Int J Physiol Pathophysiol Pharmacol 2013;5(3):161-168
www.ijppp.org /ISSN:1944-8171/IJPPP1306002

Original Article

Modulation of nicotinic acetylcholine receptors in native sympathetic neurons by ethanol

Zheman Xiao[1*], Fan Zhu[2*], Zuneng Lu[1], Songqing Pan[1], Jingjing Liang[1], Jiangquan Zheng[3], Zhongchun Liu[1]

[1]Department of Neurology, Institution of Neuropsychiatry Research, Renmin Hospital of Wuhan University, Jiefang Road 238, Wuhan 430060, Hubei, PR China; [2]School of Basic Medical Science, Wuhan University, Hubei, PR China 430071; [3]Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences, 27 Taiping Road, Beijing, PR China. [*]These authors are contributed equally to the work.

Received June 13, 2013; Accepted June 28, 2013; Epub September 10, 2013; Published September 15, 2013

Abstract: Objective: To determine whether actions on nicotine acetylcholine receptors (nAChRs) contribute to ethanol's depressant effects on the autonomic nervous system. Methods: The acute effects of ethanol on nAChRs were examined in primary cultured superior cervical ganglion (SCGs) by whole-cell patch clamp recordings. After the whole-cell configuration was formed, drugs diluted to various concentrations with extracellular solution were applied directly to single neurons. Results: Held at -70 mV, ethanol significantly and reversibly inhibited nicotine-evoked currents (I_{Nic}) with a maximum inhibition rate of ~80% and an IC_{50} of 232.88±40.66 mM. At 50 mM, ethanol accelerated the slow decay, but did not affect the quick decay and rising time of I_{Nic}. There was neither use-dependence nor voltage-dependence of ethanol on suppressing I_{Nic} in SCGs. Conclusion: Ethanol inhibited the whole-cell I_{Nic} significantly, probably through noncompetitive inhibition at the binding sites outside of the cell membrane.

Keywords: Ethanol, superior cervical ganglion, nicotine, desensitization, nicotine acetylcholine receptors

Introduction

Ethanol is one of the most commonly used psychotropic drugs, and the small ethanol molecule displays wide and complex effects by interacting with biological membranes and proteins. The alcohol modulation of many channels and receptors has been studied extensively; for example, it has been studied in sodium channels [1], BK channels [2], NMDA receptors [3], GABA-A receptor chloride channel complexes [4], and glycine receptor channels [5], as well as in the inhibition of Ca^{2+} channels [6, 7]. In the case of nicotinic acetylcholine receptors (nAChRs), the action of alcohol is more complex.

Alcohols appear to have two predominant actions on the nAChR. Ethanol and other short-chain n-alkanols potentiate ion current or ion flux through these receptor-channels. In addition to alcohol-induced potentiation of nAChR function, an additional channel-blocking action of alcohols has been described. Different nAChR has different response to different Alcohols [8]. The present research aimed to investigate the effect of ethanol to superior cervical ganglion neurons (SCG), the direct target to ethanol.

Ligand-gated ion channels are important regulators of neuronal excitability and are likely molecular targets for anesthetics and alcohols acting on in the central nervous system. Peripheral nicotinic acetylcholine receptors, such as the ligand-gated ion channels found in the central nervous system, are sensitive to anesthetics [9]. nAChR is the best characterized member of the superfamily of cysteine-loop ligand-gated ion channels [10]. Similar to alcohol, nicotine (NIC) is a substance that is widely used and is present in smoking and chewing tobacco products. Approximately 29.9% of the population in China admitted to using a form of tobacco product (the principal method of nicotine intake) in 2008.

It is a well-known fact that both alcohol and nicotine are frequently used simultaneously; it has been reported that alcoholics commonly

engage in heavy cigarette smoking [11-13]. From a toxicology standpoint, it is therefore important to understand how these drugs interact to exert their effects on the organ tissues and cells. Currently, there is evidence to suggest that nicotine, when administered in conjunction with alcohol, may be capable of protecting against alcohol-mediated deleterious effects. In an in vitro model system [14], found that pretreatment of primary cultures of cerebellar granule cells with nicotine was capable of protecting the cells against alcohol-induced cytotoxicity and that this effect was blocked by nAChR antagonists. Alternately, in vivo studies have suggested that nicotine-mediated attenuations of alcohol-induced damage may be a result of nicotine's ability to decrease blood alcohol concentration, possibly through the action of gastric alcohol dehydrogenase [15].

