Neurotoxicity Research, 2004, VOL. 6(4). pp. 311-316

F.P. Graham Publishing Co.

Nicotine Inhibits Ethanol-induced Toxicity in Cultured Cerebral Cortical Cells

YOUSEF TIZABI[a,*], KEBRETEN F. MANAYE[b], DUANE T. SMOOT[c] and ROBERT E. TAYLOR[a]

[a]*Department of Pharmacology,* [b]*Department of Physiology and Biophysics, College of Medicine, Howard University, 520 W Street NW;* [c]*Department of Medicine, Howard University Hospital, Washington, DC 20059, USA. ytizabi@howard.edu*

(Received 14 May 2004; Revised 24 June 2004; In final form 24 June 2004)

The high incidence of smoking among alcoholics may be partially due to nicotine's ability to counteract some of the adverse effects of ethanol on motor coordination and/or cognitive functions. Neuroprotective effects of nicotine on ethanol-induced toxicity in cerebellar granular cells have been observed. In this study, we sought to determine whether similar protection is observed in neocortical cells and if so, what specific nicotinic receptor subtypes may be mediating the actions of nicotine. Primary cultures of neocortical cells were prepared from 20-day embryos obtained from time-pregnant Sprague-Dawley rats. Cells were cultured for 10 days and were then exposed for 3 days to various concentrations of ethanol with and without pretreatment with nicotine and nicotinic antagonists. Cellular toxicity was evaluated by measuring the lactate dehydrogenase level. Administration of ethanol (10-100 mM) resulted in a dose-dependent toxicity. Pretreatment with nicotine (5-20 μM) resulted in a dose-dependent protection against ethanol-induced toxicity. The effects of nicotine were blocked by pretreatment with nicotinic antagonists such as mecamylamine (1-20 μM), dihydro-beta-erythroidine (DHBE) (50 nM-1.0 μM) and methyllycaconitine (MLA) (5 nM-1 μM) in a dose-dependent manner. Compared to previous studies, higher ethanol concentrations were required to induce toxicity in neocortical *vs* cerebellar granule cells. Moreover, the effects of nicotine in the neocortical cells were blocked by lower concentrtartions of MLA, but higher concentrations of DHBE compared to cerebellar cells. Collectively, the results suggest differential sensitivity of various neuronal populations to the toxic effect of ethanol. Furthermore, protective effects of nicotine against alcohol in various regions appear to be mediated by different nicotinic receptor subtypes. The neuroprotective effect of nicotine against ethanol-induced toxicity may be a contributing factor to the high incidence of smoking among alcoholics.

Keywords: Ethanol; Nicotine; Nicotinic Receptors; Cortex; Primary Cell Culture; Neurotoxicity; Neuroprotection

INTRODUCTION

It is well documented that the incidence of smoking is very high among heavy drinkers (Kozlowski *et al.*, 1993; Shiffman and Balabanis, 1995; Daeppen *et al.*, 2000; Dawson, 2000; Romberger and Grant, 2004). Simultaneous consumption of alcohol and tobacco smoke can lead to synergistic morbidity in cancers of the head, neck and esophagus (Olson *et al.*, 1985; Johnson and Jennison, 1992; Castellsague *et al.*, 1999), or gastric ulcers (Ko and Cho, 2000). Several factors may contribute to concomitant use of alcohol and nicotine intake (smoking). These include genetic predisposition, and/or additive rewarding or reinforcing effects of alcohol and nicotine (True *et al.*, 1999; Madden and Heath, 2002; Tizabi *et al.*, 2002; Butt *et al.*, 2004; Rose *et al.*, 2004). In addition, nicotine's ability to counteract some of the adverse effects of ethanol may be a powerful incentive for alcohol consumers to increase their tobacco (nicotine) intake. For example, attenuation of alcohol-induced motor incoordination by nicotine (Dar *et al.*, 1994) may be a desirable outcome of smoking in alcoholics. Similarly, nicotine's reduction of cognitive deficits manifested as a result of heavy drinking may significantly contribute to the high incidence of smoking in alcoholics (Gould and Lommock 2003; Rezvani and Levin, 2003).

