BIOGRAPHICAL SKETCH

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NAME: Hamm, Heidi E, PhD

POSITION TITLE: Professor of Pharmacology

eRA COMMONS USER NAME (credential, e.g., agency login): HAMMHE

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Atlantic Union College, Lancaster, MA	B.A.	1973	Languages
University of Texas--Austin	Ph.D.	1980	Zoology
University of Wisconsin--Madison	Postdoctoral	1980-1983	Biochemistry

A. Personal Statement

My role in this grant is to provide expertise in the physiological outcomes of disabling the Gβγ interaction with the ternary SNARE complex. We have showed that mice that lack the receptor-mediated inhibition of secretion through Gβγ-SNARE are resistant to diet induced obesity, and we will now determine whether the beneficial metabolic effects when the mutation is present only in adrenergic neurons. My lab has been involved in signal transduction research in my laboratory since it first started in 1983, as well as in my postdoctoral work from 1980-1983. I then determined the three-dimensional structures of heterotrimeric G protein α subunits in their active and inactive conformations and in complex with the Gβγ subunits with Paul Sigler. We are currently working on the regulation of vesicular exocytosis mediated by Gi/o-coupled presynaptic receptors by Gβγ subunit binding to SNAREs. One of the most important roles of Gβγ subunits is to monitor and control secretion of hormone and neurotransmitter release by $G_{i/o}$-coupled inhibitory receptors. They do this through a dual regulation of the amount of Ca^{2+} coming in through voltage gated Ca^{2+} channels, and direct inhibition of synaptotagmin binding to SNARE proteins at the exocytotic fusion apparatus. We discovered this novel Gβγ regulation of exocytotic fusion and showed that Gβγ directly competes with synaptotagmin binding to SNAP25 and syntaxin, together with Simon Alford's laboratory. This results in an inhibition of vesicular fusion. We have created an animal model with defective Gβγ-SNARE interaction to study the role of this novel mechanism of neuromodulation in physiology and behavior. I have mentored over 20 graduate students, over 35 postdoctoral fellows, and three cardiology fellows, and many of them are in full, associate, and assistant professor positions in academia, or in scientific positions in biotech and pharma.

a. Blackmer T, Larsen EC, Takahashi M, Martin TF, Alford S, Hamm HE. 2001. G protein βγ subunit-mediated presynaptic inhibition: regulation of exocytotic fusion downstream of Ca^{2+} entry. *Science* 292,293-297.
b. Gerashshenko T, Blackmer T, Yoon EJ, Bartleson C, Hamm HE, Alford S. 2005. Gβγ acts at the C terminus of SNAP-25 to mediate presynaptic inhibition. *Nat Neurosci.* 8,597-605.
c. Zurawski Z, Thompson Gray AD, Brady LJ, Page B. Church E, Harris NA, Dohn MA, Yim Y-Y, Hyde K, Mortlock DP, Jones CK, Winder DG, Alford S, HE Hamm. Disabling Gβγ SNARE interaction in transgenic mice disrupts GPCR-mediated presynaptic inhibition leading to physiological and behavioral phenotypes. *Sci. Signaling*, 2019 Feb 19;12(569). pii: eaat8595.
d. Ceddia, R. P., Zurawski, Z., Thompson Gray, A.D., Adegboye, F., Shi, F., Liu, D., McGuinness, O. P., Collins, S., H. E. Hamm. Impairment of the Gβγ-SNARE brake on exocytosis enhances insulin action, protects against diet-induced obesity, and promotes adipocyte browning. biorXiv/2020/069138.

B. Positions and Honors

POSITIONS

1983-l984 Asst. Prof., Dept. of Visual Science, School of Optometry, Indiana University, Bloomington, IN.

1984-1996 Asst., Assoc. and Full Prof., Dept. of Physiol. and Biophys., Univ. Ill. Chicago Coll. of Medicine.
1996-2000 Prof., Dept. of Mol. Pharm. and Biol. Chem., Northwestern Univ. School of Medicine.
2000–2014 Professor and Chair, Department of Pharmacology, Vanderbilt University Medical Center.
2014-present Professor, Department of Pharmacology, Vanderbilt University Medical Center.

