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ORIGINAL PAPER

Persistent overexpression of SERCA2a affects bladder functions under physiological conditions, but not in bladder outlet obstruction-induced sub-acute pathological conditions

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Abstract A functional impairment of the bladder and heart in a decompensated state caused by a pressure overload is accompanied by a decrease in the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2). The beneficial effects of SERCA2 overexpression in preserving cardiac functions have been previously reported. The aim of the present study was to investigate the effects of overexpressed SERCA2 on bladder functions under physiological and pathological conditions using partial bladder outlet obstruction (BOO) in SERCA2a transgenic Wistar rats (TG). Bladder cystometry and western blot analysis were performed using the wild-type Wistar rats (WT), TG, and BOO models (WTBOO and TGBOO). Persistent overexpression of SERCA2 induces reduced bladder compliance without hypertrophy in TG. BOO induces reduced bladder compliance and hypertrophy in WT and TG in the sub-acute phase, but persistent overexpression of SERCA2a in TG does not aggravate the bladder compliance and hypertrophy. In conclusion, SERCA2a overexpression affects bladder functions under physiological conditions, but not in BOO-induced sub-acute pathological conditions.

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Introduction

The contractile activity of smooth muscle depends on cytosolic calcium (Ca^{2+}) concentration, which is regulated by transporters and pumps at cellular and organelle membranes. The sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) lowers cytosolic Ca^{2+} concentration and thus relaxes the contracted muscles. SERCA2a is expressed in slow skeletal, cardiac, and smooth muscles, and SERCA2b is expressed in all cell types [1, 2].

Quantitative changes in the expression of SERCA2 with correlations to functional alterations have been reported both in experimental animal models and in the human failing hearts [3]. The SERCA2 is decreased in pressure overload-induced cardiac hypertrophy in the decompensated phase. The overexpression of SERCA2a protein preserves an inotropic effect under the same conditions [4]. In rabbit bladders that were subjected to long-term (for 4 weeks) partial bladder outlet obstruction (BOO), the impairment of bladder function was associated with decreased SERCA2 protein expression levels [5, 6]. Similarly, obstructive dysfunction secondary to symptomatic benign prostatic hyperplasia in men is also partially characterized by the decreased SERCA2 protein expression level [7]. These data suggest that the functional impairment of heart and bladder in a decompensated phase due to pressure overload is associated with decreased SERCA2 protein levels.

On the other hand, pressure overload induced contrasting organ-specific effects in the heart [8] and bladder [9] of mice heterozygous for the SERCA2 allele despite equally

decreased SERCA2 protein levels. In this model, the onset of heart failure with transverse aortic coarctation was accelerated and morbidity/mortality ratios were greatly increased [8]. In the same BOO murine model, bladder hypertrophy was suppressed and in vivo whole bladder contractility was improved [9]. Thus, in contrast to cardiac muscle, deletion of a SERCA2 allele confers protection against bladder hypertrophy. The aim of the present study was to investigate how overexpressed SERCA2 affects bladder functions under physiological and pathological conditions by applying the BOO model in SERCA2a-transgenic rats [10].

Materials and methods

Ethical information

All experiments were conducted in accordance with the institutional guidelines approved by the Nara Medical University Institutional Animal Care and Use Committee.

Animals and study design

We established transgenic Wistar rats overexpressing SERCA2a (TG) as previously reported in detail [10]. Wild-type female Wistar rats (WT) ($n = 10$) and female TG ($n = 10$) weighing 250–300 g (10–13 weeks old) were used in the present study. Cystometry was performed under conscious conditions.

After cystometry, the rats were sacrificed, the bladders isolated, and then divided into two segments at the median sagittal plane. The posterior part of the segment was used for histochemistry and the rest of the segment was used for western blot analysis. Immunostaining was enforced in an isolated bladder to confirm the expression and distribution of SERCA2. Membrane protein fractions isolated from the whole homogenates were used for western blot analysis using a SERCA2-specific monoclonal antibody. In addition, similar experiments were conducted in partial bladder outlet obstruction (BOO) models of WT (WTBOO, $n = 5$) and TG (TGBOO, $n = 5$) weighing 270–350 g (12–15 weeks old).

Cystometry

The rats were anesthetized with isoflurane (Escain; Mylan, Tokyo, Japan). A midline abdominal incision was made and a transvesical catheter with a fire-flared tip (PE-50) was inserted into the dome of the bladder and secured with a silk thread for bladder filling and pressure recording. A three-way stopcock was connected to the transvesical catheter to monitor bladder pressure. Cystometry was performed in conscious conditions using a Bollman cage. In order to restrict the use of invasive procedures under conscious states, the connective tissues and other accessory tissues surrounding the bladder were not removed during in vivo measurements. Saline was continuously infused from the transvesical catheter at room temperature for at least 2 h at a rate of 0.1 mL/min to record cystometrograms. During cystometry, basal pressure (BP: the lowest pressure during filling phase), threshold pressure (TP: pressure immediately before micturition contraction), maximum pressure (MP: maximum pressure during micturition phase), relaxation time (RT: interval from MP to BP), micturition interval (MI: interval between MP and the next MP, proportional to voided volume), voided volume (VV), and post-void residual (PVR) were measured by using a PowerLab unit and Chart software (PowerLab 6; AD Instruments, Colorado Springs, CO, USA). Saline voided from the urethral orifice during onetime micturition was collected and its volume was measured as VV. The illustrations of these parameters are shown in Figs. 1 and 6 (below). PVR is the residual saline volume in the bladder. Immediately after voiding, the infusion was stopped and PVR was measured by drainage of the intravesical fluid. We calculated bladder compliance, which expresses the change of bladder capacity per cmH_2O, by using the formula [(VV + PVR)/(TP − BP)]. In the field of urology, compliance is routinely measured as an index of bladder storage function. Since the bladder can be extended at lower intra-bladder pressure, higher compliance indicates a better storage function. Non-voiding contractions (vesical pressure increases before each micturition without the expulsion of the fluid) higher than 5 cmH_2O were used as a surrogate for detrusor overactivity (DO) [11]. In BOO models, the appearance of DO indicates appropriate pressure overloading due to BOO. DO was defined as positive if non-voiding contractions appeared three or more times before each micturition.

