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Aripiprazole, a novel antipsychotic drug, preferentially increases dopamine release in the prefrontal cortex and hippocampus in rat brain

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Abstract

Aripiprazole,7-{4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butyloxy}-3,4-dihydro-carbostycil (OPC-14597), a novel atypical antipsychotic drug, is a dopamine D2 receptor partial agonist with functional 5-HT_{2A} receptor antagonist, and 5-HT_{1A} receptor partial agonist properties as well. Other atypical antipsychotic drugs, e.g. clozapine, but not typical antipsychotic drugs, e.g. haloperidol, produce significant increases in dopamine and acetylcholine release in the medial prefrontal cortex in rats, effects believed to be related to the ability to improve cognitive function. The increase in the medial prefrontal cortex dopamine release by the atypical antipsychotic drugs has been shown to be partially inhibited by *N*-[2[4-)2-methoxyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride (WAY100635), a selective 5-HT_{1A} receptor antagonist. Aripiprazole, 0.1 and 0.3 mg/kg, significantly increased dopamine release in the hippocampus. Moreover, aripiprazole, 0.3 mg/kg, slightly but significantly increased dopamine release in the medial prefrontal cortex but not in the nucleus accumbens. These increases were significantly inhibited by WAY100635. By contrast, aripiprazole, 3.0 mg/kg and 10 mg/kg, significantly decreased dopamine release in the nucleus accumbens but not the medical prefrontal cortex. However, aripiprazole 10 mg/kg significantly decreased dopamine release in the both regions. Aripiprazole had no effect on acetylcholine release in the medial prefrontal cortex, hippocampus, or nucleus accumbens at any dose, except for 3.0 mg/kg, which decreased acetylcholine release in the nucleus accumbens only. Aripiprazole, 0.3 mg/kg, transiently potentiated haloperidol (0.1 mg/kg)-induced dopamine release in the medial prefrontal cortex but inhibited that in the nucleus accumbens. The present study demonstrated that aripiprazole, at low doses of 0.1 and 0.3 mg/kg, increases dopamine release in the medial prefrontal cortex and hippocampus. It also suggests that the function of both the medial prefrontal cortex and hippocampus may contribute to the ability of aripiprazole to improve negative symptom and cognition.

Keywords: Aripiprazole; Antipsychotic; Dopamine; Serotonin; Prefrontal cortex; Hippocampus

1. Introduction

Aripiprazole,7-{4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butyloxy}-3,4-dihydro-carbostycil (OPC-14597)] is a novel atypical antipsychotic drug, which has been shown to be efficacious for both positive and negative symptoms, as well as cognition in patients with schizophrenia and to produce minimal extrapyramidal side effects (Carson et al., 2000; Kane et al., 2002; Cornblatt, personal communication, 12/2002). Aripiprazole has been shown to be a partial dopamine D2 receptor agonist in vitro as well as in vivo (Kikuchi et al., 1995; Lawler et al., 1999; Burris et al., 2002) whereas other atypical antipsychotic drugs such as clozapine, risperidone, quetiapine, olanzapine, melperone, and ziprasidone are D2 receptor antagonists (Meltzer and McGurk, 1999).

Although aripiprazole has high affinity for D2 receptors in rat striatum, frontal cortex, and limbic forebrain (0.5 nM), it has been reported to behave as a functionally selective partial agonist which may, depending upon the cellular milieu, display partial agonist, full agonist, or antagonist actions at D2 receptors (Lawler et al., 1999; Shapiro et al., 2003). Thus, aripiprazole has been found to have an antagonist action at post-synaptic D2 receptors, as indicated that (1) it inhibits apomorphine-induced stereotyped behavior and hyperlocomotion in rats without producing stereotypy or increasing locomotion by itself (Kikuchi et al., 1995); (2) it

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produces a two-fold increases in the level of serum prolactin (Inoue et al., 1996); (3) it antagonizes the effect of dopamine receptor agonist tailpexole on yawning in rats (Fujikawa et al., 1996); (4) it increases dopamine metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) levels in the prefrontal cortex and striatum (Semba et al., 1995); and (5) it displays D2 receptor antagonism in several in vitro model systems as well (Lawler et al., 1999; Shapiro et al., 2003). Electrophysiological studies have also confirmed that aripiprazole inhibits the dopaminergic suppression of the firing of neurons in the nucleus accumbens by postsynaptic D1 and D2 receptor antagonism (Amano et al., 1995). On the other hand, aripiprazole has been found to be a partial agonist at D2 presynaptic autoreceptors in vivo, as indicated by its ability to inhibit the gamma-butyrolactone-induced increase in dopamine synthesis rate in mouse forebrain (Kikuchi et al., 1995; Semba et al., 1995). In addition, aripiprazole has also been found in selected in vitro model systems to behave as a partial agonist (Lawler et al., 1999; Burris et al., 2002; Shapiro et al., 2003). It is unique among atypical antipsychotic drugs in that it is also a potent D3 and D4 receptor partial agonist (Shapiro et al., 2003). Like other atypical antipsychotic drugs, e.g. clozapine, quetiapine and ziprasidone, aripiprazole has partial 5-HT_{1A} receptor agonist effect (Jordan et al., 2002; Shapiro et al., 2003) as well as 5-HT_{2A} receptor antagonist action (Burris et al., 2002; Jordan et al., 2002).

