EFFECT OF PHENOBARBITAL AND BENZONAL ON OXIDATION OF SUCCINATE AND α-KETOGLUTARATE BY RAT BRAIN MITOCHONDRIA

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Barbiturates, used in anticonvulsant therapy, and in particular, phenobarbital, have a rotenone-like action on the respiratory chain of mitochondria (MCh) and inhibit NADH-dehydrogenase, which leads to an increase in the degree of reduction of pyridine nucleotides [6, 10]. Meanwhile an increase in the concentrations of ATP and acid phosphatase (AP) [16] and a decrease in the concentration of all metabolites of the tricarboxylic acid cycle (TAC) [12] are observed in brain tissue. Rising levels of NADH and ATP activate the oxidation of succinate, one of the most important energy-producing systems of the brain. However, data in the literature on the effect of barbiturates on succinate dehydrogenase (SDH) activity and on utilization of succinic acid (SA) in the brain MCh are contradictory [5, 8, 10, 15].

In view of the positive correlation between the degree of regulatory inhibition of SDH, developing against the background of activation of the enzyme, and the seizure threshold [7], it was decided to study the effect of phenobarbital and its structural analog, the original Soviet anticonvulsant benzonal, which has no hypnotic action, on oxidation of succinate by isolated rat brain MCh in experiments *in vivo* and *in vitro*. Changes in NAD-dependent respiration by these preparations were estimated by the use of α-ketoglutarate (α-KG) as the substrate.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 200-220 g. The drugs were used in the form of a suspension in 1% starch mucilage and injected intraperitoneally into the animals in a dose of ED_{50} in the metrazol convulsion inhibition test: phenobarbital 12.5 mg/kg and benzonal 10 mg/kg. The animals were decapitated 40 min after injection of the drug. Brain MCh were isolated by differential centrifugation, using methods preserving their native properties [2, 11]. The isolation medium was: 0.3 M mannitol, 0.01 M Tris-buffer, 2×10^{-4} M EDTA (pH 7.4). Respiration of MCh was studied polarographically in different metabolic states according to Chance [9]. The incubation medium was: 0.17 M mannitol, 4×10^{-2} M KCl, 5×10^{-3} M KH_2PO_4, 0.01 M Tris-buffer, 2×10^{-4} M EDTA (pH 7.4). The concentration of SA and α-KG in the polarographic cells was 6×10^{-3} M, and ADP was added in a concentration up to $\times 10^{-4}$ M. To estimate "pure" NAD-dependent respiration of MCh, together with α-KG the competitive SDH inhibitor malonate was added to the incubation medium in a concentration of 3×10^{-3} M, for up to 70% of the oxygen utilized on addition of α-KG may be used up by oxidation of succinic acid, produced from α-KG during the action of α-KG-dehydrogenase, the GABA-shunt, and the Krebs–Cohen dismutation reaction, in MCh [3].

In the experiments in vitro the drugs were added to the incubation medium in the following concentrations: phenobarbital 4×10^{-9}-4×10^{-4} M, benzonal 2×10^{-9}-2×10^{-4} M.

The results were analyzed by Wilcoxon's test for comparison of pairs [1].

EXPERIMENTAL RESULTS

When the metabolic effects of the anticonvulsants were analysed it was assumed that the most important parameters of the state of the SA oxidation system are not only the rate of utilization of the substrate by MCh in Chance's state 3, but also the increase in this rate after addition of SDH activators to the medium, whereby inhibition of the enzyme can be revealed. This inhibition accompanied hyperactivation of the SA oxidation system and develops on account of the action of endogenous regulators (oxaloacetic acid, serotonin) on SDH [9, 11].

