Metabolic Correction of Gas Exchange Disturbances in Rats with Barbiturate Coma

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Krebs cycle intermediates normalized gas exchange and decreased the mortality rate in rats with barbiturate coma. Treatment with other substrates including glucose and products of glycolysis was ineffective. Oxygen inhalation had no effect on oxygen consumption and indexes of external respiration. Our results suggest that deficiency of endogenous intermediates of the Krebs cycle, but not disturbances in oxygen mass transfer, serves as a limiting factor for oxygen consumption in rats with barbiturate coma.

Key Words: *barbiturates; coma; oxygen consumption; limiting factor*

Narcotic effect of barbiturates is accompanied by reduction of O_2 consumption [2-4]. The role of this phenomenon in the pathogenesis of barbiturate coma remains unclear. Published data show that barbiturate coma impairs external respiration [3] and cerebral blood flow [10,11]. Disturbances in gas exchange are believed to be associated with the limiting effect of oxygen mass transfer on its consumption. Artificial ventilation and vasoactive drugs are used for the treatment of patients with barbiturate coma [2]. However, barbiturates *in situ* [7,8] and *in vitro* suppress metabolic activity of the brain tissue [13], decrease the arteriovenous gradient of O_2 concentration, and reduce O_2 consumption [9]. These changes reflect impaired O_2 extraction from the blood in tissues. This probably results from their inability to satisfy energy requirements, which is partly due to substrate deficiency. Our previous studies showed that barbiturate coma is accompanied by disturbances in substrate supply [4]. Here we studied whether the observed changes serve as a limiting factor under these conditions. The possibility of metabolic correction of disturbances accompanying barbiturate coma was evaluated.

MATERIALS AND METHODS

Experiments were performed on female albino rats weighing 100-120 g. O_2 consumption was measured as described elsewhere [4]. Acute poisoning with barbiturates was produced by intraperitoneal injection of sodium thiopental (ST) in a dose of 75 mg/kg. Pure O_2 was pumped into a respirometric chamber to study the effect of oxygenotherapy on gas exchange. Minute ventilation (VE) was measured in ST-narcotized rats using a sealed mask, valve box, and respirometric burette. The device was calibrated with a gas counter. The respiratory volume was calculated as the ratio between VE and number of respiratory movements per 1 min. The duration of sleep was estimated as the time of lateral posture. The effect of test substances on survival rate of animals with thiopental coma was determined by the percent of protection. This index was calculated as the difference between survival rates in the treated and control groups (in %). Substrates of cell respiration, sodium benzoate, and nicotinic acid were injected intraperitoneally in a dose of 5 mmol/kg (1 ml per 100 g body weight) 30 min after intraperitoneal administration of ST. Control animals received an equivalent volume of NaCl. The solutions were equivalent by Na^+ content.

Intergroup differences in parametric indexes were evaluated by Student's *t* test. Intergroup differences in survival rates were estimated by exact Fisher test.

RESULTS

ST in a dose of 75 mg/kg produced coma without sensorimotor reflexes (except for corneal reflex), which

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persisted for 1 day after treatment. The rate of O_2 consumption decreased to ~30% and remained practically unchanged for 0.5-2 h. However, VE progressively decreased. By the 2nd hour, VE was 0.25-fold lower than 30 min after treatment. Sodium succinate increased O_2 consumption in narcotized rats by 1.4 times, but had no effect on pulmonary ventilation (Fig. 1). Treatment with Krebs cycle intermediates, including sodium salts of succinate, malate, α-ketoglutarate, citrate (Fig. 2, *2*, *4*, *6*, *7*, *10*, *17*), dimethyl succinate (Fig. 2, *3*), and glutamate (Fig. 2, *13*, *14*), intensified gas exchange in animals. Bioenergetic substrates (not intermediates of the Krebs cycle) pyruvate, acetate (Fig. 2, *5*, *9*), and glucose (Fig. 2, *11*, *12*) did not improve gas exchange. Carbonic acids and their salts (not bioenergetic substrates), sodium benzoate and nicotinic acid, did not modulate gas exchange (Fig. 3, *8*, *15*).

Oxygenotherapy for 3 h had no effect on O_2 consumption, duration of sleep, and mortality rate (5 of 8 treated rats and 3 of 8 control rats). This procedure did not modulate the effect of sodium succinate (Fig. 2, *16*, *17*).

Various intermediates of the Krebs cycle, including dimethyl succinate and glutamate, increased the survival rate of rats with thiopental coma. Other substances were ineffective in this respect (Table 1). Sixfold administration of sodium succinate at 0.5-h intervals accelerated normalization of gas exchange (compared to single treatment and control animals, Fig. 3). Repeated and single injection of this substance increased the survival rate of animals (5 of 7 rats and 6 of 7 rats, respectively). It should be emphasized that all control animals (n=7) died over the 1st day after the onset of coma.