Western blot analysis

The membrane protein fractions were isolated from the frozen whole bladder including smooth muscles, connective tissues, and mucosal tissues. The frozen bladders were homogenized in 1 mL of TSE buffer containing 10 mmol/L Tris–HCl, pH 7.4, 0.32 mol/L sucrose, 1 mmol/L EGTA, 5 mmol/L NaN_3, 10 mmol/L β-mercaptoethanol, 20 mmol/L leupeptin, 0.15 mmol/L pepstatin A, 0.2 mmol/L phenylmethanesulfonyl fluoride, and 50 mmol/L NaF with a Polytron homogenizer (NS-310E; Micotec) and centrifuged at 1,000*g* for 10 min. The supernatants were centrifuged at 100,000*g* for 60 min at 4 °C. The pellets obtained following this latter step consists of cellular membrane fractions and were processed for immunoblotting of SERCA2. The same amounts of membrane proteins

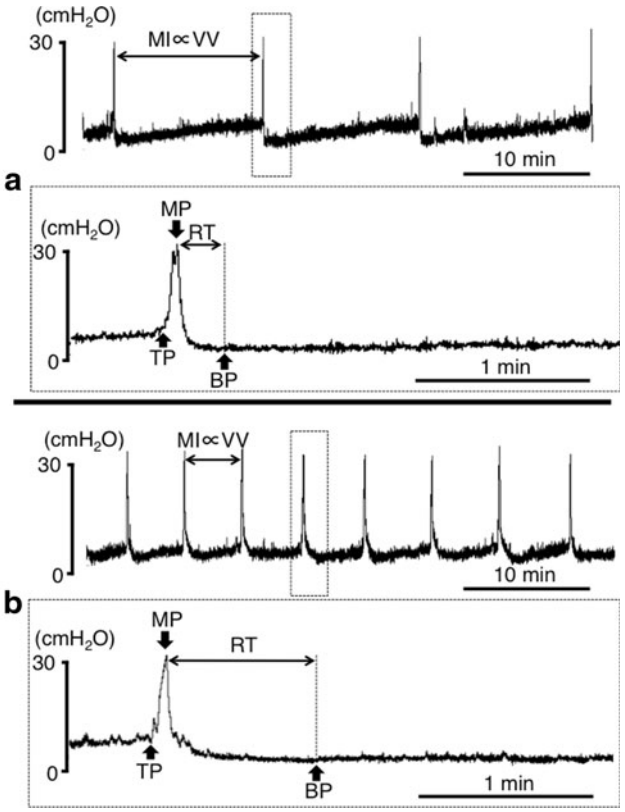

Fig. 1 Typical charts of cystometry of WT (**a**) and TG (**b**). TG (**b**) showed reduced MI and increased RT than that of WT (**a**). The trace surrounded by *dotted square* in each *upper trace* was expanded 14-fold only in time axis and corresponds to each lower trace in (**a**) and (**b**). MI ∝ VV denotes MI is proportional to VV. *MP* maximum pressure, *RT* relaxation time, *BP* basal pressure, *TP* threshold pressure, *MI* micturition interval, *VV* voided volume

(5 μg/lane) were separated on sodium dodecyl sulfate–polyacrylamide gels (7.5 %) in a gel apparatus (Hoefer TE 22 Mini Tank Transfer Unit; GE), and transferred to polyvinylidene difluoride membranes. The membranes were blocked (ECL Blocking Agent; GE) and then incubated with anti-SERCA2 antibody (1:1,000 dilution; Thermo). The antigens were detected by the luminescence method (ECL Prime western blotting detection reagent; GE) with peroxidase-linked anti-rabbit immunoglobulin G (IgG) (1:400,000 dilution). After immunoblotting, the film was scanned with a scanner, and the intensity of the whole bands was calculated by NIH image analysis. SERCA2 protein levels of TG, WTBOO, and TGBOO were normalized to that of WT (=1.0) and expressed as fold change.

Azan staining and immunohistochemistry (histological analysis)

A 0.5 (width) × 0.5 cm (height) sample of the posterior bladder wall was embedded in paraffin. Paraffin blocks were cross-sectioned in 5 μm thickness. Azan staining was performed to evaluate fibrosis in the bladder wall. The SERCA2 antibody for immunolocalization were obtained commercially (catalog no. AB 3625; Abcam). Paraffin sections were blocked with 1 % BSA (catalog no. 820451; MP Biomedical) in Tris-buffered saline (TBS: Tris–HCl 0.05 mol/L; NaCl 0.15 mol/L, PH 7.6) for 1 h and incubated with anti-SERCA2 in blocking solution overnight at 4 °C. Slides were subjected to three 5-min washes, incubated with anti-rabbit IgG catalog no. E 0432; DAKO) at room temperature, and washed with TBS three times for 5 min each. Photographs were taken using a microscope (PROVIS AX-80; Olympus, Tokyo, Japan). The thickness of the bladder smooth muscle layer was measured using photomicrographs.

