—Notes—

Ethanol Similarly Induces Ascorbic Acid Release in the Prefrontal Cortex and Striatum of Freely Moving Mice

Yue HOU,[a,b] Chunfu WU,*[,b] Jingyu YANG,[b] Xiang HE,[a] and Tao GUO[a]

[a]*General Hospital of Shenyang Military Region, 110016, Shenyang, P.R. China, and*
[b]*Shenyang Pharmaceutical University, 110016, Shenyang, P.R. China*

(Received March 31, 2006; Accepted May 23, 2006)

Previous studies have shown that acute systemic administration of ethanol induced striatal ascorbic acid (AA) release in mice and rats. Undercutting the prefrontal cortex completely eliminated ethanol-induced AA release in rat striatum. In the present study, *in vivo* brain dialysis coupled with high performance liquid chromatography (HPLC)-electrochemical detection was used to evaluate the effect of ethanol on the release of AA in the prefrontal cortex, compared to that in the striatum of freely moving mice. The results showed that ethanol (4.0 g/kg *i.p.*) similarly induced AA release in the prefrontal cortex and striatum of freely moving mice.

Key words——ascorbic acid; ethanol; prefrontal cortex; striatum

INTRODUCTION

In addition to the action on behaviors, the interaction of ethanol with neurotransmission in the central nervous system is well documented. For example, ethanol can affect the synthesis, release and catabolism of many neurotransmitters[1—3] and modulators.[4—6] It has been found that ethanol at low doses selectively increases dopamine release from nucleus accumbens of rats, and at higher doses also increase dopamine release from striatum.[1] Ethanol increases 5-HT release from nucleus accumbens and decreases 5-HT contents in the hippocampus, striatum and hypothalamus.[2] Systemic administration of ethanol showed dual modulatory effects on acetylchline release in rat hippocampus depending on the dose and the site of administration.[7]

AA (Fig. 1) 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.[8,9] It has been shown recently that AA acts not only as an antioxidant, but also as a neuromodulator in the central nervous system.[10,11] For example, AA can block the D-amphetamine-induced stereotypy and potentiate the cataleptic effect of haloperidol in rats.[12—14] It directly alters striatal dopamine binding sites[15—17] and inhibits the binding of dopamine antagonists to dopamine receptors.[18] Extracellular AA concentrations in the brain change in response to various drug treatments.[5,19—22]

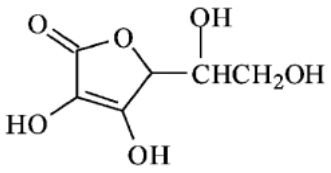

Fig. 1. Molecular Structure of Ascorbic Acid

There are also reports dealing with the interaction between ascorbic acid and ethanol in the central nervous system. Exogenous administration of sodium ascorbate antagonizes ethanol-induced impairment of swimming in mice[23] and raises the survival rate in ethanol-intoxicated mice.[24] Pre-treatment with AA potentiates ethanol-induced loss of righting reflex in mice.[25] More recently, it has been shown that acute administration of ethanol significantly enhanced the release of AA in the striatum of freely moving rats and mice.[5,26] Our previous study has also shown that ethanol significantly enhanced the release of AA in the accumbens nucleus.[27] However, whether ethanol has similar effect on the release of endogenous AA in the prefrontal cortex has not been reported. Thus, in the present study, we investigate whether ethanol has similar effect on the release of endogenous AA in the prefrontal cortex, compared to the striatum.

MATERIALS AND METHODS

Animals Female and male Swiss mice weighing

*e-mail: wucf@syphu.edu.cn

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 12L : 12D light cycle (light on 06 : 30). 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.

Drugs Ethanol (purity 99.5%) was purchased from Shenyang Reagents Co., China, and diluted with saline to 20% before use. Ethanol was injected intraperitoneally at a dose of 4.0 g/kg.[26]

Implantation of the Dialysis Probe and Brain Dialysis 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 either the striata (coordinates: A+0.6 mm, V−3.5 mm) or the 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.[26]

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. Test solutions (saline or ethanol) were administered when the baseline of AA output was stable in last three samples. At the end of the experiments, the position of the dialysis fiber was verified and the data were discarded if the fiber was positioned incorrectly.