It was reported recently that ethanol-induced toxicity in the cerebellar granular cells may be blocked by pretreatment with nicotine (Tizabi *et al.*, 2003). Since cerebellum is intimately involved in motor coordination, it was suggested that neuroprotective effects of

*Corresponding author. Tel.: +1 202-806-9719; Fax: +1 202-806-4453; E-mail: ytizabi@howard.edu
ISSN 1029 8428 print/ ISSN 1476-3524 online.

nicotine on cerebellar cells may contribute to concomitant use of alcohol and nicotine (Tizabi *et al.*, 2003). Cerebral cortex, on the other hand, is intimately involved in cognitive processing. Thus, in this study, we sought to determine whether nicotine may protect against ethanol-induced toxicity in neocortical cells, and if so, which nicotinic receptor subtypes may be mediating the actions of nicotine.

METHODS

Materials

Culture media, fetal bovine serum (FBS), fetal horse serum (HS), newborn calf serum, 5-fluro-5-deoxyuridine (Fudr), antibiotics, Dubecco's medium, mecamylamine (M), nicotine (N), dihydro-beta-erythroidine (DHBE) and methyllycaconitine (MLA) were purchased from Sigma (St Louis, MO). Ethanol (E) 100% (200 proof) was purchased from Warner Graham Co. (Cockeysville, MD). CytoTox 96 cytotoxicity kit for measurements of lactate dehydrogenase (LDH) was purchased from Promega (Madison, WI).

Cell Cultures

Cerebral cortical cells were prepared from 18-20-day embryos obtained from time-pregnant Sprague-Dawley rats as described by Kawahara *et al.* (2001). For each experiment, the fetus from one or two pregnant rats were utilized. Briefly, the embryos were removed from the dams, their brain isolated, and the entire cerebral cortex was dissected out under sterile condition. Following removal of the meninges, the tissue was placed in 0.15 unit/ml papain solution (PBS containing 0.02% L-cystein, 0.02% bovine serum albumin, 0.5% glucose, 50 units/l Pent-Strep). The cells were then mechanically dissociated and placed in culture medium (Dubecco's modified eagle medium supplemented with 5% horse serum, 5% newborn calf serum, and 1 mM sodium pyruvate). Cells were seeded at 5 x 10^5 cells/well in 96-well microplates (100 µl/well) precoated with 10 µg/ml poly-L-lysine. The plates were placed in a 37°C sealed incubator (5% CO_2). Three to five days later the cultures were treated with 50 µM Fudr for 3 days to prevent glial mitosis. During the remainder of the culture period, the medium was changed twice weekly using the incubation buffer.

Treatments

Similar treatments that were previously performed on cerebellar granular cells (Tizabi *et al.*, 2003), were conducted here on cortical cells in order to facilitate comparison between the two cell types.

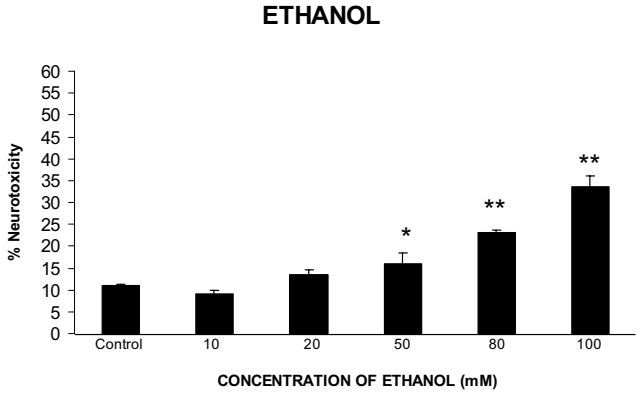

FIGURE 1 Effect of various concentrations of ethanol (E) on cellular toxicity in primary culture of cortical cells. Values are mean ± SEM. n = 8/group. *P <0.05 *vs* control. **P <0.01 *vs* control.

Effects of Ethanol

On culture day 10 the medium was changed once again, however, at this time no serum was present in the medium. Cells were then exposed to various concentrations of ethanol (10-100 mM). Three days later, the media was removed and the concentrations of LDH, determinant of cell toxicity, were measured as described below.

