The calculated/predicted f_b values of the cephalosporins from the neural network model are in good accordance with the respective experimental ones. For the training set of 20 cephalosporins and the test set of 3 cephalosporins, root mean squared errors (RMSE) between experimental f_b values and calculated/predicted f_b values are only 0.036 and 0.045, respectively. The polar molecular surface areas (S_H, $S_{O,N}$, or the sum of S_H and $S_{O,N}$) are clearly related to the capacity of a compound to form hydrogen bonds. They have been widely used to predict human intestinal absorption (Clark 1999; Fu et al. 2005a), blood-brain barrier penetration (Fu et al. 2005b), corneal permeability (Fu et al. 2001), and so on. Since hydrogen bonding is one of main factors responsible for the plasma protein binding, the polar molecular surface areas are also good predictors of plasma protein binding.

Some physicochemical parameters such as pKa, octanol/water or octanol/buffer partition coefficient are usually used to predict plasma protein binding of drugs (Gobburu and Shelver 1995; Morris and Bruneau 2000). These physicochemical parameters are determined experimentally. The major advantage of the current technique is that the predictors, MW, S_H, and $S_{O,N}$, can be easily calculated from the chemical structure of the drug, thus making preliminary physicochemical studies unnecessary and prediction of plasma protein binding convenient.

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Shenyang Pharmaceutical University, Shenyang

Similar effects of clozapine and olanzapine on ethanol-induced ascorbic acid release in the prefrontal cortex of freely moving mice

YUE HOU, CHUN FU WU, JING YU YANG, PEI FEI GU, XIU LI BI, FANG DAI

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Dr. C. F. Wu, Department of Pharmacology, Shenyang Pharmaceutical University, Wenhua Road 103, Shenyang 110016, P.R. China
wucf@syphu.edu.cn

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Previous studies have shown that acute systemic administration of ethanol induced ascorbic acid (AA) release in mouse striatum and prefrontal cortex. Clozapine and olanzapine showed similar effects on ethanol-induced AA release in mouse striatum. However, their effects on ethanol-induced AA release in mouse prefrontal cortex have not been reported. Thus, their effects on this neurochemical event were further investigated in the present study. The results showed that ethanol (4.0 gkg i.p.) significantly stimulated AA release in the prefrontal cortex by about 200 of baseline in mice. Clozapine and olanzapine, at the dose of 1.0 mgkg s.c., had no effect on basal AA or ethanol-induced AA release. However, both drugs, at the dose of 10 mgkg s.c., significantly inhibited ethanol-induced AA release. The present study demonstrated for the first time that similar actions were exhibited by clozapine and olanzapine for the regulation of ethanol-induced AA release in the mouse prefrontal cortex.

Ascorbic acid (AA) is a normal constituent of the brain, and its concentration in the brain of several mammalian species, including man, is higher than that in any other organ with the exception of the adrenal cortex (Mefford et al. 1981; Schenk et al. 1982). It has been shown recently that AA acts not only as an antioxidant, but also as a neuromodulator in the central nervous system (Grunewald 1993; Rebec and Pierce 1994). For example, AA directly alters striatal dopamine binding sites (Dorris 1987; Hadjiconstantinou and Neff 1983; Kayaalp et al. 1981) and inhibits the binding of dopamine antagonists to dopamine receptors (Heikkila et al. 1982). More recently, it has been shown that acute administration of ethanol significantly enhanced the release of AA in the striatum and prefrontal cortex of freely moving mice (Hou et al. 2005, 2006). Further studies have also shown that clozapine and olanzapine similarly regulated ethanol-induced AA release in mouse striatum (Hou et al. 2005). However, their effects on ethanol-induced AA release in mouse prefrontal cortex have not been reported. Thus, in the present study, their effects on this neurochemical event were further investigated.

