

## ROLE OF UROTHELIAL AND SUBUROTHELIAL NERVE GROWTH FACTOR IN HUMAN BLADDER FUNCTION

### Hypothesis / aims of study

Nerve growth factor (NGF) is involved in many active processes and may contribute to bladder dysfunction in conditions such as spinal cord injury, urethral obstruction or chronic inflammation. In animals, it has been linked to hyperactive voiding, detrusor overactivity, alterations in afferent excitability, and mechanical stretch. Thus NGF seems to be involved in abnormal afferents and sensation especially, and perhaps also in detrusor overactivity or underactivity. Because urothelial tissue has neuronal-like properties, and afferent nerve endings are located in or just beneath it, the concentration of NGF in (sub)urothelium may be important for bladder function.

We therefore decided to test whether NGF concentration in human bladder urothelium or suburothelium was related to detrusor overactivity, bladder sensation, detrusor contractility, or other aspects of lower urinary tract function. By studying only females we eliminated the possible confounding effect of urethral obstruction.

### Study design, materials and methods

Subjects were women over 20 y of age, enrolled in a study of detrusor ultrastructure. Study procedures were approved by the Institutional Review Board. Exclusion criteria included multiple sclerosis, Alzheimer's or other dementing disease, diabetes mellitus, bladder carcinoma, pelvic irradiation, prostatectomy, abdominoperineal resection, disc disease, detrusor-sphincter dyssynergia, alcohol abuse, current urinary tract infection, pregnancy, anaphylactic reaction to local anesthetic, current anticholinergic medication or ASA that could not be stopped for the study, and current coumadin.

Baseline evaluation included medical history, physical examination, 3-day bladder diary, and comprehensive videourodynamics. Subjects were then classified either as "cases," with detrusor overactivity on urodynamics and one or more of precipitancy (sudden urge to void); frequency ( $\geq 9$ ), nocturia ( $\geq 2$ ); or as "controls" without detrusor overactivity and without these symptoms. Subjects who could not be classified did not participate.

Biopsies were taken 15-20 mm superior to the left and right ureteric orifices, under lidocaine anesthesia, with cold cup forceps under direct vision. Superficial biopsies containing mainly (sub)urothelium were immersed in HBSS with protease inhibitor, homogenized (left and right combined), and frozen at  $-80^{\circ}\text{C}$ .

On later analysis a whole cell lysate was prepared by combining both cytosol and membrane protein fractions. Protein concentrations were determined using the BCA protein assay. Samples were assayed in duplicate by ELISA. Tissue NGF values were normalized against the protein concentrations of each sample.

Spearman's rank correlation coefficient was used to examine bivariate associations between normalized NGF concentration and variables representing detrusor overactivity ("cases" versus "controls"), bladder sensation (strong desire to void and maximum cystometric capacity), and detrusor contractility (isovolumetric detrusor pressure, contractility index  $\text{PIP}_1$ , and residual urine after free uroflow). We also examined associations with age and voiding diary variables (mean and maximum voided volumes, and mean daytime voiding frequency).

### Results

The subjects comprised 27 cognitively competent, fully functional female volunteers (mean age 52 y, range 22-82 y). 12 were "cases" with detrusor overactivity and 15 were "controls".

NGF concentration was negatively (but not significantly) associated with detrusor overactivity ( $r = -0.22$ ,  $P = 0.3$ ) and 2 measures of detrusor contractility (isovolumetric pressure:  $r = -0.26$ ,  $P = 0.2$ ; and  $\text{PIP}_1$ :  $r = -0.16$ ,  $P = 0.4$ ). It was weakly associated with 4 measures related to bladder sensation: volume at strong desire to void and maximum cystometric capacity ( $r = -0.20$ ,  $P = 0.3$ ;  $r = -0.30$ ,  $P = 0.14$ ), and maximum single voided volume and mean daytime

voided volume on bladder diary ( $r = -0.29$ ,  $P = 0.16$ ;  $r = -0.23$ ,  $P = 0.3$ ). The negative correlations imply a positive association with sensation. There was no significant association with age ( $P = 0.6$ ) or post-void residual urine ( $P = 0.9$ ).

NGF levels were near-significantly but negatively associated with voiding frequency ( $r = -0.36$ ,  $P = 0.08$ ). In principle, voiding frequency depends on sensation and fluid output. Because sensation cannot easily account for the negative sign, we examined the association of NGF with 24-hour urine output on bladder diary. It was highly significant ( $r = -0.50$ ,  $P = 0.01$ ). This same trend was present in subgroups both with and without detrusor overactivity.

#### Interpretation of results

Elevated NGF levels in human urothelium/suburothelium are weakly associated with increased bladder sensation as measured during cystometry, and this is consistent with the known neuronal-like properties of urothelial tissue. In our population of adult females however there is no evidence that elevated NGF levels are associated with detrusor overactivity or increased detrusor contractility, at least at the point of time at which we measured them.

Our most striking finding is that NGF levels in superficial bladder biopsies are significantly lower in subjects with higher 24-hour urine output. As urine accumulates in the bladder, the wall is extended and the urothelial surface area increases. The total area created each day is proportional to the total urine output in 24 hours. This involves an active process (trafficking), in which vesicles are transported to the urothelial surface and open up into the bladder, so releasing their contents and increasing urothelial surface area. If the vesicles contain NGF, then filling of the bladder would lead to transport of NGF to the surface and its release into the urine. Alternatively NGF may be required for movement of the vesicles. In either case bladder filling would tend to deplete superficial NGF levels.

#### Concluding message

Elevated NGF levels in human urothelium/suburothelium are weakly associated with increased bladder sensation, consistent with the neuronal-like properties of this tissue. They are not clearly associated with detrusor overactivity or detrusor contractility. NGF levels are markedly lower in urothelium/suburothelium from subjects with higher 24-hour urine output. These data suggest a possible role for NGF in the regulation of membrane protein trafficking during bladder filling.

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