Acute administration of atypical antipsychotic drugs, e.g. clozapine, risperidone, olanzapine, quetiapine and ziprasidone, produces greater increases in extracellular dopamine and acetylcholine release in the medial prefrontal cortex than in the nucleus accumbens, a component of the limbic system (Moghaddam and Bunney, 1990; Kuroki et al., 1999; Ichikawa et al., 2002a,b,c). It is well known that both of these neurotransmitters play a critical role in cognitive function in primates, including man (William and Goldman-Rakic, 1995; Meltzer and McGurk, 1999). The increased release of dopamine in the medial prefrontal cortex has been suggested to contribute to the ability of these atypical antipsychotic drugs to improve cognitive dysfunction and negative symptoms (Kuroki et al., 1999; Meltzer, 1999). The increase in the medial prefrontal cortex dopamine release by these atypical antipsychotic drugs has been reported to be partially but significantly inhibited by *N*-[2[4-)2-methoxyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexaneca rboxamide trihydrochloride (WAY100635), a 5-HT_{1A} receptor antagonist (Rollema et al., 2000; Ichikawa et al., 2001), even though only clozapine, quetiapine and ziprasidone are 5-HT_{1A} receptor partial agonists. Thus, stimulation of 5-HT_{1A} receptors, directly or indirectly, appears to be necessary for maximal increase in cortical dopamine release, induced by the atypical antipsychotic drugs (Ichikawa et al., 2002a,c).

Using in vivo microdialysis, aripiprazole, 2.5 mg/kg, i.p., was reported to have no effect on dopamine release but increases DOPAC and HVA in the medial prefrontal cortex in rat, whereas at doses of 10 and 40 mg/kg, it produced a dose-dependent decrease in extracellular dopamine release in the frontal cortex (Semba et al., 1995). No other atypical antipsychotic drug has been reported to decrease dopamine release in the medial prefrontal cortex, an effect which might be expected to impair cognition assuming that levels of dopamine release in the cortex were not elevated prior to treatment. The possibility that aripiprazole might increase dopamine release in the medial prefrontal cortex at lower doses has not been studied. Therefore, in the present study, using in vivo microdialysis, the effect of a wide range of doses of aripiprazole (0.1–10 mg/kg) on dopamine and acetylcholine release in the medial prefrontal cortex and nucleus accumbens was studied. Since atypical antipsychotic drugs are sometimes clinically used in combination with typical neuroleptic drugs, e.g. haloperidol, with the goal of enhancing their ability to improve psychotic symptoms, it was of interest to also study the effect of the combination of aripiprazole and haloperidol on dopamine release in the medial prefrontal cortex and nucleus accumbens.

In addition, there is extensive evidence that the hippocampus is essential for declarative (storage) memory, one of the key cognitive measures which is impaired in schizophrenia (Eichenbaum et al., 1999; Fernandez et al., 2002; Seidman et al., 2002). Dysfunction in dopaminergic and cholinergic neurotransmission within the hippocampus may contribute to the cognitive deficits, especially long-term memory impairment, in patients with schizophrenia. There is evidence that atypical antipsychotic drugs, clozapine, olanzapine, risperidone and ziprasidone, significantly increase dopamine and acetylcholine release in the hippocampus in rat (Shirazi-Southall et al., 2002; Chung et al., in preparation). The beneficial effects of the atypical antipsychotic drugs to improve long term memory deficits in schizophrenia (Meltzer and McGurk, 1999; Harvey and Keefe, 2001), may be related to these and other effects in the hippocampus. Therefore, in the present study, the effect of aripiprazole on dopamine and acetylcholine release in this region was also determined.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley albino rats (Zivic-Miller Laboratories, Porterville, 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" (NIH publication No. 85-23, revised 1985) were followed.

2.2. Surgery and microdialysis

Rats were anesthetized with intraperitoneal injection of a combination of xylazine (6 mg/kg; Rompun, Shawnee Mission, KS) and ketamine hydrochloride (70 mg/kg, Ketaset, Fort Dodge Laboratories, Fort Dodge, IA) and mounted in a stereotaxic frame (Stoetling, Wood Dale, IL). Stainless guide cannula (21-gauge) with a dummy probe were placed and fixed by cranioplastic cement (Plastic One, Roanoke, VA) onto the cortex dorsal to the medial prefrontal cortex, hippocampus and the nucleus accumbens. Rats received dual probe implantation for the medial prefrontal cortex, nucleus accumbens or hippocampus (coordinates: A +3.2, L +0.8 (10 °C inclination), V −5.5 mm; A +2.0, L +1.5 to +1.7, V −7.5 mm and A −5.6, L +5.0, V −7.0 mm, respectively, relative to bregma). The incision bar level was 3.0 mm, according to the atlas of Paxinos and Watson (1998). The length of exposed dialyzing membrane was 2 mm (for the medial prefrontal cortex and nucleus accumbens) or 3 mm (for the hippocampus).

