

Cystometric parameters and the activity of signaling proteins in association with the compensation or decompensation of bladder function in an animal experimental model of partial bladder outlet obstruction

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Abstract. We conducted this study to determine whether the degree of detrusor contractility is associated with the compensation or decompensation of bladder function depending on the residual volume (RV) during the first two weeks after the onset of partial bladder outlet obstruction (BOO). Moreover, we also examined whether the degree of the phosphorylation and expression of signaling proteins [AMP-activated kinase (AMPK), extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) and protein kinase C (PKC)] is associated with the prevalence of compensation or decompensation of bladder function. Twenty-seven female Sprague-Dawley (SD) rats were randomly assigned to either the sham-operated group (n=7) or the group with partial bladder outlet obstruction (BOO) (n=20). We then measured cystometric parameters from three reproducible micturition cycles and averaged the results for a comparison between the two groups. Based on a cut-off value of a mean RV% of 25%, we subdivided our experimental animals into two subgroups: the subgroup with bladder compensation (mean RV%, <25%) and the subgroup

with bladder decompensation (mean RV%, >25%). Our results indicated that the degree of detrusor overactivity (DO) was associated with the compensation or decompensation of bladder function depending on the RV during the first two weeks after the onset of BOO in an animal experimental model of partial BOO. Moreover, we also demonstrate that AMPK and ERK1/2 are involved in the compensation or decompensation of bladder function. Furthermore, our results suggest that PKC is not involved in two-phase bladder contraction. Alterations in the activities of signaling proteins, such as AMPK and ERK1/2 may prove to be helpful in the treatment of patients with voiding difficulty.

Introduction

Benign prostatic hyperplasia is a leading cause of partial bladder outlet obstruction (BOO) in elderly men. This may eventually lead to the occurrence of detrusor dysfunction (1). Animal experimental studies have demonstrated that the bladder undergoes three sequential stages (hypertrophy, compensation and decompensation) in partial BOO (2). Detrusor overactivity (DO) then occurs during the filling phase. This is followed by the transition from compensation to decompensation, characterized by decreased detrusor contractility during the voiding phase (3). To date, a number of studies have shown that changes occur in detrusor contractility in partial BOO (2,4-7). However, little is known about the changes in detrusor contractility that occur due to the compensation and decompensation of bladder function during the filling and voiding phase.

Animal experiments have indicated that the DO is an indicator of a rapid but transient contraction of the bladder smooth muscles during the filling phase, thus termed as a phasic contraction (8). The contractile response of the bladder shows a biphasic pattern during the voiding phase: initial phasic contraction and prolonged sustained tension, thus termed as a tonic contraction. Presumably, this may play a role in efficient

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voiding and the resulting residual volume (RV) (9,10). Thus, the bladder smooth muscles undergo two phasic contractions followed by one tonic one, involving different types of kinetics (8). However, little is known about the underlying mechanisms through which the transition from the compensation to decompensation of bladder function occurs. Certain studies have suggested that the decreased microvascular perfusion and the impaired energy metabolism are involved in the alterations in detrusor contractility (11,12).

As shown in the contraction of all the other smooth muscles, the bladder smooth muscles are contracted in an adenosine triphosphate (ATP)-dependent manner as a main source of metabolic energy (8). Each smooth muscle cell contains only a small amount of ATP and is unable to import ATP. This strongly suggests that signaling pathways are involved in the control of the uptake and subsequent use of ATP (13). In further detail, it has been suggested that the AMP-activated kinase (AMPK) plays a key role in regulating the energy balance and thereby mediating the unlimited supply of ATP (14). To date, however, no studies have proposed a theory to explain the mechanisms through which energy is supposedly supplied to the bladder. Furthermore, AMPK is definitely involved in the pathophysiology of various cardiovascular diseases, such as pathological cardiac hypertrophy or heart failure (13). It has also been reported to be present in the bladder (15). However, little is known about its role in the ATP-dependent process in the bladder.

Previous studies have indicated that there are alterations in the degree of the expression of signaling proteins, such as extracellular signal-regulated kinase (ERK) and protein kinase C (PKC) (16,17). These signaling proteins are involved in the transduction of intracellular signals and the control of cell growth and differentiation in response to mechanical stimuli in BOO. This phenomenon is termed as mechanotransduction (18). Furthermore, it has been suggested that ERK and PKC are also involved in the AMPK phosphorylation pathway in the heart (19,20). However, little is known about their involvement in the AMPK phosphorylation pathway in the bladder.

