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EFFECT OF CEREBRAL ISCHEMIA ON CALCIUM TRANSPORT IN ISOLATED BRAIN MITOCHONDRIA

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INTRODUCTION

Isolated brain mitochondria accumulate calcium ions in an energy-linked process /Carafoli and Lehninger, 1971, Łazarewicz et al., 1974/. In the cell, mitochondria are able to accumulate rapidly large amounts of calcium, regulating the concentration of these ions in cytosol on a normally very low level of about 10^{-6} M /Baker, 1972/. Calcium ions act antagonistically to magnesium, and as it results from the works of Bygrave /1967/, Takagaki /1968/, Huttunen /1969/, Kennedy and Weiss /1959/ and Vignais et al., /1971/, at an increased calcium to magnesium concentration ratio it comes to serious disturbances of carbohydrate, protein and phospholipid metabolism. Mitochondrial calcium transport has therefore a regulative meaning for the cell physiology, and alterations of this transport may lead to metabolic perturbations. In our previous studies /Łazarewicz et al., 1972/ we have observed the uncoupling of oxidative phosphorylation in ischemic brain mitochondria, which we relate to the effect of free fatty acids /FFA/, released in the brain in ischemic conditions. As the processes of ion transport in mitochondria are closely related to the mechanism of oxidative phosphorylation, we undertook the study of the effect of ischemia on calcium transporting ability of brain mitochondria.

MATERIALS AND METHODS

Postdecapitative ischemia was realised by incubation of the decapitated animal head at 37°C up to 5 min. Guinea pig brain mitochondria were isolated by the method of Ozawa et al., /1966/. Calcium accumulation linked to succinate oxidation supported by rotenone or ATP+Mg^{2+} /Łazarewicz and Hamberger, 1974/ was estimated by measuring the activity of $^{45}Ca^{2+}$ remaining in the medium after the sedimentation of mitochondria, as described by Carafoli and Lehninger /1967/. Oxygen consumption was measured with Clark electrode, parameters of oxidative phosphorylation were calculated as described previously /Łazarewicz et al., 1972/. Mitochondrial lipids were extracted according to Folch et al., /1957/ and separated by thin-layer chromatography. Phospholipid content was estimated by phosphorus assay. Fatty acids were identified and determined by the use of gas chromatography. Protein was estimated by the method of Lowry et al., /1951/.

RESULTS AND DISCUSSION

The effect of 5 min postdecapitative ischemia on calcium accumulation by mitochondria in vitro is shown in Table 1. In conditions of massive loading of Ca^{2+} /Lehninger et al.,1967/, the mitochondrial capacity for calcium decreased considerably in consequence of ischemia. This was even more marked in the case of limited calcium transport linked to succinate oxidation in the presence of rotenone, where mitochondria accumulated only 40% of the added calcium.The presence of $ATP+Mg^{2+}$ reversed however this effect.

Table 1. EFFECT OF ISCHEMIA ON Ca^{2+} UPTAKE IN ISOLATED BRAIN MITOCHONDRIA

	Control	Ischemia 5'
Massive uptake /nmoles·mg prot.$^{-1}$/	773,6 ± 52,0	499,8 ± 60,0
Limited uptake + Rotenone /%Ca^{2+} accumulated/	97,4 ± 0,6	39,9 ± 3,9
Limited uptake + $ATP+Mg^{2+}$ /%Ca^{2+} accumulated/	98,1 ± 0,3	97,8 ± 0,4

For massive Ca^{2+} uptake mitochondria were incubated in the medium containing:80mM NaCl, 10mM Tris-HCl /pH 7,4/,10mM succinate,10mM $MgCl_2$,4mM Na-phosphate/pH 7,4/,3mM ATP,3mM $CaCl_2$ and 1,3 mg of mitochondrial prot. per ml, for 10 min at 30°C. Incubation for limited uptake was performed in the medium containing 80mM NaCl, 10mM Tris-HCl /pH 7,4/,10mM succinate, 1 mM Na-phosphate /pH 7,4/, 0,1mM $CaCl_2$ and 2,5 uM rotenone or 1mM ATP+10mM $MgCl_2$+oligomycin 1 ug/ml, with 1,3mg of mitochondrial protein per ml at 25°C for 3 min.
Values represent means ±S.E.M. from four experiments.

