EFFICIENCY OF PARATHYROID HORMONE DEPENDENT MAGNESIUM AND PHOSPHATE TRANSPORT IN MITOCHONDRIA*

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It is now well established that the addition of highly purified preparations of parathyroid hormone to isolated mitochondria results in increased magnesium phosphate uptake by this organelle (Sallis et al., 1963; Sallis et al., 1965; Rasmussen et al., 1964). This transport reaction is closely coupled to and is powered by the oxidative phosphorylation system (Sallis et al., 1963; Sallis et al., 1965). The approximate site of the energy tap from the phsophorylation chain has been established by means of inhibitor studies (Sallis et al., 1965). Several workers have been interested in the relationship of respiration and phosphate transport in mitochondria and have attempted to establish a quantitative relationship between the two phenomena. It has been disturbing that repeated measurements by us and others (Sallis et al., 1963; Fang and Rasmussen, 1964) revealed that the efficiency of the parathyroid hormone dependent transport in terms of moles of ions transported per atom of oxygen

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consumed continued to be low. Thus, Fang and Rasmussen (1964) reported that the ratio of inorganic phosphate accumulated to oxygen consumed varied between 0.45 and 1.2. These investigators surmised that the low efficiency found was due to loss of phosphate from the mitochondria during the uptake process. By making a few assumptions, they attempted to correct for the efflux and were then able to calculate a P_i/O of 1.8 - 2.4. During the course of our experiments, we observed that in an all Tris medium (devoid of Na^+ and K^+) parathyroid hormone gave much improved transport of phosphate with no increase in respiratory response (Sallis and DeLuca, 1966). It therefore appeared that a reasonable P/O and Mg/O ratio could be obtained using this medium. Indeed Mg/O ratios approaching 3.0 and P_i/O ratios approaching 2.0 with NAD linked substrates could be obtained.

METHODS AND MATERIALS

The experiments were performed using rat liver mitochondria prepared and incubated as previously described (DeLuca and Engstrom (1961). The incubation medium contained 880 μmoles sucrose, 80 μmoles of Tris phosphate, pH 7.4, 40 μmoles of $MgCl_2$, 20 μgrams of oligomycin, 400 μg purified parathyroid hormone and various substrates such as 240 μmoles Tris pyruvate -- 6 μmoles of Tris malate, or 60 μmoles Tris succinate, or 120 μmoles of Tris β-hydroxybutyrate, or 60 μmoles of Tris L-glutamate. The total volume was 6.0 ml.

The reaction was initiated by adding a mitochondrial suspension (1.4 mg of mitochondrial nitrogen) to the medium which was contained in a 25 ml Erlenmeyer flask. The vessels were incubated with shaking at 30°C. A 1 ml aliquot of the sample was taken to measure oxygen consumption. An oscillating platinum electrode (Oxygraph -- Gilson Medical Electronics, Middleton,

Wis.) was employed for measurements of mitochondrial respiration. Control and hormone treated samples were incubated for equal lengths of time (3-8 minutes). Samples were removed from the flask, pipetted onto celite pads, and washed under water pump suction with 0.25 M sucrose as previously described (Sallis et al., 1963). The pads were dried in an oven at 110°C for 1 hour. Phosphate was extracted from the pad with 5 N H_2SO_4 and was determined by the method of Fiske and SubbaRow (1925). Magnesium was assayed in the Perkin-Elmer atomic absorption spectrometer (model No. 214) after extraction with 0.1 N HCl. Parathyroid hormone was prepared from fresh frozen beef parathyroid glands. Defatted glands were extracted with 70% aqueous phenol (Aurbach, 1959) and a TCA powder made (Rasmussen and Westall, 1957). The TCA powder was subjected to carboxymethyl cellulose chromatography by a procedure similar to that used by Friedman and Munson (1959).

