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Research Article

Atpase Activity of Rabbit Bladder Muscle and Mucosa: Effect of EDTA

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Abstract

Introduction

The urinary bladder like all smooth muscles depends on adenosine triphosphate (ATP) for energy for all biochemical processes including contraction, neurohumeral transmission, secretion, and calcium translocation. ATPase is the enzyme that breaks down ATP to ADP and Pi with the release of energy utilized for the biochemical processes just mentioned. Studies have demonstrated that calcium storage and release from the sarcoplasmic reticulum play an important role in the contractile response of the rabbit urinary bladder to both field stimulation (mediated via neurotransmitter release) and carbachol (direct muscarinic stimulation). In view of the importance of ATPase and calcium in urinary bladder smooth muscle function, we studied the effect of EDTA (divalent ion chelator) on ATPase activity in the bladder smooth muscle and mucosa.

Methods

Eight mature male NZW rabbits were used for these studies. Each rabbit was anesthesized with isoflurane. Under sterile conditions, the urinary bladder was emptied, freed from fat and connective tissue excised and weighed. The bladder body was separated from the bladder base-urethra at the level of the ureteral orifices. The bladder body was opened longitudinally and separated by blunt dissection into muscle and mucosal compartments and each compartment frozen in liquid Nitrogen and stored at -800C for biochemical evaluation. Microsomal preparations were prepared from muscle and mucosal samples and utilized in our studies on ATPase activity in the presence and absence of EDTA.

Results

1) The bladder smooth muscle has a significantly higher concentration of calcijm – magnesium specific ATPase activity than the mucosa, whereas the mucosa has a higher concentration of sodium-potassium ATPase activity than the smooth muscle. 2) The maximal ATPase activity of the muscle was significantly higher than the activity of the mucosa. 3) The ATPase activities of both muscle and mucosa were linear with time over the first 5 minutes, and then the curve progressively decreases up to the 20 minute time period.4) EDTA at both 6 and 12 mM chelate both the calcium and magnesium in the assay resulting in a significant and similar decreases in ATPase activity. Under both concentrations of EDTA the mucosa still has significantly lower activity than the smooth muscle.

Keywords: ATP (adenosine triphosphate); ATPase; Calcium; Magnesium; EDTA; Rabbit

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Introduction

The urinary bladder is a smooth muscle organ composed of primarily two components: The smooth muscle that expands to store urine at low intravesical pressure, and then expels urine through a coordinated and sustained contraction; and the mucosa (internal lining) which protects the smooth muscle from both infection and penetration of caustic substances within the urine [1-5]. On an intracellular basis, detrusor contraction depends upon the interaction of the contractile proteins actin and myosin which in turn depends upon a phasic increase in intracellular free calcium translocated both from intracellular storage sites in the sarcoplasmic reticulum (SR) and from the extracellular

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space through the cell wall [6-9].In regard to bladder contraction, although there is aninitial phasic response to stimulation, bladder emptying requires a sustained increase in intravesical pressure during urine expulsion [10-12]. Prior studies from our laboratory and others demonstrated that the contractile response of the bladder to neuronal and muscarinic stimulation depends upon a rise in intracellular free calcium, resulting from both calcium influx from extracellular spaces and calcium release from intracellular stores (calcium-induced calcium release) [13-15]. The participation of intracellular calcium in the response to stimulation involves thapsigargin-sensitive calcium release from the sarcoplasmic reticulum (SR) and calcium uptake into the SR by SR calcium activated ATPase (SERCA). Thapsigargin, a specific inhibitor of SERCA, has marked effects on the contractile response of the bladder to stimulation [9, 16-20]. The other important enzyme required for contraction is ATPase. This enzyme breaks down ATP to ADP + Pi with the release of energy. This energy is then used by the smooth muscle cell to support the interaction of actin and myosin resulting in contraction [21-24]. Other functions of ATPase include neurotransmitter release, and calcium translocation. In the mucosa, the release of energy by ATPase is used to support cellular hyperplasia and hypertrophy, and secretion [21-24].

Methods

Animals

All studies were approved by the IACUC and R&D Committees of the Stratton VA Medical Center.

The New Zealand White rabbit (NZW) is an excellent model for the study of bladder function and dysfunction[25, 26]. The rabbit bladder is of sufficient size to allow easy urodynamic evaluation and its response to FS and pharmacologic agents is consistent from animal to animal. In addition, rabbits can be housed in sufficient numbers to perform statistically significant experiments.

