Partial Outlet Obstruction in Rabbits: Duration Vs Severity: A Review

Robert M Levin, Catherine Schuler, Connor Callaghan and Robert E. Leggett

Author details:

Robert M Levin
Senior Research Career Scientist (Stratton VA Medical Center)
Professor Emeritus (Albany College of Pharmacy and Health Sciences; Stratton VA Medical Center), United States
Email: robert.levin2@va.gov

Catherine Schuler
Research Associate
Stratton VA Medical Center
United States
Email:catherine.schuler@va.gov

Connor Callaghan
Student Research Assistant
Albany College of Pharmacy and Health Sciences
United States
Email:Connor.Callaghan@acphs.edu

Robert E. Leggett
Research Associate
Stratton VA Medical Center
United States
Email:robert.leggett@va.gov

Abstract:

Background: Obstructive bladder dysfunction (OBD) is a common medical problem. More than 80% of males older than 50 years of age have varying degrees of OBD secondary to benign prostatic hyperplasia.

Methods: For the studies presented in this review article, we utilized a rabbit model of partial outlet obstruction in which we surgically place a silk ligature loosely around the catheterized urethra of a male rabbit for various periods of time.

Results: Our studies to date have demonstrated that OBD is initiated by four specific pathological processes: **1)** Selective postsynaptic denervation and defective neuro-humoral transmission; **2)** mitochondrial damage and intracellular metabolic dysfunction; **3)** sarcoplasmic reticulum (SR) damage; and **4)** calcium dysregulation resulting in an increase in basal intracellular free calcium and the activation of specific calcium-activated proteases and lipases.

Conclusions: From the studies described in this review, we have developed the following hypotheses concerning the etiology of OBD in both our rabbit model of OBD and OBD in men: **(A)** The shift from compensated bladder function to mild decompensation is primarily due to intracellular calcium overload of the smooth muscle cells and the activation of proteolytic and lipolytic enzymes. **(B)** The shift from mild obstructive bladder dysfunction through severe dysfunction is primarily due to oxidative stress and the generation of oxidative free radicals resulting in oxidative damage to the same cellular and subcellular membrane systems as described above.

1) Introduction:

The function of the urinary bladder is to collect and store urine at low intravesical pressure and then, periodically, to expel the urine via a highly coordinated and sustained contraction [1, 2]. Bladder function depends upon several factors including state of innervation, vascularization, structure of the organ as a whole, contractile response of the smooth muscle (SM) elements to autonomic stimulation, availability of metabolic energy (cytosolic adenosine triphosphate [ATP] and mitochondrial oxidative phosphorylation) and the density and distribution of connective tissue in the detrusor. These factors are intimately connected, and an alteration in one factor can induce substantial adaptive changes in the others [1, 2].

Obstructive bladder dysfunction (OBD) is a common medical problem. More than 80% of males older than 50 years of age have varying degrees of OBD secondary to benign prostatic hyperplasia [3].

2) Methods:

All methods utilized in these experiments were approved by the IACUC and R&D Committees of the Stratton VA Medical Center. New Zealand White rabbits were divided into 4 equal groups. Groups 1-3 received partial outlet obstructions by surgically placing a silk ligature loosely around the catheterized urethra of an anesthetized rabbit. The rabbits from each group were allowed to recover for variable periods of time. The rabbits in group 4 received sham surgery. At the end of each time period, the rabbits were anesthetized with isoflorane, the bladders excised, weighed, and three isolated full thickness strips were taken for contractility studies. The balance of the bladders were separated by blunt dissection into bladder smooth muscle and mucosa, frozen in liquid nitrogen and stored at -80^{o}C for a variety of biochemical tests.

In regard to the contractility studies [4], each isolated strip was mounted in individual 15 ml baths containing warmed oxygenated Tyrodes solution and allowed to equilibrate for 30 minutes. A passive length-tension curve, which is a measure of the compliance of the strip (mg of tension per mm of length), was generated and contractile responses (maximal tension generation [mg tension/cross sectional area] and maximal rate of tension generation [mg tension generated/sec]) to field stimulation (FS) (32 Hz) were used to determine the passive length that allows for maximal active tension generation. At this length, the responses to FS at 2, 8, and 32 Hz, ATP (1mM), carbachol (20 μM), potassium chloride (KCl) (120 mM) were determined. Each strip was washed three times at 15 minute intervals with fresh warmed oxygenated buffer between pharmacological additions.