Materials and methods

Cell preparation

The SCGs were dissociated from the of p1 Wistar rats of either sex in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23). In brief, freshly dissected SCGs were transferred to a 35-mM culture dish containing dissection solution. The ganglia were individually trimmed to remove the peripheral nerves and excessive tissues under a Zeiss operating microscope in DMEM (Gibico, Carlsbad, CA, USA) with trypsin (5 mg/mL, Sigma-Aldrich, St. Louis, MO, USA). After enzymatic treatment for 30 to 40 min at 37°C equilibrated with 95% air and 5% CO_2, the suspension was centrifuged at 500×*g* for 2 min. Next, the ganglia were rinsed with stop solution, and SCGs were mechanically dissociated by trituration in a fire-polished Pasteur pipette. The pellet was gently resuspended in DMEM containing insulin (1 mg/mL, Sigma-Aldrich), transferrin (1 mg/mL, Sigma-Aldrich), penicillin (100 U/ml), streptomycin (100 U/ml) and nerve growth factor (50 ng/ml; Alomone Labs, Jerusalem, Israel). The cell suspension was plated into a 24-well culture plate with a poly (D-lysine)-coated (50 μg/ml; Sigma-Aldrich) glass coverslip (12-mm diameter; Fisher Scientific Co., Fair Lawn, NJ, USA) in each well and incubated with air containing 5% CO_2 at 37°C. Half of the growth medium was changed every 3 days.

Electrophysiological recordings

At 3-7 days after plating, SCGs were bathed with physiological solution containing 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 3 mM $CaCl_2$, 10 mM glucose, and 10 mM HEPES; pH was adjusted to 7.3 with NaOH, osmolarity was adjusted to 310 mOsm/L with glucose. Nicotine-evoked currents were measured from cultured SCGs with a conventional whole-cell patch clamp technique. Data were acquired with an Axopatch 1-D. The pClamp 8.2 software package was interfaced to a Digidata 1322A acquisition board (Axon Instruments, Inc., Foster City, CA), and signals were filtered at 5 kHz, digitized at 10 kHz and stored in a PC for analysis. Patch electrodes were pulled from borosilicate capillaries using PP-830 (Narishige CO., Tokyo, Japan) micropipette puller, and the tips were heat-polished. The pipette resistances were 2-4 MΩ when filled with 140 mM KCl, 10 mM HEPES, 2 mM Na_2ATP, and 10 mM EGTA intracellular solution; pH was adjusted to 7.3 with KOH and osmolarity was adjusted to 300 mOsm/L with glucose. The membrane potential was held at -70 mV.

Applications of agonists and antagonists

All drugs were applied by a puff pipette, which was connected to a pressure ejector (BH-2, Medical Systems Corp.). N_2 pressure was adjusted to approximately 50-60 kPa. The puff pipette consisted of 3 microtubes. One of microtubes was filled with the agonist nicotine (Sigma-Aldrich) as the control group. The other microtubes were filled with a mixture of the same concentration of the agonist and different concentrations of ethanol. The diameter of a microtube was 5-10 μm. The distance between the pipette and the recorded neuron was 20-30 μm. The duration of drug application was 1 s, and the interval time between the applications was 3 min. We had detected that the 3 min interval was sufficient for nAChRs recovering from the desensitization and the residual inhibition of ethanol. After each application of a drug, the pipette must be moved out of the extracellular solution at once to avoid a possible diffusion of the drugs.

