Ammonia Potentiates the Lethal Effect of Ethanol on Rats

V. L. Rejniuk, T. V. Schafer, and Ju. Ju. Ivnitsky

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 145, No. 6, pp. 688-690, June, 2008
Original article submitted November 13, 2007

Blood ammonia concentration increased in the portal vein (by 1.4 times) and inferior vena cava (caudal to the renal vein inflow, by 2.2 times; and cranial to the hepatic vein inflow, by 2.5 times) of rats 3 h after intragastric administration of 16.57 M ethanol solution (446 mmol/kg, ≈1.4 $LD_{50/48\ h}$). Ammonia concentration in mixed blood samples (post-decapitation) increased by 39%. The rate of ammonia accumulation was 3-fold higher in an intraperitoneal lavage solution. Three-hour exposure of ethanol-treated animals to atmospheric ammonia (0.84-1.07 mg/liter, i.e. ≈$^1/_8LC_{50}$ for intact rats) was followed by a 2.4-fold increase in blood ammonia concentration as compared to specimens of the ethanol group. Ammonia inhalation potentiated the lethal effect of ethanol (dose variation factor 0.81), suppressed external respiration, and decreased oxygen consumption. Our results indicate that kinetic changes in endogenous ammonia have an adverse effect on the outcome of alcohol intoxication in rats.

Key Words: *ethanol; endogenous ammonia; atmospheric ammonia; hyperammonemia*

Disturbances in the ammonia detoxification function of the liver are a manifestation of ethanol cytotoxicity. Urea synthesis is suppressed in people with mild alcohol intoxication [4] and in rats after 5-day substitution of drinking water for ethanol solution [5]. Five-day administration of ethanol to rats is accompanied by an increase in blood ammonia concentration [7]. Metabolic changes in the small intestinal mucosa of rats are observed under similar conditions and result in barrier dysfunction for ammonia [8]. These changes in combination with impaired ammonia detoxification in the liver contribute to increased release of intestinal ammonia into systemic circulation. The role of endogenous ammonia in the pathogenesis of acute alcohol intoxication remains unknown. It is necessary to evaluate the influence of exogenous ammonia-induced hyperammonemia on the effect of ethanol.

Military Medical Academy, St. Petersburg, Russia. ***Address for correspondence:*** vladton@mail.ru. V. L. Rejniuk

Here we studied the effect of ammonia inhalation in nonlethal toxic dose on mortality rate in rats with acute alcohol intoxication.

MATERIALS AND METHODS

Experiments were performed on male outbred rats weighing 180-220 g and obtained from the Rappolovo nursery. The animals were divided into 2 groups of intact and ethanol-treated specimens. The loss of sensorimotor responses (including the corneal reflex) served as the model of coma. Ethanol was administered intragastrically in a dose of 446 mmol/kg (≈1.4 $LD_{50/48\ h}$), which caused death of 17% animals over 3 h. Administration of ethanol in the specified minimum dose was followed by the development of coma in all animals over 3 h after ethanol treatment. Ethanol solution (16.57 M) was administered in a volume of 26 ml/kg. Control animals received an equivalent volume of water. The animals were deprived of food for 24 h, but received water *ad libitum* to provide ethanol flux into the intestine.

0007-4888/08/1456-0741

In series I, we studied the effect of ethanol on blood ammonia concentration in the portal vein and inferior vena cava. Twelve rats from each group survived by the 3rd hour of the study. The inferior vena cava (caudal to the renal vein inflow; and cranial to the hepatic vein inflow) and portal vein were punctured after 3 h. During each puncture, blood samples (1 ml) were collected into heparinized syringes and deproteinized with trichloroacetic acid. Ammonia concentration was measured spectrophotometrically using Nessler's reagent. Intact rats were treated with sodium thiopental in a dose of 75 mg/kg to avoid traumatic stress. The blood was sampled after 1-2 min (fall on the side). Published data show that blood ammonia concentration in rats remains practically unchanged over 30 min after administration of thiopental in the specified dose [6].

