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Differential effects of acute administration of haloperidol and clozapine on ethanol-induced ascorbic acid release in rat striatum

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Abstract

Antipsychotic drugs were initially considered to act predominantly through their antagonism at dopamine D_2-like receptors. However, reports have demonstrated that the typical neuroleptic drug haloperidol and the atypical neuroleptic drug clozapine showed differential actions in clinical, behavioral and biochemical studies. Since ascorbic acid has a potential usefulness in psychological therapeutics, the present study investigates the actions of these two drugs on ethanol-induced ascorbic acid release in the striatum in order to help explain the different mechanisms of these drugs. The results showed that clozapine, at the doses of 15 and 30 mg/kg, i.p., had no effect on basal ascorbic acid release. However, a synergistic tendency at a dose of 15 mg/kg and a significant synergism at a dose of 30 mg/kg were observed on ascorbic acid release when clozapine was used with ethanol. In contrast, haloperidol, at the doses of 0.5, 1.0 and 2.0 mg/kg, i.p., administered alone did not affect the basal release of striatal ascorbic acid, and when used together with ethanol had neither a potentiating nor an antagonizing effect on ethanol-induced ascorbic acid release. Chlorpromazine, a nonselective dopamine receptor antagonist, at the dose of 5 mg/kg, i.p., affected neither the basal nor the ethanol-induced ascorbic acid release. Ritanserin, a 5-HT_2 receptor antagonist, at the dose of 1 mg/kg, s.c., significantly antagonized ethanol-induced ascorbic acid release. These results demonstrate that clozapine dose-dependently potentiates the stimulatory effect of ethanol on striatal ascorbic acid release and this effect of clozapine may not be related to its dopamine D_2 receptor antagonism.

Keywords: Clozapine; Haloperidol; Ritanserin; Ascorbic acid; Ethanol; Microdialysis; (Rat)

1. Introduction

The action of antipsychotic drugs was initially considered to predominantly involve the antagonism of dopamine D_2-like receptors (Carlsson, 1978). However, accumulated data have demonstrated the differential actions of some antipsychotic drugs with the property of dopamine D_2 receptor antagonism in clinical, behavioral and biochemical studies. For example, although used for the management of psychotic symptoms, haloperidol, a typical neuroleptic, has a high risk of causing extrapyramidal effects (EPS), such as tardive dyskinesia and parkinsonism, in patients (Casey, 1997) and catalepsy in experimental animals (Dorris and Dill, 1986). In contrast, clozapine, an atypical neuroleptic which is more effective than other neuroleptic drugs at reducing the positive, negative and disorganized symptoms of schizophrenia, rarely produces EPS (Lieberman et al., 1989).

It is well known that the antipsychotic potency of ''typical'' neuroleptics is related to their affinity for the dopamine D_2 receptor (Creese et al.,1983). Recent studies have shown that the ''atypical'' drugs, such as clozapine, bind to a range of neurotransmitter receptors, such as dopamine receptors, 5-HT receptors, muscarinic receptors and adrenoceptors (Guo et al., 1995). More recently, it has been reported that clozapine also antagonizes α_2-adrenoceptors (Hertel et al., 1999) and regulates $GABA_A$ receptors (Farnbach-Pralong et al., 1998). Moreover, haloperidol and clozapine differentially affect the excitatory amino acid system (Meador-Woodruff et al., 1996; Duncan et al., 1998). These findings make it more difficult to understand the relationship between the pharmacological properties of antipsychotic drugs and their clinical actions.

It has been shown recently that ascorbic acid acts a neuromodulator in the central nervous system. Biochemically, ascorbic acid inhibits the binding of dopamine to

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homogenates of neostriatal tissue (Rebec and Pierce, 1994) and inhibits the binding of dopamine antagonists (Kimura and Sidhu, 1994). Behaviorally, ascorbic acid blocks amphetamine-induced focused stereotypy (Tolbert et al., 1979) and locomotion (Heikkila et al., 1981). Dopamine receptor agonists can stimulate the release of endogenous ascorbic acid in rat striatum (Pierce and Rebec, 1990), and dopamine receptor antagonists can block amphetamine-induced striatal ascorbic acid release (Oh et al., 1989). These observations strongly suggest the close functional relationship between the central dopaminergic system and ascorbic acid release.

