Hyperammoniemia in Rats with Barbiturate Coma

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Sodium thiopental in the comatogenic (but not soporogenic) dose caused hyperammoniemia in rats. Blood ammonium level increased 3-fold within 3 h and 5-fold within 18 h. Blood urea level increased by one-third within 18 h against the background of unchanged creatinine level and hematocrit. Urinary excretion of ammonium did not decrease, while its release with exhaled air increased, indicating intensification of ammonium formation in the body. Barbiturate coma did not change the slope of curves of dose-dependent increase of ammonium or urea levels in the blood of rats injected with ammonium acetate, which attested to the absence of appreciable disorders in the ammonium detoxifying function of the liver. Ammonium hyperproduction could be caused by gastrointestinal stasis verified by X-ray examination and confirmed by correlation between blood urea level and stool retention in narcotized rats.

Key Words: *thiopental coma; gastrointestinal stasis; ammonium; urea; creatinine*

Inhibition of gas exchange and thermogenesis in barbiturate coma (BC) can be partially caused by exhaustion of the tissue pool of the Krebs cycle intermediates [2,3], which probably results from intensification of α-ketoglutarate and oxaloacetate amination under conditions of hyperammoniemia development. The content of the gastrointestinal tract serves as the main source of ammonium in the body; ammonium formation can many-fold increase within several minutes. Intestinal stasis detected in rats and horses with BC can promote this hyperproduction [4]. Despite decreased cardiac output, the intestinal bloodflow in BC increases [6], which can promote ammonium absorption. Hyperammoniemia accompanies shock of different origin, including shock caused by pentobarbital. Lethal hyperammoniemia in a patient with ureterosigmoidostomy during therapeutic induction of BC was reported [5].

Here we evaluated the effect of acute barbiturate intoxication in rats on ammonium formation in the gastrointestinal tract, its excretion, and blood content.

MATERIALS AND METHODS

Female albino rats (100-120 g) were divided into control (intact) and experimental (injection of sodium thiopental, ST) groups. Hematocrit, blood content of ammonium, urea, and creatinine were measured in animals injected with ST in doses of 0.8 or 1.0 LD_{50} 18, 3, and 0.5 h before decapitation; the effects of ST (1.0 LD_{50}) on the gastrointestinal motility, urinary excretion of nitrous metabolites and exhalation of ammonium were evaluated by X-raying; the effects of ST (0.8 or 1.0 LD_{50}) on changes in blood levels of ammonium and urea after injection of ammonium acetate (AA) were evaluated. All drugs were injected intraperitoneally (10 ml/kg); controls were injected with water in the same volume.

Blood samples for hematocrit evaluation were taken; the remaining part of blood was deproteinized with trichloroacetic acid and used for chemical studies. In order to separate the urine from feces and collect it into tubes with trichloroacetic acid, the

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TABLE 1. Blood Ammonium Level (μmol/liter) in Rats after Injection of ST (*M*±*m*; *n*=6)

ST dose (fracture of LD_{50})	Time after ST injection, h			
	0 (control)	0.5	3.0	18.0
0.8	103±21	116±22	133±16	197±28*
1.0	70±16	135±19	287±59*	375±102*

Note. Here and in tables 2, 3: *$p<0.05$ compared to the control.

animals were put into metabolic boxes (Nalgene) for 18 h. Ammonium was evaluated by the method of microdiffusion followed by acidometric titration. Ammonium was captured by sulfuric acid from atmospheric air, let through the box with the animal during 0.5 h, and measured by titration. Blood and urinary urea was measured using Allwax Diagnosticum kit, creatinine was assayed after Jaffe.

For evaluation the gastrointestinal tract motility, barium sulfate suspension was infused through a gastric tube 0.5 h before ST injection (1.0 LD_{50}). After injection of ST (0.5, 3, 6, and 18 h postinjection) experimental and control animals were placed into plastic penals connected in pairs and studied in a Siemens Iconos R200 digital X-ray device.

Ammonium acetate (0, 2, 4, 6, or 8 mmol/kg) was injected intraperitoneally 2 h after ST in order to evaluate the ammonium detoxifying function of the liver. After 0.5 h the rats were decapitated and blood content of ammonium and urea were measured. Rectal temperature was measured with a mercuric thermometer, O_2 consumption as described previously [1].

The significance of differences in the mean value in the groups was evaluated using Student's *t* test and Mann—Whitney *U* test.

