



HAL
open science

Biased GPCR signaling by the native parathyroid hormone–related protein 1 to 141 relative to its N-terminal fragment 1 to 36

Karina A Peña, Alex D White, Sofya Savransky, Ignacio Portales Castillo, Frédéric G Jean-Alphonse, Thomas J Gardella, Ieva Sutkeviciute, Jean-Pierre Vilardaga

► To cite this version:

Karina A Peña, Alex D White, Sofya Savransky, Ignacio Portales Castillo, Frédéric G Jean-Alphonse, et al.. Biased GPCR signaling by the native parathyroid hormone–related protein 1 to 141 relative to its N-terminal fragment 1 to 36. *Journal of Biological Chemistry*, 2022, 298 (9), pp.102332. 10.1016/j.jbc.2022.102332 . hal-03854828

HAL Id: hal-03854828

<https://hal.science/hal-03854828v1>

Submitted on 16 Nov 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Biased GPCR signaling by the native parathyroid hormone-related protein 1 to 141 relative to its N-terminal fragment 1 to 36

Received for publication, January 11, 2022, and in revised form, July 28, 2022. Published, Papers in Press, August 4, 2022,

<https://doi.org/10.1016/j.jbc.2022.102332>

Karina A. Peña^{1,†}, Alex D. White^{1,†}, Sofya Savransky^{1,2,†}, Ignacio Portales Castillo³, Frédéric G. Jean-Alphonse¹, Thomas J. Gardella³, Ieva Sutkeviciute¹, and Jean-Pierre Vilardaga^{1,*}

From the ¹School of Medicine, Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, USA; ²Graduate Program in Molecular Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, USA; ³Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA

Edited by Kirill Martemyanov

The parathyroid hormone (PTH)-related protein (PTHrP) is indispensable for the development of mammary glands, placental calcium ion transport, tooth eruption, bone formation and bone remodeling, and causes hypercalcemia in patients with malignancy. Although mature forms of PTHrP in the body consist of splice variants of 139, 141, and 173 amino acids, our current understanding on how endogenous PTHrP transduces signals through its cognate G-protein coupled receptor (GPCR), the PTH type 1 receptor (PTHR), is largely derived from studies done with its N-terminal fragment, PTHrP₁₋₃₆. Here, we demonstrate using various fluorescence imaging approaches at the single cell level to measure kinetics of (i) receptor activation, (ii) receptor signaling *via* G_s and G_q, and (iii) receptor internalization and recycling that the native PTHrP₁₋₁₄₁ displays biased agonist signaling properties that are not mimicked by PTHrP₁₋₃₆. Although PTHrP₁₋₃₆ induces transient cAMP production, acute intracellular Ca²⁺ (iCa²⁺) release and β-arrestin recruitment mediated by ligand-PTHR interactions at the plasma membrane, PTHrP₁₋₁₄₁ triggers sustained cAMP signaling from the plasma membrane and fails to stimulate iCa²⁺ release and recruit β-arrestin. Furthermore, we show that the molecular basis for biased signaling differences between PTHrP₁₋₃₆ and properties of native PTHrP₁₋₁₄₁ are caused by the stabilization of a singular PTHR conformation and PTHrP₁₋₁₄₁ sensitivity to heparin, a sulfated glycosaminoglycan. Taken together, our results contribute to a better understanding of the biased signaling process of a native protein hormone acting in conjunction with a GPCR.

PKC signaling cascades. Developments in recording GPCR-signaling cascade in individual cells in real time using optical approaches during the decade of the '00s (1, 2) have revealed that PTH₁₋₃₄ and PTHrP₁₋₃₆ differ markedly by the duration and cellular localization of the cAMP response (3). Brief stimulation with PTHrP₁₋₃₆ induces only transient cAMP production from the cell surface that is rapidly desensitized upon recruitment of β-arrestins (βarrs), cytosolic adapter proteins that canonically act to occlude further G protein coupling and promote translocation of the ligand-receptor complex from the cell surface to early endosomes. In contrast, PTH₁₋₃₄ causes an additional sustained phase of cAMP generation *via* PTH-PTHR-βarr complexes that remain active in early endosomes. Thus, this distinction in the spatiotemporal cAMP profiles of PTH and PTHrP was proposed to be the underlying determinant responsible for their biological specificity.

