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Original Article

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

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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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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,

Silver Spring, MD, USA) was used for the immunohistochemical calculations.

Real-time quantitative reverse transcription polymerase chain reaction

Total RNA was prepared using a Trizol reagent (Invitrogen, Carlsbad, CA, USA) and incubated. The gene expression of caveolin-1, caveolin-3, and NGF in the bladder tissue was analyzed by real-time polymerase chain reaction using inventoried TaqMan assays from Applied Biosystems (Life Technologies, Grand Island, NY, USA). The assay codes were Rn00755834-m1 (caveolin-1), Rn00755343-m1 (caveolin-3), and Rn01533872-m1 (NGF). 18S (Hs99999901-s1, Applied Biosystems) was used as an endogenous control to allow for relative gene expression quantification. Thermal cycling and fluorescence detection were performed with an ABI Prism 7900HT Sequence Detection System (Applied Biosystems). The data were calculated with $2[^{-\Delta\Delta}C(T)]$ [9].

Electron microscopy

The bladder tissue samples were fixed with 4% cacodylate-buffered glutaraldehyde (0.15M, pH 7.2). After fixation, the tissue was dehydrated in graded ethanol and propylene oxide and subsequently embedded in Spurrs resin (EMS, Hatfield, PA, USA). The ultra-thin sections (~90 nm) were cut with a RMC ultramicrotome MT-7000 (RMC, Tucson, AZ, USA) and mounted on copper grids, which were stained with 10% uranyl acetate and lead citrate as contrasting agents. The ultrastructure determination was performed with a JEOL 1400 transmission electron microscope (JEOL, Tokyo, Japan) at ×30,000, and the number of caveolae was separately counted in four random areas (of 2.5 cm^2) of the bladder smooth muscle [10]. The means of the caveolae counted in the bladder smooth muscle cells were used for the statistical analysis.

Statistical analysis

The data are presented as the mean ± standard deviation and were analyzed statistically using a one-way analysis of variance test followed by the Tukey test. Pearson correlations were applied to calculate the correlation between caveolins and NGF. All data were analyzed with Prism 5 software for the statistical analysis (GraphPad, San Diego, CA, USA). Values were considered significant at $p < 0.05$.

Results

The *postpartum*+AUR group has a significantly shorter inter-contraction interval, a lower voided volume and a greater residual volume compared with the *postpartum* group, but the

Table 1
Cystometric results in the experimental rats.

Group	*Postpartum*	*Postpartum*+AUR	*Postpartum*+AUR+SNS
	($n = 10$)	($n = 10$)	($n = 10$)
RV (μL)	436.24 ± 58.31	1015.22 ± 413.19*	178.48 ± 103.91**
VV (μL)	1648.12 ± 147.73	832.33 ± 392.79*	1837.75 ± 502.74**
ICI (s)	948.33 ± 96.23	493.68 ± 183.11*	970.77 ± 252.77**

* $p < 0.05$ indicates a significant difference between the *postpartum* and the *postpartum*+AUR rats.
** $p < 0.05$ indicates a significant difference between the *postpartum*+AUR and the *postpartum*+AUR+SNS rats.
AUR = acute urinary retention; ICI intercontraction interval; RV = residual volume; SNS = sacral nerve stimulation; VV = voided volume.

Table 2
External urethral sphincter electromyography results in the experimental rats.