To date, it has been well established that there is a temporal difference in the onset of compensation of bladder function, followed by its decompensation; animal experimental studies have revealed that the compensation of bladder function occurs within two weeks following the onset of BOO and decompensation subsequently occurs. In a preliminary animal study, however, the decompensation also occurred one week following the onset of BOO. This suggests that the compensation or decompensation prevalently occurs due to a difference in the degree of AMPK activity rather than a temporal difference in the time point of the onset between the two events (7).

Given the above background, we conducted this study to examine whether the degree of detrusor contractility is associated with the compensation or decompensation of bladder function depending on the RV during the first two weeks after the onset of BOO in an animal experimental model of partial BOO based on three cystometric parameters: the degree of DO, maximal pressure (MP) and RV%. We speculated that the degree of DO and MP are indicators of the two phasic contractions of the bladder during the filling and voiding phases, respectively, and the RV% is an indicator

of tonic contraction during the voiding phase. Moreover, we also examined whether the degree of the phosphorylation and expression of signaling proteins (AMPK, ERK1/2 and PKC) is associated with the prevalence of bladder compensation or decompensation.

Materials and methods

Experimental animals. For the current experimental study, 27 female Sprague-Dawley (SD) rats weighing 230-260 g were obtained from a commercial breeder (Orient Bio Inc., Seongnam, Korea) and then kept in a vivarium with free access to water and food with diurnal light cycling. Urethral constriction was induced as previously described (7). All the experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the Korean National Institutes of Health, for which the study protocol was approved by the Ethics Committee of the Inha University College of Medicine (INHA 11108-116).

We randomly assigned the SD rats into two groups: the sham-operated group (n=7) and the group with partial BOO (n=20). The sham-operated group comprised SD rats that underwent sham operation (no BOO was induced) and served as the control group. Moreover, the group with BOO comprised SD rats who underwent surgery to induce partial BOO.

For the induction of partial BOO, the SD rats in the group with BOO were anesthetized with ketamine (Ketamine 50[®]; Yuhan Corp., Korea; 75 mg kg⁻¹ intraperitoneally) and xylazine (Rompun[®]; Bayer Korea Ltd., Korea; 15 mg kg⁻¹ intraperitoneally). Through a lower midline incision, the bladder was approached and the proximal urethra exposed. A 3/0 Novafil (monofilament polybutester; Davis & Geck, Wayne, NJ, USA) ligature was placed around the urethra and then tied after a steel rod of 0.9 mm in diameter was intraluminally placed. After the knot was tied, the steel rod was removed. This was followed by the repositioning of the bladder and the closure of the abdominal wall.

To perform a cystometric analysis in the SD rats in both groups, a catheter was placed in the bladder for the measurement of intravesical pressure (IVP), followed by the recording of the intraabdominal pressure (IAP), three days prior to the cystometric analysis, as previously described (21,22). In further detail, a polyethylene catheter (PE-50; Becton Dickinson, Parsippany, NJ, USA) with a cuff was placed in the bladder dome through a lower abdominal incision. Moreover, an abdominal balloon (Latex; Dawoo Medical, Incheon, Korea) was placed around the cuff of the catheter and then displaced proximal to the bladder. This was followed by the connection to another catheter using a silk tie. These catheters were then tunneled subcutaneously and then anchored to the skin of the back with a silk ligature and their free end was sealed.

Cystometric analysis. The conscious rats were placed in a metabolic cage (Nalgene metabolic cage; Nalge Co., Rochester, NY, USA) without restraint. For cystometric analysis, the indwelling IVP catheter was attached to a two-way valve that was connected to a pressure transducer (Research Grade Blood Pressure Transducer; Harvard Apparatus, Holliston, MA, USA), as well as an infusion pump (PHD 22/2000 programmable syringe pump; Harvard Apparatus). Moreover,

Table I. Summary of cystometric parameters.

