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Presidential Address

Parathyroid Hormone — Quo vadis?

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Introduction

Parathyroid hormone (PTH) is unique among several hundred hormones, because it is a hormone for calcium. What hormone does not require calcium for its secretion and action? What cell does not require calcium for proliferation, movement, excitation and secretion? PTH which is directly involved in the control of calcium metabolism at both the whole body and cellular level may therefore be called hormone of hormones. It may therefore be appropriate to ask "Parathyroid Hormone—Quo vadis? Where are you going?", because PTH represents all the hormones.

Aging and Parathyroid Hormone

All creatures living on land are in a calcium-deficient state compared to the more primitive form of life living in calcium-rich seawater. As they age in such calcium deficient state, the degree of calcium deficiency naturally becomes more pronounced. Although serum calcium does not fall remarkably with advance in age, serum parathyroid hormone rises progressively with age.^{1~3)} Calcium deficiency may be the major factor to cause an increase of PTH secretion, possibly through repeated, transient and mild decrease of serum ionized calcium. Progressive fall of renal function with age leading to a decrease of 1,25(OH)₂vitamin D synthesis may also contribute to an increase of PTH secretion in aging. Aging thus causes secondary hyperparathyroidism as chronic renal failure does.^{4, 5)} The terminal serum PTH peak appearing late in the life of rat heralding the impending death may be explained by progressive renal failure, in view of the prevention by low protein diet which prolongs the life span probably through a reduction of the deleterious hyperperfusion.⁴⁾ Since PTH is the most important factor in maintaining body calcium economy and constant serum calcium level by mobilizing calcium from the bone and stimulating renal biosynthesis of 1,25(OH)₂ vitamin D essential for intestinal calcium absorption, its increase with advance in age may indicate an active compensatory response of the living organism against calcium deficiency progressing along with aging.

As shown in Fig. 1, the degree of calcium deficiency increases with age, along with a decrease of total calcium in the body and bone calcium content, leading to osteoporosis. Serum PTH increases with aging. When PTH mobilizes calcium from the bone, some of the calcium released from the bone enters the soft tissue especially blood vessel and brain, providing a background for hypertension, arthrosclerosis and senile dementia. PTH is thus

a pace-maker for aging, and may therefore be called a hormone for aging. Since aging is always associated with progressive calcium deficiency, PTH may also be called a hormone of calcium deficiency.

Fig. 1.

Lifespan of Parathyroid Hormone

Parathyroid hormone is also unique in its role leading to the recognition of hormone heterogeneity in peripheral blood. The group of Berson and Yalow^{6~7)} was the first to recognize this phenomenon by using two kinds of antibodies to measure PTH in human peripheral blood. The half-life of PTH in blood was quite different between values obtained by different assays. One antibody recognizing a rapidly disappearing hormone was evidently directed to the N-terminal portion or intact hormone, whereas the other recognizing a slowly disappearing hormone was presumably directed to the C-terminal portion or fragments containing it.

The concept of hormone was basically challenged by this epochmaking observation. A hormone is not an absolute, chemically defined identity any more. It is rather a series of substances undergoing changes comparable to a life cycle, just like a human being under a continuous process of growth, maturation, aging and death. A hormone is synthesized, processed, secreted, distributed through the blood stream and finally brought to the receptor of the target cell. After binding to the receptor, it is again processed, internalized, degraded and re-utilized. Following this cycle of change, it is evidently difficult to define which is

the real hormone. The substance which is mainly extracted from the secreting gland is frequently thought to be the hormone, but this does not readily apply to hormones which are short-lived in the secretory gland. Major circulating form again cannot be readily defined as the hormone, in view of the heterogeneity described above. Could a hormone be defined as the most active substance? The distinction between hormone and active fragment again becomes difficult to make. Thus the true nature of a hormone is a great challenge to endocrinologists until the details of processing and degradation of the hormone is clarified. Parathyroid hormone provides the best example of this broad and dynamic definition of a hormone. Splitting of PTH is already started in the parathyroid cells, so that fragments are secreted alone with intact hormones, necessitating the broad new definition of a hormone.⁸⁾

Parathyroid Hormone Metabolism

Protein and peptide hormones are usually metabolized through hydrolysis by proteases, classified into two major categories as shown in Table 1. Final degradation of hormones into inactive fragments may be carried out by random, non-specific and unlimited hydrolysis, usually not under a metabolic control. Since no physiological action of the generated fragment is expected, the only physiological significance of such random hydrolysis of a hormone is a control of the life span of the hormone in circulating blood, indirectly regulating the hormone action. By stimulating or suppressing degradation, the action of a hormone may be inhibited or augmented respectively.