Data analysis

The peak amplitude, and the exponential decay time constant (τ) of the whole-cell currents

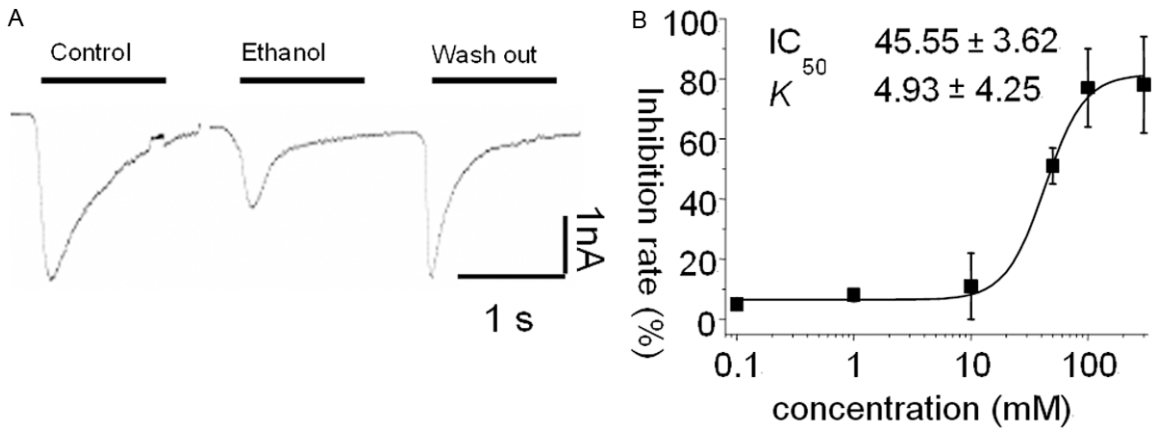

Figure 1. Inhibition and its reversibility by ethanol on nicotine-evoked currents in cultured superior cervical ganglions (SCGs). A: Representative whole-cell inward currents elicited by brief nicotine pulses (1 s) on a single SCG before (left) and after (middle) the application of ethanol. Note that the current amplitude completely recovered after 3 min of washout (right). B: Mean normalized inhibition rate with increasing ethanol concentrations. Data fitted to a Bolzmann equation with IC_{50}=45.55±3.62 mM (P<0.05, n=7).

were determined using the pCLAMP 8.2 program. The software Origin 7.0 (MicroCal Software, Inc.) was used for curve fitting and graphic display. Data were obtained from 7 cells taken from at least three different rats for each experiment. Data were shown as means±S.E.M. Statistical analysis was conducted by one-way analysis of variance (ANOVA) for multiple comparisons between two groups and by paired-t test for comparisons in the presence and absence of ethanol using the SPSS software. Differences were considered to be statistically significant at *P* values of less than 0.05.

IC_{50} values corresponded to the concentration of inhibiting agent causing a 50% reduction in the current evoked by a pulse of nicotine near the EC50 value (80 μM). The nicotine-evoked currents in the presence of the ethanol were measured at -70 mV and normalized to the amplitude of the current elicited by nicotine alone. IC_{50} was fitted with the Boltzmann Equation by the pCLAMP 8.2 program.

Results

Concentration-dependent blockade of ethanol on the nicotine-evoked current

Robust inward currents were produced when the receptors were stimulated with 80 μM nicotine at a holding potential of -70 mV. The stimulation lasted for 1 s, and the interval between stimulations was 3 min.

The agonist-induced current inhibited by ethanol (I) was compared with the control current (I_0) at -70 mV, and the inhibitory rate (%) was equal to $I*100/I_0$. When mixtures of nicotine and different concentrations of ethanol were puffed onto the neuron, the amplitudes of nicotine-induced currents were obviously depressed with a maximum inhibition rate of ~80%, and an IC_{50} of 45.55±3.62 mmol/L (**Figure 1**). The differences among the inhibitory rates induced by various concentrations of ethanol were significant (*P*<0.001). The results showed that ethanol could dose-dependently block neuronal nAChRs.

Effect of ethanol on kinetics of neuronal nAChRs

To observe the effect of ethanol on the desensitization of nAChRs, the application time was prolonged to 15 s, and the interval was accordingly increased to 6 min. The membrane potential was held at -70 mV. The effect of the ethanol at its IC_{50} concentration (50 mM) on the rise, and the decay phase of the currents was studied. The currents recorded in the presence of the ethanol (50 mM) were normalized at their peak amplitude to the corresponding control current (**Figure 2**). The rise time (10-90%) of an 80 μM nicotine-evoked current ranged from 81.2 to 213.1 ms (143.45±13.77 ms, *n*=10) and was not affected significantly by ethanol at its IC_{50} concentration (193.76±11.2 ms, *n*=10; *P*>0.05).