RESULTS

The addition of 200 µg of purified parathyroid hormone to the isolated mitochondria resulted in the uptake of about 2 µmoles of phosphate per 0.7 mg of mitochondrial nitrogen as previously reported. The shorter incubation period, 3-8 minutes, used in this study resulted in the accumulation of 0.2 - 0.4 µmoles of inorganic phosphate under the same conditions. When NAD linked substrates were used, the ratio of phosphate accumulated to oxygen consumed was about 1.5 and the magnesium to phosphate ratio was also about 1.5. However, when succinate was the substrate the ratio of phosphate accumulated to oxygen consumed dropped to 0.66. There is an apparent increase in the magnesium to phosphate ratio but this increase is not significant ($P < 0.10 > 0.05$). The magnesium to oxygen ratios were about

2.5 or approaching 3.0 when pyruvate-malate or β-hydroxybutyrate were used as substrates, while the ratio was 1.2 when succinate was the substrate. The hormone increased oxygen consumption in the mitochondria as expected (see Table 1).

TABLE 1

The Effect of Parathyroid Hormone Upon Magnesium Phosphate Transport and Oxygen Consumption of Rat Liver Mitochondria

Substrate System	P/O	Mg/O	Mg/P_i	mμ atoms Oxygen Consumed		
				Control	PTH	Difference
Pyruvate-Malate	1.44 ± 0.11*	2.51 ± 0.32	1.75 ± 0.19	145 ± 7	276 ± 7	131 ± 6
β-Hydroxybutyrate	1.57 ± 0.20*	2.38 ± 0.36	1.50 ± 0.10	147 ± 3	307 ± 18	160 ± 16
Succinate	0.66 ± 0.11‡	1.18 ± 0.11	1.90 ± 0.16	181 ± 5	413 ± 11	232 ± 16

*Average of 5 ± SE

‡Average of 4 ± SE

The incubation medium contained 880 μmoles sucrose, 80 μmoles of Tris phosphate, pH 7.4, 40 μmoles of $MgCl_2$, 20 μgrams of oligomycin, 400 μgrams purified parathyroid hormone. The substrates were present at the following levels: 240 μmoles Tris pyruvate -- 6 μmoles of Tris malate, or 60 μmoles Tris succinate, or 120 μmoles of Tris β-hydroxybutyrate. The total volume was 6.0 ml and contained 1.4 mg of mitochondrial nitrogen. The incubation time was maintained at 5 minutes.

DISCUSSION

The results reported here demonstrate that magnesium to oxygen ratios approaching 3 for NAD linked substrates are possible in parathyroid hormone stimulated mitochondria. Such values were made possible by excluding the monovalent cations sodium and potassium from the medium. Rasmussen et al. (1964) have reported a parathyroid hormone dependent transport of potassium ion into mitochondria. It may be that the low Mg/O ratios obtained previously were merely due to an unmeasured transport of potassium ion. However, potassium ion transport would not explain the low values obtained when sodium ion replaces potassium ion since Rasmussen et al. could not show a parathyroid hormone dependent transport of sodium ion. Furthermore, in our hands a parathyroid hormone stimulation of respiration related to potassium ion transport could not be demonstrated. Another possible explanation is that the potassium ion and/or sodium ion interfere with the precipitation of magnesium phosphate in the mitochondria. If this interference should prove to be the explanation, potassium ion would be more effective than sodium ion in this regard. Whatever the mechanism, clearly these monovalent cations do interfere with the hormonal stimulation of magnesium and phosphate transport and disposition in mitochondria.

It is tempting to suggest on the basis of the present results that one high energy phosphate is utilized to transport one magnesium ion. Unfortunately this would be an oversimplification. It seems certain that the ratio of 3.0 is a minimum figure since testing for efflux of phosphate ion during the measurement of phosphate transport as did Fang and Rasmussen revealed a significant loss of phosphate ion during the incubation period. In addition it has not been established whether or not the hormonal system

is anion or cation oriented. If it is anion oriented, then the lower value of 2 would apply. In any case more reasonable transport efficiencies by the parathyroid hormone dependent systems can now be obtained experimentally.

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