Diabetic Complications Consortium

Application Title: Thermosensitive TRPM8 channels and diabetic erectile dysfunction

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1. Project Accomplishments:

The aim of this project was to investigate the function of cold-sensing TRPM8 channel in internal pudendal artery with respect to erectile function. Studies were carried out examining expression levels of the channels in vascular preparations and the pharmacological activation of the channels to produce relaxation was characterized.

Erectile dysfunction is often associated with reduced levels of nitric oxide (NO). Thus, we performed a study characterizing a mouse model of erectile dysfunction associated with a mutation in luteinizing hormone receptor which is critical for fertility.

In addition to experimental studies, two literature reviews and one guidelines paper were submitted for publication (see below).

2. Specific Aims:

Specific aim 1: To investigate the expression (Figure 1) and mechanism of action of TRPM8 activation in the pudendal artery and corpus cavernosum from diabetic mice (db/db) and their nondiabetic controls (Figure 2).

Specific aim 2: To confirm the mechanism of action of TRPM8 activation in db/db mice using a knockdown strategy to reduce TRPM8 channel expression in tissues isolated form db/db mice and their controls.

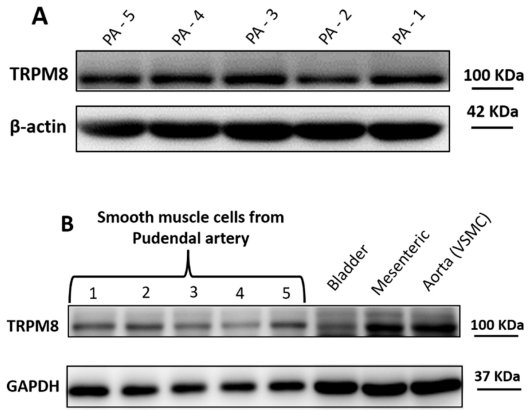

Figure 1. TRPM8 channel expression in isolated arteries and vascular smooth muscle cells (VSMCs). TRPM8 expression analyzed in internal pudendal artery (IPA, A) and VSMCs from IPA (B) using Western blot technique.

Vascular dysfunction in diabetes and obesity: focus on TRP channels. Transient receptor potential (TRP) superfamily consists of a diverse group of non-selective cation channels that has a wide tissue distribution and is involved in many physiological processes including sensory perception, secretion of hormones, vascular reactivity, and cell cycle modulation. In blood vessels, TRP channels are present in endothelial cells, vascular smooth muscle cells, perivascular adipose tissue (PVAT) and perivascular sensory nerves, and these channels have been implicated in the regulation of vascular tone, vascular cell proliferation, vascular wall permeability and angiogenesis. Additionally, dysfunction of TRP channels is associated with cardiometabolic diseases, such as

diabetes and obesity. Unfortunately, the prevalence of diabetes and obesity is rising worldwide, becoming an important public health problems. These conditions have been associated, *highlighting* that obesity is a risk factor for type 2 diabetes. As well, both cardiometabolic diseases have been linked to a common disorder, vascular dysfunction. In this review, we briefly consider general aspects of TRP channels, and we focus the attention on TRPC (canonical or classical), TRPV (vanilloid), TRPM (melastatin), and TRPML (mucolipin), which were shown to be involved in vascular alterations of diabetes and obesity or are potentially linked to vascular dysfunction. Therefore, elucidation of the functional and molecular mechanisms underlying the role of *TRP* channels in vascular dysfunction in diabetes and obesity are important for the prevention of vascular complications and end-organ damage, providing a further therapeutic target in the treatment of these metabolic diseases.

Specific aim 3: To test the hypothesis that chronic administration of a TRPM8 agonist (menthol and icilin, osmotic minipump) in diabetic leads to the prevention and improvement of ED.

Other studies:

Constitutive LH receptor activity impairs NO mediated penile smooth muscle relaxation. Timely activation of the luteinizing hormone receptor (LHCGR) is critical for fertility. Activating mutations in LHCGR cause familial male-limited precocious puberty (FMPP) due to premature synthesis of testosterone. A mouse model of FMPP ($KiLHR^{D582G}$), expressing a constitutively activating mutation in LHCGR, was previously developed in our laboratory. $KiLHR^{D582G}$ mice became progressively infertile due to sexual dysfunction and exhibited smooth muscle loss and chondrocyte accumulation in the penis. In this study, we tested the hypothesis that $KiLHR^{D582G}$ mice had erectile dysfunction due to impaired smooth muscle function. Apomorphine-induced erection studies determined that $KiLHR^{D582G}$ mice had erectile dysfunction. Penile smooth muscle and endothelial function were assessed using penile cavernosal strips. Penile endothelial cell content was not changed in $KiLHR^{D582G}$ mice. The maximal relaxation response to acetylcholine and the nitric oxide donor, sodium nitroprusside, was significantly reduced in $KiLHR^{D582G}$ mice indicating an impairment in the nitric oxide (NO)-mediated signaling. Cyclic GMP (cGMP) levels were significantly reduced in $KiLHR^{D582G}$ mice in response to acetylcholine, sodium nitroprusside and the soluble guanylate cyclase stimulator, BAY 41-2272. Expression of NOS1, NOS3 and PKRG1 were unchanged. The Rho-kinase signaling pathway for smooth muscle contraction was not altered. Together, these data indicate that $KiLHR^{D582G}$ mice have erectile dysfunction due to impaired NO-mediated activation of soluble guanylate cyclase resulting in decreased levels of cGMP and penile smooth muscle relaxation. These studies in the $KiLHR^{D582G}$ mice demonstrate that activating mutations in the mouse LHCGR cause erectile dysfunction due to impairment of the NO-mediated signaling pathway in the penile smooth muscle.