Mature forms of PTH and PTHrP are originally synthesized and secreted as 84 aa and 141 aa proteins, respectively. Early reports demonstrating that their respective N-terminal part, PTH₁₋₃₄ and PTHrP₁₋₃₆, retain their full capacity to stimulate adenylyl cyclase in cAMP accumulation assays led to the utilization of these N-terminal fragments in most studies. Indeed, it was PTH₁₋₃₄ and PTHrP₁₋₃₆ that were used in the aforementioned work that revealed differences in the time courses and subcellular locations of cAMP production by these two peptides. In contrast to these earlier findings of transient signaling by PTHrP₁₋₃₆, a recent publication proposed sustained endosomal cAMP generation induced by full-length PTHrP₁₋₁₄₁ (4). The authors employed a combination of radioimmunoassays and chemical inhibitors to suggest that PTHrP₁₋₁₄₁ induces prolonged cAMP signaling in an endocytosis-dependent manner analogous to that observed for PTH₁₋₃₄; however, cAMP experiments were performed in the presence of phosphodiesterase inhibition, which provided a measure of the cumulative levels of cAMP produced during a defined time interval, as opposed to the dynamic levels of cAMP that result from the net effects of its production and breakdown. Furthermore, the chemical compounds utilized to inhibit endocytosis generated inconsistent results with experiments

Upon its activation, the parathyroid hormone (PTH) receptor (PTHR) triggers both G_s/cAMP/PKA and G_q/Ca²⁺/

[†] These authors contributed equally to this work.

* For correspondence: Jean-Pierre Vilardaga, jpv@pitt.edu.

Present address for Frédéric G. Jean-Alphonse: Physiologie de la Reproduction et des Comportements (PRC), Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement (INRAE), Centre National de la Recherche Scientifique (CNRS), Institut Français du Cheval et de l'Équitation (IFCE), Université de Tours, 37380 Nouzilly, France. (F.G.J.-A.).

