

Sexual Differences of the Inhibitory Effect of Ethanol on Gastrointestinal Motility: *In Vivo* and *In Vitro* Studies

Ke-Jing Liu¹, Shuang-Lian Wang¹, Dong-Ping Xie¹, Pei-Yi Liu², Paulus S. Wang²,
and Chuan-Yong Liu¹

¹Department of Physiology, Medical School of Shandong University
Jinan 250012, Shandong, P.R. China

and

²Department of Physiology, School of Medicine
National Yang-Ming University,
Taipei 11221, Taiwan, R.O.C.

Abstract

Female brain is more sensitive to the acute exposure of ethanol. This study aimed to investigate the sexual difference of the ethanol-induced inhibition of gastrointestinal motility. Wistar rats were fasted and allowed drinking water only 12 - 18 h before the experiments. In the *in vivo* experiments, by using an oral radiochromium motility marker, the liquid gastric emptying and intestinal transit was measured 30 min after ethanol treatment. In the *in vitro* study, strips of stomach and duodenum smooth muscle were suspended in organ baths containing Krebs solution, and their isometric contractions were also examined. Systemic administration of ethanol (2 g/kg, i.p.) significantly inhibited the gastric emptying and intestinal transit, and the effect on female rats turned out to be greater than that on the male rats ($P < 0.05$). In an *in vitro* study, ethanol (0.38×10^{-3} M - 1.34×10^{-3} M) inhibited the motility of gastric antrum and duodenum in rats of both sexes, but there was no sexual difference in the inhibitory effect of ethanol on muscle strips. We concluded that sexual difference of the ethanol-induced inhibition of gastrointestinal motility was not resulted from the smooth muscle itself.

Key Words: sexual differences, ethanol, gastrointestinal motility

Introduction

Acute intoxication with ethyl ethanol affects many systems in the human body. Gastrointestinal symptoms, such as nausea, vomiting, and diarrhea, often accompany acute ethanol intoxication. Experimental evidence indicated that acute exposure of ethanol inhibited the motility of esophagus (6), stomach (7, 14), intestine (10), sphincter of Oddi (13), and recta (1) *in vivo* and *in vitro*.

The gastrointestinal motility is controlled by local mediators (enteric nervous system, local hormones) as well as systemic factors (central nervous system, systemic hormones, and autonomic nervous system). Data of *in vitro* experiments demonstrated

that the ethanol could inhibit the gastrointestinal motility independent of systemic factors (10, 13). *In vivo* study showed that capsaicin sensitive afferent fibers mediated the inhibitory effect of ethanol on gastric emptying and small intestinal transit (5). So, it seems that *in vivo*, central nervous system, and autonomic nervous system also inhibit ethanol on gastrointestinal motility.

Systemic administration of ethanol affected the metabolism and function of the brain, and the brain of female animals or women was more sensitive to ethanol (4, 16). The gastrointestinal motility is under the control of central nervous system (3). Sexual difference of the inhibitory effect of ethanol on gastrointestinal motility remains unknown.

The aim of the present study was to compare the effects of acute treatment of ethanol on the motility of stomach and intestine in both sexes and to test if there were sexual differences in the ethanol induced inhibition of gastrointestinal motility *in vivo* and *in vitro*.

Materials and Methods

Animal Preparation

Male and female Wistar rats (250 - 350 g) were used in both *in vivo* and *in vitro* studies. To exclude the effects of female sex hormones (estrogen, progesterone) and the estrous cycle on the gastrointestinal motility *in vivo*, the abdominal ovariectomy was conducted on the female rats 15 days before the experiment. The animals were fasted but allowed drinking water 12 - 18 h before the experiments.