Bladder outlet obstruction model

After induction of isoflurane anesthesia, the bladder was exposed via a suprapubic midline incision. Once the ureters were located, a 2-0 silk suture was placed below the bladder neck. To make partial outlet obstruction constant, a 20G needle was placed side-by-side outside the urethra, the silk suture was tied around the urethra attached to the needle, and the needle was later removed. The incision was closed with an absorbable suture. Cystometry, western blot analysis and histological analysis were performed at 2 weeks after the operation.

Statistical analysis

The overall comparisons among groups were performed using SPSS software (IBM SPSS Statistics, Chicago, IL, USA). All data are expressed as the mean ± SD. Multiple comparisons were performed by one-way analysis of variance (ANOVA) with a post hoc LSD test. The Mann–Whitney test was used to compare unpaired individual bladder weights and cystometric parameters. For each cystometric parameter, five micturition data in each rat were averaged. A P value of <0.05 was considered statistically significant.

Results

WT versus TG

Cystometry and macroscopic observations of the bladder

The body and bladder weights were not significantly different between WT and TG. VV in TG was significantly smaller than in WT (Table 1). Cystometric parameters in TG showed significantly reduced bladder compliance (Table 1), reduced MI (10.7 ± 3.3 vs. 16.8 ± 4.1 min) and

Table 1 Body weights, bladder weights, VV, PVR and bladder compliance in WT, TG, WTBOO and TGBOO

	WT ($n = 10$)	TG ($n = 10$)	WTBOO ($n = 5$)	TGBOO ($n = 5$)
Body weights (g)	277 ± 33	294 ± 23	310 ± 27	324 ± 16
Bladder weights (mg)	136 ± 32	143 ± 17	486 ± 22**	507 ± 29$^{\#}$
Voided volume (W) (mL)	1.41 ± 0.12	1.1 ± 0.19**	0.54 ± 0.11**	0.61 ± 0.21$^{\#}$
Post void residual (PVR) (mL)	0	0	1.36 ± 0.26**	1.4 ± 0.2$^{\#}$
Bladder compliance (mL/cmH_2O)	0.174 ± 0.05	0.119 ± 0.06*	0.107 ± 0.04*	0.106 ± 0.03

Values are presented as the mean ± SD

WT wild type, *TG* transgenic, *WTBOO* bladder outlet obstruction model of WT, *TGBOO* bladder outlet obstruction model of TG, *n* number of animals

* $P < 0.05$ vs. WT, ** $P < 0.001$ vs. WT, $^{\#}P < 0.001$ vs. TG

increased RT (173.5 ± 67.3 vs. 48.1 ± 18.2 s) than in WT (Figs. 1, 2). The macroscopic observations of representative bladders of TG (b) and WT (a) without any stretch were shown in Fig. 3.

Histological analysis

Azan staining and immunohistochemistry was performed in three rats of WT and TG groups. Histological analysis using Azan staining (Fig. 4a, b) revealed that the thickness of the bladder smooth muscle was not significantly different between WT and TG (1.12 ± 0.05 vs. 1.15 ± 0.04 mm). Neither smooth muscle hypertrophy nor fibrosis was observed in TG bladders. Immunohistochemistry (Fig. 4c, d) identified SERCA2 immunoreactivity only in the cytoplasm of the smooth muscle cells, and was weakly positive in the WT (Fig. 4c-a) and strongly positive in the TG (Fig. 4d-b). SERCA2 immunoreactivity was negative in interstitial cells and the mucosal layer (Fig. 4c-a, d-b).

Western blot analysis

Western blot analysis of SERCA2 protein expression was performed in four rats from WT and TG groups. The expression of SERCA2 protein was significantly higher in TG than that in WT (5.1 ± 1.0 vs. 1.0-fold change) (Fig. 5).

Therefore, we suggest that the overexpression of SERCA2 affects the bladder function, leading to reduced MI, increased RT, reduced VV, and reduced compliance.

WTBOO versus TGBOO

Cystometry

The characteristic differences between WTBOO and TGBOO, and those of WT and TG, were identified. Bladder weights in WTBOO and TGBOO were significantly larger than those in WT and TG, respectively. VVs in WTBOO and TGBOO were significantly smaller than those in WT and TG, respectively. PVRs were observed in WTBOO and TGBOO, whereas PVRs were not observed in WT and TG (Table 1). In WTBOO, MP (63.7 ± 10.4 vs. 30 ± 3.7 cmH_2O) and TP (19.2 ± 5.8 vs.

