Effects of chronic ethanol consumption on rat upper gastrointestinal system: Functional and histologic findings

Yusufhan Yazir, Melih Tugay, Zafer Utkan, Tijen Utkan

**Abstract**

The purpose of the present study was to determine the effect of chronic alcohol consumption on reactivity of esophageal tunica muscularis mucosae (TMM) and lower esophageal sphincter (LES) smooth muscle. Six male rats in alcohol-fed group received ethanol (7.2% v/v) in a modified liquid diet for 4 weeks. Two control groups were used; six rats in the standard diet-fed group received rat chow and water for 4 weeks. Six rats in sucrose-fed group were given sucrose and received a liquid diet. The smooth muscle reactivity of TMM and LES strips from ethanol-fed and control animals was evaluated in organ chambers. Also histologic study was undertaken to show effects of chronic alcohol consumption. Maximum contractile responses of TMM to KCl and carbachol were decreased in the ethanol-fed group compared to the control groups. In LES, isoproterenol- and papaverine-induced relaxant responses were similar in the ethanol-fed and control groups. Relaxant responses to serotonin were decreased in the ethanol-fed group compared to the control groups. In TMM, isoproterenol- and papaverine-induced relaxant responses were similar in the ethanol-fed and control groups. In TMM, isoproterenol- and papaverine-induced relaxant responses were similar in the ethanol-fed and control groups. Relaxant responses to serotonin were decreased in the ethanol-fed group compared to the control groups. Our findings suggest that chronic alcohol consumption impairs relaxant and contractile responses of both TMM and LES smooth muscle and it may contribute to gastroesophageal reflux commonly seen after alcohol binges.

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**Introduction**

Alcohol influences the motor activity in the esophagus, lower esophageal sphincter (LES), stomach, and small bowel and has direct effects on the mucosa of the upper tract. Previously, it has been reported that both acute and chronic ethanol consumption may cause esophageal dysmotility and LES pressure changes in human subjects (Bujanda, 2000). Moreover acute ethanol in vivo decreases both the pressure of the LES and the amplitude of contractions of the smooth muscle of the esophagus (Keshavarzian et al., 1994). It is designated that acute administration of ethanol inhibits the function of excitable tissues including nerves and muscles (Keshavarzian et al., 1991).

Although the effects of chronic alcohol consumption have been well described for several tissues, such as cardiovascular (Utkan, Yildiz, et al., 2001), urinary (Utkan, Erden, et al., 2001b) and gastrointestinal system (Franke, Tyssen, & Singer, 2005; Tyssen & Singer, 2003), the mechanisms underlying impaired smooth muscle contractility are poorly understood. It is well established that muscarinic acetylcholine receptors and nitric oxide are important in esophageal physiology (Goyal & Chaudhury, 2008; Toda & Herman, 2005). Recently, authors showed that acute ethanol inhibits carbachol-induced contractility of the LES and lower esophageal body, suggesting NO is a mediator for the inhibitory effect of ethanol on LES but not lower esophageal body (LEB) contractility (Keshavarzian, Jacyno, Urban, Winship, & Fields, 1996). In this background, our objective was to determine whether chronic alcohol exposure alters in vitro reactivity of LES and esophageal smooth muscle may lead to gastroesophageal reflux. These experiments were conducted on isolated smooth muscles to eliminate extrinsic effects.
Materials and methods

Animals and experimental designs

Adult male Wistar rats (200–250 g) were obtained in the Experimental Medical Research and Application Center (DETAB, Kocaeli University, Kocaeli) at temperature- and humidity-controlled room (22 ± 3 °C and 62 ± 7%, respectively) in which a 12–12 h light–dark cycle was maintained (08:00–20:00 h light). Rats were individually housed in metal cage and divided into three groups; standard diet-fed rats (n = 6), sucrose-fed rats (n = 6), and ethanol-fed rats (n = 6). Rats in ethanol-fed group were fed a liquid diet fortified with vitamins to which ethanol was added in increasing amounts, i.e. 5% v/v ethanol during week 1 and 7.2% ethanol during weeks 2–4, as previously described (Uzbay & Kayaalp, 1995). Sucrose-fed animals were pair fed on an isocaloric liquid diet containing sucrose as a caloric substitute for ethanol. In this group, the calorie content of the liquid diet was adjusted to match that of the ethanol-exposed group. The rats received a modified liquid diet with or without ethanol ad libitum. No extra chow or water was supplied. The liquid diet was prepared daily and animals received the diet at the same time of the day (11:00 h). The rats were weighed every day, and daily ethanol intake measured and expressed as grams per kilogram per day. Standard diet-fed rats received standard rat chow and tap water ad libitum. Ethical approval was granted by the Kocaeli University Animal Research Ethics Committee (Kocaeli, Turkey, Project number: REC 11–11).