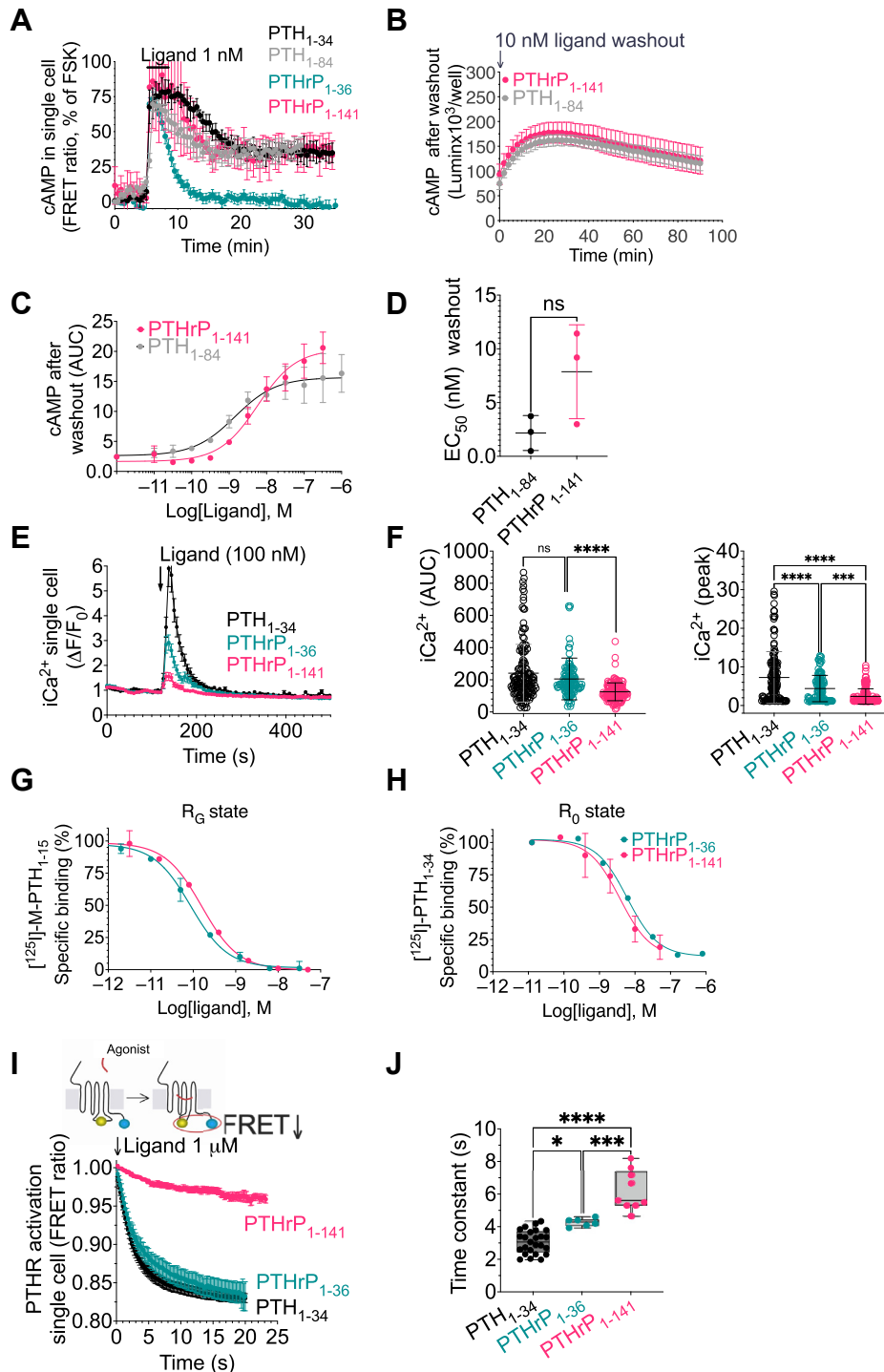


Figure 1. Signaling properties of PTHrP₁₋₁₄₁. *A*, time courses of cAMP in single HEK293 cells stimulated for 30 s with 1 nM ligands. Data are the mean ± SEM of *n* = 37 (PTHrP₁₋₃₆), *n* = 21 (PTHrP₁₋₁₄₁), *n* = 6 (PTH₁₋₈₄), and *n* = 7 (PTH₁₋₃₄) cells. *B*, time courses of cAMP in HEK-293 cells after washout of ligands measured by Glo-sensor assay. Data are the mean ± SEM of *n* = 3 experiments. *C* and *D*, relationship between cAMP responses in HEK-293 cells after washout of a range of ligand concentrations (*C*) and corresponding EC₅₀ values (*D*). Data represent the integrated response determined by measuring the area under the curve of experiments shown in panel (*B*) and are the mean ± SD of *n* = 3 experiments. *E*, intracellular Ca²⁺ mobilization measurements in single HEK-293 cells stably expressing PTHR. Data are the mean ± SEM for *n* = 44 (PTH₁₋₃₄), *n* = 42 (PTHrP₁₋₃₆), and *n* = 48 (PTHrP₁₋₁₄₁) cells. *F*, scatter plots with the mean ± SD of data shown in panel (*C*). *****p* < 0.0001 determined by one-way ANOVA with Tukey–Kramer post hoc test. (*G* and *H*) competition binding at equilibrium with [¹²⁵I]-PTH₁₋₁₅ and [¹²⁵I]-PTH₁₋₃₄ as radioligands to detect the R_G (*G*) and R₀ (*H*) states of PTHR, respectively. Data are mean ± SD from *N* = 2 independent experiments with duplicate wells for each concentration. *I* and *J*, kinetics of PTHR activation. Normalized activation kinetics of PTHR determined by FRET ratio changes from HEK293 cells expressing the receptor sensor (scheme) (*G*), and time constant (*t*) of PTHR activation determined by fitting curves in panel (*A*) to a monoexponential decrease (*H*). Mean ± SEM of *n* = 25 (PTH₁₋₃₄), *n* = 6 (PTHrP₁₋₃₆), and *n* = 9 (PTHrP₁₋₁₄₁) cells. **p* = 0.0114, ****p* = 0.0003, and *****p* < 0.0001 determined by one-way ANOVA with Tukey–Kramer post hoc test. PTH, parathyroid hormone; PTHR, PTH receptor.

