EFFECTS OF PARATHYROID HORMONE AND OTHER PROTEINS IN VITRO ON MITOCHONDRIAL METABOLISM

G. D. Aurbach[1], Bette A. Houston[1], and John T. Potts, Jr.[2]

Received July 19, 1965

It has been reported (DeLuca et al. 1962, Fang, et al. 1963, Sallis, et al. 1963a, 1963b, and Aurbach et al. 1964) that parathyroid hormone (PTH) in vitro stimulates ion transport and respiration in mitochondria. Sallis and DeLuca (1964) further found that the hormone in vitro stimulated mitochondrial ATPase and inhibited ^{32}Pi-ATP exchange reaction. These several findings suggested that the hormone acted physiologically by controlling ion transport in mitochondria. Tests for specificity showed that many non-parathyroid polypeptides or proteins were inert and lent credence to the suggestion that the hormonally induced effects on mitochondria were specifically and uniquely mediated by parathyroid hormone. However, in contrast to the previous reports our recent experiments, evolving from efforts to gain maximal sensitivity from these in vitro systems, show that several non-hormonal proteins cause similar effects and raise concern that the previously described effects on mitochondria may not be specifically related to the mechanism of action of parathyroid hormone.

MATERIALS AND METHODS

The ^{32}Pi-ATP exchange reaction was carried out as described by Sallis and DeLuca (1964); the method of Sugino and Miyoshi (1964) was used

1/ National Institute of Arthritis and Metabolic Diseases

2/ National Heart Institute, National Institutes of Health, Bethesda, Md.

to separate phosphate from ATP. Inorganic phosphate was measured by the procedure of Chen et al. (1956). The other methods used in these studies were described in the previous reports. The rate of release of $^{14}CO_2$ from succinate 1,4-^{14}C was used as a measure of the respiratory rate of mitochondrial suspensions. Each result presented is the average of duplicate or quadruplicate determinations. The preparation of parathyroid hormone used was a pure polypeptide (Potts and Aurbach, 1965). Two parathyroid proteins, devoid of hypercalcemic or phosphaturic activity in vivo, called C_1 and C_2, were purified from parathyroid extracts by gel filtration and chromatography on carboxymethylcellulose. Samples of polylysine were gifts of Drs. Berton Pressman and Herbert Sober. Ribonuclease (RNase), protamine, histone and lysozyme were commercial products. Protamine was dialyzed before use to remove sulfate. Mitochondria were prepared from the kidneys or livers of rats by methods previously described.

RESULTS

In initial studies large concentrations of two physiologically inert parathyroid proteins, C_1 and C_2, caused no significant stimulation of respiration. Later tests, however, showed that at lower concentrations proteins C_1 and C_2 stimulated respiration of liver mitochondrial suspensions (Figure 1). Note that at high concentrations these non-hormonal parathyroid proteins either cause no effect or inhibit respiration. Since several components in crude parathyroid extracts are basic proteins, and since it is known that the basic proteins polylysine, protamine and histone (Schwartz, 1965) and ribonuclease (Hanson, 1959) stimulate respiration of mitochondrial suspensions, it seemed important to test several other non-hormonal basic proteins. It was found that polylysine and protamine as well as proteins C_1 and C_2 and parathyroid hormone stimulate respiration in mitochondria (Table I) as measured