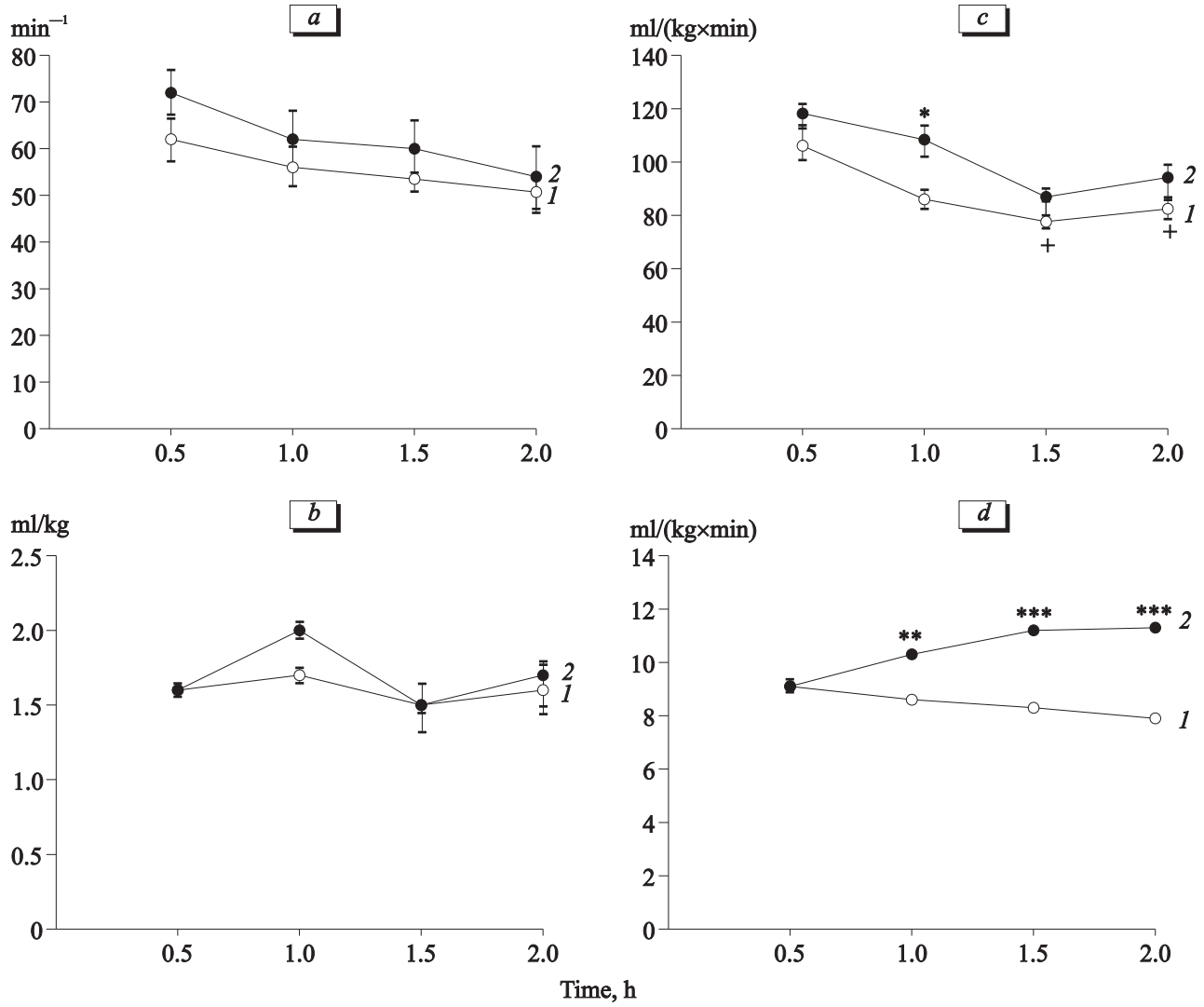

Fig. 1. External respiration and gas exchange in rats (n=11) with thiopental coma receiving NaCl (*1*) and sodium succinate (*2*). Number of respiratory movements (*a*), respiratory volume (*b*), minute volume of ventilation (*c*), and oxygen consumption (*d*). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared to NaCl (control); $^{+}p<0.05$ compared to the index recorded 0.5 h after injection of sodium thiopental.