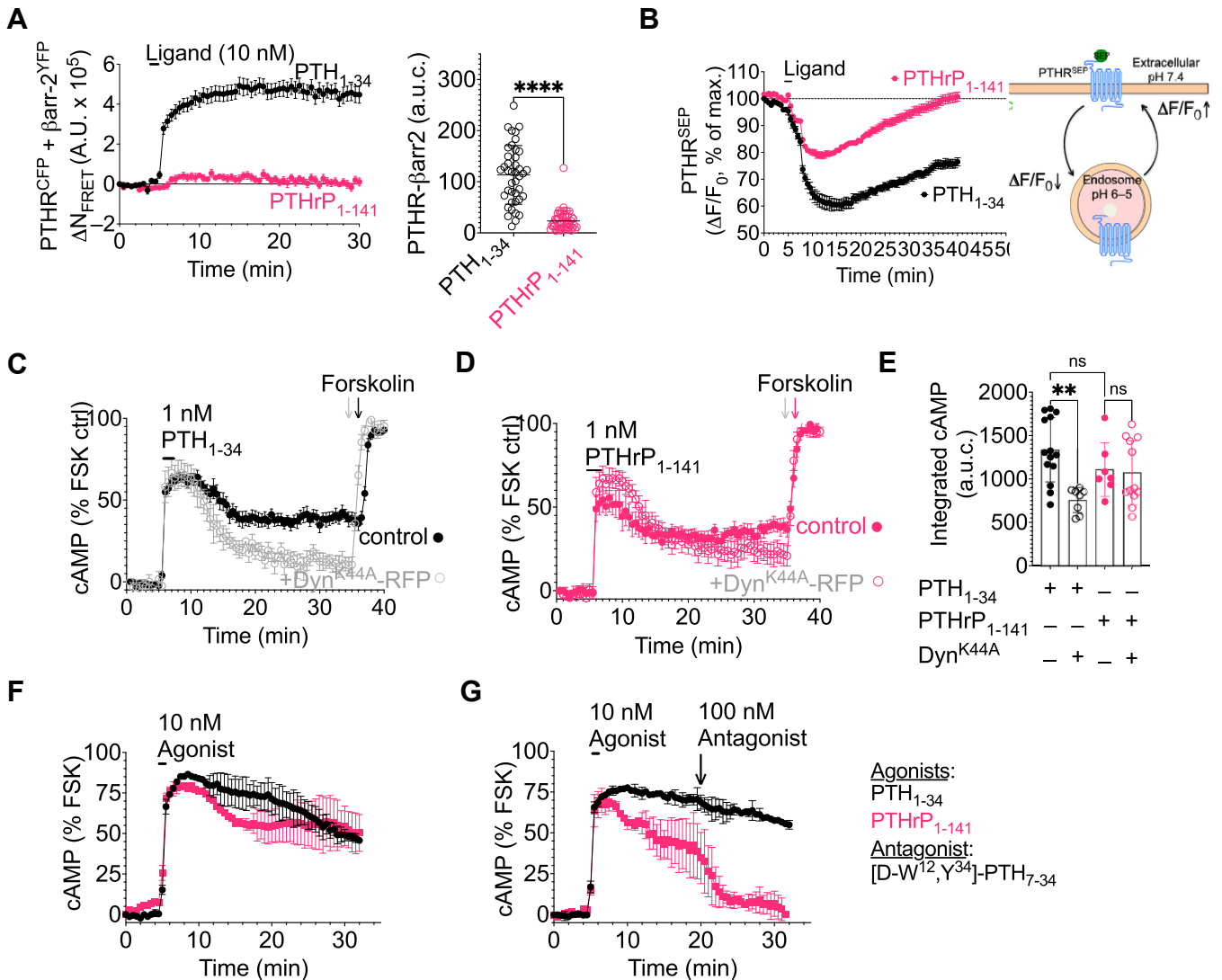


Figure 2. Endosomal cAMP signaling by PTHrP₁₋₁₄₁. *A*, time course of β-arrestin interaction with PTHR in HEK293 cells transiently expressing PTHR^{CFP} and βarr-2^{YFP} treated with 10 nM PTH₁₋₃₄ (black) or PTHrP₁₋₁₄₁ (red) for 30 s. Data are the mean ± SEM for $n = 40$ (PTH₁₋₃₄) and $n = 49$ (PTHrP₁₋₁₄₁) cells. The scatter plot shows the mean ± SD of the integrated response determined by measuring the area under the curve (a.u.c.) **** $p < 0.0001$ by *t* test. *B*, time courses of internalization and recycling of PTHR tagged with super-ecliptic pHluorin (PTHR^{SEP}) in response to 100 nM ligand measured by time-lapse confocal microscopy in single cells. The schematic illustrates the measured values. Data are mean ± SEM from $n = 12$ (PTH₁₋₃₄) and $n = 51$ (PTHrP₁₋₁₄₁) cells. *C–E*, time courses of cAMP in single HEK-293 PTHR cells transiently expressing with DynK44A^{RFP} compared to control in response to PTH₁₋₃₄ (*C*) and PTHrP₁₋₁₄₁ (*D*). Data are the mean ± SEM for $n = 14$ cells (PTH₁₋₃₄ control), $n = 9$ cells (PTH₁₋₃₄ Dyn^{K44A}), $n = 8$ (PTHrP₁₋₁₄₁ control), and $n = 12$ (PTHrP₁₋₁₄₁ Dyn^{K44A}) cells. *E*, the scatter plot represents the area under the curve (a.u.c.) corresponding to individual values and the mean ± SD. ** $p = 0.0017$ determined by one-way ANOVA with Tukey–Kramer post hoc test. *F*, time courses of cAMP in single HEK-293 PTHR cells stimulated for 30 s with 10 nM PTH₁₋₃₄ (black) or PTHrP₁₋₁₄₁. Data are the mean ± SEM of $n = 32$ (PTH₁₋₃₄) cells and $n = 46$ (PTHrP₁₋₁₄₁) cells. *G*, similar experiments as in panel (*E*) with addition of cell-impermeable PTHR antagonist 15 min after washout of PTH₁₋₃₄ or PTHrP₁₋₁₄₁. Data are the mean ± SEM of $n = 50$ (PTH₁₋₃₄) cells and $n = 37$ (PTHrP₁₋₁₄₁) cells. PTH, parathyroid hormone; PTHR, PTH receptor.

