Taiwanese Journal of Obstetrics & Gynecology 54 (2015) 671–677

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

# Effects of sacral nerve stimulation on *postpartum* urinary retention-related changes in rat bladder

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ARTICLE INFO

*Article history:*
Accepted 21 August 2014

*Keywords:*
caveolin
cystometric study
nerve growth factor
sacral nerve stimulation
urinary retention

ABSTRACT

*Objective:* To examine the effect of sacral nerve stimulation (SNS) on the urodynamic function and molecular structure of bladders in rats following acute urinary retention (AUR) after parturition.
*Material and methods:* Thirty primiparous rats were divided into three groups: *postpartum*, *postpartum*+AUR, and *postpartum*+AUR+SNS. AUR was achieved by clamping the distal urethra of a rat for 60 minutes. The *postpartum*+AUR+SNS group received electrical stimulation 60 minutes daily for 3 days after AUR. In addition to cystometric studies and external urethral sphincter electromyography, the expression of caveolins and nerve growth factor (NGF) and caveolae number in bladder muscle were analyzed.
*Results:* The *postpartum*+AUR group has significantly greater residual volume than the *postpartum* group, but the residual volume decreased significantly after SNS treatment. The *postpartum*+AUR group had significantly lower peak voiding pressure, a longer bursting period and lower amplitude of electromyograms of external urethral sphincter activity than the *postpartum* and *postpartum*+AUR+SNS groups. The *postpartum*+AUR rats had higher NGF expression, lower caveolin-1 expression, and fewer caveolae in bladder muscle compared with the *postpartum* rats. Conversely, the caveolin-1 expression and caveolae number increased, and the NGF expression decreased after SNS treatment.
*Conclusion:* Bladder dysfunction after parturition in a rat model caused by AUR may be restored to the non-AUR structural and functional level after SNS treatment.


## Introduction

*Postpartum* urinary retention (PUR) after vaginal delivery is not an uncommon event and has a reported incidence ranging from 1.7% to 17.9% [1]. Because of hormonal changes during pregnancy, the bladder is hypotonic with an increased postvoid residual volume after delivery [2,3]. Several studies have reported that PUR is associated with various obstetric factors, including epidural analgesia, instrument delivery, perineal trauma, long labor, and primiparity [2,4,5]. Persistent urinary retention is the principal complication of PUR and should be managed with intermittent self-catheterization [4]; women with PUR frequently experience mixed lower urinary tract symptoms of voiding and storage problems that cannot be solved by intermittent catheterization alone. In the recent years, sacral nerve stimulation (SNS) has become a treatment option for patients suffering from urgency incontinence and nonobstructive urinary retention refractory to conservative treatment [6], but its exact physiological mechanism of action is not fully understood. Our previous study showed that bladder dysfunction immediately *postpartum* in a rat model caused by acute urinary retention (AUR) is related to the expressions of caveolin and nerve growth factor (NGF) and number of caveolae in bladder muscle cells [7]. Hence, this study was conducted to examine the effect of SNS on urodynamic function and the molecular structure of bladders in rats following AUR after parturition.

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http://dx.doi.org/10.1016/j.tjog.2015.10.001

## Materials and methods

### *Experimental design*

The studies were performed on 30 nulliparous Sprague–Dawley rats weighing 300–330 g. They were obtained from the Laboratory Animal Centre of our country and divided into three groups: *postpartum*, *postpartum*+AUR and *postpartum*+AUR+ SNS. On the 3rd *postpartum* day, AUR was achieved by clamping the distal urethra for 60 minutes in 20 rats. Ten of these 20 AUR rats received SNS treatment for 3 days. Cystometric studies and external urethral sphincter electromyography (EUS-EMG) were performed in all rats at 6 days *postpartum*. After the bladders were harvested and stained, the expression of the caveolins and NGF and the number of caveolae in the bladder muscle cells were analyzed. All the protocols were approved by Chang Gung Memorial Hospital Animal Care and Use Committee.

### *AUR*

AUR was induced by infusing 3 mL (0.6 mL/min) of saline with an infusion pump through a urethral catheter after clamping the distal urethra with an aneurysm clip. The obstruction was sustained for 60 minutes, and the bladder was then allowed to drain, as described by Saito et al [8].