It has been proposed that it is a common property of all indirect dopamine receptor agonists to stimulate endogenous ascorbic acid release (Pierce and Rebec, 1990). Ethanol, being partially an indirect dopamine receptor agonist, also stimulates ascorbic acid release in the striatum (Svensson et al.,1992; Wu et al., 1998a). It is reported that ascorbic acid potentiates the actions of haloperidol in experimental animals, suggesting a potential usefulness of ascorbic acid in psychological therapeutics (Rebec et al., 1985; Dorris and Dill, 1986). Since the pharmacological implication of drug-induced endogenous ascorbic acid release is not clear at present, it is interesting to investigate whether the stimulated release of endogenous ascorbic acid by drugs, such as ethanol, plays role in psychological therapy. In studies of the relationship between the dopaminergic system and ethanol-induced ascorbic acid release, we observed that the ability of ethanol to induce ascorbic acid release in the striatum could be differentially affected by different dopaminergic agents (Liu et al., 1997). Since haloperidol and clozapine are representative typical and atypical dopamine receptor antagonists, respectively, further investigation of the differential actions of these two drugs on ethanol-induced ascorbic acid release in the striatum may be beneficial to highlight the different mechanisms of these drugs in antipsychotic treatments.

2. Materials and methods

2.1. Animals

Female Wistar rats weighing 200–250 g were used in the experiments. The rats were provided by the Experimental Animal Center of Shenyang Pharmaceutical University. The rats were housed under standard conditions with food and water ad libitum and maintained on 12L:12D cycle. All animal use procedures were in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China on November 14th, 1988. The experiments were carried out under approval of the Committee of Experimental Animal Administration of the University.

2.2. Implantation of the dialysis probe and brain dialysis

Rats were anesthetized with chloral hydrate (350 mg/kg i.p.) and implanted with Hospal AN 69 dialysis fibers (310 μm o.d., 200 μm i.d., Dasco, Bologna, Italy) transversally through the striata according to the following coordinates: A +1.5 mm from bregma, V −5.6 mm from occipital bone (Paxinos and Watson, 1982). The procedure used to prepare and implant the dialysis probe was essentially the same as that described previously (Consolo et al., 1987; Wu et al., 1988).

Brain dialysis was performed about 24 h after probe implantation in freely moving rats. Ringer's solution (147 mM NaCl, 2.2 mM $CaCl_2$ and 4 mM KCl) was pumped through the dialysis probe at the constant rate of 5 μl/min. After a 30-min washout, the dialysis samples were collected every 10 min and analyzed. Test solutions (saline, ethanol or drugs) were administered when the baseline of ascorbic acid output was stable in last three samples. At the end of the experiments, the position of the dialysis fiber was verified by visual examination, and the data were discarded if the fiber was positioned incorrectly.

2.3. Analysis procedure

Dialysate sample (20 μl) was injected into a high-performance liquid chromatograph with electrochemical detection. A reversed-phase column (ODS C-18, 5 mm, Dalian Chem-Physic. Institute, China) was used with the mobile phase composed of 155.6 mM NaCl and 0.54 mM EDTA-Na_2 with 1.5 mM tetrabutylammonium bromide as an ion-pairing agent. The mobile phase was pumped with a LC-10A pump (Shimadzu, Japan) at a flow rate of 1.0 ml/min. The detector (641 amperometric detector, Metrohm, Switzerland) was set at +0.6 V. The retention time for ascorbic acid under these conditions was about 4 min. The sensitivity of the ascorbic acid assay was 2 ng per sample (Wu et al., 1998a).

2.4. Drugs

Haloperidol hydrochloride (Shanghai Haipu Pharmaceutical Factory, Shanghai, China) and chlorpromazine hydrochloride (Dandong Pharmaceutical Factory, Dandong, China) were dissolved in saline. Clozapine (Changzhou Pharmaceutical Factory, Changzhou, China) was dissolved in 0.3 M HCl in saline. Ritanserin (RBI, Natick, USA) was dissolved in dimethyl sulfoxide (DMSO). Ethanol (AR) was purchased from Shenyang Reagents Co., China, and diluted with saline to 20% before use. Ethanol was injected intraperitoneally at a dose of 3.0 g/kg as previously reported (Wu et al., 1998a).