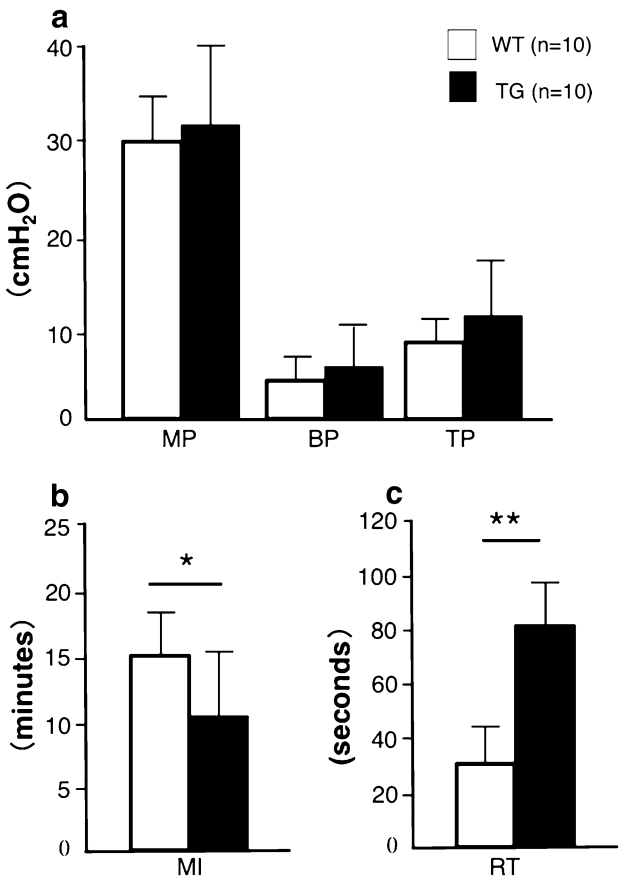

Fig. 2 Mean cystometric parameters. The TG group (*solid column*) showed significantly reduced MI (**b**) and increased RT (**c**) than those of the WT group (*open column*). *$P < 0.05$, **$P < 0.001$. *MP* maximum pressure, *BP* basal pressure, *TP* threshold pressure, *MI* micturition interval, *RT* relaxation time

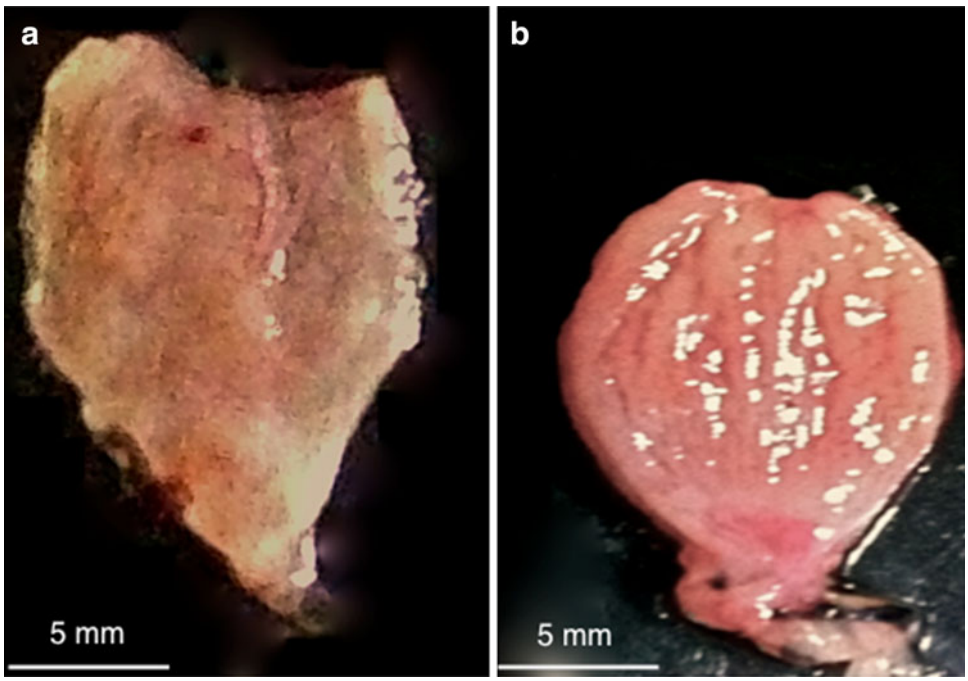

Fig. 3 Macroscopic observations of representative WT (**a**) and TG (**b**) bladders. The anterior wall of the bladder was sectioned at the median sagittal plane. The lumen is arranged at the front, the dome arranged at the top, the neck arranged at the bottom

8.6 ± 2.3 cmH_2O) were significantly higher than those in WT; in TGBOO, MP (60.5 ± 11 vs. 31.8 ± 5.1 cmH_2O) and TP (19.8 ± 5.7 vs. 10.7 ± 3.9 cmH_2O) were significantly higher than those in TG. However, there were no significant differences in the parameters such as MI in proportion to VV, MP, and TP (Fig. 6c, d), between WTBOO and TGBOO, suggesting that the bladder function in the BOO models was not affected by transgenic SERCA2.

Histological analysis

Azan staining and immunohistochemistry was performed in three rats from each WTBOO and TGBOO. Azan staining indicated that the bladder wall was thickened by smooth muscle hypertrophy and fibrosis in both WTBOO and TGBOO models (Fig. 7). In WTBOO, the thickness of the bladder smooth muscle was significantly larger than that in WT (2.08 ± 0.13 vs. 1.12 ± 0.05 mm); in TGBOO, the thickness of the bladder smooth muscle was significantly larger than that in TG (2.06 ± 0.175 vs. 1.15 ± 0.04 mm). However, there were no significant differences in the thickness of the bladder smooth muscle between WTBOO and TGBOO models (2.08 ± 0.13 vs. 2.06 ± 0.175 mm). In immunohistochemistry (Fig. 8a, b), SERCA2 immunoreactivity was identified only in the cytoplasm of the smooth muscle cells, and was strongly positive in WTBOO and TGBOO (Fig. 8a-a, b-b). Immunoreactivity for SERCA2 was negative in interstitial cells and the mucosal layer (Fig. 8a-a, b-b).

Western blot analysis

Western blot analysis of SERCA2 protein expression was performed in four rats of each group of WT, WTBOO, and TGBOO (Fig. 9). The SERCA2 protein expression was significantly higher in WTBOO and TGBOO than in WT (5.2 ± 0.67, 4.6 ± 1.1 vs. 1.0-fold change). There were no significant differences in the SERCA2 protein expression between the WTBOO and TGBOO models (5.2 ± 0.67 and 4.6 ± 1.1-fold change), suggesting that levels of overexpressed SERCA2 protein in TG was not further affected by elevation of pressure in the BOO model.

