The Inhibition of Respiration and Phosphorylation in Kidney Mitochondria by Parathyroid Hormone Administered in Vivo*

(Received for publication, September 23, 1965)

DAVID V. COHN, ANNA F. SMAIL, AND ROBERT LEVY
From the Veterans Administration Hospital and the University of Missouri at Kansas City School of Dentistry, Kansas City, Missouri 64106, and University of Kansas Medical School, Kansas City, Kansas 66103

SUMMARY

Parathyroid hormone administered in vivo to rabbits produced a metabolic alteration in kidney cortex mitochondria, evidenced by diminished respiratory rate, respiratory control, and P:O ratio with a series of substrates including pyruvate plus fumarate, succinate, α-ketoglutarate, malate, citrate, and isocitrate. The adenosine triphosphate-inorganic phosphate exchange reaction rate was decreased and adenosine triphosphatase activity was increased compared to control mitochondria. These changes did not become apparent until about 12 hours after injection, were undiminished by 2.4 hours, and could be elicited with as little as 200 U.S.P. units of hormone. Under identical conditions liver mitochondria were unaffected. Diphospho- and triphosphopyridine nucleotides in combination frequently elevated the respiration in parathyroid-treated mitochondria close to or above control levels with citrate and isocitrate but not with the remaining substrates and had no effect on respiratory control with any of the substrates. The effects of the hormone could not be duplicated by elevating and maintaining in otherwise normal rabbits serum calcium concentrations at twice normal level for 6 hours. It is concluded that the effects produced by parathyroid hormone on kidney are multiple in nature and related to the Ca++-raising potency of the preparations.

METHODS

White New Zealand rabbits (6 weeks old) were treated by subcutaneous injection in groups of three with 750 U.S.P. units of parathyroid hormone and were killed 14 to 24 hours later by exsanguination, together with a similar group of control animals. Other time schedules and dosages which were used are specified in the text. The parathyroid hormone was prepared by gel filtration as described by Aurbach and Potts (17) and was assayed in the parathyroidectomized rat (18). Three different batches, with potency varying from 1000 to 2000 U.S.P. units per mg of

Although it is generally agreed that parathyroid hormone influences calcium and phosphate metabolism by increasing the renal excretion of phosphate and by acting directly on bone cells to cause the dissolution of mineral (1), the underlying biochemical mechanisms by which these processes occur are little understood.

Studies in which parathyroid hormone has been added in vitro to liver and kidney mitochondria indicate that the hormone influences an ion transport system closely associated with the mitochondrial phosphorylation chain (2-9). When the hormone is administered to the animal, or following its addition to tissue maintained in culture, there is a generalized inhibition of oxidative metabolism in bone and liver (10, 11) and the accumulation of metabolic intermediates (12, 13). One of the intermediates, citrate, is of particular interest since it has a high affinity for Ca++. It has been suggested that a large amount of citrate formed by bone cells as a result of parathyroid hormone action could lead to dissolution of the mineral phase by chelation (14). Recently, Costello and Darago (15) reported that in kidney mitochondria the oxidation of citrate and isocitrate, but not succinate, was inhibited following treatment of the animals with parathyroid hormone. Oxidation could be restored by the addition of diphospho- or triphosphopyridine nucleotides to the assay system (16). Liver mitochondria were unaffected, a point of interest since liver, unlike kidney, is not considered to be a target organ of parathyroid hormone (1).

In the present study the respiration of kidney mitochondria derived from parathyroid hormone-treated animals was examined with several substrates in order to determine if the inhibition of oxidation was confined to citrate and isocitrate or was a generalized phenomenon, as in bone (10). In addition it was hoped to learn if alterations in oxidative phosphorylation occur that are similar to those found when parathyroid hormone is added in vitro to mitochondria. The present report shows that parathyroid hormone had an extensive inhibitory effect on the respiration of a number of substrates in addition to citrate and isocitrate. This inhibition was accompanied by a decrease in oxidative phosphorylation as established by several criteria.

* This investigation was supported in part by Research Grant DE-01523 from the National Institute of Dental Research, National Institutes of Health, United States Public Health Service.
protein, were employed in these studies. In several experiments commercial parathyroid extract ("injection parathyroid," Lilly) was used. No difference in action between the commercial and purified preparations was noted.

