THE INFLUENCE OF PARATHYROID HORMONE UPON GLUTAMATE OXIDATION IN ISOLATED MITOCHONDRIA*

Marie Fang, Howard Rasmussen, Hector F. DeLuca,
and Richard Young

Department of Biochemistry, University of Wisconsin
Madison 6, Wisconsin

Received January 10, 1963

It is generally accepted that the parathyroid hormone is involved in the regulation of calcium and phosphate metabolism. However, little is known concerning the biochemical basis of its actions. In recent years attention has been directed toward its effects upon carbohydrate and energy metabolism in bone. Bone slices obtained from parathyroid treated animals have been found to exhibit a higher rate of aerobic glycolysis as compared to those obtained from control animals, (Cohn et al., 1961, Vaes et al., 1962). It cannot be concluded at present that this alteration represents the primary metabolic effect of the hormone. Another possibility has been suggested from recent studies on the effect of this hormone upon calcium exchange in isolated mitochondria. It has been found that parathyroid hormone, added in vitro, stimulates the release of calcium from mitochondria (DeLuca et al. 1962). In view of the close association between calcium exchange and electron transport in mitochondria (DeLuca and Engstrom, 1961, Vasington and Murphy, 1962), a study of the effects of parathyroid hormone upon various parameters of mitochondrial oxidative metabolism

*Supported by grants A-5762 and A-5800 from the U. S. Public Health Service.

was initiated. The purpose of this report is to describe a specific effect of parathyroid hormone upon non-phosphorylative oxidation in kidney and liver mitochondria.

METHODS

Young Rolfsmeyer rats weighing 120-150 g were maintained on a low calcium diet for four days before being subjected to parathyroidectomy. Three to four hours after the operation the animals were killed by decapitation. The liver or kidneys were removed immediately and placed in ice-cold isotonic sucrose. Mitochondria were prepared as previously described (DeLuca and Engstrom, 1961). Mitochondrial suspensions, equivalent to 0.7 mg N, were incubated at 30°C in a medium containing 20 μmoles $MgCl_2$, 2 μmoles ATP, 30 μmoles substrate, 40 μmoles phosphate buffer pH 7.3, and 410 μmoles sucrose in a final volume of 3.0 ml. To study phosphate requirement, 40 μmoles Tris buffer pH 7.4 replaced the phosphate buffer. When indicated 2-20 μg of oligomycin B* were added in 0.1 ml of ethyl alcohol. Oxygen consumption was measured by standard manometric techniques employing a Warburg apparatus with air as the gas phase. The center well of the vessels contained 0.2 ml of 10% KOH adsorbed on filter paper. Following deproteinization of the incubation mixture with 20% perchloric acid, phosphate was determined by the method of Gomori (Gomori, 1941). P/O ratios were calculated in the usual fashion (DeLuca et al., 1957). Parathyroid hormone was prepared by gel filtration (Rasmussen and Craig, 1962). It and the other peptide hormones were dissolved in distilled water or 0.01 M acetic acid. After a ten minute equilibration period they were added from the side arm to the main chamber of the reaction vessel except in the

*Oligomycin B was generously supplied by Professor F. M. Strong.

studies of oxidative phosphorylation when they were added initially to the main chamber, and 1 mg hexokinase (Type III-Sigma Co) and 50 μmoles of glucose were added from the side arm.

RESULTS

When kidney or liver mitochondria were incubated with glutamate and excess phosphate acceptor (Hexokinase and glucose), the addition of parathyroid hormone (100 μg per flask) induced a 20% increase in oxygen uptake, and a fall in phosphorylation with a consequent fall in P/O ratio from 3.0 to as low as 0.7. When oligomycin, an inhibitor of oxidative phosphorylation, was added to mitochondria, the basal rate of oxygen consumption was diminished and phosphorylation inhibited. Under these circumstances the hormone produced a marked stimulation of oxygen consumption without reestablishing phosphorylation. The degree of this stimulation and the requirements for its demonstration are shown in Fig. 1. The stimulation of glutamate oxidation in oligomycin-inhibited mitochondria required the presence of external ATP, Mg^{++} and inorganic phosphate, but was unaffected by calcium or by the vitamin D status of the animal from which the mitochondria were isolated. This is in contrast to the effect of the hormone upon calcium release which does require the presence of vitamin D for its expression (DeLuca *et al.*, 1962).

Similar results were obtained in oligomycin-inhibited mitochondria when succinate replaced glutamate as the substrate.

The effect of the hormone on oligomycin-inhibited mitochondria was prevented by inhibitors of electron transport (cyanide, azide, and antimycin A) and by uncouplers of oxidative phosphorylation (2,4-dinitrophenol, dicumarol, Warfarin and azide). The latter compounds produced a stimulation of respiration and it was not possible to demonstrate a further stimulation by the hormone.