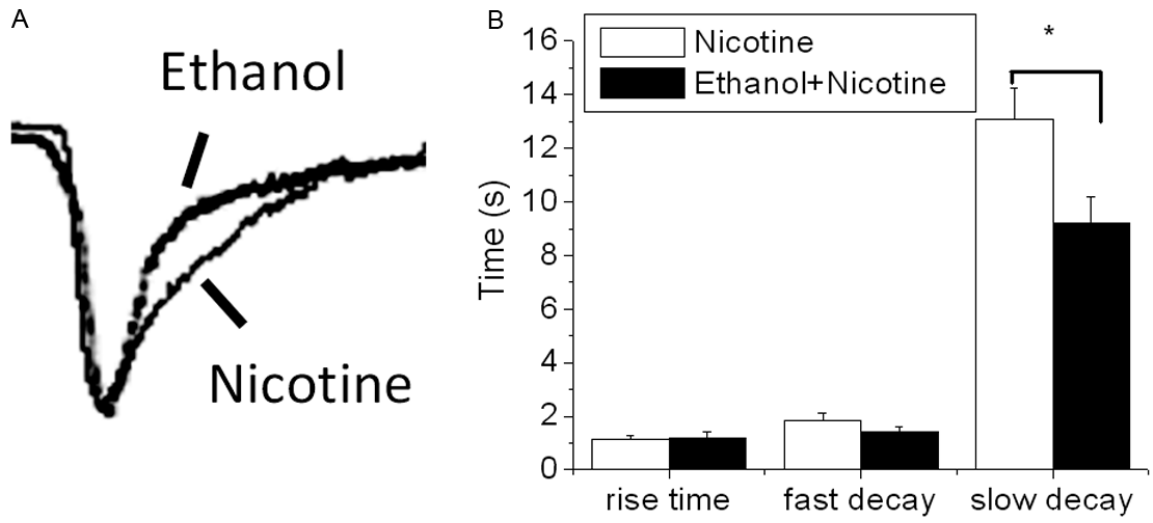

Figure 2. Effect of ethanol on the rise and decay times of nicotine-evoked currents. A: Representitive recordings evoked by the application (15 s) of nicotine (80 μM) in the absence and the presence of 50 mmol/L ethanol to a cell held at -70 mV. B: The bar graph shows a comparison of the rise time and decay time constants of the nicotine-induced currents evoked in the absence and presence of ethanol. *Ethanol significantly decreases the time of slow decay ($P<0.05$) without affecting the rise time and fast decay. The results are presented as means±S.E.M. (n=10).