Group	*Postpartum*	*Postpartum*+AUR	*Postpartum*+AUR+SNS
	($n = 10$)	($n = 10$)	($n = 10$)
PVP (cmH_2O)	55.46 ± 4.85	33.99 ± 18.93*	57.19 ± 3.36**
BP (s)	8.26 ± 2.53	17.38 ± 4.94*	11.88 ± 3.72
SP (s)	0.19 ± 0.02	0.12 ± 0.02*	0.26 ± 0.07**
AP (s)	0.05 ± 0.01	0.08 ± 0.02*	0.05 ± 0.01**
SP/SP + AP (%)	79.43 ± 1.78	61.96 ± 9.57*	79.68 ± 6.96**
Amplitude (mV)	0.12 ± 0.02	0.06 ± 0.02*	0.18 ± 0.06**

* $p < 0.05$ indicates a significant difference between the *postpartum* and the *postpartum*+AUR rats.
** $p < 0.05$ indicates a significant difference between the *postpartum*+AUR and the *postpartum*+AUR+SNS rats.
AP = active period; AUR = acute urinary retention; BP = total electromyogram bursting period; PVP = peak voiding pressure; SNS = sacral nerve stimulation; SP = silent period.

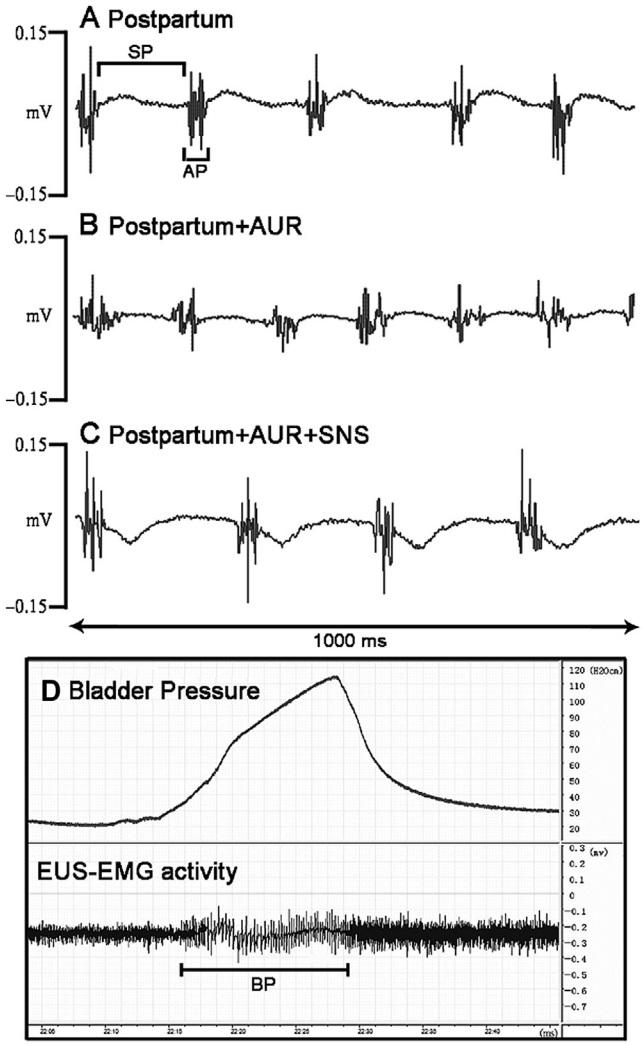

Figure 1. (A–C) The external urethral sphincter electromyogram (EUS-EMG) activity is recorded during a continuous suprapubic infusion cystometric measurement in *postpartum* rats. The individual bursting period is composed of active and silent periods. The *postpartum*+AUR group has significantly increased in bursting period and lower amplitude than the other two groups. (D) The typical pattern of bladder pressure (top tracing) and EUS-EMG activity (bottom tracing) is recorded during a cystometric study. EUS-EMG activity in (A–C) is at a faster time scale than in (B). AP = active period; BP = total EMG bursting period; SP = silent period.

intercontraction interval and voided volume increased, and the residual volume decreased significantly after SNS treatment (Table 1). The *postpartum*+AUR group has significantly lower peak voiding pressure, a longer duration of the bursting period, a lower ratio of SP/SP+AP during the bursting period, and a lower amplitude of EUS-EMG activity than the *postpartum* and *postpartum*+AUR+SNS groups (Table 2 and Figure 1).

