PROLONGED ADRENERGIC STIMULATION INDUCES DETERUSOR OVERACTIVITY AND INCREASES BLADDER NOXIOUS INPUT. EXPERIMENTAL STUDY IN THE RAT.

Hypothesis / aims of study
In normal conditions sympathetic drive into the urinary bladder induces detrusor relaxation during storage phase, through $\beta_3$-adrenoreceptors. During voiding phase, sympathetic activity is usually suppressed, therefore facilitating detrusor contraction. However, prolonged, intense sympathetic stimulation of urinary bladder seems to produce opposite results. Spontaneous hypertensive rats, known to have a strong sympathetic activity exhibit detrusor overactivity, at least, in part dependent of the Rho Kinase pathway (1). The effect of a prolonged sympathetic drive on sensory input arriving from the bladder was never investigated. Nevertheless, recent studies showed the expression of adreno-receptors in nociceptive sensory afferents, suggesting that sympathetic drive modulates sensory input arising from the urinary bladder (2).

In the present experimental study we evaluate the effect of chronic adrenergic stimulation on bladder activity and nocuous input arriving from the bladder.

Study design, materials and methods
Female rats were daily injected subcutaneously (s.c.) with 0.1 ml of saline (n=4), 0.05 mg/Kg of phenylephrine (PHE) (n=4), or 0.25 mg/Kg of PHE (n=4). Fifteen days after the animals were anaesthetized with urethane and cystometries were performed. The bladder was exposed through a low abdominal line and a 21-gauge needle was inserted in the bladder dome. After a period of 30 min for stabilization, saline was infused at a constant rate of 6 ml/h while the urethra was left unobstructed for urine expulsion. Bladder contractions were registered for 90 minutes using a pressure transducer. Animals were then perfused through the ascending aorta with 250 ml of Tyrode’s solution followed by 750 ml of 4% paraformaldehyde. Spinal cord segments L6 were collected and post-fixed for 4 h and cryoprotected overnight in sucrose 30% in phosphate buffer. Cord segments were cut in a freezing microtome in 40 $\mu$m sections. Every first and third spinal cord sections were immunoreacted against c-Fos. The immunoreaction was visualized using the ABC peroxidase-conjugated method using diaminobenzidine tetrahydrochloride as chromogen. Positive c-fos cells in dorsal horn L6 segment were counted.

Results
Animals treated with saline had a frequency of bladder contractions of 0.64±0.34 per minute while animals treated with 0.05 mg/kg, PHE had 0.97±0.44 (p<0.05) and animals treated with 0.25 mg/kg PHE had 1.6±0.25 (p<0.05). In L6 spinal cord sections of saline treated animals scarce c-Fos positive cells were observed bilaterally in the superficial dorsal horn, intermediolateral grey matter and around the central canal. In PHE treated animals a more intense c-Fos reaction was observed in the superficial dorsal horn. The average number of cells in superficial dorsal horn increased from 13.4±0.91 in animals treated with saline to 25.1±0.07 in animals treated with 0.05 mg/kg of PHE (p<0.05) and 32.2±1.65 in animals treated with 0.25 mg/kg of PHE (p<0.05).

Interpretation of results
Our data suggest that prolonged adrenergic stimulation increases detrusor activity and generates an increment in nocuous input from the urinary bladder as demonstrated by the increase of c-Fos expression in L6 spinal cord dorsal horn, an area of major nociceptive input landing. These findings combined with the recently observed sympathetic nerve fiber sprouting close to sensory fibers in chronic inflammatory conditions (3) suggest that the sympathetic autonomic system modulates sensory input, including that arising from the urinary bladder.

Concluding message
Abnormal, excessive sympathetic stimulation of the bladder may induce detrusor overactivity and enhance nociceptive bladder input. These findings may be relevant during chronic inflammatory conditions.
References

Specify source of funding or grant Funded by INComb FP7 HEALTH project no 223234
Is this a clinical trial? No
What were the subjects in the study? ANIMAL
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained? Yes
Name of ethics committee Faculty of Medicine of Porto, Portugal