Injection of phenobarbital into the animal increased all rates of succinate oxidation by

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TABLE 1. Effect of Phenobarbital and Benzonal on Respiration of Rat Brain MCh (M ± m; 4-6 experiments)

Experimental conditions	Parameter	Phenobarbital — Substrate: SA	Phenobarbital — Substrate: SA + β-HBA	Phenobarbital — Substrate: α-KG	Phenobarbital — Substrate: α-KG + malonate	Benzonal — Substrate: SA	Benzonal — Substrate: SA + β-HBA	Benzonal — Substrate: α-KG	Benzonal — Substrate: α-KG + malonate
Control	V_{4h}	41,9±1,6	52,6±0,4	20,2±0,4	15,2±1,1	42,3±1,9	52,8±0,7	26,4±0,9	18,8±0,6
	V_3	88,0±3,8	105,1±0,6	54,1±2,3	35,7±0,8	101,5±6,2	105,6±2,6	62,7±5,2	44,7±3,1
	V_{4o}	37,3±2,1	48,5±4,0	18,2±1,2	13,8±0,7	36,0±2,4	49,1±1,4	21,5±1,1	18,3±0,9
	АДФ/О	2,1±0,1	1,9±0,2	3,1±0,2	3,5±0,3	1,8±0,1	1,9±0,1	3,8±0,1	3,4±0,3
Drug	V_{4h}	48,7±2,9*	58,9±0,6*	23,6±2,1	16,8±1,1	43,4±0,9	58,5±0,6*	26,4±1,2	19,3±1,1
	V_3	104,3±5,0*	126,5±1,1*	62,4±2,9*	44,2±2,2*	87,2±5,1*	118,8±5,4*	63,0±4,1	46,0±2,7
	V_{4o}	41,3±2,2*	53,3±2,1	20,7±1,7	15,2±1,8	33,9±1,9	55,6±1,1*	21,3±2,0	18,2±1,3
		2,1±0,1	2,1±0,1	3,0±0,1	3,6±0,2	1,9±0,1	1,8±0,1	3,6±0,2	3,8±0,2
$100\cdot\frac{V_3\text{ (drug)} - V_3\text{ (control)}}{V_3\text{ (control)}}$		+19.9	−20,5	+15,4	+23,6	−14,1	+12,6	—	—

Legend. Respiration rates expressed in nanogram-atoms O_2/min/mg protein. *$P_i < 0.05$ compared with control.

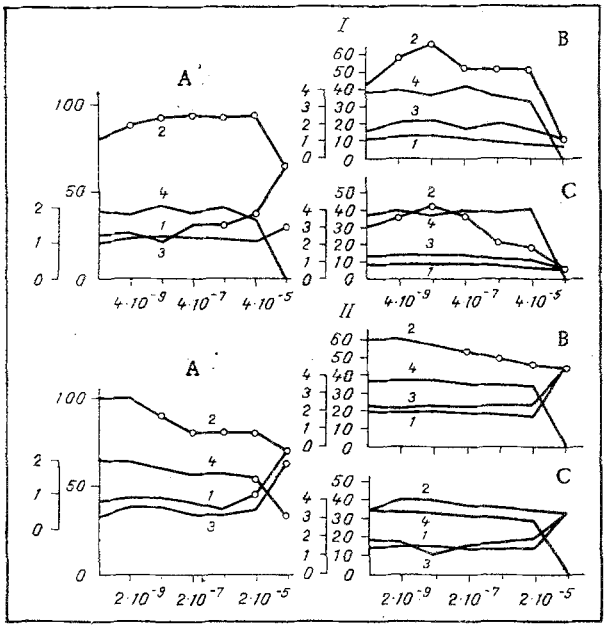

Fig. 1. Effect of phenobarbital (I) and benzonal (II) on respiration of rat brain MCh. Abscissa, concentration of drug (in M); ordinate, respiration rate of MCh (in ng-atoms O_2/min/mg protein). Ordinate: on left — ADP/O ratio. Oxidation substrates: A) SA; B) α-KG; C) α-KG + malonate. Circles indicate $P < 0.05$ compared with initial values. 1) Respiration rate before addition of ADP (V_{4p}); 2) respiration rate during utilization of ADP (V_3); 3) respiration rate after utilization of ADP (V_{4o}); 4) ADP/O ratio.

brain MCh (Table 1). The combined use of SA and the SDH activator β-hydroxybutyric acid (β-HBA) revealed an even greater difference in the respiration rates of MCh of the control animals and of rats receiving the drug. In both cases coupling of oxidative phosphorylation, characterized by the ADP/O ratio, was unchanged by the action of the drug. The use of α-KG and α-KG + malonate as substrate also was accompanied by an increase in the respiration rates of MCh under the influence of phenobarbital with no change in the degree of coupling of oxidative phosphorylation.

