Published in final edited form as:

Exp Mol Pathol. 2012 April ; 92(2): 210–216. doi:10.1016/j.yexmp.2012.01.006.

Calcineurin and Akt expression in hypertrophied bladder in STZ-induced diabetic rat

Guiming Liu^{a,b,*}, Mei Li^{a,c}, and Firouz Daneshgari^{a,b}

^aGlickman Urological and Kidney Institute and Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44195, USA

^bUniversity Institute, University Hospital Case Medical Center and Department of Urology, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA

^cDivision of Infectious Disease, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA

Abstract

Diabetes causes significant increases in bladder weight but the natural history and underlying mechanisms are not known. In this study, we observed the temporal changes of detrusor muscle cells (DMC) and the calcineurin (Cn) and Akt expressions in detrusor muscle in the diabetic rat. Male Sprague–Dawley rats were divided into 3 groups: streptozotocin-induced diabetics, 5% sucrose-induced diuretics, and age-matched controls. The bladders were removed 1, 2, or 9 weeks after disease induction and the extent of hypertrophy was examined by bladder weights and cross sectional area of DMC. Cn and Akt expression were evaluated by immunoblotting. Both diabetes and diuresis caused significant increases in bladder weight. The mean cross sectional areas of DMC were increased in both diabetic and diuretic animals 1, 2, or 9 weeks after disease induction. The expression levels of both the catalytic A (CnA) and regulatory B (CnB) subunits of Cn were increased at 1 and 2 weeks, but not at 9 weeks. Expression of Akt was similar among control, diabetic, and diuretic rat bladder at all time points. In conclusion, diabetes and diuresis induce similar hypertrophy of detrusor muscle during the first 9 weeks, indicating that bladder hypertrophy in the early stage of diabetes is in response to the presence of increased urine output in diabetes. Our results suggest that the Cn, but not the Akt signaling pathway may be involved in the development of bladder hypertrophy.

Keywords

Calcineurin; Akt; Bladder; Hypertrophy diabetes

© 2012 Elsevier Inc. All rights reserved.

*Corresponding author at: University Institute, University Hospital Case Medical Center, Department of Urology, Case Western Reserve University School of Medicine, 10900 Euclid Avenue, Cleveland, OH 44106, USA. guiming.liu@case.edu (G. Liu).

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Introduction

Diabetes mellitus (DM) seriously affects multiple organ systems, including the urinary bladder. In some series, 52% of randomly evaluated diabetic patients were found to have urologic symptoms (Ioanid et al., 1981). Our previous studies showed that diabetes-related polyuria induced time-dependent increase of bladder weight, and the main source was detrusor smooth muscle (DMC) (Liu and Daneshgari, 2006). The increased detrusor smooth muscle may be due to hypertrophy or hyperplasia or both of smooth muscle cells. Hypertrophy refers to an increase in the size of the cell while hyperplasia refers to an increase in the number of cells. However, the temporal changes of DMC and mechanisms of such changes remain unknown, whereas a detailed characterization of hypertrophy in heart diseases has been studied (Heineke and Molkentin, 2006).

Cardiac hypertrophy occurs in response to long-term increases in hemodynamic load (Heineke and Molkentin, 2006). Hemodynamic overloads, including the volume overload (elevated preload) and the pressure overload (elevated afterload), increase ventricular stroke work (Carabello et al., 1992; Gorgulu et al., 2010; Kato et al., 1996; Wojciechowski et al., 2010). The chronically increased ventricular stroke work demands of overloaded states are compensated by the development of ventricular hypertrophy. Pressure overload results in the development of concentric hypertrophy where parallel sarcomere replication produces increased wall thickness; volume overload results in series sarcomere replication and eccentric hypertrophy (Carabello et al., 1992; Gorgulu et al., 2010; Kato et al., 1996; Wojciechowski et al., 2010). Cardiac hypertrophy is at least in part responsible for the symptoms of congestive heart failure that develop in patients with chronic overload hypertrophy (Carabello et al., 1992).

