STIMULATION BY PARATHYROID HORMONE OF THE MITOCHONDRIAL UTILIZATION OF REDUCED PYRIDINE NUCLEOTIDE

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Fang et al. (1963) reported that parathyroid hormone stimulates oxygen consumption by mitochondria supplied with glutamate or succinate as substrate. They suggested that this effect was dependent on the enhanced mitochondrial uptake of phosphate that occurred under identical conditions (Sallis et al., 1963a; 1963b). With oligomycin-blocked mitochondria, hormonal stimulation of either oxygen consumption or phosphate uptake required addition of ATP.

During an investigation of these in vitro effects, we observed that parathyroid hormone stimulated the formation of $^{14}CO_2$ from succinate-1,4-^{14}C incubated with liver or kidney mitochondria. The effect was discernible at hormone concentrations of 7×10^{-9} to 5×10^{-6} M, and was not dependent on added ATP in the presence of oligomycin. Measurement of reduced pyridine nucleotide suggested that the parathyroid hormone effect on the mitochondrial system is brought about by stimulating oxidation of the DPNH produced by succinate. An account of these findings is presented in this report.

Parathyroid hormone (PTH)* was obtained as a highly purified polypeptide by partition on columns of Sephadex G-100 (Aurbach and Potts, 1964). Mitochondria were prepared from the livers of rats according to the method of DeLuca and Engstrom (1961); the 0.25M sucrose used throughout contained

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* A stock solution of 10^{-4} M parathyroid hormone in water was used; when lower concentrations of the hormone were required, the preparation was diluted with a solution made by mixing one part plasma of parathyroidectomized rats and 70 parts phosphate buffer. Other hormones were commercial products or gifts of Dr. Peter Condliffe or Dr. Martin Petersen.

1 mM EDTA. The reaction mixture (shown in Figure I) was incubated at 30° in plastic vials in a shaking incubator; $^{14}CO_2$ was collected and measured by the method of Fain et al. (1963). Reduced pyridine nucleotide was determined using the Aminco-Bowman spectrofluorometer.

Results: Parathyroid hormone, 7 x 10^{-9} M to 5 x 10^{-6} M, markedly stimulated the release of $^{14}CO_2$ with succinate-1,4-^{14}C as substrate; the rate of $^{14}CO_2$ evolution, shown in Figure I, was related to the concentration of hormone. The pH and concentrations of succinate, phosphate, and Mg were optimal for hormonal stimulation in this system, and the reaction rate ($^{14}CO_2$ evolved) was proportional to the concentration of mitochondrial protein (in the range of 0.25 to 4.0 mg protein/ml) with either the control or stimulated reaction. The effect was markedly reduced without added phosphate; adding Mn partially substituted for Mg. Neither oligomycin nor DPN was required, but the relative effect of hormone was greater

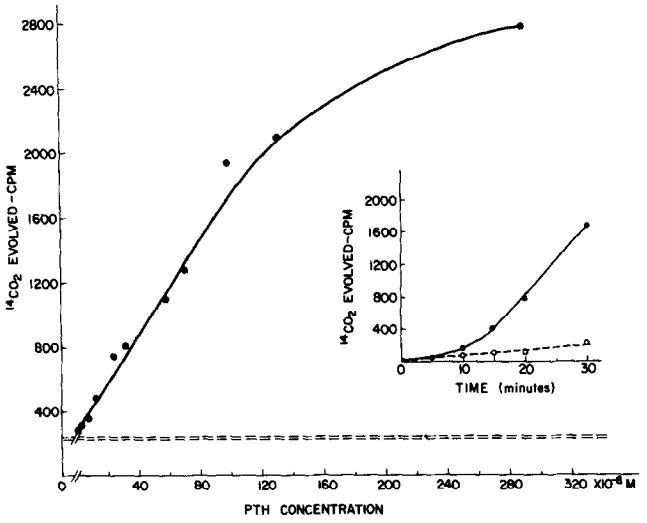

Figure 1. $^{14}CO_2$ released in response to parathyroid hormone with 30 minute incubations; the lowest concentration of hormone tested (7 x 10^{-9} M) caused significant stimulation above control. The horizontal dotted lines represent the standard error determined for the control reaction rate. Insert: rates of control (open circles) or stimulated reaction (closed circles) with 1.4 x 10^{-6} M PTH. The control rate gradually increased with incubations prolonged beyond 40 minutes. The reaction mixture contained the following: sucrose, 160 mM; potassium phosphate, pH 7.6, 32 mM; oligomycin, 0.0066 mM; DPN, 0.8 mM; succinate, 8.0 mM (containing 120,000 cpm succinate-1,4-^{14}C); $MgCl_2$, 5.3 mM and mitochondrial protein, 0.8 to 1.6 mg in a final volume of 1.0 ml.

when these compounds were included. The results caused by adding dinitrophenol or calcium to the medium are shown in Table I.