In the experiments in vitro phenobarbital also increased the rate of phosphorylating respiration of MCh, utilizing SA, over the concentration range 4×10^{-9}–4×10^{-5} M (Fig. 1). The change to a higher concentration (4×10^{-4} M) caused a sharp decrease in V_3 but a simultaneous increase in V_{4o}, and a fall of the ADP/O ratio to zero, evidence of uncoupling of oxidative phosphorylation by the drug. During oxidation of α-KG the rate of phosphorylating respiration of MCh increased under the influence of phenobarbital, just as in the case of oxidation of SA, within the range of concentrations of the drug from 4×10^{-9} to 4×10^{-8} M, after which it fell, though it remained higher than the control level until the concentration of 4×10^{-5} M was used. The change to a phenobarbital concentration of 4×10^{-4} M was characterized by a a fall of V_3 to the level of the resting respiration rate and by a simultaneous fall of the ADP/O ratio to zero. The use of malonate together with α-KG reduced the ability of phenobarbital to activate phosphorylating respiration of MCh and revealed the rotenone-like action (RLA) of the drug, starting with a concentration of 4×10^{-6} M. Evidently the increase in the rate of oxidation of α-KG by phenobarbital, abolished by the SDH inhibitor malonate, also serves as the initial manifestation of RLA and reflects the more intensive utilization of SA, produced from α-KG, by MCh. This hypothesis was confirmed in analogous experiments in which rotenone was added to the incubation medium. In a dose of 0.66 μg/ml, insufficient to depress NAD-dependent respiration of MCh, rotenone increased the rate of their oxygen consumption in Chance's state 3 during oxidation of SA by 12%. Evidently activation of oxidation of succinate by MCh is a more sensitive indicator of the RLA of phenobarbital than its inhibition of NAD-dependent respiration.

These data explain the increase in the rates of oxidation of SA and α-KG, observed in the experiments *in vivo*, without any decrease in the ADP/O ratio on administration of an anticonvulsant dose of phenobarbital to the rat by its RLA. The incomplete abolition of increased oxidation of α-KG by malonate probably reflected the insufficient effectiveness of the inhibitor during stimulation of SDH. The inhibition of SDH, developing under the influence of phenobarbital simultaneously with an increase in the rate of SA oxidation, which was abolished by β-HBA, was evidently due not only to oxaloacetic acid, but also to serotonin accumulating in the brain during phenobarbital therapy [8].

Injection of benzonal into the rats was accompanied, unlike the action of phenobarbital, by a decrease in the rate of succinate oxidation by brain MCh in Chance's state 3 (Table 1). Addition of β-HBA together with SA to the incubation medium abolished the inhibitory action of the drug. The respiration rate of MCh when oxidating α-KG and α-KG + malonate was unchanged. Irrespective of the type of substrate used, benzonal, like phenobarbital, did not change the ADP/O ratio.

Benzonal *in vitro* inhibited succinate oxidation over nearly the whole range of concentrations (2×10^{-8}–2×10^{-4} M). With an increase in the concentration of the drug in the incubation mixture to 2×10^{-5}–2×10^{-4} M uncoupling of oxidative phosphorylation took place: a decrease in V_3 and ADP/O, an increase in V_4 (Fig. 1). During oxidation of α-KG, benzonal inhibited activated respiration of MCh moderately (by 25% only in a concentration of 2×10^{-4} M). Just as during oxidation of SA, the drug in a concentration of 2×10^{-4} M uncoupled oxidative phosphorylation (a fall of ADP/O, a rise of V_4). Under conditions of oxidation by MCh of a substrate of α-KG + malonate, benzonal did not exhibit the RLA characteristic of phenobarbital: the drug caused virtually no change in the respiration rate of MCh in Chance's state 3; benzonal exhibited its uncoupling action in a concentration of 2×10^{-4} M. Consequently, inhibition of oxidation of α-KG in MCh by benzonal was due to fall in the rate of succinate utilization.

Phenobarbital and benzonal, in anticonvulsant doses, thus inhibit brain energy production; however, the action of phenobarbital is linked with inhibition of NAD-dependent respiration, whereas the action of benzonal is linked with inhibition of succinate oxidation.

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