The caveolin-1 immunoreactivity and the caveolae number in the bladder muscle significantly decreased in the *postpartum*+AUR rats compared to the *postpartum* rats; both consecutively increased after SNS treatment (Figures 2 and 3). The NGF immunoreactivity significantly increased in the rats with *postpartum*+AUR but consecutively decreased after SNS treatment. After applying Pearson correlations to calculating the correlation between caveolins and NGF among the three groups, we discovered that there was no significant correlation between caveolins and NGF. The caveolin-1 mRNA expression was significantly decreased in the *postpartum*+AUR rats compared to the *postpartum* rats, but

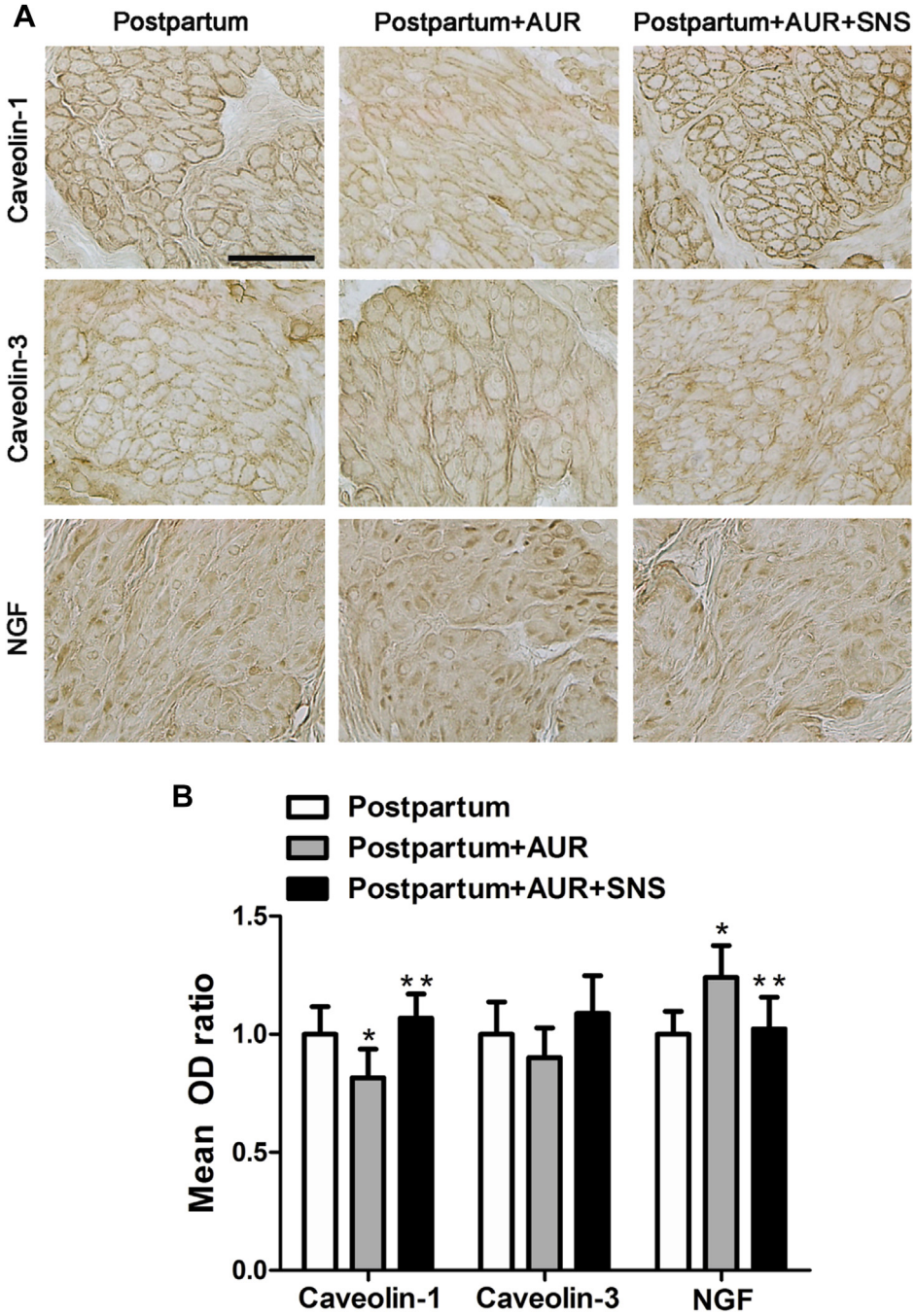

Figure 2. (A) The expression of nerve growth factor (NGF), caveolin-1 and caveolin-3 immunoreactivity in the bladder are exhibited in three different groups. The caveolin-1 expression significantly decreased in the *postpartum*+AUR rats compared to the *postpartum* rats, but consecutively increased after the sacral nerve stimulation (SNS) treatment. Conversely, the NGF expression significantly increased in the *postpartum*+AUR rats but consecutively decreased after the SNS treatment. The bar