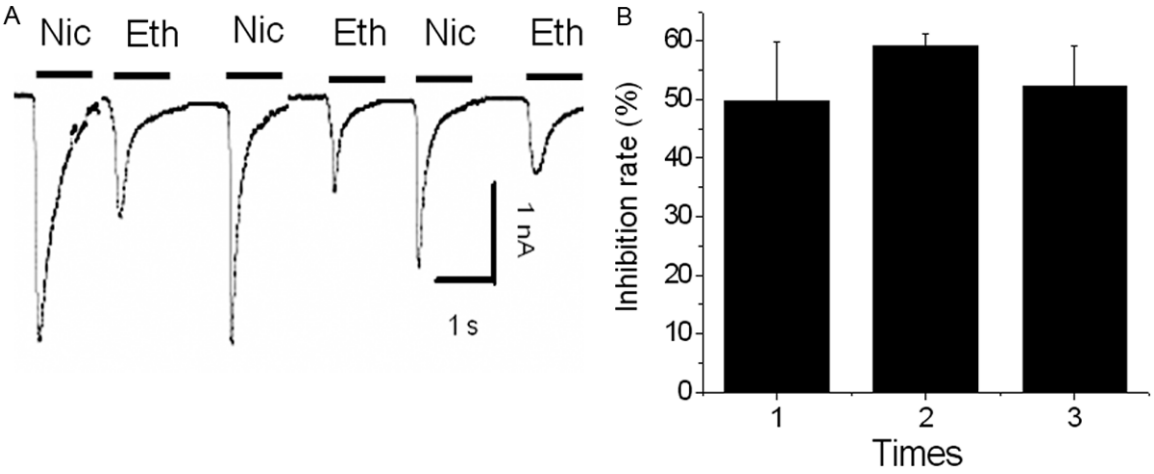

Figure 3. Use dependence of the inhibitory effect of ethanol on nicotine-evoked currents. A: Three pulses of the mixture of ethanol (50 mM) and nicotine (80 μM) separated by three pulses of the control nicotine (80 μM, 1 s) were delivered to the representative cell every 3 min. Nic refers to nicotine, Eth refers to the mixture of ethanol (50 mM) and nicotine. B: The bar graph shows that the inhibitory effect of ethanol in nicotine-induced currents is not use-dependent (n=7).

The decay rate of the nicotine-induced currents basically displayed the desensitization rate of the nAChRs. We fitted the descendent phases of the currents with a double-exponential curve. The results showed that the fast decay time constants ($\tau 1$) of the currents were intact from 1.83±0.38 s to 1.36±0.38 s ($n=7$, $P>0.05$) in the presence of ethanol, while the slow decay time constants ($\tau 2$) changed from 13.1±2.17 s to 9.23±1.98 s ($n=7$, $P<0.05$). This finding confirmed that ethanol could only accelerate the slow decay rate of nicotine-induced currents.

Use-independent blockade of nAchRs by ethanol

To study the use-dependence phenomenon of inhibition by ethanol, we measured the responses to six pulses (1 s) held at -70 mV and spaced 3 min apart. Three pulses of the ethanol (50

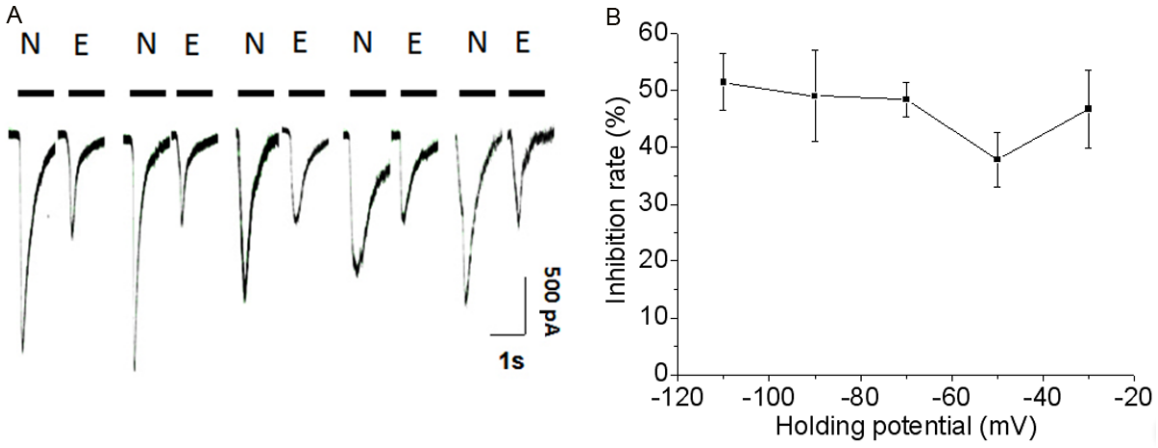

Figure 4. Voltage dependence of the inhibitory effect of ethanol in nicotine-evoked currents. A: Representitive current traces evoked by the application of nicotine (N, 80 μM) or ethanol (E, 50 mM) plus nicotine (80 μM) to the cells held at various potentials of -110, -90, -70, -50 and -30 mV. B: The relationship between the holding potential and the ratio of the amplitudes evoked in the presence or absence of ethanol at the corresponding holding potential is summarized for four cells on the right. Symbols and bars represent the mean±S.E.M. (n=7).

mM) and nicotine (80 μM) mixture were separated by three control pulses of nicotine (80 μM). The nicotine-induced current inhibited by ethanol (I) was compared with the preceding control current (I_0). It could be observed that in spite of the difference in current magnitude obtained with the mixture and control, the inhibition rate is roughly the same (49.8±10.53, 59.1±2.0, 52.23±7.89, P>0.05, n=7), regardless of the use time (**Figure 3**).