Analysis Procedure AA contents in the samples were measured by HPLC with electrochemical detection (Fig. 2). An aliquot of 20 μl dialysis samples was injected directly into HPLC system. A reversed-phase column (ODS, 5 μm, Shimadzu, Japan) 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.[5] The mobile phase was pumped with LC-10A pump (Shimadzu, Japan) at a flow rate of 1.0 ml/min. The

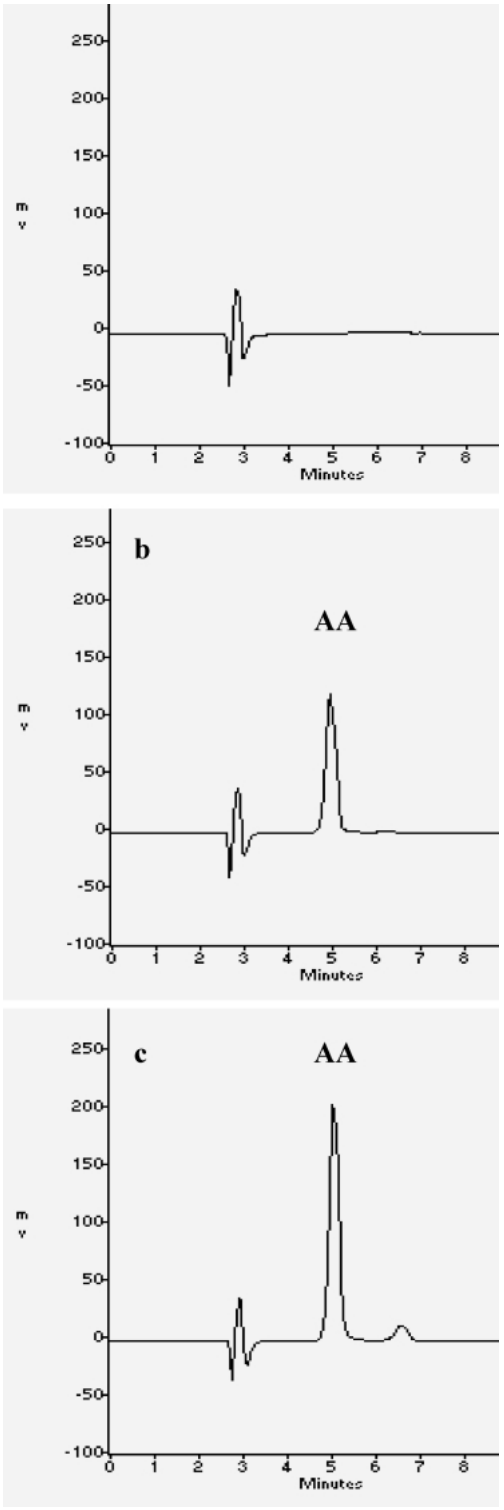

Fig. 2. Representative Chromatograms of HPLC with Electrochemical Detection of Ringer's Solution (a), the Standard Solution of AA (b), and Dialysate Sample from Mouse Prefrontal Cortex (c)

detector (L-ECD-6A, Shimadzu, Japan) equipped with a glassy carbon working electrode and an Ag/AgCl reference electrode was set at +0.7 V. The cur-

rent produced was monitored by a chromatography workshop (TianMei Co., China).

Statistical Analysis 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. 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%.

RESULTS AND DISCUSSION

Ethanol, at the dose of 4 g/kg *i.p.*, induced a significant increase in AA release both in mouse prefrontal cortex and striatum. The greatest effect was observed 30 min after ethanol administration, with the AA levels being about 100% higher when compared with the saline control group (striatum: $F_{1,22}=48.67$, $p<0.001$, Fig. 3; prefrontal cortex: $F_{1,19}=60.27$, $p<0.001$, Fig. 4).