showing no reduction of sustained cAMP responses induced by PTHrP₁₋₁₄₁ or PTH₁₋₃₄, while others showed only reduction for PTHrP₁₋₁₄₁ but not for PTH₁₋₃₄. Reduction of PTH₁₋₃₄-induced sustained cAMP response by blocking receptor endocytosis is expected given this ligand's established ability to signal *via* internalized PTHR from early endosomes (3, 5–9). These considerations motivated the necessity to implement alternative methods that permit analysis of real-time cAMP response kinetics in real time in single cells. The results unveil the mechanism by which PTHrP₁₋₁₄₁ engages in sustained signaling and how this differs from the transient effects observed with the N-terminal fragment PTHrP₁₋₃₆.

Results and discussion

We utilized FRET to record real-time courses of cAMP production in single HEK293 cell stably expressing PTHR (HEK-PTHR). We found that brief stimulation with PTHrP₁₋₁₄₁ induced a sustained cAMP response that was similar in both magnitude and duration to that induced by PTH₁₋₈₄ or PTH₁₋₃₄ and clearly distinct from the short-lived cAMP response mediated by PTHrP₁₋₃₆ (Figs. 1A and S1). We next applied Glo-sensor cAMP accumulation assays to verify that time courses of sustained cAMP production mediated by the two native hormones, PTH₁₋₈₄ and PTHrP₁₋₁₄₁, were similar (Fig. 1B) and without a significant difference in the hormone concentration

ACCELERATED COMMUNICATION: Native PTHrP signaling

dependence (Fig. 1, C and D). We observed a striking inability of PTHrP₁₋₁₄₁ to efficiently induce the release of intracellular calcium (iCa²⁺) from the endoplasmic reticulum (Fig. 1, E and F), indicating defective Gq activation by PTHrP₁₋₁₄₁. We have previously shown that Gq activation is required for endosomal cAMP generation by PTH₁₋₃₄ (9), suggesting a differential location of cAMP generation by this ligand. Moreover, the molecular basis for the failure of PTHrP₁₋₁₄₁ to mimic cAMP and iCa²⁺ signaling responses mediated by PTHrP₁₋₃₆ were unlikely to be caused by different binding affinities to either G protein coupled (R_G) or uncoupled (R₀) states of PTHR (Fig. 1, G and H) but were rather due to the stabilization of a distinct receptor conformation. We tested this theory by using cells expressing a FRET-based PTHR sensor (scheme in Fig. 1I). Time-resolved determination of intramolecular FRET changes recorded from single cells allows the analysis of the kinetics of receptor activation in response to ligand binding (1). A decrease in FRET mediated by an agonist reflects receptor switching from an inactive to an active conformation, and distinct time-constants of receptor activation measured for a saturating concentration of agonists indicate the stabilization of distinct signaling receptor conformations (1, 3, 10). As expected, perfusion of a saturating concentration of PTH₁₋₃₄, PTHrP₁₋₃₆, or PTHrP₁₋₁₄₁ to individual cells triggered a decrease in FRET; however, the significantly distinct time constants (τ) for receptor activation indicated the stabilization of distinct PTHR conformations (Fig. 1, I and J).

To assess the role of β arr recruitment, we measured PTHR- β arr interactions *via* FRET in cells transiently expressing PTHR^{CFP} and β arr-2^{YFP}. The β arr2 isoform was randomly selected, given that earlier studies demonstrated that PTH₁₋₃₄ and PTHrP₁₋₃₆ displayed equal potencies (EC₅₀ values) for recruitment of both β -arr1 and β -arr2 (8, 11, 12).

Consistent with previous studies, addition of PTH₁₋₃₄ resulted in significant association of β arr with the receptor that was stably maintained following ligand washout (Fig. 2A). In contrast, analogous experiments using PTHrP₁₋₁₄₁ failed to promote this interaction (Fig. 2A), suggesting that the sustained signaling observed for PTHrP₁₋₁₄₁ occurs in a β arr-independent manner. This finding led us to test the role of receptor internalization, a key step in PTHR endosomal signaling. Measurements of receptor internalization and recycling in single cells stably expressing PTHR^{SEP}, the PTHR N-terminally tagged with a pH-sensitive GFP (super-ecliptic pHluorin SEP) that exhibits fluorescence intensity reduction in the acidic environment encountered in endosomes (scheme in Fig. 2B), showed reduced internalization and faster recycling in response to PTHrP₁₋₁₄₁ or PTHrP₁₋₃₆ when compared to PTH₁₋₃₄ (Figs. 2B and S2). We next determined whether internalized PTHrP₁₋₁₄₁-PTHR can signal *via* cAMP. We have previously shown that expression of a dominant-negative dynamin mutant, DynK44A, effectively blocks translocation of PTH-PTHR complexes from the cell surface and blunts the sustained phase of cAMP generation without affecting the forskolin response (3, 8). Accordingly, we compared the cAMP response following brief stimulation with PTHrP₁₋₁₄₁ in HEK-PTHR control cells and those transiently expressing DynK44A fused to a red fluorescent protein (DynK44A^{RFP}) (Fig. 2C). Strikingly, blockade of receptor internalization significantly reduced the magnitude and duration of cAMP production by PTH₁₋₃₄ (Fig. 2, C and E) but had no effect on cAMP mediated by PTHrP₁₋₁₄₁ (Fig. 2, D and E), indicating that native PTHrP does not promote sustained signaling in an endocytosis-dependent manner. We recently reported on the development of G_s-biased PTH analogs that

