COMPARATIVE INVESTIGATIONS ON THE OXIDATION OF PYRUVATE IN LIVER AND BRAIN MITOCHONDRIA

H. A. KUNZ*

Department of Physiology, Hallerianum, University of Berne (Switzerland)

In connection with investigations on the role of thiamine in the excitation process of peripheral nerve[1], we have examined in detail the oxidation of pyruvate in liver and brain mitochondria (rat, pigeon), which is dependent on the presence of thiamine pyrophosphate (cocarboxylase). In the comparison of the two organs, we have come across conspicuous differences, which we wish to discuss here with regard to the central position of pyruvate in cell oxidation.

METHOD

The preparation of liver mitochondria was carried out according to the method of LEUTHARDT AND MÜLLER[2], the isolation of brain mitochondria following that of BRODY AND BAIN[3] (second modification). The experimental animals (albino rats of 150–200 g and pigeons of 250–300 g) were fasted for 24 hours before the experiment. Oxygen consumption was measured manometrically in a Warburg apparatus at 38° with air in the gas chamber. The incubating medium was made up as follows: pyruvate 0.01 *M*; magnesium chloride 0.003 *M*; adenosine triphosphate 0.001 *M*; potassium phosphate buffer 0.05 *M* (pH 7.5); potassium chloride to isotonicity; 0.5 ml of a 25% homogenate of liver or brain in isotonic potassium chloride or 0.25 *M* sucrose solution, respectively, corresponding to 125 mg of liver or brain tissue (wet weight) per sample (volume of each sample: 3 ml); 0.5 ml of mitochondria suspension, corresponding to 400 mg of liver or 800 mg of brain tissue. When cerebrum and cerebellum were used without the brain stem[3], only a small increase in oxygen consumption was observed, or none at all, in comparison with a similar wet weight of whole brain. The experiments were therefore carried out with the entire brain. Diphosphopyridine nucleotide was not included in the samples of brain mitochondria[3], because we could not ascribe to it any effect on pyruvate oxidation.

RESULTS

We first measured the effects of the individual components of the incubating medium on pyruvate oxidation (taken as O_2-consumption) in homogenate and mitochondria suspension of the same liver or of the same brain material. Marked differences in the respiration of the homogenate and mitochondria suspension of the two organs could be found. Liver shows a significant rise in the oxygen consumption of the homogenate, when magnesium ions are added (Fig. 1) and, even more noticeably, a complete dependence of the respiration of the mitochondria on magnesium ions. The earliest effect on pyruvate oxidation in rat-liver mitochondria is observed at a concentration of $1 \cdot 10^{-1}$ *M* Mg^{++}, and at $3 \cdot 10^{-3}$ *M* Mg^{++} the maximum enzyme activity is reached. Brain homogenate or mitochondria suspension, on the other hand, show no reduction

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Present address: Dr. H. A. Kunz, Pharmakologisches Institut der Universität Bern, Freiburgstrasse 30.

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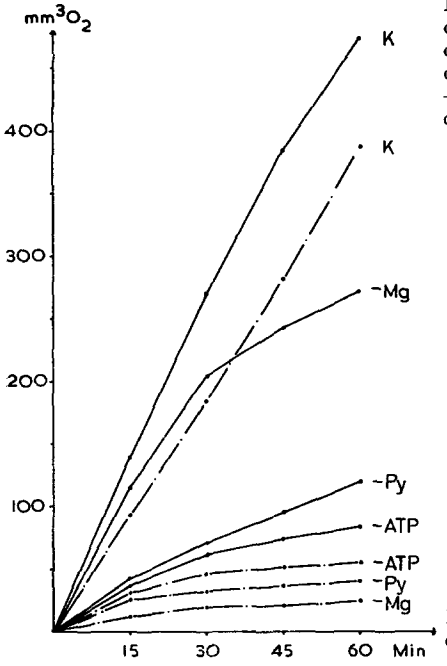

Fig. 1. Pyruvate oxidation in homogenate and mitochondria of the same rat liver. Ordinate: oxygen consumption in mm^3. Abscissa: time in minutes. For composition and total volume of the samples see text. — homogenate; —·— mitochondria; K = complete control sample; –Mg = without magnesium; –ATP = without adenosine triphosphate; –Py = without pyruvate.