Effects of Nicotine and Ethanol

To evaluate the effects of nicotine pretreatment on ethanol-induced toxicity, the same experiment as above was repeated, except in this case, 5-15 min before administration of ethanol (100 mM), the cells were treated with various concentrations of nicotine (5-20 µM). Additional controls were obtained by treating various wells with nicotine only. Again, after three days the concentration of LDH in the wells was determined.

Effects of Nicotinic Antagonists, Nicotine and Ethanol

To determine whether the effects of nicotine were mediated by nicotinic receptors, a series of experiments as above were conducted, except in these cases, 5-15 min before administration of nicotine (20 µM) or ethanol (100 mM), cells were treated with various concentrations of nicotinic antagonists: mecamylamine (1-20 µM), DHBE (1.0 nM-1.0 µM) and MLA (5.0 nM-5.0 µM). Mecamylamine is a non-competitive nicotinic receptor antagonist that may block all nicotinic receptors, whereas DHBE and MLA are competitive antagonists with selectivity for high (α_4 or β_2 containing) and low affinity (e.g. α_7 containing) nicotinic receptor subtypes, respectively.

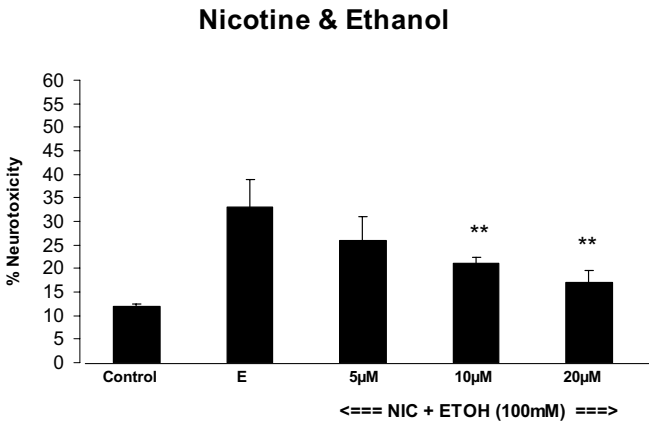

FIGURE 2 Effect of pretreatment with various concentrations of nicotine (NIC) on ethanol (E)-induced toxicity in primary culture of cortical cells. Ethanol concentration was maintained at 100 mM. Values are mean ± SEM. n = 8/group. $^{**}P < 0.01$ *vs* E.

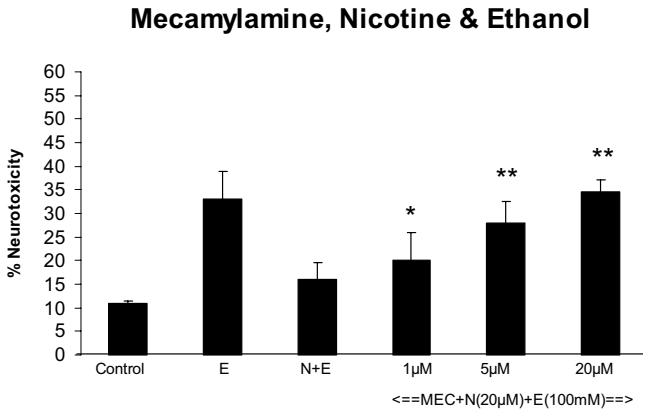

FIGURE 3 Effect of pretreatment with various concentrations of mecamylamine (MEC) on nicotine (N) protection against ethanol (E)-induced toxicity in primary culture of cortical cells. Ethanol concentration was maintained at 100 mM and nicotine concentration was maintained at 20 μM. Values are mean ± SEM. n = 8/group. $^{*}P <0.05$ *vs* N + E. $^{**}P <0.01$ *vs* N + E.

LDH Measurement

LDH activity released from the neuronal cells was measured by a commercially available cytotoxicity detection kit. Two controls were performed in each experimental setup. The first was a background control which provided information on LDH activity contained in the fresh culture medium. The absorbance value obtained in this control was subtracted from all other values. The second control was measured as the LDH released from the non-treated cells. This provided information on spontaneous LDH release which was used as control values in the figures. Total culture medium were collected and centrifuged to remove contaminating cells and cellular debris. For the actual assay, 50 μl aliquots of the media were transferred to a fresh 96 well flat-bottom plate. To each well, 50 μl of LDH assay reagent was added and the mixture was incubated for 30 min at room temperature and the absorbance of samples was measured at 490 nm (Hajimohammadreza *et al.*, 1995).