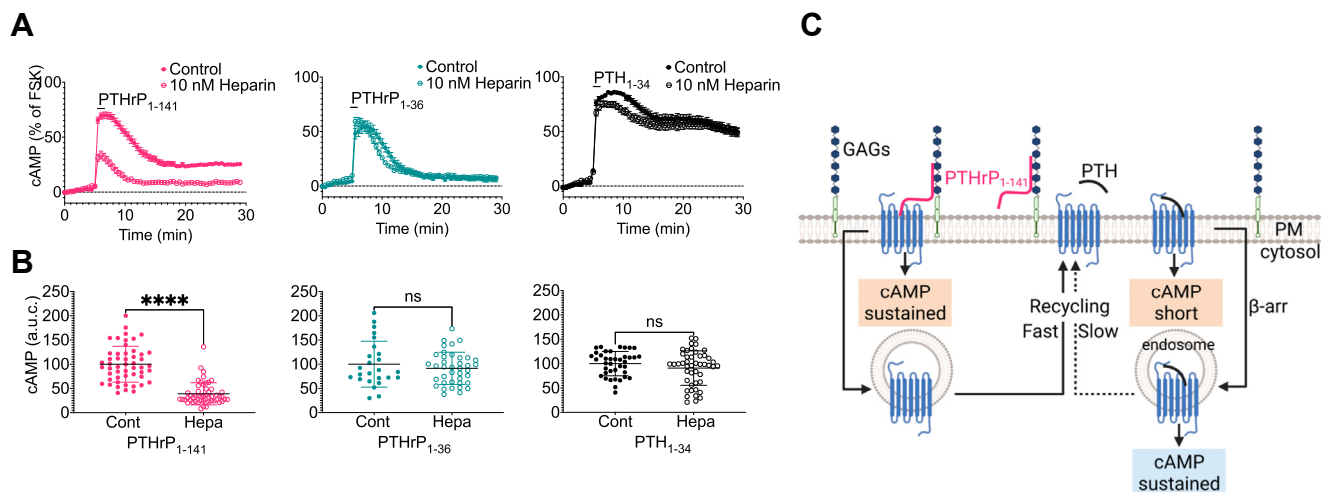


Figure 3. Effect of heparin on cAMP production. A, cAMP time courses in single HEK-293 PTHR cells in response to 1 nM ligands preincubated with 10 nM heparin. Data are the mean \pm SEM of $n = 51$ (control) and $n = 52$ (heparin) cells for PTHrP₁₋₁₄₁; $n = 25$ (control) and $n = 39$ (heparin) cells for PTHrP₁₋₃₆; $n = 39$ (control) and $n = 46$ (heparin) cells for PTH₁₋₃₄. The statistical analysis is in Table 1. B, corresponding scatter plots representing the area under the curve (a.u.c.) of individual values from (A). **** $p < 0.0001$ determined by *t* test. C, proposed mechanism for location-biased signaling of native PTHrP₁₋₁₄₁. The continuous cAMP signaling mediated by PTHrP₁₋₁₄₁ can be controlled by plasma membrane-anchored glycosaminoglycans that hypothetically retain PTHrP₁₋₁₄₁ at the cell surface thus permitting reactivation of recycled receptors. Created with BioRender.com. PTH, parathyroid hormone; PTHR, PTH receptor.

