haloperidol treatment (Purdon et al. 2000; Purdon 2000).

All experiments in the present study were conducted in the presence of 0.3 μM of the AChesterase inhibitor neostigmine in the perfusion solution. This concentration is within the range, i.e. 0.1–1 μM, regularly used in most microdialysis studies to decrease elimination and increase detectability of ACh in the dialysate. It has become increasingly apparent though that inclusion of an AChesterase inhibitor in the perfusion solution may affect the obtained pharmacological responses both quantitatively and qualitatively. This has been clearly illustrated by studying the effects of muscarinic receptor antagonists and AChesterase inhibitors on ACh outflow in the brain (Kawashima et al. 1991; Messamore et al. 1993a, 1993b; Moor et al. 1998), and on the effects of dopaminergic agents on striatal ACh efflux (DeBoer and Abercrombie 1996; Acquas and Fibiger 1998). On the other hand, physiologically relevant ACh responses appear to be independent of the concentration of AChesterase inhibitors in the perfusion medium (Moor et al. 1998; Himmelheber et al. 1998). To the extent that the effects of the antipsychotic drugs tested on hippocampal ACh are not mediated through actions at muscarinic or dopaminergic receptors (see below), it is inferred that the detected differences are not skewed by the presence of an AChesterase inhibitor in the perfusate. In fact, preliminary studies in our laboratory have revealed that at least the effects of olanzapine on ACh

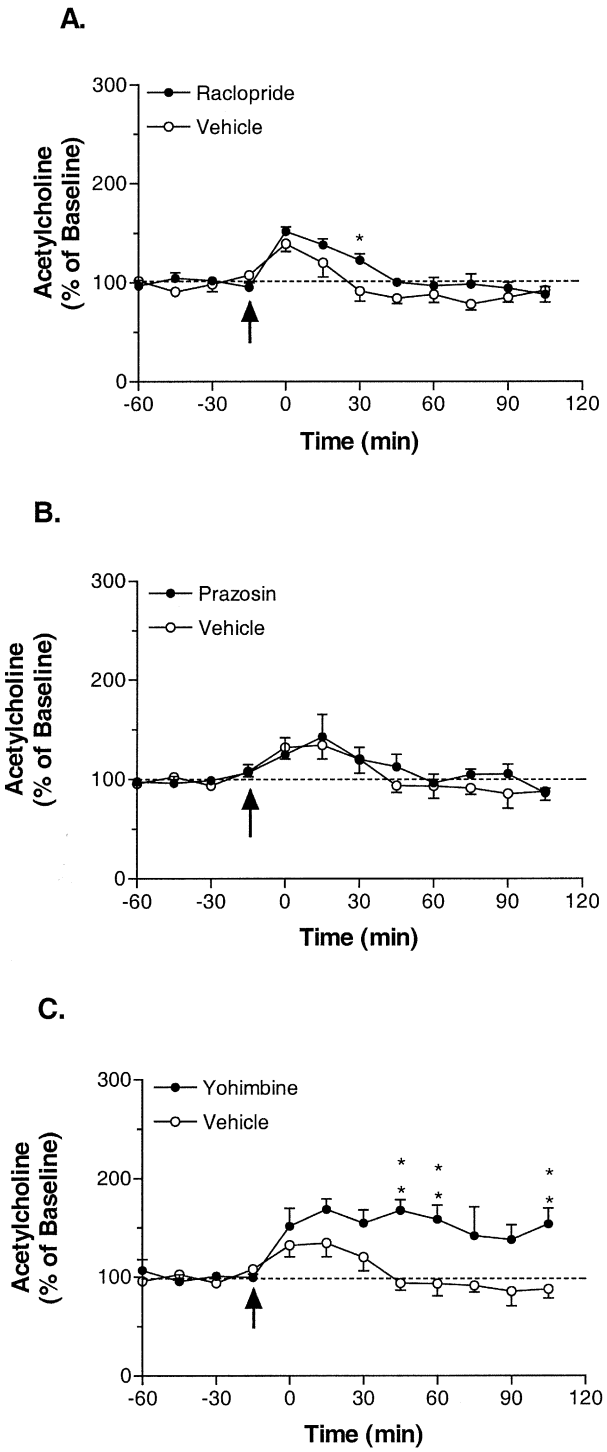

Figure 6. Panel A: Effects of raclopride (1mg/kg, SC, n = 9) or vehicle (SC, n = 6) on dialysate concentrations of acetylcholine from the hippocampus. Panel B: Effects of prazosin (1mg/kg, SC, n = 5) or vehicle (SC, n = 7) on dialysate concentrations of acetylcholine from the hippocampus. Panel C: Effects of yohimbine (5mg/kg, SC, n = 6) or vehicle (SC, n = 7) on dialysate concentrations of acetylcholine from the hippocampus. Data are presented as mean ± S.E.M. percent changes of baseline values. *$p < .05$, ** $p < .01$ compared with vehicle; arrows indicate last baseline sample.

