

Parathyroid hormone and skeletal muscle cells

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ABSTRACT

Synthesized by the parathyroid glands, parathyroid hormone (PTH) is a single-chain, 84-amino acid polypeptide that controls calcium homeostasis by increasing serum levels, regulating calcium absorption in the small intestine (indirectly thanks to the action of calcitriol), renal reabsorption and removal from the bone matrix. It exerts its classical action mainly by interacting with PTH receptor type 1, a G protein-coupled receptor that is expressed in bone and kidney and activates different signaling pathways. Disorders of the parathyroid glands most commonly present with abnormal serum calcium concentrations: persistent high blood calcium levels are associated with hyperparathyroidism, whereas reduced blood calcium levels are associated with hypoparathyroidism. Both diseases are characterized by muscular dysfunction and myopathies. Even though numerous works suggest an effect of PTH on skeletal muscle, and PTH receptors have been identified in this tissue, knowledge of the cellular and molecular mechanisms of action of this hormone in skeletal muscle is very poor. It is hypothesized that skeletal muscle may be a target for PTH and that its receptors may mediate the effects of PTH. To support these hypotheses and obtain better and more specific understanding for future therapies, it needs to be established, using *in vitro* cellular models, whether PTH can affect skeletal muscle cell proliferation and differentiation.

KEYWORDS

Parathormone, skeletal muscle, parathyroid hormone receptors, parathyroid disorders.

Parathyroid hormone, receptors and signaling pathways

Parathyroid hormone or parathormone (PTH) is a single-chain, 84-amino acid polypeptide synthesized and secreted mainly by the parathyroid glands, i.e., four endocrine glands located, in the neck, on the dorsal part of the thyroid. PTH is cleaved from the precursor pre-pro-PTH (115 aa) and stored in granules which can be secreted in a circadian and pulsatile fashion or intracellularly degraded. The biological activity of intact PTH is associated with its 1-34 amino-terminal portion^[1].

PTH is essential for keeping serum calcium concentrations within narrow limits; these concentrations are detected by the calcium-sensing receptor expressed in the parathyroid cell membrane. When the extracellular ionized calcium concentration is reduced (hypocalcemia), PTH synthesis and secretion are increased and this mobilizes calcium from bone tissue and stimulates the kidney tubules to reabsorb calcium from urine; furthermore, PTH stimulates the kidney tubules to produce calcitriol (1,25-dihydroxyvitamin D), the most active form of vitamin D, from calcidiol (25-hydroxyvitamin D), a less active form of vitamin D. Calcitriol helps to increase serum calcium concentrations because it stimulates the absorption of calcium from the gastrointestinal tract (Fig. 1)^[2].

PTH also inhibits the reabsorption of phosphate by the kidney tubules, thereby decreasing serum phosphate concentrations. Therefore, PTH is the major mediator of calcium and phosphate metabolism and it carries out its action through interactions with receptors in two principal target organs: kidney and bone^[2].