In Vivo Study: Measurement of Gastric Emptying and Gastrointestinal Transit

Gastric emptying and gastrointestinal transit were measured as described by Pu *et al.* (11). Fifteen min after the systemic treatment of ethanol (2 g/kg in 1 ml normal saline (NS), i.p.) or NS (1 ml, i.p.), rats were intubated *via* a catheter (PE-205, ID 1.67 mm, OD 2.42 mm, Clay-Adam, Parsippany, NJ, USA) with normal saline (3 ml/kg) containing $\text{Na}_2^{51}\text{CrO}_4$ (0.5 $\mu\text{Ci/ml}$) and 10% charcoal. The test meal was continuously stirred before intubation. Air (0.5 ml) was used to flush the residual charcoal suspension in the catheter into the rats. Fifteen min later, the rats were decapitated and the stomach and attached small intestine immediately exposed by laparotomy. After ligation of the esophagogastric, gastroduodenal, and ileocecal junctions, the whole stomach and small intestine were carefully removed and placed on a wooden board to observe the leading edge of the charcoal in the intestine. The small intestine was then divided into 10 equal segments and the radioactivity in the stomach and each segment of small intestine was measured in an automatic gamma counter (1470 Vizard, Pharmacia, Turku, Finland). Gastric emptying was measured by determining the amount of labeled chromium contained in the small intestine 15 min after intubation, expressed as a percentage of the amount given. Intestinal transit was assessed by calculating the geometric center of distribution of the radioactivity with the 10 segments by summation of the radioactivity in each segment multiplied by the segment number.

In Vitro Study: Recording of the Gastroduodenal Motility

The muscle strips of stomach and duodenum

was prepared as prescribed by Xie *et al.* (17). Male or female Wistar rats, weighing 250 - 350 g, were fasted for 24 h and then sacrificed. The gastric antrum and duodenum were removed. The segments of the gastric antrum and duodenum were opened along the mesentery. Muscle strips (8 \times 2 mm) were cut, parallel to either the circular or the longitudinal fibers, and named circular muscle (CM) and longitudinal muscle (LM). The mucosa on each strip was carefully removed. The muscle strip was suspended in a tissue chamber containing 5 ml Krebs solution (37 °C) and bubbled continuously with 95% O_2 and 5% CO_2 . One end of the strip was fixed to a hook on the bottom of the chamber. The other end was connected to an external isometric force transducer (JH-2, Hangtian Company, Beijing, China). Motility of muscle strips (under an initial tension of 1 g) in 4 tissue chambers was simultaneously recorded on multiple recording system (SMUP-PC, Jialong Company, Shanghai, China). Experiments were conducted after 1 h equilibration.

Experiment Protocol for Ethanol Study

In vivo Experiments :

Group 1, male rats were treated with normal saline (NS) (2 ml, i.p.) ($n = 6$)

Group 2, male rats were treated with ethanol (2 g/kg diluted into NS, intraperitoneal, i.p.) ($n = 6$). The dose of ethanol is selected according to the similar work of other group (12, 18).

Group 3. Ovariectomized female rats were treated with NS (2 ml, i.p.) ($n = 6$)

Group 4. Ovariectomized female rats were treated with ethanol (2 g/kg diluted into NS, i.p.) ($n = 6$)

In vitro Experiments:

Group 1 - 5. Muscle strips of male rats were treated with NS, ethanol 0.38×10^{-3} M, 0.63×10^{-3} M, 0.95×10^{-3} M, 1.43×10^{-3} M, respectively. ($n = 8$ in each group)

Group 6-10. Muscle strips of female rats were treated with NS, ethanol 0.38×10^{-3} M, 0.63×10^{-3} M, 0.95×10^{-3} M, 1.43×10^{-3} M, respectively. ($n = 8$ in each group)

During the recording of the contraction of the muscle strips, the integration of the curve was calculated simultaneously by software (MFlab, Jialong Company, Shanghai, China). The motility of the muscle strip was measured by the integration, and that before the ethanol was regarded as the baseline.