Eight mature male NZW rabbits were used for these studies. Each rabbit was sedated with ketamine/xylazine (25 mg/10 mg, im) with surgical anesthesia being maintained with isoflurane (1-3%). Under sterile conditions, the urinary bladder was emptied, freed from fat and connective tissue excised and weighed. The bladder body was separated from the bladder base-urethra at the level of the ureteral orifices. The bladder body was opened longitudinally and separated by blunt dissection into muscle and mucosal compartments and each compartment frozen in liquid Nitrogen and stored at -800C for biochemical evaluation.

Tissue Preparation

Frozen bladder muscle and mucosa tissue were weighed and thawed in 50 mM Tris (pH 7.4) on ice and homogenized for 10 s with a Polytron at 20 mg/ml. The homogenate was filtered through gauze, centrifuged at 2,000 g for 25 min to obtain cell/nuclear membranes which were discarded. Next, the supernatant was centrifuged at 13,300 g for 30 min to remove mitochondria. Microsomes containing the sarcoplasmic reticulum and synaptic membranes were pelleted by centrifugation at 105,000 g for 60 min, re-suspended at the original volume in homogenization buffer. This suspension was utilized for ATPase assays.

Protein concentrations for this fraction was quantitated using the Micro BCA Protein Assay Kit (Thermo Scientific, Rockford, IL USA).

ATPase Assay

Aliquots of this particulate fraction were incubated at 37o C as follows: 50 mM Tris, 2mM $CaCl_2$, 2 mM $MgCl_2$,0 - 12mM EDTA, 1 mM ATP and 375 µl tissue extract (pH 7.4; total volume 0.5 ml). At the end of the 0 -20 minute incubation, the reaction was stopped by adding 0.5 ml 12.5% trichloroacetic acid. The reaction tubes were centrifuged at 1,000 g for 5 min to remove protein precipitates. Inorganic phosphate was determined spectrophotometrically at 650 nm by adding 0.5 ml of 0.18 M ferrous sulfate dissolved in 1% ammonium molybdate. Results were expressed as nano-moles phosphate per mg protein per minute. For statistical comparison, the data was normalized to 0 minutes = 100.

Results

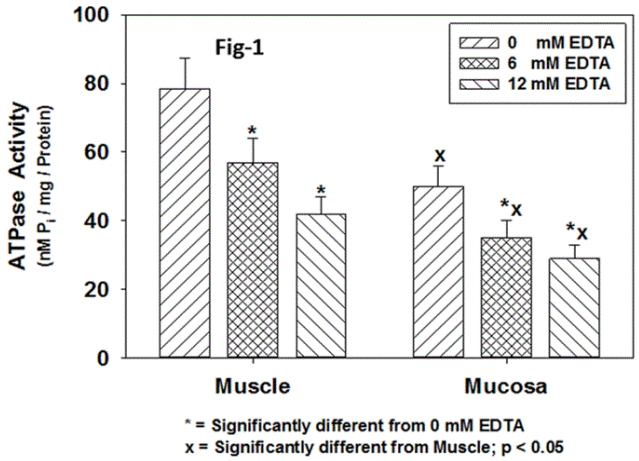

Figure 1. Maximum ATPase activity for bladder body at 0 minutes for 0 mMEDTA and for 12 mM EDTA. Each bar is the mean +/- SEM of 8 individual preparations. * = Significantly different from 0 mM EDTA; X = Significantly different from Muscle; $p < 0.05$.

Figure 1 shows the maximum ATPase activity for bladder body at 0 minutes for 0 EDTA and for 12 mM EDTA. The activity of the bladder smooth muscle was significantly higher than the mucosa.

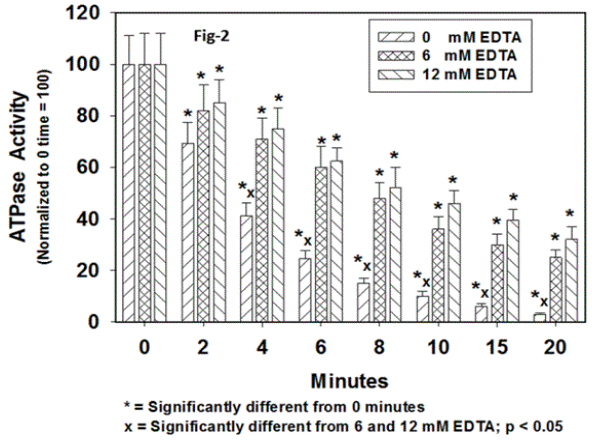

Figure 2. ATPase activity as a function of time for bladder body smooth muscle for 0, 6, and 12 mMEDTA.Each bar is the mean +/- SEM of 8 individual preparations. * = Significantly different from 0 mM EDTA; X = Significantly different from 6 and 12 mM EDTA; $p < 0.05$.