Mitochondria from renal cortex and liver were prepared in 0.25 m sucrose as described by Schneider (10). The final suspending medium usually contained 0.001 m Versene, but omission of this did not alter the results. Oxygen uptake and respiratory control (defined as ratio of respiration when ADP is not limiting to that when it is (20)), were determined with a vibrating platinum electrode (Oxygraph, Gilson Medical Electromedics, Middleton, Wisconsin). In addition, respiration and P:O ratios were measured in a differential Warburg-type manometric apparatus. ATPase activity was determined under the conditions described by Lardy and Wellman (21), and the exchange of inorganic phosphate-P32 with ATP was measured according to Dallam and Hamilton (22). Radioactivity was assayed in a liquid scintillation detector.

**Table I**

**Effect of parathyroid hormone on respiration and respiratory control of kidney and liver mitochondria**

The reactions were performed in the Oxygraph by adding 0.1 to 0.2 ml of mitochondrial suspension containing 1 to 4 mg of protein to 1.5 ml of a buffer composed of 12.5 mM potassium phosphate, pH 7.4, 5 mM MgSO4, and 200 mM sucrose. Subsequent 0.05-m1 additions of 15 μmoles of the appropriate substrate and 0.5 to 5.0 μmoles of ADP were then made. Rates were measured for 1 to 2 min. The temperature was 25°. Experiments A and C were performed with purified hormone; Experiment B with commercial parathyroid extract.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADP</td>
<td>Respiratory control</td>
</tr>
<tr>
<td></td>
<td>mmoles/min/mg protein</td>
<td>mmoles/min/mg protein</td>
</tr>
</tbody>
</table>

Experiments A and B were performed with purified hormone; Experiment C with commercial parathyroid extract.

Succinate dehydrogenase activity was measured spectrophotometrically with phenazine methosulfate (23), and malate dehydrogenase and isocitrate dehydrogenase were determined spectrophotometrically by DPNH oxidation (24) and TPN reduction (25), respectively. The determination of oxidized and reduced pyridine nucleotide (DPN + TPN and DPNH + TPNH) was conducted as described by Lowry, Roberts, and Kapphahn (26). Inorganic phosphate was assayed by the method of Fiske and SubbaRow (27), protein by the method of Lowry et al. (28), and Ca++ by flame photometry.

**Results**

**Effect of Parathyroid Hormone on Respiration and Respiratory Control**—The rabbits did not appear to be adversely affected by the treatment with parathyroid hormone and the kidneys appeared to be grossly normal. Serum calcium concentrations increased 2 to 5 mg/100 ml. No changes in respiration were observed at 1, 2, and 8 hours following injection. After 12 to 14 hours maximal inhibition became apparent and remained unchanged after 24 hours. When, in one study, individual groups of rabbits received 0, 50, 100, 200, 400, and 750 units of hormone, respectively, the mitochondria from the first three groups were unaffected by the treatment, whereas the mitochondria from the remaining groups all showed the changes reported below and to the same extent.

Table I lists data from three representative experiments which illustrate the inhibitory action of parathyroid hormone on respiration of kidney mitochondria and its lack of effect on liver mitochondria. In kidney, with fumarate + pyruvate, succinate, α-ketoglutarate, malate, citrate, or isocitrate as substrate, the respiratory control of experimental mitochondria in the presence of ADP ranged from one-fifth to two-thirds that of the control preparations. The respiration of the experimental mitochondria in the absence of added ADP was variable in different experiments and was either unchanged or up to twice that of the controls. As a result of these changes in rate, the calculated respiratory control ratio was markedly reduced. In contrast to these results, Table I shows that the treatment with parathyroid hormone had no demonstrable effect on liver mitochondria.

**Effect of Parathyroid Hormone on Phosphorylation**—In order to determine whether or not the depressed respiratory control was related to an altered level of phosphorylation, manometric studies of phosphorylation were conducted and ATPase activity and ATP-Pi exchange rates were determined.

Fig. 1 shows that oxidation and phosphorylation for a series of substrates were substantially lowered by parathyroid hormone. In these experiments the P:O ratios declined from 1.0 to 0.4 unit. In four separate studies it was observed that the decrease in P:O ratio was always less than 1 unit for succinate and approximately 1 unit for the other substrates.

Table II lists typical studies in which the ATPase activities of kidney and liver mitochondria were measured after the animals were treated with parathyroid hormone. It was observed consistently in 13 such experiments that without any additions to the basal incubation medium, the ATPase activity of the experimental mitochondria was from 2 to 3 times greater than that in the control preparations. Similarly the ATPase activity with Mg++ present was always greater than the control level but with 2,4-dinitrophenol the activity was unchanged. When the basal activity (Mg++ or dinitrophenol not added) is subtracted from
The effect of parathyroid hormone treatment in phosphorylation was further evidenced by a marked decrease in the ATP-P exchange rate. As shown in Table III, mitochondria from parathyroid hormone-treated animals were from one-half to one-quarter as active as the control mitochondria.