Statistical Analysis

Each experiment was performed eight times and each treatment condition was carried out in duplicate or triplicates. Data were analyzed by one-way analysis of variance using the Sigma Plot software. When a main significance was detected, Newman-Keuls *post hoc* test was used to determine which groups differed. The significance level was set, a priori, at $p<0.05$.

RESULTS

Effects of Ethanol

Figure 1 depicts the effects of various concentrations of ethanol (10-100 mM) on primary culture of neocortical cells. Exposure of the cells to ethanol for 3 days resulted in a dose-dependent toxicity as determined by LDH levels. Maximum toxicity (approximately 33%) was observed with the highest (100 mM) ethanol concentration.

Effects of Nicotine on Ethanol-Induced Toxicity

Figure 2 depicts the effects of pretreatment with various concentrations of nicotine (5-20 μM) on ethanol-induced cytotoxicity in primary culture of neocortical cells. In this and subsequent studies, the highest ethanol concentration (100 mM) that caused the highest toxicity was used. Exposure of the cells to nicotine prior to ethanol, dose-dependently attenuated ethanol-induced toxicity. The effects of ethanol were maximally blocked by 20 μM nicotine. Nicotine by itself, at concentrations used, did not have any effect on cell viability. Higher concentrations of nicotine (50 μM and above), however, resulted in significant toxicity (data not shown).

Effects of Mecamylamine and Nicotine Combination on Ethanol-Induced Toxicity

Figure 3 depicts the effects of pretreatment with various concentrations of mecamylamine (1-20 μM) on nicotine's inhibition of ethanol-induced cytotoxicity in primary culture of neocortical cells. In this and subsequent studies, the highest effective dose of nicotine (20 μM) that blocked ethanol-induced toxicity was used. Pretreatment of the cells with mecamylamine, dose-dependently blocked the effects of nicotine. Total inhibition of nicotine effect was achieved with 20 μM mecamylamine. Mecamylamine, at concentrations

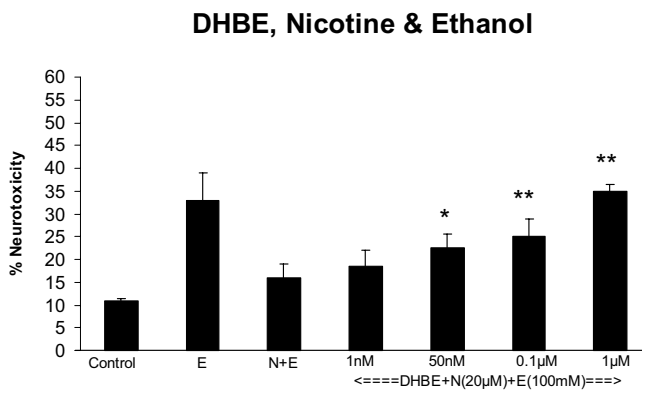

FIGURE 4 Effect of pretreatment with various concentrations of dihydro-beta-erythroidine (DHBE) on nicotine (N) protection against ethanol (E)-induced toxicity in primary culture of cortical cells. Ethanol concentration was maintained at 100 mM and nicotine concentration was maintained at 20 μM. Values are mean ± SEM. n = 8/group. *P < 0.05 *vs* N + E. **P < 0.01 *vs* N + E.

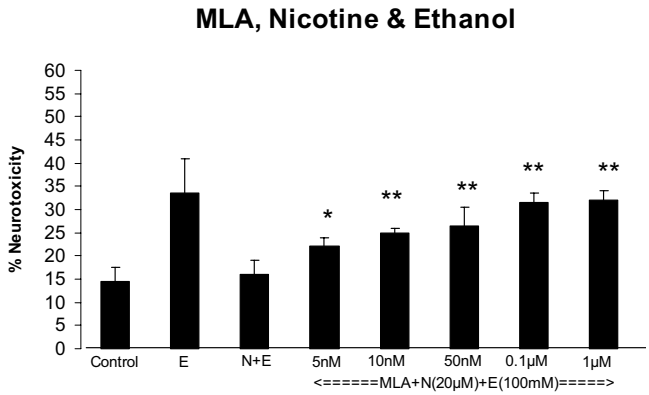

FIGURE 5 Effect of pretreatment with various concentrations of methyllycaconitine (MLA) on nicotine (N) protection against ethanol (E)-induced toxicity in primary culture of cerebellar granule cells. Ethanol concentration was maintained at 100 mM and nicotine concentration was maintained at 20 μM. Values are mean ± SEM. n= 8/group. *P <0.05 *vs* N + E. **P <0.01 *vs* N + E.

used, did not have any effect on cell viability by itself, nor did it affect ethanol-induced toxicity (data not shown).