Figure 2 shows the ATPase activity as a function of time for bladder body smooth muscle for 0, 6, and 12 mM EDTA. It is clearly demonstrated that the enzyme activity at all times (0-20 Minutes) is highest for the 0 EDTA and significantly lower for both 6 and 12 mM EDTA. ATPase activity progressively falls toward 0 by 20 minutes for all concentrations of EDTA.

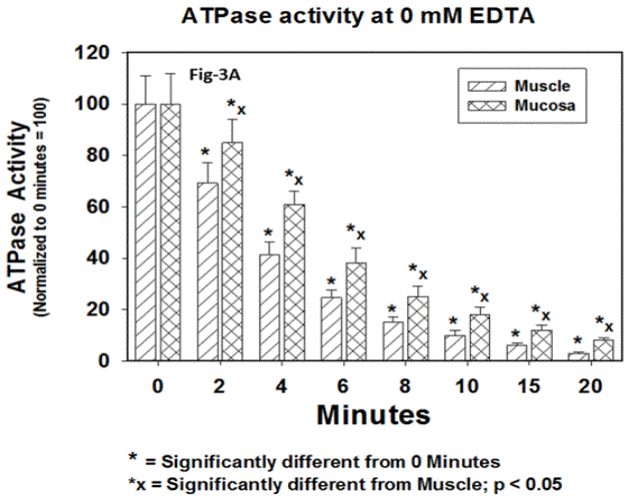

Figure 3A. Comparison of muscle and mucosa at 0 mMEDTA.Each bar is the mean +/- SEM of 8 individual preparations. * = Significantly different from 0 Minutes; X = Significantly different from Muscle; $p < 0.05$.

Figure 3A shows the comparison of muscle and mucosa at 0 mM EDTA. At all times except for 0 minutes, the ATPase activity for the mucosa was significantly lower for the mucosa than for the muscle. It should be noted that for comparative purposes, both curves are normalized to 100 at the 0 time period. Without normalization, the ATPase activity for the mucosa was also significantly lower than the activity for the muscle.

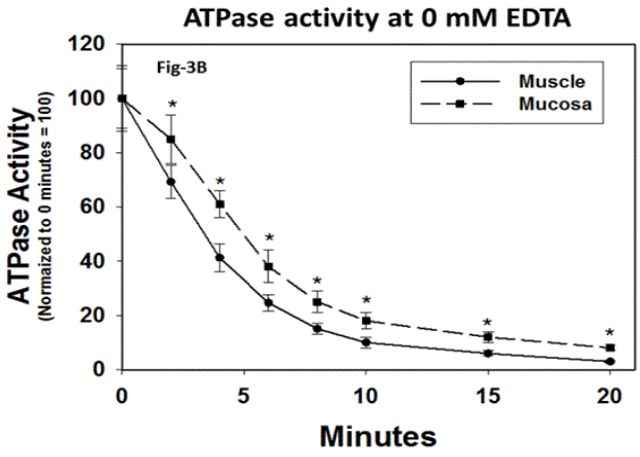

Figure 3B. Comparison of muscle and mucosa at 0 mM EDTA as a linear function of time.* = Significantly different from Mucosa; $p < 0.05$.

Figure 3B shows the comparison of muscle and mucosa at 0 mM EDTA as a linear function of time. Both curves are linear for 5 minutes, and then the rate of ATPase activity decreases progressively for the last 15 minutes.

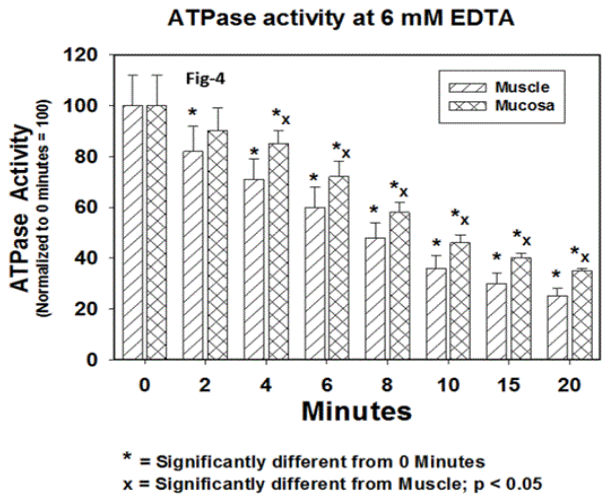

Figure 4. Comparison of muscle and mucosa at 6 mMEDTA.* = Significantly different from 0 Minutes; X = Significantly different from Muscle; $p < 0.05$.