3) Results:

3.1) Stages of rabbit bladder response to obstruction [5, 6]:

The progressive response to partial bladder outlet obstruction (PBOO) in rabbits can be divided into 3 phases:

A) An **initial response** to surgical induction of PBOO lasting 1-14 days and characterized by bladder distension followed by a progressive increase in mass to a stable level. There is no physiological correlate to this initial response, and the studies proposed are not involved in the study of this time period.

B) Compensated bladder function immediately follows the "initial phase" and lasts a variable length of time. This period is characterized by relatively stable bladder mass and normal or even increased contractile responses to FS, carbachol and KCl stimulation. Increased intracellular

free calcium concentration parallels the increased contractile responses [7, 8].

C) Decompensated bladder function:

At some point, the functional ability of the bladder to contract and empty begins to degenerate, and the organ becomes "decompensated." Decompensation is a process characterized by progressive deterioration in contractility and function (i.e., ability to generate pressure and empty), a further increase in bladder mass and a progressive decrease in the volume- fraction of smooth muscle (SM) elements in the bladder wall. The end result is either an organ with a thick fibrous wall, low capacity, poor compliance, and little or no contractile function; or a dilated bladder with a thin fibrous wall, high capacity and little or no contractile function.

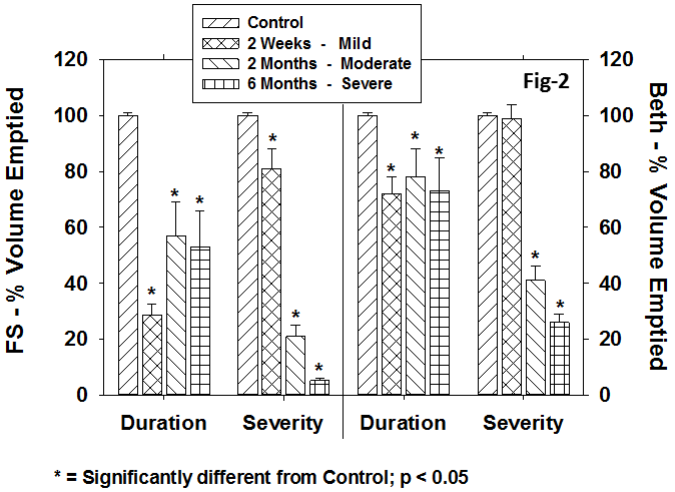
3.2) Compensation versus decompensation:

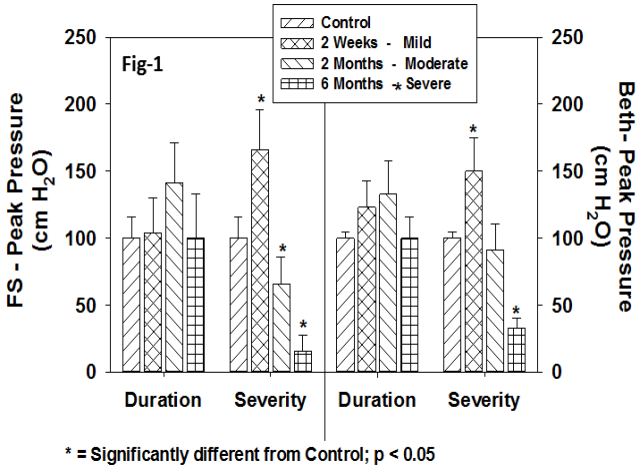
Results of experiments in which mild partial outlet obstruction was studied longitudinally (up to 6 months) showed that the level of bladder decompensation was related to both the magnitude of the increase in bladder mass and the level of contractile dysfunction but not directly to the duration of obstruction.

