Intensification of Ammonium Diffusion from Rat Gastrointestinal Tract during Acute Barbiturate Intoxication

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In rats with acute sodium thiopental intoxication, ammonium concentration in the caecal contents was at the lower boundary of control values, while accumulation of ammonium in lavage solution injected intraperitoneally was 50-70% accelerated. Blood ammonium level did not change 3 h after sodium thiopental injection in a dose inducing sopor, but increased 3-fold during coma modeling. Intragastric administration of gentamicin (antibiotic poorly absorbed from the gastrointestinal tract) 2-fold reduced ammonium concentration in the caecal contents and prevented hyperammoniemia during induction of barbiturate coma. Hence, increased permeability of the gastrointestinal wall for ammonium promotes the development of hyperammoniemia in rats during induction of barbiturate coma.

Key Words: *blood ammonium; caecal contents ammonium; barbiturate coma; peritoneal lavage solution*

Hyperammoniemia not associated with blood concentration or inhibited urinary excretion of NH_3 or its elimination with exhaled air develops in rats during simulation of barbiturate coma [3]. Hyperammoniemia was observed against the background of gastrointestinal stasis. We tested the hypothesis on the involvement of gastrointestinal stasis in the development of hyperammoniemia in barbiturate coma.

MATERIALS AND METHODS

The study was carried out on female albino rats (100-120 g). The animals were taken into experiment after 24-h fasting. In experimental series I, accumulation of NH_3 in 0.9% NaCl solution injected intraperitoneally (50 ml/kg) 2.5 h after sodium thiopental (ST) was evaluated. Experimental animals were intraperitoneally injected with ST in doses of 75 and 85 mg/kg; controls received water (10 ml/kg). Laparotomy was carried out after 3 h (under chloroform narcosis in the control), lavage fluid was collected, centrifuged, and NH_3 was measured in the supernatant. Each group consisted of 2-3 animals per experiment and at least 6 in a series of 3 experiments. In experimental series II, the effects of ST in doses of 75 and 85 mg/kg and gentamicin sulfate (GS; antibiotic poorly absorbed from the intestine) on NH_3 levels in the blood and caecal contents were evaluated. Gentamicin was administered intragastrically (10 ml 0.4% solution/kg) 4 times: 48, 24, 3 h and 5 min before ST. Controls received water. The rats were decapitated 3 h after ST injection, the blood was deproteinized with trichloroacetic acid, and NH_3 was measured in the supernatant. Caecal content was weighed, suspended in 4-fold volume of 0.9% NaCl, and NH_3 was evaluated. The groups consisted of 6 rats in all experimental series.

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TABLE 1. Accumulation of NH_3 in Lavage Solution Injected Intraperitoneally to Rats with Acute ST Intoxication ($M\pm m$; n=6)

Group	Volume of lavage solution in abdominal cavity, ml/kg	NH_3 concentration in lavage solution, μM	NH_3 accumulation rate in lavage solution, μmol/(kg×min)
Control (H_2O)	32.8±2.0	213±12	0.24±0.03
ST, 75 mg/kg	45.2±2.7	248±18	0.38±0.05*
ST, 85 mg/kg	47.4±1.0	258±13*	0.41±0.03**

Note. *p<0.05, **p<0.01 compared to the control.

The concentrations of NH_3 in the blood and lavage solution were evaluated by the microdiffusion method followed by acidometric titration [1,3]. The rate of NH_3 accumulation in lavage solution was estimated. In order to measure NH_3 concentration in the caecal contents, 2 ml suspended contents was placed into the main space of two Warburg's conical vessels. The lateral sleeves contained 1 ml saturated K_2CO_3 solution, central glasses contained sorbent for NH_3 (0.1 ml 0.25 M H_2SO_4). After sealing, K_2CO_3 was mixed with the suspension immediately in one of the vessels and after 0.5 h incubation in Warburg's device at 37°C in the other. After 24 h the sorbent was titered *in situ* with 0.5 M NaOH solution using a microdispenser with a 1-μl graduating mark and NH_3 concentration in the caecal contents was estimated.

The significance of intergroup differences between the means was evaluated using Student's t test.

RESULTS

Accumulation of NH_3 in the lavage solution during barbiturate intoxication was accelerated by 50-70% due to an increase in concentration of ammonium and volume of unabsorbed solution. A trend to a direct relationship between this effect and ST dose was noted (Table 1). Intoxication virtually did not modify the weight of caecal contents and NH_3 concentration in it. We observed a trend to accumulation of NH_3 during incubation, which was more pronounced in intact animals. The contents of the caecum was evacuated slower in rats treated with GS, NH_3 concentration in it being 2-fold lower. GS treatment virtually abolished the trend to NH_3 accumulation in samples during their incubation (Table 2). Blood NH_3 level virtually did not change after injection of ST in a dose of 75 mg/kg, while after the dose of 85 mg/kg it increased almost 3-fold. No hyperammoniemia was noted in animals narcotized after GS treatment: their blood NH_3 level was 2-fold lower than after 85 mg/kg dose of ST alone (Table 2).

Since NH_3 concentration in the lavage solution increased (Table 1), accumulation of NH_3 in the abdominal cavity depended on intensification of NH_3 transfer through the gastrointestinal wall. Since the diffusion surface and concentration gradient remained unchanged (not increased), this could be due to acceleration of ATP-dependent ionic transport and nonionic diffusion in the large and small intestine [4]. NH_3 concentration in the caecal lumen is by 2 orders of magnitude higher than in the lavage solution, therefore, the contribution of nonionic diffusion seemed to predominate. The direc-

TABLE 2. NH_3 Concentration in Caecal Contents and Blood of Rats Injected with ST and GS ($M\pm m$; n=6)

Group	Caecal contents weight index, g/kg	Caecal NH_3 concentration, mM		Blood NH_3 concentration, μM
		before incubation	after 0.5 h incubation	
Control (H_2O)	8.4±1.1	32±3	41±4	55±14
GS	14.4±0.9*	16±3*	18±2*	46±15
H_2O+ST, 75 mg/kg	9.7±1.5	29±3	36±3	41±14
GS+ST, 75 mg/kg	10.1±1.5	19±2+	20±3+	38±10
H_2O+ST, 85 mg/kg	9.2±1.1	31±3	37±3	158±10*
GS+ST, 85 mg/kg	21.8±4.4*+	14±3*+	10±4*+	78±14+

Note. *p<0.05 compared to the control; +p<0.05 compared to injection of the corresponding ST dose without GS.

tion of changes in NH_3 transfer to mesothelial and endothelial surfaces obviously coincided. Considering the linear relationship between the portocaval blood NH_3 concentration gradient and NH_3 level in the portal vein [2], increased permeability of the gastrointestinal wall for NH_3 can be a mechanism of hyperammoniemia in rats during modeling of barbiturate coma.

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