The development of limited calcium transport and oxidative phosphorylation alterations during ischemia is presented in Fig.1. As it may be seen, calcium accumulation is abruptly decreased within the period between the 1st and the 2nd minute of ischemia. The drop of oxygen consumption during succinate oxidation and the decrease of ADP/O ratio occurs relatively softly, while the decrease of respiratory control index, which is the best measure for the coupling of oxidative phosphorylation, has the dynamics identical to the altered calcium transport curve.
A marked uncoupling is achieved abruptly in the period between the 1st and 2nd minute of ischemia. So the alterations of calcium transport in ischemic mitochondria could be related not to the inhibition of respiration, but to the uncoupling of oxidative phosphorylation. As it was shown in our previous studies /Łazarewicz et al., 1972/ the uncoupling of oxidative phosphorylation in ischemic mitochondria is accompanied by the releasing of FFA and comes from this. Brain ischemia lasting 5 min results in the accumulation in brain mitochondria of about 40 nmoles of FFA per 1 mg of protein. Exogenous unsaturated fatty acids in concentrations similar to those occurring in ischemic mitochondria produce in control mitochondria alterations similar to that observed after ischemia.

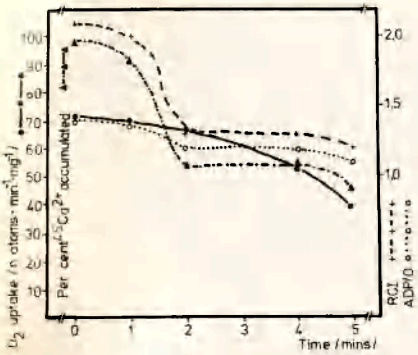

Fig.1. Calcium uptake and oxidative phosphorylation in brain mitochondria after different periods of ischemia.
Brain mitochondria were incubated in the medium for limited Ca^{2+} uptake with succinate+rotenone, as described in Table 1. The medium for oxidative phosphorylation contained: 0,3M mannitol, 10mM KCl, 10mM Tris-Cl, pH 7,4, 5mM K-phosphate, pH 7,4, 0,2mM EDTA, 10mM succinate, 2,5µM rotenone, 333 µM ADP and 1mg of mitochondrial protein in a total volume of 1,5 ml.

As it appears from Table 2 in brain mitochondria during 5 min of ischemia it comes to the decrease of the content of both studied phospholipids: phosphatidylcholine and phosphatidylethanolamine and to a twofold increase of free fatty acids concentration, especially the unsaturated ones: oleic and arachidonic.

Table 2. EFFECT OF ISCHEMIA ON PHOSPHOLIPIDS AND FREE FATTY ACIDS IN BRAIN MITOCHONDRIA

	Phospholipids		Free fatty acids			
	PC	PE	16:0	18:0	18:1	20:4
Change, % of control	80,6	90,4	87,8	185,0	227,2	194,1

Brain mitochondria, control and after 5' ischemia were treated as described under Methods. Phospholipids are: phosphatidylcholine /PC/, phosphatidylethanolamine /PE/, fatty acids: palmitic /16:0/, stearic /18:0/, oleic /18:1/ and arachidonic /20:4/.

As it is presented in Fig.2 oleate inhibits at a different degree the transport linked to succinate oxidation, supported by rotenone or ATP. At oleate concentrations corresponding to the content of FFA in ischemic mitochondria, calcium accumulation in the presence of rotenone is strongly inhibited, but the transport in the presence of ATP is not altered.

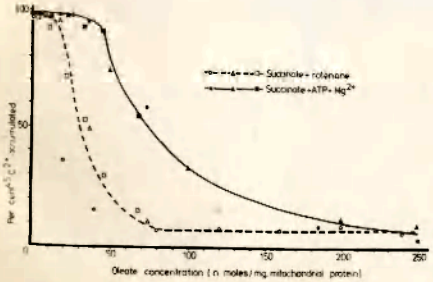

Fig.2. Effect of oleate on Ca^{2+} transport in brain mitochondria.
Brain mitochondria /1,3 mg of protein per ml/ were incubated in the medium for limited Ca^{2+} uptake, as described in Table 1, with various concentrations of Na-oleate. The points are individual results from three experiments.

These results resemble the effect of ischemia on mitochondrial Ca^{2+} uptake /Table 1/, and suggest that free fatty acids released as the result of phospholipid hydrolysis in brain ischemic mitochondria, could be responsible for the inhibition of calcium transport in these mitochondria. In our conviction the observed alterations in calcium transport in ischemic brain mitochondria in vitro correspond to the situation occurring in vivo in the cell. Ischemia would then result in a rise of cytoplasmic calcium ion concentration, leading to perturbations in the metabolism of lipids, carbohydrates and proteins. In this aspect calcium ions would be one of the elements of pathogenesis of postischemic brain abnormalities.

CONCLUSIONS

1. The accumulation of calcium in mitochondria isolated from ischemic brains is importantly decreased. These changes are parallel to the uncoupling of oxidative phosphorylation.
2. Free fatty acids, released in brain mitochondria during ischemia as the result of phospholipid degradation are most probably inhibitors of both oxidative phosphorylation and calcium transport in brain mitochondria.
3. The alterations of calcium transport in brain may lead to a rise of intracellular calcium ion concentration, which could be one of the important elements of postischemic brain cells damage.

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