HONORS AND PROFESSIONAL RESPONSIBILITIES

Keynote Address, Gordon Research Seminar on Phosphorylation and G Protein Mediated Signaling Networks, University of New England, June 2, 2018.
Member, Panel on Early Translational Research Needs in Blood Science Sponsored by the Division of Blood Diseases and Resources, NHLBI. Sept 11, 2017
2015 Robert R. Ruffolo Career Achievement Award in Pharmacology given by the American Society for Pharmacology and Experimental Therapeutics (ASPET).
Member, External Review Panel (ERP) for the Translational Research Centers in Thrombotic and Hemostatic Disorders (TRC-THD) Program, The National Heart, Lung, and Blood Institute, 2013-.
Ariens Award, Dutch Pharmacological Society (NVF), Ariens Society Annual Meeting, Lunteren, The Netherlands, 2012.
Aileen M. Lange and Annie Mary Lyle Chair in Cardiovascular Research, Department of Pharmacology, Vanderbilt University Medical Center.2012-present.
2011 Women to Watch, Nashville Medical News and Nashville Health Care Council.
Earl W. Sutherland, Jr. Chair, Department of Pharmacology, Vanderbilt University Medical Center, 2000-2012.
Peer Review Advisory Committee, National Institutes of Health, 2007-2011; Member, Advisory Council, Center for Scientific Review, 2011-2012
President, ASBMB, 2006-2008; Secretary 1995-1998; Chair, Program Committee 1998 Annual Meeting.
Scientific Advisory Board, CAESAR, Max Planck Society, 2009-2014.
Board of Directors, Keystone Symposia on Mol and Cell Biology, 2011-2017; Keystone SAB 2008-2017.
FASEB Science Policy Committee Peer Review Subcommittee, 2006-2010, Board Advisor 2007-08
Newmark Award Lecture in Biochemistry. “How do receptors catalyze G protein activation?” University of Kansas, Lawrence, Kansas, October 8, 2007.
The National Academies Committee on Prospering in the Global Economy of the 21st Century, 2005
HHMI Review Board, 2004, 2008.
Stanley Cohen Award, Outstanding Contributions to Research, Vanderbilt University, April, 2003.
Fritz Lipmann Memorial Lectureship, 92nd annual meeting ASBMB, Orlando Florida, April, 2001.
Grable Investigator, Distinguished Investigator Award, NARSAD, 2003
Board of Scientific Counselors, NHLBI, NIH, 1998-2002.
NIH Visual Sciences C Study Section, regular member, 1991-1995; NIH Reviewers Reserve, 1995-1998.
Lee & Robert Peterson Distinguished Investigator Award, NARSAD,1997.
Biophysical Society Councilor, 1993-1997.
Editorial Boards: (1) J. Biol. Chem., 1994-1999, (2) Biochemistry, 1994-1998 (3) Mol. Pharm., 1994-2009
Chair, Gordon Conference on Cyclic Nucleotides and Protein Phosphorylation, 1995.
Biochemistry Organizing Committee, Association for Research in Vision and Ophthalmology, 1990-1993.
Glaxo Cardiovascular Discovery Award, 1989-1991.
National Science Foundation Research Opportunities for Women Career Development Award, 1987-1989.

C. Contribution to Science

1. G protein structure and signaling

G proteins are switch proteins; they are normally inactive, but a receptor that has received a specific signal can activate them, leading to changes in the activity of enzymes that produce second messengers such as cAMP and calcium. G protein signaling cascades are key regulators of many physiological processes, including development, differentiation, and regulation of cell division. In brain, many neurotransmitters and neuromodulators mediate a myriad of functions by activating G protein cascades. Research in my lab is aimed at understanding how G proteins become activated, how they in turn activate effector enzymes, and how they turn off. We determined the sites of interaction between proteins using a method of decomposing the proteins into small synthetic peptides and determining which peptides blocked interaction sites. To understand the process more fully, we used X-ray crystallography to solve the three-dimensional structures of G proteins in their inactive (GDP bound), and activated (GTPγS-bound) forms in collaboration with Paul Sigler’s group.