Discussion

The present studies reveal the effects of overexpression of SERCA2a in basal and BOO models using transgenic Wistar rats overexpressing SERCA2a (TG).

First, we compared the basal bladder functions of WT and TG. Changes in cystometric parameters in TG such as reduced MI, reduced VV, reduced compliance, and increased RT were observed. Furthermore, the bladder wall in TG was not thickened, and neither smooth muscle hypertrophy nor fibrosis was found. A recent study has suggested that a troponin system cooperates along with myosin light chain kinase (MLCK) signals in the detrusor smooth muscle [12], especially at lower intracellular Ca^{2+} concentrations. This explains the detrusor muscle contraction in TG under physiological conditions, since

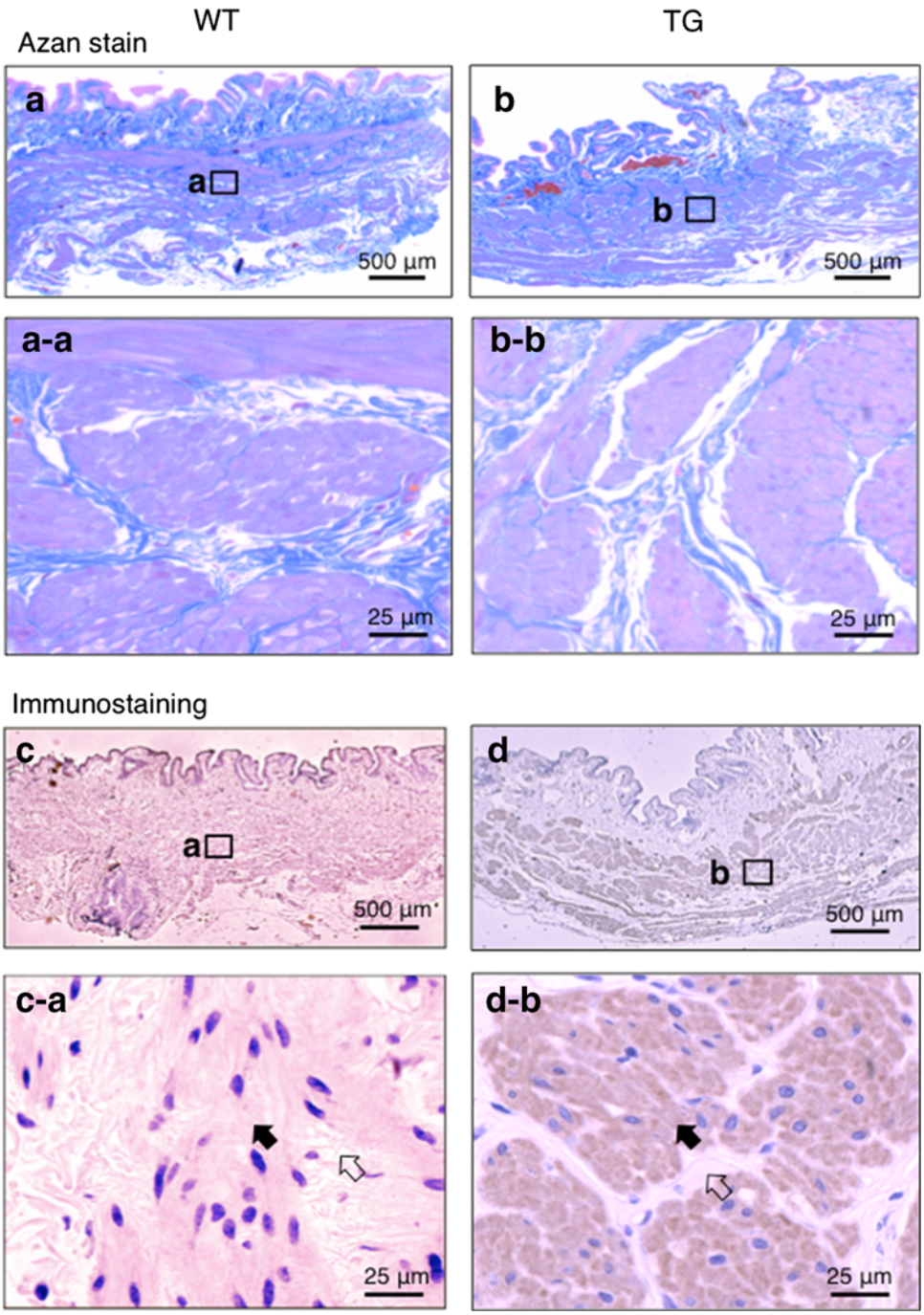

Fig. 4 Azan stain and immunohistochemistry for SERCA2 in WT (**a**, **c**) and TG (**b**, **d**) bladders. Photographs **a**, **b**, **c** and **d** are at ×20. Photographs **a-a** (indicated by *small box* in **a**), **b-b** (indicated by *small box* in **b**), **c-a** (indicated by *small box* in **c**), and **d-b** (indicated by *small box* in **d**) are enlarged at ×400. The 0.5 (width) × 0.5 cm (height) sample of the posterior bladder wall was embedded in paraffin. Paraffin blocks were cross-sectioned in 5 μm thickness. The upper side is the mucosal layer. SERCA2 immunoreactivity was identified only in the cytoplasm of the smooth muscle cells (*solid arrows* in **c-a** and **d-b**), weakly positive in WT (**c-a**) and strongly positive in TG (**d-b**). SERCA2 immunoreactivity was negative in interstitial cells (*open arrows* in **c-a** and **d-b**) and the mucosal layer

overexpression of SERCA2a may facilitate Ca^{2+} uptake into SR, leading to lower intracellular Ca^{2+} concentrations. Thus, we report here that the facilitation of the bladder contraction in turn impairs the bladder compliance.