There was one striking difference between the hormonal effect and that produced by 2,4-dinitrophenol and the other uncouplers. The hormonal stimulation of glutamate oxidation was not apparent in the absence of inorganic phosphate (Fig. 1) whereas that produced by 2,4-dinitrophenol was.

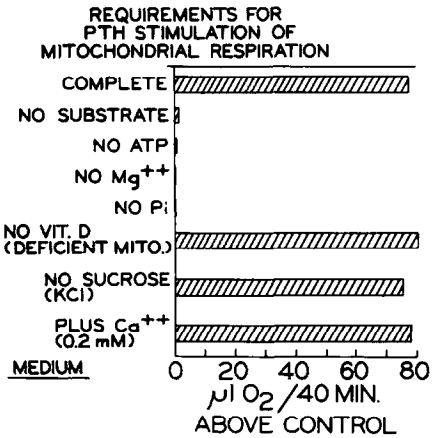

Fig. 1. The requirements for parathyroid hormone stimulation of glutamate oxidation. The values recorded are the microliters of oxygen consumed above the control value. In the absence of hormone this control value was approximately 20 µl/40 min. The complete medium contained 30 µmoles L-glutamate, 10 µmoles Mg^{++}, 40 µmoles phosphate buffer pH 7.3, 2 µmoles ATP, 10 µg oligomycin B, 410 µmoles sucrose, 100 µg parathyroid hormone, and liver mitochondria equivalent to 0.7 mg N.

A dose-response relationship was observed between hormone concentration and respiratory response. The response was specific for parathyroid hormone (Fig. 2). None of a variety of other peptide and amine hormones produced a similar effect.

DISCUSSION

The present results indicate that parathyroid hormone stimulates glutamate oxidation in isolated mitochondria with an apparent uncoupling of oxidative phosphorylation. Superficially this uncoupling is similar to that produced by 2,4-dinitrophenol. However, it differs in one striking respect. The hormonal

response requires the presence of inorganic phosphate whereas that due to 2,4-dinitrophenol does not.

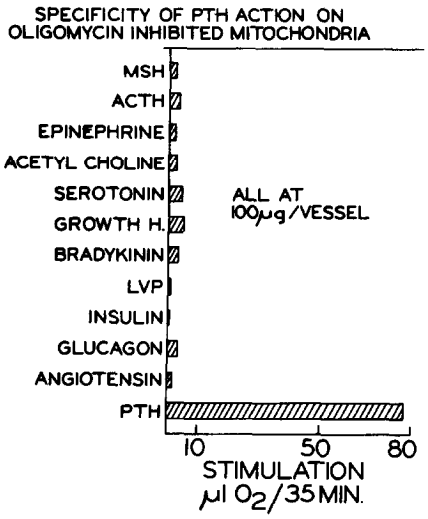

Fig. 2. Specificity of hormone action upon glutamate oxidation in oligomycin inhibited mitochondria. MSH = melonocyte stimulating hormone, ACTH = adrenocorticotropin, LVP = lysine vasopressin, PTH = parathyroid hormone. One hundred µg of each hormone was added per flask.

There is much to suggest that the primary hormonal effect is a stimulation of phosphate uptake (Sallis, DeLuca and Rasmussen, 1963) and that the increased rate of electron transport is a corollary or consequence of this phosphate translocation. The relationship between these two hormonal effects remains to be elucidated, but one of the obvious questions which must be investigated is the possibility that one or more of the phosphorylated intermediates in oxidative phosphorylation are involved.

The physiologic significance of these observations remain to be established. However, they are of interest in view of the recent attention directed toward the effects of this hormone upon various parameters of energy metabolism.

REFERENCES

Cohn, D. V. and Forscher, B. K., Biochim. Biophys. Acta, 52, 596 (1961).
DeLuca, H. F., Gran, F. C., Steenbock, H. and Reiser, S., J. Biol. Chem., 228, 469 (1957).
DeLuca, H. F. and Engstrom, G. W., Proc. Natl. Acad. Sci., 47, 1744 (1961).
DeLuca, H. F., Engstrom, G. W. and Rasmussen, H., Proc. Natl. Acad. Sci., 48, 1604 (1962).
Gomori, G., J. Lab. Clin. Med., 27, 955 (1941).
Rasmussen, H., Am. J. Med., 30, 112 (1961).
Rasmussen, H. and Craig, L. C., Biochim. Biophys. Acta, 56, 332 (1962).
Sallis, J. D., DeLuca, H. and Rasmussen, H., Biochem. Biophys. Res. Comm., 10, 266 (1963).
Vaes, G. M., and Nichols, G. Jr., Endocrinol., 70, 546 (1962).
Vasington, F. D. and Murphy, J. V., J. Biol. Chem., 237, 2670 (1962).