Figure 4 shows the comparison of muscle and mucosa at 6 mM EDTA. At all times except for 0 minutes, the ATPase activity for the mucosa was significantly lower for the mucosa than for the muscle.

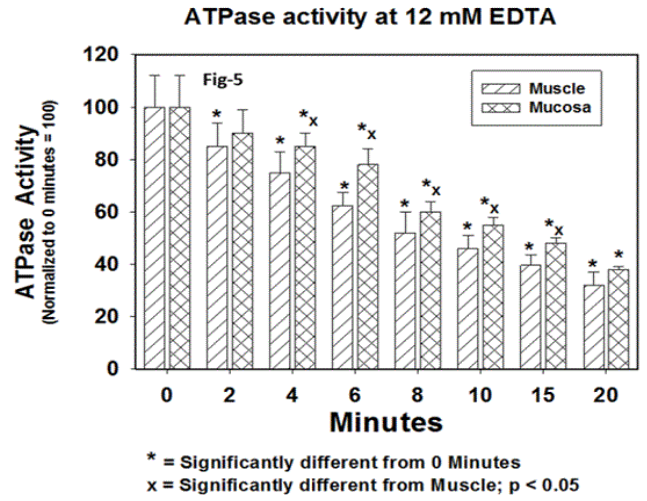

Figure 5. Comparison of muscle and mucosa at 12 mM EDTA. * = Significantly different from 0 Minutes; X = Significantly different from Muscle; $p < 0.05$.

Figure 5 shows the comparison of muscle and mucosa at 12 mM EDTA. At all times except for 0 minutes, the ATPase activity for the mucosa was significantly lower for the mucosa than for the muscle. The activities of the muscle and mucosa at 12 mM EDTA were not significantly different from that shown at 6 mM EDTA.

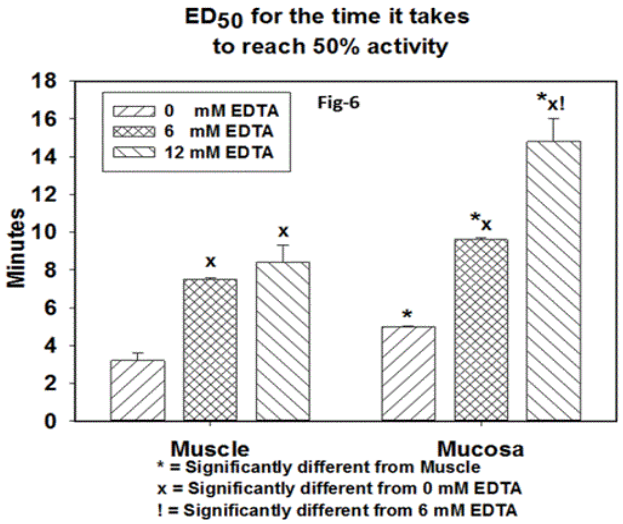

Figure 6. ED_{50} (Time it takes to reach 50% activity) for the muscle and mucosa at 0, 6, and 12 mM EDTA. * = Significantly different from Muscle; X = Significantly different from 0 Minutes; 1 = Significantly different from 6 mM EDTA; $p < 0.05$.

Figure 6 shows the ED50 (Time it takes to reach 50% activity) for the muscle and mucosa at 0, 6, and 12 mM EDTA. For all concentrations of EDTA the mucosa had a significantly higher ED50 than the muscle, and for both tissues, the ED50 increased with increasing concentrations of EDTA, especially for the mucosa.

Discussion

The urinary bladder can be divided into two components, the mucosal epithelium (urothelium) and the underlying detrusor smooth muscle. The urothelium provides a protective non-adherent surface that protects the bladder from bacterial adhesion to the bladder wall and acts as a permeability barrier against the penetration of caustic so1utes in the urine [1,27,28], thus protecting the smooth muscle elements from damage [4,29,30]. The symptoms of several urological disorders (interstitial cystitis, recurrent infections, incontinence) have been ascribed to defects in mucosal function, and may be related directly to damage to the anti-adherent properties of the urothelium and its function as a permeability barrier [29-32].