Processing of a hormone, on the other hand, is a specific, limited and metabolically controlled process to produce active fragments with significant physiological function. This may occur not only in the hormone-secreting cells, but also in the target cells after

Table 1. Hydrolysis of protein and peptide hormones.

	Degradation	Processing
Mode of Hydrolysis	Random	Limited
Selectivity	Non-specific	Specific
Metabolic Control	Controlled	Uncontrolled
Product	Useless	Active
Site	Lysosome	Membrane Cytosol
Physiological Significance	Control of Hormone Half Life in Blood	Control of Hormone Action by Active or Inhibitory Fragments

binding of the hormone to the receptor. Parathyroid hormone metabolism occurs in the parathyroid gland, kidney, liver bone and blood cells.^{9~13)} Cathepsins B and D were identified as enzymes responsible for the specific and limited PTH-hydrolysis in the liver, parathyroid glands and kidney.^{14~16)}

Unique PTH-hydrolyzing Enzyme in Rat Kidney Cell Cytosol

Orimo et al^{17~18)} were the first to demonstrate inactivation of parathyroid hormone by rat kidney utilizing a bioassay. Fujita et al^{19~20)} and Maruyama et al²¹⁾ used ¹²⁵I-labelled PTH to study the enzymatic mechanism of PTH hydrolysis by rat kidney. A microsomal endopeptidase with pepsin-like substrate specificity was partially purified. This enzyme, preferentially hydrolyzing PTH, however, hydrolyzed ¹²⁵I-PTH into small fragments in a random and unlimited fashion, being activated by Ca. In the course of investigating the specificity and function of PTH-hydrolyzing enzyme in various fractions of rat kidney cell, inhibition of ¹²⁵I-labelled PTH hydrolysis by native unlabelled PTH was tested. Failure of suppression of ¹²⁵I-labelled PTH hydrolysis by unlabelled PTH suggested an extremely high substrate specificity of PTH-hydrolyzing enzyme and a distinction made by the enzyme between labelled and unlabelled PTH. Different enzymes are apparently responsible possibly due to some change in the configuration of the substrate hormone molecule. Responses to inhibitors were also different between these two enzymes. Rat kidney cell 10⁵ × g supernatant corresponding to the cytosolic fraction was found to possess a potent activity specifically hydrolyzing native unlabelled PTH in a limited hydrolysis at neutral pH.²²⁾ While kidney cathepsin D shown by Zull et al¹⁶⁾ to split PTH in a limited hydrolysis was activated by ATP, the newly demonstrated rat renal cell cytosolic enzyme was inhibited by ATP, glutathione and calcium ion. The molecular weight appears to be large, similar to the high molecular weight cytosolic protease described by Tanaka et al.²³⁾ The unique property of

Table 2.

PTH ases

Source	Fraction	Enzyme	Site of Cleavage
Bovine Liver & Parathyroids	Lysosome	Cathepsin B	36-37
Bovine Liver & Kidney	Lysosome	Cathepsin D	34-35 37-38
Rat Kidney	Cytosol	High Molecular Weight Neutral Peptidase	
UMR-106 (Osteoblasts) OK (Proximal Tubule)	Membrane	Chymotrypsin-like Endopeptidase	23-24 34-35

this enzyme is illustrated by the failure of inhibition by trypsin inhibitor, PMSF, E64, leupeptin, chymostatin and pepstatin. This enzyme hardly hydrolyzes several synthetic analogues containing MCA except for Leu-MCA and Z-Phe-Arg-MCA. When Z-Phe-Arg-MCA was used as the substrate, this enzyme was activated by ATP and GSH, clearly distinguishing itself from the neutral PTH ase.²⁴⁾ The cell origin of this enzyme, however, remains to be established. Through studies on PTH degradation by intact kidney and bone cell line. (Table 2)