It has been reported that the overexpression of SERCA2a also facilitates the contractility of the urethral smooth muscle, thereby decreasing the stress-induced urinary incontinence [13]. However, the facilitation of the urethral contraction may increase the urethral resistance during micturition associated with incomplete relaxation. Therefore, the overexpression of SERCA2a may cause some detrimental effects on the basal function of the lower urinary tract.

In contrast, the overexpression of SERCA2a in the heart displayed different effects from those observed in the bladder. It has been reported that overexpression of SERCA2a improved hemodynamic parameters in baseline conditions and in pressure-overloaded failing transgenic rat hearts [4]. We have also recently reported that normal hearts in similar transgenic rat overexpressing SERCA2a

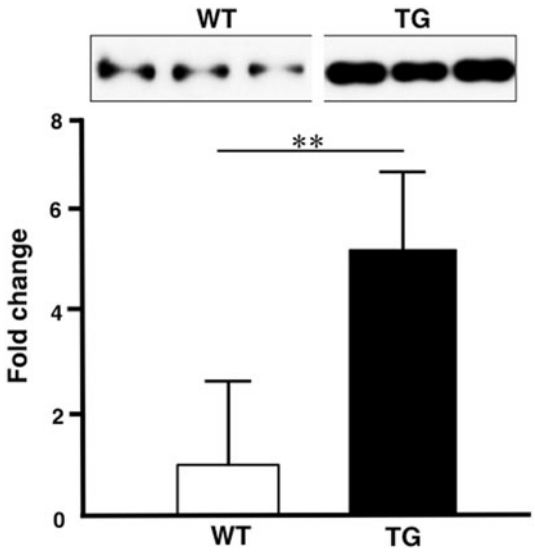

Fig. 5 Western blot analysis for detection of SERCA2 levels in WT and TG. *Upper panel* each representative band from three WT and three TG. *Lower panel* mean (+SD) fold change in the expression of SERCA2 protein in four WT and four TG. The densitometry measurements were performed on the whole band. The SERCA2 protein levels in TG were normalized to that of WT (=1.0) and expressed as fold change. The expression of SERCA2 protein in smooth muscles was significantly higher in TG (*solid column*) than that in WT (*open column*). $**P < 0.001$

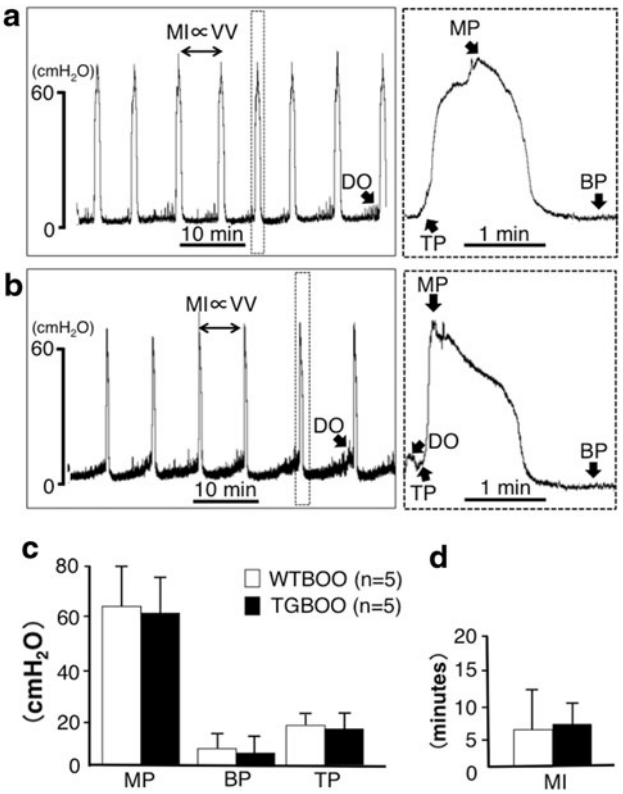

Fig. 6 Typical charts of cystometry in WTBOO (**a**) and TGBOO (**b**) and mean values (+SD) of cystometric parameters (**c**, **d**) in WTBOO (*open column*; $n = 5$) and TGBOO (*solid column*; $n = 5$). The trace surrounded by *dotted square* in each *left trace* was expanded 13-fold only in time axis, and corresponds to each *right trace* in (**a**) and (**b**). MI ∝ VV denotes MI is proportional to VV. WTBOO and TGBOO showed reduced MI and increased pressure micturition pattern than those in WT and TG, respectively (see Fig. 2), but there were no significant differences in all mean cystometry parameters between WTBOO and TGBOO. *MP* maximum pressure, *BP* basal pressure, *TP* threshold pressure, *MI* micturition interval, *DO* detrusor overactivity

show multiple beneficial effects on the left ventricular mechanical works and energetics under 300 beats per minutes (bpm) pacing [10]. Therefore, the overexpression of SERCA2a enhanced the basal cardiac function by facilitating contractility, but never caused any detrimental effect, in contrast to the bladder where it impaired the bladder storage function.