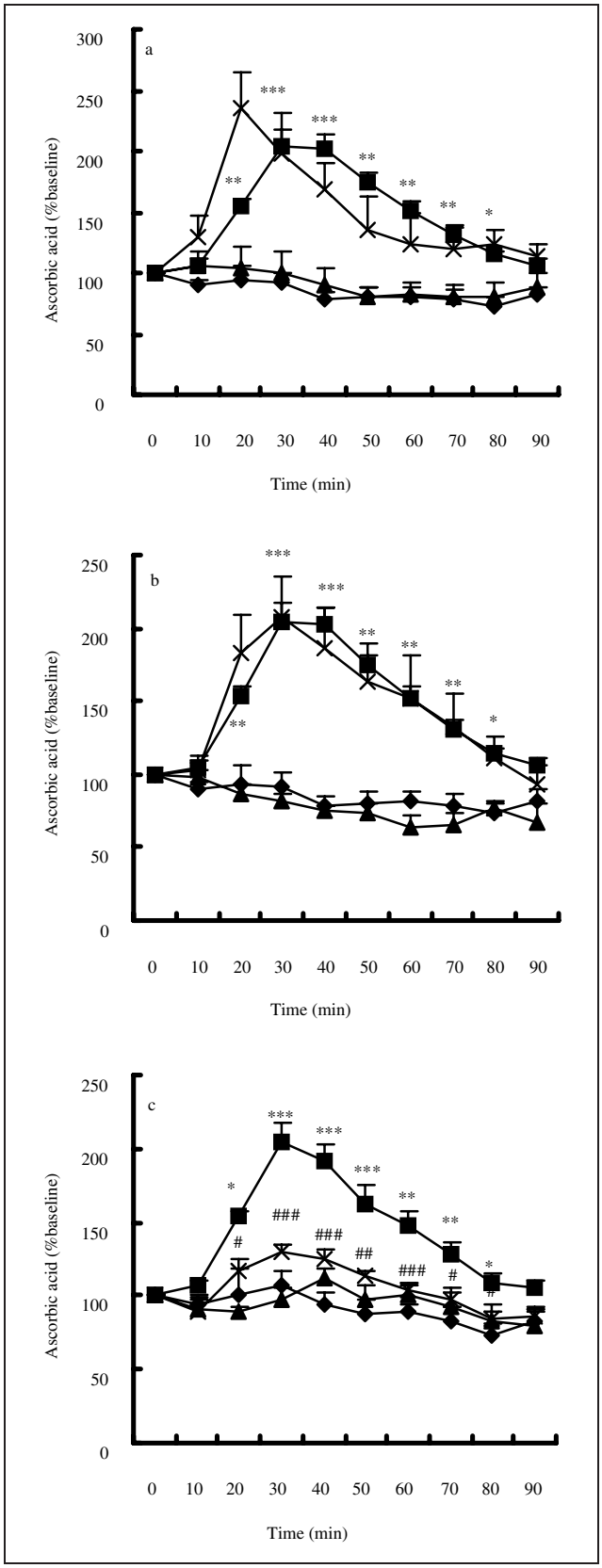

Fig. 1: Effect of clozapine on ethanol-induced ascorbic acid (AA) release in mouse prefrontal cortex. Clozapine was administered subcutaneously, at the dose of 1 mg/kg (a), 3 mg/kg (b) and 10 mg/kg (c), 10 min before ethanol administration (4.0 g/kg, i.p.). AA release is expressed as the percentage change from baseline. Data shown are means ±S.E.M. for 5–7 mice. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the corresponding control group. $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$ compared with the corresponding ethanol group. (—◆—: saline; —■—: ethanol 4.0 g/kg; —▲—: clozapine; —×—: clozapine + ethanol 4.0 g/kg)