Previous studies indicated the involvement of calcineurin (Cn)-nuclear factor of activated T-cells (NF-AT) signaling in this process of cardiac hypertrophy (Molkentin et al., 1998). In addition, Cn signaling has been shown to be involved in bladder outlet obstruction induced detrusor hypertrophy (Clement et al., 2006; Nozaki et al., 2003). Recently, there were some reports which linked signaling through the phosphoinositide-3-kinase (PI3K)/Akt pathway to induction of cardiac hypertrophy (Naga Prasad et al., 2003). PI3K activates the Akt serine/threonine kinase and enable its activation. Akt mediates a wide variety of cellular responses including growth, proliferation and survival (Lawlor and Alessi, 2001). Mammalian genomes contain three Akt genes, Akt1, Akt2 and Akt3. Akt is phosphorylated at its two regulatory phosphorylation sites, T308/S473 in Akt1, T309/S474 in Akt2 and T305/S472 in Akt3. Akt1-null mice have a 20% reduction in body size, whereas Akt2-null mice do not show any reductions in body growth (Chen et al., 2001; Cho et al., 2001). Akt3-deficient mice present a selective 25% decrease in brain size (Easton et al., 2005). Expression of activated Akt in cardiac muscle of mice was found to be sufficient to induce hypertrophy (Shioi et al., 2002).

Similarly to hemodynamic overloads in cardiac hypertrophy, urodynamic overloads in diabetic bladder, including mainly volume overload (increased urine production (Liu and Daneshgari, 2006)) and probably pressure overload (decreased urethral relaxation (Liu et al., 2008; Yang et al., 2007)), are the potential inducers of hypertrophy. We hypothesize that Cn

and Akt signaling may play roles in the hypertrophic response of the bladder to diabetes-associated polyuria.

In this study, we aimed to observe the temporal changes of DMC and the Cn and Akt expression in detrusor muscle in the diabetic rat.

Materials and methods

Experimental animals

Male Sprague–Dawley rats matched by date of birth (290 to 310 g, 10 weeks-old, Harlan), were used in this study. The animals were randomly allocated to three groups: diabetics (n=18), diuretics (n=18), and age-matched controls (n=18). Each group was subsequently divided into three subgroups of 6 rats for evaluation at 1 week, 2 weeks or 9 weeks after induction. Diabetes was induced by intraperitoneal injection of streptozotocin (STZ, 50 mg/kg dissolved in 0.1 M citrate buffer, pH 4.5), and diuresis was induced by addition of 5% sucrose to their drinking water. Blood glucose levels were measured with the ACCU-CHEK system (Roche Diagnostics Corporation, Indianapolis, IN) 72 h after administration of STZ and at the time of sacrifice to confirm diabetes (blood glucose >350 mg/dl). At designed time points, animals were sacrificed by a single intraperitoneal injection of pentobarbital (200 mg/kg). The urinary bladder was removed at the level of the bladder neck, weighed and sectioned at the equatorial midline (largest diameter). The bottom half was placed on ice. Tunica adventitia and urothelium were removed under a surgical microscope, and the smooth muscle layer was frozen for immunoblotting. The dome half was allowed to equilibrate for 15 min at room temperature in Krebs' buffer, then embedded in Tissue-Tek® O.C.T. Compound (Torrance, CA), frozen in liquid nitrogen, and stored in –80 °C freezer for immunofluorescence staining. The composition of the Krebs solution was as follows (in mM): 133 NaCl, 4.7 KCl, 2.5 CaCl₂, 16.3 NaHCO₃, 1.35 NaH₂PO₄, 0.6 MgSO₄, and 7.8 Dextrose (Hall et al., 2002). All experimental protocols were approved by our Institutional Animal Care and Use Committee.

