A PERSONAL PERSPECTIVE ON 50 YEARS OF PTH RESEARCH AT MGH:

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Historical Overview

Hyperparathyroidism

Osteoporosis

Hyperparathyroidism
On A New Gland In Man And Several Mammals

Ivar Sandström

A translation from the Swedish by Dr. Carl M. Seipel of Stockholm. From *Upsala Lakareforeneings Forhandlingar*, 1879-80, 15, 444-471
Ivar Sandström illustration

After Nordenström
Debates over Function of Parathyroids

- 1880 Sandström
  - Clearly recognized four (parathyroid) glands
    - no means to test function

- 1891-1895 Gley
  - thyroidectomy causes tetany

- 1896-1900 Vassele and Generale
  - parathyroidectomy not thyroidectomy causes tetany

- 1900-1910 debate continues

- 1910 general agreement key role of parathyroids in tetany
1910-1925 Cause of Tetany (mechanism) debated

- Hypocalcemia is the cause
  - Oral, parenteral calcium reverses tetany

- Methyl quanidine intoxication is the cause
  - Found in urine after parathyroidectomy
  - Causes tetany like Sx when given to animals

- Source of confusion-
  - Gland extracts did not reverse the tetany
At the University of Alberta in Edmonton, Canada, James Bertram Collip discovers that highly potent parathyroid extract restores blood calcium to normal limits in parathyroidectomized dogs (*J. Biol. Chem.* 63:395, 1925).

“The normal function of the parathyroid gland would appear to be related to... direct control of the calcium level of the blood...”

“. . .these glands have no function to them other than that of regulation of calcium metabolism.”
Chronology of Major Advances in PTH Research

- Parathyroid glands found in the human as separate entities from thyroid
  - Function unknown

- Parathyroid gland function debated
  - Tetany after parathyroidectomy
    - Cause - hypocalcemia vs methyl guanidine

1900-1925

- Active gland extract purified
  - Calcium regulation established

1927-1950s

- Pathophysiology of hormone excess and deficiency defined
  - Hyper- and
How Hyperparathyroidism was Discovered and Treated

Uncharted Seas

by Fuller Albright

with Read Ellsworth

Edited by Lynn Loriaux, M.D., Ph.D.

Kalmia Press, Portland, Oregon 1990
Enlarged glands in von Recklinghausen's (now known to be severe HPT)

Enlarged parathyroids in osteomalacia

Uncertainty whether enlarged parathyroids were the cause or consequence of the bone disease (Erdheim)

First successful parathyroidectomy with cure after failed parathyroid transplant (Mandl, 1925)
North American School: Function, Not Structure

**CASE OF CAPTAIN CHARLES MARTELL**

1925: Collip
Extraction active principle of parathyroids - elevates calcium in animals

1926: Links between bone disease, high blood calcium and PTH excess
DuBois, Bellevue
Confirmed at MGH, many unsuccessful OP. High calcium intake in C.M. – bones heal, but kidney stones develop

1932: Mediastinal adenoma found and removed. Death soon after, however, from complications.
40 years ago the diagnosis of hyperparathyroidism was accepted as established only after successful parathyroidectomy.

Today, diagnosis is accepted as convincing when PTH assay is (inappropriately) elevated in the absence of secondary causes.

Disease now usually mild, guidelines established to evaluate need for surgery.
Asymptomatic Hyperparathyroidism: More recent developments

- Significant skeletal improvement post parathyroidectomy confirmed with reliable methods
- Increased incidence of vertebral as well as non-vertebral fractures confirmed
- More intense renal evaluation could predict/detect nephrolithiasis
Asymptomatic Hyperparathyroidism (Continued)

- No clear data re cardiovascular and neurocognitive complications
- Surgical advances (MIP with imaging) more favorable benefit vs convenience/risk
- Guidelines tightened re skeletal and renal complications
PTH for Osteoporosis: A Paradox

- How did it happen?
  - Early findings long neglected
  - Interest renewed after 40 years
  - Research advances needed
  - Role academic/industrial partnership

- How does it work?