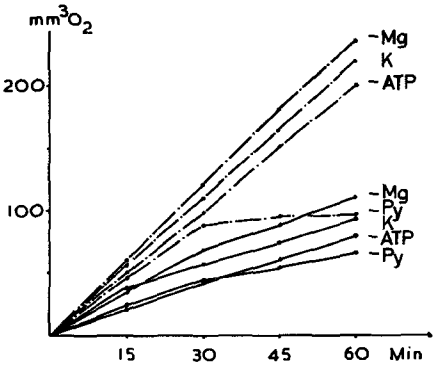

Fig. 2. Pyruvate oxidation in homogenate and mitochondria of the same brain tissue (3 rats). For explanation of symbols see Fig. 1.

in pyruvate oxidation in a magnesium-deficient medium (Fig. 2). Further, the respiration of brain homogenate and mitochondria suspension is very slightly reduced in the absence of adenosine triphosphate and decreases by only 30–50 % when oxidizable substrate (pyruvate) is deficient, while the respiration of liver homogenate and particularly liver-mitochondria suspension displays a very marked dependence on the adenosine triphosphate and pyruvate content of the incubating medium. To determine whether this difference in the pyruvate oxidation of brain and liver mitochondria occurs only in the rat or is generally true of warm-blooded animals, we carried out similar experiments on pigeon liver and brain. In these we observed the same results, except that the effect of addition of magnesium ions on pyruvate oxidation was less in pigeon-liver mitochondria than in the rat.

A second conspicuous difference between brain and liver mitochondria was observed in the ability of certain thiamine compounds, *viz.* thiamine phosphate, thiamine pyrophosphate (cocarboxylase) and acetylthiamine, to affect pyruvate oxidation by liver mitochondria. Even in high concentrations thiamine itself inhibits pyruvate oxidation only slightly or not at all. Cocarboxylase shows a stronger suppression than thiamine phosphate, a surprising finding, which perhaps could be explained on the basis of results which indicate that a thiamine pyrophosphate compound is the coenzyme of oxidative decarboxylation[4].

Further experiments have shown that the depressing effect depends upon the amount of mitochondria in contact with the high cocarboxylase concentration. For example, as shown in Fig. 4, the inhibition is decreased in samples containing the double volume of mitochondria, and in samples with a 4-fold volume of mitochondria suspension there is no inhibitory action at all.

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In analogous experiments with brain mitochondria, on the other hand, we were unable to observe any inhibition of pyruvate oxidation, even with high concentrations of the above-mentioned compounds. Similarly, oxythiamine and neopyrithiamine, *i.e.* thiamine-like compounds, which strongly suppress the oxidation of pyruvate in liver mitochondria[1], show no effect on the respiration of brain mitochondria in comparable concentrations. Similar differences exist between the mitochondria of pigeon liver and brain.

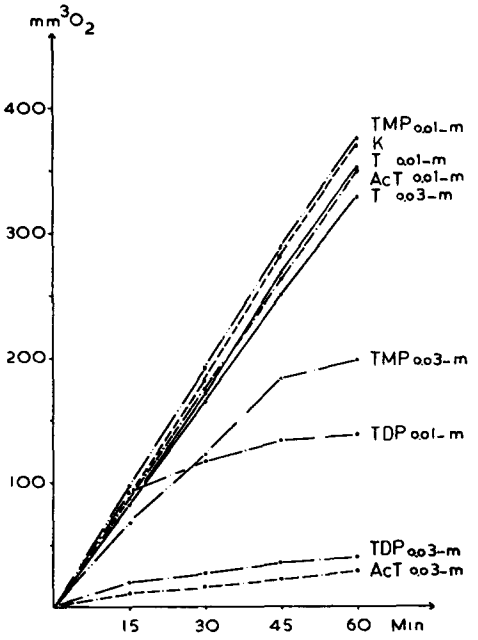

Fig. 3. Effect of thiamine and thiamine compounds on pyruvate oxidation in mitochondria of rat liver. The samples contained 0.5 ml of mitochondria suspension, pyruvate (0.01 *M*), $MgCl_2$ (0.003 *M*), ATP (0.001 *M*), K-phosphate buffer pH 7.5 (0.05 *M*), KCl to isotonicity. Thiamine (T), thiamine phosphate (TMP), thiamine pyrophosphate (TDP) and acetylthiamine (AcT), adjusted to pH 7.5 with KOH, were added immediately before the experiment. Total volume of samples: 3 ml.

