

## BLADDER OVERACTIVITY INDUCED BY CHRONIC PUDENDAL NERVE LIGATION INJURY IS ASSOCIATED WITH INCREASED EXPRESSION OF NERVE GROWTH FACTOR AND ACTIVATION OF $\alpha_1$ -ADRENOCEPTORS IN THE RAT BLADDER

### Hypothesis / aims of study

Overactive bladder (OAB) syndrome is a highly prevalent global condition that affects millions of people worldwide. Our hypothesis is that partial pudendal nerve injury induced by child delivery, episiotomy or advanced age may be one of the potential risk factors for OAB. To elucidate whether pudendal nerve injury induced by chronic nerve ligation can affect bladder function, we investigated changes in bladder activity under a conscious condition with or without capsaicin pretreatment, expression of nerve growth factor (NGF) in the bladder and contractility of detrusor muscle strips in rats.

### Study design, materials and methods

Female Sprague-Dawley rats underwent bilateral pudendal nerve ligation (PNL) near the internal iliac vessels with a suture of 5-0 silk or sham operation. After 4 weeks, conscious cystometry was performed to evaluate bladder activity during saline infusion. The bladder were then dissected and prepared for ELISA to measure NGF levels, which were standardized by tissue protein levels. In another group of rats, conscious cystometry was performed after pretreatment with capsaicin (125 mg/kg, s.c.) 4 days before the experiments to examine whether PNL-induced OAB was mediated by activation of capsaicin sensitive C-fiber afferent pathways. In addition, concentration-dependent contractions induced by carbachol ( $10^{-8}$  to  $10^{-4}$  M), a muscarinic receptor agonist, and phenylephrine ( $10^{-7}$  to  $10^{-3}$  M), an  $\alpha_1$ -adrenoceptor agonist, were examined in transverse muscle strips of the bladder lateral wall. Contractile responses were expressed as a percent of the response to 80mM KCl, and contractile forces were adjusted with the formula, weight / (length x 1.05), where 1.05 is the assumed density of muscles.

### Results

The mean intercontraction interval (ICI) (PNL:  $482.0 \pm 23.9$  sec vs. sham:  $608.1 \pm 24.3$  sec) and mean voided volume (VV) (PNL:  $0.30 \pm 0.03$  ml vs. sham:  $0.41 \pm 0.05$  ml) were significantly decreased in PNL rats compared with sham rats without capsaicin pre-treatment (Table 1), but not significantly different in rats with capsaicin pretreatment (Table 2). The other cystometric parameters were not significantly altered in PNL rats with or without capsaicin pretreatment. NGF levels were significantly increased in the bladder from PNL rats compared with sham rats (PNL:  $67.6 \pm 7.6$  pg/ $\mu$ g vs. sham:  $44.8 \pm 3.2$  pg/ $\mu$ g). In detrusor muscle strip studies, phenylephrine-induced contractile responses ( $EC_{50}$ ; PNL:  $2.2 \pm 1.3 \times 10^{-6}$ M vs. sham:  $6.8 \pm 1.4 \times 10^{-6}$ M) and forces ( $E_{max}$ ; PNL:  $1.09 \pm 0.10$  g/mm<sup>2</sup> vs. sham:  $0.69 \pm 0.07$ g/mm<sup>2</sup>) were significantly increased in PNL rats compared with sham rats (Fig.2) whereas carbachol-induced contractile responses ( $EC_{50}$ ; PNL:  $4.7 \pm 1.2 \times 10^{-6}$ M vs. Sham:  $4.0 \pm 1.1 \times 10^{-6}$ M) and forces ( $E_{max}$ ; PNL:  $5.40 \pm 0.29$  g/mm<sup>2</sup> vs. Sham:  $5.83 \pm 0.71$ g/mm<sup>2</sup>) were not significantly different (Fig.1).

### Interpretation of results

These results indicate that chronic pudendal nerve ligation induces urinary frequency associated with increased NGF levels and enhanced activity of  $\alpha_1$ -adrenoceptors in the bladder. Because previous studies have demonstrated that increased NGF levels in the bladder can activate C-fiber afferent pathways to induce bladder overactivity [Ref. 1] and that increased activity of  $\alpha_1$ -adrenoceptors in the bladder contributes to bladder overactivity in a rat model of bladder outlet obstruction [Ref. 2], increased NGF levels and enhanced activity of  $\alpha_1$ -adrenoceptors could be involved in the emergence of OAB in rats with pudendal nerve ligation injury.

### Concluding message

Chronic pudendal nerve injury induced by child delivery, episiotomy or advanced age that can increase NGF levels and activity of  $\alpha_1$ -adrenoceptors in the bladder might be one of the potential risk factors for OAB.

