Diabetic Complications Consortium

Application Title: The role of neuropilin 2 in urinary and gastrointestinal complications in diabetes

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1. Project Accomplishments:

The overall goal of our project is to explore the potential for targeting neuropilin 2 to maintain contractility of the bladder and/or colon in the setting of diabetes, and to understand molecular determinants of the consequences of Nrp2 inhibition. The rationale for the project comes from published studies from our group showing that genetic deletion of Nrp2 in smooth muscle was associated with an increase in evoked contraction in bladder and colon. To accomplish this goal, we have implemented a model of diabetes in our group based on administration of a high fat diet. As outlined below, the diet has proven effective at inducing hyperglycemia and at evoking functional changes in both bladder and colon. One challenge, discussed below in relation to the specific aims, has been an unusually low number of mice of appropriate genotype emerging from our mouse breeding. Since the entire project is dependent on generation of mice harboring the smooth muscle-specific Cre driver and a homozygous floxed Nrp2 allele (SM22α-Cre;Nrp2$^{f/f}$ mice), we have experienced a significant delay in initiating feeding studies. This in turn has impacted the timely generation of data.

2. Specific Aims:

Specific Aim 1: Determine the impact of Nrp2 targeting on bladder and gut contractility in diabetes.

Results: We have successfully implemented a model of diabetes based on administration of high fat diet. As shown in Figure 1, mice on HFD show robust weight gain and elevated blood glucose levels over the course of the feeding period.

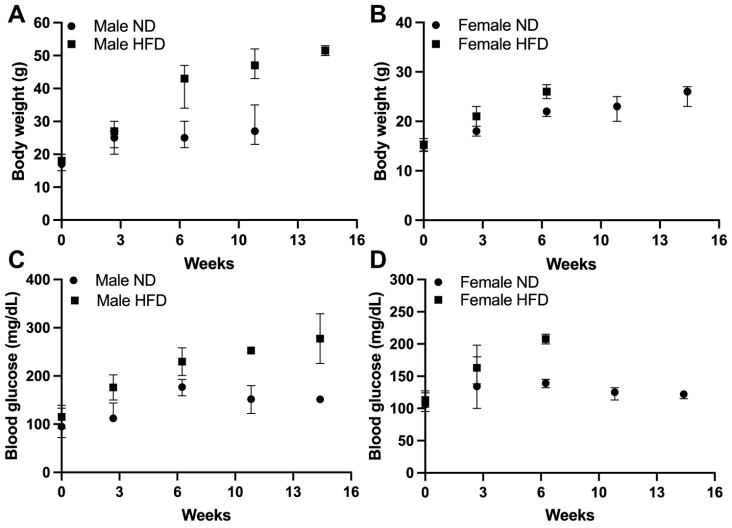

Figure 1: Elevated body weight and blood glucose in high fat diet-fed mice. SM22α-Cre;Nrp2$^{f/f}$ mice were fed normal diet (ND) or high fat diet (HFD) for the indicated time. Male mice on HFD (n=9) show increasing body weight (A) and blood glucose over time compared to male mice on ND (n=7). Female mice on HFD (n=5) show increased body weight and blood glucose compared to mice on ND (n=5), although to a lesser extent than male mice.

Given the breeding challenges noted above, together with the extended duration of high fat diet feeding required to evoke diabetes, we have functional data on only a small number of Nrp2-intact and Nrp2-deficient mice to report. Bladder or colon tissues were harvested from male mice treated 1 week prior with vehicle (VEH) or OHT to activate Cre recombinase to effect Nrp2 gene deletion. As previously reported by us, mice fed a normal diet showed increased contractility following deletion of Nrp2. An increase in evoked contraction was observed in bladder strips from Nrp2-deficient mice in response to stimulation with the muscarinic receptor agonist carbachol, ATP, KCl or electrical field stimulation, when compared to bladder tissues from Nrp2-intact mice on the normal diet (Figure 2).

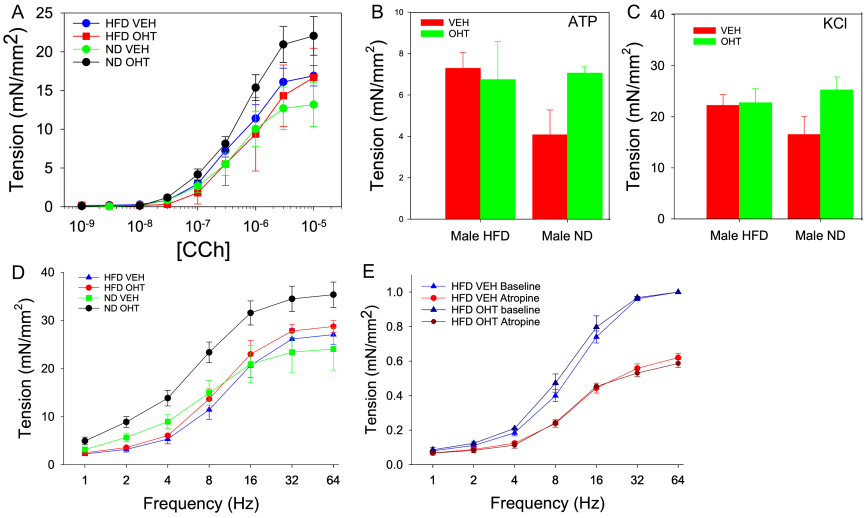

Figure 2: Impact of high fat diet-feeding on bladder contractility in male mice. Evoked contraction of bladder strips from Nrp2-intact mice (VEH) versus Nrp2-deleted mice (OHT) was assessed in response to carbachol (CCh)**(A)**, ATP **(B)**, potassium chloride (KCl)**(C)** or electrical field stimulation **(D)**. The contribution of muscarinic receptor activation to the EFS response was assessed by incubation with the muscarinic receptor antagonist atropine **(E)**. In all cases, tension is normalized to tissue cross-sectional area.

In contrast, however, deletion of Nrp2 in mice fed a high fat diet did not increase evoked contraction of bladder strips compared to Nrp2-intact mice. Overall, however, tension generation was higher in tissues from Nrp2-intact mice on high fat diet compared to Nrp2-intact mice fed normal diet, consistent with compensated function. Treatment of strips with the muscarinic receptor antagonist atropine diminished contraction to a comparable extent in tissues from Nrp2-intact or Nrp2-deficient mice on high fat diet consistent with the similar degree of evoked contraction. Similar findings were obtained from colon in that tissues from Nrp2-deficient mice fed a normal diet showed increased evoked contraction in response to stimulation with carbachol or KCl when compared to bladder tissues from Nrp2-intact mice (data not shown). Interestingly, in tissues from mice fed a high fat diet, deletion of Nrp2 was associated with minimal evoked contraction compared to Nrp2-intact tissues. Given the relatively low number of mice tested in functional assays, however, we will reserve judgement on interpretation of these findings.

Specific Aim 2: Interrogate neuropilin 2-regulated signaling networks in diabetes.

Results: We are currently banking tissue specimens from diet-fed mice for transcriptomics and proteomics analysis, but have no data on this aim at the current time.

3. Publications:

None.