2.5. Statistical analysis

Statistical analysis was carried out by using SAS software (SAS Institute, Cary, NC). To assess the significance

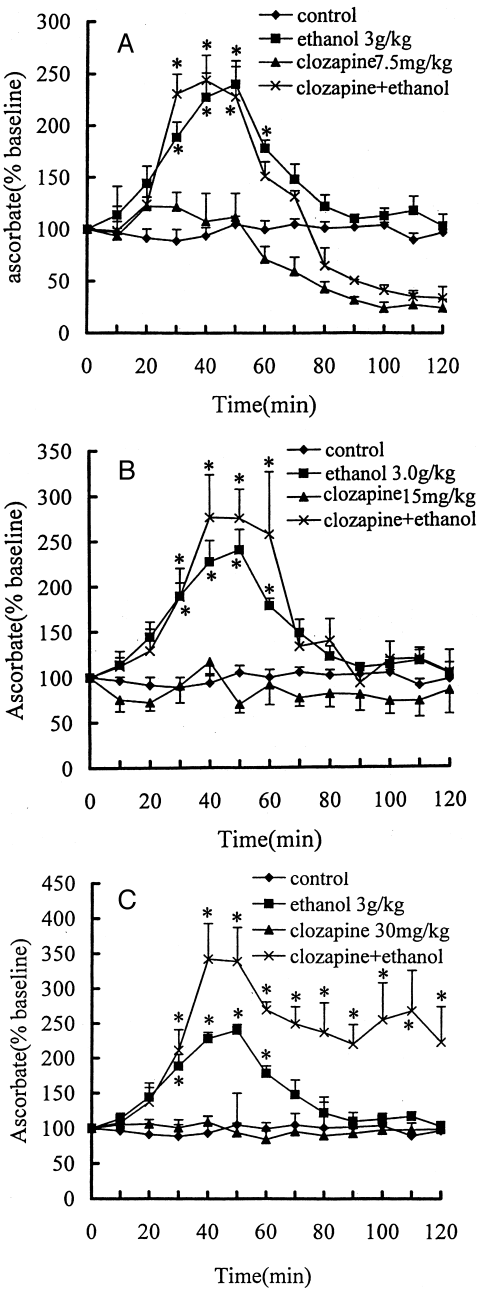

Fig. 1. Effect of clozapine on ethanol-induced ascorbic acid release in rat striatum. Clozapine was administered intraperitoneally, at the doses of 7.5 mg/kg (A), 15 mg/kg (B) and 30 mg/kg (C), 10 min before ethanol administration (3 g/kg, i.p.). Ascorbic acid release is expressed as the percentage change from baseline. Data shown are means ± S.E.M. for 5–8 rats. $^{*}P < 0.05$ compared with saline control group.

of differences between groups, summed effects of drugs over the course of an experiment were used to compare treatment area under the curve (AUC) by multifactor analysis of variance (ANOVA) followed by Fisher's least-significant difference post hoc tests. Two-way ANOVA was used to evaluate the interaction between drug treatment and ethanol groups. Ascorbic acid values are expressed as the percentage changes compared with the respective basal value, which was the mean of three consecutive samples within a variation of 10%.

3. Results

Ethanol, at the dose of 3 g/kg, i.p., induced a significant increase in ascorbic acid release in the striatum. The