Group/subgroup	BP	TP	MP	BC	MV	RV	MI
Sham (n=6)	10.1±0.72	24.5±3.6	65.5±7.7	1.27±0.12	1.24±0.11	0.03±0.03	3.90±0.35
BOO (n=16)	15.4±1.9	44.0±3.2 ^b	88.1±9.5	2.26±0.24 ^a	1.46±0.17	0.80±0.28	6.88±0.76 ^a
CM (n=10)	17.0±2.5 ^b	40.3±4.5 ^a	103.4±12.9 ^a	1.91±0.22	1.81±0.20	0.10±0.04	5.81±0.67
DCM (n=6)	12.7±2.7	50.1±3.6 ^b	62.7±3.3 ^c	2.84±0.46 ^a	0.88±0.03 ^{b,e}	1.96±0.44 ^{b,d}	8.67±1.51 ^a

Values are expressed as the means ± SEM. ^aP<0.05 and ^bP<0.01 compared with the sham-operated group (unpaired Student's t-test). ^cP<0.05, ^dP<0.01 and ^eP<0.001 compared with CM (unpaired Student's t-test). BP, basal pressure (cmH₂O); TP, threshold pressure (cmH₂O); MP, maximal pressure (cmH₂O); BC, bladder capacity (ml); MV, micturition volume (ml); RV, residual volume (ml); MI, micturition interval (min); BOO, group with bladder outlet obstruction; CM, subgroup with bladder compensation; DCM, subgroup with bladder decompenation.

it was also directly connected to another pressure transducer. Thus, the IVP was recorded synchronously with the micturition volume (MV) using a fluid collector connected to a force displacement transducer (Research Isometric Transducer; Harvard Apparatus). This was followed by the infusion of saline into the bladder at a rate of 20 ml/h at room temperature in both groups. Subsequently, data recording and analysis were performed using Acq Knowledge 3.8.1 software (Biopac Systems Inc., Goleta, CA, USA) at a sampling rate of 100 Hz and an MP150 data acquisition system (Biopac Systems Inc.).

In the current experimental study, we measured cystometric parameters, such as the degree of DO and MP from three reproducible micturition cycles and then averaged the results for a comparison between the two groups.

Subgroup analysis. Based on a cut-off value of a mean RV% of 25%, we subdivided our experimental animals into two subgroups: the subgroup with bladder compensation (mean RV%, <25%) and the subgroup with bladder decompenation (mean RV%, >25%). Thus, we attempted to examine whether detrusor contractility is associated with the compensation or decompenation of bladder function depending on the RV during the first two weeks after the onset of BOO.

Protein assay by western blot analysis. In the current study, we assayed the levels of signaling proteins, such as AMPK, ERK1/2 and PKC using the BCA protein assay kit (Pierce, Rockford, IL, USA). The signaling proteins were resolved by 8-12% SDS-PAGE and then transferred onto PVDF membranes (Millipore, Milford, MA, USA) and their activity was blocked in TBS-T buffer (40 mM Tris-HCl pH 7.4, 25 mM NaCl, 0.1% Tween-20) containing 5% skim milk. This was followed by the incubation of the membranes with primary antibodies (Cell Signaling Technology, Danvers, MA, USA) against the signaling proteins and their respective phosphorylated forms. Following incubation with the primary antibodies, the PVDF membranes were incubated with goat anti-rabbit-HRP conjugated secondary antibodies (Santa Cruz, CA, USA). This was followed by the visualization of the specific protein bands using ECL reagent (Pierce). The degree of the alteration in the phosphorylation of each signaling protein was expressed as the ratio of the level of the phosphorylated form to that of the non-phosphorylated one.

Statistical analysis. All data are expressed as the means ± standard errors of the mean (SEM). We performed a simple regression analysis using Pearson's correlation coefficient to identify the correlations between the frequency and degree of DO and MP. Moreover, we also performed the Shapiro-Wilk W test to examine whether the urodynamic parameters followed the normal distribution. Statistical significance was analyzed using an unpaired Student's t-test or one-way analysis of variance (ANOVA), accompanied by Tukey's post-hoc test for multiple comparisons. Statistical analysis was performed using GraphPad Prism software version 5.03, 2009 (Graph Pad Software, San Diego, CA, USA). A P-value <0.05 was considered to indicate a statistically significant difference.