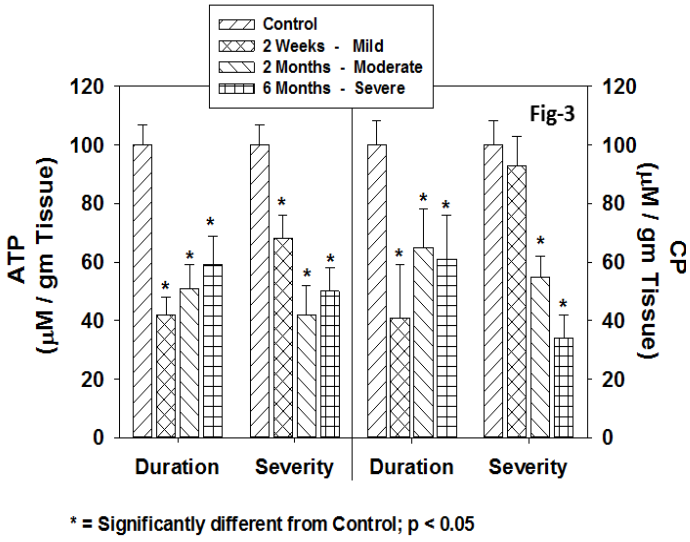
These first studies utilized an in- vitro whole bladder model [9, 10] to evaluate bladder contraction by measuring both the increase in bladder pressure to stimulation by field stimulation FS and bethanechol (Beth) and the percent of bladder volume emptied. Following contractile studies we evaluated the effect of PBOO on the concentration of the high energy phosphates adenosine triphosphate (ATP) and creatine phosphate (CP).

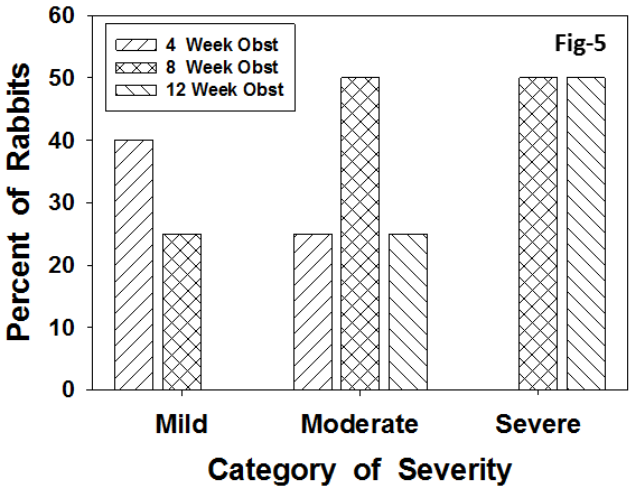
Figure 1 displays the contractile responses of the whole bladder to FS and Beth both as a function of duration of obstruction and severity. For both forms of stimulation, there were very close correlations between contractile dysfunctions and severity but not duration.

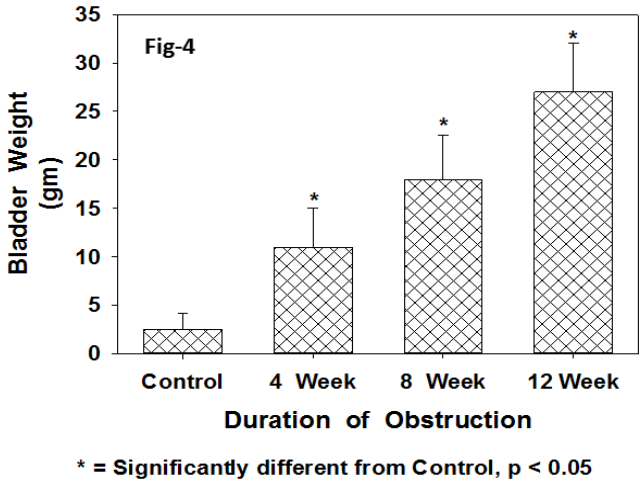
Figure 2 displays the % volume emptied to FS and Beth both as a function of duration and severity. Similar to peak pressure generation, there were very close correlations between % volume emptied and severity but not to duration. Two of the best bio-markers for mitochondrial and metabolic function are the concentrations of ATP and CP.











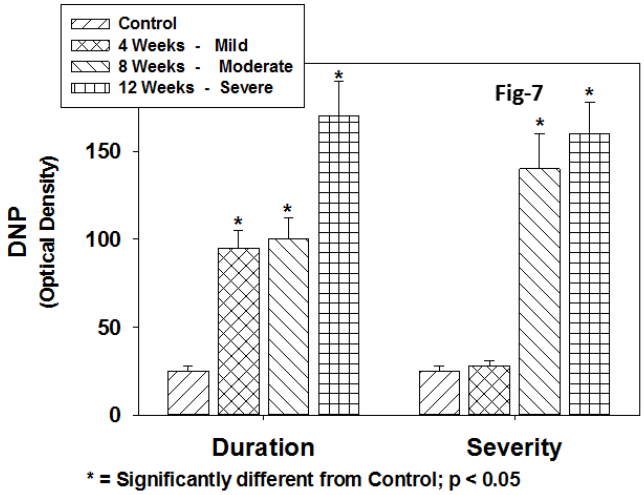


Figure 3 displays the concentrations of ATP and CP both as a function of duration of obstruction and severity. Similar to peak pressure and % volume emptied, there were very close correlations between both high energy phosphate studies and severity.