efflux in the hippocampus are independent of the concentration of neostigmine in the perfusion solution. Whether this is unequivocally the case for the effects of other antipsychotic drugs on ACh outflow in hippocampus and in other regions of the brain remains to be shown.

The selection of doses used for both typical and atypical antipsychotic drugs tested in this study was based on previous ex vivo and in vivo receptor binding studies (Schotte et al. 1996; Zhang and Bymaster 1999). For example, the ID_{50} (dose required to block 50% of the radioligand binding) values of olanzapine, risperidone, haloperidol and chlorpromazine for occupying D2 receptors in the brain of the rat are 0.6, 0.2, 0.12 and 0.5 mg/kg, respectively (Zhang and Bymaster 1999). Similarly, the occupancy of 5-HT_2 receptors is estimated to be greater than 50% with the doses of the atypical antipsychotic drugs used, in view of the fact that the ID_{50} values are 0.15, 2.2 and 0.1 mg/kg for olanzapine, clozapine and risperidone, respectively (Zhang and Bymaster 1999). Ziprasidone was tested at the 3 mg/kg dose that achieves approximately a 70% total receptor occupancy for the 5-HT_{2A} receptor subtype and a 20% total receptor occupancy for the D_2 receptors (Schotte et al. 1996; Zhang and Bymaster 1999). In addition, the 3 mg/kg dose of ziprasidone was selected in the present study, because doses in this range are usually used in other biochemical and pharmacological animal studies (see e.g. Rollema et al. 2000). Thus, it is estimated that the doses of the antipsychotic compounds tested result in a greater than 50% occupancy of D_2, 5-HT_2, or both receptors in the brain. Analogously, the doses of the receptor selective compounds used in the present study were selected on the basis of ex vivo and in vivo occupancy data for the respective receptors, if available (Schotte et al. 1996; Zhang and Bymaster 1999). In the absence of binding data, data from other pharmacological and biochemical studies revealing activity at the respective receptors were used to select relevant doses (see e.g. Nomikos et al. 1992; Andersson et al. 1994, 1995; Nomikos et al. 1994; Acquas et al. 1998; Bentley et al. 1999; Grottick et al. 2000; Marcus et al. 2000). For example, based on a number of previously published preclinical data (Nomikos et al. 1994; Andersson et al. 1995; Acquas and Fibiger 1998), it is estimated that full occupancy and blockade of D2 receptors in the brain is achieved by the dose of raclopride used.

The results of the present study show that the atypical antipsychotic drugs olanzapine and clozapine increase ACh efflux in the hippocampus to a greater extent than the classical neuroleptics haloperidol, thioridazine and chlorpromazine, and the atypical antipsychotic drugs risperidone and ziprasidone. Previous microdialysis studies have shown that clozapine increases ACh release in the prefrontal cortex and the nucleus accumbens to a greater extent than classical