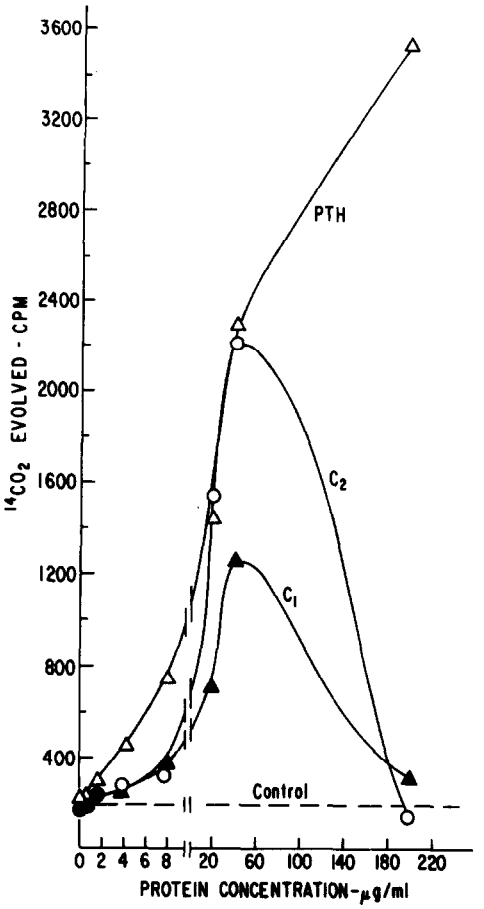

Fig. 1 Rate of degradation of succinate-1,4-^{14}C to $^{14}CO_2$ by liver mitochondria as a function of the concentration of parathyroid hormone or proteins C_1 or C_2. High concentrations of proteins C_1 and C_2 are ineffective or actually inhibitory. Medium contained sucrose 160 mM; potassium phosphate pH 7.6, 32 mM; oligomycin 0.0066 mM; DPN 0.8 mM; succinate 8 mM (with 120,000 CPM succinate-1,4-^{14}C); $MgCl_2$ 5.3 mM; and mitochondria equivalent to 0.8 to 1.6 mg. of protein in a total volume of 1.0 ml. Incubations were carried out for 30 minutes at 30°.

by the evolution of $^{14}CO_2$ from radioactive succinate. These results prompted us to test the effects of polybasic proteins on other systems heretofore shown to be sensitive to parathyroid hormone. The original publication of DeLuca, Engstrom, and Rasmussen (1962) showed that

TABLE I

Effect of Proteins on Mitochondrial Degradation of Radiosuccinate to $^{14}CO_2$

Additions	$^{14}CO_2$ Evolved cpm
None	243
Parathyroid Hormone	1520
C_1	892
C_2	717
Polylysine	657
Protamine	304
Ribonuclease	247
Lysozyme	193
Histone	191

Reaction conditions: see Fig. 1; added proteins were at a final concentration of 20 µg/ml.

parathyroid hormone induced the release of calcium from kidney mitochondria. As shown in Figure 2 and Table II, this result has been confirmed; however, this was not an effect unique to parathyroid hormone. Ribonuclease and histone, as well as proteins C_1 and C_2 were active. Polylysine inhibited uptake.

Phosphate transport in mitochondria is sensitive to parathyroid hormone, but here again specificity was lacking. This is illustrated in Figure 3; in agreement with the reports of Sallis, et al. (1963a,b) we found that parathyroid hormone sharply increased phosphate uptake. However, proteins C_1 and C_2 as well as protamine were also effective.

The recent report of Sallis and DeLuca (1965) shows that parathyroid hormone stimulates ATPase activity of liver mitochondria and inhibits ^{32}Pi-ATP exchange reaction. These findings were also amply confirmed