Three to five days after cannulation, a dialysis probe was implanted into the medial prefrontal cortex, hippocampus or nucleus accumbens under slight anesthesia with isoflurane (Metofane, Pitman-Moore, Mundelein, IL). Rats were then housed individually overnight in a dialysis cage. After the overnight perfusion at 0.4 μl/min of the probe, the flow was increased to 1.5 μl/min. One hour later, the dialysate samples were collected every 30 min. The perfusion medium was Dulbecco's phosphate-buffered saline solution (Sigma, St. Louis, MO) including Ca^{2+} (138 mM NaCl, 8.1 mM Na_2HPO_4, 2.7 mM KCl, 1.5 mM KH_2PO_4, 0.5 mM MgCl, 1.2 mM $CaCl_2$, pH 7.4). After stable baseline values in the dialysates were obtained, each rat received two injections, vehicle/aripiprazole, WAY100635/aripiprazole or aripiprazole/haloperidol. The locations of the dialysis probes were verified at the end of each experiment by brain dissection. The procedures applied in these experiments were approved by the Institutional Animal Care and Use Committee of Vanderbilt University in Nashville, TN, where the present studies were completed.

2.3. Biochemical assay

2.3.1. Determination of dopamine

Dialysate samples were directly applied onto a high-pressure liquid chromatography (HPLC) with electrochemical detection, and analyzed with a Millennium chromatogram manager (Waters, Milford, MA). Dopamine was separated (BDS Hypersil 3 μm C18, 1.0 × 100 mm; Keystone Scientific, Bellefonte, PA) at 35 °C maintained by column heater (LC-22C Temperature Controller; BAS, West Lafayette, IN). The mobile phase consisted of 48 mM anhydrous citric acid and 24 mM sodium acetate trihydrate containing 0.5 mM EDTA-Na_2, 10 mM NaCl, 2 mM dodecyl sulfate sodium salt and 17% (v/v) acetonitrile, adjusted to pH 4.8 with concentrated NaOH, and was pumped (0.05 ml/min) by LC-10AD (Shimadzu, Kyoto, Japan). A unijet working electrode (MF-1003, BAS) was set at +0.58 V (LC-4C, BAS) versus an Ag/AgCl reference electrode. Reagents used were analytical or high-performance liquid chromatography grade.

2.3.2. Determination of acetylcholine

The method has been described previously (Ichikawa et al., 2002a). In brief, dialysate samples are directly injected onto the liquid chromatography/electrochemistry (LCEC) system assisted by a chromatography manager (Millennium; Waters, Milford, MA), and analyzed for acetylcholine. Acetylcholine is separated on a coiled cation exchanger acetylcholine column (analytical column) (Sepstik 10 cm ID 530 °C 1.0 nm; BAS), followed by the post-IMER (immobilized enzyme reactor) (BAS) which consists of choline oxidase (ChO)/ acetylcholinesterase. Acetylcholine is hydrolyzed by acetylcholinesterase to form acetate and choline in the post IMER, and then choline is oxidized by ChO to produce betaine and hydrogen peroxide (H_2O_2). H_2O_2 is detected and reduced to H_2O on a Unijet amperometric detector cell with a peroxidase-redox-coated glassy carbon electrode (MF-9080; BAS), set at +100 mV (LC-4C; BAS) versus Ag/AgCl reference electrode. This reduction is analyzed with the detector (LC-4C; BAS) as signal indicating acetylcholine in the chromatogram.

2.4. Drugs

Aripiprazole (GlaxoSmithKline) was dissolved in 45% 2-hydroxypropyl-β-cyclodextrin (HBC) (Research Biochemical, Natick, MA). WAY100635 (Sandoz, Basel, Switzerland) were dissolved in deionized water. Haloperidol (McNeil, Spring House, PA) was dissolved in a small amount of 0.1 M tartaric acid and the pH was adjusted to 6–7 with 0.1 N NaOH. Vehicle or drugs in a volume of 1.0 ml/kg were administered s.c. to randomly assigned rats.

2.5. Data analysis

Only results derived from healthy rats with correctly positioned dialysis probes were included in the data analysis. Mean pre-drug baseline levels (time −60, time −30 and time 0) were designated as 100%. Following a significant overall repeated measures analysis of variance (ANOVA) (treatment × time), Fisher's protected least significant difference post hoc pairwise comparison and one-way ANOVA (StatView® 4.5 for the Macintosh) were used to determine group differences. A probability of $p < 0.05$ was considered significant in this study. All results are given as mean ± S.E.M.

3. Results

3.1. Effects of aripiprazole on dopamine release

As shown in Fig. 1, aripiprazole, 0.3 mg/kg, slightly but significantly *increased* extracellular dopamine concentrations in the medial prefrontal cortex ($F(1,15)=11.53$, $P=0.0009$, $n=9$ and 7) but not in the nucleus accumbens ($F(1,15)=0.73$, $P=0.40$, $n=9$ and 7). Aripiprazole, 0.3 mg/kg, significantly *increased* dopamine release in the hippocampus ($F(1,12)=4.26$, $P=0.04$, $n=8$ and 5). Moreover, 0.1 mg/kg of aripiprazole transiently *increased* dopamine release in the hippocampus at 60 min ($P<0.001$) and 150 min ($P<0.01$). Aripiprazole, 1 mg/kg, had no effect on dopamine release in the medial prefrontal cortex, hippocampus and nucleus accumbens ($F(1,9)=0.78$, $P=0.38$, $n=5$ and 5; ($F(1,11)=2.59$, $P=0.11$, $n=5$ and 7; $F(1,12)=0.321$, $P=0.57$, $n=6$ and 7, respectively). Aripiprazole, 3 mg/kg, *decreased* dopamine release in the nucleus accumbens ($F(1,11)=7.014$, $P=0.009$, $n=5$ and 7) without affecting that in the medial prefrontal cortex (Fig. 1). As shown in Fig. 2, aripiprazole, 10 mg/kg, ($F(1,12)=5.25$, $P=0.009$, $n=6$ and 7) significantly *decreased* dopamine release in the nucleus accumbens but to a less extent than the 3 mg/kg dose. Aripiprazole, 10 mg/kg, transiently *decreased* cortical dopamine release ($F(1,10)=7.05$, $P<0.01$, $n=6$ and 5).

