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PROSPECT

Does PTH Have a Direct Effect on Intestine?

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Abstract Dogma for the past three decades has dictated that parathyroid hormone (PTH) has no direct effect on intestine with regard to calcium or phosphate absorption, but rather that PTH acts to promote the synthesis of a hormonally active form of vitamin D, namely 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$]. However, diverse laboratories have each provided some evidence to suggest PTH does indeed have a direct effect on intestine. We will briefly review the evidence for biological effects, biochemical effects, and the presence of intestinal receptors for PTH, and conclude with the implications for biomedical research. J. Cell. Biochem. 86: 29–34, 2002. © 2002 Wiley-Liss, Inc.

Key words: calcium; receptors; signal transduction; enterocytes; phosphate

HISTORICAL PERSPECTIVE

Within the context of the endocrine physiology of calcium homeostasis, it is logical to postulate an effect of parathyroid hormone (PTH) on intestine in analogy to the actions of the vitamin D metabolite, 1,25$(OH)_2D_3$: an increase in serum calcium is due to coordinate actions on enhancing reabsorption from kidney, bone mineral mobilization, and uptake of new dietary sources of the cation from intestine. Indeed, this was proposed [Munson, 1960], and work in the early 1970s appeared to support the postulate, particularly in rat intestine [Birge and Gilbert, 1972, 1974; Burdette et al., 1972; Birge et al., 1974]. The situation quickly became more complicated as the details of vitamin D metabolism emerged. It is now well known that PTH stimulates the production of 1,25$(OH)_2D_3$, which in turn promotes calcium and phosphate absorption in the intestine. Thus, actions of PTH in vivo are difficult to separate from the actions of 1,25$(OH)_2D_3$. Moreover, the biosynthetic actions of 1,25$(OH)_2D_3$ on the cellular components of the transport pathway, are necessary to produce a responsive, biochemically competent cell [Nemere, 1996a], as amply demonstrated in VDR knockout mice [Amling et al., 1999]. The absence of biochemical competence in the cell precludes the action of PTH in intestine (see below). Another methodological approach was the use of the everted gut sac. While useful for collection of some data, it is not appropriate for assessing the effects of PTH in vitro: the receptors for this normally blood-borne hormone would be difficult to reach unless introduced directly to the vasculature for delivery to the basal lateral surface.

The seemingly final argument for the lack of an effect of PTH in intestine was the inability to document the existence of a receptor. A contributing factor to the lack of specific binding was the tendency to remove intestinal tissue from exsanguinated, anoxic animals; anoxia promotes not only the destabilization of epithelial cell lysosomes, but also the release of digestive enzymes from the contiguous pancreas. The end result would be extensive proteolysis and loss of low abundance extracellular molecules such as membrane receptors.

BIOLOGICAL ACTIONS OF PTH IN INTESTINE

Ion Uptake and Transport

The first conclusive demonstration that PTH has a direct effect in intestine was accomplished

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by removing the organ from anesthetized rats, and carefully chilling the tissue prior to enterocyte isolation by chelation protocols [Nemere and Szego, 1981a,b]. In this system, it was demonstrated that 1 pM bPTH(1–34) enhances ^{45}Ca uptake, relative to controls, over a 20 min time period with a maximum at 15 min after hormone treatment [Nemere and Szego, 1981a]. Sixteen years later, Picotto et al. [1997] reported a similar effect of physiological concentrations on calcium influx in isolated enterocytes. However, while Nemere and Szego [1981a] observed a biphasic dose-response curve, with inhibition of calcium uptake at nanomolar concentrations, Picotto et al. [1997] found significant stimulation even at micromolar concentrations of hormone. Interestingly, an investigation into the effects of aging on PTH-mediated calcium uptake in isolated rat intestinal cells indicated a greater response in enterocytes isolated from old animals than from young animals [Massheimer et al., 2000].

The effects of PTH on intestine were expanded to include observations in another species (chick), and to demonstrate transport, rather than cellular uptake. Perfusion of isolated duodenal loops with 65 pM bPTH(1–34) results in enhanced ^{45}Ca transport within 12 min, and reaches an apparent maximum within 40 min [Nemere and Norman, 1986]. The perfused duodenal loop system also yields a biphasic dose response curve [Nemere and Norman, 1986], a property that is common for membrane receptors [de Meyts, 1976]. Moreover, the response to bPTH(1–34) is blocked by simultaneous perfusion with the competitive inhibitor bPTH(3–34) amide [Nemere and Norman, 1986].