indicates 25 μm. (B) The value in the figure is the ratio of the optical density of the *postpartum*+AUR or the *postpartum*+AUR+SNS rats to that of the *postpartum* rats. * $p < 0.05$ indicates a significant difference between the *postpartum* and the *postpartum*+AUR rats. ** $p < 0.05$ indicates a significant difference between the *postpartum*+AUR and the *postpartum*+AUR+SNS rats.

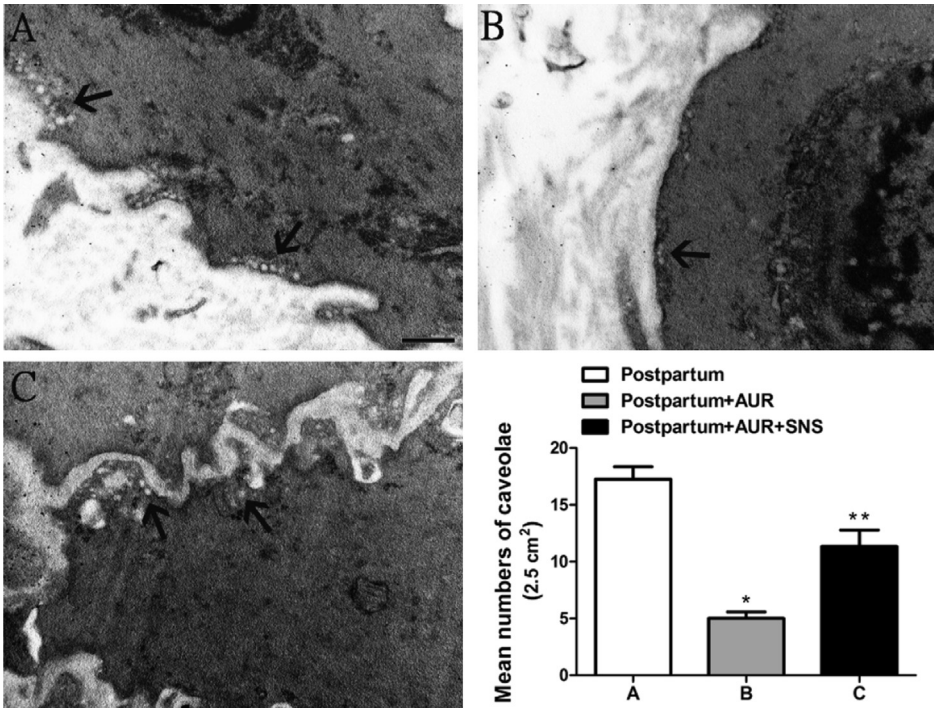

Figure 3. Electron microscopy shows that the number of caveolae in the bladder significantly decreased in the *postpartum*+AUR rats compared to the *postpartum* rats; the caveolae number consecutively increased after the sacral nerve stimulation (SNS) treatment. Examples of the caveolae are highlighted with arrows in A–C. The bar indicates 0.5 μm × 30,000. * $p < 0.05$ indicates a significant difference between the *postpartum* and the *postpartum*+AUR rats. ** $p < 0.05$ indicates a significant difference between the *postpartum*+AUR and the *postpartum*+AUR+SNS rats.

consecutively increased after SNS (Figure 4). Conversely, the expression of NGF mRNA is significantly increased in the *postpartum*+AUR rats compared to the *postpartum* rats, but consecutively decreased after SNS treatment.

