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EXPERIMENTAL MEDICINE

Properties of Brain Mitochondria in Conditions of Ischemia and Nembutal Anaesthesia in Guinea-Pigs

by

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Summary. The investigations concerned mitochondrial ability of oxidative phosphorylation, activity of mitochondrial ATPases, free fatty acids (FFA) content, and the process of swelling of the mitochondria. Barbiturate anaesthesia was shown to attenuate the disturbance of mitochondrial metabolic function due to anoxia. The *in vitro* studies demonstrate that the action of acetylcholine is similar to that of narcosis. Acetylcholine added to the medium decreases the amount of released fatty acids and improves RCI coefficient values. It is suggested that acetylcholine may be one of the factors responsible for the observed "protective" effect of barbiturate anaesthesia.

The disturbances in oxidative phosphorylation (see [12]) concomitant to anoxia and manifesting themselves in changing ADP/O index and mitochondrial ATPases activity were due to an increase of FFA concentration. At the same time it was proved that nembutal narcosis enhances the resistance of brain mitochondria to oxygen deficiency. Since the available literature provides little information on this phenomenon, the present work was intended to investigate the effect of anoxia on mitochondrial metabolism in conditions of barbiturate narcosis (defined as high-energetic state of cells [3]). Particular attention was paid to energetic properties of the brain mitochondria, mitochondrial ATPase activity and FFA content in conditions of postdecapitation ischemia applied to anaesthetized and non-anaesthetized guinea-pigs. Moreover, susceptibility of the mitochondria from the two experimental groups to swelling and disintegrating effect of exogenic fatty acids was studied, as was the possible effect of the acetylcholine accumulated in the brain during narcosis.

Methods

Experiments were made on 180—250-g guinea-pigs. The animals were anaesthetized with nembutal introduced intraperitoneally in doses 35 mg/kg body weight. After 30 min the animals were decapitated and their heads kept at 37°C for 0.5—3 min, then (up to 15 sec) the brain was removed from cranium and placed into a cold isolation medium containing 0.3 M mannitol and 0.1 mM EDTA of pH 7.4.

Mitochondrial fraction was isolated from the cortex of cerebrum by the method of Ozawa *et al.* [15]. Oxidative phosphorylation of the mitochondria was investigated by the polarographic method

et Chance [5] with the use of the Clark oxygen electrode. 5 mM potassium glutamate was used as a respiratory substrate. The results are expressed as RCI and ADP/0 coefficients calculated by the graphic method. Activity of mitochondrial ATPases endogenous, Mg- and DNP-dependent was expressed in terms of P_i increment after the 15-min incubation of the mitochondria in a medium containing 2 mM ATP. Phosphorus was determined by the Fiske-Subbarow method and protein by the Lowry method. Fatty acids were extracted and separated by thin-layer chromatography after Bazan [4] and determined colorimetrically by the Duncomb method in the modification of Itaya and Ui [10]. Swelling of the mitochondria was measured spectrophotometrically at 520 nm.

Results

Figs. 1 and 2 present values of ADP/0 and RCI coefficients of the mitochondrial fractions from the cerebral cortex of the control and experimental guinea-pigs depending on the duration of ischemia. It is seen that the mitochondria from the control animals became more uncoupled than those from the animals which had previously been treated with nembutal. After 3-min ischemia ADP/0 and RCI values decreased by 30 and 43%, respectively, whereas the mitochondria from the anaesthetized animals lost only 18 and 14% of the initial values of ADP/0 and RCI, respectively. The observed differences occurred in each series of the experiments and were statistically significant.