### *SNS*

Under isoflurane anesthesia, the rats were placed in a prone position. After disinfection, an incision was made through the skin at the S1–S3 level. Coated wire electrodes (wire diameter: 50 μm; A-M systems, Carlsborg, WA, USA) were implanted at the bilateral S2 or S3 neural foramina through a 30G needle. The correct position was confirmed by twitching of the rear leg from increasing the electric current in the wire connected to the electronic stimulator (Model YLS-9A; Chsin Medical Instrument Co., Taichung, Taiwan). The wire was fixed to the dorsal coccygeus by 5-0 silk and tunneled subcutaneously to the neck, where it exited the skin; the incision was closed. The SNS group received electrical stimulation after bladder distension with the following protocol: stimulation time 60 minutes; intensity 2–4 V; pulse duration 0.2 ms; and frequency 20 Hz. Electrical stimulation was applied 60 min/d for 3 days.

### *Suprapubic tube implantation*

All rats received suprapubic tube implantation 1 day prior to the cystometric studies. Under isoflurane anesthesia, a midline abdominal incision was made to expose the bladder. A polyethylene catheter (PE-50 tubing with a flared tip) was implanted into the bladder through the dome. A purse string suture was tightened around the catheter, and the catheter was subcutaneously tunneled into the neck. The catheter was plugged until used, and the skin and abdominal incisions were closed.

### *Conscious cystometric studies*

One day after suprapubic tube implantation, the animals were placed in metabolic cages (Medical Associates, St Albans, VT, USA) to undergo conscious cystometric studies according to methods used in a previous study [8]. All the bladder pressures were referenced to the air pressure at the level of the bladder. The pressure and force transducer signals were amplified and digitized for the data collection. The bladder was filled with room temperature 0.9% saline at 5 mL/h, and the changes in the weight of the urine collection were recorded. Because the specific gravity of rat's urine is very close to 1, the weight of the urine is equivalent to the volume of the urine. Thus, the voided and residual amount of urine was presented as volume (μL). After stabilization, the data for five representative micturition cycles were collected to analyze the cystometric parameters. The means of the collected data were reported for the analysis, with the status of rat blinded. The following cystometric variables were investigated: the intercontraction interval, the voided volume and the residual volume. Cystometry Analysis version 1.05 (Catamount Research and Development, St Albans, VT, USA) was used for cystometric analysis.

### *EUS-EMG measurement*

The rats were anesthetized with urethane and placed in a copper mesh isolation cage in a supine position, and an incision was made through the skin in the pubic area. The pubic bone was dissected by scissors, and the urethra exposed. Parallel bipolar electrodes was fixed and placed on the mid-urethra for the EUS-EMG recording. A bladder catheter was connected to the syringe pump (Infors CH-4130l; Infors, Laurel, MA, USA) and the pressure transducer. The EUS-EMG and pressure transducer signals were amplified and digitalized for computer data collection (Medical Associates). The bladder was filled with room temperature 0.9% saline at 5 mL/h through the bladder catheter. The means of the collected data were reported for analysis. Various EUS-EMG parameters of each rat were determined after 3–5 voiding cycles, including the peak voiding pressure, the duration of a single active period (AP) and duration of a single silent period (SP) during the bursting period (BP), the SP/SP+AP ratio, and the mean amplitude of AP. The EMG activity analysis was blinded to the status of the rat. MedLab Data Acquisition System Version 6 (Meiyi Ltd., Nanjing, China) was used for the EUS-EMG analysis.

### *Tissue preparation*

After the EUS-EMG testing, the rats were sacrificed and the bladders harvested. The dissected bladders were fixed in an optimal cutting temperature compound, frozen in powdered dry ice and stored at −70°C. The bladders were subjected to cryosectioning at −18°C with the sections mounted on glass microscope slides coated with saline (Muto Pure Chemical, Tokyo, Japan).

### *Immunohistochemistry*

The frozen bladder sections of 10-μm thickness from each animal were mounted on a slide glass, fixed with 4% paraformaldehyde and washed in phosphate buffered saline. After blocking the endogenous peroxidase activity, the nonspecific antibody binding was suppressed, and the slides were incubated overnight at room temperature with a rabbit polyclonal antibody directed against the NGF at a 1:250 dilution (Chemicon International, Temecula, CA, USA) or with mouse monoclonal antibodies directed against caveolin-1 or caveolin-3 at a 1:100 dilution (BD Transduction Laboratories, Lexington, KY, USA). After washing with a buffer, the sections were immunostained by the avidin–biotin peroxidase method using the Vectastain Elite Kit (Vector Laboratories, Burlingame, CA, USA) with 3-3-diaminobenzidine plus hydrogen peroxide as the chromogen. The negative control slides were prepared from identical tissue blocks by omitting the specific primary antibodies and using normal, nonimmune serum supernatant from the identical sources. The ratio of the optical density of the *postpartum*+AUR or the *postpartum*+AUR+SNS rats to that of the *postpartum* rats was determined in the NGF, caveolin-1, and caveolin-3 analyses. Image-Pro Plus Software (Media Cybernetics,