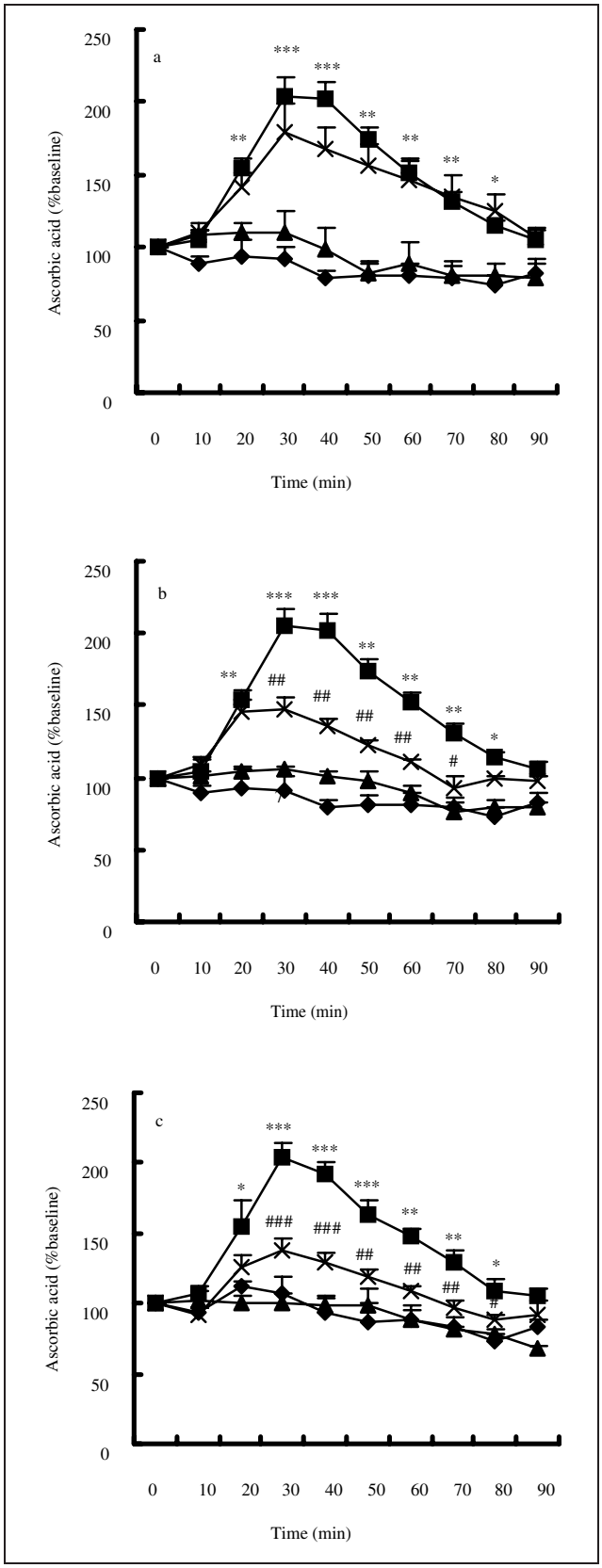

Fig. 2: Effect of olanzapine on ethanol-induced ascorbic acid (AA) release in mouse prefrontal cortex. Olanzapine was administered subcutaneously, at the dose of 1 mg/kg (a), 3 mg/kg (b) and 10 mg/kg (c), 10 min before ethanol administration (4.0 g/kg, i.p.). AA release is expressed as the percentage change from baseline. Data shown are means ±S.E.M. for 5–7 mice. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the corresponding control group. $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$ compared with the corresponding ethanol group. (—◆—: saline; —■—: ethanol 4.0 g/kg; —▲—: olanzapine; —×—: olanzapine + ethanol 4.0 g/kg)

Ethanol, at the dose of 4 g/kg i.p., induced a significant increase in AA release in mouse prefrontal cortex. The greatest effect was observed 30 min after ethanol administration, with the AA levels being about 100% higher than those in the saline control group ($F_{1, 18} = 60.27$, $P < 0.001$, Fig. 1).