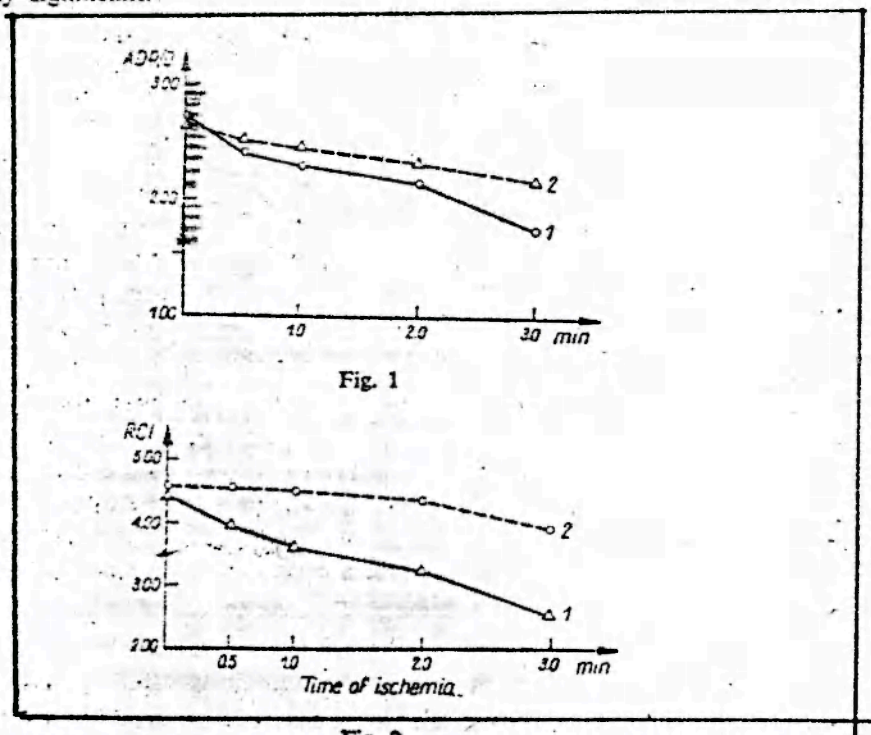

Fig. 1

Fig. 2

Effect of ischemia on coefficient ADP/0 (Fig. 1) and RCI (Fig. 2) of mitochondrial fraction control (*1*) and anaesthetized (*2*) animals

Oxidative phosphorylation in mitochondrial fraction was measured in medium containing — mannitol 0.3 M; KCl 10 mM; Tris-Cl 10 mM pH 7.4; K-phosphate buffer 5 mM, pH 7.4; EDTA 0.2 mM; glutamate potassium 5 mM; ADP 333 μM and 1 mg mitochondrial protein in total volume of 1.5 ml. Temperature 25°

Fig. 3 presents activities of endogenous, Mg- and DNP-dependent mitochondrial ATPases in correlation with ischemia. In this case a distinct effect of barbiturate narcosis on the mitochondrial enzymes activity is seen. In the brain mitochondria of the anaesthetized animals endogenous and Mg-dependent ATPases are less stimulated and ATPase DNP-dependent less inhibited than those of control animals. The initially slight difference in the activities of Mg- and DNP-dependent ATPases increases in the course of ischemia. The activity was markedly less differential in experimental than in control animals. Moreover, barbiturate narcosis attenuated ischemia-

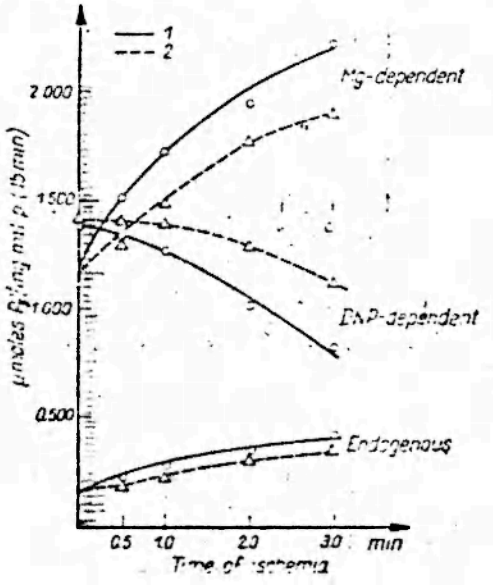

Fig. 3. Effect of ischemia on ATPase activity of mitochondrial fraction from brain control (*1*) and anaesthetized (*2*) guinea-pigs

Incubation medium: KCl 0.075 M; mannitol 0.1 M; Tris 0.05 M, Tris-Cl 0.05, pH 7.4; EDTA 0.001 M; ATP 0.002 M and 0.5 mg mitochondrial protein in total volume 1.5 ml

