Signalling from parathyroid hormone

S.C. Tovey, S.G. Dedos and C.W. Taylor

Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1PD, U.K.

Abstract

PTH (parathyroid hormone), acting via type 1 PTH receptors, is a major regulator of plasma [Ca$^{2+}$]. The G-protein, $G_s$, is an essential component of the sequence linking PTH to plasma Ca$^{2+}$ regulation, but the relative importance of intracellular signals, including Ca$^{2+}$ and cAMP, that lie downstream of $G_s$ is not resolved.

**PTH (parathyroid hormone)**

PTH is the key regulator of plasma Ca$^{2+}$ homoeostasis and bone remodelling in mammals [1,2]. A decrease in plasma [Ca$^{2+}$] is detected by a Ca$^{2+}$-sensing receptor on chief cells in the parathyroid gland leading to release of PTH. This is a single-chain polypeptide with 84 amino acid residues, although only the first 34 are required for most biological effects [1,2]. Longer-term changes in PTH synthesis are modulated by other Ca$^{2+}$-regulating hormones. PTH, through its effects on kidney, bone and the intestines, increases plasma [Ca$^{2+}$]. In kidney, PTH promotes Ca$^{2+}$ reabsorption from the distal tubules, inhibits phosphate reabsorption in the proximal tubules and increases the activity of the enzyme 1α-hydroxylase. This enzyme is responsible for biosynthesis of 1,25-dihydroxyvitamin D$_3$, which stimulates Ca$^{2+}$ and phosphate absorption from the intestines. In bone, PTH primarily acts on osteoblasts, causing the release of factors that activate osteoclasts and consequently promote Ca$^{2+}$ resorption [1,2]. The importance of PTH is evident from the variety of common diseases, such as hyperparathyroidism, associated with defective control of PTH release, and the demonstration that pulsatile delivery of PTH is an effective treatment for osteoporosis [1,2].

**PTH receptors**

There are two mammalian PTH receptors [PTHR1 (type 1 PTH receptor) and PTHR2] that differ in their distributions and ligand specificities [3,4]. Both receptors belong to the class II (or class B) family of G-protein-coupled receptors. The family also includes receptors for glucagon, calcitonin, secretin and vasoactive intestinal peptide [1,2], many of which share with PTH receptors an ability to increase intracellular cAMP and [Ca$^{2+}$] [1,3,4]. PTHR1 is highly expressed in bone and kidney and is activated equally by PTH and PTHR1 (PTH-related peptide) [1,4]. The latter is thought to have a role in regulating bone formation during embryogenesis [1,2]. PTHR2, which shares approx. 70% sequence similarity with PTHR1, is expressed mainly in brain and is activated solely by PTH [3]. Mutations of PTHR1 are associated with genetic disorders of the skeleton, such as Jansen’s metaphyseal chondrodysplasia and the perinatally lethal Blomstrand’s chondrodysplasia [1,2]. PTHR1 is thus a potentially important target for treating disorders of Ca$^{2+}$ homoeostasis and the skeleton.

**Signalling from PTH receptors**

Activation of PTH receptors in bone and kidney cells causes activation of the G-protein, $G_s$, and so stimulation of adenylate cyclase and formation of cAMP [1–4]. This sequence is important in mediating the effects of PTH on plasma Ca$^{2+}$ regulation: at least some forms of pseudohypoparathyroidism are associated with loss of one functional allele of the $G_s$ subunit, and these patients fail to respond normally to PTH [1,2]. Activation of adenylate cyclase is thought to be responsible for most cellular responses to PTH. The subsequent activation of PKA (protein kinase A; also known as cAMP-dependent protein kinase) is responsible for activating a variety of transcription factors and signalling cascades, such as the pro-proliferative MAPK (mitogen-activated protein kinase) pathway [5]. Activated PTH receptors can also couple with other signalling pathways, including activation of PLC (phospholipase C), PKC (protein kinase C), PLD (phospholipase D) and PLA$_2$ (phospholipase A$_2$) and $G_s$-mediated inhibition of adenylate cyclase activity [1,2]. The physiological significance of activating these ‘alternative’ signalling pathways is less clear, but may reflect responses to different ligands or the diverse function of PTH receptors in different tissues. In bone, many of the cAMP-independent effects of PTH are thought to be mediated by PKC [1,2]. Activation of PKC has been shown to occur via both PLC-independent and -dependent mechanisms, with the former specifically requiring residues 29–32 of PTH [6]. However, PTH-mediated activation of the PLC/PKC pathway remains controversial, with conflicting reports in bone, kidney and other tissues [4,7–11]. In some studies of bone and kidney, PTH activates PLC, resulting in the production of DAG (diacylglycerol) and IP$_3$ (inositol 1,4,5-trisphosphate) [4,7,8]. DAG activates PKC.