These were the first structures of a heterotrimeric G protein, which revealed for the first time the structure of the helical domain, an insert with respect to the ras family of small GTPases. We caught a glimpse of the self-inactivating process in another crystal form, the transition state analog, $G\alpha.GDP.AlF_4^-$. Soon after, structures of the $\beta\gamma$ subunit and the heterotrimeric G protein were solved. These high-resolution structural studies allowed us to postulate specific hypotheses regarding mechanisms of receptor:G protein interaction and activation, G protein subunit association-dissociation and effector activation.

a. Noel JP, Hamm HE, Sigler PB. 1993. The 2.2 A crystal structure of transducin-α complexed with GTPγS. *Nature* 366,654-663.

b. Lambright DG, Noel JP, Hamm HE, Sigler PB. 1994. Structural determinants for activation of the α-subunit of a heterotrimeric G protein. *Nature* 369,621-628.

c. Sondek J, Bohm A, Lambright DG, Hamm HE, Sigler PB. 1996. Crystal structure of a G-protein $\beta\gamma$ dimer at 2.1A resolution. *Nature* 379,369-374.

d. Lambright DG, Sondek J, Bohm A, Skiba NP, Hamm HE, Sigler PB. 1996. The 2.0 A crystal structure of heterotrimeric G protein. *Nature* 379,311-319.

2. Mathematical modeling of the spatio-temporal dynamics of visual transduction

Signaling pathways initiated by GPCRs have long been the focus of mathematical and biological modelers. The overwhelming majority of these models have for simplified their respective systems by assuming a well-stirred cytosol. This removes all spatio-temporal aspects involved, reducing the complexity of the interplay between the cell surface and volume. To capture the systems behavior of the biochemical and physiological machinery working together, we have collaborated with mathematician modelers, engineers, computational biologists and biologists. The mathematicians used the approach of homogenized limits and concentrated capacity to capture the spatial localization in the cell of each component of the signaling pathway as well as the temporal changes in physiological state. We implemented biochemical and electrophysiological data from experiments as model parameters for understanding the system behavior of rod phototransduction in low light. We determined that diffusion of second messengers in the cytoplasm acts as a variability suppressor to confer stereotypical single photon responses in rods. The model captured the importance of incisures in rods and also showed that the kinetics of rhodopsin deactivation regulates recovery as well as reproducibility of the photon response. Finally, we systemically investigated which factors are most important to reduce the variability of the single photon response. We use the model to run “virtual” experiments, to rapidly screen many scenarios using simulations, and as an aid to design “real” biochemical and electrophysiological experiments, on rods and cones from genetically modified animals. We also used the model to understand how rods adapt to light.

a. Andreucci D, Bisegna P, Caruso G, Hamm HE, DiBenedetto E. 2003. Mathematical model of the spatio-temporal dynamics of second messengers in visual transduction. *Biophys J* 85, 1358-1376.

b. Bisegna P, Caruso G, Andreucci D, Shen L, Gurevich VV, Hamm HE, DiBenedetto E. 2008. Diffusion of the second messengers in the cytoplasm acts as a variability suppressor of the single photon response in vertebrate phototransduction. *Biophys J* 94, 3363-3383. PMC2292384

c. Caruso G, Bisegna P, Lenoci L, Andreucci D, Gurevich VV, Hamm HE, DiBenedetto E. 2010. Kinetics of rhodopsin deactivation and its role in regulating recovery and reproducibility of rod photoresponse. *PLoS Computational Biol* 6, e1001031. doi: 10.1371/journal.pcbi.1001031. PMC3002991

d. Caruso G, Bisegna P, Andreucci D, Lenoci L, Gurevich VV, Hamm HE, DiBenedetto E. 2012. Identification of key factors that reduce the variability of the single photon response. *Proc Natl Acad Sci USA* 108, 7804-7807. PMC3093507

3. Biophysical and structural studies of G protein signaling

Dynamic measures of the conformational changes underlying signaling require a number of solution biophysical methods, such as fluorescence and electron paramagnetic resonance. We use the approach of engineering in Cys residues into Cys-less mutant G proteins that can then be targeted with fluorescent groups or nitroxides. Such engineered G protein molecules can be monitored constantly as they undergo conformational changes during the activation and inactivation process. Double Cys mutants can be used in double electron-electron resonance (DEER) experiments to provide information about changes in intra- or inter-molecular distances during signaling, and cross-linking studies can assess the relevance of the conformational changes for the signaling process. Time-resolved fluorescence studies are being used to track fast conformational changes to investigate a number of structural problems in signal transduction. How does a G protein-coupled receptor interact with the heterotrimeric G protein? What are the contact sites, and how does it work at a distance to catalyze GTP/GDP exchange? What is the path through the molecule that

ultimately causes GDP release? Upon GTP binding to the G protein α subunit, what drives dissociation from the receptor? These studies have been published in a number of papers.

a. Oldham WM, Van Eps N, Preininger AM, Hubbell WL, Hamm HE. 2007. Mapping allosteric connections from receptor to the nucleotide-binding pocket of heterotrimeric G proteins. *Proc Natl Acad Sci USA* 104,7927-7932.

