ABSTRACT # 526

L6-S1 SPINAL NERVES STIMULATION REDUCES MICTURITION FREQUENCY IN ANESTHETIZED RATS WITH CYCLOPHOSPHAMIDE-INDUCED CYSTITIS.

François Giuliano[1,3] *, Pierre Denys[2], Laurent Alexandre[1], Jacques Bernabé, Julien Allard. [1] PELVIPHARM

* giuliano@cyber-sante.org

Laboratories, Gif-sur-Yvette, France [2] CHU Raymond Poincaré, Garches, France [3] Research Group in Urology, Medical university of Paris South, Le Kremlin-Bicêtre, France

ABSTRACT

Introduction and Objective: Spinal nerve stimulation (SNS) effectively reduces urinary frequency associated with interstitial cystitis (IC). Nevertheless, the mechanisms by which SNS can exert its positive effect on this enigmatic pathology are unknown. In a model of IC in rat, we aimed to establish the relationship between the intensity of the electrical stimulation of the spinal nerves, the subtype of fibers recruited, and the resulting effect on micturition frequency.

Methods: Experiments were carried in isoflurane-anesthetized male Wistar rats. Cystitis was induced by intraperitoneal delivery of cyclophosphamide (CYP, 150 mg/kg, 48 h before the experiment). Neurograms were performed by placing a recording electrode on the pelvic nerve and a stimulating electrode on either the L6 or S1 ipsilateral spinal nerves (intensity range : 25 µA to 4 mA). Two intensities were chosen from the neurograms and systematically tested for SNS in control and CYP treated animal during transvesical cystometry. Combined analysis of the effect of CYP and SNS was performed with 2 way ANOVA.

Results: CYP treatment resulted in a significant increase in the frequency of voiding contractions and non-voiding contractions. There was no noticeable difference in the neurograms generated from the S1 and L6 spinal nerves, and between CYP and control rats. Intensities of 200 µA (Aδ-fiber specific) and 2 mA (Aδ+C-fiber specific) were chosen for SNS. Continuous electrical stimulation (ES) of the L6/S1 spinal nerves at 200 µA, 20 Hz, marginally reduced the frequency of voiding contractions in control rats (6.3±0.8 to 4.0±0.8 during the ES, p=0.26). In contrast, it significantly reduced the frequency of voiding contractions in CYP-treated rats (from 10.9±2.2 to 6.2±1.1 during the ES, p=0.03). Similarly, it barely affected the frequency of non-voiding contractions in control rats and significantly reduced the frequency of non-voiding contractions in CYP-treated rats. ES of the L6/S1 spinal nerves at 2 mA resulted in the suppression of voiding contractions, and was accompanied by continuous leakage of urine at the urethral meatus. **Conclusions:** ES of the L6/S1 spinal nerves at an intensity allowing recruitment of Aδ-fibers, but not C-fibers, lowered the number of voiding contractions in CYP treated rats to a level non significantly different from the value observed in control rats. These results support the ability of ES of the L6/S1 spinal nerves to reduce bladder overactivity in a pathophysiological model of chemical irritation of the bladder.

BACKGROUND

- Electrical stimulation of sacral spinal nerves inhibits inappropriate reflex contractions in the bladder in humans (1).
- Bladder overactivity can be inhibited by stimulating afferents conveyed by pudendal and dorsal penile nerves (2-3).
- The implication of afferent C-fibers in the generation of the overactive bladder has been demonstrated in rat models of spinal cord injury [4] and interstitial cystitis induced by cyclophosphamide intraperitoneal delivery [5].
- The positive effects of SNS on the overactive bladder may be due to an inhibition of the firing of afferent C-fibers, a proposal which was experimentally supported in a rat model of chronic spinal cord injury [6] but not in a rat model of interstitial cystitis induced by bladder HCl instillation [7].

OBJECTIVES

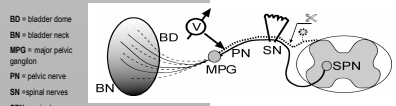
➢To assess the efficacy of spinal nerve stimulation on an overactive bladder model induced by cyclophosphamide in the rat, a model in which a clear participation of afferent C-fiber has been demonstrated.

➢To establish the relationship between the intensity of stimulation of spinal nerves and the subtype of fiber (C or Aδ) recruited .

METHODS

❖ **Neurograms:**

Stimulation : single pulse of 0.2 ms duration ES of the S1 or L6 spinal nerves at variable intensities (25 µA to 3 mA) in control and CYP treated rats
Recording : bipolar electrode on the ispilateral pelvic nerve (gain, 10000; Low pass, 10 KHz; High pass, 10 Hz)
Neurograms were established by averaging of 25 successive single pulses



❖**Cystometry:**

Transvesical cystometry (rate of perfusion, 50 µl/min)
Performed 48H00 after 150 mg/kg cyclophosphamide (Cyp) or corresponding volume of saline