Immunofluorescence staining and morphometric analysis

Frozen OCT-embedded bladder tissues were sectioned into 8 µm-thick sections. Sections were treated with 4% paraformaldehyde for 30 min, then placed in 10% normal goat serum with 0.2% Triton X-100 for 30 min, and then incubated with rabbit α-smooth muscle actin antibody (AB 1982, 1:500, Chemicon; Temecula, CA) overnight at 4 °C in a humid chamber. The slides were rinsed with PBS and then incubated with fluorescein-conjugated Alexa 488 sheep anti-rabbit IgG (1:5000; Molecular Probes, Eugene, OR), including 10 µg/ml Rhodamine Wheat Germ Agglutinin (WGA) which can bind to N-acetylglucosamine or chitobiose for glycoprotein staining of membrane. Slides were washed and mounted with medium containing DAPI (Vector Laboratories). Fluorescent images were obtained using a laser confocal microscope.

Immunofluorescence stained sections at equatorial sections of bladder were used to determine mean intracellular cross-sectional area of a single detrusor muscle cell. Fig. 1A is the representative overlaid image of immunofluorescence staining of α-smooth muscle actin

(green), membrane (red), and nuclei (blue) in the longitudinal sections of smooth muscle cells in bladder. The immunoreactive α -smooth muscle actin cells were used for analysis. Four regions of longitudinally oriented smooth muscle cells at 3, 6, 9, and 12 o'clock of equatorial sections of bladder were chosen for quantification. The intracellular cross-sectional area of each cell in a region was determined and a mean value was calculated. Then the mean of four regions was calculated to represent the intracellular cross sectional area of a single cell in each animal. The immunofluorescence images were analyzed with Image-Pro Plus 5.1 image analysis software (Media Cybernetics, Silver Spring, MD). The software can automatically distinguish regions stained with different colors and accurately measure the areas by counting the pixels and converting pixels to number of square micrometers. Fig. 1B shows the cell membranes stained in red. The area within the membrane of DMC was measured by tracing the internal edges of the cell. Fig. 1C shows the red circle along the perimeter of the cell produced by the software automatically and used for measuring the intracellular cross-sectional area of each cell. In all cases, the processing of images was performed by the same investigator unaware of treatment group assignments.

Immunoblotting

Frozen detrusor muscle tissues were homogenized in buffer containing 20 mM Tris-HCl, 1% Triton X-100, 100 mM NaCl, 0.5% NP-40 and protease inhibitors. Protein concentration was determined by the detergent-compatible Bio-Rad DC protein assay. Proteins were separated by SDS-PAGE. Equal amounts of protein extract (40 μ g) from control, diabetic, and diuretic groups at the same time point were distributed to the same gel (4–15% linear gradient 18 well gel, Bio-Rad Laboratories) to reduce any other non-treatment effects. The proteins then were transferred to nitrocellulose membranes. The membranes were blocked with 5% milk in PBS containing 0.05% Tween-20 and probed with a primary antibody in the blocking buffer, then incubated with the appropriate secondary antibodies (Jackson ImmunoResearch, West Grove, PA). The bands were visualized using enhanced chemiluminescence and Kodak Biomax film. Membrane was also incubated with anti β -actin antibodies (A 5441, 1:100000, Sigma-Aldrich, Saint Louis, Missouri). The primary antibodies used were rabbit-anti-CnA (1:100) and rabbit-anti-CnB (1:100) from Biomed Technology (Stoughton, MA), rabbit-anti-Akt (1:1000), rabbit-anti-Akt (Thr308) (1:1000), and mouse-anti-Akt (Ser473) (1:1000) from Cell Signaling Technology (Danvers, MA). Band intensities were evaluated using Image J software. Beta-actin was used as a loading control. The band intensity quantification was normalized to β -actin.

Statistical analysis

All data is expressed as the mean plus or minus standard error of the mean (SEM). Comparisons of measurements among the diabetic, diuretic and control groups at the same time point were performed with the One-way ANOVA test, followed by Newman-Keuls multiple comparison (Graph Pad 4.0 software).