In series II, ammonia accumulation in the intraperitoneally injected solution of 0.9% NaCl (50 ml/kg) was studied 2.5 h after administration of ethanol. Each group consisted of 7 rats. Laparotomy was performed 3 h after ethanol administration (0.5 h after administration of lavage solution). Lavage solution was quantitatively collected. Ammonia concentration was measured in the centrifugate. To avoid traumatic stress, intact animals were treated with chloroform vapors 30-40 sec before laparotomy. Previous studies showed that blood ammonia concentration remains unchanged under these conditions [9].

In series III, experimental animals were maintained in the atmosphere of pure air or ammonia for 3 h (1.07 and 0.84 mg/liter ammonia at the beginning and end of a 3-h period, respectively; $\approx {}^{1}/_{8}$ LC_{50}) [1]. The method of ammonia inhalation was described previously [2]. Ethanol was administered in a dose of 240, 275, 309, 378, 446, 515, or 583 mmol/kg. Each dose of ethanol was tested on 6 animals (18 rats for 446 mmol/kg ethanol). The state of animals was monitored for 2 days (maximum period of mortality). The mortality rate was estimated over 3 and 48 h. The average life span (ALS) of animals was evaluated. Gas exchange parameters and blood ammonia concentration were measured in some rats of the ethanol group (446 mmol/kg) and intact animals. Oxygen consumption was estimated before and 3 h after ethanol administration [3]. The respiratory rate (RR) was determined. The rats survived by the 3rd hour were decapitated. Ammonia concentration was measured in trunk blood.

The significance of intergroup differences was evaluated by Student's *t* test. The dose-effect relation for ethanol was studied by means of Statistica+2005 software. Graphic data were processed with Origin 7.0 software. The dose variation factor (DVF) was calculated as follows: $DVF=LD_{50\ treatment}/LD_{50\ control}$).

RESULTS

Autopsy of animals was performed 3 h after ethanol administration and revealed the presence of colorless fluid and gas in the stomach and small intestine. Vessels of the portal vein bed were plethoric. Blood ammonia concentration in the portal

TABLE 1. Ammonia Concentration in Blood Samples from the Portal Vein and Inferior Vena Cava of Rats 3 h after Administration of Ethanol in a Dose of 446 mmol/kg (*M*±*m*, *n*=12)

Group	Ammonia concentration, μM		
	portal vein	inferior vena cava	
		caudal to the renal veins	cranial to the hepatic veins
Intact rats	168±16	141±17	110±6^{+}
Ethanol administration	238±20*	304±21**$^{+}$	276±17**

Note. *$p \leq 0.05$ and **$p \leq 0.001$ compared to intact animals; $^{+}p \leq 0.01$ compared to the portal vein.

TABLE 2. Ammonia Accumulation in the Lavage Solution during Acute Ethanol Intoxication (*M*±*m*, *n*=7)

Group	Volume of non-sorbed solution in the peritoneal cavity, ml/kg	Ammonia concentration in the lavage solution, μM	Rate of ammonia accumulation in the lavage solution, μmol/kg/min
Intact rats	31.6±2.7	190±40	0.21±0.06
Ethanol administration	32.8±2.7	577±61**	0.68±0.12*

Note. *$p \leq 0.05$ and **$p \leq 0.01$ compared to intact animals.

TABLE 3. RR and Oxygen Consumption in Rats 3 h after Administration of 446 mmol/kg Ethanol and Ammonia Inhalation ($M\pm m$, n=10)

Group	RR, min^{-1}		Oxygen consumption, mmol/kg/min	
	basal level	after 3 h	basal level	after 3 h
Intact rats	110±17	102±16	1.19±0.06	1.08±0.07
Ethanol administration	117±6	85±7*	0.98±0.07	0.43±0.05**
Ethanol administration and ammonia inhalation	129±6	59±9*+	0.93±0.11	0.24±0.04**++

Note. Significant differences: *$p\leq0.05$ and **$p\leq0.001$ compared to the basal level; +$p\leq0.05$ and ++$p\leq0.001$ compared to the ethanol group.

vein and inferior vena cava (caudal to the renal veins; and cranial to the hepatic veins) of treated rats was higher than in intact animals (by 1.4, 2.2, and 2. 5 times, respectively). The transhepatic gradient of blood ammonia concentration was not revealed in rats with alcohol intoxication (Table 1). The rate of ammonia accumulation in the lavage solution increased by 3 times (Table 2).