Table 1
Effect of heparin on cAMP production

Ligands	Control	Hep, 1 nM	<i>p</i> Value	Control	Hep, 10 nM	<i>p</i> Value
PTHrP ₁₋₁₄₁	100 ± 37 (33)	51 ± 30 (24)	< 0.0001	100 ± 37 (51)	38 ± 22 (52)	< 0.0001
PTHrP ₁₋₃₆	100 ± 26 (15)	129 ± 44 (9)	0.056	100 ± 47 (25)	92 ± 33 (39)	0.39
PTH ₁₋₃₄	100 ± 44 (43)	91 ± 31 (23)	0.38	100 ± 25 (39)	91 ± 35 (46)	0.18

The area under the curve (a.u.c) from data in Figure 3. Mean value ± SD of (N) experiments with *p* values determined by *t* test. Abbreviations: Hep, heparin.

stimulate sustained cAMP production exclusively from the cell surface due to retention of active ligand–receptor complexes at the cell surface. This was experimentally confirmed *via* cAMP time courses using a cell-impermeable PTHR antagonist, which completely abolished the sustained phase of cAMP generation for G_s-biased peptides but not for PTH₁₋₃₄, consistent with its ability to signal from intracellular compartments (8). We thus utilized this same approach to test whether PTHrP₁₋₁₄₁ likewise induces prolonged cAMP signaling *via* ligand–receptor complexes that are localized to the cell surface. Indeed, addition of the cell-impermeable antagonist at 15 min following agonist washout rapidly reduced cAMP levels to baseline in cells treated with PTHrP₁₋₁₄₁ but had no effect in those stimulated with PTH₁₋₃₄ (Fig. 2, F and G). These findings demonstrate that PTHrP₁₋₁₄₁ promotes sustained cAMP responses *via* active ligand–receptor complexes localized to the cell surface, which appear inconsistent with experiments showing receptor internalization.

To reconcile this apparent incompatibility, we hypothesized that the highly positively charged domain of PTHrP₁₋₁₄₁ (⁸⁸KKKKGKPGKRKEQKKRRTR¹⁰⁸), not present in PTHrP₁₋₃₆ or PTH, permits the hormone to attach to the cell surface *via* interactions with polyanionic glycosaminoglycans (GAGs) present on membrane glycoproteins such as heparan sulfate proteoglycan. Consistent with this theory was the significant reduction in the magnitude and duration of cAMP production in response to PTHrP₁₋₁₄₁ in the presence of soluble heparin used as a decoy to prevent potential PTHrP₁₋₁₄₁ and GAGs interactions (Fig. 3, A and B). The selective effect of heparin was verified by its lack of inhibitory action on cAMP induced by either PTH₁₋₃₄ or PTHrP₁₋₃₆ (Fig. 3A, and Table 1).

Collectively, these data prompt a reinterpretation of our previous understanding on how hormones act on the PTHR by providing compelling evidence that native PTHrP₁₋₁₄₁ is biased toward sustained PTHR signaling *via* cAMP at the plasma membrane. The results support a model where PTHrP₁₋₁₄₁ stabilizes an active receptor conformation that impairs βarr coupling and Gq signaling possibly through the interaction with GAG. Future experiments are needed for an extended characterization of PTHrP and GAG interaction as a possible means to reactivate recycled receptor by the cell surface–anchored hormone (Fig. 3C).

Experimental procedures

Materials and methods are detailed in SI Appendix.

Data availability

Source data are stored in Excel 2013 and will be deposited in the institutional repository of the University of Pittsburgh (<http://d-scholarship.pitt.edu/>).

Supporting information—This article contains supporting information (1, 5, 6, 10, 13–18).

Acknowledgments—We thank Dr T. John Martin for providing the PTH₁₋₁₄₁ protein to initiate this work.

Author contributions—I. S. and J. P. V. conceptualization; J. P. V. methodology; K. A. P., F. G. J. A., T. J. G., and J. P. V. validation; K. A. P., A. D. W., S. S., I. P. C., and F. G. J. A. investigation; A. D. W. and J. P. V. writing—original draft; K. A. P. and J. P. V. writing—review & editing; S. S. visualization; T. J. G. and J. P. V. supervision.

Funding and additional information—This research was supported by grant Award Numbers T32GM133332 (to S. S.) from the National Institute of General Medical Sciences (NIGMS), DK116780 and DK122259 (to J. P. V.) from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), United States of the US National Institutes of Health (NIH), United States, R01HD100468 from the NIH, and grant number CHE-9808188 (to the Center of Molecular Analysis, Department of Chemistry, Carnegie Mellon University) from the National Science Foundation (NSF), United States. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Conflict of interest—The authors declare that they have no conflicts of interest with the contents of this article.

Abbreviations—The abbreviations used are: βarr, β-arrestin; GAG, glycosaminoglycan; PTH, parathyroid hormone; PTHR, PTH receptor.