Second, we compared the bladder remodeling following BOO, in WT (WTBOO) and TG (TGBOO) models. The changes in cystometric parameters in WTBOO and TGBOO models showing reduced MI and decreased VV than those in WT and TG appeared to be causally related to the presence of PVR and reduced bladder compliance. DO was identified in the storage phase and MP in the micturition phase was markedly increased both in WTBOO and TGBOO models, indicating the enhancement of afferent nerve activity associated with BOO and the compensatory contraction of the detrusor muscle. There were no significant changes in cystometric parameters between WTBOO and TGBOO models (see Fig. 6c; Table 1), although these changes are typical as the BOO model. Therefore, the overexpression of SERCA2a did not affect the BOO-induced bladder dysfunction in the sub-acute phase 2 weeks following BOO.

The bladder remodeling following BOO was identified by the increased bladder weights, thickened bladder wall, and increased fibrosis in WTBOO and TGBOO, and was not different between WTBOO and TGBOO. The structural changes in the bladder wall, which are different from TG, may be responsible for the reduced bladder compliance in WTBOO and TGBOO. Therefore, the overexpression of SERCA2a also did not affect BOO-induced bladder remodeling in the sub-acute phase.

The amounts of SERCA2 protein were markedly increased in WTBOO and TGBOO compared with the levels in the WT, but were not different between WTBOO and TGBOO. This is an interesting new finding, although the detailed mechanism is still unknown. In the rabbit BOO model, 2–4 weeks after the BOO procedure, an increased expression of SERCA2 has been reported [14]. Thus, the contractility of the detrusor muscle would be preserved in this BOO model. However, the contractility of the detrusor muscle has already been enhanced in the TG model. Therefore, a further increase in the expression of SERCA2 might be unnecessary or it is plausible that the expression of SERCA2 had already reached its maximum.

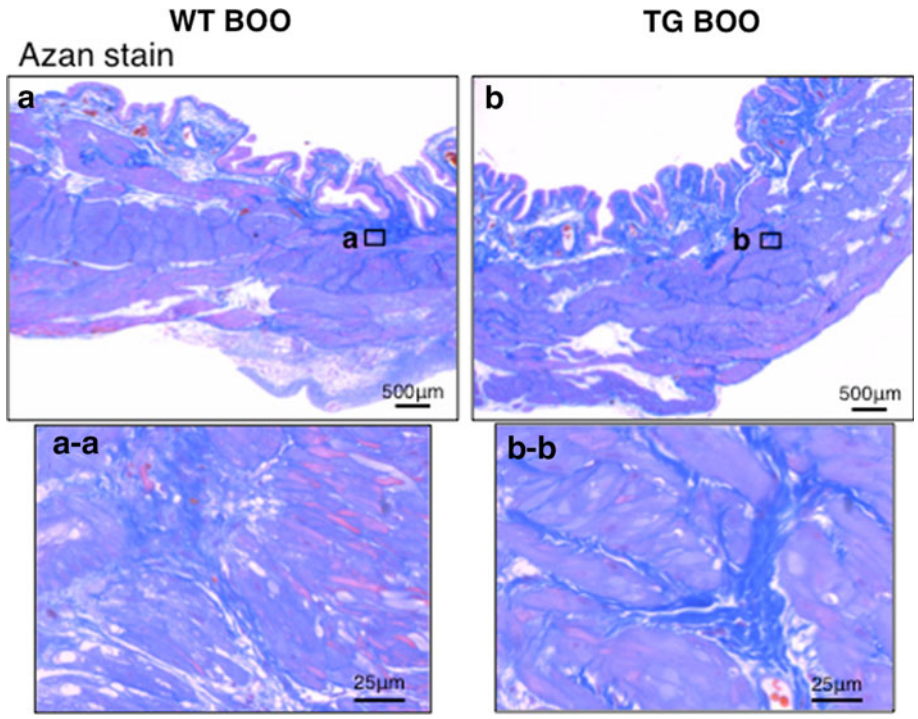

Fig. 7 Azan stain in the bladder of WTBOO (**a**) and TGBOO (**b**). Photographs **a** and **b** are at ×20. Photographs **a**-**a** (indicated by *small box* in **a**) and **b**-**b** (indicated by *small box* in **b**) are enlarged at ×400. The 0.5 (width) × 0.5 cm (height) sample of the posterior bladder wall was embedded in paraffin. Paraffin blocks were cross-sectioned in 5 μm thickness. The upper side is the mucosal layer. The bladder walls in WTBOO and TGBOO were thickened by hypertrophied smooth muscles and fibrosis