**Effect of Pyridine Nucleotide and Other Factors on Respiration**—Because Costello and Darago (16) reported that the inhibition in respiration induced by parathyroid hormone could be overcome with DPN or TPN, these and other cofactors were tested singly and in combination with several substrates for their effect on respiratory rate. Our data only partially confirm the findings of the previous investigators. With both experimental and control mitochondria, DPN and TPN, singly or in combination, had a slight to moderate stimulatory effect on those substrates in which respiratory chain oxidation is pyridine nucleotide-linked but had no effect on flavin-linked succinate (Table IV). Thus with fumarate + pyruvate, citrate, isocitrate, or α-ketoglutarate,

**TABLE II**

<table>
<thead>
<tr>
<th>ATPase Activity</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity*</td>
<td>Increase</td>
<td>Activity*</td>
</tr>
<tr>
<td>Experiment A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>22</td>
<td>43</td>
</tr>
<tr>
<td>Mg++</td>
<td>36</td>
<td>66</td>
</tr>
<tr>
<td>None</td>
<td>130</td>
<td>132</td>
</tr>
<tr>
<td>Dinitrophenol</td>
<td>108</td>
<td>66</td>
</tr>
<tr>
<td>Experiment B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>21</td>
<td>73</td>
</tr>
<tr>
<td>Mg++</td>
<td>42</td>
<td>92</td>
</tr>
<tr>
<td>Dinitrophenol</td>
<td>158</td>
<td>151</td>
</tr>
</tbody>
</table>

* Millimicromoles of inorganic P released per min per mg of protein.
† Over activity with no addition.

that when Mg++ or dinitrophenol was present, it appears that the Mg++-activated ATPase was unchanged or increased slightly, whereas the dinitrophenol-activated ATPase consistently declined. This suggests that the ATPase that was stimulated by the hormone was that which could be stimulated by dinitrophenol and is considered to be involved in the phosphorylation process (29).

**FIG. 1.** The effect of parathyroid hormone on oxygen uptake and P:O ratio of kidney mitochondria. Each reaction vessel contained 0.1 or 0.2 ml of mitochondrial suspension (1.2 to 3.4 mg of protein) in 3.0 ml of a reaction mixture containing 30 μmoles of substrate, 40 μmoles of MgSO₄, 120 μmoles of glycylglycine buffer, pH 7.4, 2 μmoles of ATP, 0.02 mg of crystalline hexokinase, 60 μmoles of glucose, and approximately 400 μmoles of sucrose. The center well contained 0.2 ml of 5% KOH and a filter paper fan. The reaction was started by adding the glucose and hexokinase from one side arm and was stopped by adding H₂SO₄ from a second side arm. The gas phase was air and the temperature was 30° C, one side arm and was stopped by adding H₂SO₄ from a second side arm.
Effect of Parathyroid Hormone on Mitochondria

Vol. 241, No. 4

FIG. 2. The effect of pyridine nucleotide on the respiration of control and experimental (parathyroid hormone-treated (PTH)) mitochondria. Portrayed are tracings of the rates of oxygen consumption obtained in the Oxygraph. The "spikes" on the curves are artifacts caused by addition of reactants and mixing and have been reduced in size in this figure to conserve space. The general experimental conditions are those described in the text and Table I. At each of the places indicated by a "spike" one of the following was added: substrate (S), citrate or fumarate + pyruvate, 15 pmoles; ADP, 0.5 pmoles; DPN + TPN (PN), 2.0 pmoles of each. The respiratory rates per mg of mitochondrial protein for each segment of the curves are indicated by the affixed numbers.

DPN + TPN increased the respiratory rate up to 50%. The degree of stimulation with these substrates varied in different experiments and bore no discernible relationship to the extent of inhibition of respiration. In general, however, the relative effect of the pyridine nucleotides on respiration was greatest with citrate and isocitrate, and with these substrates the respiratory rate was elevated close to or in some cases above the control level. This latter situation is portrayed in Fig. 2. The total mitochondrial alteration was not simply a result of a deficiency of pyridine nucleotide, however, since these cofactors failed to correct the difference in rate between the control and experimental mitochondria with the remaining substrates. Moreover, as shown in Fig. 2, even when, in the case of citrate, DPN + TPN stimulated respiration above the control level, the alteration evidenced by loss of respiratory control was not corrected. These curves show that with the experimental mitochondria, pyridine nucleotide increased the respiratory rate irrespective of the presence of ADP. Of further interest in these experiments was the finding that there was no difference in pyridine nucleotide content between control and experimental tissue as revealed by direct assay of the mitochondria and original homogenates from which these mitochondria were prepared.