Sodium succinate increased O_2 consumption, but did not intensify external respiration. Moreover, the decrease in pulmonary ventilation was not accompanied by reduction of O_2 consumption (Fig. 1). These data indicate that the intensity of external respiration is not a limiting factor for O_2 consumption during acute poisoning with ST. A 5-fold increase in partial O_2 pressure in the inspired air should produce a comparable rise in O_2 pressure in the arterial blood [1], but had no effect on O_2 consumption (Fig. 2, *16*). There-

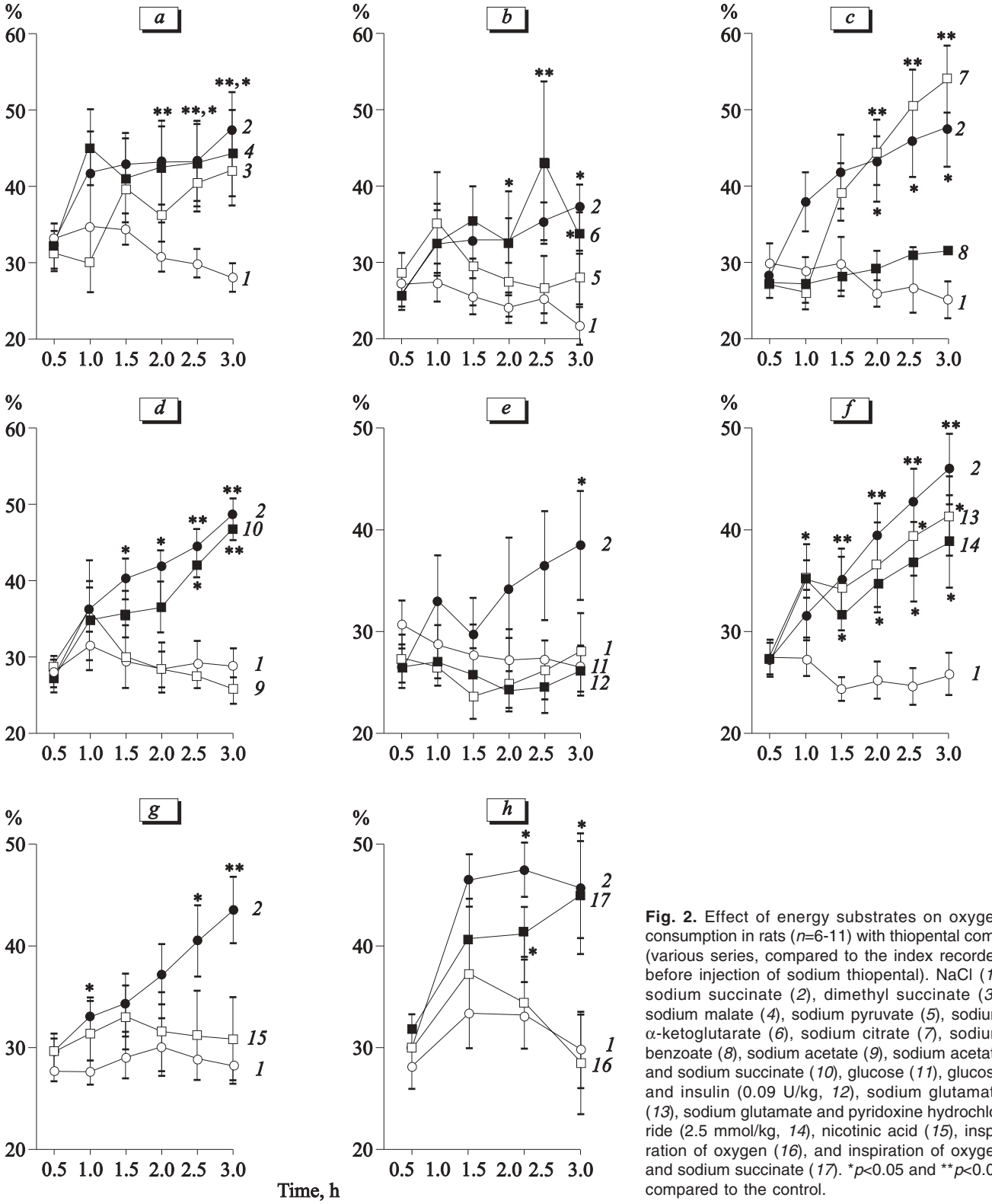

Fig. 2. Effect of energy substrates on oxygen consumption in rats (n=6-11) with thiopental coma (various series, compared to the index recorded before injection of sodium thiopental). NaCl (*1*), sodium succinate (*2*), dimethyl succinate (*3*), sodium malate (*4*), sodium pyruvate (*5*), sodium α-ketoglutarate (*6*), sodium citrate (*7*), sodium benzoate (*8*), sodium acetate (*9*), sodium acetate and sodium succinate (*10*), glucose (*11*), glucose and insulin (0.09 U/kg, *12*), sodium glutamate (*13*), sodium glutamate and pyridoxine hydrochloride (2.5 mmol/kg, *14*), nicotinic acid (*15*), inspiration of oxygen (*16*), and inspiration of oxygen and sodium succinate (*17*). *p<0.05 and **p<0.01 compared to the control.

fore, the system of oxygen mass transfer does not play a limiting role.