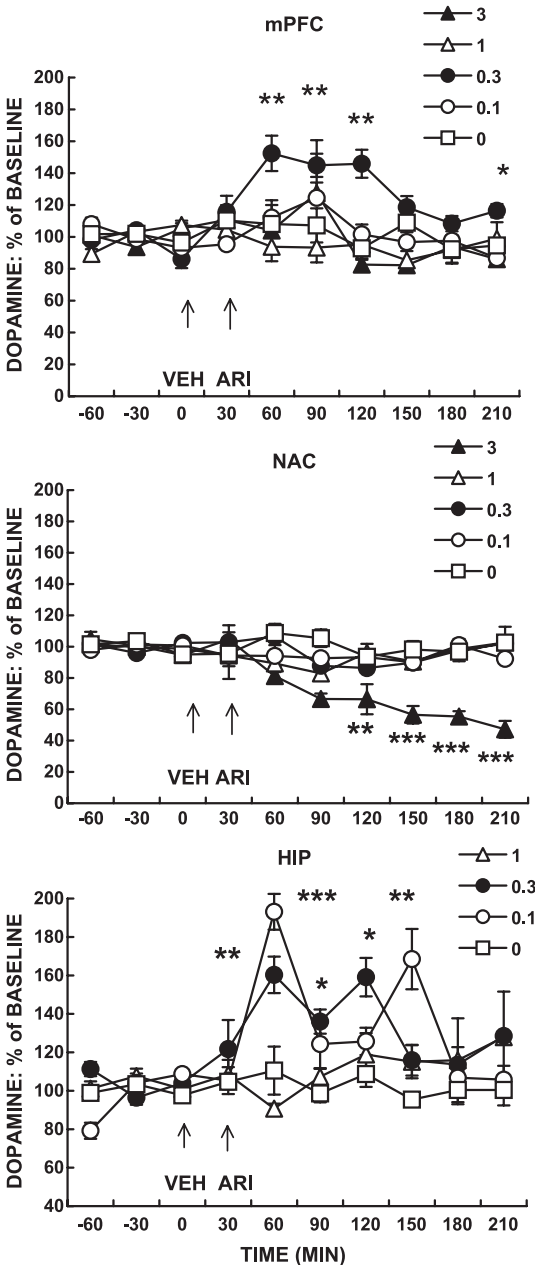

Fig. 1. Time course effects of aripiprazole (0.1–3 mg/kg, s.c.) on extracellular dopamine levels in the medial prefrontal cortex, nucleus accumbens, and hippocampus. Data are means ± S.E.M. of the dialysate dopamine levels, expressed as a percentage of each predrug baseline dopamine value. *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared to vehicle group. The arrows indicate drug injection times.

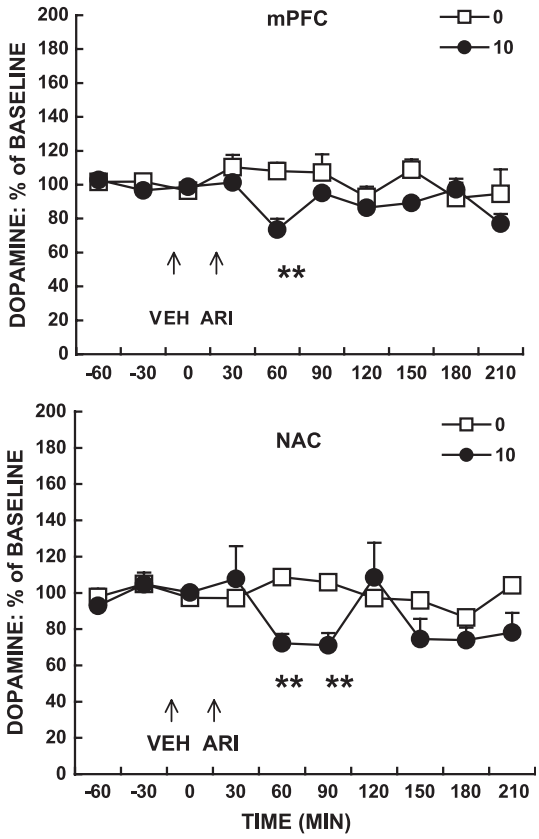

Fig. 2. The effect of aripiprazole (10 mg/kg, s.c.) on extracellular dopamine levels in the medial prefrontal cortex and nucleus accumbens. Data are means ± S.E.M. of the dialysate dopamine levels, expressed as a percentage of each predrug baseline dopamine value. **$P<0.01$ compared to vehicle group. The arrows indicate drug injection times.

