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A G Protein–Linked Receptor for Parathyroid Hormone and Parathyroid Hormone–Related Peptide

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The complementary DNA encoding a 585–amino acid parathyroid hormone–parathyroid hormone–related peptide (PTH-PTHrP) receptor with seven potential membrane-spanning domains was cloned by COS-7 expression using an opossum kidney cell complementary DNA (cDNA) library. The expressed receptor binds PTH and PTHrP with equal affinity, and both ligands equivalently stimulate adenylate cyclase. Striking homology with the calcitonin receptor and lack of homology with other G protein–linked receptors indicate that receptors for these calcium-regulating hormones are related and represent a new family.

PTH REGULATES CA AND P METABOLISM by binding to specific G protein–coupled receptors in bone and kidney (*1*), thereby activating adenylate cyclase and phospholipase C (*2, 3*). PTHrP, which shares 8 of 13 NH_2-terminal residues with PTH, causes the hypercalcemia of malignancy syndrome (*4, 5*). Both peptides appear to bind to the same ~80-kD receptor glycoprotein (*5–9*).

A random-primed, size-selected cDNA library (1.2×10^7 independent transformants) was constructed in pcDNAI (*10*) from opossum kidney (OK) cells (*3*). COS-7 cells were transfected with 26 plasmid pools, each representing ~10,000 independent colonies, and screened by [^{125}I]PTHrP(1-36) photoemulsion autoradiography (*11, 12*). Two independent clones, OK-O and OK-H, were isolated; each contained ~1.8-kb inserts.

Both receptors, transiently expressed in COS-7 cells, bound PTH(1-34) and PTHrP(1-36) equivalently [K_d (dissociation constant) ≈ 4 nM, compared to ~0.5 nM in native OK cells] when either peptide was used as radioligand (Fig. 1). PTH(3-34) and PTH(7-34) bound with progressively lower affinities with these expressed receptors (K_d ≈ 17 and 230 nM, respectively), showing affinities similar to their affinities in native OK cells (~5 and ~100 nM, respectively). Scatchard analysis revealed $>10^6$ receptor copies per transfected COS-7 cell (20% average transfection efficiency, range 10 to 40%). When OK-O and OK-H were stably expressed in LLC-PK$_1$ porcine renal cells at a density of 10,000 to 100,000 receptors per cell, both of the receptors' apparent K_d's for PTH(1-34) were ~0.3 nM (n = 10) (*13*). Therefore, the lower affinity of these receptors for PTH(1-34) and PTHrP(1-36) in COS-7 cells most likely reflects inefficient G protein coupling. Both peptides stimulated cAMP accumulation in COS-7 cells expressing either OK-O or OK-H equivalently [ED_{50}'s of ~0.4 nM (Fig. 1)] and had similar ED_{50}'s for both ligands in native OK cells. Northern blot analysis of total and poly(A)$^+$ RNA from OK cells revealed an apparently single, approximately 3.0-kb hybridizing species (*14*).

Nucleotide sequencing (*15*) of both strands of OK-O revealed an open reading frame encoding a 585–amino acid protein [figures 2B and 4 in (*16, 17*), respectively]. OK-H is identical to OK-O with the exception of one additional nucleotide at the 3′ end and a deletion of 16 nucleotides at the 5′ end. Further, one of six consecutive G's (nucleotides 1629 to 1634) is deleted and causes a shift in reading frame. Therefore, the resulting 515–amino acid protein encoded by OK-H is shorter than OK-O, and its last eight residues (508 to 515) differ. The region including these multiple G's was amplified from genomic OK cell DNA by polymerase chain reaction and cloned into pcDNAI. Because six individual clones all contained six G's, OK-H is likely to represent a cloning artifact.

Searches for similar sequences in nucleic acid and protein databases showed no related proteins: comparison of the deduced peptide sequence of OK-O with those of 120 reported G protein–linked receptors revealed less than 10% conservation of 35 "signatures," features that are at least 80% conserved among these other receptors (*18, 19*).