Effects of DHBE and Nicotine Combination on Ethanol-Induced Toxicity

Figure 4 depicts the effects of pretreatment with various concentrations of DHBE (1.0 nM-1.0 μM) on nicotine's inhibition of ethanol-induced cytotoxicity in primary culture of neocortical cells. Pretreatment of the cells with DHBE dose-dependently blocked the effects of nicotine. DHBE at 50 nM, reduced nicotine's effect by 33%, and at 1.0 μM totally blocked nicotine's effect. DHBE, at concentrations used, did not have any effect on cell viability by itself, nor did it affect ethanol-induced toxicity (data not shown).

Effects of MLA and Nicotine Combination on Ethanol-Induced Toxicity

Figure 5 depicts the effects of pretreatment with various concentrations of MLA (5 nM-1.0 μM) on nicotine's inhibition of ethanol-induced cytotoxicity in primary culture of neocortical cells. Pretreatment of the cells with MLA dose-dependently blocked the effects of nicotine. MLA at the lowest concentration (5 nM), reduced nicotine's effect by approximately 34% ,and at 0.1 μM completely blocked nicotine's effect. MLA at concentrations used, did not have any effect on cell viability by itself, nor did it affect ethanol-induced toxicity (data not shown).

DISCUSSION

The results of this study indicate that ethanol-induced toxicity in cultured cerebral cortical cells can be attenuated by nicotine pretreatment. The effects of nicotine, in turn, can be blocked by nicotinic antagonists. These findings are similar to our previous findings in primary cultures of cerebellar granule cells (Tizabi *et al.*, 2003). However, two major differences are noted between neocortical and cerebellar cells. First, neocortical cells are less susceptible to the toxic effects of ethanol. This is because at 20 mM concentration, ethanol did not result in any significant toxicity in neocortical cells but did cause significant toxicity (approximately 23%) in cerebellar granule cells. Moreover, at the highest ethanol concentration (100 mM), the maximum toxicity in the neocortical cells was approximately 33%, whereas in the cerebellar cells it was approximately 42%. Second, the protective effects of nicotine in neocortical cells appear to be mediated primarily via low affinity nicotinic receptors, whereas in cerebellar cells the high affinity nicotinic receptors may play a major role. Thus, MLA which is a selective antagonist of low affinity nicotinic receptors, at 0.1 μM, almost completely blocked the nicotine effect in the neocortical cells, whereas the same concentration resulted in approximately 50% inhibition of the nicotine effect in the cerebellar cells. Conversely, DHBE which is a selective antagonist for the high affinity nicotinic receptors, at 50 nM blocked approximately 33% of the nicotine effect in neocortical cells, whereas the same concentration of DHBE resulted in almost total block of nicotine effect in cerebellar granule cells.

Considerable data suggest that some of the central actions of alcohol may be mediated through nicotinic receptors. For example, the stimulatory effects of alcohol on the mesolimbic dopaminergic pathway may be

at least partially mediated through central nicotinic receptors (Blomqvist *et al.*, 1996; Ericson *et al.*, 1998; Soderpalm *et al.*, 2000; Tizabi *et al.*, 2002; Larsson and Engel, 2004). In addition to their involvement in a variety of physiological functions (Decker and Meyer, 1999; Leonard and Bertrand, 2001; Picciotto, 2003), nicotinic receptors are believed to play an important role in neurogenesis as well as neuronal maturation and neuroprotection (Winzer-Serhan and Leslie, 1997; Kihara *et al.*, 1998; Opanashuk *et al.*, 2001; Zanardi *et al.*, 2002; Shaw *et al.*, 2003; Stevens *et al.*, 2003; Takada *et al.*, 2003; Tizabi *et al.*, 2003).

