Introduction

Objectives: Ischemic vascular disease remains a significant healthcare and economic burden in the United States and worldwide. Despite major advances in our knowledge of diverse underpinnings of ischemic disease, fully effective therapies remain elusive and, in turn, its morbidity and mortality persist. A historically ignored facet of ischemic pathology is localized tissue acidosis, typically considered merely as a metabolic byproduct. Further complicating our understanding of the impact of tissue acidosis is regional heterogeneity between intracellular, extracellular, and plasma membrane acid/base levels. The objective of this project is to advance precision in assessing acid/base status in order to determine its regulatory impact on vascular ischemia. Built on preliminary in vivo and in vitro data using wild type (WT) and pH-sensing G protein-coupled receptor (GPR)-deficient models, this project will establish capacity of our newly developed GPR-tethered pH reporter SNAP in the assessment of acid/base status discretely at membrane GPR microdomains in ischemic tissues. Aim 1 will establish functionality of GPR-SNAP in synthetic heterogeneous liposomal membranes and Aim 2 will determine translational relevance of GPR-SNAP in WT and GPR-deficient vascular cells and intact arteries. Methods: Biological functionality of GPR-SNAP will be examined using cell-free liposomal membranes of varying compositions with/without GPR over-expression (Aim 1) and primary vascular cells and intact arteries from WT and GPR-deficient mice under normal or ischemic conditions (Aim 2). Comprehensive pH assessment in cytosolic, extracellular, and membrane preparations will be performed. Anticipated Results & Conclusions: Using artificial and in vitro live cell membranes, results are expected to demonstrate functionality of GPR-SNAP as an advanced and precise pH biosensor in vascular ischemia and will highlight GPR-SNAP as a next generation pH sensor for discrete determination of acid/base status in broad ischemic pathologies.

Abstract

A family of pH-sensing G protein-coupled receptors (GPCRs) has highlighted the importance of focal membrane acid/base status in (patho)physiology. pH-sensing GPR68 identified in vascular smooth muscle (VSM), yet its role is vascular physiology & pathology including ischemia is not known. To address these gaps in our knowledge, we synthesized first- and second-generation pH reporters and conjugated them to extracellular GPR68 we will examine their capacity to sense GPR68-specific membrane pH in cell-free, in vitro, and in vivo models under normal or ischemic/acidic conditions.

Methods

Aim 1: Biological functionality of GPR-SNAP in synthetic heterogeneous liposomal membranes and primary vascular cells and intact arteries from WT and GPR-deficient mice under normal or ischemic conditions. Results & Discussion

SNAP was able to bind extracellular His of GPR68 across tissue loops may limit activation of GPR68 following acidic protonation. Alternative: generate third-generation GPR68-tethered pH probe

Aim 1: Artificial liposomal membranes +/- cholesterol, phosphatase pools; modified lipid compositions/lipid rafts; modified cortical cytoskeletal components. Cohorts: veh/SNAP in buffer; veh/SNAP in liposomes/buffer; control FLAG-plasmid; GPR68-FLAG plasmid. FACs to obtain pure membrane populations. Ratiometric fluorescent pH measurements at various times.

Aim 2: In vitro studies

WT control and GPR68-deficient mouse primary VSM cells +/- veh/SNAP. FACs to obtain pure SNAP-expressing cell populations.

Conclusions

Tissue acidosis, particularly spatial pH heterogeneity, are overlooked facets of ischemic disease, a highly significant healthcare and financial burden. pH-sensing GPR68 highlights importance of membrane acid/base balance. Anchoring a pH reporter to GPR68 will allow determination of acidosis discretely at membrane GPR68 microdomains. This work advance precision in our capacity to evaluate acid/base status in normal or ischemic tissues and will highlight its mechanistic impact en route to more targeted therapies.

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