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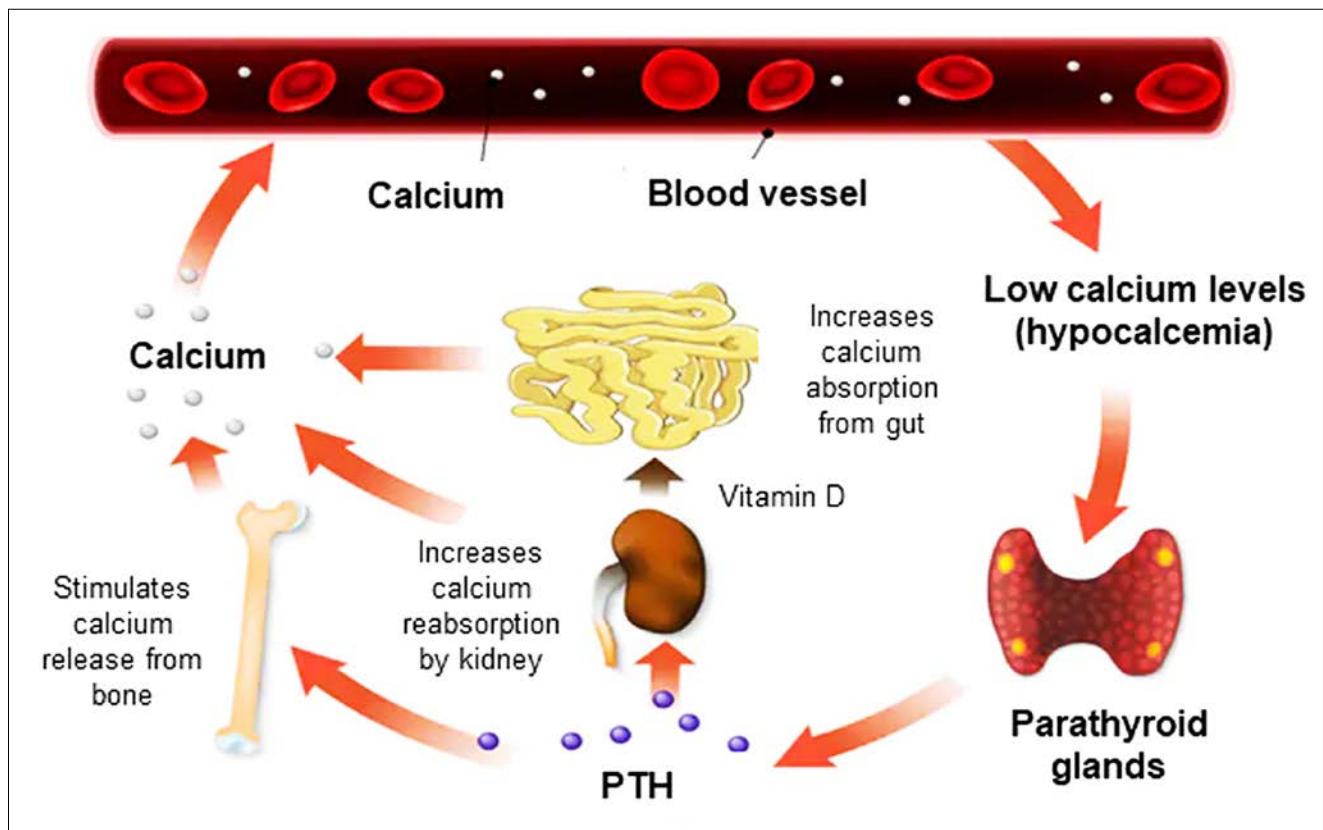
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The classical actions of PTH 1-84 are mediated through PTH receptor type 1 (PTHr1), a G protein-coupled receptor expressed on the surface of osteoblasts and osteocytes in bone and in tubular cells in the kidney, although it is also present in other tissues, such as the intestine and mammary glands^[3]. Stimulation of PTHr1 leads to $G\alpha_s$ -mediated activation of the adenylyl cyclase/cyclic AMP (cAMP)/protein kinase A (PKA) signaling pathway. PTHr1 is also linked to $G\alpha_q$ -mediated activation of the phospho-lipase/protein kinase C signaling cascade, the $G\alpha_{12/13}$ -phospholipase D/RhoA pathway, and the extracellular signal-regulated kinase $1/2$ mitogen-activated protein kinase signaling cascade, the latter via G protein-dependent and G protein-independent/ β -arrestin-dependent mechanisms^[4].

PTHr1 is also activated in response to PTH-related peptide (PTHrP), which was first isolated in 1987 in the search for a causative agent of hypercalcemia of malignancy^[5]. For both PTH and PTHrP, the 1-34 amino-terminal sequence (N-terminal structure) interacts with the cell-surface receptor PTHr1, and is necessary for their action, activating the above-mentioned signaling cascades at target tissues.

These two ligands have very different physiologic func-

Figure 1 PTH regulates calcium homeostasis: it exercises control of calcium metabolism by regulating its mobilization from the bone, absorption in the small intestine and reabsorption at the renal level.



tions. PTH, secreted by the parathyroid glands, is a circulating hormone that acts on target cells in bone and kidney to regulate levels of calcium and phosphate in the blood and enhance bone remodeling, as evidenced by slow bone turnover in individuals with PTH deficiency^[6]. In contrast, PTHrP in normal physiology, is produced in many tissues, acts locally, and has broad roles in cell differentiation and proliferation, for example in skin cells, breast cells, and the differentiation of chondrocytes and osteoblasts^[5,7].

In humans, PTH potently activates a second PTH receptor, called PTHR2, bringing about cAMP accumulation, whereas PTHrP is almost inactive. PTHR2 is abundantly expressed in the central nervous system (hypothalamus), but also in the pancreas and placenta^[8].

A third PTH-recognizing receptor has been identified in zebra fish and broadly shows the same pharmacological profile as PTH1R^[8].

Parathyroid diseases

Disorders of the parathyroid glands most commonly present with abnormalities of serum calcium concentration. The resulting diseases are characterized by signs and symptoms associated with the persistent presence of high blood calcium levels (hypercalcemia) associated with primary hyperparathyroidism (PHPT), or reduced blood calcium levels (hypocalcemia) associated with hypoparathyroidism (HypoPT)^[9].