RESULTS

The rat status after injection of 0.8 LD_{50} ST was soporous: despite lateral posture, they retained the audiomotor reflex and local defense reaction to painful stimulation; no lethal outcomes were recorded. Blood ammonium content tended to increase; this increase became significant after 18 h. Injection of 1.0 LD_{50} ST caused BC (sensorimotor reflexes were absent except the corneal reflex). Blood ammonium content increased 3-fold within 3 h and 5-fold within 18 h (Table 1). Hematocrit

TABLE 2. Excretion of Nitrous Metabolites with Urine and Defecation (per kg body weight over 18 h) after Injection of ST in a dose of 1.0 LD_{50} (*M*±*m*; *n*=14)

Parameter	ST	Control
Volume of urine, ml	33±6	48±10
Ammonium, mmol	1.12±0.23	0.92±0.17
Urea, mmol	11.0±1.8	13.7±1.7
Creatinine, μmol	38±7	55±3
Defecation, g	2.9±1.5*	13.0±3.8

TABLE 3. Hematocrit and Blood Concentrations of Nitrous Metabolites in Rats 18 h after Injection of ST in a Dose of 1.0 LD_{50} (*M*±*m*; *n*=14)

Parameter	ST	Control
Hematocrit, %	45±2	45±2
Ammonium, mmol/liter	375±102*	70±16
Urea, mmol/liter	11.4±0.6*	8.7±0.6
Creatinine, μmol/liter	56±3	50±3

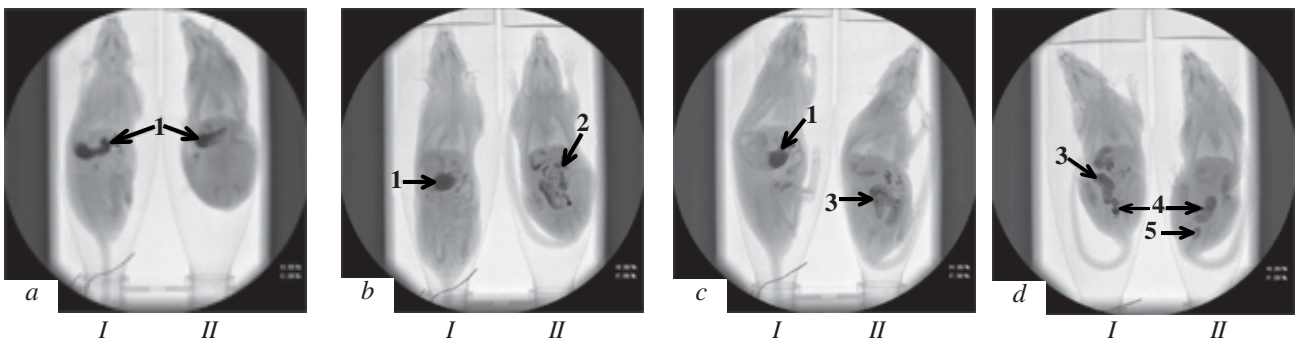

Fig. 1. Effect of ST on the movement of barium sulfate in the rat gastrointestinal tract 1 (*a*), 3.5 (*b*), 6.5 (*c*), and 18.5 h (*d*) after barium sulfate administration. *I*) 1.0 LD_{50} ST injected 0.5 h before barium sulfate; *II*) intact rat after barium sulfate administration. *1*) stomach; *2*) small intestine; *3*) caecum; *4*) sigmoid colon; *5*) rectum.