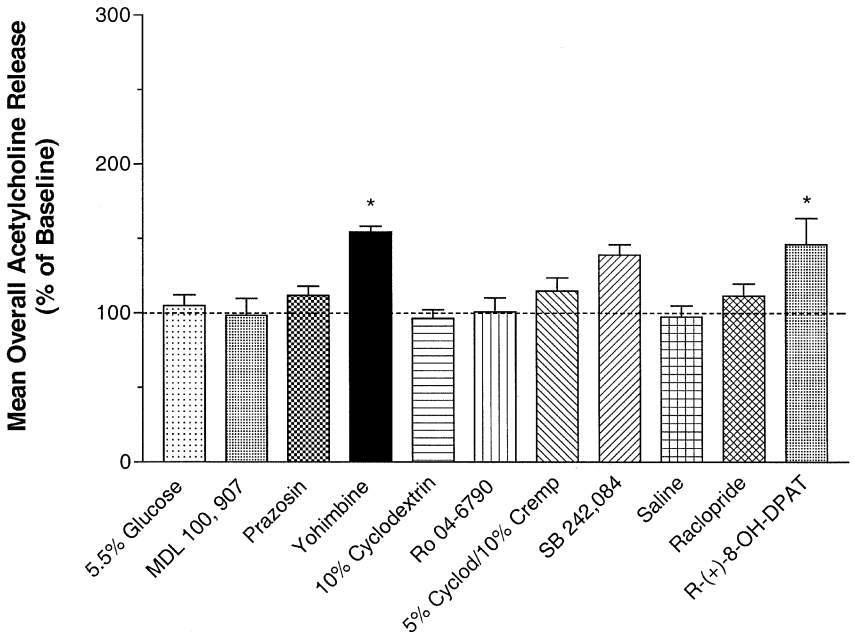

Figure 7. Effects of receptor selective compounds and their respective vehicle on acetylcholine outflow in the hippocampus. Each bar represents the mean ± S.E.M. percent change of baseline values over a 2-h postinjection interval. Data were analyzed with an 1-way analysis of variance, followed by the Newman-Keuls test; $*p < .05$ compared with the respective vehicle.

neuroleptics (Parada et al. 1997; Moore et al. 1999), whereas clozapine and other antipsychotic drugs studied variably affect ACh release in the striatum (Imperato et al. 1993; Ueda et al. 1995; DeBoer and Abercrombie 1996; Ikarashi et al. 1997; Parada et al. 1997; Moore et al. 1999). The effect of clozapine on ACh release appeared to be more pronounced in the prefrontal cortex than in the nucleus accumbens or the striatum (Parada et al. 1997). The present results indicate that effective antipsychotic drugs are clearly differentiated on the basis of their effects on ACh outflow in the hippocampus, although the reasons for this differential response between olanzapine and clozapine in one hand and all the other antipsychotic drugs in the other cannot be easily understood. Differences in the ability of the tested antipsychotic drugs to affect D_2 receptors could not entirely account for the observed differences in ACh outflow, because all the antipsychotic drugs tested (with the exception of ziprasidone and clozapine) block D_2 receptors to a considerable degree (see above). In addition, full D_2 receptor blockade achieved by raclopride results only in a modest increase in hippocampal ACh (this study). These results are in agreement with those of earlier microdialysis studies testing the effects of other D_2 receptor antagonists on ACh efflux in the brain (Imperato et al. 1993; Russi et al. 1993; DeBoer and Abercrombie 1996; Ikarashi et al. 1997; Acquas and Fibiger 1998; Moore et al. 1999). Similarly, under the present experimental conditions, administration of olanzapine, clozapine, risperidone and ziprasidone results in a considerable blockade of 5-HT_2 receptors (see above). Thus, differences in the degree of blockade of 5-HT_2 receptors elicited by the antipsychotic drugs tested could not solely account for the observed differential ACh responses. In consonance with this notion, administration of the selective 5-HT_{2A} and 5-HT_{2C} receptor antagonists, at pharmacologically relevant doses, failed to affect significantly ACh outflow in the hippocampus (this study). Similar results have previously been reported with other 5-HT_2 receptor antagonists, e.g. ketanserin, ritanserin, and mesulergine, which exhibit varying degrees of 5-HT_{2A} vs. 5-HT_{2C} receptor selectivity (Hirano et al. 1995; Consolo et al. 1996; Zhelyazkova-Savova et al. 1999). Interestingly, both clozapine and olanzapine, in contrast to other antipsychotic drugs tested, appear to show high affinity for the 5-HT_6 receptors (Roth et al. 1994; Bymaster et al. 1999), and 5-HT_6 receptor antagonists purportedly increase cholinergic function in the brain (Bentley et al. 1999). Accordingly, selective action at the 5-HT_6 receptors could account for the pronounced effects of clozapine and olanzapine on hippocampal ACh outflow, as compared with the other antipsychotic drugs. However, administration of the 5-HT_6 receptor antagonist Ro 04-6790 did not significantly affect ACh efflux in the hippocampus, a finding that argues against a significant involvement of 5-HT_6 receptors in the detected differential ACh responses. It is noteworthy that antipsychotic drugs exhibit varying degrees of antagonism at α_1 adrenoceptors (Cohen and Lipinski 1986). In view, however of the present and earlier (Acquas et al. 1998) findings that the α_1 adrenoceptor antagonist prazosin failed to affect hippocampal ACh concentrations, it is concluded that α_1 adrenoceptor antagonism does not significantly contribute to the displayed differences in the hippocampal ACh responses. On the other hand, it has been well established that 5-HT_{1A} receptor agonistic action results in a significant increase in ACh outflow in the brain (Izumi et al. 1994; Wilkinson et al. 1994). This