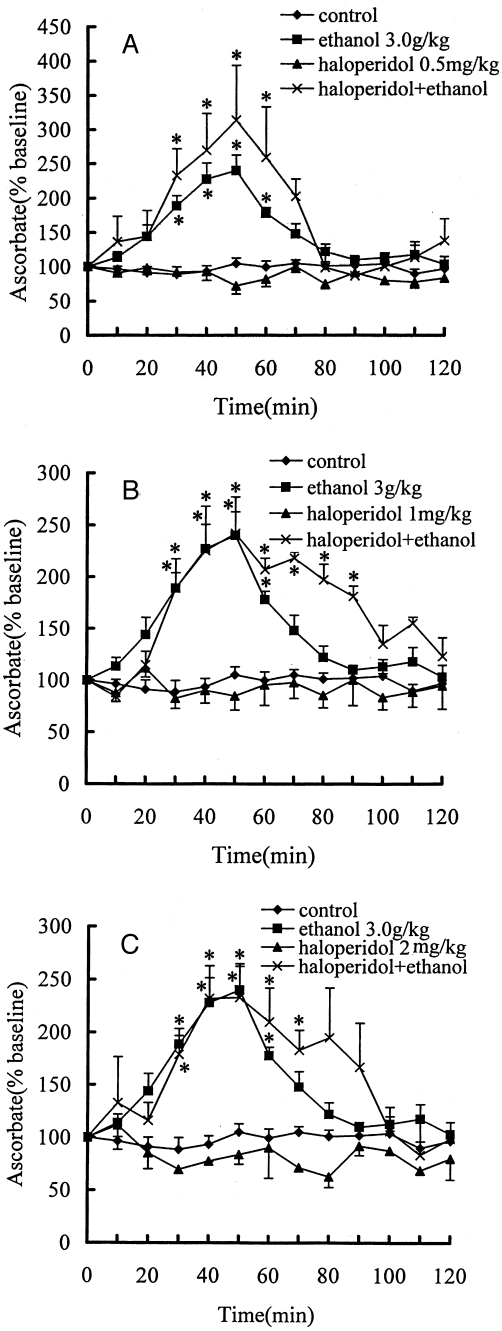

Fig. 2. Effect of haloperidol hydrochloride on ethanol-induced ascorbic acid release in rat striatum. Haloperidol hydrochloride was administered intraperitoneally, at the doses of 0.5 mg/kg (A), 1 mg/kg (B) and 2 mg/kg (C), 10 min before ethanol administration (3 g/kg, i.p.). Ascorbic acid release is expressed as the percentage change for baseline. Data shown are means ± S.E.M. for 5–8 rats. $^{*}P < 0.05$ compared with saline control group.

greatest effect was observed 50 min after ethanol administration, with the ascorbic acid level being more than 150% higher than baseline (see Fig. 1, ethanol group).

3.1. Effect of clozapine

Clozapine, at the dose of 7.5 mg/kg, i.p. administered alone, decreased striatal ascorbic acid release ($F(1,15) = 4.46$, $P = 0.056$) 50 min after drug administration. When it was used with ethanol, it neither potentiated nor antagonized the increasing effect of ethanol on ascorbic acid release ($F(1,14) = 0.77$, $P = 0.396$). However, when the effect of ethanol diminished, clozapine still decreased ascorbic acid release (Fig. 1A). When the dose was increased to 15 and 30 mg/kg, clozapine did not affect basal ascorbic acid release (Fig. 1B,C). However, a synergistic tendency was observed on ascorbic acid release when 15 mg/kg of clozapine was used with ethanol ($F(1,14) = 4.55$, $P = 0.054$) (Fig. 1B). Furthermore, a significant synergism was obtained on ascorbic acid release when 30 mg/kg of clozapine was used with ethanol ($F(1,14) = 8.88$, $P = 0.009$) (Fig. 1C). These results show that clozapine dose-dependently potentiated the stimulatory effect of ethanol on striatal ascorbic acid release.

3.2. Effect of haloperidol

Haloperidol, at the doses of 0.5, 1.0 and 2.0 mg/kg, i.p., administered alone did not affect the basal release of striatal ascorbic acid. When it was used together with ethanol, neither a potentiating nor an antagonizing effect was observed on ethanol-induced ascorbic acid release ($F(1,15) = 1.65$, $P = 0.225$ for haloperidol 0.5 mg/kg × ethanol; $F(1,15) = 1.59$, $P = 0.231$ for haloperidol 1.0 mg/kg × ethanol; $F(1,15) = 2.91$, $P = 0.116$ for haloperidol 2.0 mg/kg × ethanol) (Fig. 2A,B,C).