Figure 1 compares the maximal results obtained for calcium uptake in isolated rat enterocytes in response to PTH, with the optimal response for calcium uptake in chick enterocytes, and transport in the perfused chick duodenal loop. As would be expected, uptake in isolated cells is substantially less than net transport. Indeed, lack of excessive ^{45}Ca accumulation indicates the continued viability of the isolated cells and the ability to extrude the cation after uptake.

A somewhat surprising finding with the perfused duodenal loop system is that PTH also stimulates phosphate *transport*/*absorption* [Nemere, 1996b]. Conventional wisdom has suggested that PTH stimulates phosphate *excretion* in kidney. Thus, enhanced phosphate transport in intestine represents a divergent action. Phosphate transport also exhibits a biphasic dose-response curve [Nemere, 1996b], although apparent maxima and range of optimal concentrations differ from that found for calcium transport [Nemere and Norman, 1986].

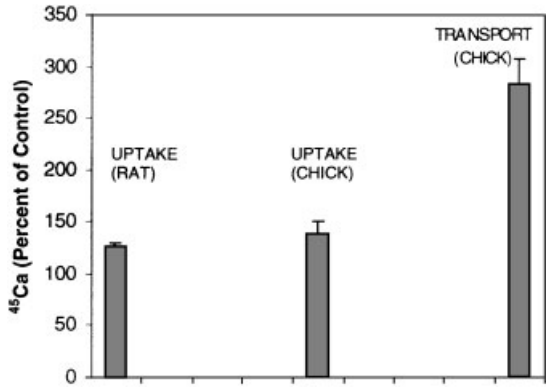

Fig. 1. Comparison of PTH effects on calcium uptake in isolated intestinal cells from rats or chicks and calcium transport in the perfused chick duodenal loop. Data are presented as mean ± SEM for values obtained at optimal times after addition of hormone (1 pM PTH for rat enterocytes, 15 min; 65 pM for chick enterocytes, 10 min; 65 pM for chick duodenal loop, 40 min).

Activation of Signal Transduction Pathways

Rat intestinal cells isolated from either young (3–6 months) or old (20–24 months) male rats, respond differently to PTH in G-protein coupled activation of adenylate cyclase (AC), phospholipase C (PLC), protein kinase A (PKA), and protein kinase C (PKC) [Massheimer et al., 2000; Gentili et al., 2001]. Our interpretation of the data presented by Massheimer et al. [2000] and Gentili et al. [2001], is that PTH in young rats evokes increased intracellular calcium concentrations through activation of voltage dependent calcium channels by cAMP/PKA and via calcium release from the endoplasmic reticulum evoked by PLCβ/IP_3. In contrast to the response in young rats, old rats respond to PTH by increasing voltage dependent calcium uptake, but lack the PLCβ/IP_3 dependent effect on intracellular calcium release. Besides having an effect on enterocyte calcium transport capacity, PTH stimulates duodenal cell proliferation in young rats via activation of mitogen-activated protein kinase (MAPK) isoforms ERK1

and ERK2. The mechanisms leading to the activation of MAPK, ERK1, and ERK2 are not fully clear, but indicate that cAMP and Ca^{2+} play important roles upstream in the signaling cascade leading to ERK1 and ERK2 activation [Gentili et al., 2001].

In isolated chick intestinal epithelial cells, PTH stimulates the activation of PKA between 1–10 min after addition, relative to vehicle controls [Nemere, 1999]. By comparison, PTH has no stimulatory effect on PKC activity [Nemere, 1999]. This difference between two species (chick and rat), suggests that PTH activation of PKA is a central event in the transport of calcium, while activation of PKC in the rat may represent a branch point after interaction with the receptor, responsible in part for the pleiotropic actions of the peptide hormone. The related observation that vitamin D-deficient chicks lack components of the cAMP pathway [Corradino, 1976; Nemere and Campbell, 2000] may explain why high levels of PTH fail to correct deficient calcium absorption in the intestine of rachitic animals.