2,4-dinitrophenol and $MgCl_2$ were added, as shown, to the medium in concentration of 0.1 mM and 3 mM, respectively. Temperature 25°, incubation time 15 min

-induced FFA release (Fig. 4). After 3 min of ischemia FFA content in mitochondrial fraction of brain of the anaesthetized animals was by about 20% smaller than in in the controls which proves the mitochondria-"protecting action" of barbiturate narcosis. Investigation of the effect of exogenic oleate on the swelling of mitochondria (Figs. 5 and 6 in [8]) also confirm the action of narcosis caused by oxygen deficiency. The rates of swelling and shrinking of mitochondria after the ATP addition are smaller in experimental than in control animals.

All these results indicate that *in vivo* barbiturate narcosis increases resistance of mitochondria to the damaging effect of exogenic fatty acids, ischemia and the ageing process.

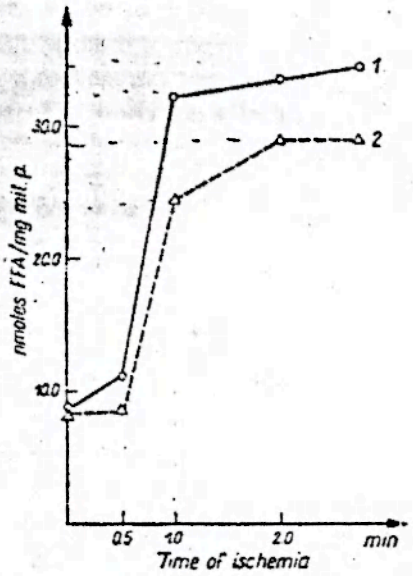

Fig. 4. Effect of ischemia on liberation FFA in mitochondrial fraction from brain control (*1*) and anaesthetized (*2*) guinea-pigs

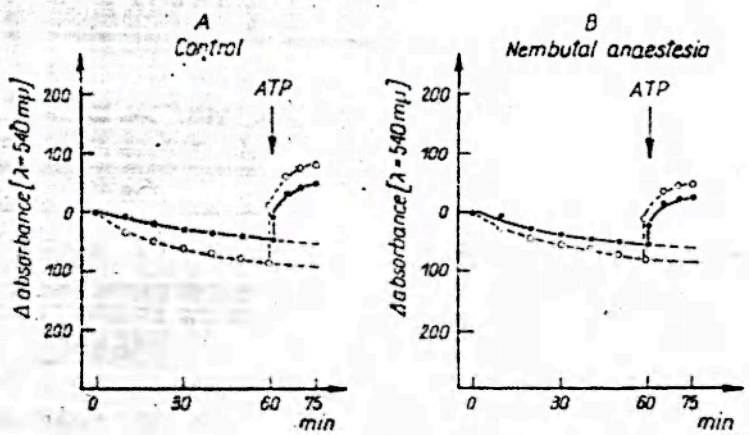

Fig. 5. Effect of oleate on swelling of mitochondrial fraction from guinea-pig brain

— — — oleate, — + oleate

Incubation medium for mitochondrial swelling contained: KCl 0.15 M; Tris-Cl 20 mM, pH 7.4, and 0.5 mg mitochondrial protein in total volume of 3.0 ml. Incubation was started by addition of the mitochondrial suspension and was carried out at 25° for 75 min in thermostatized glass cells. Absorbancy was measured at 520 mμ with SP-500 Unicam spectrophotometer. Sodium oleate if added was used at 10 μM concentration. For contraction 5 mM ATP 3 mM $MgCl_2$ was added to each sample

The distinct increase in acetylcholine in conditions of barbiturate narcosis observed by Księżak *et al.* [11] is specific of central nervous system. The results presented below suggest its possible role in the increased resistance of the brain of

anaesthetized animals to FFA-induced breakdown. When added to mitochondria 0.5 mM acetylcholine in the presence of 10^{-4} M eserine, as evidenced by the quantity of free fatty acids released in the ageing, stabilizes the mitochondrial membranes (Table I). After incubation with acetylcholine FFA content is lower than in the control.