Statistical Analysis

The data were expressed as the mean value \pm S.E.M. The treatment means were tested for homogeneity using one-way analysis of variance, and the significance of any difference between means was

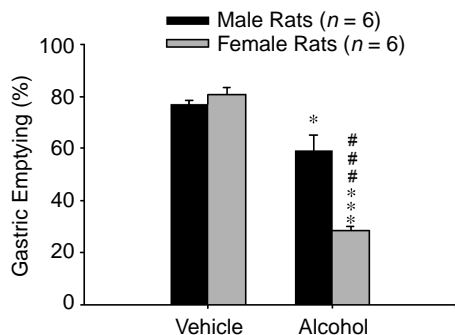


Fig. 1. The inhibitory effect of ethanol on gastric emptying in male and female rats. * $P < 0.05$, *** $P < 0.001$ vs. vehicle control; ### $P < 0.001$ between the male and female rats.

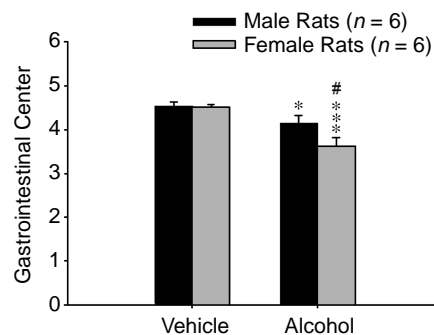


Fig. 2. The inhibitory effect of ethanol on intestinal transit in male and female rats. * $P < 0.05$, *** $P < 0.001$ vs. vehicle control; # $P < 0.05$ between the male and female rats.

tested using Duncan's multiple range test (15). A difference between two means was considered to be statistically significant when P was smaller than 0.05.

Results

Sex Difference of the Inhibitory Effect of Ethanol on Gastric Emptying

Ethanol (2 g/kg, i.p.) significantly decreased the gastric emptying in both male and ovariectomized female rats (Fig 1). Fifteen min after the ethanol treatment, the gastric emptying of the male rats is $59.01 \pm 6.23\%$, significantly lower than that of the vehicle control group ($76.83 \pm 1.66\%$, $P > 0.05$). (Fig. 1).

After the treatment of ethanol (2 g/kg, i.p.), the gastric emptying of ovariectomized female rats was decreased from $80.69 \pm 2.76\%$ (vehicle control) to $28.42 \pm 1.60\%$ (ethanol treatment group) ($P < 0.05$, Fig. 1). The gastric emptying of male vehicle group ($76.83 \pm 1.66\%$) did not differ significantly from that of the ovariectomized female vehicle group ($80.69 \pm 1.60\%$) ($P > 0.05$, Fig. 1). Fifteen min after the ethanol treatment (2 g/kg, i.p.), the gastric emptying of the female rats is significantly lower than that of the male rats ($P < 0.05$, Fig. 1).

Sex Difference of the Inhibitory Effect of Ethanol on Intestinal Transit

In the group of male rats, systemic administration of ethanol (2 g/kg, i.p.) significantly decreased the gastrointestinal transit. Fifteen min after the ethanol treatment, the gastrointestinal center was 4.15 ± 0.17 , lower than that of the vehicle control (4.54 ± 0.09 , $P < 0.05$, Fig. 2).

Treatment of ethanol (2 g/kg, i.p.) also significantly decreased intestinal transit of the

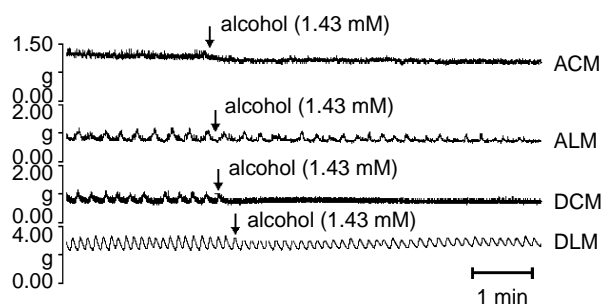


Fig. 3. Original recording of the muscle strip motility before and after ethanol treatment. It showed that the motility of gastric and duodenum motility *in vitro* decreased after ethanol treatment. ACM, antrum circular muscle; ALM, antrum longitudinal muscle; DCM, duodenum circular muscle; DLM, duodenum longitudinal muscle.

ovariectomized female rats. Fifteen min after ethanol administration, the gastrointestinal center is 3.63 ± 0.18 , lower than that of the vehicle control (4.51 ± 0.07 , $P < 0.001$, Fig. 2).