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Key words: calcium, cAMP, G-protein, inositol 1,4,5-trisphosphate, intracellular signalling, parathyroid hormone.

Abbreviations used: DAG, diacylglycerol; HK293, human embryonic kidney cell; IP$_3$, inositol 1,4,5-trisphosphate; IP$_{3}$-R, IP$_3$ receptor; MAPK, mitogen-activated protein kinase; NHERF2, Na$^{+}$/H$^{+}$-exchanger regulatory factor 2; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PTH, parathyroid hormone; PTHR1, type 1 PTH receptor.

*To whom correspondence should be addressed (email cwt1000@cam.ac.uk).*
and IP₃ causes Ca²⁺ release from intracellular stores. In bone, PLC activation has been studied using a mutant PTH receptor incapable of stimulating IP₃ formation, but capable of normal cAMP signalling [12]. When expressed in mice, this mutant receptor led to abnormalities in bone development, such as enhanced chondrocyte proliferation and delayed ossification. This suggests that the normal function of PTH-mediated PLC signalling may be to slow proliferation and promote chondrocyte differentiation, thereby opposing the CAMP-mediated actions of PTH [13].

Which pathway predominates may depend on the tissue, ligand or other factors. For example, the NHERF2 (Na⁺/H⁺-exchanger regulatory factor 2), via a PDZ domain interaction with the C-terminal intracellular tail of PTHR1, has been shown to increase the formation of IP₃, but decrease the formation of cAMP in response to PTH [14]. The relative expression of NHERF2 may determine which pathway predominates in a particular tissue. After activation, the PTH receptor is rapidly phosphorylated by GRKs (G-protein-coupled-receptor kinases), which then facilitate association with β-arrestins, resulting in receptor inactivation and internalization. The association of β-arrestins with PTH receptors also leads to a prolonged activation of the MAPK signalling pathway [15].

**PTH receptors and intracellular Ca²⁺ signals**

PTH has been shown to increase intracellular Ca²⁺ by both activating Ca²⁺ entry and promoting Ca²⁺ release from intracellular stores. In bone, kidney and smooth-muscle cells, PTH activates Ca²⁺ influx across the plasma membrane via cAMP and PKA-dependent activation of voltage-operated Ca²⁺ channels [16–18]. In kidney and bone cells, PTH has also been shown to release Ca²⁺ from intracellular stores by a mechanism that remains unclear [4,7–11,19–22]. Some reports suggest that activation of endogenous or heterologously expressed PTH receptors results in Ca²⁺ release via activation of Gq and PLC and the subsequent production of IP₃ [4,7,8]. However, it has also been demonstrated that PTH can mediate Ca²⁺ release independently from the activation of Gq and PLC and even in the presence of an IP₃ receptor (IP₃ antagonist) [9–11,19–22]. The most dramatic effect of PTH on Ca²⁺ release has been shown in osteoblast-like cells expressing endogenous PTHR1 and HEK-293 cells (human embryonic kidney cells) heterologously expressing PTHR1. In both systems, PTH, without itself causing Ca²⁺ mobilization, massively potentiates Ca²⁺ release in response to stimulation of Gq-coupled receptors [10,11,22]. PTH-mediated potentiation of Ca²⁺ release, evoked by stimulation of the Gq-linked muncaricin M₃ receptor, in HEK-293 cells is illustrated in Figure 1. In these cells, PTH neither causes IP₃ formation nor effects IP₃ formation in response to muscarinic receptor stimulation [10]. The potentiated response is blocked by the IP₃ antagonist heparin and PTH also potentiates responses to a cell-permeant form of IP₃ [10,22]. Together, these results suggest that PTH potentiates responses to Gq-coupled stimuli by sensitizing IP₃-R to IP₃ [22]. The sensitization of IP₃-R is insensitive to PKA inhibition, suggesting that phosphorylation of IP₃-R is not required [10,22]. The signal that couples activation of PTHR1 with the sensitization of IP₃-R remains unclear, nor is the physiological role of potentiation understood.

In osteoblast-like cells, PTH potentiates Ca²⁺ responses to extracellular nucleotides, resulting in increased activation of the CREB (cAMP-response-element-binding protein). This in turn leads to increased transcription of the c-fos proto-oncogene that has been strongly implicated in driving osteoblast proliferation and differentiation [11]. This suggests that, in bone at least, potentiated responses may provide a novel mechanism for integrating the effects of systemic PTH and local signals like ATP [11].

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References


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