Although our results implicate nicotinic receptors in protective effects of nicotine, they do not support a role for these receptors in ethanol-induced toxicity. This is because nicotinic antagonists, at concentrations that blocked nicotine effects, were ineffective in modifying the effects of ethanol. Our findings, however, add to the well documented neurochemical, behavioral and metabolic interactions between ethanol and nicotine and are in agreement with general findings of a neuroprotective effect of nicotine or nicotinic agonists in ethanol-induced toxicity (Prendergast *et al.*, 2000; Penland *et al.*, 2001; Li *et al.*, 2002; Tizabi *et al.*, 2003). The exact mechanism(s) of the neuroprotective effects of nicotine against alcohol-induced toxicity is not known. However, various intermediaries in neuroprotective effects of nicotine against other neurotoxins have been identified. These include activation of fibroblast growth factors or growth-promoting enzymes such as Jnaus kinase 2 (Belluardo *et al.*, 2000; Shaw *et al.*, 2003), inactivation of L-type calcium channels (Stevens *et al.*, 2003) or inhibition of oxidative stress (Newman *et al.*, 2002; Guan *et al.*, 2003). On the other hand, alcohol-induced toxicity may be mediated by the release of cytochrome *c* from mitochondria or a decrease in mitochondrial membrane potential, excess Ca++ influx, free radical formation, or apoptosis (Bhave *et al.*, 1997; Gruol *et al.*, 1998; Huentelman *et al.*, 1999, Li *et al.*, 2002). Hence, it would be of considerable interest to determine which of the above or other novel mechanisms (Kostrzewa and Segura-Aguilar, 2003) are specifically involved in ethanol-induced toxicity and nicotinic protection against such damage.

In summary, alcohol-induced cytotoxicity in primary cultures of neocortical cells is blocked by pretreatment with nicotine. The effects of nicotine, in turn may be blocked by nicotinic antagonists, implicating both high and low affinity nicotinic receptors. The neuroprotective effect of nicotine against ethanol-induced toxicity in neocortical cells provides support for the hypothesis that the high incidence of smoking among alcoholics may be at least partially due to pharmacodynamic interactions between nicotine and alcohol. Further in-vivo studies are required to confirm this hypothesis in regard to cerebral cortical functions.

Acknowledgment

Supported by NIAAA (U56 AA014643) and NIH /NIGMS (2SO6 GM08016-33).

References

Belluardo N, G Mudo, M Blum and K Fuxe (2000) Central nicotinic receptors, neurotrophic factors and neuroprotection. *Behav. Brain Res.* **113**, 21-34.

Bhave SV and PL Hoffman (1997) Ethanol promotes apoptosis in cerebellar granule cells by inhibiting the trophic effect of NMDA. *J. Neurochem.* **68**, 578-586.

Blomqvist O, M Ericson, DH Johnson, JA Engel and B Soderpalm (1996) Voluntary ethanol intake in the rat, effects of nicotinic acetylcholine receptor blockade or subchronic nicotine treatment. *Eur. J. Pharmacol.* **314**, 257-267.

Butt CM, NM King, JA Stitzel and A Collins (2004) Interaction of the nicotinic cholinergic system with ethanol withdrawal. *J. Pharmacol. Exp. Ther.* **308**, 591-599.

Castellsague X, N Munoz, E DeStephani, CG Victora, R Castellotto, PA Rolon and MJ Quintana (1999) Independent and joint effects of tobacco smoking and alcohol drinking on the risk of esophageal cancer in men and women. *Int. J. Cancer* **82**, 657-664.

Daeppen JB, TL Smith, GP Danko, L Gordon, NA Landi, JI Nurnberger Jr, KK Bucholz, E Raimo and MA Schuckit (2000) Clinical correlates of cigarette smoking and nicotine dependence in alcohol-dependent men and women. The Collaborative Study Group on the Genetics of Alcoholism. *Alcohol. Alcohol.* **35**, 171-175.

Dar MS, ER Bowman and L Chunxiao (1994) Intracerebellar nicotinic-cholinergic participation in the cerebellar adesoinergic modulation of ethanol-induced motor incoordination in mice. *Brain Res.* **644**, 117-127.

