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SPECIFICITY OF THE ACTION OF PARATHYROID HORMONE UPON MITOCHONDRIAL METABOLISM

CYANOGEN BROMIDE-DERIVED PARATHYROID-HORMONE PEPTIDES

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SUMMARY

The mitochondrial response to cyanogen bromide-treated parathyroid hormone was studied as a means of testing further the relationship between the structure and the effects *in vitro* of this hormone. The treated hormone and appropriate control hormone were tested in a standard bioassay and in a mitochondrial assay system *in vitro*.

Reaction of more than 90 % of the methionine residues in the hormone resulted in total inactivation of the hormone both *in vivo* and *in vitro*. This result disagrees with previously published data.

INTRODUCTION

The initial studies[1–4] of the effects of parathyroid hormone upon respiration and ion transport in isolated mitochondria led to the idea that these effects were highly specific and related to the physiologic effects this hormone produces *in vivo*. However, the specificity of the mitochondrial response to parathyroid hormone was subsequently questioned by AURBACH, HOUSTON AND POTTS[5,6], who found that several non-hormonal basic polypeptides were capable of inducing some of the same changes in mitochondrial metabolism produced by parathyroid hormone. In addition, POTTS AND AURBACH[7] and POTTS, AURBACH AND SHERWOOD[8] reported that treatment of the purified bovine parathyroid polypeptide with CNBr, according to the method of GROSS AND WITKOP[9], led to a total loss of its effect *in vivo*, but did not destroy its ability to alter mitochondrial metabolism *in vitro*. This, if true, would cast considerable doubt upon the mitochondrial responses to parathyroid hormone as being specific and of physiologic significance. However, further investigations of this problem of hormonal specificity in our own laboratory[10,11] have shown that, under appropriate experimental circumstances, it is possible to distinguish clearly between the effects of parathyroid hormone and those of a number of other basic polypeptides upon mitochondrial metabolism. Furthermore, four chemically modified derivatives of bovine parathyroid hormone have been prepared and their comparative activities examined *in vivo* and *in vitro*[11]. With each of these derivatives, there was a comparable

loss of activity *in vivo* and *in vitro*. This latter result contrasted so sharply with the initial reports of POTTS AND AURBACH[7] and POTTS, AURBACH AND SHERWOOD[8] that we deemed it essential to reinvestigate the effect of CNBr treatment upon the acitivity of bovine parathyroid hormone both *in vivo* and upon mitochondrial metabolism *in vitro*.

MATERIALS AND METHODS

Parathyroid hormone was prepared by a modification of the method of HAWKER, GLASS AND RASMUSSEN[12] using Sephadex G-75 rather than Sephadex G-100 in the initial gel filtration of the trichloroacetic acid precipitate prepared by the urea–HCl–cysteine method.

A modification of the method of GROSS AND WITKOP[9] was used to prepare cyanogen bromide peptides. The parathyroid hormone was dissolved in 0.01 M acetic acid containing 1/1000 (v/v) 2-mercaptoethanol, and allowed to remain at 20° for 12 h. This solution was then frozen and lyophilized. Immediately upon release of the vacuum under N_2, 3 ml of 0.1 M HCl containing 97 mg of CNBr was added to the lyophilized hormone, the solution cooled and the air evacuated from the tube. The vacuum was released under N_2, and the reaction allowed to proceed for 18 h. The reaction mixture was then diluted with 0.01 M acetic acid, frozen and lyophilized. The lyophilized powder was dissolved in 15 ml of 0.01 M acetic acid, frozen and lyophilized. This was repeated 4 times. The hormone was then redissolved in 0.01 M acetic acid containing 1/1000 (v/v) 2-mercaptoethanol and allowed to remain at 20° for 18 h. The preparation was frozen and lyophilized. An appropriate control was prepared as described above, deleting the CNBr from the HCl. Two-dimensional thin-layer chromatography was carried out using 20 cm × 25 cm glass plates as support and cellulose as adsorbent. The solvent for the first system was butanol–acetic acid–water (70:60:20, by vol.), and that for the second, butanol–pyridine–water (85:50:40 by vol.). 100-μg samples were spotted for each chromatography.

Amino acid analyses were performed on a single-column system[13] employing a Technicon amino acid analyzer (Technicon Chromatography Corp., Ardsley, N.Y.) equipped with a digital read-out integrator (Model CRS 10AB, Infotronics Corp., Houston, Texas).