TABLE I

$^{14}CO_2$ Evolved in 30 Minutes

Incubation Medium*	Control CPM	+PTH 1.4×10^{-6} M CPM
Complete	125	1,380
Minus Oligomycin	267	950
Minus DPN	97	321
Minus Mg	313	844
Minus Mg + Mn	247	1,370
Minus Pi + Tris pH 7.6	75	93
Complete	300	1,970
+Dinitrophenol 3×10^{-6} M	536	2,620
+Dinitrophenol 2×10^{-5} M	1800	1,510
Complete	218	2,200
+Ca 2×10^{-4} M	396	1,210
+Ca 5×10^{-4} M	868	1,270
+Ca 2×10^{-3} M	985	850

* See Figure I

Addition of amytal (1.7×10^{-3} M), antimycin (2.5×10^{-7} M), or dicumarol (8×10^{-6} M) markedly reduced or abolished the hormonal effect. The most active hormonal preparation in the mitochondrial system was the most homogeneous.[+]

Thin layer chromatography of the reaction media showed that less malate accumulated in the hormone-stimulated reaction than in the control reaction (Table II). It was also found that the hormone caused significant stimulation of $^{14}CO_2$ release from malate-1,4-^{14}C (Table III); note that

[+] This material was prepared by chromatography of the Sephadex-purified product on carboxymethylcellulose (Potts and Aurbach, 1964), a procedure that removed several contaminants representing 20-30% of the protein in the hormone isolated by gel filtration. Digestion of parathyroid hormone with trypsin destroyed the activity of the hormone in vivo and resulted in complete loss of activity of the hormone system. None of twelve other compounds (oxytocin, vasopressin, thyroxin, acetylcholine, epinephrine, growth hormone, prolactin, ACTH, intermedin, gonadotropin, insulin and thyrotropin) caused significant stimulation of $^{14}CO_2$ release from radio-succinate.

addition of succinate caused a decreased conversion of malate-^{14}C to $^{14}CO_2$ but allowed greater hormonal stimulation. Fluoroacetate did not inhibit the hormonal effect with either malate or succinate as substrate. There

TABLE II

Analysis of Reaction Media after 30 Minute Incubation*

	Control μMoles	+PTH 2 x 10^{-6} M μMoles
Succinate disappeared	0.64	0.57
Malate found	0.30	0.15
CO_2 released	0.044	0.16

*In this experiment succinate concentration was reduced to 1mM; other components as in Figure 1. Succinate and malate were measured by radioassay after separation by thin layer chromatography on silica gel in n-pentanol saturated with 44% formic acid. The same specific activity of malate (or succinate) was obtained whether recovered from the control or the stimulated reaction.

TABLE III

$^{14}CO_2$ Released from Malate 1,4-^{14}C in Response to Parathyroid Hormone 2 x 10^{-6} M

Experiment	Control CPM	+PTH CPM
1	139	859
2	255	564

In Experiment 1 the reaction mixture contained 5mM succinate, 5mM malate (160,000 cpm) and 2.5 mM fluoracetate. Other components as in Figure I.

In Experiment 2 succinate was omitted; 8mM malate and 2.5 mM fluoracetate were used. Other components as in Figure I.

was no stimulation of $^{14}CO_2$ release using carboxyl-labeled pyruvate or acetate as substrate. Although several of the enzymes (in the pathway: succinate$\rightarrow CO_2$) were tested, we did not find enzymatic activities to be directly affected by PTH. However, it was discovered that PTH caused accelerated oxidation of DPNH (determined as a decrease in fluorescence) added to the routine reaction medium (Figure II). Hormone-induced oxidation of endogenous reduced pyridine nucleotide was also observed with the routine reaction mixture without added DPN or DPNH.