3.2. Effect of WAY100635 pretreatment on aripiprazole-induced dopamine release

WAY 100635, the selective 5-HT_{1A} receptor antagonist, at a dose of 0.2 mg/kg, 30 min prior to aripiprazole,

completely attenuated the ability of aripiprazole, 0.3 mg/kg, to increase dopamine release in both the medial prefrontal cortex ($F(1,14)=26.3$, $P<0.0001$, $n=9$ and 6) and hippocampus ($F(1,11)=505$, $P=0.02$, $n=8$ and 4). WAY100635, 0.2 mg/kg, by itself, had no significant effect on dopamine release in the medial prefrontal cortex ($F(1,13)=0.59$, $P=0.45$, $n=7$) or hippocampus ($F(1,10)=0.47$, $P=0.974$, $n=6$ and 5) (Fig. 3).

3.3. Effect of haloperidol on aripiprazole-induced dopamine release

Pretreatment with aripiprazole, 0.3 mg/kg, transiently but significantly potentiated the ability of haloperidol, 0.1 mg/kg, to increase dopamine release in the medial prefrontal cortex ($F(1,13)=10.29$, $P=0.002$, $n=9$ and 5), whereas it inhibited the haloperidol (0.1 mg/kg)-induced dopamine release in the nucleus accumbens ($F(1,12)=18.96$, $P<0.001$, $n=8$ and 5) (Fig. 4).

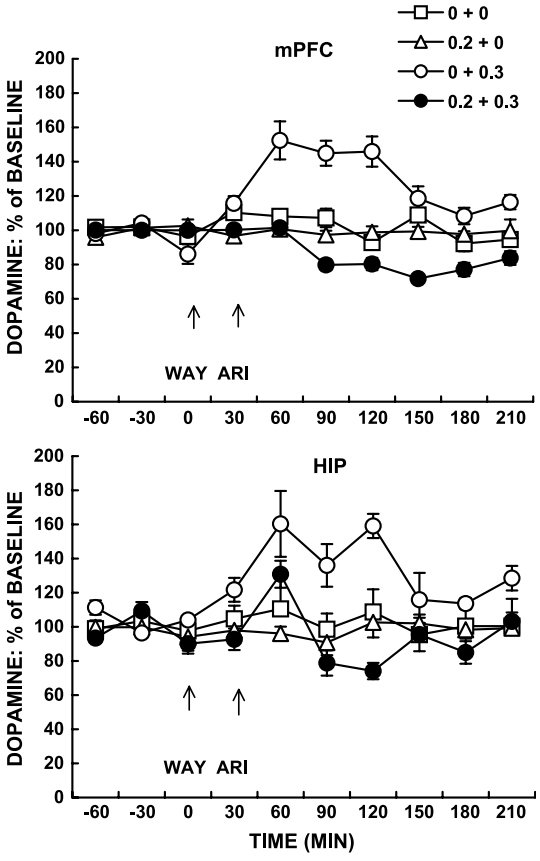

Fig. 3. The effect of WAY 100635 (0.2 mg/kg, s.c.), a selective 5-HT_{1A} receptor antagonist, on aripiprazole (0.3 mg/kg, s.c.)-induced dopamine release in the medial prefrontal cortex and hippocampus. Rats were pretreated with WAY 100605 30 min prior to administration of aripiprazole. Data are means ± S.E.M. of the dialysate dopamine levels, expressed as a percentage of each predrug baseline dopamine value. The arrows indicate drug injection times.

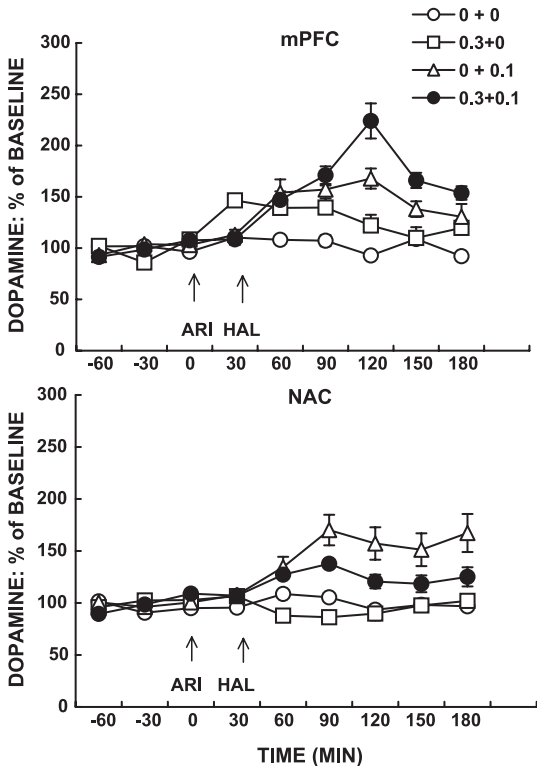

Fig. 4. The effect of aripiprazole (0.3 mg/kg, s.c.) on haloperidol (0.1 mg/kg, s.c.)-induced extracellular dopamine levels in the medial prefrontal cortex and nucleus accumbens. Rats were pretreated with aripiprazole 30 min prior to administration of haloperidol. Data are means ± S.E.M. of the dialysate dopamine levels, expressed as a percentage of each predrug baseline dopamine value. The arrows indicate drug injection times.

