PARATHYROID HORMONE STIMULATION OF PHOSPHATE UPTAKE BY RAT LIVER MITOCHONDRIA*

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During the course of our studies upon the effect of parathyroid hormone upon mitochondrial metabolism, it was noted that the parathyroid hormone stimulated non-phosphorylative oxidation. This effect, which was highly specific for this peptide hormone, required the presence of an oxidizable substrate, magnesium ions, inorganic phosphate and external ATP (Fang et al., 1962). The dependence upon inorganic phosphate immediately suggested the possibility that the hormonal stimulation of respiration might be related to an alteration of phosphate exchange in the mitochondria. This possibility was further suggested by a recent report describing a phosphate transport system in heart mitochondria coupled to respiration (Brierley, Bachmann and Green, 1962). It is the purpose of this paper to report an effect of parathyroid hormone upon the uptake of inorganic phosphate by liver mitochondria.

METHODS

Liver mitochondria, isolated as previously described (Fang et al., 1963), equivalent to approximately 0.7 mg N were incubated with shaking at 30°C in a medium containing 40 μmoles sodium phosphate buffer pH 7.4 labeled with 0.2 μc P^{32}, 590 μmoles sucrose, 20 μmoles $MgCl_2$, 30 μmoles

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L-glutamate, 2 μmoles ATP (Pabst) and 10 μg oligomycin B** in a total volume of 3.0 ml. After an initial 5 minute incubation, 200 μg pure parathyroid hormone was added in 0.002 N acetic acid.

At the indicated times, a 1.0 ml aliquot of the reaction mixture was removed and rapidly filtered by suction through a pad of celite held on a perforated planchet to collect the mitochondria, a technique originally described by Azzone and Ernster (1961). The celite retained mitochondria were washed immediately under suction with two 1.0 ml portions of ice-cold isotonic sucrose. The planchets containing the celite pad were dried at 110°C for 1 hour and the radioactivity measured with a thin end-window Geiger counter. The celite pad was extracted with 5 N H_2SO_4, and inorganic phosphate was determined on the extract by the method of Fiske and SubbaRow (1925).

RESULTS

As evident from Figure 1, parathyroid hormone stimulated the uptake of phosphate into the mitochondria. It was clear that the uptake of radioactivity was correlated with a net accumulation of phosphate. As much as 1 μmole of phosphate was taken up per mg mitochondrial protein. This is similar to the quantity of phosphate taken up by heart mitochondria as reported by Brierley et al. (1962). The uptake of inorganic phosphate under the influence of hormone appeared complete after 15 minutes of incubation. In the absence of hormone, there was no further increase in phosphate uptake after 5 minutes.

A number of other peptide and protein hormones, at equal or higher concentrations, were ineffective in this system((Table 1).

DISCUSSION

In a previous study, parathyroid hormone was found to stimulate non-phosphorylative oxidation in oligomycin inhibited mitochondria (Fang et al. 1963). This stimulation required the presence of substrate, magnesium ions, ATP and inorganic phosphate. Under the same conditions, the hormone stimulates

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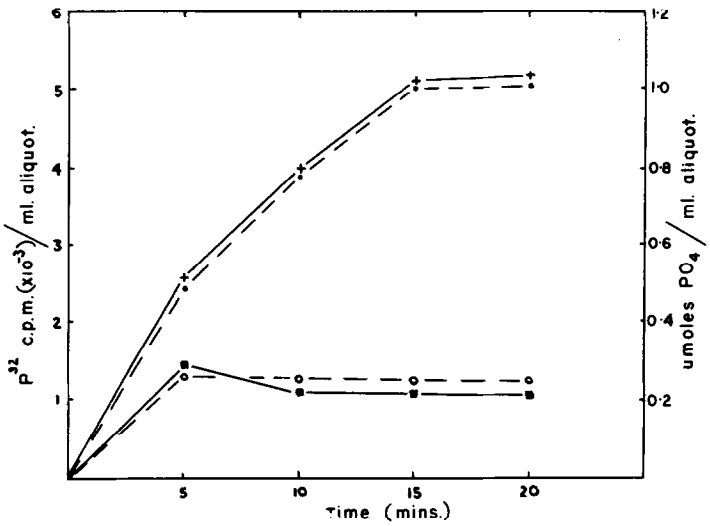

Figure 1. Parathyroid hormone stimulation of P^{32}-labeled inorganic phosphate uptake by liver mitochondria.

Inorganic Pi^{31} no hormone o ---- o; o ---- o Pi^{31} 200 µg parathyroid hormone; P^{32} no hormone ——— ; + ——— + P^{32}, 200 µg parathyroid hormone.

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the uptake of inorganic phosphate by liver mitochondria. Preliminary studies indicate that the uptake of phosphate is dependent on substrate, magnesium ions and ATP. It was interesting that there was no significant phosphate transport in the absence of hormone unlike the findings of Brierley, et al. (1962) with heart mitochondria. However, the reaction mixture used by them is quite different from that used in this study. For example, a much higher magnesium concentration and no external ATP were used in their study. In addition, a difference may exist between liver and heart mitochondria with respect to their capacity to accumulate phosphate. Thus, a more thorough examination of the two systems may prove valuable.

The stimulation of phosphate uptake by parathyroid hormone appeared highly specific. In other experiments the effect of the hormone could also be demonstrated in liver from rats deficient in vitamin D. This contrasts with the previous observations that the effect of the hormone on the release of calcium from mitochondria was dependent upon the presence of vitamin D (DeLuca, Engstrom and Rasmussen, 1962). It is important to note that the hormonal

Table 1

Specificity of Parathyroid Hormone Action on Phosphate Uptake

Addition*	Mitochondrial P^{32} c.p.m. ($\times 10^{-3}$)
None	3.51
Parathyroid hormone	20.31
Adrenocorticotropic hormone	3.81
Angiotensin	4.05
Growth hormone	3.54
Glucagon	4.05
Insulin	3.45
Lipid mobilizing hormone	3.57
Lysine vasopressin	4.41
Melanocyte stimulating hormone	3.51
Oxytocin	3.06
Triiodothyronine	3.51

*200 μg hormone added per 3.0 ml reaction mixture.

stimulation of non-phosphorylative oxidation was also independent of vitamin D. The exact relationship between the hormonal stimulation of non-phosphorylative respiration and of phosphate uptake by mitochondria is not known, but because of the similarity of requirements, it seems likely that the two are related. In fact, the most reasonable explanation is that the increased oxygen consumption is secondary to the increased phosphate uptake. If this were the case, the relationship might be explained by one of several possibilities. The uptake of phosphate could require energy derived from a high energy intermediate of oxidative phosphorylation, or an intermediate in oxidative phosphorylation could serve as the carrier for the translocation of phosphate. On the other hand, the increased level of phosphate within the mitochondria might in some

way uncouple electron transport from the phosphorylative machinery. These possibilities are currently under investigation.

The physiologic significance of the present results and the previous observation on calcium exchange remains obscure. However, there is a striking similarity between the hormonal stimulation of calcium and phosphate movements across the mitochondrial membrane, and the hormonal stimulation of renal tubular calcium reabsorption and phosphate secretion. This fact, coupled with the highly specific nature of these *in vitro* effects indicate that in all likelihood, the effects upon the mitochondria are related in some fashion to the effects of the hormone in the intact organism. However before this can be established numerous questions must be answered. In any case, it is evident that isolated mitochondria provide the best model currently available for the further study of calcium and phosphate transport and of the biochemical basis for the actions of vitamin D and parathyroid hormone.

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