

Reply to: The G protein preference of orexin receptors is currently an unresolved issue

Received: 15 January 2023

Accepted: 15 May 2023

Published online: 01 June 2023

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REPLYING TO J. P. Kukkonen *Nature Communications* <https://doi.org/10.1038/s41467-023-38764-3> (2023)

Kukkonen challenges our interpretation of data in Figure 5d of our paper¹. In this experiment, we co-transfected HEK293 cells with CMV-driven overexpression plasmids for OX₂R and G_{o1l} along with a pGlo-Sensor cAMP reporter construct, and measured diminished cAMP levels with OX₂R agonists after forskolin stimulation. Results from this experiment showed a weak reduction in cAMP, without a characteristic low-nanomolar EC₅₀ for either TAK-925 or Orexin B (in contrast to the parallel inositol phosphate accumulation assay with overexpressed OX₂R and G_{o1l}). We acknowledge the validity of Kukkonen's arguments that the cAMP data could also be explained by a combination of signaling through different G protein subtypes and/or regulation by PDEs. We also appreciate his arguments that the G protein selectivity or promiscuity exhibited by OX₂R will depend on the cell line and experimental setup, and has not been definitively established in CNS neurons.

Reference

1. Yin, J. et al. Molecular mechanism of the wake-promoting agent TAK-925. *Nat Commun* **13**, 2902 (2022).

Acknowledgements

Portions of the original research were supported by the National Institutes of Health (R01NS097594 to J.K.D.B. and D.M.R.; R35GM116387 to D.M.R.), the Welch Foundation (I-1422 to J.K.D.B.; I-1770 to D.M.R.; I-1944 to X.B.), the Edward Mallinckrodt, Jr. Foundation (Scholar Award to D.M.R.), and the Julie and Louis Beecherl, Jr., Chair in Medical Science at The University of Texas Southwestern (to J.K.D.B.). The original OX₂R-mGsqiN cryo-EM data were collected at The University of Texas Southwestern Medical Center Cryo-EM Facility, which is funded by the CPRIT Core Facility Support Award RP170644.

Author contributions

J.Y., Y.K., M.F., J.K.D.B., and D.M.R. designed the original research. J.Y., Y.K., K.C., Y.S., R.R., and P.L. performed experiments. J.Y., Y.K., A.P.M., K.C., E.K., A.O., T.K., Y.M., Y.S., R.R., P.L., X.B., J.K.D.B., and D.M.R. analyzed data. J.Y., Y.K., A.P.M., M.S., J.K.D.B., T.K., M.F., and D.M.R. wrote the original paper. Authors from The University of Texas Southwestern Medical Center (J.Y., K.M., R.R., P.L., X.B., J.K.D.B., D.M.R.) obtained structural data for OX₂R-mGsqiN bound to TAK-925. Authors from Takeda Pharmaceutical Company Limited (Y.K., A.P.M., M.S., E.K., A.O., T.K., Y.M., Y.S., M.F.) obtained structural data for OX₂R-Gi1 bound to TAK-925. All authors contributed to this reply.

Competing interests

J.Y., K.C., R.R., P.L., X.B., J.K.D.B., and D.M.R. declare no competing interests. Y.K., A.P.M., M.S., E.K., A.O., T.K., Y.M., Y.S., and M.F. are current or former employees of Takeda Pharmaceutical Company Limited.

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Peer review information *Nature Communications* thanks the anonymous reviewers for their contribution to the peer review of this work.

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