Results

General characteristics. In the current study, there were 10 SD rats (n=10) (62.5%) in the subgroup with bladder compensation and 6 SD rats (n=6) (37.5%) in the subgroup with bladder decompenation (Fig. 1).

Moreover, the degree of compliance was significantly lower in the group with BOO compared with the sham-operated group (0.38±0.01 ml/cmH₂O vs. 9.16±0.83 ml/cmH₂O) (P<0.0001). However, there was no significant difference in the degree of compliance between the subgroup with bladder decompenation and the subgroup with bladder compensation (0.39±0.02 ml/cmH₂O vs. 0.38±0.02 ml/cmH₂O) (P>0.05).

Changes in cystometric parameters. As compared with the sham-operated group, in the group with BOO, we observed a significant increase in threshold pressure (TP) (P<0.01), bladder capacity (BC) (P<0.05) and micturition interval (MI) (P<0.05). As compared with the subgroup with bladder compensation, the subgroup with bladder decompenation showed a significant decrease in MP (P<0.05) and MV (P<0.001) and a significant increase in RV (P<0.01). However, there were no significant differences in other cystometric parameters, including BP, TP, BC and MI between the subgroup with bladder compensation and the subgroup with bladder decompenation (Table I).

No DO occurred during the filling phase in the sham-operated group. However, DO occurred in 87.5% (14/16) of the SD rats in the group with BOO. In the group with BOO, the mean frequency and degree of DO were 1.42±0.27 min⁻¹

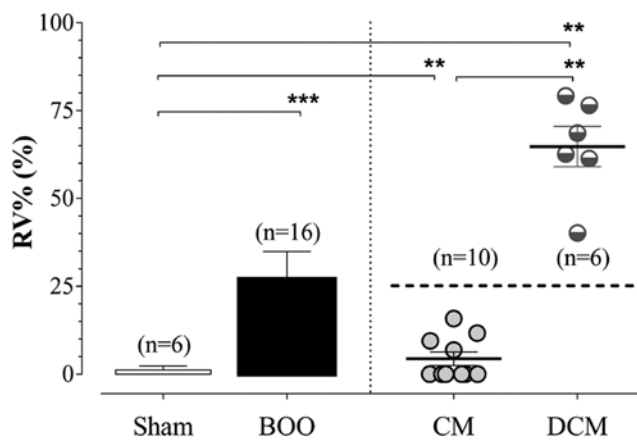


Figure 1. Analysis of the two subgroups: the subgroup with bladder compensation and the subgroup with bladder decompensation. Each bar represents the mean \pm SEM. The two scatter dot plots on the right indicate the subgroups with bladder compensation (CM; $n=10$) and decompensation (DCM; $n=6$), respectively. The horizontal dashed line indicates a cut-off value of a mean RV% of 25%. Sham, sham-operated group; BOO, group with bladder outlet obstruction.

and 6.75 ± 1.39 cm H₂O, respectively. In addition, the frequency of DO was significantly lower in the subgroup with bladder decompensation as compared with the subgroup with bladder compensation. There was no significant difference observed in the degree of DO between the subgroup with bladder decompensation and the subgroup with bladder compensation (Fig. 2).

Univariate analysis revealed a significant positive correlation between the frequency and degree of DO ($r=0.73$, $P<0.05$) and MP ($r=0.64$, $P<0.05$) and a significant negative correlation between the frequency of DO and RV% ($r=0.50$, $P<0.05$). However, a significant negative correlation was observed between MP and RV% in the subgroup with bladder decompensation ($r=0.88$, $P<0.01$), which was not observed in the subgroup with bladder compensation (Fig. 3).

Degree of expression and phosphorylation of signaling proteins. As shown by western blot analysis, there were significant differences in the degree of expression and phosphorylation of signaling proteins between the subgroup with decompensation and the subgroup with compensation (Fig. 4). That is, the degree of phosphorylation of AMPK and ERK1/2 was significantly lower in the subgroup with bladder compensation as compared with the sham-operated group and the subgroup with bladder decompensation. However, there was no significant difference between the subgroup with bladder decompensation and the sham-operated group. Furthermore, the degree of ERK1/2 expression was significantly higher in the subgroup with bladder compensation.