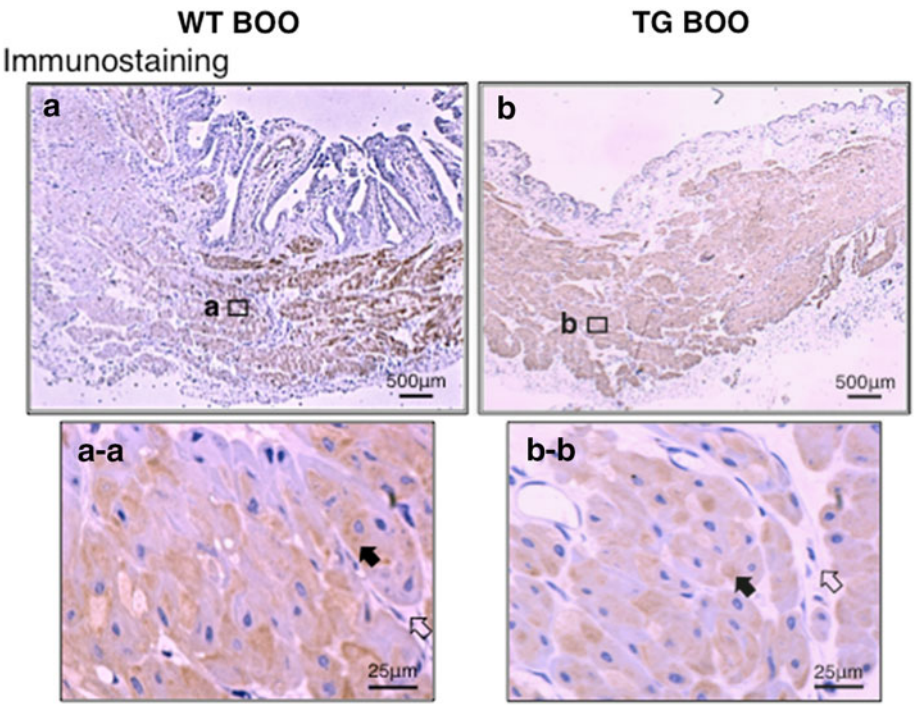

Fig. 8 Immunohistochemistry for SERCA2 in the bladder of WTBOO (**a**) and TGBOO (**b**). Photographs **a** and **b** are at ×20. **a**-**a** (indicated by *small box* in **a**) and **b**-**b** (indicated by *small box* in **b**) photographs are enlarged at ×400. The 0.5 (width) × 0.5 cm (height) sample of the posterior bladder wall was embedded in paraffin. Paraffin blocks were cross-sectioned in 5 μm thickness. The upper side is the mucosal layer. SERCA2 immunoreactivity was identified only in the cytoplasm of the smooth muscle cells (*solid arrows* in **a**-**a** and **b**-**b**), strongly positive in WTBOO (**a**-**a**) and TGBOO (**b**-**b**). SERCA2 immunoreactivity was negative in interstitial cells (*open arrows* in **a**-**a** and **b**-**b**) and the mucosal layer

A decrease in SERCA2 levels under pathological conditions with pressure overload was shown to exert organ-specific differential effects in the heart [8] and the bladder [9]. In a mouse model with decreased SERCA2 level, the cardiac function became worse following aortic stenosis [15, 16], while the bladder function was maintained following BOO [9]. A plausible reason for this may be the characteristically marked difference in the contraction frequency between the bladder (once every 1–3 h) and heart (about 400 bpm), since rapid calcium cycling is less important for the bladder function.

Increased SERCA2 level may be beneficial in the chronic phase at 4–6 weeks or longer following BOO, when the bladder dysfunction is usually decompensated in

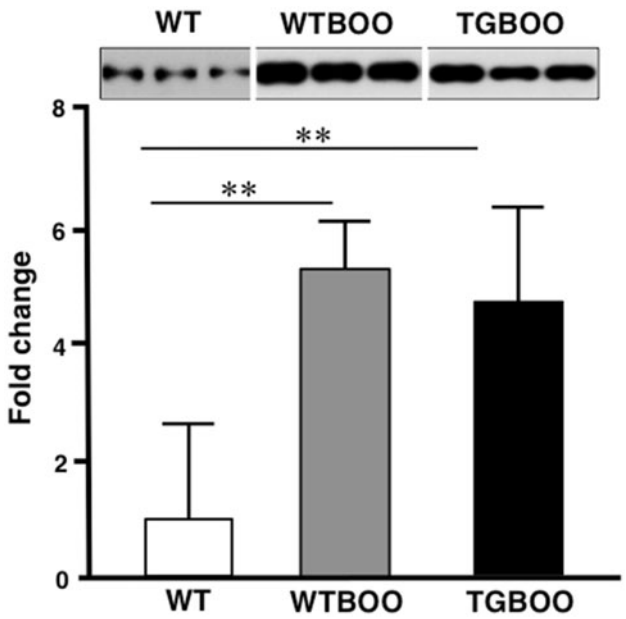

Fig. 9 Western blot analysis for SERCA2 in WTBOO, TGBOO, and WT rats. *Upper panel* each representative band from three WT, three WTBOO and three TGBOO. *Lower panel* mean (+SD) fold change in the expression of SERCA2 protein in smooth muscles was significantly higher in four WTBOO (*gray column*) and four TGBOO (*solid column*) compared to that in four WT (*open column*). The densitometry measurements were applied to the whole band. SERCA2 protein levels of WTBOO and TGBOO are normalized to that of WT (=1.0) and expressed as fold change. $^{**}P < 0.001$

WT. However, increased SERCA2 level does not exert a beneficial effect at least in the present sub-acute phase after BOO.

A recent study has suggested that Ca^{2+}/calmodulin-dependent MLCK is a major regulator of detrusor muscle contraction at high intracellular Ca^{2+} concentrations, especially under pathological conditions (probably corresponding to pressure-overloaded pathological conditions, which highly facilitate voltage-gated Ca^{2+} channels in the plasma membrane) [12]. Therefore, increased SERCA2 levels do not appear to affect the detrusor muscle contraction in WTBOO and TGBOO.

In conclusion, persistent overexpression of SERCA2a in TG affects bladder functions under physiological conditions, but not in BOO-induced sub-acute pathological conditions. Persistent overexpression of SERCA2a in TG induces reduced bladder compliance and increased relaxation time after micturition without structural changes, causing an impaired storage function. BOO induces reduced bladder compliance and hypertrophy in WT and TG in the sub-acute phase. However, persistent overexpression of SERCA2a in TG does not aggravate the bladder compliance and hypertrophy induced by BOO in comparison with WT.

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Conflict of interest The authors declare that they have no conflict of interest.

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