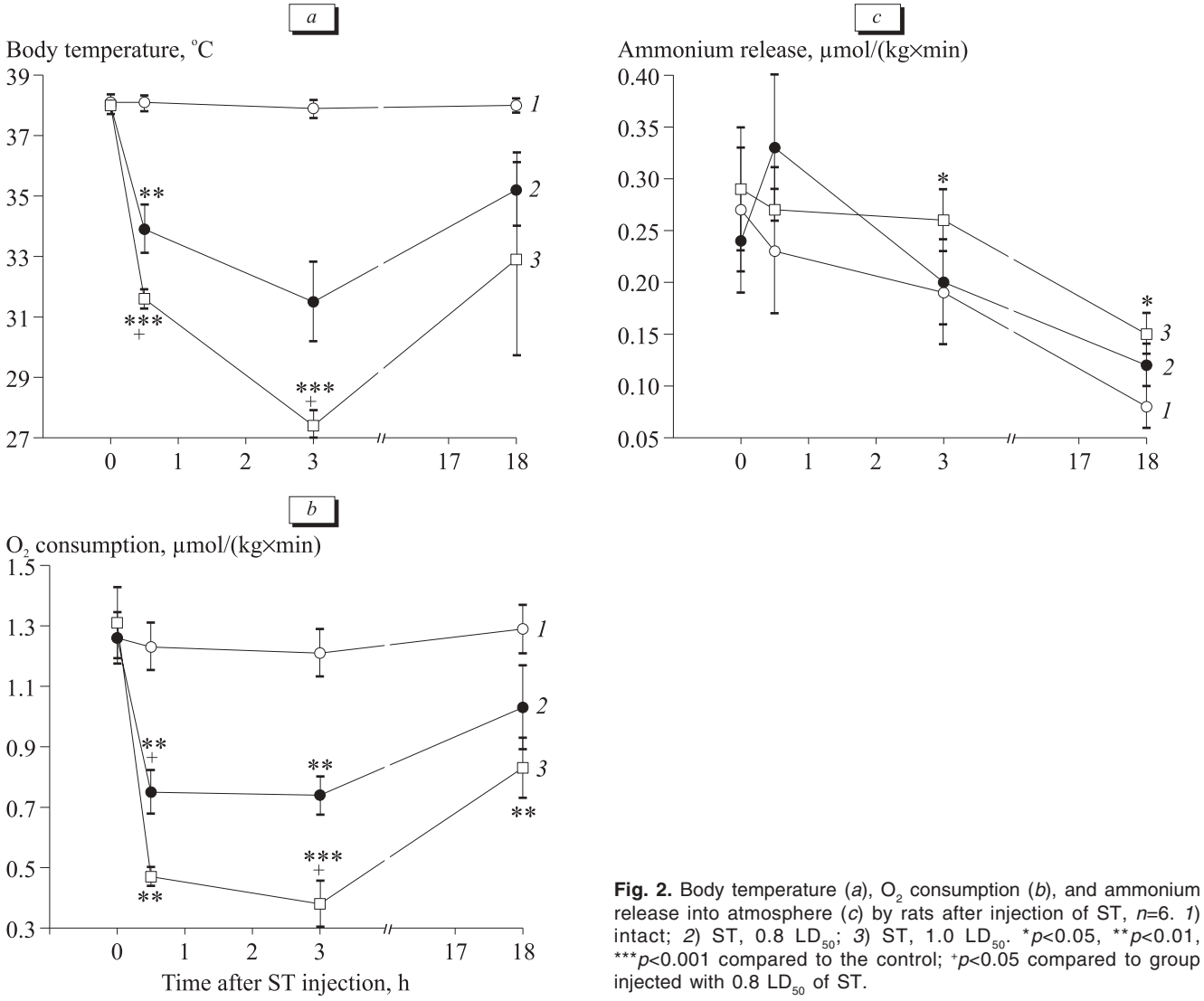

Fig. 2. Body temperature (*a*), O_2 consumption (*b*), and ammonium release into atmosphere (*c*) by rats after injection of ST, *n*=6. *1*) intact; *2*) ST, 0.8 LD_{50}; *3*) ST, 1.0 LD_{50}. **p*<0.05, ***p*<0.01, ****p*<0.001 compared to the control; +*p*<0.05 compared to group injected with 0.8 LD_{50} of ST.

and blood creatinine level did not change (data not presented).

Injection of ST in a comatogenic dose inhibited gastrointestinal motility: after 18 h the contrast agent was concentrated in the stomach and caecum, while in controls it was almost completely eliminated (Fig. 1). The weight of feces over 18 h after ST injection (1.0 LD_{50}) was 4.5 times lower than in intact animals deprived of water or fodder (Fig. 2).

Diuresis and urinary excretion of urea and creatinine tended to decrease in BC, while ammonium excretion tended to increase (Table 2). Blood ammonium content increased 5.4 times over 18 h, urea content increased by one-third, while hematocrit and creatinine concentration remained unchanged (Table 3). Blood level of urea in rats with coprostasis (13.6±1.9 mmol/liter; *n*=10) 2-fold surpassed than in animals with normal stool (5.7±0.5 mmol/liter; *n*=4; *p*<0.05).

Ammonium release into the air decreased during 18 h in both groups. The decrease was slower in rats with BC, despite hypothermia and gas exchange depression (Fig. 2). After TH injection in doses of 0.8 and 1.0 LD_{50} the slope of curves presenting the relationship between ammonium and urea levels in the blood and the dose of injected AA did not differ from that of intact animals (Fig. 3).

These data indicate that hyperammoniemia in BC is a threshold effect linked with the development of coma. Presumably, increased release of ammonium into the blood caused by gastrointestinal stasis in the absence of appreciable disorders in the ammonium detoxifying function of the liver plays the key role in the development of hyperammoniemia.