PTH Degradation by Intact Cells

In an attempt to clarify the relationship between action and degradation of PTH in a more uniform system, intact cell lines obtained from the bone and kidney tissue, the main target organs for PTH, were utilized. Both UMR-106, osteoblast-like osteosarcome cell line responded to PTH specifically through binding to a receptor. Both of these cell lines also degraded PTH in a precess of limited hydrolysis.²⁵⁾ On incubation of human PTH 1~84 with intact UMR-106 cells, distinct peaks in HPLC corresponding to 1,23,24~84, 1~34 and 35~38 were obtained, as confirmed by amino acid analysis.

The degradation of parathyroid hormone by these cell lines is apparently accomplished by an enzyme attached to the cell surface because the conditioned medium obtained through incubation of these cell lines did not possess any PTH-degrading activity. On incubation of the cell with PTH, no PTH fragments were found to be associated with cell homogenate, making the possibility of internalization of the hormone followed by degradation unlikely. Inhibition studies suggested that the enzyme on the cell surface responsible for PTH degradation apparently possessed the characteristics of chymotrypsin, inhibition by PMSP and chymostatin, and the failure of inhibition by Ep 475, leupeptin, trypsin inhibitor, elastatinal, phenanthroline, phosphoramidon, pepstatin A, chloroquine, ammonium chloride and monensin.

The PTH degradation on the cell surface, furthermore, was apparently mediated by PTH receptor, since the time course of cyclic AMP production was immediately followed by PTH fragmentation, and hPTH3-34 binding to PTH receptor inhibits both PTH degradation and cyclic AMP production. Cyclic AMP-A-kinase system, however, appeared to inhibit PTH degradation. Another important system of intracellular signal transduction, C-kinase system, on the contrary, appeared to stimulate PTH degradation. Phorbol ester mimicking C-kinase action, TPA, as well as ionophore A23187 increasing cytosolic free calcium augmented PTH degradation independently and synergistically. PTH-degrading activity by chymotrypsin-like enzyme on the cell surface of UMR-106 and OK cells thus appear to be controlled by cyclic AMP-A kinase system and intracellular calcium-C-kinase system (Fig. 2). The presence of PTH-specific degrading system on the cell surface of UMR-106 and OK cell operating through PTH specific receptor under a precise control system may confirm the inseparable relationship between PTH action and degradation, although the details of such inter-relationship remains to be elucidated. PTH degrading enzymes are summarized in Table 2. In addition to lysosomal cathepsin B and D, high molecular weight neutral

cytosolic PTH ase in rat kidney cell and chymotrypsin-like PTH ase on the surface of PTH-responsive cells UMR 106 and OK cells participate in PTH degradation. The precise physiological control of these enzymatic process connected with PTH action may suggest that PTH degradation is a specific and significant process connected with PTH action.

Mechanism of PTH Degradation

Fig. 2. Mechanism of PTH degradation and its metabolic control.

Summary

Parathyroid hormone is the most important calcium regulating hormone, especially in the process of aging. As a compensation for progressive calcium deficiency in aging, PTH secretion progressively rises. Heterogeneity of PTH in peripheral blood indicates the importance of understanding the degradation mechanism throughout the life cycle of the hormone. In addition to cathepsin B and D which degrades PTH in the liver, kidney and parathyroid gland, a new neutral PTH ase was found in the cytosolic fraction of rat kidney cells. PTH responsive bone and kidney cell lines, UMR-106 and OK cells, were found to degrade PTH by a chymotrypsin-like enzyme on the plasma membrane through a receptor mediated mechanism. This process was inhibited by cyclic AMP-A-kinase system and augmented by C-kinase system, suggesting an intimate relationship between PTH action and degradation and also a new physiological significance of PTH degradation.

Coworkers and Collaborators

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