b. Alexander NS, Preininger AM, Kaya AI, Stein RA, Hamm HE, Meiler J. 2014. Energetic analysis of the rhodopsin-G-protein complex links the α5 helix to GDP release. *Nat Struct Mol Biol*. 21,56-63. PMC3947367

c. Kaya AI, Lokits AD, ~Gilbert JA, Iverson TM, Meiler J, Hamm HE. 2014. A conserved phenylalanine as a relay between the α5 helix and the GDP binding region of heterotrimeric Gi protein α subunit. *J Biol Chem.* 289,2447524487. PMC4148873

d. Kaya AI, Lokits AD, Gilbert JA, Iverson TM, Meiler J, Hamm HE. 2016. A conserved hydrophobic core in Gα1 regulates G protein activation and release from activated receptor. *J Biol Chem* 291,19674-19686. PMC5016700

4. Regulation of synaptic transmission by G$\beta\gamma$ mediated inhibition of exocytotic fusion

We are investigating the molecular basis for interactions of $\beta\gamma$ subunits of G proteins with downstream effectors. It is known that a number of signaling pathways are regulated by free $\beta\gamma$ subunits, which are liberated by the GTP-dependent dissociation from the α subunit. Some of these are K^+ and Ca^{2+} channels, phospholipase Cβ, PI-3-kinase, certain isoforms of adenylyl cyclase, βARK, and MAP kinase cascades. Site-directed Ala scanning mutagenesis was used to characterize the molecular basis of these interactions. One of the most important roles of G$\beta\gamma$ subunits is to monitor and control the amount of hormone or neurotransmitter release from cells or synapses by Gi/o-coupled inhibitory receptors. They do this through a dual regulation of the amount of Ca^{2+} coming in through voltage gated Ca^{2+} channels, and direct inhibition of synaptotagmin binding to SNARE proteins, the exocytotic fusion apparatus. Synaptotagmin is the Ca^{2+} sensor that triggers SNARE assembly and exocytosis in response to an action potential. We showed that G$\beta\gamma$ directly competes with synaptotagmin binding to SNAP25 and syntaxin, two of the SNARE proteins. This inhibits vesicular fusion and changes vesicle fusion mode to “kiss-and-run”. We believe that this is one of the regulatory mechanisms the neural and endocrine systems use to modulate hormone and neurotransmitter release; in brain, this is a key mechanism for modulating neural circuitry in information processing. We are interested in this mechanistically and are working on understanding the details of how these proteins interact. G$\beta\gamma$ inhibits vesicular fusion even in a completely reconstituted system. We showed that removal of the C-terminal 3 amino acids from SNAP25 decreases G$\beta\gamma$-SNARE interaction and based on this finding, we generated CRISPR mice with a premature stop codon. These mice have interesting phenotypes that we are teasing apart.

a. Blackmer T, Larsen EC, Takahashi M, Martin TF, Alford S, Hamm HE. 2001. G protein $\beta\gamma$ subunit-mediated presynaptic inhibition: regulation of exocytotic fusion downstream of Ca^{2+} entry. *Science* 292,293-297.

b. Gerashshenko T, Blackmer T, Yoon EJ, Bartleson C, Hamm HE, Alford S. 2005. G$\beta\gamma$ acts at the C terminus of SNAP-25 to mediate presynaptic inhibition. *Nat Neurosci.* 8,597-605.

c. Zurawski Z, Rodriguez S, Hyde K, Alford S, Hamm HE. 2016. G$\beta\gamma$ binds to the extreme C terminus of SNAP25 to mediate the action of $G_{i/o}$-coupled G protein-coupled receptors. *Mol Pharmacol* 89, 75-83. PMC4702098

d. Zurawski Z, Thompson Gray AD, Brady LJ, Page B. Church E, Harris NA, Dohn MA, Yim Y-Y, Hyde K, Mortlock DP, Jones CK, Winder DG, Alford S, HE Hamm. Disabling Gβγ SNARE interaction in transgenic mice disrupts GPCR-mediated presynaptic inhibition leading to physiological and behavioral phenotypes. *Sci. Signaling*, 2019 Feb 19;12(569). pii: eaat8595.