After the administration of vehicle, the intestinal transit of the ovariectomized rats did not differ significantly from that of the male rats ($P > 0.05$, Fig. 2). Fifteen min after ethanol administration, the gastrointestinal center of the ovariectomized female rats was significantly lower than that of the male rats. So the inhibitory effect of ethanol on intestinal transit was greater on female than on male rats ($P < 0.05$, Fig. 2).

Inhibitory Effect of Ethanol on the Motility of the Gastric Antrum In Vitro

Ethanol (0.38×10^{-3} M - 1.43×10^{-3} M) decreased the motility of gastric antrum *in vitro* (Fig. 3). Three min after the treatment of ethanol (0.38×10^{-3} M), the circular muscle motility of the male rats decreased by

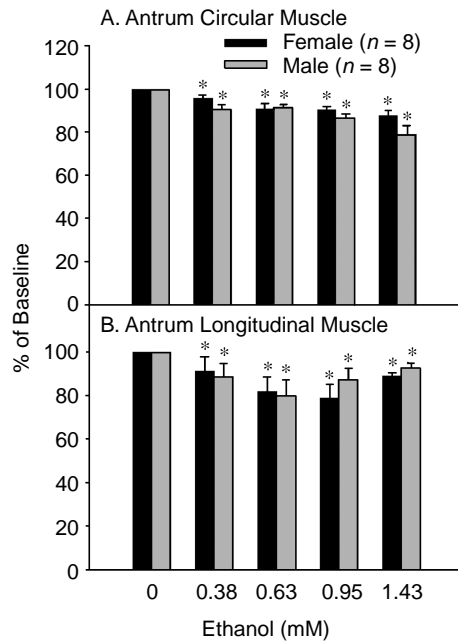


Fig. 4. (A) The inhibitory effect of ethanol on the motility of circular muscle strip of gastric antrum *in vitro*. * $P < 0.05$ vs. the data before ethanol treatment, (B) The inhibitory effect of ethanol on the motility of longitudinal muscle strip of gastric antrum *in vitro*. * $P < 0.05$ vs. the data before ethanol treatment

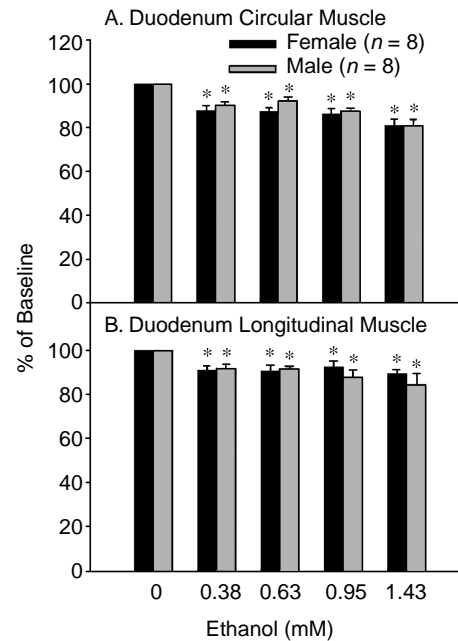


Fig. 5 (A) The inhibitory effect of ethanol on the motility of duodenum circular muscle strip *in vitro*. * $P < 0.05$ vs. the data before ethanol treatment, (B) The inhibitory effect of ethanol on the motility of duodenum longitudinal muscle strip *in vitro*. * $P < 0.05$ vs. the data before ethanol treatment

$9.8 \pm 2.4\%$ ($P < 0.05$, Fig. 4A), and that of the female rats decreased by $4.5 \pm 1.8\%$ ($P < 0.05$, Fig. 4A).

Ethanol exerted the similar effect on the motility of longitudinal antrum muscle *in vitro*. Three min after ethanol administration (0.38×10^{-3} M), the motility of muscle strips in male rats decreased by $11.5 \pm 6.2\%$ ($P < 0.05$, Fig. 4B), while in female rats it decreased by $8.9 \pm 6.7\%$ ($P < 0.05$, Fig. 4B).