Organ bath studies

At the end of the 4-week period the rats were killed using an overdose of ether. TMM strips were prepared according to Baxter, Craig, and Clarke (1991). LES strips were also prepared in a manner consistent with the method described by Coruzzi, Poli, and Bertaccini (1985). TMM and LES strips were mounted in 20 ml organ chambers for isometric tension measurement. The tissue baths contained Tyrode’s solution composed of (millimoles per liter): NaCl 114; KCl 4.7; CaCl2 2.5; MgSO4 1.2; KH2PO4 1.2; NaHCO3 2.5; glucose 11.5. The solution was gassed with 95% O2 and 5% CO2 during the study and the temperature was maintained at 37 °C. The strips were tied with silk thread to a force transducer (MAY-COM FDT 10-A, COMMAT Iletisim Co. Ankara, Turkey) at one end and fixed to a glass support at the other end. Isometric tension was recorded on a computer via four-channel transducer data acquisition system (TDA-94 COMMAT, COMMAT Iletisim Co. Ankara, Turkey) using software (Polywin 95 ver 1.0 COMMAT, COMMAT Iletisim Co. Ankara, Turkey), which also had the capacity to analyze the data. After mounting, the TMM and LES strips were allowed to equilibrate under a resting tension 0.5 g for 90 min. During this period, the bath fluid was routinely changed every 15 min. Agonists were added directly to the organ bath. At the completion of each experiment, tissues were lightly blotted and weighted. In the beginning of the in vitro experiments, first TMM and LES strips were stimulated with 80 mM KCl. In examining the contractile response to the muscarinic agonist carbachol (10−9–3 × 10−4 M), cumulative concentration–response curve was constructed in a stepwise manner after the response to the previous concentration had reached a plateau both for TMM and LES strips. Following completion of carbachol dose response curve, tissues were washed for a further 60 min and precontracted with a submaximal concentration of carbachol (10−6–3 × 10−6 M). After the contractions reached a plateau relaxation responses for TMM strips were obtained. Concentration–response relationships for serotonin (10−7–10−3 M), isoproterenol (10−7–10−4 M) and papaverine (10−6–10−4 M), was obtained for TMM in a cumulative manner.

LES strips were contracted with a submaximal concentration of carbachol (10−6–3 × 10−6 M) and after the response stabilized relaxation response to isoproterenol (10−6–10−4 M), SNP (10−6–10−4 M), nicotine (10−6–10−3 M) and papaverine (10−6–10−4 M) were obtained by cumulative additions of small aliquots of concentrated stock solutions directly to the tissue bath; drug concentrations were increased when the response to the preceding concentration was maximal.

Blood analysis

Animals were not fasted prior to blood collection. Blood ethanol concentration was determined in a drop of whole blood (5 ml per rat) collected at the time of death (in the morning 09:00–10:00 h), using the alcohol dehydrogenase method (Aeroset C8000 autoanalyzer, Abbott Diagnostics, IL) and glucose was also determined using a commercial glucose meter and glucose-sensitive dipsticks (Accutrend Alpha glucometer, Boehringer, Mannheim, Germany).

Analysis of data

Results are expressed as mean ± SEM where n equals the number of animals. The contractile force was expressed as milligrams of developed tension per milligram of tissue wet weight. The relaxant effects of agonists were expressed as a percentage of the precontracting to carbachol. Concentration–response curves were fitted by nonlinear regression with simplex algorithm and Emax (maximum response) and EC50 (i.e. the concentration for the half maximal response) values were calculated using the software of transducer data acquisition system. PD2 values (apparent agonist affinity constants; −logEC50) were calculated.