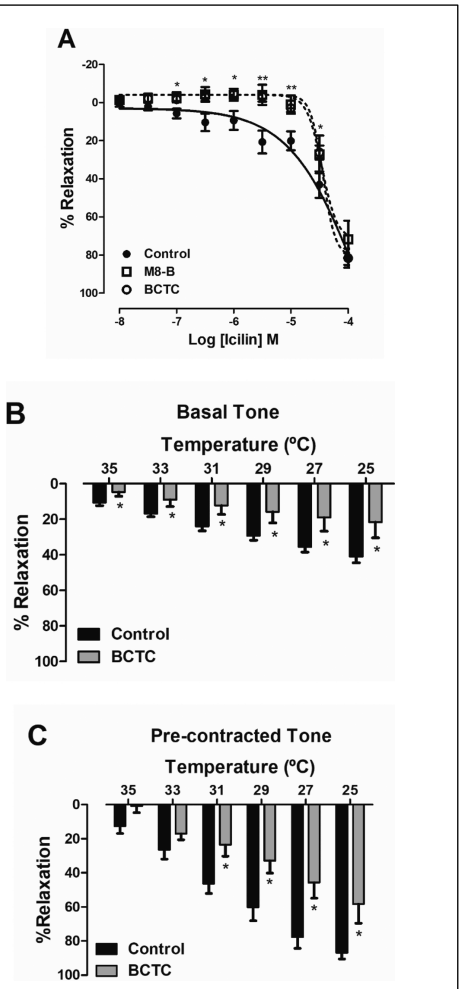

Figure 2. TRPM8 activation by cold temperature and chemical stimulus induced vasodilation in IPA. A. Log concentration-response curves showing the relaxant effect of icilin (TRPM8 activator), in the IPA pre-contracted by phenylephrine (10^{-6}M), in the absence (●, control, n=6) or in the presence of BCTC (○, 2×10^{-6} M, n=7) and M8-B (□, 10^{-6}M, n=6), both TRPM8 blockers. B. Bar graph summary showing that the reduction of temperature of PSS solution induced vasodilation of the basal (A, n=5) or contracted arterial tone (B, n=6). Both effects were reduced by BCTC (2×10^{-6} M, A and B, n= 6). Data are the mean ± SEM. The data were examined using one-way ANOVA followed by Bonferroni post-test (Fig. 2A) and Student's t tests (Fig. 2B and C). *$p < 0.05$, **$p < 0.01$ vs control/endothelium intact.

Vascular stress signaling in hypertension. Cells respond to stress by activating a variety of defense signaling pathways, including cell survival and cell death pathways. While cell survival signaling helps the cell to recover from acute insults, cell death or senescence pathways induced by chronic insults can lead to unresolved pathologies. Arterial hypertension results from chronic physiological maladaptation against various stressors represented by abnormal circulating or local neurohormonal factors, mechanical stress, intracellular

accumulation of toxic molecules and dysfunctional organelles. Hypertension and aging share common mechanisms that mediate or prolong chronic cell stress, such as endoplasmic reticulum stress and accumulation of protein aggregates, oxidative stress, metabolic mitochondrial stress, DNA damage, stress-induced senescence and pro-inflammatory processes. This review discusses common adaptive signaling mechanisms against these stresses including unfolded protein responses, antioxidant response element signaling, autophagy, mitophagy and mitochondrial fission/fusion, signaling effector stimulator of interferon genes (STING)-mediated responses and activation of pattern recognition receptors. The main molecular mechanisms by which the vasculature copes with hypertensive and aging stressors are presented and recent advancements in stress-adaptive signaling mechanisms as well as potential therapeutic targets are discussed.

Guidelines for the measurement of vascular function and structure in isolated arteries and veins. The measurement of vascular function in isolated vessels has revealed important insights into the structural, functional, and biomechanical features of the normal and diseased cardiovascular system, and has provided a molecular understanding of the cells that constitutes arteries and veins and their interaction. Further, this approach has allowed the discovery of vital pharmacological treatments for cardiovascular diseases. However, the expansion of the vascular physiology field has also brought new concerns over scientific rigor and reproducibility. Therefore, it is appropriate to set guidelines for the best practices of evaluating vascular function in isolated vessels. These guidelines are a comprehensive document detailing the best practices and pitfalls for the assessment of function in large and small arteries and veins. Herein, we brought together experts in the field of vascular physiology with the purpose of developing a consensus guide for *ex vivo* vascular function. By utilizing this document, vascular physiologists will have consistency amongst methodological approaches, producing more reliable and reproducible results.

3. Publications:

Hiremath DS, Priviero FBM, Webb RC, Ko CM and Narayan P. Constitutive LH receptor activity impairs NO mediated penile smooth muscle relaxation. Reproduction 161:31-41, 2021.

dos Anjos Moraes R, Webb RC and Silva DF. Vascular dysfunction in diabetes and obesity: focus on TRP channels. Front Physiol, in press, 2021.

Calmasini FB, McCarthy CG, Wenceslau CF, Priviero FBM, Antunes E and Webb RC. Macrophage-specific Toll like receptor 9 (TLR9) causes corpus cavernosum dysfunction in mice fed a high fat diet. J Sexual Med, in press, 2021.

Cicalese SM, da Silva JF, Fernanda Priviero F, Webb RC, Eguchi S, and Tostes RCA. Vascular stress signaling in hypertension. Circ Res, in press, 2021.

Wenceslau CF, McCarthy CG, Earley S, England SK, Filosa JA, Goulopoulou S, Gutterman DD, Isaksom BE, Kanagy NL, Martinez-Lemus LA, Sonkusare SK, Thakore P, Trask AJ, Watts SW, Webb RC. Guidelines for the measurement of vascular function and structure in isolated arteries and veins. Am J Physiol, in press, 2021.