There is no sexual difference between the effects of ethanol on male and female antral motility ($P > 0.05$, Fig. 4B).

Inhibitory Effect of Ethanol on Duodenal Motility In Vitro

Ethanol (0.38×10^{-3} M - 1.43×10^{-3} M) significantly decreased the duodenum motility of rats *in vitro*. Three min after ethanol (0.38×10^{-3} M) treatment, the motility of the circular muscle strips of the male rats decreased by $9.8 \pm 1.6\%$ ($P < 0.05$, Fig. 5A), and that of the female rats decreased by $12.3 \pm 2.2\%$ ($P < 0.05$, Fig. 5A). The inhibitory effect of the ethanol on the circular muscle strips of the two sexes does not differ significantly ($P > 0.05$, Fig. 5A).

Ethanol exerted similar effects on the duodenum longitudinal muscle. The motility of the longitudinal muscle strips in both sexes decreased three min after ethanol treatment. The muscle strip motility of male

rats decreased by $8.3 \pm 1.7\%$ ($P < 0.05$, Fig. 5B), and that of the female rats decreased by $9.3 \pm 1.9\%$ ($P < 0.05$, Fig. 5B). The inhibitory effect of ethanol on motility of the duodenum longitudinal muscle of the male rats *in vitro* did not differ significantly from that of the female rats (Fig. 5B).

Discussion

It is well known that the acute administration of ethanol inhibits the gastrointestinal motility (1, 6, 7, 10, 13). Our results demonstrated the sexual difference of the inhibitory effect of ethanol on gastrointestinal motility. The female gut was shown to be more sensitive to the inhibitory effect of ethanol than that of the male rats. This sexual difference only existed in the *in vivo* study. In the experiment *in vitro*, the difference between the two sexes disappeared.

The regulation of gastrointestinal motility is very complicated. *In vivo*, the gut is under the control of central nervous system (CNS), peripheral autonomic nervous system (ANS), systemic hormones as well as enteric nervous system (ENS) and paracrine and autocrine hormones. In the *in vitro* experiment, the muscle strips of gut is only influenced by the ENS and paracrine and autocrine hormones. The effect of CNS, ANS and systemic hormones could be excluded.

The ethanol treatment inhibited the muscle strips of gastric antrum and duodenum *in vitro*. So, it is clear that ethanol could inhibit the gut motility independent of systemic factors. These results are consistent with the report of Lu *et al.* based on the experiment on canine jejunal circular smooth muscle (10).

In the present study, the sexual difference of the inhibitory effect of ethanol on gut motility only existed in the *in vivo* preparations. Therefore, sexual difference did not seem to result from smooth muscles and was not associated with the ENS and local hormones.

Sex-related hormones, such as estrogen and progesterone, exerted influence on the gastrointestinal motility *in vivo* (2, 8). In this study, in order to exclude the effect of these hormones, all female rats were ovariectomized 14 days before the experiment. With this pretreatment, the effect of estrogen and progesterone, mainly secreted by the ovary in female animals, was excluded (9). Hence, the more profound response of the female gut to ethanol was not because of the influence of estrogen and progesterone.

We concluded that ethanol inhibited the gastrointestinal motility *in vivo* and *in vitro*. Only the *in vivo* preparation showed sexual difference to the ethanol-induced inhibition. So it seemed that the sexual difference of the inhibitory effect of ethanol on the motility of gastrointestinal motility did not result from the smooth muscles itself.

Acknowledgment

This project is sponsored by the Natural Scientific Foundation of Shandong Province (Q2004C01) and the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry and the "1020" Project of Health Institution, Shandong Province.