### References

1. J Urol 2001; 165: 975-9
2. J Urol 2002; 167: 1513-21

**Table 1.** Parameters of bladder activity during cystometry in rats without capsaicin pre-treatment

	BP (cmH <sub>2</sub> O)	TP (cmH <sub>2</sub> O)	MVP (cmH <sub>2</sub> O)	ICI (sec.)	VV (ml)	RV (ml)	VE (%)
Sham	$4.5 \pm 0.4$	$8.2 \pm 0.4$	$32.2 \pm 3.4$	$608.1 \pm 24.3$	$0.41 \pm 0.05$	$0.08 \pm 0.01$	$81.4 \pm 2.0$
PNL	$5.1 \pm 0.5$	$8.2 \pm 0.6$	$32.8 \pm 3.1$	$482.0 \pm 23.9^{**}$	$0.30 \pm 0.03^*$	$0.08 \pm 0.01$	$77.5 \pm 3.8$

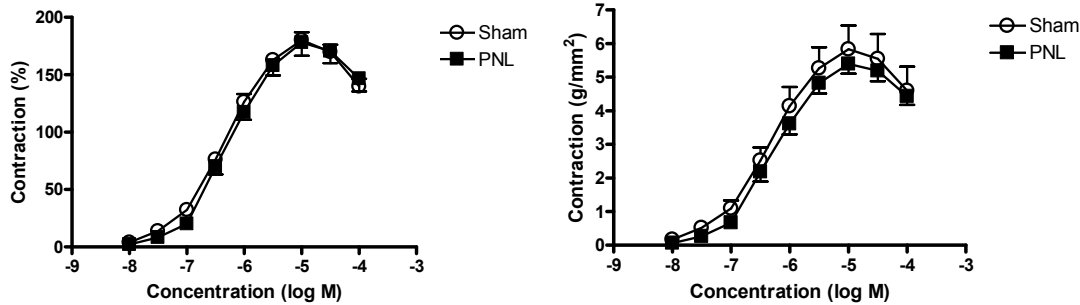
BP: basal pressure, TP: threshold pressure, MVP: maximal voiding pressure, ICI: intercontraction interval, VV: voided volume, RV: residual volume, VE: voiding efficiency

\*\*p<0.01, \*p<0.05

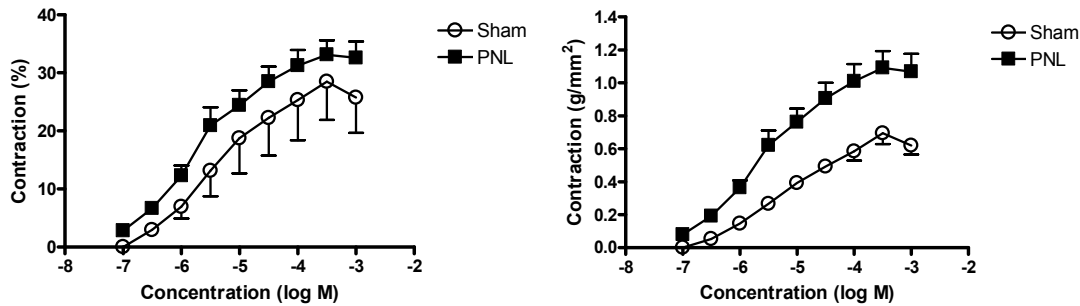
**Table 2.** Parameters of bladder activity during cystometry in rats with capsaicin pretreatment

	BP (cmH <sub>2</sub> O)	TP (cmH <sub>2</sub> O)	MVP (cmH <sub>2</sub> O)	ICI (sec.)	VV (ml)	RV (ml)	VE (%)
Sham	4.7 ± 0.1	9.7 ± 0.9	34.2 ± 1.3	729.4 ± 92.7	0.47 ± 0.07	0.06 ± 0.01	88.3 ± 2.4
PNL	5.6 ± 0.5	10.4 ± 1.0	35.1 ± 2.3	665.0 ± 15.1	0.40 ± 0.03	0.05 ± 0.01	88.1 ± 1.9

BP: basal pressure, TP: threshold pressure, MVP: maximal voiding pressure, ICI: intercontraction interval, VV: voided volume, RV: residual volume, VE: voiding efficiency



**Fig.1.** Comparison of concentration-response curves of carbachol-induced contractions (left) and contractile forces (right) in detrusor muscle strips from sham and PNL rats.



**Fig.2.** Comparison of concentration-response curves of phenylephrine-induced contractions (left) and contractile forces (right) in detrusor muscle strips from sham and PNL rats.

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**ANIMAL SUBJECTS:** This study followed the guidelines for care and use of laboratory animals and was approved by University of Pittsburgh Institutional Animal Care and Use Committee