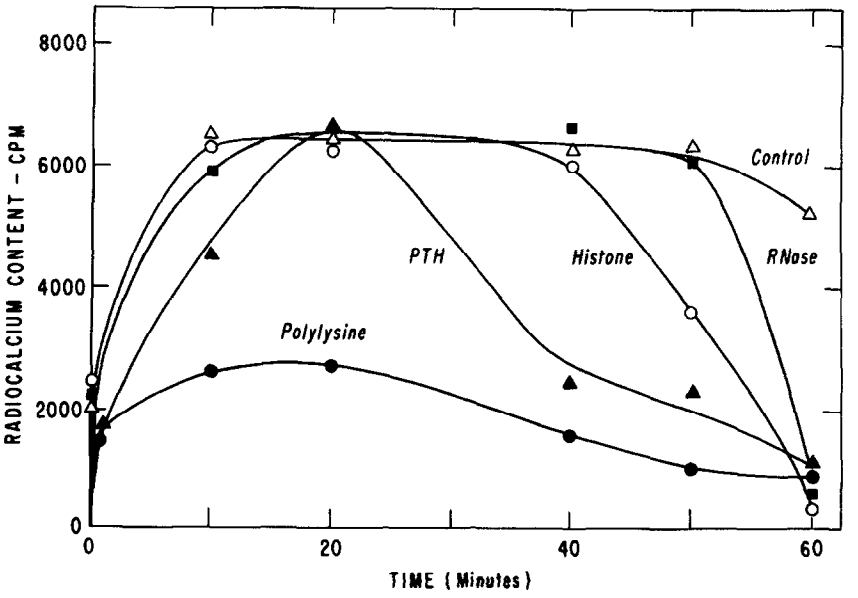

Fig. 2 Effect of several proteins on the release of calcium bound by rat kidney mitochondria in vitro. Curve marked control = no additions; other curves show the effects of proteins added to a concentration of 20 µg/ml. The ordinate indicates the amount of calcium bound by mitochondria throughout the incubation period. The medium contained: sucrose, 85 mM; potassium phosphate, pH 7.4, 12.5 mM; $MgCl_2$ 2.5 mM; ATP 0.2 mM; $CaCl_2$ (with 7300 cpm/ml. ^{45}Ca), 0.1 mM; KCl 10 mM; cytochrome C 0.02 mM; and mitochondria equivalent to 0.8 to 1.6 mg. of protein in a final volume of 1.0 ml.

TABLE II

Uptake and Release of Calcium by Kidney Mitochondria

Additions	Calcium (as ^{45}Ca) bound by mitochondria		
	10 min. cpm	50 min. cpm	60 min. cpm
None	2250	2150	2230
Parathyroid hormone	2680	194	823
C_1	2510	2240	323
C_2	2270	138	148

Incubation conditions: see Fig. 2. The concentration of each protein was 20 µg/ml. In this experiment 0.1 µMoles of $CaCl_2$ were equivalent to 2400 cpm ^{45}Ca.

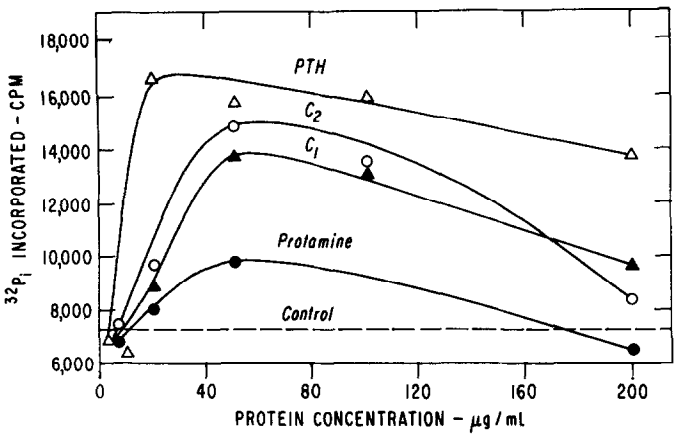

Fig. 3 Rate of phosphate accumulation by liver mitochondria as a function of the concentration of parathyroid hormone, protamine, or the proteins C_1 or C_2. The reaction medium contained sucrose, 190 mM, potassium phosphate, pH 7.3, 13.3 mM (containing 136,000 cpm/ml. of ^{32}P), $MgCl_2$, 6.7 mM, sodium glutamate, 10 mM and mitochondria equivalent to 0.8 to 1.6 mg. of protein in a final volume of one ml. Incubations were carried out for 25 minutes at 30°.