References

- Berenson, M.M. and Avner, D.L. Ethanol inhibition of rectosigmoid motility in humans. *Digestion* 22: 210-215, 1981.
- Chen, T.S., Doong, M.L., Chang, F.Y., Lee, S.D. and Wang, P.S. Effects of sex steroid hormones on gastric emptying and gastrointestinal transit in rats. *Am. J. Physiol.* 268: G171 - G176, 1995.
- Countee, R.W. Extrinsic neural influences on gastrointestinal motility. *Am. Surg.* 43: 621-626, 1977.
- Hommer, D.W. Male and female sensitivity to ethanol-induced brain damage. *Ethanol Res. Health* 27: 181-185, 2003.
- Izbeki, F., Wittmann, T., Jancso, G., Csati, S. and Lonovics, J. Inhibition of gastric emptying and small intestinal transit by ethanol is mediated by capsaicin-sensitive afferent nerves. *Naunyn Schmiedeberg's Arch. Pharmacol.* 365: 17-21, 2002.
- Keshavarzian, A., Urban, G., Sedghi, S., Willson, C., Sabella, L., Sweeny, C. and Anderson, K. Effect of acute ethanol on esophageal motility in cats. *Alcohol. Clin. Exp. Res.* 15: 116-121, 1991.
- Knights, L.C., Maurer, A.H., Wikander, R., Krevsky, B., Malmud, L.S. and Fisher, R.S. Effect of ethyl ethanol on motor function in canine stomach. *Am. J. Physiol. (Gastrointest. Liver Physiol.)* 262: G233-G230, 1992.
- Liu, C.Y., Chen, L.B., Liu, P.Y., Xie, D.P. and Wang, P.S. Effects of progesterone on gastric emptying and intestinal transit in male rats. *World J. Gastroenterol.* 8: 338-341, 2002.
- Lo, M.J. and Wang, P.S. Relative and combined effects of estradiol and prolactin on corticosterone secretion in ovariectomized rats. *Chinese J. Physiol.* 46: 103-109, 2003.
- Lu, G., Sarr, M.G. and Szurszewski, J.H. Effects of ethyl ethanol on canine jejunal circular smooth muscle. *Dig. Dis. Sci.* 42: 2403-2410, 1997.
- Pu, H.F., Huang, W.J., Tseng, W.M., Wang, S.W., Liu, Y.W., Doong, M.L. and Wang, P.S. Effects of juice from *Morinda citrifolia* (Noni) on gastric emptying in male rats. *Chinese J. Physiol.* 47: 169-74, 2004.
- Rossetti, Z.L. and Crespi, F. Inhibition of nitric oxide release *in vivo* by ethanol. *Alcohol. Clin. Exp. Res.* 28: 1746-1751, 2004.
- Sari, R., Palcolgyi, A., Rakonczay, Z. Jr., Takacs, T., Lonovics, J., Czako, L., Szivassy, Z. and Hegyi, P. Ethanol inhibits the motility of rabbit sphincter of Oddi *in vitro*. *World J. Gastroenterol.* 20: 3470-3474, 2004.
- Sim, S.S., Choi, J.C., Min, D.S., Rhie, D.J., Yoon, S.H., Hahn, S.J., Kim, C.J., Kim, M.S. and Jo, Y.H. Effect of ethanol on spontaneous phasic contractions of cat gastric smooth muscle. *Scand. J. Gastroenterol.* 37: 23-27, 2002.
- Steel, R.D. and Torrie, J.H. *Principles and Procedures of Statistics*. McGraw-Hill, New York (1960).
- Webb, B., Burnett, P.W. and Walker, D.W. Sex differences in ethanol-induced hypnosis and hypothermia in young Long-Evans rats. *Alcohol. Clin. Exp. Res.* 26: 695-704, 2002.
- Xie, D.P., Chen, L.B., Liu, C.Y., Zhang, C.L., Liu, K.J. and Wang, P.S. Arecoline excites the colonic smooth muscle motility via M₃ receptor in rabbits. *Chinese J. Physiol.* 47: 89-94, 2004.
- Yoon, S.S., Kwon, Y.K., Kim, M.R., Shim, I., Kim, K.J., Lee, M.H., Lee, Y.S., Golden, G.T. and Yang, C.H. Acupuncture-mediated inhibition of ethanol-induced dopamine release in the rat nucleus accumbens through the GABAB receptor. *Neurosci. Lett.* 369: 234-238, 2004.