3.4. Effect of aripiprazole on acetylcholine release

Aripiprazole, 0.1, 0.3 and 1 mg/kg, had no effect on acetylcholine release in the medial prefrontal cortex, nucleus accumbens and hippocampus. However, at 3 mg/kg, it significantly decreased acetylcholine release in the nucleus accumbens ($F(1,12)=9.51$, $P=0.003$, $n=8$ and 5) but not in the medial prefrontal cortex and hippocampus (Fig. 5).

4. Discussion

The main findings in this study are that aripiprazole, 0.1 and 0.3 mg/kg, significantly increased dopamine in the hippocampus, and that 0.3 mg/kg of aripiprazole, slightly but significantly increased dopamine release in the medial prefrontal cortex, but not the nucleus accumbens. Secondly, as is the case with other atypical antipsychotic drugs, the increase in dopamine release in the medial prefrontal cortex and hippocampus produced by aripiprazole, 0.3 mg/kg, were significantly inhibited by WAY 100635, 0.2 mg/kg. We also found that pretreatment with aripiprazole, 0.3 mg/kg, potentiated the haloperidol, 0.1 mg/kg-induced dopamine release in the medial prefrontal cortex, but inhibited its

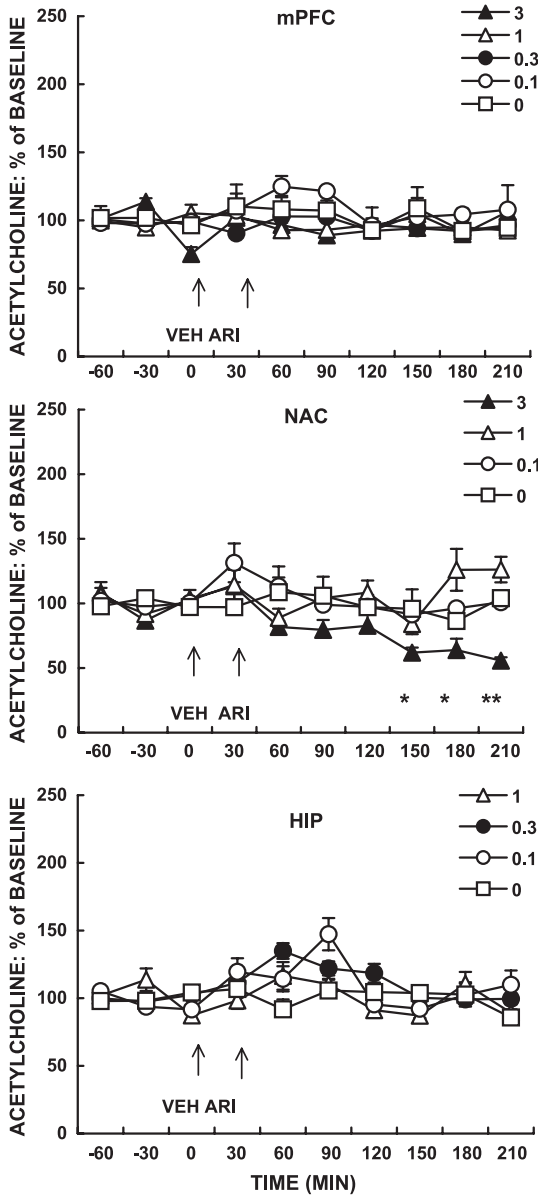

Fig. 5. Time course effects of aripiprazole (0.1–3 mg/kg, s.c.) on extracellular acetylcholine levels in the medial prefrontal cortex, nucleus accumbens, and hippocampus in the absence of acetylcholinesterase inhibition. Data are means ± S.E.M. of the dialysate acetylcholine levels, expressed as a percentage of each predrug baseline acetylcholine value. *$P<0.05$, **$P<0.01$ compared to vehicle group. The arrows indicate drug injection times.

ability to increase dopamine release in the nucleus accumbens. Aripiprazole, 3 and 10 mg/kg, significantly decreased dopamine release in the nucleus accumbens. In addition, aripiprazole, at doses of 0.1, 0.3 and 1 mg/kg, had no effect on acetylcholine release in the medial prefrontal cortex, hippocampus, or nucleus accumbens whereas aripiprazole, 3 mg/kg, decreased acetylcholine release in the nucleus accumbens but not the medial prefrontal cortex or hippocampus. In this regard, aripiprazole was dissimilar to other atypical antipsychotic drugs.