TABLE III

ATPase Activity of Liver Mitochondria

Additions	µMoles Pi Released
None	0.22
PTH	0.68
Polylysine	0.67
C_2	0.60
C_1	0.44
Ribonuclease	0.43
Protamine	0.43
Lysozyme	0.23
Histone	0.21

The medium contained 60 mM Tris - 33 mM cacodylate - 33 mM acetate buffer pH 6.7; 5 mM ATP, 1 mM $MgCl_2$, 50 mM sucrose and liver mitochondria equivalent to 0.5 to 1.0 mg. of protein in a final volume of 0.5 ml. The proteins added were at a final concentration of 40 µg/ml.; incubations were carried out for 20 minutes at 30°.

TABLE IV

Inhibition of ^{32}Pi-ATP Exchange Reaction by Certain Proteins

Additions	AT^{32}P Found cpm
None	501
Parathyroid hormone	31
C_1	114
C_2	19
Polylysine	19
Protamine	43
Ribonuclease	126
Lysozyme	632
Histone	473

The reaction medium contained Tris pH 7.4, 60 mM, sucrose 25 mM, sodium phosphate (^{32}Pi 8 x 10^5 cpm/ml.) pH 7.4, 8 mM, ATP 8 mM and 0.8 to 1.6 mg. mitochondrial protein in a final volume of one ml. Incubations were for 15 minutes at 30°; parathyroid hormone and other proteins were used at a final concentration of 80 µg/ml.

(Table III and Table IV); however, once again basic proteins other than parathyroid hormone were active.

DISCUSSION

It is apparent that in addition to parathyroid hormone, several non-hormonal basic proteins stimulate mitochondrial respiration, ion transport, and ATPase, but inhibit the ^{32}Pi-ATP exchange reaction. Thus these effects may not be so uniquely a measure of the biological actions of parathyroid hormone as the previous reports suggested.

Rasmussen et al. (1964) have also isolated from parathyroid extracts a protein that is inert in vivo but stimulates mitochondrial

transport of phosphate *in vitro*. They postulated that this protein might be yet another biologically important factor produced by parathyroid glands. The latter speculation may not be warranted in view of the uncertain specificity of protein effects on mitochondria. All of our tests completed to date show that the non-hormonal parathyroid proteins C_1 and C_2 are unrelated either biologically, immunologically, or chemically (by amino-acid analysis) to the parathyroid hormone.

Further rigorous studies are necessary to precisely determine whether any effects of parathyroid hormone on mitochondria validly reflect the mechanism of action of the hormone and whether it is sound to use the *in vitro* tests as bioassay tools.

REFERENCES

Aurbach, G. D., Houston, B. A., and Potts, J. T., Jr., Biochem. Biophys. Res. Commun. 17, 464 (1964).

Chen, P. S., Toribara, T. Y., and Warner, H., Anal. Chem. 28, 1756 (1956).

DeLuca, H. F., Engstrom, G. W., and Rasmussen, H., Proc. Nat'l. Acad. Sci., U.S. 48, 1604 (1962).

Fang, M., Rasmussen, H., DeLuca, H. F., and Young, R., Biochem. Biophys. Res. Commun. 10, 260 (1963).

Hanson, J. B., J. Biol. Chem. 234, 1303 (1959).

Potts, J. T., Jr., and Aurbach, G. D., in The Parathyroid Glands: Ultrastructure, Secretion and Function, 1965, in press.

Rasmussen, H., Sze, Y., and Young, R., J. Biol. Chem. 239, 2852 (1964).

Sallis, J. D., DeLuca, H. F., and Rasmussen, H., Biochem. Biophys. Res. Commun. 10, 266 (1963a).

Sallis, J. D., DeLuca, H. F., and Rasmussen, H., J. Biol. Chem. 238, 4098 (1963b).

Sallis, J. D., and DeLuca, H. F., J. Biol. Chem. 239, 4303 (1964).

Schwartz, A., J. Biol. Chem. 240, 939 (1965).

Sugino, Y., and Miyoshi, Y., J. Biol. Chem. 239, 2360 (1964).