The expression of PKC was decreased in both the subgroup with bladder compensation and the subgroup with bladder decompensation, significantly lower as compared with the sham-operated group. Moreover, there was no significant difference observed in the degree of phosphorylation of PKC between the subgroup with bladder compensation and the subgroup with bladder decompensation (Fig. 4). These find-

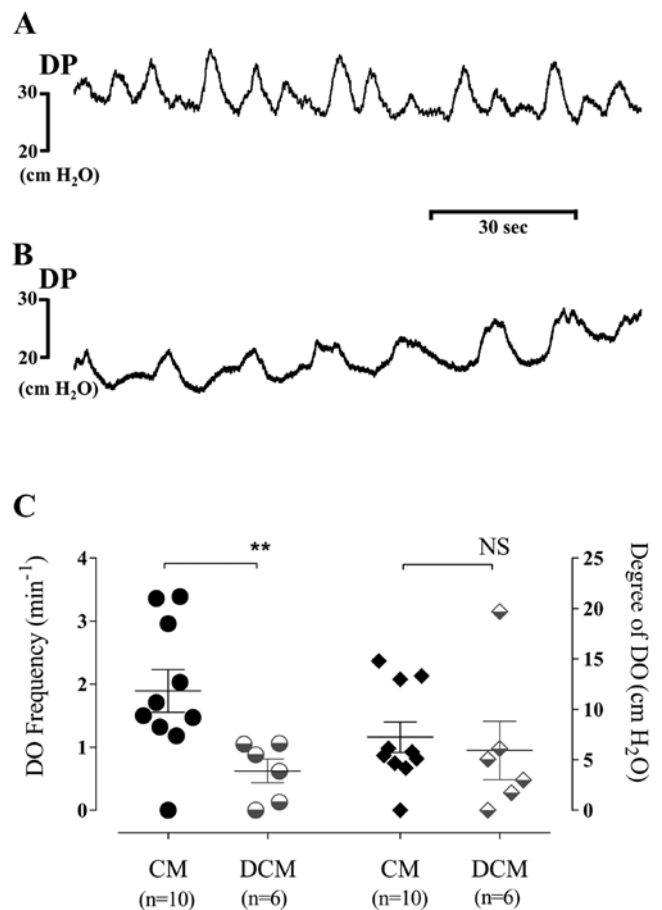


Figure 2. Parameters associated with detrusor overactivity (DO). (A and B) Representative tracings and (C) mean values of the frequency (left two columns) and degree of DO (right two columns) in (A) the subgroup with bladder compensation (CM) and (B) the subgroup with bladder decompensation (DCM). NS, not significant.

ings indicate that PKC is not involved in two-phase bladder contraction.

Discussion

The decompensation of the bladder in partial BOO is postulated to be a process of progressive deterioration in contractility and voiding function of the bladder smooth muscles (8,23). However, its definition has not been fully established in a clinical and experimental setting. Levin *et al* defined the decompensation of the bladder as the condition where the mean bladder contractility was smaller by 80% as compared with normal controls when there were contractile responses of the isolated bladder strips to stimulation in an *in vitro* setting (24). However, this does not apply to our animal model. Patients are clinically diagnosed with voiding problems based on a RV of >100 ml when the normal bladder capacity is 400 ml (25). We therefore divided our experimental animals based on a cut-off value of a mean RV of 25%. Thus, our study differs from previous reports.

Presumably, the initial contractile response of the urinary bladder determining the MP of the bladder may be associated with the intracellular concentration of ATP. It is also hypothesized that the subsequent tonic phase may be associated with