Drugs

Following chemicals were obtained from Sigma Chemical (St Louis, MO): Carbachol chloride, isoproterenol bitartrate, serotonin creatinine sulfate, nicotine, sodium nitroprusside and papaverine hydrochloride. In the high K+ solution NaCl was exchanged for equimolar amounts of KCl. Drugs were prepared daily in distilled water and kept in ice during the course of experiments.

Histochemistry

The entire stomach, including portions of the esophagus and duodenum, was removed. The stomach and esophagus were opened by an incision along the long curvature, continuing through the LES and esophagus. The LES was identified visually at the end of the esophagus. LES and esophagus was split form the junction into two pieces. Almost 0.3–0.5 cm of LES pieces from the end were cut for histological studies. The samples of LES were fixed in neutral buffered formaline, consisting of 10% formaldehyde and pH 7.4, for 24 h and room temperature. After dehydration in ethanol, tissues were cleared in xylene and embedded in paraffin wax. Subsequently, sections at 5 μm thickness were transversely cut and collected on polylysine coated glass slides. The best oriented slides were chosen for the study. Tissue sections were deparaffinized in xylene and rehydrated in a graded series of ethanol and stained with hematoxylin and eosin. Respectively, the samples were dehydrated, cleared and mounted with entellan on glass slides. Sections were examined by light microscopy.
Immunohistochemistry

Serial sections at 3 μm thickness were collected on poly-L-lysine coated slides (Menzel-Glaser, Germany) and incubated over night at 56 °C. Briefly, tissue sections were deparaffinized in xylene and rehydrated through a graded series of ethanol. To unmask antigens, an antigen-retrieval procedure was performed by treating the samples in 10 mM citrate pH 6.0, in a microwave oven at 600 W for 5 min, twice and left to cool for 20 min at room temperature. After three washes in phosphate buffered saline (PBS; pH 7.4), endogenous peroxidase activity was quenched by 3% hydrogen peroxide in methanol H2O2 for 15 min and again washed three times in PBS. Afterwards, sections were incubated in a blocking serum (Histostatin plush kit broad spectrum, Invitrogen, CA) for 10 min at room temperature to block non-specific binding. Subsequently, sections were incubated over night at room temperature with rabbit polyclonal anti-nNOS (Invitrogen, CA) at 1:100 dilution. Sections were then incubated in a humidified chamber. Negative control incubations were performed by replacing the primary antibody with the appropriate non-immune IgG in the same concentrations. Sections were washed three times in PBS and incubated with the biotinylated secondary antibodies (Histostatin plush kit broad spectrum, Invitrogen, CA) for 20 min at room temperature. After three washes with PBS, the sections were incubated with peroxidase labeled streptavidin (Histostatin plush kit broad spectrum, Invitrogen, CA) for 10 min. Peroxidase activity was visualized with 3-amino-9-ethylcarbazol (AEC) chromogen in large-volume AEC substrate (AEC red substrate kit, Invitrogen, CA). All incubations were performed in a moist chamber at room temperature, using PBS for washes between incubation steps. The sections were counterstained with Mayer’s hematoxylin (Invitrogen, CA) and mounted with Clearmount (Invitrogen, CA) on glass slides. The same immunostaining procedure was applied to all slides under the same condition simultaneously. Slides were examined under light microscope (Olympus BX 50) and photomicrographs were taken with Leica DM 100 (Leica DFC 290HD). All samples were treated with exactly the same protocol. Two independent observers graded the staining intensity on a semi-quantitative scale ranging from no expression (−), very weak (1+), moderate (2+), strong (3+) to very strong (4+) expression. The concordance between the grading of two observers was 87%.

Statistical analysis

Statistically significant differences among groups were calculated by one-way ANOVA with post-hoc Tukey’s–Kramer test. The score of immunoreactivity was assessed by the Kruskal Wallis Test. Probabilities of less than 5% (p < 0.05) considered significant.

Results

Body weights, ethanol consumption, blood ethanol and glucose level

Daily ethanol consumption of the rats was in a range of 11.3–14.6 g/kg. Before beginning treatment, standard diet-fed rats had a mean body weight of 224.2 ± 8.4 g, sucrose-fed rats weighed 220.8 ± 8.4 g, and ethanol-exposed rats weighed 220.7 ± 7.8 g. After 4 weeks’ ethanol treatment, rats’ body weight (419.2 ± 9.4 g) was similar that of standard diet-fed (429.2 ± 8.2) and sucrose-fed rats (414.2 ± 9.7). Mean blood ethanol level was 78.5 ± 9.81 mg/dl at the time of sacrifice. No ethanol was detected in the two control groups. There was no change in the blood glucose levels in standard diet-, sucrose-, and ethanol-fed groups (165.3 ± 16.7 mg/dl; 174.2 ± 17.4 mg/dl; and 176.1 ± 19.8 mg/dl, respectively).