OK-O likely contains the entire coding region of the PTH-PTHrP receptor. The ATG codon (nucleotides 114 to 116) probably encodes the initator methionine because it is in frame with an upstream stop-codon (nucleotides 99 to 101) and is preceded by a purine at position 111 (*20*). The receptor is predicted to contain ten hydrophobic regions (*21*); domains I through VII are putatively membrane-spanning (Fig. 2A). Hydrophobic region A probably represents a signal peptide. Region B is unlikely to be membrane-spanning, because such an assignment would place all potential N-glycosylation sites inside the cell. Hydrophobic region C is predicted to reside within the unusually long, first putative extracellular loop. This assignment allows positive charges to line the intracellular borders of several of the

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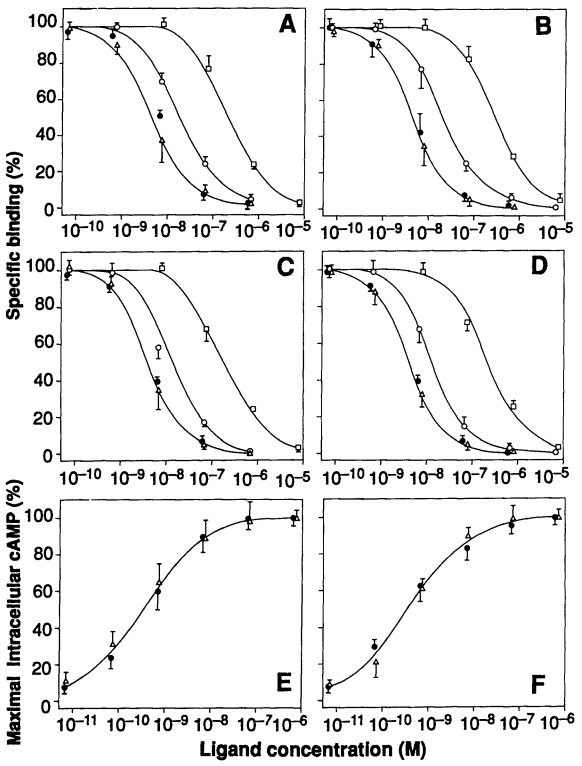


Fig. 1. Biological properties of COS-7 cells expressing OK-O (**A**, **C**, and **E**) and OK-H (**B**, **D**, and **F**). COS-7 cells were transfected in dishes (15 cm wide) before replating into 24-well plates (~50,000 cells per well). We performed radioreceptor assays as described (*6*), using either [^{125}I]PTH(1-34) (A and B) or [^{125}I]PTHrP(1-36) (C and D) in the absence or presence of increasing concentrations of competing PTHrP(1-36) (●), PTH(1-34) (△), PTH(3-34) (○), or PTH(7-34) (□). Specific binding of [^{125}I]PTH(1-34) and [^{125}I]PTHrP(1-36) was 12.9 ± 1.4% and 10.8 ± 2.1%, respectively, for OK-O, and 14.4 ± 4.5% and 18.3 ± 0.7%, respectively, for OK-H; nonspecific binding was 1.6 ± 0.6% for either ligand. No radioligand was displaced by salmon CT, bPTH(53-84), or insulin (up to 10^{-6} M). Intracellular cAMP accumulation was measured (*6*) in COS-7 cells expressing OK-O (E) or OK-H (F) after stimulation with PTH(1-34) (●) and PTHrP(1-36) (△) (10^{-11} to 10^{-6} M). No increase in cAMP was seen when COS-7 cells expressing CDM-8 alone were treated with either ligand (up to 10^{-6} M). Mean ± SD of at least two independent experiments shown.