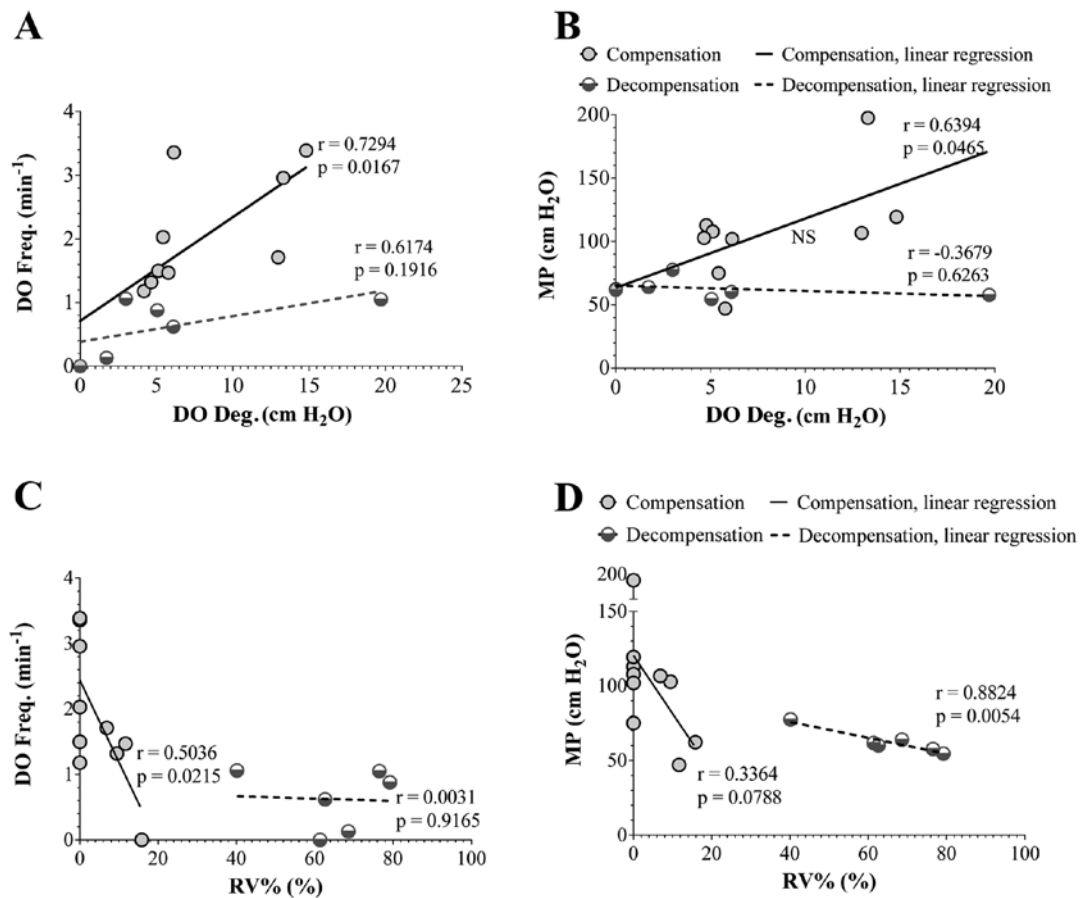


Figure 3. Correlation between (A) the degree (Deg.) and frequency (Freq.) of detrusor overactivity (DO) and (B) maximal pressure (MP) and correlations between the (C) residual volume percentage (RV%) and the frequency of DO and (D) maximal pressure (MP).