Rat liver mitochondria for assay *in vitro* of the hormone preparations were prepared by a modification of the method of SCHNEIDER[14] using 0.37 M sucrose containing 0.1 mM EDTA and centrifugation at 480 × *g* for 10 min, 4340 × *g* for 20 min and 7710 × *g* for 10 min, respectively, in a Sorvall RC-2 centrifuge. The assay was carried out as previously described[11]. The assay *in vivo* of the hormone preparations was performed by a modification of the method of MUNSON[15] using thyroparathyroidectomized rats.

RESULTS

Treatment of bovine parathyroid hormone with CNBr resulted in the specific destruction of more than 90% of the methionine residues when the product was analyzed by amino acid analysis (Table I). Concomitant with this destruction was the appearance of a new ninhydrin-positive peak in the amino acid chromatogram

TABLE I

AMINO ACID COMPOSITION OF PURIFIED BOVINE PARATHYROID HORMONE BEFORE AND AFTER TREATMENT WITH CNBr

Amino acid	*Residues per mole*	
	Parathyroid hormone	*CNBr-treated parathyroid hormone*
Cysteic acid	0.00	0.00
Asp	8.10	8.65
Thr	0.78	0.96
Ser	6.60	7.10
Glu	10.95	11.34
Pro	N.D.*	N.D.*
Gly	5.09	4.99
Ala	6.60	6.73
Val	6.03	5.96
Met	0.74	0.00
Ile	2.70	2.88
Leu	7.65	9.03
Tyr	0.77	0.80
Phe	2.25	1.92
Lys	7.52	9.23
His	2.84	2.11
Arg	5.70	5.19

* Not detected because of technical reasons.

between serine and glutamic acid, the position where homoserine is normally found. There was no significant change in the content of any other amino acids.

The treated hormone was also analyzed by two-dimensional thin-layer chromatography, and found to contain 3 new ninhydrin-positive spots, none of which corresponded to the original hormonal polypeptide, and a faint spot in the same position as the native hormone.

When the peptides derived from CNBr-treated hormone were tested *in vitro*,

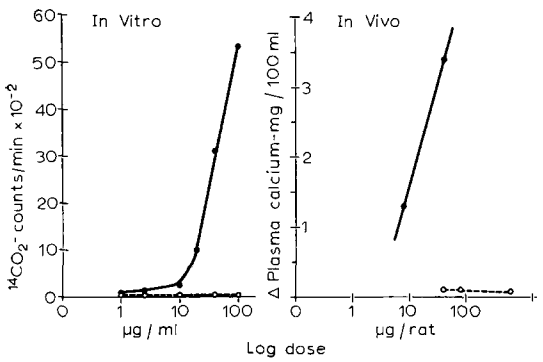

Fig. 1. The dose-response relationship observed in a mitochondrial assay system *in vitro* (left) measuring hormone stimulation of succinate decarboxylation in a medium containing Mg^{2+} and phosphate and in the standard rat assay *in vivo* (right) measuring change in plasma calcium 6 h after intraperitoneal injection of test samples when either parathyroid hormone (●—●) or CNBr-treated parathyroid hormone (○ – – – ○) was employed.

they were inactive at a dosage of 100 μg/ml even though the hormone put through the entire procedure, except exposure to CNBr, possessed considerable activity even at 5 μg/ml and produced a striking effect at 100 μg/ml (Fig. 1). *In vivo*, there was a concomitant loss of hormone activity.

DISCUSSION

The present results clearly show that, under the conditions of our experiments, CNBr treatment of bovine parathyroid hormone leads to nearly complete loss of the two methionine residues in the native hormone without altering the content of the other amino acids; this change is accompanied by evidence of nearly complete cleavage of the native molecule into a mixture of smaller peptides; and none of these peptides possesses biological activity when tested *in vivo* or *in vitro*. The latter conclusion is opposite to that of POTTS, AURBACH AND SHERWOOD[8], but agrees with previous evidence that other chemical alterations of the methionine residues lead to nearly total loss of biological activity *in vivo* and *in vitro*[11,16].

It is not possible at present to resolve the difference between the present evidence and the report of POTTS, AURBACH AND SHERWOOD[8]. This is particularly difficult because no experimental details were given in their report and it is possible that a difference in experimental technique is the basis for these conflicting results. Nevertheless, the present results are reproducible and constitute further support for the notion that the effect of the parathyroid hormone upon the functional properties of the mitochondrial membrane is highly specific.

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