Results

General characteristics

General physical characteristics of the animals are summarized in Table 1. The initial mean body weight was similar for all 3 groups, but the diabetic group weighed less than the corresponding diuretic and control groups 1 week, 2 weeks and 9 weeks after induction ($P<0.01$). The mean blood glucose levels of the diabetic rats were ~4 times higher than those of control and diuretic rats. However, there were no significant differences in body weights and blood glucose levels between control and diuretic animals ($P>0.05$). The bladder weights increased markedly in both the diabetic and diuretic rats compared with controls. There were no significant differences in bladder weights between the diabetic and diuretic animals at any of the time points.

Immunofluorescence stained image morphometric analysis

Immunofluorescence stained α -smooth muscle actin image showed longitudinal and latitudinal muscle bundles in the bladder wall. Fig. 1A is the representative image of immunofluorescence staining of α -smooth muscle actin (green) in the longitudinal sections of smooth muscle cells at equatorial section of a urinary bladder in a control rat. Membrane was visualized with a rhodamine labeled WGA (red) (Fig. 1B). Nuclei were visualized with DAPI (blue).

Fig. 1D–F contain representative images of cross sectional cell membrane glycoproteins (red) in equatorial sections of urinary bladders in age-matched control, diabetic and diuretic rats 9 weeks after induction. Quantitative analysis of the mean of intracellular cross-sectional area of longitudinal sections of smooth muscle cells at equatorial section is shown in Fig. 1G. The mean cross sectional area of a single DMC in a normal rat is approximately 22–24 μm^2 . The average area of a DMC was increased significantly (1.6–1.8 times of control) 1, 2, and 9 weeks after induction in diabetic and diuretic rats compared with corresponding controls ($P<0.01$). There were no significant differences between diabetic and diuretic rats in the mean cross sectional area of a single DMC at any of the time points ($P>0.05$).

Changes in CnA and CnB expression in detrusor muscle

Western blot analysis revealed that Cn expression changed in a time-dependent manner in diabetic and diuretic rats. CnA and CnB expression 1 week (Fig. 2) and 2 weeks (Fig. 3) after diabetes and diuresis induction were significantly increased compared with that of corresponding controls, but were not different from the corresponding controls at 9 weeks (Fig. 4).

Changes in Akt expression in detrusor muscle

Immunoblotting analysis revealed that no changes in total Akt among these groups (Figs. 2, 3, 4). In addition, there were no differences in the phosphorylation of Akt at either the threonine 308 or the serine 473 residues (Figs. 2, 3, 4).

Discussion

In the present study, we investigated the temporal changes of DMC and the possible underlying mechanisms of these alterations. Our results showed that the mean cross sectional areas of DMC were increased in both diabetic and diuretic animals as early as 1 week after disease induction. Cn was expressed in detrusor smooth muscle and its expression increased 1 week and 2 weeks after diabetes induction. However, Akt, which also was expressed in the smooth muscle, did not change in the diabetic animals within the investigated period. Diuresis produced similar results to the diabetic group.

Diuresis, induced by feeding 5% sucrose instead of water to animals, causes significant increases in bladder weight, but does not affect the body weight or serum glucose concentration (Longhurst et al., 1990). Diuresis alone can induce many of the effects associated with diabetes, including bladder hypertrophy, increased contractility, and increased capacity (Tammela et al., 1995). Therefore, the use of a diuretic group is crucial to distinguish morphological changes produced by diabetes from those possibly induced by the pure effect of increased urine output. Our previous study showed feeding water including 5% sucrose in rat can induce the similar increased fluid intake and urine output as in a diabetic rat (Liu and Daneshgari, 2006).