Figures 1-3 are adapted from the study published in reference [9]. There is a close relationship between bladder weight following PBOO with severity of dysfunction but not with duration of obstruction, thus we differentiate the status of the obstructed rabbit bladder (state of compensation/decompensation) by both bladder mass and by the comparative contractile responses of the bladder to various forms of stimulation [9, 11]. Our next sets of experiments utilized 32 rabbits divided into 4 equal groups of 8 rabbits each. Group 1 was controls (sham operated) while groups 2-4 were obstructed for 4, 8, and 12 weeks respectively [12-14].

Figure 4 shows the progressive increase in bladder weight. Please note the rather large standard errors which indicate the large variability in weights for each duration.

Figure 5 shows the percent of rabbits in the three groups of severity for each duration. Although it is clearly true that the longer rabbits are obstructed the greater proportion of them shift from mild through severe decompensation, individual rabbits may remain compensated or at mild decompensation for prolonged periods of time. This is very similar to progressive decompensation in men with OBD. That is to say, the level of obstructive dysfunction is not directly related to size of the prostate or how long individual men have had BPH. Some men with extremely large prostates have no urological problems while some men with small prostates have severe dysfunction.

3.3) Criteria for defining the level of compensation - decompensation: In **compensated** bladders, the average response to all forms of in vitro stimulation averaged >80% of control.

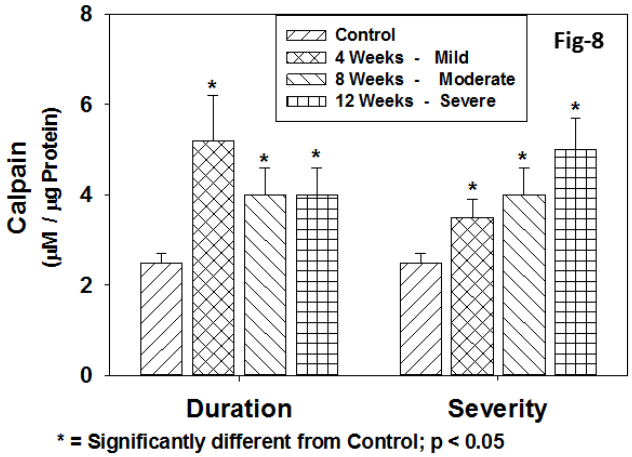
Mild decompensation is defined as the average response to all forms of stimulation which is >60% and <80% of the control responses.

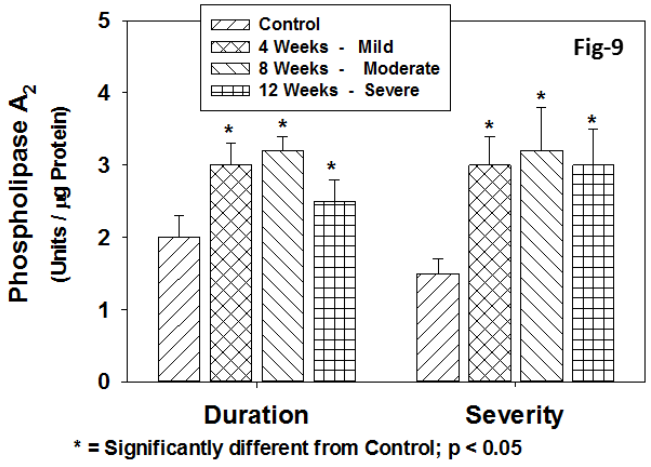
Intermediate decompensation is defined as having an average contractile function of >20% and <60% of control.

Severely decompensated bladders exhibit maximal responses averaging <20% of control.

Figure 6 displays the nitrotyrosine (NT) concentration as a function of both duration and severity. At all durations of obstruction, the concentration of NT was significantly increased especially in the 12 week group. However, there was no increase in NT in the mild severity group. There was a strong progressive increase in NT in the moderate and severe groups.