Dawson DA (2000) Drinking as a risk factor for sustained smoking. *Drug Alcohol Depend.* **59**, 235-249.

Decker MW and MD Meyer (1999) Therapeutic potential of neuronal nicotinic acetylcholine receptor agonists as novel analgesics. *Biochem. Pharmacol.* **58**, 917-923.

Ericson M, O Blomqvist, JA Engel and B Soderpalm (1998) Voluntary ethanol intake in the rat and the associated accumbal dopamine overflow are blocked by ventral tegmental mecamylamine. *Eur. J. Pharmacol.* **358**, 189-196.

Gould TJ and JA Lommock (2003) Nicotine enhances contextual fear conditioning and ameliorates ethanol-induced deficits in contextual fear conditioning. *Behav. Neurosci.* **117**, 1276-1282.

Gruol DL, AE Ryabinin, KL Parsons, M Cole, MC Wilson and Z Qiu (1998) Neonatal alcohol exposure reduces NMDA induced Ca^{2+} signaling in developing cerebellar granule neurons. *Brain Res.* **793**, 12-20.

Guan ZZ, WF Yu and A Nordberg (2003) Dual effects of nicotine on oxidative stress and neuroprotection in PC12 cells. *Neurochem. Int.* **43**, 243-249.

Hajimohammadreza I, AW Probert, LL Coughenour, SA Borosky, PA Boxer, FW Marcoux and KKW Wang (1995) Inhibition of

calmodulin dependent protein kinase-II attenuates excitotoxic amino acid mediated neuronal death. *J. Neurosci.* **15**, 4093-4101.

Huentelman MJ, CM Peters, WE Ervine, SM Polutnik and P Johnson (1999) Ethanol has differential effects on rat neuron and thymocyte reactive oxygen species levels and cell viability. *Comp. Biochem. Physiol. Pharmacol. Toxicol. Endocrinol.* **124**, 83-89.

Johnson, KA and KM Jenninson (1992) The drinking-smoking syndrome and social context. *Int. J. Addict.* **27**, 749-792.

Kawahara M, M Kato and Y Kuroda (2001) Effects of aluminum on the neurotoxicity of primary cultured neurons and on the aggregation of ß-amyloid protein. *Brain Res. Bull.* **55**, 211-217.

Kihara T, S Shimohama, M Urushitani, H Sawada, J Kimura, T Kume, T Maeda and A Akaike (1998) Stimulation of alpha4 beta2 nicotinic acetylcholine receptors inhibits beta-amyloid toxicity. *Brain Res.* **792**, 331-334.

Ko JK and CH Cho (2000) Alcohol drinking and cigarette smoking, a "partner" for gastric ulceration. *Zhonghua Yi Xue Za Zhi (Taipei)* **63**, 845-854.

Kostrzewa RM and J Segura-Aguilar (2003) Novel mechanisms and approaches in the study of neurodegeneration and neuroprotection. A review. *Neurotoxicity Res.* **5**, 375-383.

Kozlowski LT, JE Henningfield, RM Keenan, H Lei, G Leigh, LC Jelinek and CA Haertzen (1993) Patterns of alcohol, cigarette, and caffeine use in two drug-abusing populations. *J. Subst. Abuse Treat.* **10**, 171-179.

Larsson A and JA Engel (2004) Neurochemical and behavioral studies on ethanol and nicotine interactions. *Neurosci. Biobehav. Rev.* **27**, 713-720.

Leonard S and D Bertrand (2001) Neuronal nicotinic receptors, from structure to function. *Nicotine Tobac. Res.* **3**, 203-223.

Li Y, EM Meyer, DW Walker, WJ Millard, YJ He and MA King (2002) Alpha7 nicotinic receptor activation inhibits ethanol-induced mitochondrial dysfunction, cytochrome c release and neurotoxicity in primary rat hippocampal neuronal cultures. *J. Neurochem.* **81**, 853-858.

Madden PA and AC Heath (2002) Shared genetic vulnerability in alcohol and cigarette use and dependence. *Alcohol Clin. Exp. Res.* **26**, 1919-1921.

Newman MB, GW Arendash, RD Shytle, TT Bickford and PR Sanberg (2002) Nicotine=s oxidative and antioxidant properties in CNS. *Life Sci.* **71**, 2807-2820.