Our previous studies demonstrated that the primary source of the increased bladder weight was the detrusor smooth muscle (Liu and Daneshgari, 2006). The present study showed the mean cross sectional area of a single longitudinal DMC in normal rat is 22–24 μm^2 . The mean area of DMC increased significantly, as early as 1 week after induction in diabetic and diuretic rats relative to controls, were approximately ~1.6–1.8 times of control, and remained stable between 2 and 9 weeks after induction. However, the bladder weight increased 1 week after induction of diuresis or diabetes, and the bladder weight continued to increase up to 9 weeks in both groups. These results indicate that the hyperplasia of DMC may also play a role in the increased bladder weight in addition to hypertrophy. Eika et al. demonstrated that diabetes-induced diuresis stimulates DNA synthesis and cell proliferation in bladder tissue (Eika et al., 1993). In addition, the gradual increase of other components of bladder wall, like urothelium, also contributes to the increased bladder weight (Liu and Daneshgari, 2006). There were no significant differences between diabetic and diuretic rats in the mean area of DMC at any time points, indicating that the hypertrophy is a likely response to polyuria in the early stage of diabetes. The functional significance of the detrusor smooth muscle hypertrophy is that it may be involved in the generation of altered detrusor pressure as part of a compensatory and/or pathophysiological response to the increased urine load.

The mechanisms involved in triggering tissue hypertrophy are not fully understood. Urodynamic overloads in diabetic bladder are the potential inducers of hypertrophy. In addition, it has been reported that a high filling rate is a primary factor responsible for the induction of hypertrophy of the bladder smooth muscle (Saito et al., 1994). Acute overdistention induced a 5-fold increase in ^3H -thymidine incorporation in the bladder body (Tong et al., 1992). However, the molecular mechanisms of such hypertrophic changes remain unknown.

The Ca^{2+} -dependent serine/threonine protein-phosphatase calcineurin was identified as a central pro-hypertrophic signaling molecule in the myocardium (Molkentin et al., 1998). Calcineurin consists of a 57–61-kDa catalytic subunit (CnA) and a 19-kDa regulatory subunit (CnB). CnB carries the binding site of Ca^{2+} , which enables calmodulin binding to CnA. The activation of Cn occurs through the binding of calcium/calmodulin, which displaces an autoinhibitory domain of the CnA subunit. The well characterized substrate of Cn is the transcription factor NFAT (Crabtree and Olson, 2002). NFAT has been shown to be both necessary and sufficient for mediating cardiac hypertrophy (Molkentin et al., 1998). Calcineurin dephosphorylates multiple serine residues near the N termini of NFAT proteins leading to their translocation from the cytoplasm to the nucleus where they engage a variety of transcription factors and activate Cn-responsive genes.

The current results show that Cn was expressed in bladder smooth muscle. The expression pattern of CnA, the catalytic subunit, and CnB, the regulatory subunit, changes from being significantly greater than control at 1 week and 2 weeks to being not significantly different at 9 weeks. These changes in CnA expression in diabetic and diuretic animals are similar to those in the pressure-overloaded cardiohypertrophy (Lim et al., 2000) and bladder outlet obstruction induced detrusor hypertrophy (Clement et al., 2006; Nozaki et al., 2003). Interestingly, the changes of CnB expression are not consistent with a previous study in bladder outlet obstruction induced detrusor hypertrophy (Nozaki et al., 2003), which showed that CnB expression was unchanged throughout the development of hypertrophy. However, CnB protein expression was found to increase in dilated cardiomyopathy compared with non-failing controls (Diedrichs et al., 2004). Our results suggest that a Cn-mediated signaling pathway may be involved in the development of detrusor hypertrophy.