PHPT is an endocrine disorder, characterized by hypercalcemia combined with inappropriately normal or elevated PTH levels which are associated with serious skeletal and renal complications. It is caused by excessive synthesis and secretion of PTH by one or more of the four parathyroid glands and it leads to reduction of bone mineral density (BMD), deterioration of bone microarchitecture, and an increased risk of fractures given the catabolic action of high levels of PTH on bone. Patients also present complex patterns of symptoms consisting of muscular fatigue, myopathy, various neuropsychiatric and cardiovascular manifestations and kidney stones^[10,11]. The definitive cure for PHPT is surgical removal of the hyper-functioning parathyroid tissue, which leads to normalization of the biochemical indices^[12].

HypoPT, on the other hand, is a rare endocrine disorder caused by chronic deficiency or absence of PTH, usually a complication of anterior neck surgery, although it can also occur as a genetic or autoimmune disorder^[13]. Hypocalcemia, hyperphosphatemia and low or undetectable PTH levels can cause muscle symptoms like cramps, paresthesias and numbness, life-threatening arrhythmias, laryngospasm, bronchospasm and seizures^[14]. Moreover, chronic deficiency of PTH leads to a profound reduction in bone remodeling, with consequent abnormalities in bone density, microarchitecture and bone strength. Many patients with HypoPT complain of reduced quality of life (QoL), in particular describing symptoms of cognitive dysfunction, collectively described as “brain fog”^[15]. Although conventional treatment of HypoPT with calcium

supplements and active vitamin D can be sufficient to maintain serum calcium levels, high doses may be required, adding to the risk of long-term soft tissue calcifications^[14].

Treatment with PTH peptides makes it possible to reduce calcium and active vitamin D administration, and obtain an improvement of neuromuscular symptoms compared with standard therapy^[16]. Probably the increased control of serum calcium levels obtained with PTH peptides plays a role, although other, still poorly understood, mechanisms are possibly involved too. Therefore, future studies will aim to uncover potential mechanisms of action of PTH on neuromuscular tissues.

Replacement therapy with recombinant human PTH (1-84) represents a major step in the therapeutics of this disease^[17,18]. Theoretically, PTH (1-84) is more attractive as a replacement hormone in hypoparathyroidism because the full-length peptide is exactly what is missing in this disease. In 2015, in the United States, the FDA approved recombinant human PTH (1-84) for the management of hypoparathyroidism and many clinical studies regarding its impact on QoL are still ongoing^[13,19-21].

PTH effects on skeletal muscle

PTH is essential for the maintenance of calcium homeostasis through its actions able to regulate bone remodeling. PTH can indeed promote both bone formation and bone resorption, through its direct effects on osteoblasts and indirect actions on osteoclasts. The final effect, anabolic or catabolic, on bone mass depends on the duration and periodicity of the PTH exposure; bone resorption predominates when continuous exposure to high levels of PTH persists, whereas administration of low and intermittent doses of PTH leads to a net increase in bone mass^[22,23].

As calcium has a central role in muscle function, it is conceivable that altered serum calcium levels affect skeletal muscle tissue.

Moreover, since bone and muscle are connected with each other, interacting anatomically and biochemically, they are considered to be a single functional system, and their relationship is essential for the physiology of the entire body. The catabolic and anabolic actions of PTH on the skeleton are undoubtedly well known, but it is equally important to investigate and clarify the effects of PTH treatment on the muscle tissue that is functionally linked to the bones.

A recent article by Sato *et al.* reports the effects of human recombinant PTH (1-34) on bone and skeletal muscle in a rat model of osteoporosis and muscle atrophy. Their data showed that monotherapy with PTH (1-34), at a dosage of 30 µg/kg body weight three times per week, significantly increased proximal and distal femoral BMD and the percentage of skeletal muscle mass in ovariectomized, tail-suspended rats^[24]. Another article reported that PTH (1-34) and growth hormone prevented disuse osteopenia and sarcopenia in rats^[25] while, in another study, PTH (1-34) treatment significantly improved muscle weakness in a dystrophin-deficient mdx mouse model^[26]. Although the effects of PTH (1-34) remain unknown in humans, it may have an anabolic effect on muscle atrophy and muscle

weakness.