The effects of aripiprazole, 0.3 mg/kg, on cortical dopamine release are similar to what has been reported for other atypical antipsychotic drugs (Ichikawa et al., 2001; Kuroki et al., 1999; Moghaddam and Bunney, 1990; Volonte et al., 1997; Rollema et al., 2000), although the magnitude of the increase of aripiprazole is less than that of the other atypical antipsychotics. It is unclear if this slight increase, were it to occur in patients, would be sufficient to improve negative symptom and cognition. The effect of aripiprazole (0.3 mg/kg) to increase dopamine release in the medial prefrontal cortex is most consistent with its weak antagonism of D2 receptors, in combination with functional 5-HT_{2A} receptor antagonism and/or 5-HT_{1A} receptor partial agonism, as will be discussed. It has been reported that low (0.1 mg/kg) but not high (1.0 mg/kg) dose of haloperidol slightly increased cortical dopamine release (Kuroki et al., 1999; Bonaccorso et al., 2002). Aripiprazole, at low dose of 0.1–0.5 mg/kg, was reported, like haloperidol, 0.5 mg/kg, to inhibit the effect of the dopamine receptor agonist talipexole on yawning in rats (Fujikawa et al., 1996), suggesting that at this dose range, aripiprazole exhibits weaker agonistic and stronger antagonistic effects (Fujikawa et al., 1996). In addition, at a low dose of 2.5 mg/kg, aripiprazole increased DOPAC and HVA in the medial prefrontal cortex and striatum (Semba et al., 1995). The inability of aripiprazole at dosages higher than 0.3 mg/kg to increase cortical dopamine release in the rat suggests that the extent of D2 receptor blockade needed to increase dopamine release with a partial D2 receptor agonist occurs within a narrow range, analogous to the narrow range of D1 receptor activation needed for optimal working memory performance in the non-human primate (William and Goldman-Rakic, 1995). The ability of atypical antipsychotic drugs which are full D2 receptor antagonists, e.g. clozapine, to increase dopamine release in the medial prefrontal cortex is evident over a much wider range of doses. Blockade of the effect of aripiprazole, 0.3 mg/kg, to increase extracellular dopamine in the medial prefrontal cortex by WAY100635 is compatible with the 5-HT_{1A} receptor partial agonist properties of aripiprazole and the role of 5-HT_{1A} receptor agonism in the release of cortical dopamine (Rollema et al., 2000; Ichikawa et al., 2001). A behavioral study also is consistent with aripiprazole having 5-HT_{1A} receptor agonist properties in vivo (Marona-Lewicka and Nichols, 2003). These results suggest that 5-HT_{1A} receptor agonism plays a role in the action of aripiprazole comparable to that in other atypical antipsychotic drugs (Millan et al., 1998; Ichikawa et al., 2002c).

The effect of higher doses of aripiprazole, 3 and 10 mg/kg, to decrease dopamine release in the nucleus accumbens is consistent with its dopamine receptor agonist action as other dopamine receptor agonists, e.g. quinpirole, a dopamine D2/3 receptor agonist, also decrease dopamine release in the nucleus accumbens (Barnes et al., 1990; Yamada et al., 1994; See, 1994). The D3 receptor preferring agonist PD128907 ((+)-*trans*-3,4,4*a*,10*b*-tetrahydro-4-propyl-2*H*,5*H*-[1]benzopyrano[4,3*b*]-1,4-oxasin-9-ol) has been shown to decrease

dopamine release in the rat cortex. It is noteworthy that aripiprazole is also a D3 receptor agonist (Pugsley et al., 1995). We also studied the effect of high dose aripiprazole, 10 mg/kg, on dopamine release. Our results are consistent with those of Semba et al. (1995) who reported that aripiprazole, 10 and 40 mg/kg, decreases cortical dopamine release, although the decrease found in the present study was small in comparison. The 40 mg/kg dose did not significantly affect extracellular DOPAC or HVA levels, whereas the 2.5 and 10 mg/kg increased extracellular DOPAC and HVA levels in the medial prefrontal cortex (Semba et al., 1995). However, the clinical release of these higher doses of aripiprazole on cortical dopamine release is uncertain, since the aripiprazole dose in man is approximately 0.15–0.5 mg/kg/day (Carson et al., 2000; Kane et al., 2002). The decrease in extracellular dopamine in the ventral striatum (nucleus accumbens), at doses of 3 and 10 mg/kg reported here, is consistent with the decrease in dopamine release in the dorsal striatum reported by Semba et al. (1995) at doses ≥ 2.5 mg/kg, suggesting that aripiprazole, in that dose range, has an agonist action at D2/D3 autoreceptors in both the dorsal and ventral striatum. Decreased dopamine release in the ventral striatum, by diminishing excessive dopamine release, may contribute to decreasing psychotic symptoms in patients with schizophrenia (Laruelle et al., 1996). However, a decrease in dopamine release in the cortex might produce a worsening of cognition or negative symptom. Aripiprazole has been referred to as a dopamine system stabilizers, e.g. a drug which can enhance dopaminergic activity when it is diminished or suppress it when it is increased (Stahl, 2001a, b). The enhancement of dopaminergic activity may be based on D2 receptor partial agonism when dopamine release is low as well as dopamine release in the cortex or hippocampus. It would be expected to diminish dopaminergic activity when dopamine release at D2 receptors is increased.