Voltage-independent blockade of nAchRs by ethanol

To determine whether the ethanol binding site on AChRs is located externally or within the ion channel, we studied the inhibition of nicotine-currents by ethanol as a function of membrane potential. The effect of the membrane potential on the inhibition of ethanol was observed at various holding membrane potentials. The drugs were puffed for 1 s, and the interval between puffs was 3 min.

The inhibition rates of ethanol on nicotine-evoked current at different holding potentials (-110 mV, -90 mV, -70 mV, -50 mV and -30 mV) were 51.46±5.98%, 49.04±8.23%, 48.39±3.04, 37.86±4.78%, and 46.73±6.78%, respectively (P>0.05, n=7). The statistical results revealed that the interaction between the change of membrane potentials and the inhibition rate was not significant, which meant that the blockade of ethanol on neuronal AChRs was not notably affected by the hyperpolarization of the membrane potentials (**Figure 4**).

Discussion

In the present research, ethanol dose-dependently depressed the nicotine-induced currents. In the presence of ethanol (50 mM), the slow decay time constants of nicotine-induced currents decreased remarkably (P<0.05), while the fast decay was intact. This result means that ethanol could speed up the desensitization of the SCG neuronal nAChRs. The phenomenon of desensitization meant a reversible decline in the conductance of responses to the agonists. In the presence of an antagonist interacting with the agonist action site, a number of the receptors will be inhibited and stay in a refractory condition, while other receptors may still be competitively excited by agonists. For the antagonists that act competitively on the recognition sites of nAChRs, their influence on the receptor activities such as channel opening, closing, and desensitization are short-lived in the presence of agonists. However, allosteric antagonists can influence the desensitization of receptors, even in the presence of agonists because their binding sites on neuronal nAChR are different from agonist recognition sites [16]. As ethanol could speed up the decay of nicotine-induced current, i.e., accelerate the desensitization of neuronal nAChR, it

was deduced that ethanol might depress nAChR by interacting with allosteric sites, rather than acetylcholine recognition sites.

It was found that ethanol's inhibition of nicotine-activated currents was independent of membrane voltage between -110 and -30 mV, with no statistically significant differences being observed between the inhibition rates of the peak currents (**Figure 4**). The fact that the I-V relationships remained unaltered suggests that ethanol inhibits nicotinic currents by a mechanism other than blocking the channel core [17].

The use dependence of blocking ACh receptors has been attributed to drug trapping in the receptor channel [18]. Inhibition of nicotine-evoked currents by ethanol at 50 mM was use-independent, which indicated that the interactions of ethanol with nAChRs were on the extracellular domain.

Our previous research has shown that ethanol (100 mmol/L) inhibits the Ca^{2+} influx through the voltage-gated calcium channel (VDCC) and the ionophore channel, as well as the voltage-gated sodium channel (VDSC) [1, 6]. It is well-known that 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP), a nicotinic acetylcholine receptor (nAChR) agonist [19], nicotine [20] and Ach [21] induce calcium and sodium influx. Inhibition of nAChR function decreases Ach induced calcium and sodium influx. It was speculated that the inhibition of VDCC and VDSC is a consequence of inhibiting nAChRs.

Several α and β subunits that can contribute to the formation of neuronal nAChRs have been cloned. The α2-6 subunits appear to participate with the β2-4 subunits in forming neuronal nicotinic receptors that are insensitive to the antagonist α-bungarotoxin. These receptors appear to be formed from the confluence of α and β subunits, sometimes with more than one α subunit type per receptor. Two α subunits (7 and 8) have been identified that form the α-bungarotoxin sensitive neuronal nicotinic receptors [22]. These subunits can form fully functional homomeric receptor channels in heterologous expression systems and may do so in neurons, as well. One notable feature of the neuronal α-bungarotoxin sensitive receptors is the high calcium permeability of their channels. Superior cervical sympathetic ganglion neurons of rat express predominantly α7, α3, and α4 subunits [23]. Therefore, this study's results might be explained by α7-mediated inhibition of VDCC by ethanol. Some investigations have concentrated on examining alcohol effects on the neuronal nAChR subtypes including the α-bungarotoxin-sensitive α7 subunit. These studies indicate that ethanol inhibits the function of these receptors at pharmacologically relevant concentrations. It might be the mechanisms ethanol inhibiteed nAChR in SCGs [8]. Further experiments may be necessary to confirm this hypothesis.