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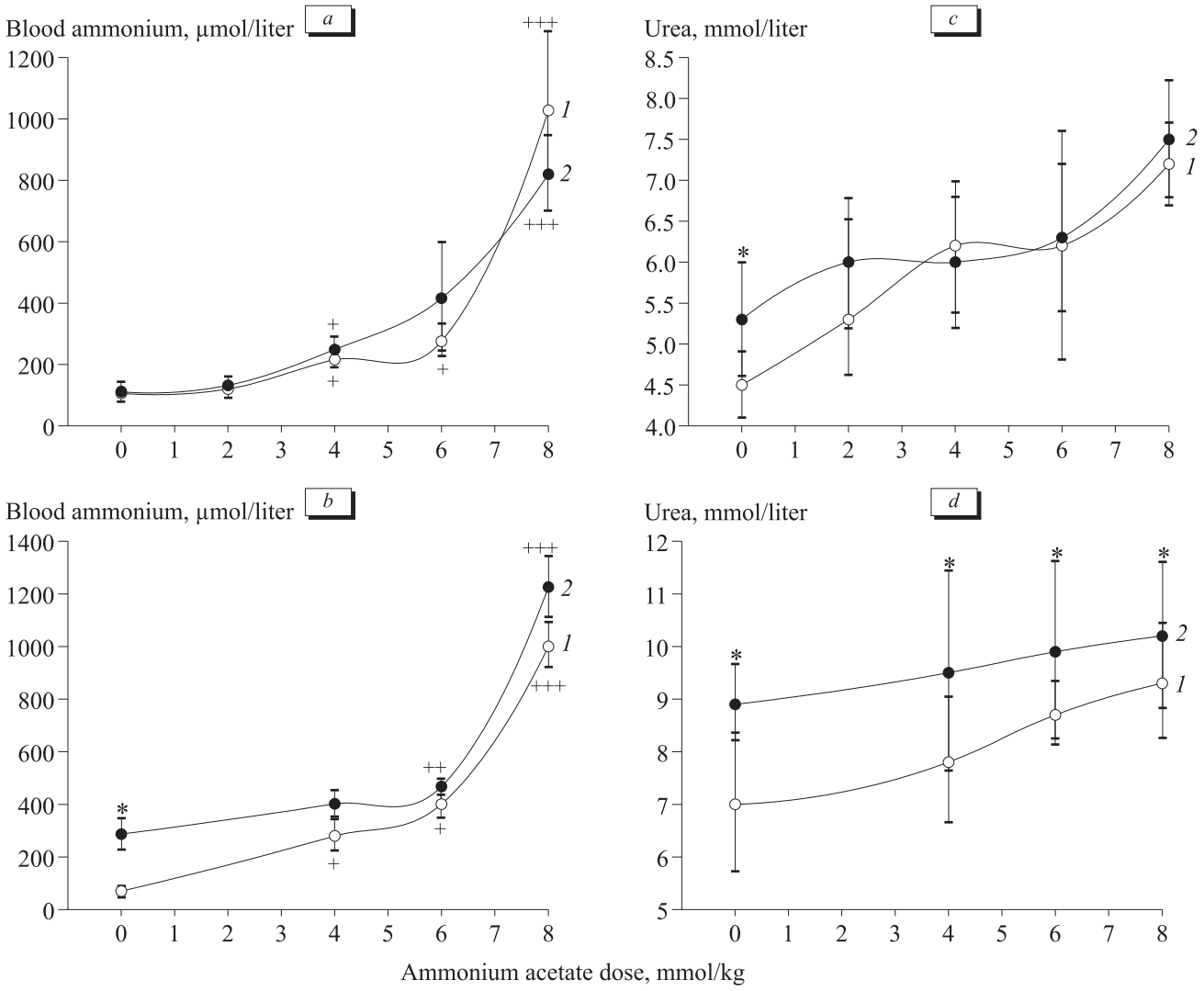

Fig. 3. Effect of AA on blood levels of ammonium (*a*, *b*) and urea (*c*, *d*) in intact animals and animals narcotized by ST in doses of 0.8 LD_{50} (*a*, *c*) and 1.0 LD_{50} (*b*, *d*) 2 h before AA injection, *n*=6; *1*) intact; *2*) ST. **p*<0.05 compared to the control; ^{+}p<0.05, ^{++}p<0.01, ^{+++}p<0.001 compared to group injected no AA.

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