Figure 7 displays the DNP conc entration as a function of both duration and severity. At all durations of obstruction, the concentration of DNP was significantly increased especially in the 12 week group. However, there was no increase in DNP in the mild severity group. There was a significant increase in DNP in both the moderate and severe groups. **Figure 8** displays the calcium activated protease calpain concentration as a function of both duration and severity. At all durations of obstruction, the concentration of calpain was significantly increased. However, there was a progressive significant increase from the mild through severe groups.





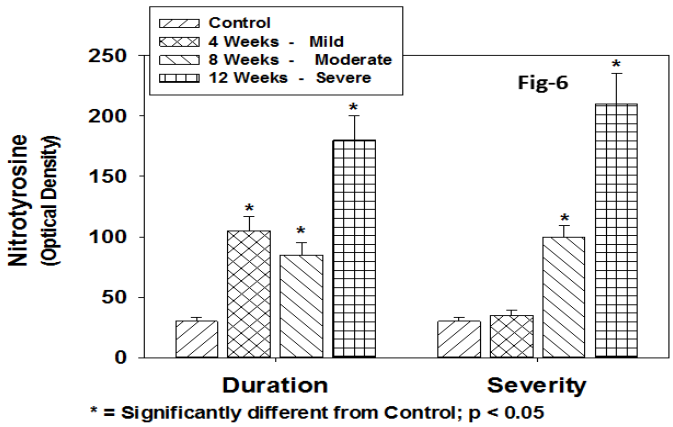
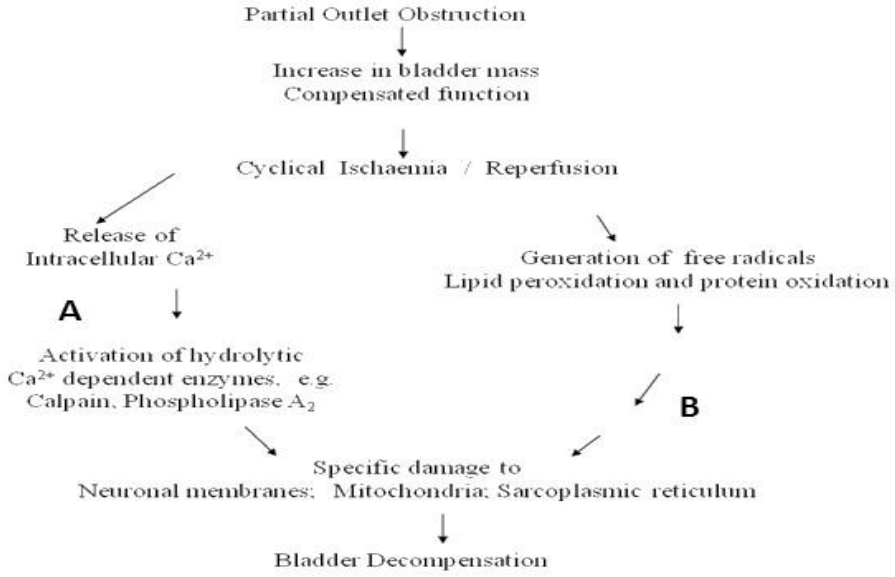


Figure 9 displays the calcium activated lipase phospholipase A_2 (PLA_2) concentration as a function of both duration and severity. At all durations and severities of obstruction, the concentration of PLA_2 was significantly increased.

Figures 4-9 are adapted from the studies published in references [12, 14, 15]. One of the most interesting findings was found in the mild obstruction group (independent of duration) which showed no increase in NT or DNP indicating that at this level of decompensation there was no increase in oxidative stress (free radical damage). The second most interesting finding was that there were significant increases in both calcium activated proteases (calpain) and lipases (PLA_2) indicating intracellular calcium overload. In summary, studies completed to date have demonstrated that OBD is initiated by four specific pathological processes:

1) Selective postsynaptic denervation and defective neurohumoral transmission [16, 17];
2) mitochondrial damage and intracellular metabolic dysfunction [18, 19];
3) sarcoplasmic reticulum (SR) damage; and
4) calcium dysregulation resulting in an increase in basal intracellular free calcium [7, 20] and the activation of specific calcium-activated proteases and lipases. The following schematic presents our view of the progression of obstructive bladder dysfunction (men and rabbits: **3.4) Sequence of events leading to bladder decompensation [12]**