TABLE 1

Effect of 0.5 mM acetylcholine on liberation of FFA during ageing of mitochondrial fraction from guinea pig brain

Exp.	Control	Incubation (60 min 37°C)	
		— Ach	+ Ach
	nmoles FFA/mg mitochondrial protein		
1	25.8	34.1	25.8
2	22.6	27.7	18.1
3	34.4	44.4	29.3

Acetylcholine raises RCI 3.6 to 5.5 (Table II), coupling increases by about 50%, which is mainly due to a decrease in oxygen consumption in the 4th energetic stage of mitochondria. But the observed decrease in ADP/0 from 2.48 to 2.18 was statistically almost significant.

TABLE II

Effect of 0.5 mM acetylcholine on RCI and ADP/O of mitotochondrial fraction from guinea pig brain [5]

ADP/O		RCI	
— Ach	+ Ach	— Ach	+ Ach
2.48 ± 0.15	2.18 ± 0.11	3.62 ± 0.23	5.50 ± 0.37

Discussion

Anaesthesia decreases tissue respiration and by decreasing the oxygen demand of the central nervous system cells makes possible a longer survival of animals in hypoxia [2, 17]. This corroborates the supposition that hypothermy is that factor of narcosis which is responsible for this phenomenon [2, 13]. But narcosis is high-energetic state of the cells which is characterized by a decreased rate of ATP consumption which results in its accumulation in the brain [3, 7]. The observed less-damaging effect of ischemia on the mitochondrial fractions of the anaesthetized animals may be due to the protective effect of ATP facilitating the incorporation of phosphorous and fatty acids to phospholipids of the mitochondrial membranes [18].

The "protective" effect of anaesthesia on the energetic metabolism of the cells may also be caused by its stabilizing action on the mitochondrial membranes as it inhibits the activity of membrane phospholipases [16].

The stabilizing effect of anaesthetics on membrane has also been described by Ohki [14] and Allison [1]. Anaesthetics, when applied in low concentrations *in vivo*, may react with polar groups of phospholipid membranes by inhibiting the binding of Ca^{++} ions on the surface of these membranes. This process not only results in decreased excitability of these membranes, but also causes their stabilization.

In the present work it was shown that barbiturate narcosis "protects" to some extent the brain mitochondria against the damaging effect of oxygen deficiency and ageing in that they considerably decrease amount of the released fatty acids. Moreover, the results suggest the possibility that acetylcholine actively participates in preserving the integrity of the membrane structure, presumably by stimulating phospholipid resynthesis [6, 9, 19]. The experiments made with acetylcholine indicate that the rate of releasing the fatty acids decreases with ageing and that energetic activity of the mitochondria increases.

The results obtained complement the data concerning the observed [8] "protective" effect of anaesthesia on preserving the energetic ability of the mitochondria. It was shown in [8] that the mitochondria of anaesthetized animals preserve a higher degree of coupling after their incubation with oleate and that their ageing is slower than in the controls.

Participation of acetylcholine in an increased phospholipid resynthesis may be connected with utilizing the energetic potential of ATP accumulated during narcosis.

Further investigations on mechanisms of protective action of barbiturate anaesthesia, taking into account the role of acetylcholine or other compounds specific of the central nervous system (biogenic amines), is underway.

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Д. Маевска, А. Громэк, Я. Строшнайдэр, Свойства митохондрий мозга морских свинок в условиях ишемии и нембуталовой анестезии

Содержание. Исследованы способности митохондрий к оксидативной фосфорилации, активности митохондриальных АТФаз, содержание свободных жирных кислот, а также процесс набухания митохондрий. Доказано, что барбитуровый наркоз смягчает вызванные недостатком кислорода расстройства биохимической функции митохондрий. Проведенные *in vitro* исследования с ацетилхолином указывают, что его влияние подобно наркозу. Добавленный ацетилхолин к среде уменьшает количество освобождающихся жирных кислот, а также улучшает коэффициент RCI в митохондриях. Обсуждается возможность участия ацетилхолина как одного из коэффициентов, ответственных за наблюдаемый эффект барбитуровой анестезии.