PTH RECEPTORS IN INTESTINE

mRNA

The PTH/PTHrP receptor (PTHR) has a broad tissue distribution, and expression of PTHR mRNA has been demonstrated in many "nonclassical" PTH target tissues [Urena et al., 1993; Li et al., 1995; Watson et al., 2000]. In rat small intestine, PTHR transcripts have been established to be present in the villus epithelium, intestinal crypt cells, interstitial cells, and in the surrounding smooth muscle layer [Urena et al., 1993; Li et al., 1995; Watson et al., 2000]. The intestinal cells expressing transcripts for PTHR also express mRNA for PTHrP [Selvanayagam et al., 1991; Urena et al., 1993; Li et al., 1995; Watson et al., 2000], indicating the possibility of both autocrine and paracrine actions of PTH/PTHrP.

Binding of Peptide

The presence of mRNA for the PTH receptor in intestine is often an indication that the protein product of translation also exists. Functional receptor protein was demonstrated to be present in isolated chick basal lateral membranes using ^{125}I-labeled [$Nle^{8,18}$,Tyr^{34}]bPTH (1–34) amide [Nemere, 1996b]. Binding studies indicate a Kd of 0.2 nM, and unlabeled peptide is able to displace iodinated ligand 33, 80, and 90% when present at 10-, 100-, or 1000-fold excess, respectively [Nemere, 1996b].

Immunocytochemistry

Immunoreactive PTHR has been demonstrated in the villus epithelium, intestinal crypt cells, interstitial cells, and in the surrounding smooth muscle layer in the rat intestine [Watson et al., 2000]. Each of the cell types exhibit nuclear staining as well as immunoreactive PTHR in the plasma membrane [Watson et al., 2000]. Although mRNA for PTHrP is expressed in both the apical epithelium of the villi and in the intestinal crypts, no immunoreactivity for the peptide is detected at either location [Watson et al., 2000].

Thus, intestinal epithelial cells possess PTH receptors as demonstrated by three criteria: presence of mRNA, specific, saturable binding of ligand, and immunoreactive staining. The inescapable conclusion is that PTH is capable of acting on enterocytes through receptor-mediated actions.

PHYSIOLOGICAL IMPORTANCE OF PTH ACTION IN INTESTINE

Until recently, the focus of the biomedical community has been on the effects of excessive PTH secretion that occur over long time periods (e.g., in hyperparathyroidism). Inevitably, this leads to bone resorption. More recently, attention has focused on the anabolic effects of low doses of PTH on bone, whether administered continuously or intermittently in rodents [Alexander et al., 2001; Zhou et al., 2001; Suzuki et al., 2002], and in the treatment of osteoporosis in humans [Bilezikian and Kurland, 2001; Fujita, 2001]. Some of the anabolic effects have been related to the mid-region of PTH (fragment 28–48), since the N-terminal fragment is known to stimulate osteoclast activity [Kim et al., 2002]. However, the N-terminal fragment PTH(1–34) when administered in vivo, is known to increase bone mineral density [Suzuki et al., 2002], and this may in part be due to stimulation of calcium and phosphate uptake in intestine [Nemere and Szego, 1981a,b; Nemere and Norman, 1986; Nemere, 1996b].

In addition to the rapid effects on intestinal calcium and phosphate transport, PTH potentiates the production of calcium binding proteins

in the presence of $1,25(OH)_2D_3$ [Parkes and Reynolds, 1978] and enhances proliferation of intestinal cells [Gentili et al., 2001; Ye et al., 2001]. These long-term effects, which ultimately result in a higher transport capacity for calcium and phosphate through enhanced production of biochemically competent enterocytes, are proposed to be mediated by activation of the MAPK signaling cascade and nuclear localization of the PTHR/PTHrP hormone-receptor complex. Indeed, an inhibitor (PD098059) for MAPK kinase (MEK) blocks the PTH mediated increase in rat enterocyte proliferation [Gentili et al., 2001]. Moreover, a rat gastrointestinal epithelial cell line (IEC-6) transfected with PTHrP lacking the nuclear localization signal loses the ability of PTHrP to induce proliferation in IEC-6 intestinal epithelial cells [Ye et al., 2001].

Thus, understanding the actions of PTH in intestine will open new approaches to therapeutic support of clinical cases manifesting an imbalance in calcium homeostasis. A potentially exciting pharmacological development will be to find receptor ligands that promote mineral absorption in intestine, while minimizing bone resorption and maximizing bone formation.

