OXIDATION OF SUCCINATE BY RAT BRAIN MITOCHONDRIA DURING EXPERIMENTAL SEIZURES

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Seizures are accompanied by considerable expenditure of high-energy compounds by the brain followed by their compensation [14]. The kinetic advantage of succinate from the point of view of energy generation for functional hyperactivity [2] suggests that it may participate in the maintenance of paraoxysmal brain discharges in epilepsy, but no special investigation of this matter has been made.

The aim of this investigation was to study the effect of a metrazol seizure of succinate oxidation by isolated rat brain mitochondria (MHC) during different seasons of the year.

EXPERIMENTAL METHOD

Male noninbred albino rats and Wistar rats weighing 200-220 g were used. Seizures were induced by intraperitoneal injection of metrazol in doses of 100 and 130 mg/kg. The total fraction of brain MCH was isolated at 10,000g by methods enabling the native state of the MCH to be preserved in the form of associates [3, 11]. In particular, leaving out washing and using mild suspension of MCH without additional dilution with medium enabled a protein concentration of 45-50 mg/ml to be achieved in the suspension and a final concentration in the cell of 2.5-3.0 mg/kg. The isolation medium consisted of 0.3 M mannitol, 0.01 M Tris-HCl, 2×10^{-4} M EDTA (pH 7.4). The respiration rate of MCH in different metabolic states was measured polarographically by the method in [8]: in the active metabolic state during phosphorylation of ADP, in the resting state before and after phosphorylation. The incubation medium was: 0.17 M mannitol, 4×10^{-2} M KCl, 5×10^{-3} M KH_2PO_4, 0.01 M Tris-HCl, 2×10^{-4} M EDTA (pH 7.4). The oxidation substrate was succinic acid (SA) in a concentration of 6 nM. ADP was added to the cell to a concentration of 0.2 mM. Reserves of endogenous SA (ESA) were estimated as the malonate-sensitive respiration fraction during utilization of β-hydroxybutyric acid (β-HBA) [4]. Glutamate, isocitrate, α-glycerophosphate (α-GP), and β-HBA, in concentrations of 3 mM if added separately or 1 mM if added together, were used as succinate dehydrogenase (SDH) activators. Addition of the activators revealed latent inhibition of SDH, which is observed in experimental stress and in some pathological states [5, 9, 12] and is aimed at reducing mitochondrial respiration, when at a higher level than in intact animals (limitation of hyperactivity). In mild forms of stress inhibition of SDH is abolished by "mild" activators glutamate and isocitrate, probably due to removal of oxaloacetic acid (OAA) from SDH. In severe stress inhibition of SDH is not abolished by glutamate and isocitrate (they may even inhibit phosphorylating respiration a little), but addition of β-HBA or α-GP stimulates the enzyme strongly.

The results were analyzed by Wilcoxon's test for matched pairs [1]. Typical results of individual experiments are given in Figs. 1 and 2.

EXPERIMENTAL RESULTS

The sensitivity of rats to metrazol was found to vary in different seasons. In summer injection of 100 mg/kg of the drug caused the appearance of clonic spasms after 1-2 min, changing rapidly into tonic convulsions (generalization). In winter, the dose of metrazol to give the same effect had to be increased to 130 mg/kg. The convulsions developed more slowly (after 3-8 min).

Comparison of the states of MCH from intact animals also revealed significant seasonal differences, evidently causing differences in the animals' reactivity to seizures (Fig. 1).

