Effect of Atmospheric Ammonia on Mortality Rate of Rats with Barbiturate Intoxication

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Ammonia inhalation (0.84-1.07 mg/liter, 3 h) was accompanied by a 65% increase in ammonia concentration in mixed blood of intact rats. This treatment did not cause death of intact animals, but potentiated the lethal effect of sodium thiopental and inhibited external respiration and O_2 consumption in animals. The resistance of rats to the lethal effect of barbiturate tended to decrease under conditions of experimental hyperammonemia induced by intraperitoneal injection of ammonium acetate in a nonlethal dose (6 mmol/kg). Our results indicate that potentiation of the toxic effect of barbiturates by atmospheric ammonia is related to its resorptive effects.

Key Words: *sodium thiopental; atmospheric ammonia; blood ammonia; ammonium acetate; mortality*

Barbiturate coma in rats is accompanied by hyperammonemia and increased excretion of ammonia with expired air [6]. Hyperammonemia stimulates amination of keto acids, which probably contributes to depletion of the tissue pool of Krebs cycle intermediates during barbiturate coma [3]. Hyperammonemia can led to the development of brain edema due to accumulation of glutamine [4] and programmed neuronal death due to glutamate accumulation [5]. The anticholinesterase effect of ammonia [7] can modulate external respiration.

Deactivation of ammonia is an energy-requiring process. The role of endogenous ammonia in barbiturate intoxication remains unclear. This problem can be solved by studying the effect of hyperammonemia of different nature after barbiturate treatment.

Here we studied the effect of ammonia inhalation in a nonlethal toxic dose on the course and outcome of barbiturate intoxication in rats.

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MATERIALS AND METHODS

Female albino rats weighing 100-120 g were divided into control (intact animals) and experimental groups (ammonia treatment). Ammonia was inhaled or injected intraperitoneally (ammonium acetate, AA). During inhalation, the rats were placed in a sealed chamber (0.7 liters). The chamber was ventilated with air at a flow rate of 15 liters/h. Air was delivered through a flask containing 20 ml 2.5% aqueous solution of ammonia. Ammonia concentration in the gas mixture (1.07 mg/liter, initial concentration; 0.84 mg/liter, 3 h after the start of treatment) was estimated by titration of an absorbing agent (sulfuric acid solution) at the chamber outlet. Under these conditions, the mean concentration of ammonia in air was $^1/_8$ LC_{50} [2]. Control animals were placed into the same chamber ventilated with air delivered through distilled water. The animals received sodium thiopental (ST) immediately before placement into the chamber.

Previous experiments showed that intraperitoneal injection of AA in doses of 4-6 mmol/kg to rats led to a short-term (up to 40 min) increase in

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blood ammonia concentration, which peaked 10-15 min postinjection. The AA dose of 6 mmol/kg is nonlethal, but the dose of 8 mmol/kg is close to LD_{100}. For hyperammonemia modeling, AA in a dose of 6 mmol/kg was administered 3 times (0.5, 1.5, and 2.5 after treatment with 75 mg/kg ST). Both drugs were injected intraperitoneally (10 ml/kg). Control rats received an equivalent volume of water. Immediately after ST administration, the animals were maintained at room temperature (18-20°C) or kept in a ventilated box (20°C) for 3 h. These conditions provided constant body temperature [1].

O_2 consumption in the organism was estimated [2]. The rats surviving by the end of the 3rd hour were decapitated, the blood was deproteinized with trichloroacetic acid, and ammonia concentration in the supernatant was measured by the microdiffusion method with subsequent acidometric titration.

The significance of intergroup differences was estimated by Student's *t* test. Differences in survival rate were evaluated by Fischer exact test. The ST dose-response dependence was analyzed by means of Statistica+2005 software.

RESULTS

Administration of ST in doses of 0.7-1.0 LD_{50} was accompanied by a 2-3-fold increase in blood ammonia concentration, which persisted for 3 h. After ammonia inhalation, blood ammonia concentration increased by 65%. These changes were insignificant in barbiturate-anesthetized animals (Fig. 1).

Inhalation of ammonia inhibited external respiration and decreased O_2 consumption. This effect was significant in animals receiving ST (Fig. 2). Ammonia and ST had an additive effect on both parameters. The animals inhaling ammonia for 3 h survived over 48-h monitoring. One third of rats inhaling ammonia and receiving ST in nonlethal doses of 57 and 62 mg/kg died over 3 h (Fig. 3).

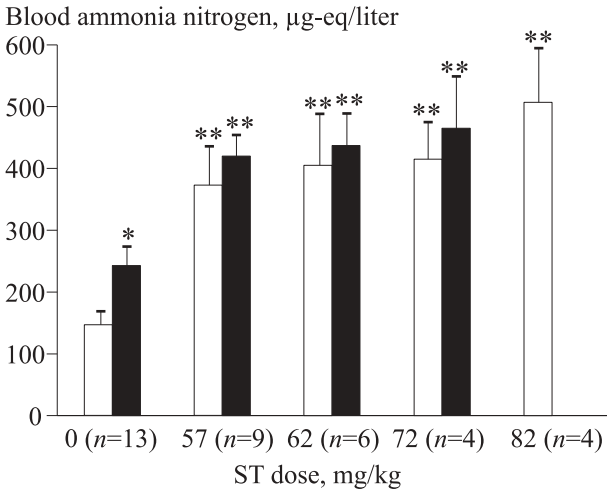

Fig. 1. Blood ammonia concentration in rats after ST administration under conditions of air breathing (light bars) or 3-h ammonia inhalation (dark bars). $p<0.05$: *compared to the control; **compared to animals not receiving ST.