Disturbances in O_2 utilization by tissues are not associated with inhibition of glycolysis [6,12]. These changes were not abolished by administration of glucose, glucose and insulin, and pyruvate (final product of glycolysis). The product of pyruvate oxidation did not prevent suppression of gas exchange (Fig. 2). Thus, this disorder was not related to abnormal oxidation of pyruvate. Moreover, oxidation of pyruvate and α-ketoglutarate is realized via the same mechanism. However, administration of α-ketoglutarate intensified gas exchange during thiopental coma. Our hypothesis that barbiturates *in situ* inhibit $NADH_2$-oxidizing enzymes was not confirmed [4]. The degree of O_2 consumption increased under the influence of not only succinate independently oxidized by NAD^+, but also of other intermediates of the Krebs cycle. These results indicate that barbiturate coma is not accompanied by inactivation of enzymes involved in the metabolic cycle or respiratory chain. Therefore, barbiturate coma is not accompanied by inactivation of enzymes for this metabolic pathway or respiratory chain. The decrease in O_2 consumption was prevented by Krebs cycle intermediates. These changes were also abolished by dimethyl succinate and glutamate that undergo transition into Krebs cycle intermediates under the influence of intracellular esterases and NAD^+-dependent L-glutamate dehydrogenase, respectively. Therefore, the content of Krebs cycle intermediates is a limiting factor for O_2 consumption during thiopental coma. These substrates and their precursors produce a positive effect on gas exchange (Fig. 2) and outcome of poisoning with barbiturates (Table 1). Our results indicate that metabolic correction of energy deficiency can be achieved via normalizing the content of these endogenous compounds.

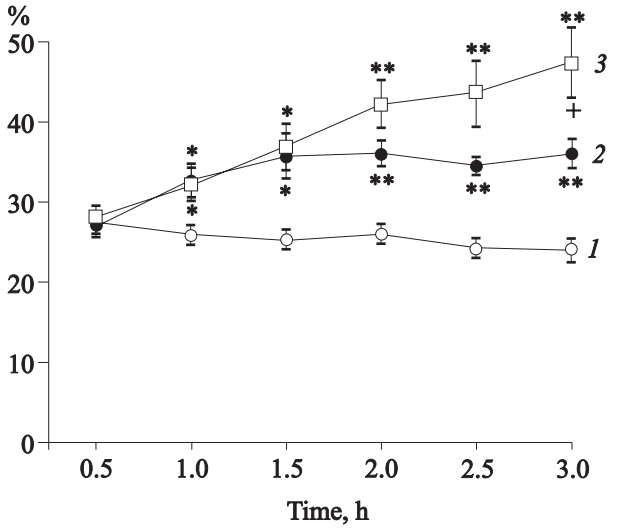

Fig. 3. Effect of single and 6-fold treatment with sodium succinate on oxygen consumption in rats (n=7) with thiopental coma (compared to the level observed before administration of sodium succinate). Sixfold treatment with NaCl at 30-min intervals (control, *1*). Treatment with sodium succinate 30 min after injection of sodium thiopental; administration of NaCl after 60, 90, 120, 150, and 180 min (*2*). *p<0.05 and **p<0.01 compared to the control; ^+p<0.05 compared to *2*.

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TABLE 1. Effect of Single Treatment with Several Substrates or O_2 Inspiration on the Survival Rate of Rats with Thiopental Coma*

Substance	Number of rats in group	Survival rate of control animals, %	Percent of protection
Sodium succinate	84	57	$+30^+$
Dimethyl succinate	6	33	$+67^{++}$
Sodium malate	6	33	$+67^{++}$
Sodium α-ketoglutarate	10	30	+10
Sodium glutamate	6	83	+17
Sodium glutamate and pyridoxine chloride (2.5 mmol/kg)	6	83	+17
Sodium benzoate	11	64	-9
Sodium acetate	6	83	-17
Sodium acetate and sodium succinate	6	83	0
Glucose and insulin (0.09 U/kg)	4	83	0
Nicotinic acid	6	83	0
O_2	8	62	-25
O_2 and sodium succinate	8	62	0

Note. *Data on mortality rate of control rats not equal to 0 and 100%. ^+p<0.01 and ^{++}p<0.05 compared to the control.

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