Previous experiments have shown that intraperitoneal injection of ethanol induced an increase in striatal AA release.[5] However, intra-striatal administration of ethanol into the striatum via dialysis probe did not increase striatal AA release,[5] suggesting the controlling mechanisms of ethanol-induced striatal AA release reside outside the striatum. Further studies demonstrated that dopaminergic[4] and glutamatergic[28] systems were involved in ethanol-induced AA release. Blockade of dopamine D2 receptors by sulpiride or inhibition of NMDA receptors by MK-801 antagonizes, whereas, blockade of dopamine D1 receptors by SCH 23390 potentiates ethanol-induced AA release in the striatum. Lesion of the substantia nigra or treatment with reserpine, which decreased the striatal dopamine levels by 95%, did not affect ethanol-stimulated AA release in the striatum, suggesting that ethanol-induced striatal AA release may not via the activation of presynaptic nigrostriatal dopaminergic pathway.[4] Deafferentation of the glutamatergic projection from cortex to striatum by undercutting the prefrontal cortex completely eliminated ethanol-induced striatal AA release, indicating the necessity of the integrity of the corticostriatal pathway in regulation of ethanol-induced striatal AA release.[28] However, whether ethanol increases AA release in prefrontal cortex has not been reported. The present study demonstrated for the first time that ethanol induced AA release in the prefrontal cortex, further suggesting a close link between ethanol-induced AA release in the prefrontal cortex and in the striatum.

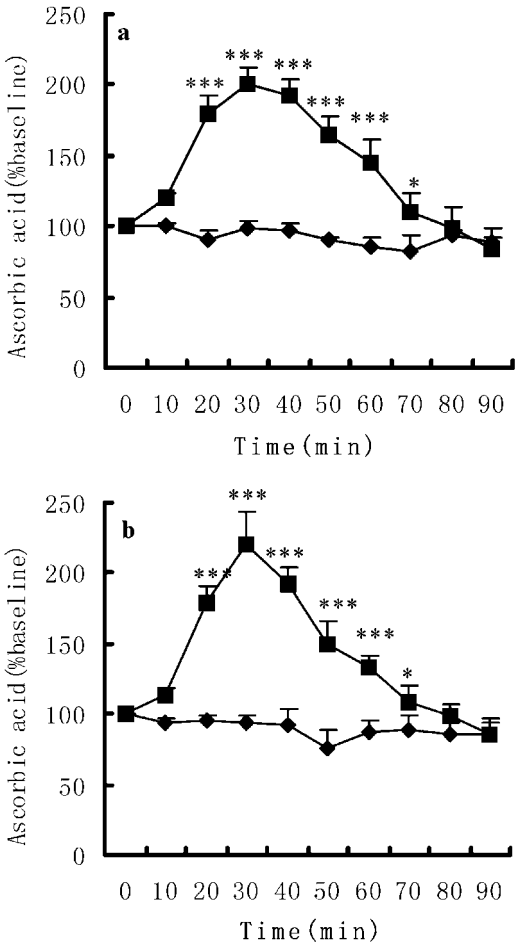

Fig. 3. Effect of Ethanol (4.0 g/kg, *i.p.*) on Ascorbic Acid (AA) Release in the Striatum of Male (a) and Female (b) Mice

AA release is expressed as the percentage change from baseline. Data shown are means ± S.E.M. for 5—8 mice. $^{*}p<0.05$, $^{***}p<0.001$ compared with the corresponding control group. (–◆– : saline, –■– : ethanol 4.0 g/kg).

There is current interest in the role of ethanol-induced oxidative stress in the injury of the central nervous system.[29] Intake of ethanol affects the activities of antioxidant enzymes such as superoxide dismutase, catalase and GSH-Px in the brain.[30] Ethanol can induce superoxide anion or hydrogen peroxide formation in animals.[31] It is believed that ascorbic acid, as a classical antioxidant, plays a major role in protecting brain against oxidative damage.[32,33] Previous studies have shown that endogenous released ascorbic