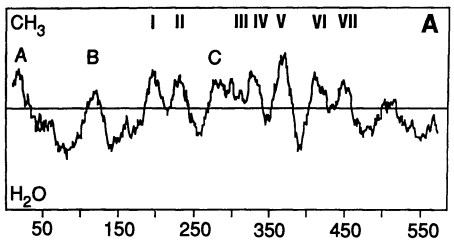
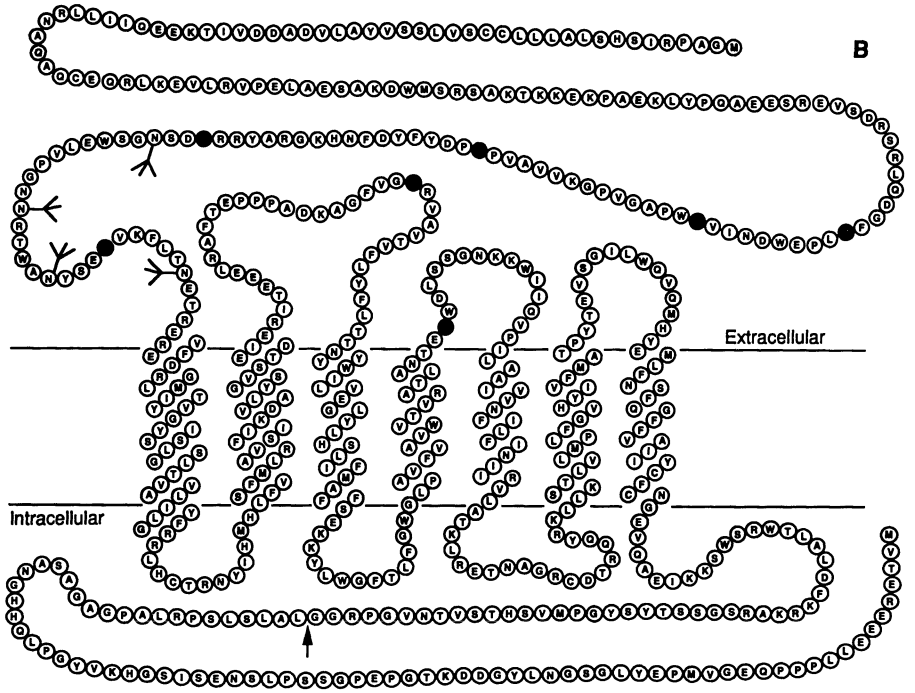


Fig. 2. (**A**) Hydrophobicity plot (*21*) of the deduced PTH-PTHrP receptor peptide sequence with a 20-residue window (*24*). (**B**) Schematic representation of the PTH-PTHrP (OK-O) receptor (NH_2 terminus at top); potential N-glycosylation sites (Ψ); cysteine residues that are conserved in the CT receptor (●) (*16*). In OK-H, residues 508 to 515 (to the left of the arrow) are WPCPSALD. The peptide ends eight residues after the arrow.



subsequent transmembrane domains (*22*) and allows those domains to share the topology suggested for the related calcitonin receptor (*16*). The designations of membrane-spanning sequences are speculative; the unique sequence of the receptor mandates a detailed analysis of its functional and topological domains. In this regard, the properties of OK-O and OK-H are informative; the identical binding characteristics of OK-O and OK-H and the equivalent potencies of the activated receptors to stimulate cAMP accumulation (Fig. 1) indicate that the 78 COOH-terminal amino acids are irrelevant to these functions of this receptor.

Comparison between the PTH-PTHrP receptor and a porcine renal calcitonin receptor (CTR) reveals a close relationship, with the strongest identity in the carboxy portion of the seventh membrane-spanning domain and its adjacent intracellular region, where 17 of 18 amino acids are identical [figure 4 in (*16*)]. Two of the four potential glycosylation sites are conserved in the CTR. Additionally, seven of eight functionally important (*9*), extracellular cysteines are conserved. Five of these residues reside proximal to the first membrane-spanning domain, and no gaps are required for their alignment between the PTH-PTHrP receptor and the CTR. The surprising structural similarity between the PTH-PTHrP receptor and the CTR suggests that these novel receptors may share functional features distinguishing them from other G protein–linked receptors.

Note added in proof: After submission of this paper, the rat secretin receptor sequence was published (*23*); its high sequence identity (30% and 42% with the CT and PTH-PTHrP receptors, respectively) indicates that it also is a member of this receptor family.

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