The blood concentration of ethanol at which most people undergo acute alcohol intoxication is approximately 100-150 mg/L (22-33 mM). A concentration higher than 400-500 mg/ml (85-100 mM) could be lethal [24]. Given that concentrations as high as 300 mM had been encountered [25, 26], the 50-mM ethanol concentration used in the present study is a clinically pathological dose. The inhibition of nAChRs by ethanol may explain the toxicity caused by alcoholism. However, it is still not clear if the toxic effects are only due to the blockage of nAChRs, and what the functional relationships of VDCC, VDSC and nAChRs are. Further studies will be required to address these questions.

Currently, there is evidence to suggest that nicotine, when administered in conjunction with alcohol, may be capable of protecting against alcohol-mediated deleterious effects [27]. In an in vitro model system, it was found that pretreatment with nicotine on cerebellar granule and cerebral cortical cells was capable of protecting against alcohol-induced cytotoxicity and that this effect was blocked by nicotinic acetylcholine receptor (nAChR) antagonists [14]. Alternately, in vivo studies have suggested that nicotine-mediated attenuations of alcohol-induced damage may be a result of nicotine's ability to decrease blood alcohol concentration, possibly through the action of gastric alcohol dehydrogenase [15].

Conclusion

In conclusion, these experiments demonstrate that a pathological dose of ethanol noncompetitively blocks nicotinic currents from nAchRs expressed on SCGs in culture. Ethanol accelerated the slow decay but did not affect the quick

decay and rising time. There was no use dependence and voltage dependence of ethanol in suppressing I_{Nic} in SCGs. This study provided the evidence that the observed toxic effects of ethanol on the SCGs nAchRs could be responsible for the autonomic dysfunction in alcoholics.

Acknowledgements

This work was supported by a NNSF grant (30900459), a national doctoral program grant (200804861046), and a junior teacher grant of Wuhan University (3082012), China.

Address correspondence to: Zhongchun Liu or Zuneng Lu, Department of Neurology, Institution of Neuropsychiatry Research, Renmin Hospital of Wuhan University, Jiefang Road 238, Wuhan 430060, Hubei, PR China. E-mail: zcliu6@whu.edu.cn (Zhongchun Liu); 13995672166@126.com (Zuneng Lu)

References

[1] Xiao Z, Lu Z, Liu Z, Liu W, Li L, Yin S, Yu S, Dong H, Zhu F. Ethanol inhibits voltage gated sodium channels in cultured superior cervical ganglion neurons. Neuroreport 2008; 19: 1773-1776.

[2] Crowder CM. Ethanol targets: a BK channel cocktail in C. elegans. Trends Neurosci 2004; 27: 579-582.

[3] Boyce-Rustay JM, Cunningham C. The role of NMDA receptor binding sites in ethanol place conditioning. Behav Neurosci 2004; 118: 822-834.

[4] Mediratta PK, Mahajan P, Sharma KK, Bhandari R, Dubey SP. Involvement of GABA-A receptor chloride channel complex in isolation stress-induced free choice ethanol consumption in rats. Indian J Exp Biol 2003; 41: 47-52.

[5] Eggers ED, Berger AJ. Mechanisms for the modulation of native glycine receptor channels by ethanol. J Neurophysiol 2004; 91: 2685-2695.

[6] Xiao ZM, Li LJ, Yu SZ, Lu ZN, Li CY, Zheng JQ. Effects of extracellular Ca^{2+} influx and intracellular Ca^{2+} release on ethanol-induced cytoplasmic Ca^{2+} overload in cultured superior cervical ganglion neurons. Neurosci Lett 2005; 390: 98-103.

[7] Oz M, Tchugunova YB, Dunn SM. Direct inhibition of voltage dependent Ca^{2+} fluxes by ethanol and higher alcohols in rabbit T-tubule membranes. Eur J Pharmacol 2001; 418: 169-176.

[8] Lovinger DM. Alcohols and neurotransmitter gated ion channels: past, present and future. Naunyn Schmiedebergs Arch Pharmacol 1997; 356: 267-282.