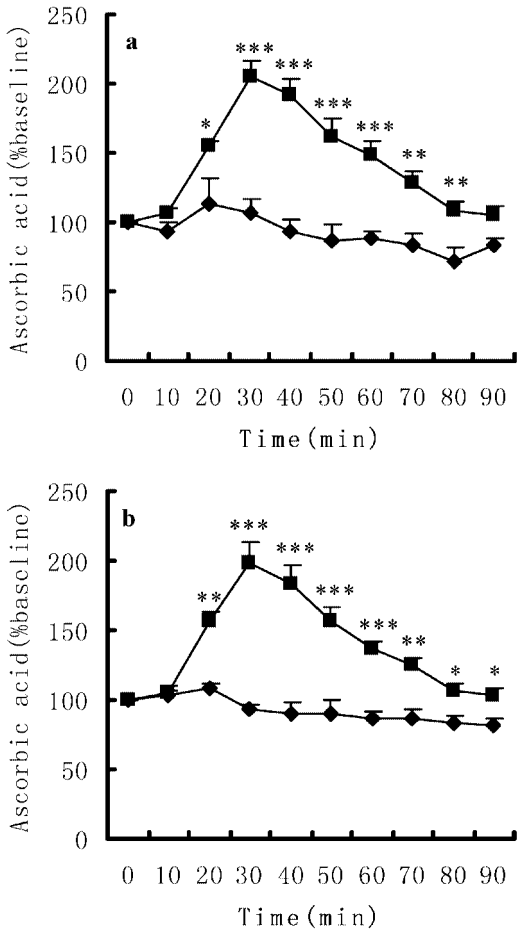

Fig. 4. Effect of Ethanol (4.0 g/kg, *i.p.*) on Ascorbic Acid (AA) Release in the Prefrontal Cortex of Male (a) and Female (b) Mice

AA release is expressed as the percentage change from baseline. Data shown are means ± S.E.M. for 5—8 mice. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared with the corresponding control group. (–◆– : saline, –■– : ethanol 4.0 g/kg).

acid plays an important role in scavenging hydroxyl radical induced by ethanol.[34] Taken together, the present study provides the evidence that stimulated ascorbic acid release in mouse prefrontal cortex and striatum has important physiological and pharmacological implications for protecting the central nervous system from the damage induced by ethanol.

Previous studies have demonstrated the existence of sex difference in responding to several stimuli.[35—37] Thus, mice of both genders were used in the present study. The results showed that there was no significant difference between female and male mice, either in the basal levels of AA or in the changes of it after acute administration of ethanol.

It is well documented that there exists a functional link between AA release and the glutamatergic system. Microinjection of glutamate into various brain regions evoked increases in extracellular AA signals.[38] Some glutamate reuptake blockers reduced or eliminated the ascorbate signals.[39,40] Striatal AA release induced by ethanol could be potentiated by the glutamate uptake L-trans-pyrrolidine-2,4-dicarboxylate,[41] and reversed by the NMDA receptor antagonist 3-(2-carboxypiperazin-4-yl) propyl-1- phosphonic acid.[42] It is also reported that uptake of released glutamate facilitates the release of AA via the glutamate/AA hetero-exchange mechanism.[43] This may partially explain the mechanism of ethanol-induced AA release in the prefrontal cortex, since administration of ethanol significantly decreasing the release of glutamate in cortical synaptosomes.[44]

In conclusion, the present study demonstrated for the first time that acute administration of ethanol increased AA release in mouse prefrontal cortex. However, the mechanisms underlying ethanol-induced endogenous AA release in the prefrontal cortex merits further investigation.

Acknowledgements This project is supported by Young Key-teacher Foundation of Ministry of Education, China.

REFERENCES

1) Imperato A., Di Chiara G., *J. Pharmacol. Exp. Ther.*, **239**, 219–228 (1986).
2) Yoshimoto K., McBride W. J., Lumeng L., Li T. K., *Alcohol*, **9**, 17–22 (1991).
3) Wang Y., Chiou A. L., Jeng C. H., Yang S. T., Lin J. C., *Pharmacol. Biochem. Behav.*, **66**, 679–685 (2000).
4) Liu W., Wu C. F., Huang M., Xiao K., *Brain Res.*, **869**, 31–38 (2000).
5) Wu C. F., Liu J., Consolo S., Liu W., *Neurosci. Lett.*, **250**, 95–98 (1998).
6) Wu C. F., Liu J., Liu W., Consolo S., Huang M., Yang J. Y., *Addict. Biol.*, **6**, 25–34 (2001).
7) Henn C., Loffelholz K., Klein J., *Naunyn Schmiedebergs Arch. Pharmacol.*, **357**, 640–647 (1998).
8) Mefford I. N., Oke A. F., Adams R. N., *Brain Res.*, **212**, 223–226 (1981).
9) Schenk J. O., Miller E., Gaddis R., Adams R. N., *Brain Res.*, **253**, 353–356 (1982).
10) Grunewald R. A., *Brain Res. Rev.*, **18**, 123–

