Diabetic Complications Consortium
January 24, 2020

Application Title: Thermosensitive TRPM8 channels and diabetic erectile dysfunction

Principal Investigator: R. Clinton Webb

1. Project Accomplishments:

Diabetes mellitus (DM) is a chronic disorder that can alter carbohydrate, protein, and fat metabolism. It is caused by the absence of insulin secretion or due to defects in insulin uptake in the peripheral tissue. DM is broadly classified under two categories, which include type 1 and type 2 diabetes.

Progress during the last year focused on two studies related to erectile dysfunction and insulin resistance characteristic of diabetes: A. Impaired corpus cavernosum reactivity in mice fed a high fat diet; and B. erectile dysfunction in spontaneously hypertensive rats, a rat strain that is insulin resistant. Below is specific information on each study.

A. TNF-α derived from macrophages impairs the corpus cavernosum reactivity in mice fed high fat diet: Role of Toll-like receptor 9 (TLR9).

Introduction: Weight gain and body mass are central to the formation and rising incidence of type 1 and type 2 diabetes. In addition, obesity and diabetes are associated with systemic inflammation. This work aimed to investigate cavernosal reactivity in TLR9 mutant mice fed a high fat diet.

Methods: Experiments were performed to characterize TLR9 signaling pathway in corpus cavernosum and its participation in obesity-induced erectile dysfunction. C57BL/6 (WT) and TLR9 mutant mice were fed either regular or high-fat diet (HFD) for 12 weeks. At the end of the treatment period, cavernosal reactivity was measured using standard myograph procedure. Signaling components of the TLR9 pathway were measured using immunoblot technique.

Results: Cavernosal contraction was increased for KCl in WT HFD mice and decreased for phenylephrine in $TLR9_{MUT}$ HFD mice. In addition, WT mice fed HFD had impaired cavernosal relaxation induced by neuronal release of NO and it was accompanied by decreased expression of nNOS in the corpus cavernosum. TNF-α and TNF-R1 expression was measured in the CC. It was found increased expression of TNF-α in the CC of WT mice fed HFD. However, in $TLR9_{MUT}$ mice fed HFD, TNF-α expression was similar to that observed in control mice fed standard chow.

Conclusion: These findings indicate that cavernosal dysfunction observed in obesity is at least in part mediated by the production of TNF-α upon activation of TLR9 expressed in the macrophages and a possible associated with the decreased systemic inflammation.

B. TRPM8 channel activation triggers relaxation of pudendal artery with increased sensitivity in hypertensive rats.

Introduction: Erectile dysfunction (ED) is frequently encountered in patients with arterial hypertension and there is a recent functional correlation between the expression of thermoreceptor channels TRPM8 (melastatin 8) and alterations in blood pressure in hypertension. The aim of this study was to investigate the function of cold sensing TRPM8 channel in internal pudendal artery (IPA) in both normotensive and hypertensive rats.

Methods: We performed experiments integrating physiological, pharmacological, biochemical and cellular techniques.

Results: TRPM8 channels are expressed in the IPA and in vascular smooth muscle cells from IPA. In addition, TRPM8 activation, by both a cooling compound icilin (82.1 ± 3.0%, n=6) and cold temperature [thermal stimulus, basal tone (25ºC, 41 ± 3.4%, n=5) or pre-contracted tone induced by phenylephrine (25ºC, 87 ± 3.6%, n=7)], induced relaxation in IPA. Furthermore, the results showed that the concentration-response curve to icilin was significantly shifted to the right in different conditions, such as: the absence of the vascular endothelium, in the presence of L-NAME (10^{-4} M), or indomethacin (10^{-5} M) or by a combination of charybdotoxin (10^{-7} M) and apamin (5x10-6 M), and Y27632 (10^{-6} M). Interestingly, icilin-induced vasodilation was significantly higher in IPA from spontaneously hypertensive (SHR, $E_{10M}{}^{-4}$ = 75.3 ± 1.7%) compared to Wistar rats ($E_{10M}{}^{-4}$ = 56.4 ± 2.6%), despite no changes in the TRPM8 expression in IPA between the strains, suggesting that the sensitivity of TRPM8 channels is higher in SHR.

Conclusions: These data demonstrate for the first time, the expression and function of TRPM8 channels in the IPA involving, at least in part, endothelium-derived relaxing factors and ROCK inhibition. Overall, this channel could potentially be a new target for the treatment of hypertension associated-ED.

2. Specific Aims:

Specific aim 1: To investigate the expression and mechanism of action of TRPM8 activation in the pudendal artery and corpus cavernosum from hypertensive rats and their controls.

We performed experiments to determine the expression levels and function of TRPM8 channels in internal pudendal arteries from spontaneously hypertensive (SHR) and normotensive Wistar rats (Figure 1). We observed that pudendal arteries from SHR were more sensitive to the relaxant effects of icilin than arteries from normotensive rats (Figure 1C).