Clozapine, at the dose of 1 and 3 mg/kg s.c., did not affect the basal level of AA or the increasing effect of ethanol on AA release (Fig. 1a, 1b). However, when the dose of clozapine was increased to 10 mg/kg s.c., it markedly suppressed ethanol-induced AA release (Fig. 1c), without affecting the basal AA level in the prefrontal cortex. Two-way ANOVA analysis showed no significant interaction between clozapine 1, 3 mg/kg and ethanol, but a significant interaction between clozapine 10 mg/kg and ethanol in the action on AA release (clozapine 1 mg/kg: $F_{1, 18} = 0.42$, $P = 0.5275$; clozapine 3 mg/kg: $F_{1, 18} = 0.13$, $P = 0.7241$; clozapine 10 mg/kg: $F_{1, 22} = 33.38$, $P = 0.0001$).

Olanzapine, at the dose of 1 mg/kg s.c., did not affect the basal or ethanol-induced AA level (Fig. 2a). However, olanzapine markedly antagonized ethanol-induced AA release at the higher doses of 3 and 10 mg/kg (Fig. 2b, 2c) without affecting the basal AA release. Two-way ANOVA analysis showed no significant interaction between olanzapine 1 mg/kg and ethanol, but a significant interaction between olanzapine 3, 10 mg/kg and ethanol in the action on AA release (olanzapine 1 mg/kg: $F_{1, 18} = 2.62$, $P = 0.1239$; olanzapine 3 mg/kg: $F_{1, 18} = 11.09$, $P = 0.0037$; olanzapine 10 mg/kg: $F_{1, 18} = 25.15$, $P = 0.0001$).

Clozapine and olanzapine belong to the relatively new class of the second generation of neuroleptic agents, the so-called atypical neuroleptics. Those drugs have a greater affinity to serotonin 5-HT_{2A} than to dopamine D_2 receptors and cause fewer EPS and improve negative symptoms, in contrast to classical neuroleptics (Bhana et al. 2001). Besides such clinical similarities, clozapine and olanzapine share many other similar properties observed in biochemical, behavioral, and electrophysiological studies. For example, long-term administration of olanzapine or clozapine inhibited stress-induced dopamine output in the rat prefrontal cortex (Dazzi et al. 2004). Olanzapine and clozapine reverse subchronic phencyclidine-induced functional hyperactivity of *N*-methyl-D-aspartate receptors in pyramidal cells of the rat medial prefrontal cortex (Ninan et al. 2003). The present study provides new data for further understanding the similar properties of clozapine and olanzapine in regulating some neurochemical events in the central nervous system.

In conclusion, the present study demonstrated for the first time that clozapine and olanzapine inhibited ethanol-induced AA release in mouse prefrontal cortex. However, the mechanisms underlying their effects merit further investigation.

Experimental

Male Swiss mice weighing 25–30 g, were used in the experiments. The animals were provided by the Experimental Animal Center of Shenyang Pharmaceutical University. The mice were housed under standard conditions with food and water *ad libitum* and maintained on a 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.

Mice were anesthetized with chloral hydrate (350 mg/kg i.p.) and implanted with Hospal AN 69 dialysis fibers (310 μm i.d., Dasco, Bologna, Italy) transversally through mouse prefrontal cortex (coordinates: A +2.0 mm, V −1.2 mm). The procedure used to prepare and implant the dialysis probe was essentially the same as that described previously (Hou et al. 2005).

Brain dialysis was performed about 24 h after probe implantation in freely moving mice. 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. AA contents in the samples were measured by HPLC with electrochemical detection as described before (Hou et al. 2005).

Test solutions (saline, ethanol or drugs) were administered when the baseline of AA output was stable in last three samples. Clozapine (Changzhou Pharmaceutical Factory, Changzhou, China) and olanzapine (Eli Lilly & Co. Ltd.) were dissolved in 0.3 M HCl in saline, after which the pH was adjusted to 5–6 with sodium hydroxide. Ethanol (Shenyang Reagents Co., China) was diluted with saline to 20% before use, and was injected intraperitoneally at a dose of 4.0 g/kg (Hou et al. 2005).

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

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