

to play an important role in impaired contractility seen in DM. Insulin and IGF is required by several cell-types including connective tissue fibroblasts and also the smooth and striated myocytes. In addition to stimulating growth, insulin and its congeners also promote the production of secondary growth factors as fibroblast growth factor (FGF) and connective tissue growth factor (CTGF) peptides. DM disrupts various growth factors signaling pathways mediated by PKC isoforms. The objective of our study was to interrogate the structural and molecular changes occurring in pelvic floor tissue seen in DM.

Methods- Samples of the levator ani striated muscle and fibroconnective tissue of the control (WT) and transgenic rat model of DM were cryosectioned and digitally imaged with an epifluorescence microscope (Leica). PKC isoforms Cy-3 (α , β , ϵ , δ , η , ξ) were examined by indirect immunofluorescence against C-terminal antigens. Sections were simultaneously doubly or triply stained to depict surface glycoproteins (Oregon green labeled wheat germ agglutinin), or actin (Texas red phalloidin) and nuclear DNA (bisbenzamide).

Results- The connective tissue sheath covering striated pelvic muscle (epimysium), individual cells, and fiber bundles (endomysium and perimysium, respectively) is severely diminished in DM. Connective tissue covered fascicles that enter the muscle, carrying nerves and vessels are also decreased in number and size in DM. This is apparent from i. the decreased staining of sialylated and glucuronylated proteins detected by the lectin and wheat germ agglutinin, ii. the thinned basophilic areas in conventional H&E stain, iii. By the attenuated autofluorescence of elastin, and iv. Decreased cell number indicated by nuclear stain. In muscle from diabetic animals the endomysial connective tissue between cells also appears particularly susceptible to rupture and premature tear. In addition, changes in expression and distribution of PKC isoforms, or mitochondrial Hsp75 suggest phenotypic remodeling of striated muscle, vascular and connective tissues in DM. The epimysium in diseased muscle is reduced to 15-45 microns compared to 65-150 microns in controls. Similarly the endomysium between muscle cells is reduced from 4.8 ± 0.3 to 2.7 ± 0.4 microns in diseased muscle.

Conclusions- These findings suggest that DM induce fundamental changes in the intracellular structure and signaling pathways of the pelvic floor tissues. These molecular changes may explain the pathological morphology and contractile dysfunction seen in lower urinary tract and pelvic floor tissues in DM.

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Title (type in CAPITAL LETTERS, leave one blank line before the text):

THE DENSITY OF PRESUMABLY SENSORY SP- AND CGRP-CONTAINING NERVE FIBERS CORRELATES WITH THE FUNCTIONAL STATUS OF THE LOWER URINARY TRACT IN PATIENTS WITH MULTIPLE SCLEROSIS. A PRELIMINARY STUDY.

Aims of Study:

Multiple sclerosis (MS) is a chronic inflammation-demyelination process of the nervous system . Lower urinary tract dysfunctions occurs in about 96% of MS patients, in 12% they occurs at the very beginning phase of the MS or even they precede the onset of MS itself. The most common functional finding in these patients is detrusor hyper-reflexia combined in some cases with detrusor-sphincter dyssynergia. It is suggested, that hyper-reflexia in patients with MS may be due to activation of C-fibers afferents. In our pilot study we decided to evaluate the functional status of the lower urinary tract in the

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patients with clinically proven MS and to check whether it may correlate with the innervation pattern of the urinary bladder.

Material and Methods:

10 patients (6 females, 2 males, mean age 37.5) with clinically proven MS were included in the study.

All patients underwent three-channel subtraction cystometry combined with the EMG recording.

Afterwards patients were anaesthetized and bladder biopsies were taken from the bladder trigone using a rigid cystoscope and biopsy forceps. A mean of two biopsies were obtained from each patient. The specimens were fixed in 4% paraformaldehyde in phosphate buffered saline (PBS) for 2 hours, rinsed in PBS and stored in 18% sucrose solution. 10- μ m-thick cryostat sections of biopsies samples were cut at the cryostat and mounted on chrome-alum-gelatine-coated glass slides. In order to reduce the non-specific background autofluorescence and non-specific staining, the sections were incubated for 10 sec in a freshly prepared mixture of 0.5 N H₂SO₄ and KMnO₄ in distilled water, thoroughly washed in distilled water (3x2 min), and transferred in 0.1 M PBS for 10 min.

Specimens were then incubated overnight with rat anti-substance P (SP) and rabbit anti calcitonin gene-related peptide (CGRP) antiserum. The immunoreaction was then visualised by incubation of sections with FITC-conjugated goat anti-rat or goat anti-rabbit IgG.

Results:

Based on the urodynamic studies two distinct groups could be distinguished. Group one (n=6) was characterized by a pronounced hypersensitivity, lowered capacity and compliance as well as by high maximum pressure of unstable detrusor (Pdet inst): volume at first sensation (FS)-98ml, Pdet/FS-23cm H₂O; Cyscap-217ml, Pdet/Cyscap-51 cm H₂O; Pdetinst-69cm H₂O /mean values/.

During voiding these patients had functional outflow obstruction caused by detrusor-sphincter dyssynergia (Q max 10.8 ml/s, degree of obstruction according to Schaffer - 2.4, post-void residual - 30 ml, mean values). In the second group (n=4) sensation capacity and compliance were normal and maximum pressure of unstable detrusor low (28cm H₂O in a mean). Voiding phase was characterized by a maximum flow rate of 9.8ml/s, degree of obstruction 2.6 and post-void residual 135 ml /mean values/.

Immunohistochemical staining of the bladder biopsies revealed moderate density of CGRP and SP containing nerve fibers in the suburothelial and submucosal layers of the group one patients urinary bladders. In the bladder biopsies taken from the group two patients single SP and CGRP containing nerve fibers were observed in the suburothelium, in the submucosal layer SP and CGRP positive nerve fibers were observed sporadically.

Discussion:

Our preliminary results demonstrate that in the MS patients functional disturbances of the lower urinary tract are reflected in the innervation pattern of the urinary bladder. It is well known that at least some of the CGRP and SP containing nerve fibers are sensory non-myelinated C-fibers responsible for an altered micturitional reflex that initiates detrusor hyper-reflexia. On the other hand, detrusor hyper-reflexia could be successfully managed by intravesical capsaicin or resiniferatoxin instillations. There is however a population of the patients who are not responding to this treatment. Our preliminary data may suggest that CGRP- and SP-containing nerve fibers density in the urinary bladder could play a role in the prediction of an outcome after capsaicin or resiniferotoxin treatment.

References:

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