Histologic and immunohistochemical examination

In the epithelium of LES, mild atrophic changes including epithelial thinning were found in some tissues obtained from alcohol-fed group with hematoxylin and eosin staining. This epithelial thinning was not observed in standard diet-fed and sucrose-fed groups.

In the standard diet-fed group, nNOS-immunoreactive neuronal cell bodies were found throughout the myenteric plexus of the LES (Fig. 1A). Similarly, immunopositive staining was found in neuronal cell bodies throughout the myenteric plexus in the sucrose-fed groups (Fig. 1B). There was no significant change in immunoreactivity between standard diet-fed and sucrose-fed group (Table 1). However, weak immunostaining was observed in myenteric plexus in alcohol-fed group (Fig. 1C) compared to standard diet-fed (p < 0.05) and sucrose-fed group (p < 0.05) (Table 1).
**Table 1**

Semi-quantitative distribution of nNOS-immunoreactive neurons of myenteric plexus in the rat LES.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Standard diet-fed</th>
<th>Sucrose-fed</th>
<th>Alcohol-fed</th>
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<tbody>
<tr>
<td>1</td>
<td>2±</td>
<td>3±</td>
<td>1±</td>
</tr>
<tr>
<td>2</td>
<td>3±</td>
<td>2±</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>3±</td>
<td>4±</td>
<td>2±</td>
</tr>
<tr>
<td>4</td>
<td>3±</td>
<td>3±</td>
<td>2±</td>
</tr>
<tr>
<td>5</td>
<td>4±</td>
<td>2±</td>
<td>1±</td>
</tr>
<tr>
<td>6</td>
<td>3±</td>
<td>4±</td>
<td>1±</td>
</tr>
</tbody>
</table>

The staining intensity was classified no expression (–), very weak (1±), moderate (2±), strong (3±) to very strong (4±) expression.

**TTM strips**

The cumulative addition of carbachol produced concentration-dependent contractions of the TMM strips, the contractility was significantly less in strips from rats in ethanol-fed group than in those from control rats (p < 0.05) (Fig. 2). However, responses to carbachol were similar in the two control groups (Fig. 2). There were no significant differences among the pD2 values of TMM strips from the groups (Table 2). The contractions elicited by 80 mM KCl were also decreased in the ethanol-fed group compared with controls (Table 2).

Isoproterenol, serotonin and papaverine produced concentration-dependent relaxation in submaximally (70–75% of maximal contraction) precontracted (10−6–3 × 10−8 M carbachol) TMM strips obtained from each group. When tissues were contracted with carbachol to assess responses to relaxant agonists, the tension induced was similar in three groups (data not shown). The relaxation in response to serotonin was significantly less in strips from rats in ethanol-fed group than in those from control rats, the concentration–response curve for serotonin was shifted to the right, with significantly lower Eₘᵢₓ and pD2 values (p < 0.05) (Fig. 3, Table 2). However, responses to serotonin were similar in the two control groups (Fig. 3, Table 2). The relaxation elicited by isoproterenol or papaverine was similar in all groups and there were no significant changes in the Eₘᵢₓ and pD2 values; nor were there significant differences in the mass of the strips used for the contractility studies (Table 2).

**LES strips**

The cumulative addition of carbachol produced concentration-dependent contractions of the LES strips; the contractility was decreased in strips from rats in ethanol-fed group than in those from control rats (p < 0.05) (Fig. 4). There were no significant differences among the pD2 values of LES strips from the groups. Also, responses to carbachol were similar in the two control groups (Table 3). The contractions elicited by 80 mM KCl were also decreased in rats in ethanol-fed group than in those from control rats (Table 3). Isoproterenol, nicotine, sodium nitroprusside, and papaverine produced concentration-dependent relaxation in submaximally (70–75% of maximal contraction) precontracted (10−6–3 × 10−8 M carbachol) LES strips obtained from each group. When tissues were contracted with carbachol to assess responses to relaxant agonists; the tension induced was similar in three groups (data not shown).