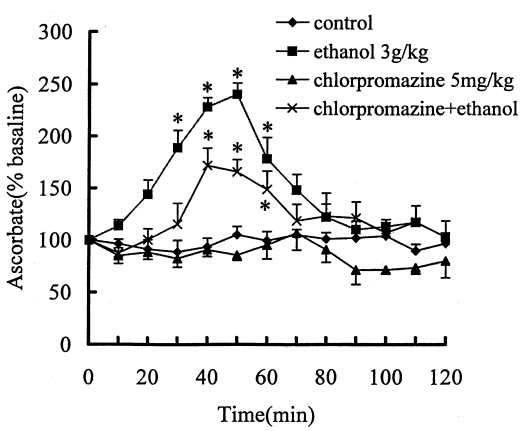

Fig. 3. Effect of chlorpromazine hydrochloride on ethanol-induced ascorbic acid release in rat striatum. Chlorpromazine hydrochloride was administered intraperitoneally, at the dose of 5 mg/kg, 10 min before ethanol administration (3 g/kg, i.p.). Ascorbic acid release is expressed as the percentage change from baseline. Data shown are means ± S.E.M. for 5–8 rats. * $P < 0.05$ compared with saline control group.

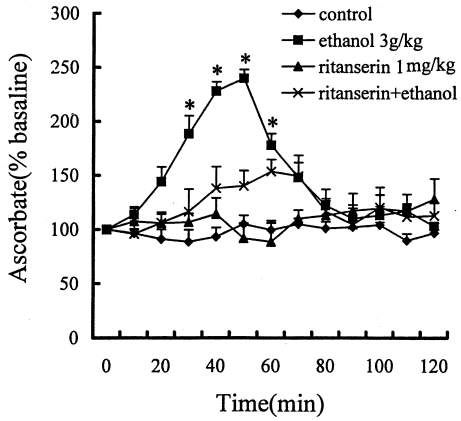

Fig. 4. Effect of ritanserin on ethanol-induced ascorbic acid release in rat striatum. Ritanserin was administered intraperitoneally, at the dose of 1 mg/kg, 10 min before ethanol administration (3 g/kg, i.p.). Ascorbic acid release is expressed as the percentage change from baseline. Data shown are means ± S.E.M. for 5–8 rats. * $P < 0.05$ compared with saline control group.

3.3. Effect of chlorpromazine

Chlorpromazine, at dose of 5 mg/kg, i.p., neither affected the basal, nor potentiated the ethanol-induced ascorbic acid release in rat striatum (Fig. 3).

3.4. Effect of ritanserin

Ritanserin, at the dose of 1 mg/kg, s.c., did not affect the basal ascorbic acid release. However, it significantly inhibited ethanol-induced ascorbic acid release when it was used in combination with ethanol ($F(1,16) = 7.37$, $P = 0.018$) (Fig. 4).

4. Discussion

The present results provide the first evidence that haloperidol and clozapine differentially regulate ethanol-induced ascorbic acid release in rat striatum. Although the link between the different effects of these two drugs and their clinical usage is not clear, these results add some new neurochemical data supporting the different mechanisms of actions of typical and atypical neuroleptics in antipsychotic treatments.

The antipsychotic potency of neuroleptic drugs, such as haloperidol, was found to relate to their blockade of dopamine D_2 receptors (Creese et al., 1983). However, besides blocking dopamine D_2 receptors (Farde et al., 1992), it has been shown recently that clozapine, an atypical neuroleptic drug which has markedly better efficacy than the typical neuroleptic drugs, acts also on other neuronal systems. Moreover, the various activities of

clozapine on different neuronal systems have been hypothesized to be important for its unique clinical profiles, which challenges the dopamine hypothesis of antipsychotic therapy.