Hyperammonemia was found in mixed blood samples after decapitation of treated rats (161±19 *vs*. 98±11 μM in intact animals; $M\pm m$; n=11; $p\leq0.05$). Ammonia inhalation was followed a 2.4-fold increase in blood ammonia concentration as compared to animals of the ethanol group (up to 383±95 μM; $M\pm m$; n=11; $p\leq0.05$). Alcohol coma was accompanied by a decrease in RR and oxygen consumption (by 17% and 2.5 times, respectively, compared to intact animals). Ammonia inhalation was followed by further decrease in gas exchange (by 31 and 44%, respectively, Table 3). The lethal effect of ethanol was potentiated. $LD_{50/3\ h}$ of ethanol in control rats and ammonia-inhaling specimens was 536 mmol/kg (confidence interval 481-591 mmol/kg) and 432 mmol/kg (391-473 mmol/kg), respectively. DVF was 0.81. The same tendency was found after ammonia inhalation. $LD_{50/48\ h}$ of ethanol was 322 mmol/kg (285-360 mmol/kg) and 284 mmol/kg (247-322 mmol/kg), respectively. DVF was 0.88. ALS of dead animals in the ammonia inhalation group tended to decrease after administration of ethanol in all doses. A significant decrease in ALS was observed after administration of ethanol in a dose of 446 mmol/kg (14.0±2.2 *vs*. 20.1±1.8 h in the control; $M\pm m$; n=17; $p\leq0.05$).

Our results indicate that intragastric administration of ethanol in the coma-inducing dose is accompanied by hyperammonemia due to increased release of intestinal ammonia into the systemic circulation. This effect depends on an increase in transperitoneal ammonia diffusion into vessels of the inferior vena cava bed (bypassing the liver). Further increase in blood ammonia concentration after inhalation of ammonia in the toxic dose (nonlethal dose for intact animals) aggravates impairment of gas exchange and potentiates the lethal effect of ethanol. Hence, kinetic changes in endogenous ammonia have an adverse effect on the outcome of alcohol intoxication in rats.

REFERENCES

1. *Hazardous Industrial Substances*, Eds. N. V. Lazarev and I. D. Gadaskina [in Russian], Leningrad (1977), Vol. 3, 7th Edition, p. 89.
2. V. L. Rejniuk, T. V. Schafer, R. V. Ovsep'yan, and Ju. Ju. Ivnitsky, *Byull. Eksp. Biol. Med*., **143**, No. 6, 634-637 (2007).
3. T. V. Schafer, Ju. Ju. Ivnitsky, and V. N. Malakhovsky, *Ibid*., **135**, No. 4, 419-422 (2003).
4. N. K. Aagaard, T. Thogersen, T. Grofte, *et al*., *Alcohol Clin. Exp. Res*., **28**, No. 5, 697-701 (2004).
5. K. Adachia, T. Matsuhashia, Yu. Nishizawaa, *et al*., *Biochem. Pharmacol.*, **50**, No. 9, 1391-1399 (1995).
6. Ju. Ju. Ivnitsky, V. L. Rejniuk, T. V. Schafer, and V. N. Malakhovsky, *Toxicol*., **224**, 184-190 (2006).
7. V. Mohanachari, K. S. Reddy, and K. Indira, *Toxicol. Lett*., **20**, No. 2, 225-228 (1984).
8. M. C. Vemuri and K. Indira, *Biochem. Med. Metab. Biol*., **36**, No. 1, 8-13 (1986).
9. A. Watanabe and Y. Kuwabara, *Res. Exp. Med. (Berl.)*, **194**, No. 3, 157-164 (1994).