133 (1993).
11) Rebec G. V., Pierce R. C., *Prog. Neurobiol.*, **43**, 537–565 (1994).
12) De Angelis L., *Curr. Ther. Res.*, **49**, 267–273 (1991).
13) White L. K., Carpenter M., Block M., Basse-Tomusk A., Gardiner T. W., Rebec G. V., *Psychopharmacology*, **94**, 284–287 (1988).
14) Dorris R. L., Dill R. E., *Pharmacol. Biochem. Behav.*, **24**, 781–783 (1986).
15) Dorris R. L., *Proc. Soc. Exp. Biol. Med.*, **186**, 13–16 (1987).
16) Hadjiconstantinou M., Neff N. H., *Neuropharmacology*, **22**, 939–943 (1983).
17) Kayaalp S. O., Rubenstein J. S., Neff N. H., *Neuropharmacology*, **30**, 409–410 (1981).
18) Heikkila R. E., Cabbat F. S., Manzino L., *J. Neurochem.*, **38**, 1000–1006 (1982).
19) Saponjic R. M., Mueller K., Krug D., Kunko P. M., *Pharmacol. Biochem. Behav.*, **48**, 161–168 (1994).
20) Brazell M. P., Mitchell S. N., Joseph M. H., Gray J. A., *Neuropharmacology*, **29**, 1177–1185 (1990).
21) Enrico P., Esposito G., Mura M. A., Fresu L., De Natale G., Miele E., Desolo M. S., Miele M., *Brain Res.*, **745**, 173–182 (1997).
22) Crespi F., Keane P. E., *Neurosci. Res.*, **4**, 323–341 (1987).
23) Busrel R. G., Lehmann A. G., *Behav. Brain Res.*, **1**, 351–356 (1980).
24) Yunice A. A., Lindeman R. D., *Proc. Soc. Exp. Biol. Med.*, **154**, 146–150 (1977).
25) Wu C. F., Zhang H. L., Liu W., *Pharmacol. Biochem. Behav.*, **65**, 413–418 (2000).
26) Hou Y., Yang J. Y., Wu C. F., Huang M., *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **29**, 83–89 (2005).
27) Gu P. F., Yang J. Y., Wu C. F., Li W., Shang Y., *Brain Res.*, **1033**, 194–201 (2005).
28) Liu J., Wu C. F., Liu W., Zhang H. L., Li C. L., *Addict. Biol.*, **4**, 272–282 (1999).
29) Bondy S. C., *Toxicol. Lett.*, **63**, 231–241 (1992).
30) Somani S. M., Husain K., Schlorff E. C., *Res. Commun. Alcohol Subst. Abuse*, **19**, 75–90 (1998).
31) Albano E., Tomasi A., Goria-Gatti L., Dianzani M. U., *Chem. Biol. Interact.*, **65**, 223–234 (1988).
32) Vatassery G. T., *Lipids*, **30**, 1007–1013 (1995).
33) Rice M. E., *Trends Neurosci.*, **23**, 209–216 (2000).
34) Huang M., Liu W., Li Q., Wu C.F., *Brain Res.*, **944**, 90–96 (2002).
35) Guo M., Wu C. F., Liu W., Yang J. Y., Chen D., *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **28**, 115–121 (2004).
36) Rodgers R. J., Cole J. C., *Physiol. Behav.*, **54**, 729–736 (1993).
37) Fundaro A., *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **22**, 147–158 (1998).
38) Ghasemzadeh B., Cammack J., Adams R. N., *Brain Res.*, **547**, 162–166 (1991).
39) Miele M., Boutelle M. G., Fillenz M., *Neuroscience*, **62**, 87–91 (1994).
40) Cammack J., Ghasemzadeh B., Adams R. N., *Brain Res.*, **565**, 17–22 (1991).
41) Wu C., *Neurosci. Lett.*, **171**, 105–108 (1994).
42) Wu C., *Neurosci. Lett.*, **157**, 111–114 (1993).
43) Yusa T., *Brain Res.*, **897**, 104–113 (2001).
44) Sabria J., Torres D., Pasto M., Peralba J. M., Allali-Hassani A., Pares X., *Addict. Biol.*, **8**, 287–294 (2003).