Olsen J, S Sabreo and U Fasting (1985) Interaction of alcohol and tobacco as risk factors in cancer of the laryngeal region. *J. Epidemiol. Community Health* **39**, 165-168.

Opanashuk LA, JR Pauly and KF Hauser (2001) Effect of nicotine on cerebellar granule neuron development. *Eur. J. Neurosci.* **13**, 48-56.

Penland S, B Hoplight, J Obernier and FT Crews (2001) Effects of nicotine on ethanol dependence and brain damage. *Alcohol* **24**, 45-54.

Picciotto MR (2003) Nicotine as modulator of behavior, beyond the inverted U. *Trends Pharmacol. Sci.* **24**, 493-499.

Prendergast MA, BR Harris, S Mayer and JM Littleton (2000) Chronic, but not acute, nicotine exposure attenuates ethanol withdrawal-induced hippocampal damage *in vitro*. *Alcohol Clin. Exp. Res.* **24**, 1583-1592.

Rezvani AH and ED Levin (2003) Nicotine-alcohol interactions and attentional performance on an operant visual signal detection task in female rats. *Pharmacol. Biochem. Behav.* **76**, 75-83.

Romberger AJ and K Grant (2004) Alcohol consumption and smoking status, the role of smoking cessation. *Biomed. Pharmacother.* **58**, 77-83.

Rose JE, LH Brauer, FM Behm, M Cramblett, K Calkins and D Lawhon (2004) Psychopharmacological interactions between nicotine and ethanol. *Nicotine Tobac. Res.* **6**, 133-144.

Shaw S, M Bencherif and MB Marrero (2003) Angiotensin II blocks nicotine-mediated neuroprotection against ß-amyloid (1-42) via activation of the tyrosine phosphatase SHP-1. *J. Neurosci.* **23**, 11224-11228.

Shiffman S and M Balabanis (1995) Associations between alcohol and tobacco, In *Alcohol and Tobacco, From Basic Science to Clinical Practice* (Fertig JB and JP Allen, Eds.), *NIAAA Res Monograph* **30**, NIH PUB95-3931, pp 17-36.

Soderpalm B, M Ericson, P Olausson, O Blomqvist and JA Engel (2000) Nicotinic mechanisms involved in the dopamine activating and reinforcing properties of ethanol. *Behav. Brain Res.* **113**, 85-96.

Stevens TR, SR Krueger, RM Fitzsimonds and MR Picciotto (2003) Neuroprotection by nicotine in mouse primary cortical cultures involves activation of calcineurin and l-type calcium channel inactivation. *J. Neurosci.* **23**, 10093-10099.

Takada Y, A Yonezawa, T Kume, H Katsuki, S Kaneko, H Sugimoto and A Akaike (2003) Nicotinic acetylcholine receptor-mediated neuroprotection by donepezil against glutamate neurotoxicity in rat cortical neurons. *J. Pharmacol. Exp. Ther.* **306**, 722-727.

Tizabi Y, RL Copeland Jr, VA Louis and RE Taylor (2002) Effects of combined systemic alcohol and central nicotine administration into VTA on dopamine release in the nucleus accumbens. *Alcohol Clin. Exp. Res.* **26**, 394-399.

Tizabi Y, M Al-Namaeh, KF Manaye and RE Taylor (2003) Protective effects of nicotine on ethanol-induced toxicity in cultured cerebellar granule cells. *Neurotoxicity Res.* **5**, 315-322.

True WR, H Xian, JF Scherrer, PAF Madden, KK Bucholz, AC Heath, SA Eisen, MJ Lyons, J Goldberg and M Tsuang (1999) Common genetic vulnerability for nicotine and alcohol dependence in men. *Arch. Gen. Psychiatr.* **56**, 655-661.

Winzer-Serhan UH and FM Leslie (1997) Codistribution of nicotinic acetylcholine receptor subunit alpha3 and beta4 mRNAs during rat brain development. *J. Comp. Neurol.* **386**, 540-554.

Zanardi A, G Leo, G Biagini and M Zoli (2002) Nicotine and neurodegeneration in ageing. *Toxicol. Lett.* **127**, 207-215.