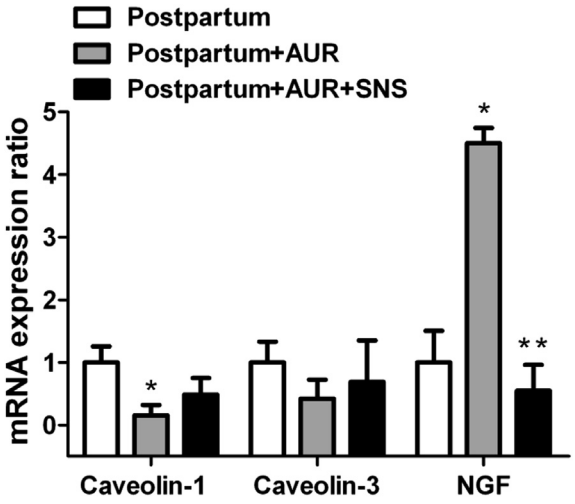

Figure 4. The ratios of the bladder mRNA signal intensities of caveolin-1, caveolin-3, and nerve growth factor (NGF) are presented. The signal intensity for the caveolin-1 mRNA was significantly decreased in the *postpartum*+AUR rats compared to the *postpartum* rats, but consecutively increased after the sacral nerve stimulation (SNS) treatment. Conversely, the signal intensity for the NGF mRNA was significantly increased in the *postpartum*+AUR rats compared to the *postpartum* rats, but consecutively decreased after the SNS treatment. * $p < 0.05$ indicates a significant difference between the *postpartum* and the *postpartum*+AUR rats. ** $p < 0.05$ indicates a significant difference between the *postpartum*+AUR and *postpartum*+AUR+SNS rats.

Discussion

In rodents, the tonic activity of the external urethral sphincter reflects the closure of the urethral outlet during urine storage, whereas the bursting activity reflects the rhythmic opening and closing of the urethral outlet to produce a pulsatile flow of urine [11,12]. Our results showed that the *postpartum*+AUR group has a significantly lower peak voiding pressure and lower amplitude of EUS-EMG activity, resulting in voiding dysfunction after the AUR insult. We hypothesize that the peaking voiding pressure might represent the function of the detrusor muscle.

The EUS-EMG measurements in our study showed that increased duration of the BP in the *postpartum*+AUR rats might indicate that the bladder tries to compensate for the increased residual urine by increasing the voiding time. Simultaneously, the SP/SP+AP ratio during BP was reduced in the *postpartum*+AUR group, which indicates that the total opening time of the urethral outlet was reduced after the AUR insult. The AUR group had the lowest amplitude of EUS-EMG activity among the *postpartum* groups, implying that the amplitude of EUS-EMG activity might be able to represent the function of the external urethral sphincter. Liu et al [12] reported that diabetic rats have a much longer BP and a lower SP/SP+AP ratio than control rats. They concluded that diabetes induces functional and anatomic abnormalities of the external urethral sphincter in rats that may cause bladder dysfunction [12].