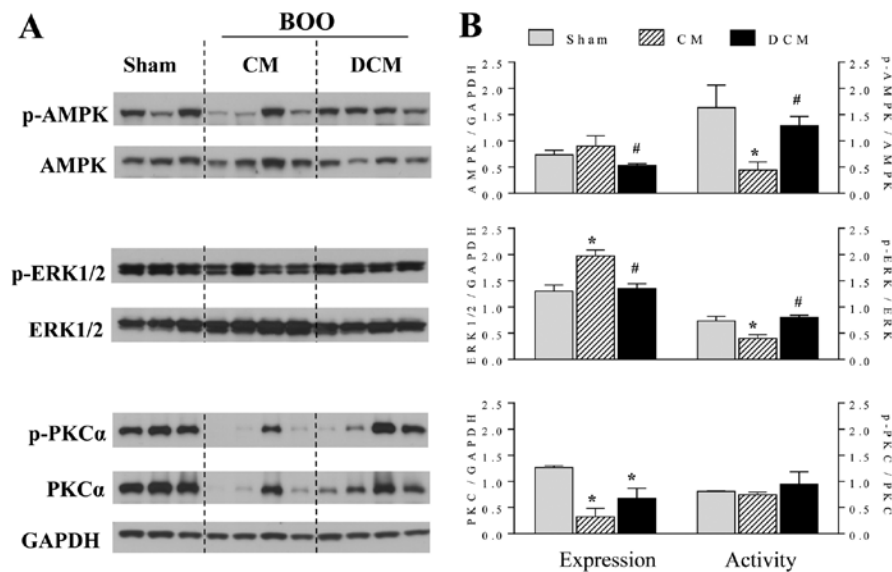


Figure 4. Western blot analysis. (A) Representative blots of protein bands using western blot analysis. (B) The degree of the expression and phosphorylation of signaling proteins. The vertical bars represent the means \pm SEM. BOO, group with bladder outlet obstruction; CM, the subgroup with bladder compensation; DCM, the subgroup with bladder decompenation. *P<0.05 compared with the sham-operated group. #P<0.05 compared with CM.

mitochondrial respiration. Partial BOO induces a marked decrease in the tissue ATP content, oxidative glucose metabolism and in the activity of mitochondrial enzymes (26). The

sustained tonic component is highly dependent on the deprivation of glucose and oxygen as compared with the peak pressure response. This explains the mechanisms through which the

voiding difficulty occurs, despite a lack of decreased DO during the early phase of decompensation (24).

AMPK is a phylogenetically conserved serine-threonine kinase that is involved in the regulation of diverse cellular pathways through which the cellular energy is consumed and chronic muscular pathology due to energy deprivation occurs (13). Previous studies have suggested that not only is AMPK activated when the cellular AMP-to-ATP ratio is increased (14), indicating the state of intracellular energy deprivation, but also that it is produced in the presence of metabolic stress involved in reducing ATP synthesis or increasing ATP consumption. There is no direct evidence indicating that AMPK is involved in the decompensation of bladder function in partial BOO. According to certain studies however, the ATP consumption is reduced in BOO and this is accompanied by an increase in AMP synthesis and a decrease in ATP synthesis (9,26). In the current study, the degree of AMPK phosphorylation was significantly lower in the compensated bladder, accompanied by the appropriate level of the contractility of bladder smooth muscles, which indicates that there was a decreased need for the production of extra ATP. Furthermore, the degree of AMPK expression was restored to the normal level in the decompensated bladder, accompanied by decreased bladder contractility. This suggests that there would be a greater need for the production of ATP for efficient voiding in the decompensated bladder as compared with the compensated one. However, this was notably observed at two weeks after the onset of BOO. Moreover, further studies are warranted to examine the changes in the degree of AMPK expression that occur during the acute period or after longer periods of time. It has been reported that ERK1/2 MAP kinase is involved in the *in vitro* generation of the force of smooth muscles in such conditions that cytosolic Ca^{2+} levels are decreased to a near resting level. However, little is known about the *in vivo* involvement of ERK1/2 MAP kinase in the function of smooth muscles (16).

PKC isoforms play a key role in the regulation of cell growth through several intracellular signaling pathways (9,20). Chang *et al* demonstrated that the degree of PKC α activity is lower in the decompensated bladder as compared with the compensated one (27). This is not in agreement with our results. Presumably, this may be due to the difference in criteria for differentiating between compensation and decompensation. Chang *et al* defined the decompensation of the bladder solely based on the daily voiding frequency and the volume of voiding without considering the residual urine at each cycle (27). In the current experimental study however, we analyzed the cystometric parameters after the removal of residual urine at the end of each cycle of awake cystometry. This may be noteworthy in that rigorous criteria for differentiating between the compensation and decompensation of the bladder should be applied. The degree of PKC expression was decreased in both subgroups (with bladder compensation and decompensation). This indicates that PKC may be involved in the dysfunctional signal transduction pathways through which the downstream targets of contraction (e.g., Ca^{2+} sensitization) are regulated. However, this warrants further investigation.

In conclusion, our results indicate that the degree of DO is associated with the compensation or decompensation of bladder function, depending on the RV during the first two weeks after the onset of BOO in an animal experimental model of partial BOO. Moreover, it can also be concluded that AMPK

and ERK1/2 are involved in the compensation or decompensation of bladder function. Our results also suggest however, that PKC is not involved in two-phase bladder contraction. Finally, alterations in the activity of signaling proteins, such as AMPK and ERK1/2 may prove to be helpful in the treatment of patients with voiding difficulty.

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