Residue "switch" revealed in PTH system

James Kling

Bone resorption and bone growth are coupled to the exposure of osteoblasts to two hormones—parathyroid hormone (PTH) and parathyroid hormone related protein (PTHrP). Low levels of both hormones stimulate bone growth, and higher levels stimulate resorption. Peptide derivatives of both hormones are currently in clinical trials as treatments for osteoporosis. However, all the current osteoporosis treatments are peptide-based inhibitors of bone resorption. The complementary therapeutic goal of restoration of bone growth has proved elusive. But new research has unveiled an important molecular "switch" governing the binding and activation selectivity of the two hormones toward two distinct receptors.

The PTH/PTHrP receptor, which is found on osteoblasts, is a seven transmembrane domain receptor that has an almost identical pharmacological profile to both hormones. The gene for that receptor is commonly expressed in the kidney and bones, but evidence of a PTH response in other tissues—and the discovery of several new mRNA species related to PTH/PTHrP in the brain and testes—led to the recent cloning and partial characterization of another, related receptor, the PTH2 receptor. The two receptors show 70% amino acid sequence homology and both stimulate cAMP production in a coupled signaling pathway. But their responses to the two hormones differ. In cells that produce the PTH/PTHrP receptor, both hormones stimulate cAMP production, but in cells that have the PTH2 receptor, only PTH is active.

Thomas Gardella and Harold Jüppner of the Endocrine Unit at Massachusetts General Hospital (Boston, MA) have investigated the structural root of this differential activation (*J. Biol. Chem.* 276:19888–19893, 1996). They transiently expressed the PTH2 receptor gene in a rat COS-7 cell line and measured interaction of the receptor with several PTH/PTH2 hybrids and analogs of the two hormones. They found that replacing histidine with isoleucine at position 5 in PTHrP stimulated cAMP activity, but the binding constant remained about 40-fold below that of PTH. That isoleucine-5 PTHrP analog binds effectively yet poorly activates the PTH2 receptor puzzles Gardella. "It may be that the analog binds rapidly, but quickly dissociates, which could result in the low apparent binding constant," he said.

That result prompted a systematic survey of hybrids of the two hormones to find another binding factor. The researchers found that a single substitution at position 23 in PTHrP—from phenylalanine to tryptophan—produced an antagonist of the receptor, binding to it without stimulating cAMP production.

Similar experiments (*Endocrinology*, 137:4217–4224, 1996) in a human embryonic kidney cell line expressing the PTH2 receptor gene confirmed that the isoleucine (or Ile5) analog of PTHrP binds to and stimulates cAMP accumulation in the PTH2 cell line, although the affinity of the analog is about 10 times less than that of PTH itself. The team—led by Michael Chorev of Harvard Medical School (Boston, MA)—also has unpublished circumstantial evidence that contradicts the findings of Gardella and Jüppner: A PTHrP analog without its first six N-terminal residues binds to and stimulates the PTH2 receptor in the kidney cell line, suggesting that position 5 controls both actions. Both research teams agree that unpredictable expression in transient cell lines may be the root of the difference. Larry Suva of Harvard Medical School, a co-author on the *Endocrinology* paper, offered one explanation: "If you're getting over-expression of the receptor, you could, through mass action, have ligands interacting with the receptor and getting slightly different signals."

In any case, said Gardella, the two papers agree on one thing: Position 5 is critical to the activation of the PTH2 receptor. The mechanism behind the switch is still uncertain, but histidine could alter the peptide conformation or directly hinder binding with the receptor through steric interactions.

Gardella thinks the histidine switch at position 5 is no accident. No physiological function has been attributed to the PTH2 receptor, but Gardella figures that it must have one. Otherwise, he says, "Nature wouldn't go to the trouble of avoiding it (by giving PTHrP a histidine 'off switch')."

The switch at position 23, if it holds up, could open a whole new avenue of research. "Having an antagonist that's specific to the PTH2 receptor but doesn't antagonize the PTH/PTHrP receptor will help to sort out the role of the PTH2," said Gardella.

James Kling is an independent science writer working in Bellingham, WA.