LD_{50} for ST in control and ammonia-inhaling rats was 83.9±2.1 and 63.6±1.5 mg/kg, respectively. The factor of dose variation was 0.76.

Animal sensitivity to the lethal effect of ST tended to increase after administration of AA. Thirty minutes after AA administration, blood ammonia concentration increased in intact rats, but only tended to increase in barbiturate-anesthetized animals. The trends to an increase in blood ammonia concentration and mortality rate were still observed when narcotized rats were maintained under isothermal conditions (Table 1).

Our results show that inhalation of ammonia and intraperitoneal injection of AA caused hyperammonemia in intact animals. The degree of hyperammonemia decreased after barbiturate inhibition of ammonia inhalation and, probably, AA resorption from the abdominal cavity. Administration of ammonia increased the mortality rate of rats with barbiturate intoxication. This effect was most pro-

TABLE 1. Three-Hour Mortality Rate and Blood Ammonia Concentration in Rats with Experimental Barbiturate Coma under Isothermal Conditions and/or AA Administration (*M*±*m*)

Preparation	Air temperature over 3 h after ST administration, °C	Number of animals		Blood ammonia nitrogen in survived rats, µg-eq/liter
		total (in the group)	died over 3 h	
Water (control)	18-20	8	—	53±19
AA		8	—	163±29*
ST		16	4 (25%)	107±12*
AA+ST		16	7 (44%)	135±26*
ST	34° C	16	1 (6%)	100±20*
AA+ST		16	3 (19%)	143±18*

Note. *$p<0.05$ compared to the control.

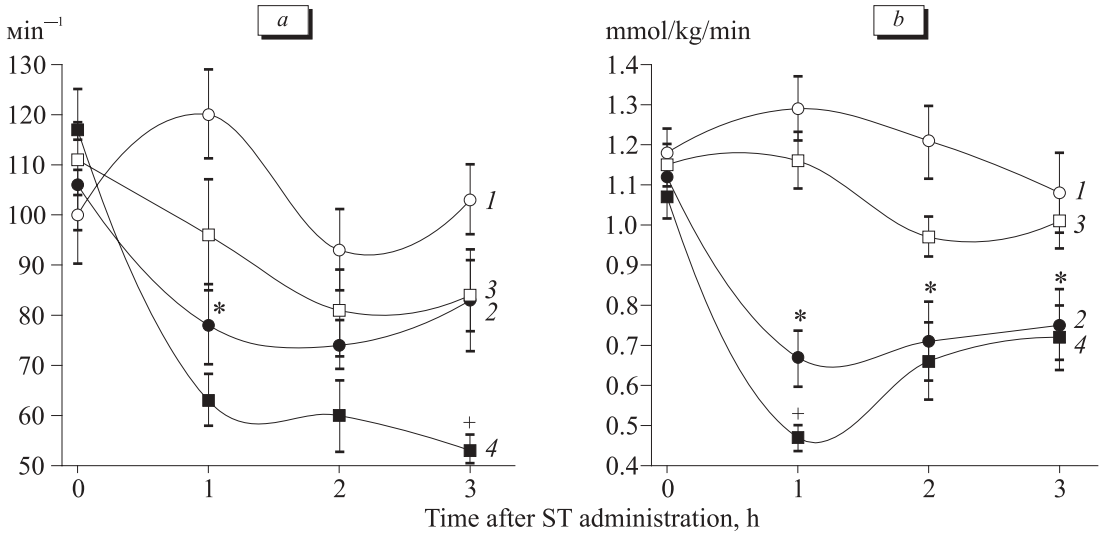

Fig. 2. Frequency of respiratory movements (*a*) and O_2 consumption (*b*) in rats after ST administration under conditions of air breathing (control) or ammonia inhalation (n=8). Intact animals (*1*); ST administration (*2*); ammonia inhalation (*3*); and ST administration followed by ammonia inhalation (*4*). p<0.05: *compared to *1*; +compared to *2*.

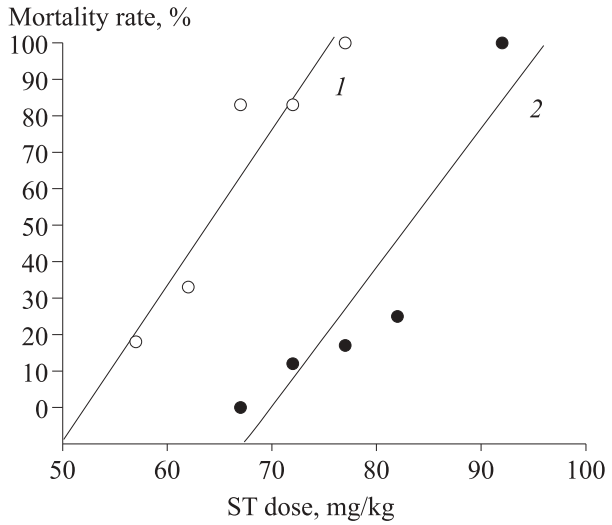

Fig. 3. Mortality rate of rats over 3 h after ST administration under conditions of air breathing (*1*) or 3-h ammonia inhalation (*2*). The number of animals per group for each dose of ST varied from 6 to 23.

nounced after inhalation of ammonia. The potentiation of the lethal effect of barbiturates can be related to both local and resorptive activity of atmospheric ammonia.

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