Other cofactors which were tested for their ability to restore respiration of the experimental mitochondria were coenzyme Q₉ (6 × 10⁻⁵ m) and cytochrome c (1 × 10⁻⁴ m) which were added singly or in combination in the presence of pyridine nucleotide. These had no effect on respiratory rate.

Control and experimental mitochondrial suspensions were tested in combination, or alternatively, supernatant fluids obtained from heat-inactivated control suspensions (85° for 5 min) were added to the experimental mitochondria. In no case was the respiratory rate greater than that predicted from simple addition of the respiratory rates of the control or experimental components of the system.

Because a limited respiratory rate due to parathyroid hormone might be due to a limited entry of the added substrates and cofactors to the mitochondria, the experimental mitochondrial suspensions were treated by sonic oscillation for periods up to 3 min in a Raytheon sonic oscillator in the original 0.25 M sucrose suspension or after resuspension in 0.03 M potassium phosphate buffer, pH 7.4. Such treatment, which might be expected to decrease a possible permeability barrier (30), did not increase the respiratory rate for citrate, succinate, or pyruvate + fumarate (the three substrates tested in this study) but instead decreased the already depressed rate slightly.

Oligomycin (5 µg per ml), 2,4-dinitrophenol (2 × 10⁻⁵ M), and CaCl₂ (1.3 × 10⁻⁴ M) did not alter the respiratory rate of experimental mitochondria while exhibiting the expected inhibitory or stimulatory action in the control mitochondria. These results indicate that the decreased respiratory rate brought about by parathyroid hormone was not a result of a block in the phosphorylation chain.

Enzyme Levels—The possibility that the treatment with para-

---

TABLE V

A. Effect of parathyroid hormone on enzymatic activity of kidney mitochondria

The general methods for measurement are described in the text or in Table I. For the enzymatic assays of malate and isocitrate dehydrogenases, the mitochondrial suspensions used to determine respiratory rate were diluted with 3 parts of H₂O₂ and were treated by sonic oscillation for 2 min before assay.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>370</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>141</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>109</td>
</tr>
</tbody>
</table>

* Millimicromoles of substrate per min per mg of protein.

B. Effect of parathyroid hormone on respiratory rate of kidney mitochondria

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Respiratory rate†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Succinate</td>
<td>119</td>
</tr>
<tr>
<td>Fumarate + pyruvate</td>
<td>65</td>
</tr>
<tr>
<td>Isocitrate</td>
<td>30</td>
</tr>
</tbody>
</table>

† Millimicromoles of O₂ per min per mg of protein.
thyroid hormone lowered the actual enzyme level in the mitochondria which in turn caused the depressed respiratory rate was tested by assaying the activity of three representative enzymes; isocitrate dehydrogenase, malate dehydrogenase, and succinate dehydrogenase. Table V lists data from a typical experiment which show that despite an inhibition in respiratory rate of 50%, the levels of activity of these enzymes were the same in the control and experimental mitochondria.

**Elevation of Serum Calcium Concentration by Injection of CaCl₂**—In order to determine whether or not the changes in respiration brought about by parathyroid hormone were secondary to the hypercalcemia which invariably existed at the time at which the animals were killed, serum calcium concentration was elevated by injection of suspensions of CaCl₂ in olive oil. This procedure has been described previously (11). In these studies the serum calcium concentration was maintained at levels up to 20 mg/100 ml for 6 hours at which time the animals were killed and mitochondria were prepared. Control animals received similar injections of sodium chloride suspensions. This treatment had no appreciable effect on respiratory rate or respiratory control.

**Discussion**

The present report indicates that parathyroid hormone administered to the intact animal produces a striking alteration in kidney mitochondrial metabolism which is evidenced by a depressed respiratory rate and level of phosphorylation. The immediate reasons for these changes are not known. Appropriate experiments seem to rule out alterations due to decreased permeability to substrate, decreased enzyme level, or diminished amount of certain cofactors in the electron transport chain. Moreover, a block in the phosphorylation chain could not account for the decrease in respiratory rate since agents such as Ca++ and 2,4-dinitrophenol did not stimulate the respiration of the experimental mitochondria. It is suggested that the treatment with hormone produced multiple biochemical alterations in the mitochondrion rather than a single change in one component, as indicated by the partial to complete restoration of respiration by DPN and TPN with citrate or isocitrate, the lack of effect of these pyridine nucleotides with the other substrates, and the inability to restore respiratory control to normal levels with any of the substrates. One possibility is that these changes reflect alteration in the morphology of the mitochondrion.