The relaxation in response to nicotine and sodium nitroprusside was significantly less in strips from rats in ethanol-fed group than in those from control rats. The concentration–response curve for nicotine or sodium nitroprusside was shifted to the right; with significantly lower Eₘᵢₓ and pD2 values (p < 0.05) (Figs. 5 and 6; Table 3). However, responses to nicotine or sodium nitroprusside

**Fig. 2**. Carbachol concentration–response curves in isolated rat TMM strips. All points show the mean ± SEM of responses obtained individual experiments on different tissues from different animals. Data expressed as milligrams of tension per mg of tissue wet weight. Number of rats in each group is shown in parentheses. *p < 0.05 compared with the standard diet- and sucrose-fed groups.

**Fig. 3**. Serotonin concentration–response curves in isolated rat TMM strips. All points show the mean ± SEM of responses obtained in individual experiments on different tissues from different animals. Data expressed as percentage of the contraction by carbachol. Number of rats in each group is shown in parentheses. *p < 0.05 compared with the standard diet- and sucrose-fed groups.
However, our aim was to determine the effects of chronic alcohol consumption during active drinking with detectable blood alcohol levels. The effect of chronic alcohol on esophageal muscle contractility during drinking was different from that of human. But some similarities exist between them such as NO-mediated LES relaxation and other neurotransmitters (Jneda & Cunnane, 2003; Yuan, Costa, & Brookes, 1998). The effect of chronic alcohol on esophageal muscle contractility during withdrawal is different from the effect of chronic alcohol consumption during active drinking with detectable blood alcohol levels. However, our aim was to determine the effects of chronic alcohol consumption on esophageal smooth muscle. Contractile and relaxant movement of the esophagus and LES lead to peristaltic activity. Esophageal and LES peristaltic activity play an important role for antireflux barrier preventing GER. There are numbers of clinical studies which showed excessive alcohol consumption has been associated with abnormal GIS motor activity or gastroesophageal reflux disease (Bujanda, 2000; Burbige, Lewis, & Halsted, 1984; Fields, Jacyno, Wasylw, Winship, & Keshavarzian, 1995; Keshavarzian, Iber, & Ferguson, 1987; Tyssen & Singer, 2003). In this respect, we studied upper GIS smooth muscle reactivity in chronic alcohol consumption model, which may partly explain impaired GIS motor activity.

Our previous study revealed impaired esophageal smooth muscle reactivity in GER model similar to present study results (Tugay et al., 2003). Keshavarzian et al. (1994) reported that acute ethanol administration caused to significant decrease in LES pressure and esophageal contraction amplitudes. This finding is consistent with our present study, which showed KCl- and carbachol-induced esophageal smooth muscle contractility inhibited after chronic ethanol intake. It is well known that acetylcholine is an important cholinergic neurotransmitter which responsible for esophageal contraction. Intracellular Ca^{2+} release causes switch in the signal-transduction pathway mediating contraction in response to acetylcholine. Additionally, KCl-induced contractile responses were similar in the two control groups (Table 3). The relaxation elicited by isoproterenol or papaverine was similar in all groups and there were no significant changes in the $E_m$ and $pD_2$ values (Table 3).

### Discussion

It is well established that rat esophageal tissue is histologically different from that of human. But some similarities exist between them such as NO-mediated LES relaxation and other neurotransmitters (Jneda & Cunnane, 2003; Yuan, Costa, & Brookes, 1998). The effect of chronic alcohol on esophageal muscle contractility during withdrawal is different from the effect of chronic alcohol consumption during active drinking with detectable blood alcohol levels. However, our aim was to determine the effects of chronic alcohol consumption on esophageal smooth muscle. Contractile and relaxant movement of the esophagus and LES lead to peristaltic activity. Esophageal and LES peristaltic activity play an important role for antireflux barrier preventing GER. There are numbers of clinical studies which showed excessive alcohol consumption has been associated with abnormal GIS motor activity or gastroesophageal reflux disease (Bujanda, 2000; Burbige, Lewis, & Halsted, 1984; Fields, Jacyno, Wasylw, Winship, & Keshavarzian, 1995; Keshavarzian, Iber, & Ferguson, 1987; Tyssen & Singer, 2003). In this respect, we studied upper GIS smooth muscle reactivity in chronic alcohol consumption model, which may partly explain impaired GIS motor activity.