Biochemical (Saller et al., 1990; Schmidt and Fadayel, 1995), electrophysiological (Ugedo et al., 1989) and behavioral (Kulikov et al. 1994) studies have demonstrated the existence of functional interactions between brain serotonergic and dopaminergic systems. Especially the high affinity of clozapine at 5-HT_2 receptors (Meltzer et al., 1989; Meltzer, 1992; Meltzer, 1999) and the agonistic property of clozapine at 5-HT_{1A} receptors (Rollema et al., 1997) provide an explanation for the unique antipsychotic profile of the atypical neuroleptic drugs. Clozapine, like ritanserin and 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin) (Pehek et al., 1993, Rollema et al., 1997), strongly increases dopamine release in the medial prefrontal cortex (Meltzer et al., 1989; Meltzer, 1992), which may locally activate dopamine D_1 and D_2 receptors. It has been demonstrated that the effect of clozapine on dopamine release in the medial prefrontal cortex is due to its blockade of 5-HT_2 receptors (Meltzer et al., 1989; Meltzer, 1992), or is, for a large part, mediated via activation of 5-HT_{1A} receptors (Rollema et al., 1997). However, this effect of clozapine cannot explain its action on ethanol-induced ascorbic acid release because in the present study we observed that ritanserin, a selective 5-HT_2 receptor antagonist, inhibited ethanol-induced ascorbic acid release, which is opposite to the ascorbic acid releasing effect of clozapine. Moreover, in another experiment we have observed that activation of 5-HT_{1A} receptors by 8-OH-DPAT also inhibited ethanol-induced ascorbic acid release (Wu et al., 1998b). Thus, although clozapine displays a significant effect on 5-HT_2 and 5-HT_{1A} receptors (Meltzer et al.,1989; Meltzer, 1992; Rollema et al., 1997), the present data suggest that its antagonistic property on 5-HT_2 and agonistic property on 5-HT_{1A} receptors is not responsible for its synergistic effect on ethanol-induced ascorbic acid release in the striatum.

It is assumed that the absence of dopamine D_2 receptor-mediated catalepsy with clozapine is due to its concomitant potent 5-HT_{2A} receptor blockade (Meltzer, 1995). Although 8-OH-DPAT and ritanserin have been shown to block dopamine D_2 receptor-mediated catalepsy and to share properties that are liable to transform a typical antipsychotic into an atypical antipsychotic (Invernizzi et al., 1988; Lucas et al.,1997), the previous and present studies have clearly shown that ritanserin and 8-OH-DPAT do not behave like clozapine in the regulation of ethanol-induced ascorbic acid release (Wu et al., 1998b). These results suggest that it is not possible to discriminate between antipsychotic drugs as typical and atypical ones by their effects on ethanol-induced ascorbic acid release.

Clozapine has a range of activities at many receptors, such as blockade of dopamine D_1, D_2, 5-HT_{2A}, muscarinic M_2, M_3, M_5 receptors and α_1- and α_2-adrenoceptors (Corbett et al., 1993; Zorn et al., 1994), activation of D_1, D_2, 5-HT_{1A}, M_1 and M_4 receptors (Bolden et al., 1992; Zorn et al., 1994; Salmi and Ahlenius, 1996; Rollema et al., 1997; Zeng et al., 1997; Ninan and Kulkarni 1998), and activation of GABAergic (Farnbach-Pralong et al., 1998) and glutamatergic neurotransmission (Daly and Moghaddam, 1993; Yamamoto et al., 1994). It also binds to dopamine D_4 receptors (Van Tol et al., 1991) and with moderate affinity to 5-HT_6 and 5-HT_7 receptors (Roth et al., 1994). However, as we know, blockade of dopamine D_2 and 5-HT_{2A} receptors, and activation of 5-HT_{1A} receptors antagonizes, whereas blockade of dopamine D_1 receptors potentiates ethanol-induced ascorbic acid release in the striatum (Liu et al., 1997; Wu et al., 1998b). Moreover, activation of GABAergic and glutamatergic neurotransmission augments extracellular striatal ascorbic acid levels (Bigelow et al., 1984; Rebec and Pierce, 1994) and activation of muscarinic M_1 and M_4 receptors decreases glutamatergic neurotransmission (Smolders et al., 1997; Rawls and McGinty, 1998). Thus, it is reasonable to assume that blockade of dopamine D_1 receptors, or activation of GABAergic and glutamatergic neurotransmission is responsible for the clozapine-induced potentiation of the ethanol-induced ascorbic acid release. However, the mechanisms of potentiation of ethanol-induced striatal ascorbic acid release by clozapine may be more complicated than those discussed here. The contribution of other receptors, such as adrenoceptors, on the effect of clozapine in this experimental model is obscure as yet. It is possible that the potentiating effect of clozapine on ethanol-induced ascorbic acid release is the outcome of the interactions of these receptors and neuronal systems. The differential effects shown by clozapine and haloperidol in the present study provide new information for explaining the clinical differences between the two agents.