Although the depressed respiration with citrate and its stimulation by pyridine nucleotides resemble the findings of Costello and Darago (15, 16), the over-all inhibition of respiration and respiratory control with all of the substrates tested reported herein does not support the concept of an exclusive control on citrate oxidation by parathyroid hormone. The reasons for the discrepancy between our data and those of Costello and Darago (15, 16) could be the result of differences in experimental design, specifically in dosage level and animal species. Whereas the former workers used rats and injected hormone at a level of 6 units per g divided into 3 doses, our results were obtained in the rabbit with a single injection at a level of 0.2 to 1.0 unit per g.

The alterations in phosphorylation induced by the administration of parathyroid hormone in vivo agree with the data of Stalls and DeLuca (8) who found that parathyroid hormone added in vitro stimulated mitochondrial ATPase and inhibited the ATP-P₄ exchange reaction. On the other hand, a stimulation of respiration (6, 7, 9, 31) has been observed when the hormone was used in vitro, in contrast to the inhibition reported in the present study. Two other important differences between the effects of parathyroid hormone in vivo and in vitro also must be cited. First, in the studies in vitro both liver and kidney mitochondria gave equivalent results, whereas only kidney mitochondria appear to change when the route in vivo was used. Second, the changes reported for the studies in vitro occur within a few minutes after addition of the hormone to the experimental system, compared to the extended time required for the mitochondrial changes to be detected when the hormone is administered in vivo. One reason for these differences might be the large amounts of hormone used in the majority of the systems in vitro (6, 7, 9), levels ranging from 40 to 400 units of hormone per 4 mg of mitochondrial protein compared to only 200 to 750 units per the whole animal in the present report. It is probable, therefore, that the changes produced by the hormone given in vivo are unrelated to the immediate effects of the hormone produced by its addition to a system in vitro.

It is still required to determine whether or not the present changes in mitochondrial metabolism are related to the physiological action of parathyroid hormone. Certain evidence suggests that they are: the effects of treatment were found in kidney, a target organ of parathyroid hormone, and not in liver, which is not considered to be a target organ; elevation of serum Ca++ concentration by injection of CaCl₂ failed to reproduce the effects in mitochondria indicating that these changes were not secondary to the hypercalcemic action of the hormone; and finally, although the hormone preparations used in this study were still heterogeneous, the changes observed appeared to be related to their calcium-raising properties. Thus, in one experiment a solution of hormone at approximately 2000 units per mg of protein which had produced the usual mitochondrial effects was kept frozen for 6 weeks. At that time it was tested in the rat and was found to have lost its biological potency and it also failed to produce any mitochondrial changes. Since the changes in mitochondria were not detected prior to 8 hours after administration of the hormone, and whereas it is well known that phosphate excretion by the kidney commences within a few minutes after treatment (32), these alterations were probably not related to phosphate excretion by the kidney. Rather, since elevation of serum Ca++ concentration occurs several hours after injection of hormone (32) and the experimental animals were invariably hypercalcemic at the time of death, it is likely that the observed changes were related to the process of Ca++ elevation. That such a biochemical separation of the immediate and long term actions of the hormone is possible is shown in studies in which the hypercalcemic, but not phosphaturic, action of parathyroid hormone is blocked with actinomycin D (32, 33).

If the present results are extrapolated to those in bone (10, 34), the decrease in conversion of Krebs' cycle intermediates to CO₂ produced by the administration of parathyroid hormone in vivo can be similarly attributed to an altered mitochondrial metabolism of bone cells. This conclusion is in contrast with certain evidence which suggests that the inhibition in bone was due to a limited entry of the substrate into the cell (10). Also it would not explain the marked change in liver cell respiration produced by similar treatment with the hormone (11), since in the present studies no change in liver mitochondrial respiration was detected.

It is now clear that parathyroid hormone can exhibit a variety of actions depending upon its route of administration, the tissue...
investigated, and possibly the dosage level (10). Further elucidation of these effects and their relationship one to the other are required for a complete understanding of the biochemical mode of action of the hormone.

Acknowledgment—We are indebted to Dr. R. D. Dallam for his assistance in establishing techniques for the studies on ATP-Pi exchange rates and for his many helpful suggestions.

REFERENCES
The Inhibition of Respiration and Phosphorylation in Kidney Mitochondria by Parathyroid Hormone Administered in Vivo
David V. Cohn, Anna F. Smaich and Robert Levy


Access the most updated version of this article at http://www.jbc.org/content/241/4/889

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/241/4/889.full.html#ref-list-1