In cardiac muscle, the PI3K/Akt pathway has been identified as another potential mediator of the response to mechanical overload (Shioi et al., 2002). We examined the changes of total Akt level and phosphorylated Akt1 at T308 and S473 in detrusor muscle in diabetic and diuretic rats. However, we did not see any changes in total Akt level or Akt1 phosphorylation in diabetes- and diuresis-induced bladder hypertrophy 1 to 9 weeks after induction. There are two possible reasons for this observation: 1) we may have missed the changes, as Akt could be activated shortly after initial polyuria-induced stretch of bladder, and then return to the baseline before 1 week or 2) the Akt signaling pathway is not involved in diabetes- and diuresis-induced bladder hypertrophy. A recent study (McMullen et al., 2003) showed that phosphoinositide 3-kinase plays a critical role for the induction of physiological, but not pathological, cardiac hypertrophy.

Conclusions

STZ-induced diabetes and 5% sucrose-induced diuresis induced rapid and marked DMC hypertrophy, which suggests that diabetes-associated polyuria contributes an important role in diabetes-induced bladder hypertrophy. The Cn signaling pathway might be involved in polyuria-induced detrusor smooth muscle hypertrophy. However, it is unlikely that the Akt signaling pathway is involved in diabetes-induced bladder hypertrophy in rat. The effects of a Cn blocker on diabetes-induced bladder hypertrophy warrants further investigation.

Acknowledgments

This study was supported by grants from a Juvenile Diabetes Research Foundation International Fellowship and the Animal Models of Diabetic Complications Consortium (U01-DK61018). We thank Kerry O. Grimberg, PhD for her medical editorial assistance.

References

- Carabello BA, Zile MR, Tanaka R, et al. Left ventricular hypertrophy due to volume overload versus pressure overload. *American Journal of Physiology*. 1992; 263:H1137–H1144. [PubMed: 1415762]
- Chen WS, Xu PZ, Gottlob K, et al. Growth retardation and increased apoptosis in mice with homozygous disruption of the Akt1 gene. *Genes & Development*. 2001; 15:2203–2208. [PubMed: 11544177]
- Cho H, Mu J, Kim JK, et al. Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). *Science*. 2001; 292:1728–1731. [PubMed: 11387480]
- Clement MR, Delaney DP, Austin JC, et al. Activation of the calcineurin pathway is associated with detrusor decompensation: a potential therapeutic target. *Journal of Urology*. 2006; 176:1225–1229. [PubMed: 16890730]
- Crabtree GR, Olson EN. NFAT signaling: choreographing the social lives of cells. *Cell*. 2002; 109 (Suppl):S67–S79. [PubMed: 11983154]
- Diedrichs H, Chi M, Boelck B, et al. Increased regulatory activity of the calcineurin/NFAT pathway in human heart failure. *European Journal of Heart Failure*. 2004; 6:3–9. [PubMed: 15012912]
- Easton RM, Cho H, Roovers K, et al. Role for Akt3/protein kinase Bgamma in attainment of normal brain size. *Molecular and Cellular Biology*. 2005; 25:1869–1878. [PubMed: 15713641]
- Eika B, Levin RM, Monson FC, et al. 3H-thymidine uptake by the rat urinary bladder after induction of diabetes mellitus. *Journal of Urology*. 1993; 150:1316–1320. [PubMed: 8371420]
- Gorgulu S, Norgaz T, Nurkalem Z, et al. Comparison of left ventricular contractility in pressure and volume overload: a strain rate study in the clinical model of aortic stenosis and regurgitation. *Echocardiography*. 2010; 27:798–802. [PubMed: 20456482]
- Hall R, Andrews PL, Hoyle CH. Effects of testosterone on neuromuscular transmission in rat isolated urinary bladder. *European Journal of Pharmacology*. 2002; 449:301–309. [PubMed: 12167473]
- Heineke J, Molkentin JD. Regulation of cardiac hypertrophy by intracellular signalling pathways. *Nature Reviews Molecular Cell Biology*. 2006; 7:589–600.
- Ioanid CP, Noica N, Pop T. Incidence and diagnostic aspects of the bladder disorders in diabetics. *European Urology*. 1981; 7:211–214. [PubMed: 7227400]
- Kato S, Koide M, Cooper G, et al. Effects of pressure- or volume-overload hypertrophy on passive stiffness in isolated adult cardiac muscle cells. *American Journal of Physiology*. 1996; 271:H2575–H2583. [PubMed: 8997318]
- Lawlor MA, Alessi DR. PKB/Akt: a key mediator of cell proliferation, survival and insulin responses? *Journal of Cell Science*. 2001; 114:2903–2910. [PubMed: 11686294]
- Lim HW, De Windt LJ, Steinberg L, et al. Calcineurin expression, activation, and function in cardiac pressure-overload hypertrophy. *Circulation*. 2000; 101:2431–2437. [PubMed: 10821822]
- Liu G, Daneshgari F. Temporal diabetes- and diuresis-induced remodeling of the urinary bladder in the rat. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology*. 2006; 291:R837–R843.
- Liu G, Lin YH, Yamada Y, et al. External urethral sphincter activity in diabetic rats. *Neurourology and Urodynamics*. 2008; 27:429–434. [PubMed: 18351620]
- Longhurst PA, Wein AJ, Levin RM. In-vivo urinary bladder function in rats following prolonged diabetic and non-diabetic diuresis. *Neurourology and Urodynamics*. 1990; 9:171–178.
- McMullen JR, Shioi T, Zhang L, et al. Phosphoinositide 3-kinase(p110alpha) plays a critical role for the induction of physiological, but not pathological, cardiac hypertrophy. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100:12355–12360. [PubMed: 14507992]