The lack of effect of haloperidol on ethanol-induced ascorbic acid release could be tentatively explained by its mixed effect on dopamine D_1 and D_2 receptors (Shen et al., 1995), since chlorpromazine, another nonselective neuroleptic acting on both dopamine D_1 and D_2 receptors, also showed no significant effect on ethanol-induced ascorbic acid release. In our another study, we observed that L-sulpiride, a selective dopamine D_2 receptor antagonist, inhibited and SCH 23390 ((*R*)-(+)8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol hemimaleate), a selective dopamine D_1 receptor antagonist, potentiated ethanol-induced ascorbic acid release. When L-sulpiride and SCH23390 were used in combination, their respective inhibiting and potentiating effects on ethanol-induced ascorbic acid release disappeared (Liu et al., 1997). Thus, it is possible that the lack of the effect of haloperidol and chlorpromazine on ethanol-induced ascorbic acid release in the striatum results from the counteraction of both effects due to simultaneous antagonism of dopamine D_1 and D_2 receptors. Clozapine, at the lower dose of 7.5 mg/kg, showed a decreased tendency to affect basal

ascorbic acid release. This may result from its mixed dopamine D_1 and D_2 receptor antagonistic property, since chlorpromazine and haloperidol at a higher dose also slightly decreased basal ascorbic acid release in the present experiments.

Recent studies have indicated that clozapine may activate the glutamatergic system. Acute administration of clozapine, but not haloperidol, increases glutamate and aspartate levels in the medial prefrontal cortex of rats (Daly and Moghaddam, 1993; Yamamoto et al., 1994) and in the serum of patitents (Evins et al., 1997), antagonizes noncompetitive NMDA receptor antagonist MK-801 ((+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*] cyclo-hepten-5,10-imine maleate)-produced discriminative stimulus cue (Corbett, 1995) and ketamine-induced brain metabolic activation (Duncan et al., 1998). It is reported that central administration of glutamate increases ascorbic acid release in the striatum (Grunewald and Fillenz, 1984). Glutamate reuptake inhibitor potentiates, whereas NMDA receptor antagonists inhibit, ethanol-induced ascorbic acid release in the striatum (Wu, 1994; Liu et al., 1999). Moreover, lesioning the frontal cortex completely eliminates ethanol-induced striatal ascorbic acid release, which strongly suggests that activation of the corticostriatal glutamatergic pathway may be responsible for the effect of ethanol on striatal AA release (Liu et al., 1999). Thus, it is plausible to assume that the potentiating effect of acute clozapine on ethanol-induced ascorbic acid release is mainly due to its activation of the corticostriatal glutamatergic pathway.

The differential effect of acute haloperidol and clozapine on ethanol-induced ascorbic acid release may also come from their different effects on glutamate neurotransmission. Single injection of haloperidol did not alter extracellular striatal glutamate levels (Daly and Moghaddam, 1994). An increase in extracellular striatal glutamate levels, however, was only observed 24 h after 21 days daily injection (Moghaddam and Bunney, 1993), or 3 days after 6 months oral administration of haloperidol (See and Chapman, 1994), suggesting that, not like clozapine, some suppressing mechanism of haloperidol on glutamatergic system may occur during chronic treatment.

In conclusion, the present study demonstrates for the first time that acute administration of clozapine and halperidol differentially affects ethanol-induced ascorbic acid release in rat striatum. It is unlikely that the mechanism of the potentiating action of clozapine on the action of ethanol is involved in its effect on dopamine D_2, 5-HT_2, 5-HT_{1A} and muscarinic receptors. However, as we know at present, blockade of dopamine D_1 receptors and activation of the GABAergic and glutamatergic systems by clozapine may play a role in this potentiating property of clozapine on the action of ethanol. These experimental results demonstrate another difference between the profiles of the atypical antipsychotic clozapine and the typical antipsychotic haloperidol. However, whether the changes in ethanol-stimulated endogenous ascorbic acid release in the striatum after drug treatment reflect the potential atypical antipsychotic profile of a drug merits extensive investigation by using more typical and atypical antipsychotic drugs.

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