5. Protease activated receptor function in the cardiovascular system

Protease-Activated Receptors (PARs) are unique among GPCRs in that they are activated by thrombin through proteolytic generation of a tethered ligand. The ability of the tethered ligand to initiate signaling through intramolecular binding has hampered the generation of potent antagonists to these receptors; partly because thrombin’s proteolytic cleavage of PAR is irreversible and the tethered ligand cannot diffuse away from the receptor. Thrombin activates PAR1 and PAR4 on human platelets to initiate signaling cascades leading to increases in $[Ca^{2+}_i]$, secretion of autocrine activators, trafficking of adhesion molecules to the plasma membrane, “inside-out” integrin activation and shape change, which all promote platelet aggregation. We uncovered a number of differences in signaling properties of PAR1 and PAR4 receptors in platelets and are also studying the synergistic actions of thrombin and collagen receptors in platelet activation.

BY studying platelets from diabetics, we seek to understand why patients with diabetes, insulin resistance and obesity have an increased tendency to form clots that cause heart attacks and strokes. Therein we documented resistance to inhibition of clinically relevant targets among diabetics and alarming differences in G-protein coupled responses depending on race. The long term goal is to understand how aberrant signaling downstream of G-protein coupled receptors contributes to the progression of cardiovascular disease.

More recently we have begun to consider PARs from a pharmacological perspective. The thrombin receptors work in a progressive manner, with PAR1 activated at low thrombin concentrations, and PAR4 recruited at higher thrombin concentrations implying distinct roles for the two receptors in thrombosis and hemostasis. Indeed, PAR4 is engaged more slowly as platelets generate thrombin and displays superior efficacy, so it may be a better pharmacological target that may reduce the thrombotic burden while leaving hemostasis intact. However, the lack of an efficient PAR4 antagonist has prevented an accurate assessment of PAR4's role, therefore we initiated efforts to target PAR4 with a small molecule. Efficacious scaffolds have been identified and current efforts are focused on improving pharmacokinetic profiles and potency. We anticipate having a tool compound that can probe the role of PAR4 in physiologically relevant contexts *in vivo.*

a. Cleator JH, Duvernay MT, Holinstat M, Colowick NE, Hudson WJ, Song Y, Harrell FE, Hamm HE. 2014. Racial differences in resistance to P2Y12 receptor antagonists in type 2 diabetic subjects *J Pharmacol Exp Ther* 351,33-43. PMC4165026

b. Duvernay MT, Temple KJ, Maeng JG, Blobaum AL, Stauffer SR, Lindsley CW, Hamm HE. 2017. Contributions of protease-activated receptors PAR1 and PAR4 to thrombin-induced GPIIbIIa activation in human platelets. *Mol Pharmacol* 91,39-47. PMC5198515

c. Duvernay MT, Matafonov A, Lindsley CW, Hamm HE. Platelet Lipidomic Profiling: Novel Insight into Cytosolic Phospholipase $A_2\alpha$ Activity and Its Role in Human Platelet Activation. Biochemistry. 2015 Sep 15;54(36):5578-88. doi: 10.1021/acs.biochem.5b00549. Epub 2015 Sep 1.PMID: 26295742

d. Bertron JL, Duvernay MT, Mitchell SG, Smith ST, Maeng JG, Blobaum AL, Davis DC, Meiler J, Hamm HE, Lindsley CW. Discovery and Optimization of a Novel Series of Competitive and Central Nervous System-Penetrant Protease-Activated Receptor 4 (PAR4) Inhibitors. ACS Chem Neurosci. 2021 Dec 15;12(24):4524-4534. doi: 10.1021/acschemneuro.1c00557. Epub 2021 Dec 2.PMID: 34855359

D. Research Support

1R01 DK109204-01 (Hamm) 04/01/2016-03/31/2021

GPCR Regulation of insulin secretion by modulation of the release machinery

Our proposal is to determine if reversing Gβγ-SNARE interaction with small molecule inhibitors can enhance glucose-stimulated insulin secretion in normal mice and in animal models of T2D.
Role: PI

This proposal is in NCE.

1R01 HL133923-01A1 (Hamm) 04/01/2017-03/31/2021

Targeting PAR4 in Thrombotic Disorders: Pharmacogenomic Approach

The overall goal of this grant is to develop a new class of anti-platelet compounds for managing the risk and treatment of thrombosis, by targeting protease activated receptor 4 (PAR4). Role: PI

1R01 NS111749-01(Hamm) 01/01/2019-31/12/2023

Regulation of exocytosis by direct Gβγ blockade of fusion

This Multi PI grant is dedicated to discovering which GPCRs regulate calcium channels and which work through the Gβγ-SNARE complex.