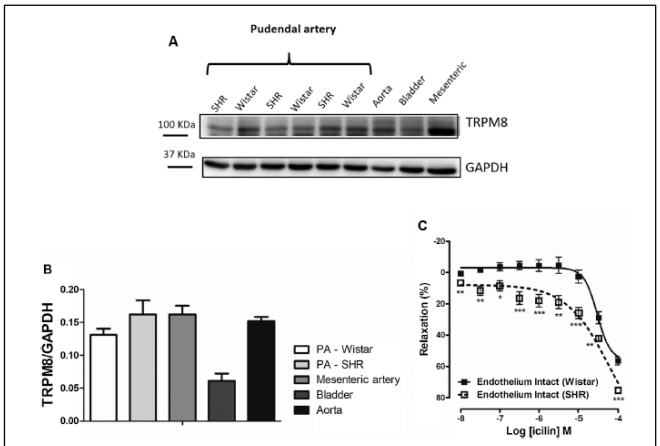

Figure 1. The sensitivity (C) but not expression (A,B) of TRPM8 channels are increased in internal pudendal artery from hypertensive rats. Expression levels for the TRPM8 channel in SHR arteries did not differ from that in arteries from normotensive rats (Figure 1A,B). A. Representative images of immunoblots for TRPM8 channels expression obtained of experiments with IPA from SHR and Wistar rats. Aorta, mesenteric artery and bladder were used as positive control. B. Protein expression analysis for TRPM8 channels normalized by GAPDH in IPA from SHR and Wistar rats (n=5). C. Logarithmic concentration response curves showing the relaxant effect of icilin in IPA from SHR (□, n=12) and Wistar (□, n=9) rats, pre-contracted by Phe (10−6 M) with functional endothelium. Values are expressed as means Å} S.E.M. The data were examined using unpaired Student's t tests. *p < 0.05, **p < 0.01, ***p < 0.001 vs Wistar rats.

Specific aim 2: To confirm the mechanism of action of TRPM8 activation is endothelium dependent.

We characterized relaxation responses to icilin in intact and endothelium-denuded pudendal arteries (Figure 2). The relaxation responses to the TRPM8 were greater in intact arteries than in those denuded of the endothelium.

Specific aim 3: To test the hypothesis that TRPM8 activation by cold temperature induces relaxation of the internal pudendal artery.

We characterized cold-induced relaxation of basal tone (Figure 3, top) and pre-contracted tone to phenylephrine (Figure 3, bottom). Under both conditions, BCTC, an inhibitor of TRPM8 channels reduced relaxation in response to reduced temperature.

3. Publications:

Silva DF, Wenceslau CF, McCarthyCG, Szasz T, Ogbi S, Webb RC. TRPM8 channel activation triggers relaxation of pudendal artery with increased sensitivity in the hypertensive rats. Pharmacol Res 47:104329 (1-11), 2019.

Priviero F, Calmasini F, Justina VD, Wenceslau CF, McCarthy CG, Antunes E, R. Webb RC. TNF-α derived from macrophages impairs the corpus cavernosum reactivity in mice fed high fat diet: role of Toll-like receptor 9 (TLR9). In preparation.

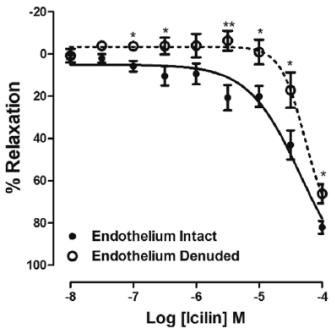

Figure 2. Endothelium-dependent and -independent relaxation induced by TRPM8 activation in internal pudendal artery. Asterisks indicate a statistically significant difference from intact responses ($p<0.05$; n=6).

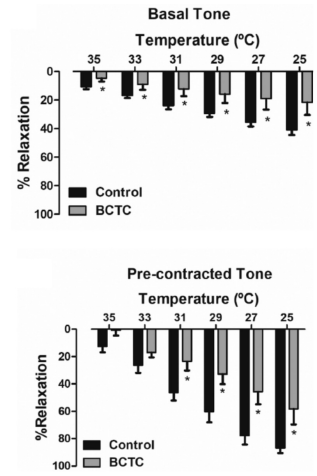

Figure 3. TRPM8 activation by cold temperature caused relaxation of pudendal arteries on basal tone (top) and in precontracted tone (bottom). Asterisks indicate a statistically significant difference from intact responses ($p<0.05$; n=6).

ADMINISTRATIVE NOTE:

This award has an approved No Cost Extension through December 31, 2020.

From: Krukowski, Ashley <AKRUKOWSKI@augusta.edu>
Sent: Wednesday, November 20, 2019 3:34 PM
To: MacVean, Kara <KMACVEAN@augusta.edu>
Cc: Willingham, Yolanda <YWILLINGHAM@augusta.edu>
Subject: RE: NIDDK00085R2C

Hi Kara,

The extension through 12/31/20 has been approved. We will update our internal records for route 00033964.

Best,

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