SUMMARY

Table I summarizes the observations described in this review and emphasizes the range of evidence for a direct effect of PTH on intestine from biological response, to signal transduction, to the presence of receptors. With these reports in mind, we present Figure 2, which illustrates the new paradigm for PTH action in the body. Aside from the two well-known target tissues, kidney and bone, we have added intestine. In keeping with the systemic effects of PTH on raising serum calcium, it can no longer be denied that PTH enhances calcium (and phosphate) absorption in intestine. As is now appreciated for steroid hormones, PTH acts not only through rapid signal transduction mechanisms to meet acute demands for dietary minerals, but also promotes nuclear effects that may include altered gene transcription and cell proliferation.

TABLE I. Summary of PTH Effects on Intestine

	Animal	Observation	References
Calcium/Phosphate transport	Chicken	Increased Ca-transport	Nemere and Norman [1986]
		Increased P-transport	Nemere [1996b]
Calcium uptake/extrusion	Rat	Na^+ dependent Ca^{2+} extrusion	Birge et al. [1974]
		Ca^{2+} uptake	Nemere and Szego [1981a,b]
		Voltage dependent Ca^{2+} uptake	Picotto et al. [1997]
Cell proliferation/ protein expression	Chicken	Increased synthesis of CaBP	Parkes and Reynolds [1978]
	Rat (IEC-6 cells)	Increased cell proliferation	Ye et al. [2001]
Signal transduction	Chicken	PKA	Nemere [1999]
		PKC	Nemere [1999]
	Rat	MAPK (ERK ½)	Gentili and de Boland [2000]; Gentili et al. [2001]
		AC	Massheimer et al. [2000]
		cAMP	Picotto et al. [1997]; Massheimer et al. [2000]; Gentili et al. [2001]
		PKA	Massheimer et al. [2000]
		PLCβ	Massheimer et al. [2000]; Gentili et al. [2001]
		PLCγ	Gentili et al. [2001]
		IP_3	Massheimer et al. [2000]
		DAG	Massheimer et al. [2000]
		PKC	Picotto et al. [1997]; Massheimer et al. [2000]
		Intracellular Ca^{2+}	Massheimer et al. [2000]; Picotto [2001]
		Tyrosine phosphorylation	Gentili et al. [2001]
Receptor expression/ PTH binding	Chicken	PTH binding to PTHR	Nemere [1996b]
	Rat	Expression of the PTHrP gene	Selvanayagam et al. [1991]; Watson et al. [2000]
		Expression of the PTH/PTHrP receptor gene and peptide	Urena et al. [1993]; Watson et al. [2000]
		Nuclear localization of the PTH/PTHrP receptor gene and peptide	Watson et al. [2000]
	IEC-6 and LoVo cells (rat and human)	Expression of PTHrP and the PTH/PTHrP receptor gene	Li et al. [1995]

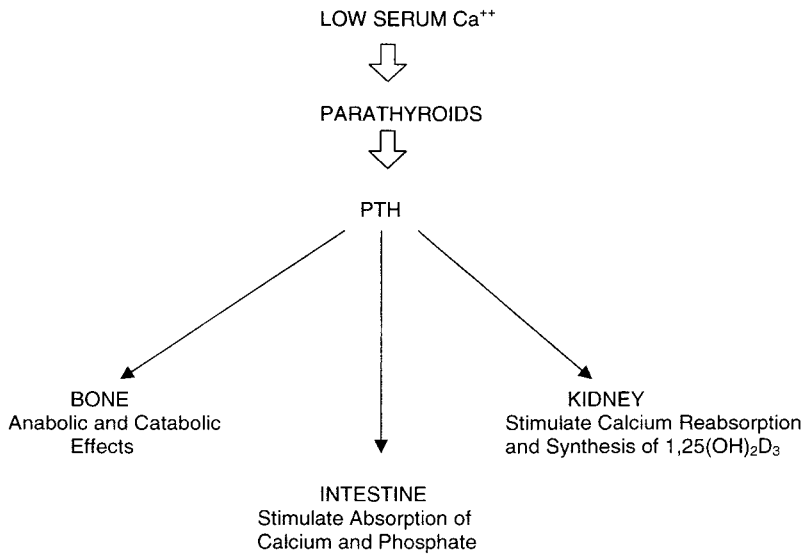

Fig. 2. Schematic presentation of PTH action on three major target tissues involved in calcium homeostasis. The arrows indicate a direct effect on intestine, kidney, and bone.

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