Haloperidol, 0.1 mg/kg, by itself, has been reported to slightly increase dopamine release in the medial prefrontal cortex but not the nucleus accumbens, and to produce no significant effect on acetylcholine release in the medial prefrontal cortex (Liegeois et al., 2002; Ichikawa et al., 2002b). The effect of haloperidol, 0.1 mg/kg but not 1.0 mg/kg, to increase dopamine release in the medial prefrontal cortex, is potentiated by pretreatment with the 5-HT_{2A} receptor antagonist M100907 ((+)-alpha-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol) or the 5-$HT_{2A/2C}$ receptor antagonist SR46349-B (*trans*-4-([3*Z*]3-(2-dimethylaminoethyl)oxyimino-3(2-flurophenyl+++)propen-1-yl]phenol hemifumarate), while the larger increase in dopamine release produced by both doses of haloperidol in the nucleus accumbens is inhibited by M100907 and potentiated by SR46349-B (Liegeois et al., 2002; Bonaccorso et al., 2002). The lower, but not the higher, dose of haloperidol would be expected to produce dopamine receptor occupancy less than 60% in the striatum (Schotte et al., 1996). Aripiprazole, 0.3 mg/kg, which increased cortical dopamine release to a similar extent as low dose haloperidol, also significantly potentiated low dose haloperidol (0.1 mg/kg)-induced dopamine release in the medial prefrontal cortex but inhibited that in the nucleus accumbens, as previously reported with M100907 and 8-hydroxy-2(di-*n*-propylamino)tetralin (8-OH-DPAT), suggesting that these effects of aripiprazole are related to its 5-HT_{2A} receptor antagonist or 5-HT_{1A} receptor agonist properties or both. The inhibitory effect of haloperidol in the nucleus accumbens may also be due, in part, to dopamine receptor agonist properties. These results suggest that aripiprazole may have its most beneficial effects on cognition and perhaps other outcome measures at relatively low range of doses. This is consistent with clinical evidence with regard to efficacy for positive and negative symptoms. The antipsychotic effect of aripiprazole is evident at doses of 0.15–0.50 mg/kg/day (Carson et al., 2000; Kane et al., 2002), comparable to the doses effective to modulate cortical dopamine release in rats reported here. Of course, pharmacokinetic difference between species may obviate such comparisons. Aripiprazole, 3 mg/kg, significantly decreased dopamine release in the nucleus accumbens. This dose is 10-fold higher than the dose which increased dopamine release in the medial prefrontal cortex and hippocampus. It is unclear whether both effects can be achieved in man within the dose range of 0.15–0.5 mg/kg/day.

The present study demonstrated that aripiprazole produced identical dose–response effects on dopamine release in the hippocampus and the medial prefrontal cortex. The finding that aripiprazole has a comparable dose–response relationship with regard to dopamine release in both regions of the brain provides additional support for the validity of the finding that aripiprazole increases dopamine release in the medial prefrontal cortex only at a low dose, 0.3 mg/kg. It appears to have a broader dose–response effect in the hippocampus. This is the first report that a partial dopamine receptor agonist such as aripiprazole increases dopamine release in the hippocampus and that this increase was inhibited by the selective 5-HT_{1A} receptor agonist, WAY 100635. We have noted similar findings for clozapine and risperidone as well (Chung et al., in preparation), suggesting that release of dopamine in the medial prefrontal cortex and hippocampus are controlled by similar mechanisms.

Clozapine, quetiapine, olanzapine, risperidone and ziprasidone increase acetylcholine release in the medial prefrontal cortex or hippocampus, or both (Ichikawa et al., 2002b; Shirazi-Southall et al., 2002). This may contribute to the ability of atypical antipsychotic drugs to improve cognitive dysfunction. However, in the present study, aripiprazole alone had no effect on acetylcholine release in the medial prefrontal cortex and hippocampus. Aripiprazole has no anticholinergic effect (Kikuchi et al., 1995) and does not interact significantly with any muscarinic receptor (Shapiro et al., 2003). Our results with aripiprazole alone suggest that acetylcholine release in the medial prefrontal cortex or

hippocampus do not contribute to the reported beneficial effects of aripiprazole on cognition in patients with schizophrenia. Our present results also suggest that the combination of 5-HT_{1A} receptor stimulation and 5-HT_{2A} receptor antagonism is not sufficient to increase acetylcholine release in the medial prefrontal cortex or hippocampus, since aripiprazole is a potent 5-HT_{1A} receptor partial agonist and 5-HT_{2A} receptor antagonist. It has been reported that 5-HT_{1A} receptor agonists, e.g. 8-OH-DPAT, ipsapirone and buspirone, increase acetylcholine release in the medial prefrontal cortex and hippocampus (Wilkinson et al., 1994; Consolo et al., 1996; Fujii et al., 1997; Ichikawa et al., 2002a). The increases in acetylcholine release by these agents has been suggested to occur through a polysynaptic mechanism, possibly including postsynaptic 5-HT_{1A} receptors and dopamine D1 receptors (Consolo et al., 1996; Ichikawa et al., 2002a). The dissociation of enhanced acetylcholine and dopamine release in the medial prefrontal cortex with aripiprazole but not the other atypical antipsychotic drugs could prove useful to determine the importance, if any, of the increase in acetylcholine release in the medial prefrontal cortex to improve cognition in patients with schizophrenia.

In conclusion, aripiprazole, at low doses ($\leq$0.3 mg/kg), preferentially increased dopamine release in the medial prefrontal cortex and hippocampus compared to the nucleus accumbens by a mechanism requiring the stimulation of 5-HT_{1A} receptors, suggesting that aripiprazole is similar to other atypical antipsychotic drugs in these regards. Aripiprazole, at high doses (>3 mg/kg) decreased dopamine release in the nucleus accumbens. These effects on dopamine release in the medial prefrontal cortex, hippocampus or nucleus accumbens may contribute to the ability of aripiprazole to improve positive and negative symptoms as well as cognition, in patients with schizophrenia.

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