Sacral neuromodulation is a routine part of the treatment for refractory nonobstructive urinary retention [13]. White et al [14] assessed the results of sacral neuromodulation in 40 patients with refractory, nonobstructive urinary retention and found 28 (70%) patients demonstrated > 50% improvement in their symptoms. SNS in partial bladder outlet obstruction (BOO) rats can prevent functional and structural changes in the detrusor muscle

without affecting detrusor contraction [15]. In this study, the AUR rats immediately *postpartum* have dysfunctional bladder signs, including shorter intercontraction intervals, lower voided volumes, and higher residual volume. Although the exact physiological mechanism of SNS action is not understood, our data demonstrate that SNS may significantly elevate the peak voiding pressure in *postpartum*+AUR rats, possibly by stimulating the innervations of and restoring the function of the detrusor muscle. Also, we hypothesize that as AUR might result from the damage to detrusor or innervated nerves in bladder or proximal urethra, SNS may enhance the pulse of the stimulation to the receptor in the arc of micturition mechanism, which in turn strengthens bladder contraction or corrects dyssynergia between the detrusor and the urethra and consequently leads to restoration of the bladder function.

The bladder can increase the production of NGF in response to bladder distension [16]. The mRNA expression of the bladder NGF was increased in the BOO rats with detrusor overactivity [17]. The mechanism of the NGF induction of the detrusor muscle overactivity is not clear, but increased NGF production may induce detrusor overactivity by the afferent nerve excitability resulting from altered ion channel expression [18]. SNS has become an established treatment option for patients with intractable detrusor overactivity in some countries [16,19]. In our experiments, the NGF immunoreactivity and mRNA expression in the *postpartum* rats were significantly increased following AUR insult and was restored to the non-AUR level after SNS treatment.

The caveolae are abundant in all muscle types [20]. The pathological disruption or changes in the number of caveolae may affect bladder contractility and contribute to the pathogenesis of bladder dysfunction [21,22]. The detrusor muscle in the rodents with BOO revealed a decrease in the number of caveolae in the plasma membrane [23]. Our data show that a number of caveolae in the bladder significantly decreased in the AUR *postpartum* rats compared with the non-AUR *postpartum* rats; the number of caveolae subsequently increased after SNS treatment.

Caveolins, the main membrane proteins of the caveolae, interacted with several signaling proteins in the caveolae [24]. Decreased expressions of caveolin-1, caveolin-2, and caveolin-3 were found in BOO-induced detrusor hypertrophy, which might contribute to changes in the signal transduction pathways [23]. However, in a recent paper, Kim et al [25] pointed out that detrusor overactivity induced by BOO causes a significant increase in the expression of caveolin-1 in the suburothelial microvasculature. Our results indicate that in rat bladder detrusor muscle, the expression of caveolin-1 immunoreactivity and mRNA was lower in the AUR than the non-AUR *postpartum* rats. Caveolin-1 expression subsequently increased after SNS treatment; caveolin-3 expression showed no significant change in the *postpartum* rats. Caveolin-1 knockout mice exhibited impaired urinary bladder contractions *in vivo* during cystometry [26]. Lai et al [26] reported that longitudinal bladder strips from caveolin-1 knockout mice were stimulated electrically and exhibited a 30–40% decrease in electrical neural contractions compared with the wild type controls.

Limitations of the present study include its relatively small animal number and lack of longitudinal or serial measurements to track the changes and improvement of urodynamic properties after SNS treatment. We also recognize the possibility that AUR may recur after SNS treatment and the effectiveness of SNS treatment declines after repeated application. Besides, the mechanism of micturition in rats is somewhat different from that of humans.

In conclusion, our results indicated that bladder dysfunction after parturition in a rat model caused by AUR is related to the expression of NGF and caveolin-1, and the caveolae in bladder muscle cells and may be restored to non-AUR structural and functional level after SNS treatment. *Postpartum* urinary retention is not an uncommon event in humans. Electrical stimulation has been popularly applied to the treatment of urinary incontinence and overactive bladder syndrome in many hospitals, but only limited data so far touch on the effect of electrical stimulation on the *postpartum* urinary retention. We hope to translate the positive result of SNS treatment to clinical use in dealing with *postpartum* urinary retention problems in the future, not only because it is effective but also because there is little concern on the safety of breast feeding by *postpartum* women from this treatment.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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