References

- Vilardaga, J. P., Bunemann, M., Krasel, C., Castro, M., and Lohse, M. J. (2003) Measurement of the millisecond activation switch of G protein-coupled receptors in living cells. *Nat. Biotechnol.* **21**, 807–812
- Vilardaga, J. P., Bunemann, M., Feinstein, T. N., Lambert, N., Nikolaev, V. O., Engelhardt, S., *et al.* (2009) GPCR and G proteins: drug efficacy and activation in live cells. *Mol. Endocrinol.* **23**, 590–599
- Ferrandon, S., Feinstein, T. N., Castro, M., Wang, B., Bouley, R., Potts, J. T., *et al.* (2009) Sustained cyclic AMP production by parathyroid hormone receptor endocytosis. *Nat. Chem. Biol.* **5**, 734–742
- Ho, P. W. M., Chan, A. S., Pavlos, N. J., Sims, N. A., and Martin, T. J. (2019) Brief exposure to full length parathyroid hormone-related protein (PTHrP) causes persistent generation of cyclic AMP through an endocytosis-dependent mechanism. *Biochem. Pharmacol.* **169**, 113627

5. Feinstein, T. N., Wehbi, V. L., Ardura, J. A., Wheeler, D. S., Ferrandon, S., Gardella, T. J., *et al.* (2011) Retromer terminates the generation of cAMP by internalized PTH receptors. *Nat. Chem. Biol.* **7**, 278–284
6. Gidon, A., Al-Bataineh, M. M., Jean-Alphonse, F. G., Stevenson, H. P., Watanabe, T., Louet, C., *et al.* (2014) Endosomal GPCR signaling turned off by negative feedback actions of PKA and v-ATPase. *Nat. Chem. Biol.* **10**, 707–709
7. Jean-Alphonse, F. G., Wehbi, V. L., Chen, J., Noda, M., Taboas, J. M., Xiao, K., *et al.* (2017) beta2-adrenergic receptor control of endosomal PTH receptor signaling via Gbetagamma. *Nat. Chem. Biol.* **13**, 259–261
8. White, A. D., Peña, K. A., Clark, L. J., Maria, C. S., Liu, S., Jean-Alphonse, F. G., *et al.* (2021) Spatial bias in cAMP generation determines biological responses to PTH type 1 receptor activation. *Sci. Signal.* **14**, eabc5944
9. White, A. D., Jean-Alphonse, F. G., Fang, F., Peña, K. A., Liu, S., König, G. M., *et al.* (2020) Gq/11-dependent regulation of endosomal cAMP generation by parathyroid hormone class B GPCR. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 7455–7460
10. Vilardaga, J. P. (2011) Studying ligand efficacy at G protein-coupled receptors using FRET. *Met. Mol. Biol.* **756**, 133–148
11. Liu, S., Jean-Alphonse, F. G., White, A. D., Wootten, D., Sexton, P. M., Gardella, T. J., *et al.* (2019) Use of backbone modification to enlarge the spatiotemporal diversity of parathyroid hormone receptor-1 signaling via biased agonism. *J. Am. Chem. Soc.* **141**, 14486–14490
12. Vilardaga, J. P., Krasel, C., Chauvin, S., Bambino, T., Lohse, M. J., and Nissenson, R. A. (2002) Internalization determinants of the parathyroid hormone receptor differentially regulate beta-arrestin/receptor association. *J. Biol. Chem.* **277**, 8121–8129
13. Hammonds, R. G., McKay, P., Jr., Winslow, G. A., Diefenbach-Jagger, H., Grill, V., Glatz, J., *et al.* (1989) Purification and characterization of recombinant human parathyroid hormone-related protein. *J Biol Chem* **264**, 14806–14811
14. Li, J., Dong, S., Townsend, S. D., Dean, T., Gardella, T. J., Danishefsky, S. J., *et al.* (2012) Chemistry as an expanding resource in protein science: fully synthetic and fully active human parathyroid hormone-related protein (1-141). *Angew Chem Int Engl* **51**, 12263–12267
15. Wehbi, V. L., Stevenson, H. P., Feinstein, T. N., Calero, G., Romero, G., and Vilardaga, J. P. (2013) Noncanonical GPCR signaling arising from a PTH receptor-arrestin-Gbetagamma complex. *Proc Natl Acad Sci U S A* **110**, 1530–1535
16. Castro, M., Dicker, F., Vilardaga, J. P., Krasel, C., Bernhardt, M., and Lohse, M. J. (2002) Dual regulation of the parathyroid hormone (PTH)/PTH-related peptide receptor signaling by protein kinase C and beta-arrestins. *Endocrinology* **143**, 3854–3865
17. Dean, T., Vilardaga, J. P., Potts, J. T., Jr., and Gardella, T. J. (2008) Altered selectivity of parathyroid hormone (PTH) and PTH-related protein (PTHrP) for distinct conformations of the PTH/PTHrP receptor. *Mol Endocrinol* **22**, 156–166
18. McGarvey, J. C., Xiao, K., Bowman, S. L., Mamonova, T., Zhang, Q., Bisello, A., *et al.* (2016) Actin-sorting nexin 27 (SNX27)-retromer complex mediates rapid parathyroid hormone receptor recycling. *J Biol Chem* **291**, 10986–11002