The parathyroid polyhormone hypothesis revisited

EF Nemeth[1]

[1]NPS Pharmaceuticals, Toronto, Ontario, Canada

The parathyroid polyhormone hypothesis holds that peptides derived from the metabolism of parathyroid hormone (PTH) (so-called C-terminal fragments) are themselves biologically active and that their effects are mediated by a novel 'C-terminal receptor.' The evidence supporting these assertions is extensive but remains inconclusive. This Commentary focuses on *in vivo* pharmacology studies that provide information relevant to understanding the physiological significance of C-terminal fragments. The more recent studies of this sort provide compelling evidence that the bioactivity of C-terminal fragments is likely to become physiologically relevant in settings of secondary hyperparathyroidism. In this condition, circulating levels of C-terminal fragments greatly exceed those of PTH. There is convincing evidence that the hypocalcemic effect of C-terminal fragments results from direct actions on the skeleton that inhibit bone resorption. On the other hand, there are few if any results of *in vivo* studies suggesting a role for C-terminal fragments in more physiological settings, at least when parameters associated with systemic calcium homeostasis are assessed.

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Correspondence: *EF Nemeth, NPS Pharmaceuticals, 101 College Street, South Tower, Suite 800, Toronto, Ontario M5G 1L8, Canada.*
E-mail: enemeth@npsp.com

The physiological significance of peptides derived from proteolysis of parathyroid hormone (PTH) has remained controversial ever since they were first detected in 1971,[1] and it continues to be an aspect of parathyroid physiology that periodically captures general interest. This field of research merited two chapters of review in the first edition of *The Parathyroids*[2,3] but not a one in the second edition. In fact, the biological activity of PTH peptide fragments receives little mention in the latter edition ('Scant mention' is more accurate. Of 863 pages of text (including pages listing references), the biological activity of C-terminal fragments receives passing mention on three. There are four separate references to this topic in the 2nd edition of *The Parathyroids*. The interested reader with time on their hands can challenge these figures, but I believe they are correct.), an omission that might have signaled a resolution to this long-standing controversy by simply dropping it in the dustbin. However, this topical research has been plucked from the trash, dusted off, and presented anew. It cleans up nicely – and there is renewed interest in the biological activity of PTH-derived peptides.

Three quite disparate experimental approaches have provided the results that drew general attention: improvements in immunoassays for PTH, engineered cell lines that lack the classical PTH receptor, and an animal model showing a robust effect of PTH peptide fragments. These recent findings were covered in the presentations of Drs Pierre D'Amour and Harald Jüppner in the second session of the Symposium entitled 'Control and Action of PTH.'

The written version of Dr D'Amour's presentation appears in this Issue.[4] With initial resistance that finally yielded to constant prodding, I agreed to author some text in lieu of Dr Jüppner and was tasked with providing a general overview of the field. General reviews and commentaries on this topic are recent and plentiful;[5–9] the most comprehensive is that by Murray *et al.*[10] This Commentary differs from these overviews by viewing the controversy regarding bioactive PTH peptides through the lens of pharmacology. The view is admittedly narrow, but it brings into focus some studies that have previously escaped attention yet contain results that address the significance of these bioactive peptides in physiological or pathophysiological settings.

As one who has watched the action in this field from the sidelines, I take some risk in commenting about it. However, the task of doing so has fallen on many in the past who accept to chair a session and they have left some guidance. That left

by Roy Greep at the Third Parathyroid Conference in1967 is some of the best, and I use it now as my safe harbor statement:

> First, I must make it clear that my overview of this highly competitive area of research will be that of an interested observer and not a contestant. That position affords me the advantage of emotional detachment and of a no-iron-in-the fire point of view. The disadvantage is that an observer cannot gain the deep insight that comes with daily involvement with the thorny aspects of problems in research.[11]

Like Greep, I too will '…look for soft spots in this total effort…' and play the devil's advocate, mostly because it is an open position. However, I start angelically by reintroducing a term that is conceptually useful and perhaps physiologically meaningful.