- Molkentin JD, Lu JR, Antos CL, et al. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell*. 1998; 93:215–228. [PubMed: 9568714]
- Naga Prasad SV, Perrino C, Rockman HA. Role of phosphoinositide 3-kinase in cardiac function and heart failure. *Trends in Cardiovascular Medicine*. 2003; 13:206–212. [PubMed: 12837584]
- Nozaki K, Tomizawa K, Yokoyama T, et al. Calcineurin mediates bladder smooth muscle hypertrophy after bladder outlet obstruction. *Journal of Urology*. 2003; 170:2077–2081. [PubMed: 14532857]
- Saito M, Longhurst PA, Murphy M, et al. Effect of slow and rapid cystometry on in vitro rat urinary bladder DNA synthesis. *General Pharmacology*. 1994; 25:1021–1025. [PubMed: 7835619]
- Shioi T, McMullen JR, Kang PM, et al. Akt/protein kinase B promotes organ growth in transgenic mice. *Molecular and Cellular Biology*. 2002; 22:2799–2809. [PubMed: 11909972]
- Tammela TL, Leggett RE, Levin RM, et al. Temporal changes in micturition and bladder contractility after sucrose diuresis and streptozotocin-induced diabetes mellitus in rats. *Journal of Urology*. 1995; 153:2014–2021. [PubMed: 7752386]
- Tong YC, Monson FC, Erika B, et al. Effects of acute in vitro overdistension of the rabbit urinary bladder on DNA synthesis. *Journal of Urology*. 1992; 148:1347–1350. [PubMed: 1404671]
- Wojciechowski P, Juric D, Louis XL, et al. Resveratrol arrests and regresses the development of pressure overload- but not volume overload-induced cardiac hypertrophy in rats. *Journal of Nutrition*. 2010; 140:962–968. [PubMed: 20335634]
- Yang Z, Dolber PC, Fraser MO. Diabetic urethropathy compounds the effects of diabetic cystopathy. *Journal of Urology*. 2007; 178:2213–2219. [PubMed: 17870107]

Fig. 1.