Discussion: Measurement of $^{14}CO_2$ evolution from succinate in this mitochondrial system afforded an apparently specific in vitro test sensitive

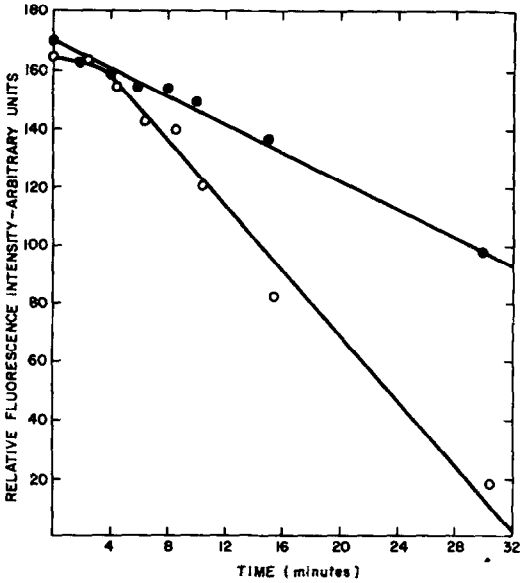

Figure 2. Rate of oxidation of DPNH, 10^{-4} M, added to routine reaction mixture without (closed circles) or with 1.4×10^{-6} M PTH (open circles). Aliquots of the reaction mixture were added to 0.01 M NaOH, and fluorescence determined with activation at 345 mμ and emission at 455 mμ.

to as little as 0.2 U.S.P. units of parathyroid hormone; this test may prove to be a precise and rapid bioassay for the hormone.

The hormonal stimulation with oligomycin-blocked mitochondria incubated with radiosuccinate was not dependent on ATP and thus occurred under conditions reported to prevent hormonal stimulation of phosphate uptake (Sallis et al., 1963a, b) and oxygen consumption (Fang et al., 1963).

Thus it is unlikely that the hormonal influence on succinate degradation is dependent on accelerated transport of phosphate into mitochondria; however, the effect reported here was markedly influenced by phosphate in the medium. We do not have an adequate explanation for the ATP requirement with oligomycin-blocked mitochondria reported by Fang, Sallis et al. Perhaps with their conditions ATP acted by maintaining the integrity of a structural factor of mitochondria. The following is a tentative interpretation of the PTH-stimulated degradation of succinate: 1) succinate causes extensive reduction of intramitochondrial pyridine nucleotide (Chance and Baltschefsky, 1958), 2) the DPNH produced by succinate is compartmented and unavailable to pyridine nucleotide-linked dehydrogenases (Chance and Hollunger, 1961), 3) oxidation of DPNH (stimulated by PTH) to DPN effectively increases the availability of DPN for malic dehydrogenase, 4) resultant from 3) there is greater conversion of malate to oxalacetate; the latter is then decarboxylated. Thus measurement of CO_2 release from succinate in this system may just be an indirect, though highly sensitive assay for the rate of re-oxidation of the DPNH produced by succinate.

Chance (1963) has observed that oxidation of DPNH occurs during active transport of calcium by mitochondria. This result may be directly comparable to the calcium-induced degradation of succinate currently reported (Table I). It is possible that the PTH-induced utilization of DPNH is a manifestation of hormonal activation of a specific ion transport system.

REFERENCES

Aurbach, G.D. and Potts, J.T., Jr., Endocrinology (In Press).
Chance, B., in "Energy-Linked Functions in Mitochondria", Ed. B. Chance, Academic Press, New York, 1963, p.253.
Chance, B. and Baltschefsky, H., J. Biol. Chem. 233: 736, 1958.
Chance, B. and Hollunger, G., J. Biol. Chem. 236: 1534, 1961.
DeLuca, H.F. and Engstrom, G.W., Proc. Natl. Acad. Sci. U.S. 47: 1744, 1961.
Fain, J. N., Scow, R.O., and Chernick, S.S., J. Biol. Chem. 238: 54, 1963.
Fang, M., Rasmussen, H., DeLuca, H.F. and Young, R., Biochem. Biophys. Res. Commun., 10: 260, 1963.
Potts, J.T., Jr, and Aurbach, G.D., in "Proceedings Second Parathyroid Symposium", Eds. R.V. Talmage and P.J. Gaillard (In Press).
Sallis, J.D., DeLuca, H.F. and Rasmussen, H., Biochem. Biophys. Res. Commun., 10: 226, 1963a.
Sallis, J.D., DeLuca, H.F. and Rasmussen, H., J. Biol. Chem. 238: 4098, 1963b.