Excess of PTH appears to have detrimental effects on skeletal muscle metabolism, since it has been shown that hyperparathyroidism is associated with muscle dysfunctions, such as muscular weakness, myopathy and impaired postural stability^[27]. Studies report that both intact PTH and its amino-terminal fragment exert their effects by affecting muscle protein metabolism, enhancing muscle proteolysis and increasing the release of alanine and glutamine, leading to alteration of amino acid metabolism^[28]. Furthermore, it has been reported that PTH administration causes biochemical derangements whose effects are responsible for protein metabolism and bioenergetic alterations in the skeletal muscle. Reduced energy production in skeletal muscle is evidenced by reductions of ATP content, mitochondrial oxygen consumption and activity of mitochondrial Mg ATPase^[29].

Abnormal muscle gene expression has been found in biopsies from patients with primary PHPT, where the ability of chronic PTH stimulation to affect certain mechanisms may explain recognized clinical consequences of PHPT, among them muscular fatigue^[10].

Levels of PTH increase with age and it has been hypothesized that the age-related change in this hormone concentration can be responsible for the loss of muscle strength and muscle mass characteristic of sarcopenia^[30].

Recent studies have shown that PTH and PTHrP can cause “browning” of white adipose tissue through activation of the PKA pathway, promoting the expression of thermogenic genes, including uncoupling protein-1 and hypermetabolism of adipose tissue. In addition, an increase of PTH/PTHrP appears to upregulate the ubiquitin-proteasome proteolytic system to degrade muscle protein and consequently bring about muscle wasting^[31].

In the last decade, the importance of skeletal muscle as a secretory organ has been highlighted. After contraction, skeletal muscle is able to produce and release, into the blood stream, a myriad of cytokines and growth factors called myokines, which can exert their effects on different organs and tissues^[32-34]. Also, skeletal muscle can represent a target organ for several hormones, and PTH is presumably one of them.

Numerous studies demonstrate that PTH affects the cell function of many organs that are not traditional targets of the hormone. Although PTH/PTHrP receptor mRNAs are highly expressed in kidney and bone, i.e., classical PTH targets that are associated with calcium homeostasis, their wide tissue distribution (in the heart, brain, liver, pancreas and other tissues, where acute exposure of PTH results in the generation of cAMP) suggests that these latter organs also contain PTH receptors, and that their response to PTH involves an interaction between this hormone and its receptors^[35,36].

The hypothesis that PTH may also have an effect on skeletal muscle is supported by the fact that mRNAs of both PTH receptors (PTHr1 and PTHr2) are expressed in this tissue, providing the molecular basis for the suggestion that skeletal muscle can be a target of PTH and that its receptors may mediate the actions of PTH and/or PTHrP that are distinct from the traditional function of PTH as a regulator of extracellular calcium concentration^[8,10,36,37].

Furthermore, data regarding the identification of PTHR1 and PTHR2 mRNAs in the brain may indicate that a lack/excess of PTH stimulation within in this organ can explain some of the neuropsychiatric manifestations, cognitive disorders and reduced QoL in patients with hypoPT and PHPT^[10].

Although a large body of evidence suggests an effect of PTH on skeletal muscle tissue, reports of *in vitro* studies of this hormone and skeletal muscle cells are very limited.

In vitro cell models are a valuable tool that may increase knowledge and understanding, at cellular and molecular levels, of the effects of PTH on skeletal muscle cells, and thus help to identify new mechanisms of action that control skeletal muscle differentiation.

A literature report showing that PTHR1 expression is required for myocyte differentiation, and that PTH accelerates myogenesis and the production of myotubes in a mouse cellular model, highlights, for the first time, the importance of PTH in skeletal muscle regeneration^[38].

Another *in vitro* study reports that PTH modulates the uptake and retention of 25-hydroxyvitamin D in skeletal muscle cells^[39].

Recently, our research group developed an *in vitro* model of skeletal muscle cells isolated from human biopsies with the aim of characterizing skeletal muscle endocrine machinery and its hormonal regulation. The analysis of PTHR1 mRNA in this model of myogenesis showed a significant increase in PTHR1 gene expression during skeletal muscle cell differentiation, supporting the possible involvement of PTH in skeletal muscle regeneration and in its function^[40].

Future directions

Considering the presence of PTH receptors in many non-traditional target organs for the hormone, including skeletal muscle, it is clearly very important to increase efforts to understand how PTH acts, not only on the regulation of bone and kidney cells, but also on skeletal myogenesis. Further studies, including *in vitro* analysis at the molecular and cellular levels, are therefore necessary for a better and more specific understanding of the effects of PTH on skeletal muscle cell proliferation and differentiation.

Furthermore, given the close relationship between bone and skeletal muscle, which together form a unit, investigation of the actions of PTH on the muscle compartment could shed new light on the interactions between these two important tissues, and enable the development of a PTH therapy for diseases characterized by skeletal muscle degeneration, such as HypoPT.

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