Effect of Sodium Succinate on Gas Exchange in Rats with Barbiturate-Induced Coma

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Injection of sodium succinate in doses of 5 or 10 mmol/kg (but not 1 mmol/kg) intensified oxygen consumption in rats with sodium thiopental-induced coma. Injection of SDH inhibitor (sodium malonate) inhibited gas exchange and abolished the effect of sodium succinate. The effect of succinate on rat survival was positive, while that of malonate was negative, but manifested only as a trend. The critical role of succinate oxidation in preventing lethal complications of barbiturate-induced coma is proved.

Key Words: *sodium thiopental; coma; sodium succinate; sodium malonate*

Barbiturate coma can be a result of accidental poisoning of a complication of anesthesia. Despite the capacity of soporific and sedative drugs to reduce oxygen consumption by cerebral cells [5], brain hypoxia is the main cause of lethal outcome and residual encephalopathy in coma caused by overdosage of soporific drugs [2]. Impairment of external respiration [4] and cerebral bloodflow [8] were described as possible mechanisms of hypoxia in acute barbiturate poisoning.

However, it can be hypothesized that barbiturates can produce various effects on energy metabolism in the brain. For instance, barbiturates in a concentration of 10^{-3} M block $NADH_2$ oxidation enzymes [1]. Due to almost 20-fold higher capacity of the nerve tissue to barbiturate accumulation in comparison with the blood [10], this level can be attained at doses inducing coma (over 10^{-4} mol/kg). Acidosis associated with the narcotic effect of barbiturates [9] can promote their penetration into cells, which provides conditions for direct inhibition of cell respiration predominantly at the level of electron-transporting complex I. It is interesting to evaluate the role of this mechanism in suppression of gas exchange characteristic of barbiturate-induced coma.

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It was previously shown that succinate oxidation by cultured glial cells is more resistant to sodium thiopental inhibition than NAD^+-dependent oxidation of substrates [7]. If this phenomenon manifests in nerve tissue *in vivo*, succinic acid oxidation in barbiturate coma can play a greater role in energy supply than normally. Since the level of endogenous succinate in tissues is below the Michaelis constant (K_m) for SDH [3], it can limit the rate of NAD^+-independent ATP resynthesis in the brain and, hence, resistance to the toxic effect of barbiturates. We investigated the effect of sodium succinate on gas exchange and survival of rats in coma caused by sodium thiopental in the absence of oxygen therapy and artificial ventilation.

MATERIALS AND METHODS

Experiments were carried out on female albino rats (100-120 g). The animals were pre-adapted to respirometry. The rate of gas exchange was measured by the method of Regnault in our modification [6]. After evaluation of the initial level of oxygen consumption the rats were intraperitoneally injected with sodium thiopental in a dose of 75 mg/kg (*i.e.* 0.28 mmol/kg). Thirty minutes after injection of thiopental oxygen consumption was measured again and sodium succinate and/or SDH inhibitor sodium malonate was injected. Controls received sodium chloride solution. All solu-

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tions were injected in a volume of 1 ml per 100 g body weight and were equivalent by sodium content in parallel experimental groups. Further measurements of gas exchange were carried out every 30 min. The results were expressed in % of the initial level of oxygen consumption. The significance of differences between the groups was evaluated using Student's *t* test. The effects of sodium succinate and/or malonate on gas exchange in intact rats were studied according to the same protocol, except the injection of sodium thiopental.

For evaluation of the effects of succinate, malonate, and their combination on rat survival, sodium thiopental was administered in doses of 65-85 mg/kg and after 30 min sodium succinate and/or malonate was injected. Animals dead within 2 days after barbiturate injection were counted. The number of animals dead before drug injections was negligible, and they were not included in the analysis. The index of survival (%) was used for evaluation of rat resistance to thiopental in each experimental group. LD_{50} was estimated for each group using regression analysis in logprobit coordinates using Excel XP software (Microsoft). Standard $S_{LD_{50}}$ error was estimated using the method of Miller and Tainter. The factor of dose alteration as a result of modifying effect was estimated as the ratio of sodium thiopental LD_{50} in experimental and control groups.

RESULTS

Sodium thiopental in a dose of 75 mg/kg rapidly and considerably inhibited gas exchange. The decrease in gas exchange to 17% of the initial level led to death of all animals receiving no treatment. Single injection of sodium succinate led to positive changes in oxygen consumption, which 2.5 h after thiopental injection was 60% more intensive than in the control (Fig. 1).