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### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Standard diet-fed</th>
<th>Sucrose-fed</th>
<th>Alcohol-fed</th>
</tr>
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<tbody>
<tr>
<td>$E_{max}$</td>
<td>$KCl$</td>
<td>14.37 ± 2.40</td>
<td>17.01 ± 1.10</td>
</tr>
<tr>
<td></td>
<td>Carbachol</td>
<td>29.80 ± 1.70</td>
<td>31.00 ± 2.00</td>
</tr>
<tr>
<td></td>
<td>Nicotine</td>
<td>91.29 ± 3.60</td>
<td>88.00 ± 3.50</td>
</tr>
<tr>
<td></td>
<td>SNP</td>
<td>57.70 ± 3.30</td>
<td>60.00 ± 5.20</td>
</tr>
<tr>
<td></td>
<td>Isoproterenol</td>
<td>52.84 ± 6.40</td>
<td>48.00 ± 4.50</td>
</tr>
<tr>
<td></td>
<td>Papaverine</td>
<td>98.88 ± 1.12</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>$pD_2$</td>
<td>Carbachol</td>
<td>6.91 ± 0.18</td>
<td>6.95 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>Nicotine</td>
<td>3.42 ± 0.03</td>
<td>3.53 ± 0.02</td>
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<tr>
<td></td>
<td>SNP</td>
<td>6.66 ± 0.49</td>
<td>6.68 ± 0.82</td>
</tr>
<tr>
<td></td>
<td>Isoproterenol</td>
<td>6.66 ± 0.15</td>
<td>6.68 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>Papaverine</td>
<td>5.10 ± 0.04</td>
<td>4.96 ± 0.03</td>
</tr>
</tbody>
</table>

Maximum response values for KCl, and carbachol ($E_{max} = g/g$ contraction), Nicotine, SNP, isoproterenol, and papaverine ($E_{max} = \%$ relaxation response after carbachol contraction), $pD_2$ values (−logEC50) for carbachol, nicotine, SNP, isoproterenol and papaverine in LES strips.

Values are arithmetic means ± SEM, *p < 0.05 (statistically significant) when compared with standard diet- and sucrose-fed groups; n = number of strips.
are mediated by direct depolarization of smooth muscle and influx of extracellular calcium through voltage-dependent calcium channels. A previous study demonstrated that acute alcohol intake impaired \(\text{Ca}^{2+}\) homeostasis in esophageal muscle (Keshavarzian et al., 1994). Therefore it is possible that in addition to intracellular \(\text{Ca}^{2+}\) release, chronic alcohol intake may perturb \(\text{Ca}^{2+}\) homeostasis by nonselective mechanisms such as voltage-dependent sarcoplasmic \(\text{Ca}^{2+}\) channels or subsequent \(\text{Ca}^{2+}\) dependent activation of esophageal smooth muscle contractile filaments similar to acute alcohol exposure. Also, in our study, both KCl- and carbachol-induced LES contractile response impaired after chronic alcohol intake. These findings suggest that chronic alcohol consumption may affect both esophageal TMM and LES smooth muscle reactivity through the mechanisms of inhibition of extra-cellular calcium ion influx.

Although various smooth muscle contractility changes after ethanol-exposure are well documented, the mechanisms of chronic ethanol consumption on esophageal and LES smooth muscle have been poorly understood. Underlying mechanisms may include direct actions of acetaldehyde, the principal metabolite of ethanol. This hypothesis is consistent with a previous study, which has been shown that acetaldehyde decreased both KCl- and norepinephrine-elicited contractions of isolated aortic rings (Brown & Savage, 1996). Furthermore, authors reported that acetaldehyde inhibits voltage-dependent calcium currents in aortic smooth muscle cells and consequently this might be responsible, in part, for the inhibitory actions of acetaldehyde in aortic vascular smooth muscle observed (Morales, Ram, Song, & Brown, 1997).