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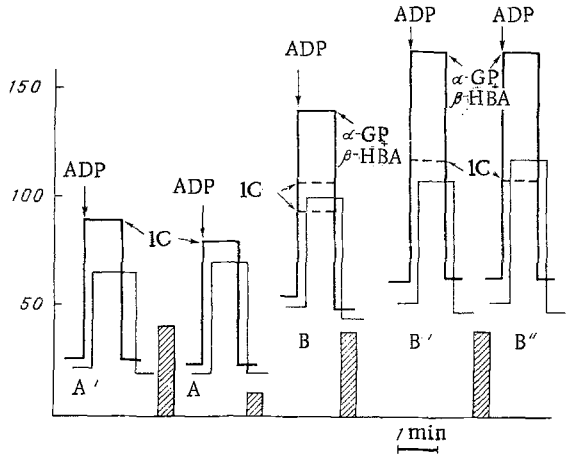

Fig. 1. Oxidation of added SA and reserve ESA in brain MCH of intact rats after seizure in summer and winter. Ordinate, rate of oxidation (in ng-atoms O_2/min/mg protein). Thin line represents succinate, bold line succinate + SDH activators. Shaded columns—ESA reserves. A A') Summer, B, B', B") winter; A, B) MCH of intact animals, A', B',B") MCH of animals after seizure. IC) Isocitrate.

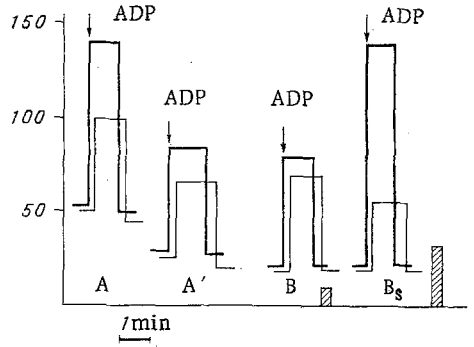

Fig. 2. Oxidation of added SA and reserve ESA in brain MCH of rats after convulsions prolonged to 40 min in winter (A) and injection of serotonin in summer (B). This line indicates succinate, bold line succinate + β-HBA and α-GP; Shaded columns — ESA reserves. A,B) MCH of intact animals, A') MCH of rat after seizure, B_S) MCH of rat after injection of 17.6 mg/kg of serotonin. Remainder of legend as to Fig. 1.

These differences were mainly manifested as elevation of the resting respiration level in winter, possibly due to increased heat production on account of oxidation of SA in MC [6]. Addition of ADP at different levels of resting respiration led to approximately the same increase in the rate of respiration in the active state, but because of the initial difference in rates the final level in winter was higher than in summer (125 ± 5 and 75 ± 5 ng-atoms O_2/min/mg respectively). The response of MCH to addition of SDH activators also differed significantly. The weaker activator isocitrate gave a small increase in respiration (14%) in summer, whereas the strong activator did not induce a greater increase. In the winter period isocitrate gave activation of phosphorylating respiration about equal to that in summer in about half of the experiments, but in the rest it caused slight inhibition, similar to that

observed during prolonged stress. Just as in severe stress, addition of strong activators (β-HBA and α-GP) in the winter period was accompanied by considerable stimulation of respiration — up to 140 ± 5 ng-atoms O_2/min/mg (Fig. 1A, B), indicating stronger inhibition of SDH in winter. The raised level of ESA in the winter period (Fig. 1) agrees with this conclusion, for when SDH activity is depressed, large stocks of succinate are preserved.

The changes described in respiration of MCH are evidence of activation of succinate oxidation in winter compared with summer. An increase in the intensity of oxidation of SA causes an associated increase in the intensity of inhibition of SDH due to OAA formation. Under these circumstances abolition of inhibition of SDH can not only activate respiration of MCH, as is observed in summer, but can also provoke its inhibition due to supercompensation of excessive OAA production. Such states of stress on the succinate-dependent energy production system are characterized by the apeearance of an activating action of β-HBA and α-GP, whose effect is probably connected with abolition of deeper inhibition of SDH induced by serotonin [5, 12]. Differences in metabolism of MCH in winter and summer evidently determine the differences we found in responses of MCH to injection of metrazol into rats at these periods. In summer (Fig. 1A) the seizure was characterized by some depression of active respiration and of the rate of phosphorylation, evidence of the appearance of mild inhibition of SDH by OAA: the "mild" SDH activator isocitrate increased the rate of respiration of MCH and revealed greater inhibition of SDH than in the control animals, at this time of year. The ESA reserves after seizures in summer reached the level of the "winter" control.