THE PARATHYROID POLYHORMONE HYPOTHESIS

The moniker 'parathyroid polyhormone' appears to have originated in a review by Lawrence Mallette[12] and it is a term that has been used rarely since. When the phrase does appear, it is in reference to PTH-related peptide (PTHrP).[13,14] However, it is a term that succinctly and accurately describes the subject matter presented at the Symposium. And it remains a heuristic term – one that should prompt further experimental investigation in order to accept it into or exclude it from our lexicon but mostly to push back or eliminate this frontier of parathyroid physiology.

The version of the parathyroid polyhormone hypothesis offered here refers specifically to PTH and consists of two distinct assertions. The first is that peptides derived from the metabolism of PTH are biologically active. The second is that the biological effects of these fragments result from actions on a novel receptor – one that is not the PTH/PTHrP receptor (PTH-R1). Although the validity of the second assertion requires that of the first, PTH peptide fragments could express their biological activity without acting on a new receptor. The validity of both assertions has rather profound implications: nothing less than a previously unrecognized ligand(s) and receptor system that contributes to parathyroid physiology.

There is certainly no lack of peptide ligand candidates. The proteolytic breakdown of PTH both within parathyroid glands and at peripheral sites (mostly within Kupffer cells of the liver) gives rise to many structurally distinct N-terminally truncated peptides with intact C-terminals (collectively: C-terminal fragments).[10] These peptide fragments circulate at levels that normally surpass and, in end-stage renal disease, greatly exceed that of PTH.[4,15,16] Although there is a general agreement that the quantitatively major peptide fragments have N-terminals originally within the mid-molecule region of the parent hormone (positions 39–53) and end at position 84,[10] the unambiguous sequence identification of the quantitatively dominant species has just recently been reported.[17] In contrast, N-terminally intact PTH peptides, like the 1–34 fragment teriparatide, are generally believed to be absent.[9,10] Only one report describes the detection and characterization of an endogenous N-terminally intact peptide fragment of PTH (PTH(1–37)[18]); if this fragment does normally circulate, its contribution to the total pool of PTH peptide fragments is quantitatively minor. In any event, the major ligand candidates at present are mid-molecule C-terminal fragments that include 'non-1–84 PTH,' generally thought to be PTH(7–84). Moreover, new peptide ligand candidates continue to be identified.[4]

C-terminal fragments are biologically active *in vitro*

Most of the evidence demonstrating biological effects of C-terminal fragments derives from *in vitro* studies and the sheer volume of such reports is staggering.[10] Typically, concentrations of C-terminal fragments several orders of magnitude higher than those that appear in the circulation are required to elicit a cellular response, but there are plenty of reports showing quite dramatic effects at peptide concentrations that are likely within the physiological range, probably within the therapeutic, and certainly correspond to the much higher levels that occur in patients with severe secondary hyperparathyroidism. The remaining uncertainties about the precise structure of C-terminal fragments will likely soon vanish when improved bioanalytical methods are combined with those of immunoassay. And there are no early indications that unambiguous structural identification of peptide fragments derived from PTH will greatly alter the conclusions drawn from *in vitro* studies.

Many of the responses affected by very low concentrations of C-terminal fragments, however, are in transformed cell lines and the limitations inherent in these cellular systems are well known. This is not to deny the insights that can be gained from *in vitro* experiments. Indeed, it can be argued that Tim Murray's observations using a rat osteosarcoma cell line[19] were the spark that ignited real interest in this field. In addition, *in vitro* studies in a transformed cell remains one of the few practical ways to tease out the molecular mechanisms underlying physiological phenomenon. However, a physiological phenomenon is what seems to be lacking when trying to make sense of the *in vitro* results. The literature is replete with reports showing that all kinds of cellular responses, in a wide variety of functionally distinct tissues, can be elicited or modulated by exposure to C-terminal fragments. The *in vivo* corollary and the physiological context to place the diverse effects observed *in vitro* is either entirely lacking or too speculative. The literature is becoming awash with cellular and molecular mechanisms that need something to explain. Gathered here for consideration are results of *in vivo* studies that might clarify this complexity.

C-terminal fragments are biologically active *in vivo*

One approach of determining the physiological significance of PTH C-terminal fragments is to perform head-to-head comparisons of PTH and teriparatide *in vivo*. The logic of this approach is simple: the exogenous administration of