Micturition is mediated through the coordinated contraction of bladder smooth muscle [1,2]. Bladder dysfunction secondary to benign prostatic hyperplasia (BPH) has-been ascribed to progressive changes in innervation and smooth muscle contraction [28,33,34]. In animal models of BPH (partial outlet obstruction), the associated bladder contractile dysfunctionis mediated by three processes: (1) progressive denervation, (2) mitochondrial damage and the associated decreased ability to generate ATP, and (3) progressive reduction [28,34]. in the ability of the sarcoplasmic reticulum to store and release calcium [35,36]. Studies indicate that disorders of mucosal function (*i.e.*, interstitial cystitis and recurrent infection) and smooth muscle function (i.e., bladder contractile failure secondary to BPH and hyperreflexia) are related to cyclical ischemia and reperfusion injury [37-39].

Ischemia and reperfusion activate calcium-dependent degradative enzymes such as calpain and phospholipase A2 that cause intracellular damage through hydrolysis of neuronal, cellular, and subcellular membranes [40, 41]. In a previous study, we characterized the free fatty acid (FFA) and phospholipid (PL) content of normal rabbit bladder muscle and mucosa, and characterized the endogenous lipase activity. The results demonstrated that (1) The basal FFA concentration of the mucosal homogenates was 5 times that of the muscle homogenates. (2) The basal PL concentrations of the two tissues were similar. (3) Sub cellular studies: FFA concentration was greatest in the mitochondrial fraction of both compartments. In the mucosa, PL concentration was significantly greater in the mitochondria and microsomes than in the other fractions; in the smooth muscle, the PL concentration was highest in the mitochondria. (4) The maximal endogenous lipase activity was 10 times higher in the mucosal homogenates than in the muscle homogenates.

These results are consistent with those of previous studies which indicate that the mucosa is metabolically more active than the resting smooth muscle, which may cause the mucosa to be significantly more sensitive than the muscle to hypoxic/ischemic damage.

Biochemically, both the urothelium and smooth muscle compartments are totally dependent upon ATP as the metabolic energy source to regulate virtually all functions of the bladder including, contraction, divalent ion transport to the SR, divalent ion transport out of the cell, secretion, urothelial hyperplasia and hypertrophy [42, 43]. ATPase is the enzyme responsible for the hydrolysis of ATP to ADP and Pi with the release of metabolic energy[21, 22, 44].

According to Wilkopedia,ATPase are a class of enzymes that catalyze the hydrolysis of ATP into ADP and Pi. This de-phosphorylation reaction releases energy, which the is then utilized to drive other chemical reactions that would not otherwise occur. This process is widely used in all known forms of life. Some such enzymes are proteins and others are soluble enzymes within the cytosol.

Two major functions of intracellular ATPase are to transport Ca^{2+} into the sarcoplasmic reticulum via SERCA (sarco-endoplasmic reticular calcium ATPase); and to transport Ca^{2+} out of the cell, through the plasma membrane (via Ca^{2+} - Mg^{2+} ATPase) [45-47]. Other forms of ATPase require Na and / or K for activity [21-24]. The current study evaluated the effect of EDTA on ATPase activity of the normal bladder muscle and mucosa. EDTA is a divalent ion chelator; thus in our system both 6 and 12 would be expected to completely inhibit divalent ion-dependent ATPase activity, without affecting other forms of ATPase [48, 49].

The current study characterized the effect of EDTA, a strong divalent ion chelator, on the microsome ATPase activities of the normal rabbit bladder muscle and mucosa. The results clearly demonstrated: 1) the maximal ATPase activity of the muscle was significantly higher than the activity of the mucosa. This is because ATP hydrolysis is required for neurotransmission, contraction, and calcium translocation into the SR and out of the cell through the cell membrane.2)The ATPase activities of both muscle and mucosa are linear with time over the first 5 minutes, and then the curve progressively decreases up to the 20 minute time period.

Bladder divalent ion Km is approximately 0.26mM [50], thus at 1 mM ATP concentration in the assay, the decrease in activity shown after 5 minutes was not due to decreased substrate, but due to the significantly reduced activity at the 5 minute mark.3)EDTA at both 6 and 12 mM chelate both the calcium and magnesium in the assay resulting in a significant and similar decrease in ATPase activity. Under both concentrations of EDTA the mucosa still has significantly lower activity than the smooth muscle. 4) At all concentrations of EDTA the mucosa had a significantly higher ED50 than the muscle, and for both tissues, the ED_{50} increased with increasing concentrations of EDTA, especially for the mucosa.

Conclusions

The maximal ATPase activity of the muscle was significantly higher than the activity of the mucosa. The ATPase activities of both muscle and mucosa are linear with time over the first 5 minutes. Bladder divalent ion Km is approximately 0.26 Mm. At both concentrations of EDTA the mucosa has significantly lower activity than smooth muscle. ED50 for both tissues increased with increasing concentrations of EDTA.

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