Metrazol convulsions in winter considerably exceeded all rates of SA oxidation (Fig. 1B', B"). In this season two types of metrazol-induced activation of respiration rates were clearly noted: moderate and maximal. In the moderate type of (up to 110 ± 5 ng-atoms O_2/min/mg) the "mild" SDH activators increased the rate of active succinate oxidation weakly, but in the maximal type (up to 120 ± 5 ng-atoms O_2/min/mg) they reduced it, but at the same time the addition of more powerful SDH activators in both cases increased the rate of respiration to 180 ± 10 ng-atoms O_2/min/mg, revealing reserves of latent enzyme activity restrained by massive inhibition of SDH. Consequently, in the winter period convulsions cause deeper inhibition of SDH than in summer, a conclusion confirmed by the high ESA reserves, despite great expenditure of the substrate during seizures (Fig. 1).

In summer and, in particular, in winter a seizure thus activates SA oxidation while at the same time increasing the reserves of factors restraining this activity, thereby inhibiting SDH. The fact that in winter, when the animals are less sensitive to metrazol, increased inhibition of SDH took place may indicate a possible anticonvulsant action of factors limiting the hyperactive oxidation of succinate. This hypothesis was tested in experiments in which 3-5 subthreshold doses of metrazol, prolonging clonic convulsions, were injected into rats in the course of 40 min. Under these circumstances elevation of the threshold to the action of each successive dose of the convulsant was observed, with very strong inhibition of respiration of MCH, which was not restored to the control level even when β-HBA and α-GP were used (Fig. 2A').

Since the serotonin level is raised in winter [10] and since this mediator produces the deepest inhibition of SDH, limits seizure activity, and potentiates the action of anticonvulsants [7], the suggestion arose that not only OAA, but also serotonin, may be involved in the mechanism of termination of seizure activity through inhibition of SDH. In fact, injection of a protective dose of serotonin (17.6 mg/kg) into rats in summer against the background of unactivated oxidation completely protected the animals against the action of metrazol even in a dose of 130 mg/kg, and induced (Fig. 2B) a massive increase in inhibition of SDH in brain MCH with an increase in the ESA reserves. These results are in good agreement with the known fact that brain serotonin level rises during convulsions [7] and during treatment with anticonvulsants [13].

Thus during metrazol-induced seizures oxidation of succinate is activated, with a simultaneous increase in the reserves of SDH restraining factors. Evidently the developing inhibition of SDH is compensatory in character, preventing injury to MCH, which may arise during hyperactive oxidation of the substrate, and limiting the supply of energy to epileptized neurons, and thus leading to termination of seizure activity.

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ACTIVITY OF THE MONOOXYGENASE SYSTEM AND RATE OF LIPID PEROXIDATION IN RAT LIVER MICROSOMES DURING REINDUCTION BY POLYCHLORINATED BIPHENYLS

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One of the mechanisms of protection of the body against the action of many foreign substances is induction of the microsomal monooxygenases of the liver; this process develops rapidly and a high level of monooxygenases is maintained for a long time, depending on the chemical nature of the inducer and the conditions of its introduction [1, 7, 9]. The possibility of periodic entry of foreign substances into the body accounts for the great interest in the study of their reinducing effects, which have so far received little investigation, especially after complete partial restoration of the original level of enzyme activity [4, 15]. In previous investigations the writers discovered some features of induction of the monooxygenase system (MOS) of the endoplasmic reticulum of the liver during acute exposure to polychlorinated biphenyls (PCB), which are widespread pollutants of the biosphere [5, 6].

The aim of this investigation was to study the functional state of MOS and also the velocity of lipid peroxidation (LPO) in rat liver microsomes during repeated administration of PCB at different times after primary induction.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing initially 200-220 g. The animals were kept on the normal animal house diet and were given food and water *ad lib*. Soval (a mixture of PCB of Soviet manufacture) was dissolved in corn oil and administered to the rats by the intragastric route (2 ml/kg body weight) in a dose of 500 mg/kg. In experimental

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