receptor activity might be of importance for the neurobiological effects of, primarily, clozapine and ziprasidone (Rollema et al. 1997, 2000). Similarly, it is unequivocally recognized that α_2 adrenoceptor antagonists increase ACh efflux in the brain (Tellez et al. 1997; Acquas et al. 1998), and that α_2 adrenoceptor antagonism may underlie some of the central actions of clozapine, risperidone, and to a lesser extent of olanzapine (Hertel et al. 1999a,b). Notwithstanding the fact that we also found significant increases in ACh concentrations in the hippocampus in response to the 5-HT_{1A} receptor agonist R-8-OH-DPAT (+100%) and the α_2 adrenoceptor antagonist yohimbine (+60%), the marked increase in hippocampal ACh after olanzapine (+1400%) cannot be attributed in its entirety to these receptor actions. Admittedly though, 5-HT_{1A} receptor agonistic action and α_2 adrenoceptor antagonistic activity may play an important role in the stimulatory effects of clozapine and risperidone, respectively, on ACh outflow in the hippocampus.

Previous microdialysis studies have shown that administration of non-selective muscarinic receptor antagonists causes marked increases in ACh concentrations in the brain (Day et al. 1991; Kawashima et al. 1991; Quirion et al. 1994; Moor et al. 1998). These effects are mainly attributed to M_2 receptor antagonism, whereas the involvement of M_1 or other muscarinic receptor activity is less obvious (Billard et al. 1995; Stillman et al. 1996). In this regard, it appears that M_1 receptor agonists invariably augment ACh release in the brain (Ogane et al. 1990; Murakami et al. 1996; Suzuki et al. 1998). Thus, M_1 receptor antagonistic action may even counteract the increase in ACh release mediated via M_2 receptor antagonism. Of the antipsychotic compounds tested, olanzapine, clozapine and thioridazine show comparable affinities for the M_1 and M_2 receptors in the nM range in vitro (Bolden et al. 1992; Bymaster et al. 1996; Arnt and Skarsfeldt 1998). Nevertheless, only olanzapine and thioridazine appear to function as full antagonists at both muscarinic receptors (Bolden et al. 1992; Bymaster et al. 1996). Importantly, based on in vivo binding data (Zhang and Bymaster 1999), the estimated occupancy of muscarinic receptors after olanzapine and clozapine is less than 10%, whereas that after thioridazine is somewhat higher, at the doses tested. Yet, olanzapine and clozapine markedly increase hippocampal ACh outflow, sharply contrasting the modest effects observed after thioridazine. In fact, even a much higher dose of thioridazine (30 mg/kg) failed to increase ACh outflow further (Southall et al. unpublished observations). Additionally, other pharmacological studies have provided substantial evidence in support of the notion that the muscarinic component of the activity of either olanzapine or clozapine in vivo is at best minimal (Arnt and Skarsfeldt 1998; Bymaster and Falcone 2000). Taken together, it appears likely that muscarinic receptor antagonism does not solely mediate the marked effects of olanzapine and clozapine on ACh efflux in the hippocampus, although such an involvement cannot understandably be excluded.

In summary, we have demonstrated by using in vivo microdialysis, that the atypical antipsychotic compounds, olanzapine and clozapine, increase ACh in the hippocampus to a far greater extent than other typical and atypical antipsychotic drugs. Selective receptor activities at 5-HT_{2A}, 5-HT_6, 5-HT_{2C}, D_2, and α_1-adrenergic receptors most likely are not solely responsible for the increases in hippocampal ACh efflux observed with either olanzapine or clozapine. On the other hand, α_2-adrenergic receptor antagonism and 5-HT_{1A} agonistic action may be of some importance to the stimulatory effect of clozapine on ACh efflux in the hippocampus. To this end, it cannot be excluded that combinations of several receptor-mediated mechanisms including those studied herein are responsible for the marked effects of olanzapine and clozapine on ACh release. In this regard, the role of other receptor systems, e.g. D_1, D_4, histaminergic, 5-HT_7, in the detected, drug selective action of clozapine and olanzapine on hippocampal ACh remains to be elucidated. Ultimately, the unique multireceptor acting profiles that clozapine and olanzapine exhibit (Bymaster et al. 2000) may be responsible for their marked effects on cholinergic neurotransmission in the brain. This distinguishing feature may in turn be important for the beneficial effects of these atypical antipsychotic agents in cognitive function in schizophrenia.

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