Confirmed by Selye

1932

*After these reports no further studies for 40 years.*

1965-1972  • NIH + MGH: The isolation, structure, and synthesis of PTH provides pure material for clinical study
1959


1960s

- Improvements in purification and protein sequence analysis

1970s

- PTHrP, PTH1R cloned
- Many PTH molecules back to fish
- PTH receptor traced to pre-vertebrates
- Genetic modification in rodents defines role of PTHrP

1980s-1990s

Hormone structure and synthesis
History of PTH as a Bone Anabolic Agent

1975
- Rapid improvement in techniques for accurate assessment of bone mass
- Resumption of clinical interest
- MGH, England and France: Trials with PTH in osteoporotic patients begin

2001
- Striking efficacy found in bone mass and fracture prevention in controlled international study

2002
- Approved by the FDA for therapy of osteoporosis

**Improve utility**

Combination with anti-resorptives

- Bisphosphonates
- Denosumab
- Other?

**Improve product**

- More Effective PTH/PTHrP Analogue
- Non-parenteral delivery of PTH peptide agonists
- 2nd-generation – orally active small molecule
- 3rd generation – downstream targets of PTH anabolic action on bone cells
94 postmenopausal osteoporotic women assigned to 2 years treatment with:

- Teriparatide
- Denosumab
- Both
**PTH for Osteoporosis: Future Directions**

**Improve utility**
Combination with anti-resorptives
- Bisphosphonates -
  - Denosumab +
- Other?

**Improve product**
- More Effective PTH/PTHrP Analogues
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PTH Research (2014- ): Improved insights into Molecular and Cellular Mechanisms of Hormone/Action

- Explains apparent paradox: Two ligands (PTH/PTHrP) serving distinctive biological functions/only one receptor

- Results in development of improved PTH/PTHrP analogs for therapeutic uses
Activation by PTH(1-14)

Aib Aib
A1 V2 S3 E4 I5 Q6 L7 M8 H9
Gln Har Ala Trp
N10 L11 G12 K13 H14

Shimizu, M 2000 J.B.C.
Shimizu, M 2001 Endocrinology
Shimizu, N 2001 J.B.C.
Shimizu, N 2004 J.B.M.R.
Shimizu, N 2005 J.B.C.
Differences in Internalization

\[ \text{GFP} \text{PTHR} + \text{PTH}(1-34)\text{TMR} \quad \text{GFP} \text{PTHR} + \text{PTHrP}(1-36)\text{TMR} \]


Other *in vitro* Evidence for Distinctive \( R^0 \) Conformation

- Resistance to GTP\( \gamma \)S in binding assays
- Protracted cellular signaling after ligand wash-out
- Biophysical studies
  - FRET
  - Spinning disc microscopy
Model of Activation by Parathyroid Hormone - Related Protein - (PTHrP)

Modified from Ferrandon S. Nature Chemical Biology, Oct. 2009

Model of Activation by Parathyroid Hormone - (PTH)

Modified from Ferrandon S. Nature Chemical Biology, Oct. 2009
Structure of M-modified PTH Analogs & Ligand Chain-Length
Required for R₀ Properties

- **PTH(1-34)**
  - J-domain binding signaling: 1
  - N-domain binding: 34

- **PTHrP(1-36)**
  - J-domain binding signaling: 1
  - N-domain binding: 36

- **M-PTH(1-14)**
  - A Aib Har W
  - Chain lengths: 1 3 10 11 12 14 19 34

- **M-PTH(1-34)**
  - A Aib Q Har A W R
  - Chain lengths: 1 3 10 11 12 14 15 34

- **M-PTH(1-14)**
  - Chain lengths: 1 3 10 11 12 14 15 36

- **PTHrP (15-36)**
  - Chain lengths: 1 3 10 11 12 14
Unexpected Developments and Surprising Turns Can Lead to New Therapies
Potential Application of R\(^0\) Conformational Selective PTH Analogs in Hypoparathyroidism

**ASBMR Abstract #F483**


A new long-acting PTH/PTHrP hybrid analog that binds to a distinct PTHR conformation has superior efficacy in a rat model of hypoparathyroidism.
Search for Small Molecule Agonists/Modifiers
Large Size of Peptide-Binding Pocket in Family B GPCRs

Unexpected Drug Binding Pocket Deep in CRFR1

Predicted peptide site

Site of T410P activating mutation in PTHR1

Hollenstein et al. 2014 Trends Pharmacol Sci. 35:12-
Evolution

- PTH, PTHrP, traced backward in time to fish - how much earlier?
- PTHrP traced back to Sea Squirt 520 million years ago

Evolutionary Conservation Indicates Biological Roles
(What Roles in Calcium Rich Environment?)

With Terrestrial Habitat, a New Challenge to Calcium Homeostasis

Parathyroid glands as source of PTH secretion first appear

PTH a secreted hormone

PTHrP an autocrine/paracrine factor

Do earlier uses persist in part? Or purely vestigial?