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EXPRESSION OF NEUROVASCULAR STRUCTURES IN THE DIABETIC MOUSE BLADDER: THE FIRST STEP IN UNDERSTANDING DIABETIC BLADDER DYSFUNCTION?

Hypothesis / aims of study

Diabetic bladder dysfunction is a recognized manifestation of autonomic neuropathy in diabetes mellitus [1] [2], and has a prevalence of diagnosis in up to 80% of individuals with diabetes. Despite the prevalence of this disorder, little research has been undertaken regarding the etiology. The aim of this study was to evaluate the expression of neurovascular structures in diabetic and non-diabetic mouse bladders. The hypothesis was that the diabetic mouse bladders would likely display increased vascularity and decreased neural structures when compared to the non-diabetic mouse bladders.

Study design, materials and methods

Three TallyHo (TH) diabetic and six C57BL/6 (C57) non-diabetic mice were sacrificed at 12 weeks of age. The bladders were immediately harvested and embedded in freezing medium. Cryostat sections (10 m) underwent immunohistochemical staining for sensory nerves and blood vessels with the following primary antibodies: guinea pig polyclonal antibody to Substance P (1:500 dilution), rabbit polyclonal antibody to TRPV1 (1:1500 dilution), and rat monoclonal antibody to CD31 (1:500 dilution). Slides were incubated with the appropriate secondary antibodies at a 1:400 dilution, and then counterstained with DAPI. Controls included an equal number of bladder sections stained with the omission of the primary antibody. With the use of ImageJ freeware, the intensity of fluorescent signal was quantitatively determined in each of four quadrants of a digital image for each bladder section (24 total TH and 48 total C57 images for each antibody) by normalizing with background signal value.

Results

All signal intensity values were averaged separately for the TH and C57 strains and then compared. Statistical analysis was performed with SAS v.9.2. P-values <0.05 were considered statistically significant. There was a significant difference in expression of all three antibodies between the TH and C57 bladders. Mean CD31 signal intensity (in fluorescence units, FU) for TH bladders was 3.77 FU (SD$\pm$0.30), and 2.96 FU (SD$\pm$0.72) for C57 bladders ($p=0.004$). The two sensory nerve antibodies displayed a divergent expression pattern, where Substance P was significantly increased in TH bladders ($p=0.02$), whereas TRPV1 was significantly increased in C57 bladders ($p<0.0001$).

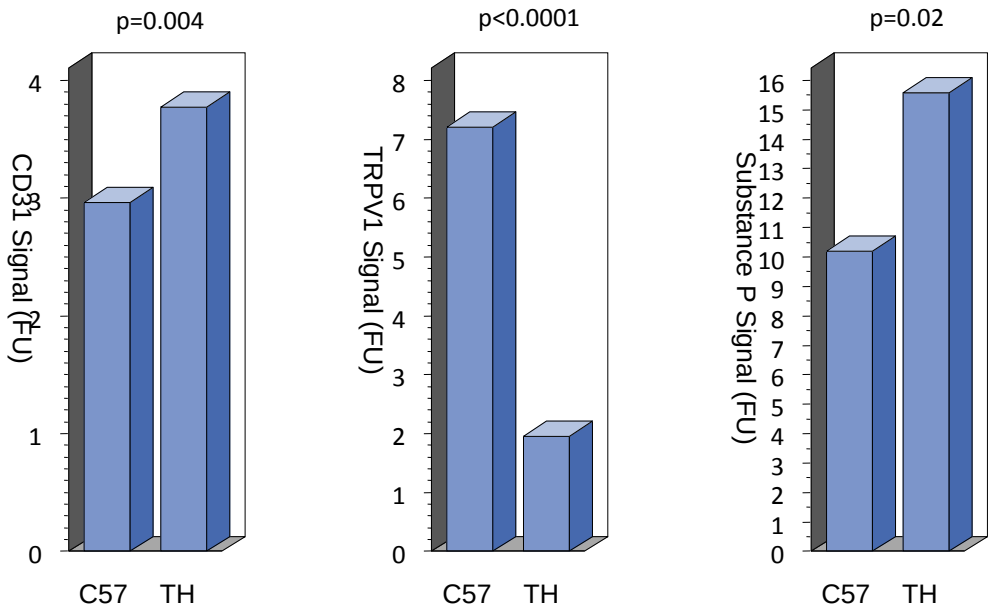
Interpretation of results

These diabetic mouse bladders display significant neurovascular alterations as compared to non-diabetic mice. This is likely a diabetes-induced manifestation in the mouse bladder consistent with neurovascular changes that are seen in other organ systems in diabetic states. Vessel damage is a common consequence seen in diabetics. The increased vascularization seen in the diabetic bladders may be a result of a reparative mechanism to increase blood flow. The divergent expression pattern of the sensory nerves may signify some of the characteristics seen in human diabetic bladder dysfunction: the increase in urgency and pain sensation capability, and a decrease in the afferent control and functional ability of the bladder [2, 3].

Concluding message

The expression pattern of CD31, Substance P and TRPV1 in these diabetic mice may mirror that of human diabetic bladders. Further studies may lead to a better understanding of the pathophysiology of diabetic bladder dysfunction.

Figure 1. Mean Signal Intensities: CD31: C57 2.96 FU (SD$\pm$0.7), TH 3.77 FU (SD$\pm$0.3); TRPV1: C57 7.21 FU (SD$\pm$5.4), TH 1.96 FU (SD$\pm$2.7); Substance P: C57 10.20 FU (SD$\pm$11.4), TH 15.57 FU (SD$\pm$6.9)



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