In the present study, we found impaired relaxant response to serotonin following chronic alcohol intake. Although it could be suggested that changes in mechanical events and intracellular levels of cAMP induced by the activation of the serotonergic receptor might be responsible for decreased serotonin response following chronic alcohol intake, the mechanism underlying this impaired relaxation requires further study. Additionally, beta receptor-mediated isopropylxanthine-induced relaxant response by nonselective mechanisms was not changed after chronic ethanol intake. Taken together proposed mechanism of impaired serotonin-induced relaxant response could be related to serotonin receptors and/or postreceptor mechanisms.

The NO pathway is well defined and NO is widely accepted that as an important transmitter of LES in different species such as human (McKirdy, McKirdy, Lewis, & Marshall, 1992), mice (Kim, Goyal, & Mashimo, 1999), rats (Farre et al., 2007), cats (Kortezova et al., 1996), opposum (Tottrup, Svane, & Forman, 1991), and canine (De Man et al., 1991), as well as human esophageal body (Freiksaitis, Tremblay, & Diamant, 1994). NO released by non-adrenergic noncholinergic nerves activates guanylate cyclase in LES, resulting in an increase in intracellular cyclic guanosine monophosphate (cGMP). The accumulation of cGMP leads to smooth muscle relaxation (Daniel, Bowes, & Jury, 2002; Jun, Lee, & Sohn, 2003). Further experiments showed that diminished nitric inhibitory neurotransmission to the LES are responsible for pathophysiological mechanism of esophageal achalasia (Mearin et al., 1993; Watanabe et al., 2002). Recently, it has been reported that nitric oxide has an important role in the alcohol-related impaired LES motor activity. It was suggested that NO seems to be a mediator of ethanol inhibition of some aspects of LES motor functions in the acute model (Keshavarzian et al., 1996). Also it has been shown that nicotine-induced relaxations are mediated mainly by NO in the LES (Dobrava, Mihzorkova, Kortezova, & Papasova, 1994). In this study, we found that chronic alcohol exposure to LES affected either potency \(\text{EC}_{50}\) or the efficacy (maximal response) of nicotine, resulting in concentration-dependent relaxant response significantly differs from that of control groups. Therefore, the most likely explanation was impaired production of NO in chronic alcohol exposure process. Tissue relaxation by the NO donor sodium nitroprusside was also decreased in ethanol-fed animals compared to that of sucrose-fed or standard diet-fed tissues. On the basis of the results, we speculated that LES tissue from chronic ethanol-fed group impaired the ability to relax via the nitric oxide/cGMP pathway following exposure to NO donor agents. Moreover we found decreased nNOS immunoreactivity in LES after chronic alcohol consumption. We believe that, the significant reduction in nNOS staining may either be due to loss of neurons that express it or/and loss of neuronal expression. This finding is consistent with the results of previous report on chronic alcohol-fed rats. Authors reported that chronic ethanol administration differentially alters the nNOS activity and the amount of NOS protein in gastrointestinal tract (Krecsmarik et al., 2006).

Another possibility is that the impairment of NO-mediated relaxation is associated with elevated glucose levels and diabetes mellitus, it might be argued that the decreased relaxation in ethanol-fed rats is a result of increased glucose levels (Bucala, Tracey, & Cerani, 1991; Utkan et al., 2009). However, this possibility is unlikely because the blood glucose levels in the present rats did not significantly changed among the three groups. These findings imply that increased glucose levels in alcohol consumption are not a mechanism responsible for the impaired NO-mediated relaxation in the alcohol treated animals.

Another theory is that acetaldehyde inhibits nitric oxide formation, which leads to impaired nitric oxide-mediated relaxation in chronic alcohol consumption. Consistently, Kim et al showed that acetaldehyde suppressed neurogenic relaxation induced by transmural electrical field stimulation in rabbit corpus cavernosum smooth muscle, and noted that increasing the acetaldehyde level seen in chronic alcoholism may contribute to male erectile dysfunction mainly by the inhibition of nitric oxide formation (Kim, Sohng, Lee, & Koh, 2000). Taking these findings together, we could be speculated that chronic alcohol consumption impairs the synthesis or availability of NO in LES smooth muscle or the ability of tissue to relax to NO.

In conclusion, our detailed study on esophageal and LES smooth muscle reactivity after chronic alcohol intake demonstrated impaired receptor-dependent and receptor-independent contractile and relaxant responses in the rat model. This observation simply supports abnormal selective and nonselective mechanisms could be causative factors for clinical findings after chronic alcohol intake.

Acknowledgments

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References