[9] Zhou QL, Zhou Q, Forman SA. The n-Alcohol site in the nicotinic receptor pore is a hydrophobic patch. Biochemistry 2009; 39: 14920-14926.

[10] Karlin A. Emerging structure of the nicotinic acetylcholine receptors. Nat Rev Neurosci 2002; 3: 102-114.

[11] Ait-Daoud N, Wiesbeck GA, Bienkowski P, Li MD, Pfützer RH, Singer MV, Lesch OM, Johnson BA. Comorbid alcohol and nicotine dependence: from the biomolecular basis to clinical consequences. Alcohol Clin Exp Res 2005; 29: 1541-1549.

[12] Little HJ. Behavioral mechanisms underlying the link between smoking and drinking. Alcohol Res Health 2000; 24: 215-224.

[13] Sher KJ, Gotham HJ, Erickson DJ, Wood PK. A prospective, high-risk study of the relationship between tobacco dependence and alcohol use disorder. Alcohol Clin Exp Res 1996; 20: 485-492.

[14] Tizabi Y, Manaye KF, Taylor RE. Nicotine blocks ethanol induced apoptosis in primary cultures of rat cerebral cortical and cerebellar granule cells. Neurotox Res 2005; 7: 319-322.

[15] Gilbertson RJ, Barron S. Neonatal ethanol and nicotine exposure causes locomotor activity changes in preweanling animals. Pharmacol Biochem Behav 2005; 81: 54-64.

[16] Bertrand D, Gopalakrishnan M. Allosteric modulation of nicotinic acetylcholine receptors. Biochem Pharmacol 2007; 74: 1155-1163.

[17] Lax P. Melatonin inhibts nicotinic currents in cultured rat cerebellar granule neurons. J Pineal Res 2008; 44: 70-77.

[18] Aracava Y, Pereira E, Maelicke A, Albuquerque E. Memantine Blocks α7* Nicotinic Acetylcholine Receptors More Potently Than N-Methyl-D-aspartate Receptors in Rat Hippocampal Neurons. J Pharmacol Exp Ther 2005; 312: 1195-1205.

[19] Kim DC, Park YS, Jun DJ, Hur EM, Kim SH, Choi BH, Kim KT. N-(4-Trifluoromethylphenyl) amide group of the synthetic histamine receptor agonist inhibits nicotinic acetylcholine receptor-mediated catecholamine secretion. Biochem Pharmacol 2006; 71: 670-82.

[20] Si ML, Lee TJ. Pb^{2+} inhibition of sympathetic alpha 7- nicotinic acetylcholine receptor-mediated nitrergic neurogenic dilation in porcine basilar arteries. J Pharmacol Exp Ther 2003; 305: 1124-1131.

[21] Bray C, Son JH, Meizel S. Acetylcholine causes an increase of intracellular calcium in human sperm. Mol Hum Reprod 2005; 11: 881-889.

[22] Couturier S, Bertrand D, Matter JM, Hernandez MC, Bertrand S, Millar N, Valera S, Barkas T,

Ballivet M. A neuronal nicotinic acetylcholine receptor subunit (alpha 7) is developmentally regulated and forms a homo-oligomeric channel blocked by alpha-BTX. Neuron 1990; 5: 847-856.

[23] Srivatsan M, Treece J, Shotts EE. Nicotine alters nicotinic receptor subunit levels differently in developing Mammalian sympathetic neurons. Ann N Y Acad Sci 2006; 1074: 505-513.

[24] Rubin E. Alcoholic myopathy in heart and skeletal muscle. New Engl J Med 1979; 301: 28-33.

[25] Berlid D, Hasselbalch H. Survival after a blood alcohol of 1127 mg/dl. Lancet 1981; 2: 363.

[26] Johnson RA, Noll EC, Rodney WM. Survival after a serum ethanol concentration of 1.5%. Lancet 1982; 8312: 1394.

[27] Taslim N, Saeed Dar M. The Role of Nicotinic Acetylcholine Receptor (nAChR) α(7) Subtype in the Functional Interaction Between Nicotine and Ethanol in Mouse Cerebellum. Alcohol Clin Exp Res 2010; 35: 540-549.