A, Representative image of immunofluorescence staining of α -smooth muscle actin (green) at equatorial section of a urinary bladder in a control rat. Membrane is visualized with a rhodamine labeled WGA (red). Nuclei are visualized with DAPI (blue). B, Confocal image of cross sectional cell membrane glycoproteins stained with rhodamine labeled WGA (red). C, Image analysis of cross-sectional area of α -smooth muscle actin-immunoreactive detrusor muscle cell. The inner area of a single detrusor muscle cell is recognized and captured by the automated digital image analyzer for measurement of the mean of cross-sectional area. White scale bar is 50 μ m. D-F. Representative images of cross sectional cell membrane glycoproteins (red) in equatorial sections of urinary bladders in age-matched control (D), diabetic (E) and diuretic (F) rats 9 weeks after induction of diabetes or diuresis, visualized with rhodamine labeled WGA. G, Quantitative analysis of cross-sectional area of single detrusor muscle cell. White scale bar is 50 μ m. Asterisk indicates significantly different when compared with the value of age-matched control group ($P < 0.01$).

Fig. 2.

CnA and CnB expression (A), Phosphorylated Akt in Ser473, phosphorylated Akt in Thr308, and total Akt expression (B) in bladder of diabetic and diuretic rat after 1 week induction and age-matched control (immunoblotting results). Each lane was from a single rat. C, Quantitative analysis of CnA and CnB expression. D, Quantitative analysis of Phosphorylated Akt in Ser473, phosphorylated Akt in Thr308, and total Akt expression. Data are presented as mean $\pm$ SE of 4–6 rats per group. Asterisk indicates significantly different when compared with the value of age-matched control group ($P<0.05$).

Fig. 3. CnA and CnB expression (A), Phosphorylated Akt in Ser473, phosphorylated Akt in Thr308, and total Akt expression (B) in bladder of diabetic and diuretic rat after 2 week induction and age-matched control (immunoblotting results). Each lane was from a single rat. C, Quantitative analysis of CnA and CnB expression. D, Quantitative analysis of Phosphorylated Akt in Ser473, phosphorylated Akt in Thr308, and total Akt expression. Data are presented as mean $\pm$ SE of 4–6 rats per group. Asterisk indicates significantly different when compared with the value of age-matched control group ($P<0.05$).

Fig. 4.

CnA and CnB expression (A), Phosphorylated Akt in Ser473, phosphorylated Akt in Thr308, and total Akt expression (B) in bladder of diabetic and diuretic rat after 9 week induction and age-matched control (immunoblotting results). Each lane was from a single rat. C, Quantitative analysis of CnA and CnB expression. D, Quantitative analysis of Phosphorylated Akt in Ser473, phosphorylated Akt in Thr308, and total Akt expression. Data are presented as mean $\pm$ SE of 4–6 rats per group.

Table 1

Body weight, bladder weight, and blood glucose levels of diabetic, diuretic and age-matched control rats.

Time point	Group	Initial body weight (g)	Final body weight (g)	Blood glucose (mg/dl)	Bladder weight (mg)
1 wk	Control	296.75±1.35	330.00±3.67	120.75±4.41	88.75±2.50
	Diabetic	292.50±2.67	286.50±2.60 [#]	464.50±10.20 [#]	125.67±4.23 [*]
	Diuretic	298.17±4.94	336.50±1.95	124.33±3.07	132.33±4.68 [*]
2 wks	Control	309.00±8.24	354.83±4.41	124.50±3.36	82.67±2.79
	Diabetic	298.43±2.67	280.86±3.94 [#]	488.00±2.54 [#]	136.71±6.67 [*]
	Diuretic	307.50±3.27	358.33±4.46	126.50±2.80	142.00±4.23 [*]
9 wks	Control	302.50±2.93	445.00±6.34	131.67±1.73	93.17±3.81
	Diabetic	303.33±5.90	268.17±3.47 [#]	458.00±8.90 [#]	159.00±11.86 [*]
	Diuretic	305.33±5.30	451.50±5.04	116.00±5.90	177.50±4.34 [*]

Values are expressed as mean plus or minus standard error of mean of 6 individual rats.

^{*} Significantly different from corresponding value in age-matched control group ($P<0.01$).

[#] Significantly different from corresponding value in age-matched control and diuretic group ($P<0.01$).