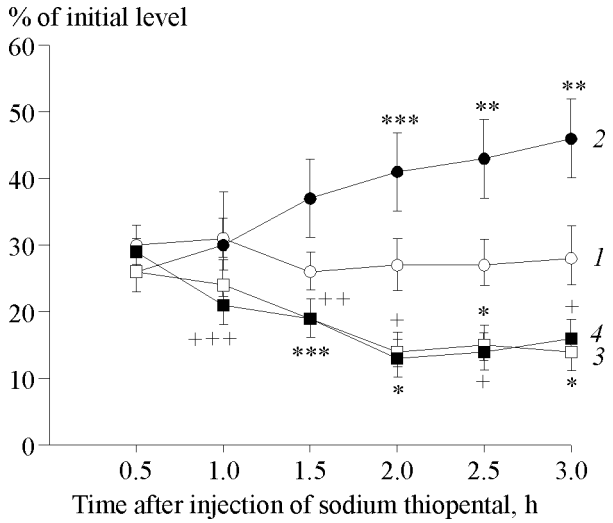

Fig. 1. Oxygen consumption by rats in coma induced by sodium thiopental after injection of sodium succinate and/or malonate. *1*) control (sodium chlorine, 20 mmol/kg); *2*) sodium succinate (5 mmol/kg)+sodium chloride (10 mmol/kg); *3*) sodium malonate (5 mmol/kg)+sodium chloride (10 mmol/kg); *4*) sodium succinate (5 mmol/kg)+sodium malonate (5 mmol/kg). **p*<0.01, ***p*<0.02, ****p*<0.05 compared to the control; ^{+}p<0.001, ^{++}p<0.01, $^{+++}$ *p*<0.02 compared to group 2.

Sodium malonate suppressed gas exchange and abolished the effect of succinate; the intensity of oxygen consumption after 2.5 h was 2-fold below the control. In animals injected with sodium malonate, the decrease in oxygen consumption to 10-17% of the initial level was not absolutely mortal.

Sodium succinate in a dose of 5 mmol/kg (but not 1 mmol/kg) intensified gas exchange in rats with thiopental-induced coma. However, increasing the dose to 10 mmol/kg did not potentiate this effect (Table 1).

Injection of sodium succinate in the same doses to intact animals did not appreciably modify gas exchange. The effect of malonate (5 mmol/kg) on gas exchange of intact rats was negligible: oxygen con-

TABLE 1. Dose-Dependent Effect of Sodium Succinate on Gas Exchange (% of Initial Oxygen Consumption) in Rats with Sodium Thiopental-Induced Coma (*M*±*m*, *n*=8)

Succinate dose		Time after drug injection, h					
		thiopental, 0.5	succinate				
			0.5	1.0	1.5	2.0	2.5
1 mmol/kg	control	31±3	36±4	32±5	35±3	38±4	35±4
	experiment	42±4	45±8	46±12	41±5	45±8	47±11
5 mmol/kg	control	35±2	40±3	36±2	34±2	31±1	35±2
	experiment	34±1	48±4	44±2**	48±2*	50±3*	60±6*
10 mmol/kg	control	38±6	35±8	37±5	36±4	34±5	33±4
	experiment	31±3	37±4	43±3	39±5	53±6***	49±6

Note. *<*p*0.001, ***p*<0.02, ****p*<0.05 compared to the control.

TABLE 2. Survival of Rats (%) after Acute Sodium Thiopental Poisoning and Treatment with Sodium Succinate and/or Sodium Malonate in a Dose of 5 mmol/kg

Group of rats	Thiopental doses, mg/kg					Thiopental LD_{50}±S, mg/kg	Factor of dose alteration
	65	70	75	80	85		
Chloride (control)	—	50 (6)	63 (8)	20(10)	0 (5)	73.90±1.62	—
Succinate	—	67 (6)	88 (8)	29 (7)	25 (4)	77.77±1.57	1.05
Malonate	17 (6)	67 (6)	38 (8)	—	—	70.87±1.10	0.96
Succinate+malonate	83 (6)	67 (6)	38 (8)	—	—	72.70±2.53	0.98

Note. The number of animals in each group is shown in parentheses.

sumption decreased by no more than 30% of the control level and returned to normal within 1 hour.

The survival of rats injected with sodium succinate during barbiturate coma was higher than in the corresponding control groups (for each dose of thiopental), which according to the nonparametric sign test attested to a stable trend ($p<0.06$) to improving rat resistance to thiopental under the effect of succinate. The resistance to thiopental in rats treated with sodium succinate tended to decrease under the effect of sodium malonate (Table 2).

Thus, single injection of sodium succinate to animals with barbiturate coma promoted normalization of oxygen consumption. This effect was not related to the stimulatory effect of the drug on the central nervous system, because it was not observed in intact animals. The obligatory condition for the manifestation of this effect was involvement of exogenous succinate in cell respiration, which is confirmed by the fact that specific SDH inhibitor sodium malonate abolished this effect. Sodium succinate intensified gas exchange in thiopental coma only when injected in the substrate doses capable of creating succinate concentration in tissues surpassing SDH K_m to attaining the maximum rate of succinate oxidation (≥5 mmol/kg).

Despite appreciable improvement of rat survival after administration of succinate, the factor of dose alteration for this drug was not high (1.05). This can be due to the fact that thiopental half-life in the body (10-12 h in humans [4]) is by one order of magnitude higher than the estimated period of oxidation of succinate injected in a dose of 5 mmol/kg. This suggests that repeated injections of succinate in cases with barbiturate poisoning can improve treatment efficiency.

Our findings suggest that death in barbiturate coma is caused by not only impairment of oxygen mass transfer to brain cells, but also inhibition of NAD^+ dependent tissue respiration. Substrate maintenance of tissue respiration in barbiturate coma suggests, along with injection of sodium succinate, oxygen therapy and/or artificial ventilation.

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