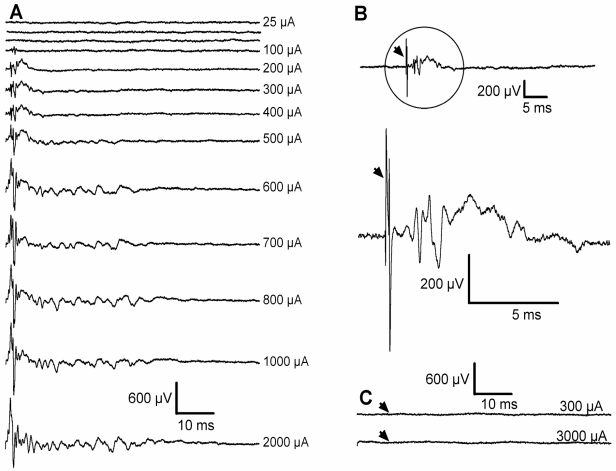
❖**Spinal nerve stimulation (SNS):**

SNS was performed monolaterally on the L6 and S1 spinal nerves
Two different intensities were performed :200 µA and 2 mA
A total of 4 groups of 8 rats was used

Pretreatment	SNS (0.2 ms duration at 20 Hz)
Saline	200 µA
Saline	2 mA
Cyp	200 µA
Cyp	2 mA

➔ SNS WAS PERFORMED DURING CONTINUOUS CYSTOMETRY FOR 45 MIN, 45 MIN AFTER THE START OF CYSTOMETRY

RESULTS

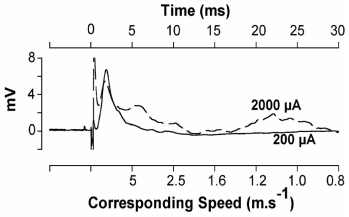


Recordings correspond to the measured intravesical pressure upon continuous perfusion of the bladder through the bladder dome in anesthetized rats, before and during electrical stimulation of the L6/S1 spinal nerves. Recordings in A and B correspond to rats pretreated with 150 mg/kg cyclophosphamide.

In A, note the decrease in the number of voiding contractions induced by ES of the spinal nerves at 200 µA.

In contrast, ES of spinal nerves at 2 mA (B) completely abolished the occurrence of bladder contractions.

Recording in C corresponds to a rat pretreated with vehicle. Note the lower frequency of bladder contractions in C compared to A and B before the electrical stimulation.

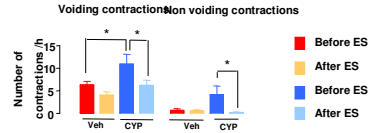


The upper axes corresponds to the time elapsed from the electrical stimulation and the lower axis gives the corresponding conduction velocities of the recorded action potentials. The fastest action potentials are the first recorded, and correspond to large and small diameter myelinated fibers. They correspond to action potentials travelling antidromically in the afferent fibers and orthodromically in the efferent fibers. The slowest action potentials are the last recorded and correspond to action potentials running antidromically in the non-myelinated, thin diameter, afferent C-fibers.

Two neurograms (i.e. average of 25 successive recordings) obtained by ES (pulse duration, 0.2 ms) of the S1 spinal nerve at 200 µA (filled line) and 2000 µA (dotted line) are represented.

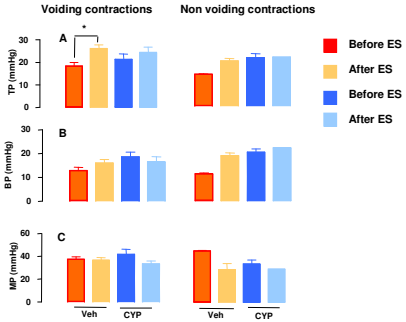
➔Overall, slow action potentials with conduction velocities lesser 2.5 m/s (corresponding to C fibers) were absent from the neurogram obtained at 200 µA, and present in the neurogram obtained at 2000 µA. There was no difference in neurograms obtained in saline or cyclophosphamide pretreated rats.

➔ 200 µA was chosen as the intensity specifically recruiting Aδ fibers, and 2000 µA as the intensity recruiting Aδ and C fibers



Effect of L6-S1 spinal nerve stimulation (200µA) on frequencies of voiding and non voiding contractions

- Bars correspond to the quantification of the number of voiding and non-voiding contractions during cystometry before and after SNS at 200 µA in 8 rats pretreated with cyclophosphamide (Cyp) and in 8 other rats pretreated with the corresponding vehicle (Veh).
- Statistical analysis was performed with 2 way ANOVA with repeated measures, followed by Student-Newman-Keul's test when applicable.*:p<0.05.



Effect of L6-S1 spinal nerve stimulation (200µA) on pressure threshold (TP), basal pressure (BP) and maximal pressure (MP) of voiding and non voiding contractions

CONCLUSION

- Rats pretreated with cyclophosphamide displayed significant bladder overactivity compared to control.
- SNS at 200 µA decreased significantly the number of voiding and non voiding contractions in rats pretreated with cyclophosphamide, but not in rat pretreated with the corresponding vehicle.
- SNS at 200 µA significantly increased threshold pressure in the group pretreated with vehicle, but left BP and MP unaffected.
- SNS at 2 mA abolished bladder contractions, precluding quantifications of basal (BP), threshold (TP) and maximal pressure (MP) of bladder contractions before versus after SNS .

Several studies have recently suggested that symptoms associated with interstitial cystitis, i.e. urinary frequency-urgency syndrome and chronic pelvic pain could also be treated using spinal nerve stimulation [8-10].
In the present study, SNS at low intensity (200 µA), recruiting specifically Ad fibers, decreased significantly the frequency of micturition, and the occurrence of non-voiding contractions in rats with cyclophosphamide-induced interstitial cystitis.

These results further demonstrate the ability of electrical stimulation of the spinal nerves, or neuromodulation, to decrease bladder overactivity in pathophysiological models, and give support to the use of SNS to treat interstitial cystitis in humans.

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