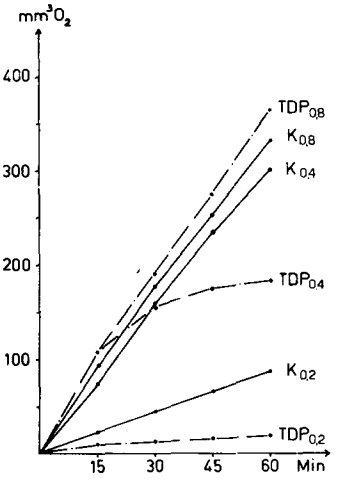

Fig. 4. Inhibition of pyruvate oxidation in rat-liver mitochondria by high concentration of thiamine pyrophosphate. For composition and total volume of the samples see Fig. 3. K = control samples; TDP = samples containing thiamine pyrophosphate (0.01 *M*). The indices mean ml mitochondria suspension per sample. For other explanations see text.

DISCUSSION

With regard to the effect of magnesium ions on pyruvate oxidation in the homogenates and mitochondria suspensions of liver, the preceding results may well indicate that magnesium ions are set free from the coenzyme-magnesium-protein complex—the enzyme system of the oxidative decarboxylation—in the mitochondria, when the cells are broken up during the homogenizing process. An equilibrium may then be set up between the magnesium ions free in solution and those bound on the enzyme. In this case, one must assume that the free magnesium ions are distributed between the soluble fraction of the mitochondria and the solution in which they are bathed. In preparing the mitochondria suspension from the homogenate, the magnesium ions in the surrounding solution are removed; this causes a further, strong dissociation of magnesium ions from the enzyme complex and thereby leads to the inactivation of

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the enzyme. The addition of magnesium ions to the homogenate, and particularly to the mitochondria, shifts the equilibrium back again to the side of the undissociated, active enzyme system, leading to the rise in pyruvate oxidation, which is seen in Fig. 1. A further role of magnesium in pyruvate oxidation is suggested by BALTSCHEFFSKY[5], who recognized obvious morphological changes in rat-liver mitochondria in a magnesium-deficient incubating medium. According to him, one function (among others) of magnesium in oxidative phosphorylation is to maintain the structure of the mitochondria. The enzyme complex of the brain mitochondria, although analogous in its components to that of liver, appears not to dissociate during the preparation procedure for reasons we are not yet able to explain. Our observation agrees well with the results of OCHOA[6], who was able to remove detectable amounts of magnesium ions from pigeon-brain homogenate only after long dialysis (or precipitation with sodium pyrophosphate).

That still further dissimilarities exist between liver and brain mitochondria is evident from the difference in the effect of the thiamine derivatives on the pyruvate oxidation in liver and brain mitochondria. The results of the foregoing experiments are perhaps best explained by assuming that these compounds penetrate the "membrane" of the brain mitochondria in only small concentrations or not at all, on account of a different structure of this "membrane" (higher lipid content?). Up till now no structural difference has been established by electron microscopy between liver and nerve cell mitochondria[7].

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SUMMARY

1. In contrast to mitochondria suspensions of mammal or bird liver (rat, pigeon), similar suspensions of brain do not require the addition of magnesium ions for the oxidative decarboxylation of pyruvate.

2. The pyruvate oxidation in liver mitochondria is inhibited by thiamine compounds (thiamine phosphate, thiamine pyrophosphate, acetylthiamine), while the same compounds, under comparable conditions, do not show any inhibitory action on brain mitochondria.

3. The experimental results are thought to indicate that the pyruvodehydrogenase of the liver, unlike that of the brain, loses magnesium ions by dissociation during the preparation of the mitochondria. The lack of inhibitory effect of thiamine compounds on the pyruvate oxidation of the brain is ascribed to a different structure and permeability of the "membrane" of brain mitochondria.

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