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Authors: J. A. Clausen, W. J. Lynch, P. Aslan and K. H. Moore
Institution: The Detrusor Muscle Laboratory, Department of Urogynaecology and Urology, St. George Hospital, University of New South Wales, Australia
Title: ALTERATIONS IN THE DISTRIBUTION OF GAP-43/P75NTR IN URINARY BLADDER FROM HUMAN URGE SYNDROME/OAB

Aims of Study:

Patients with "urge syndrome"/OAB* all suffer from an increased sensory activity in the afferent limb of the micturition reflex, regardless of whether they have incontinence i.e. unstable detrusor contractions. Previous authors found increased density of subepithelial nerves in the bladder of patients with idiopathic detrusor instability (IDI) compared to controls [1], but the reason for this remains unclear. Such findings have not been demonstrated for other afferent disorders such as sensory urgency (SU). The possibility that afferent nerves may be undergoing sprouting or remodelling has not been investigated. Growth associated protein (GAP-43) is highly expressed in neural tissue undergoing development and/or remodelling [2,3].

Furthermore, significant increases in endogenous nerve growth factor (NGF) have been measured in patients with IDI compared to control bladders, using ELISA techniques to detect the protein [4]. NGF binds to two receptors, the high affinity TrkA and low affinity $p75^{NTR}$ [5], both receptors are present in the normal bladder [2,6,7], but this has not been investigated in patients with "urge syndrome".

In this study, we have investigated the afferent innervation of patients with IDI and SU, versus controls. Our aims were to: (1) determine whether the increase in subepithelial nerve density was due to increased nerve sprouting/plasticity using GAP-43 immunohistochemistry; (2) determine whether the observed increase in endogenous NGF is associated with any alteration in the density of nerves expressing the low affinity NGF receptor, $p75^{NTR}$; and (3) compare the distribution of a general neuronal marker, protein gene product 9.5 (PGP), in the patient groups.

Methods:

Patients with refractory IDI who had not responded to >2 anticholinergic drugs with bladder training for >12 months, and in patients with severe SU who failed to respond, cystoscopy was performed to exclude other abnormalities. Control samples (biopsies) were taken from normal bladder at check cystoscopy, with Ethical Committee approval.

Samples were fixed in 4% paraformaldehyde in PBS, pH 7.2 buffer overnight, washed and cryoprotected in 30% sucrose for 24 h prior to sectioning (10 m) on a cryostat. Sections were labelled using an established protocol [8]. Quantification was done using VideoPro image analysis to measure the area of stained nerve fibres. Results were analysed using Arcus Quickstat statistical software. Up to 10 areas immediately beneath to epithelium was assessed per section, a total of 60-80 fields per sample were measured for stained nerve fibres. All control, IDI or SU data were pooled for statistical analysis. The target sample size is 10-20 cases per patient group, recruitment continues.

Results:

Biopsies were obtained from 10 control, 4 IDI and 5 SU patients. SU cases had small volumes at first desire to void (mean 95ml) and small bladder capacities (mean 245ml). In those with IDI, the mean max P_{det} was 46 cm

H_2O, range 33-70 cm H_2O.

There was a significant increase in GAP-43 (P< 0.0001, Mann-Whitney U test) subepithelial immunostaining and significant decreases in $p75^{NTR}$ and PGP 9.5 (both P<0.01) immunostaining on bladders from patients with IDI compared to controls. Immunostaining GAP-43 on bladders from patients with SU showed a significant increase (P<0.0001), and significant decreases in PGP 9.5 (P<0.0001) and $p75^{NTR}$ (P<0.05) staining, compared to controls. The median and interquartile range of these results is shown in the table below.

Table: Median areas (m^2) of urinary bladder subepithelial nerves immunostained with PGP 9.5, GAP-43 and $p75^{NTR}$ antibodies.

Patient disorder	PGP 9.5	GAP-43	$p75^{NTR}$
Controls (n = 10)	246 (132-395)	86 (54-135)	247 (139-365)
IDI (n = 4)	204 (139-307)	137 (96-215)	187 (112-350)
SU (n = 5)	138 (84-234)	110 (77-164)	211 (107-395)

Interquartile range in brackets.

Conclusions:

Our preliminary results for patients with IDI indicate a trend towards (1) increases in the densities of nerve fibres which may be involved with neural sprouting and/or remodelling and (2) decreases in the densities of nerve fibres and the $p75^{NTR}$ expression in subepithelial nerve fibres, compared to controls. Patients with SU tended towards decreased densities of (1) subepithelial nerve fibres and (2) the $p75^{NTR}$ expression in subepithelial nerve fibres, compared to controls. Nevertheless the majority of the nerve fibres observed in these patient samples appeared to be undergoing sprouting and/or remodelling.

*OverActive Bladder (OAB).

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