A = Calcium Overload -- B = Oxidative Stress

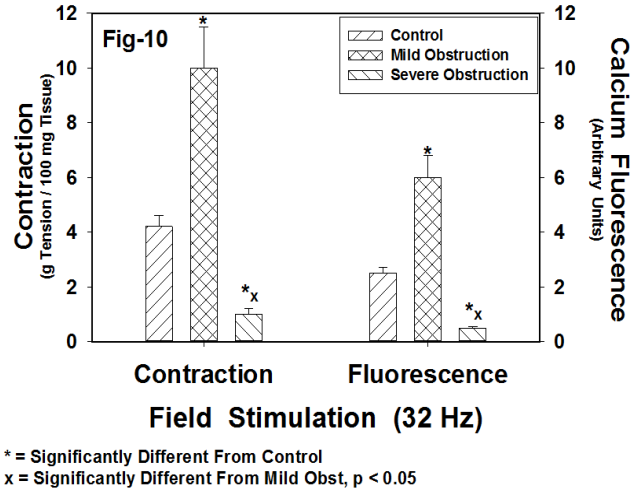
As demonstrated above, recent data indicates that in both compensation and mild decompensation (early stages of obstruction), there is no increase in free radical products (oxidative stress) such as DNP, NT or malondialdehyde (MDA) [12] but there are significant increases in the calcium-activated proteases and lipases including calpain and PLA_2 activity (calcium overload) [14]. This is shown in the left side (A) of the above schematic. This correlates very well with our surface spectrofluorometry studies (on rats) which demonstrated that in mild obstruction there was a direct correlation between the increased contractile responses to FS, bethanechol (cholinergic agonist) and KCl (musculotropic agonist) and increased intracellular free calcium responses [8, 21]. In these studies, we utilized a Surface Spectrophotometer that could measure simultaneously contractile responses to various forms of stimulation within a thin strip of bladder smooth muscle and intracellular free calcium by pre- incubating the strip with the Ca^{2+} fluor FURA-2AM

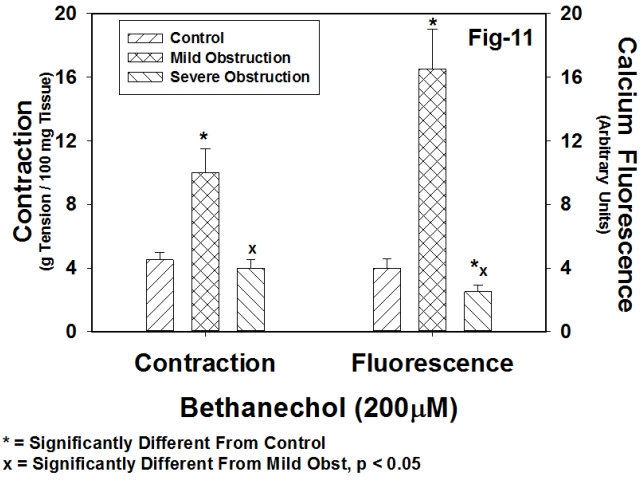
Figure 10 displays the correlation of the contractile response to FS (32 Hz) with intracellular free Ca^{2+} [8, 21]. There were very significant increases in both the contractile response to FS and the intracellular free Ca^{2+} concentration with mild obstruction whereas there were significant decreases from control with severe obstruction.

Figure 11 displays the correlation of the contractile response to bethanechol (200 μM) with intracellular free Ca^{2+} [8, 21]. There were very significant increases in both the contractile response to FS and the intracellular free Ca^{2+} concentration with mild obstruction whereas there both parameters decreased to control levels or below with severe obstruction. Virtually identical results were obtained for KCl and ATP stimulation. **Figures 10 and 11** were adapted from references [8, 21]

4) Discussion and Conclusions:

This leads us to believe that the initiation of bladder decompensation (from normal function to compensated to mild decompensation) results from intracellular calcium overload and activation of calcium activated proteases, lipases and phospholipases. The primary targets for these degenerative enzymes are the cellular and subcellular membranes of the synapse, nerve cell, mitochondria and sarcoplasmic reticulum as these are the organelles which are most sensitive to obstructive and hydrolytic damage [22, 23].





Calpains are a family of calcium activated cysteine proteases localized both in the cytosol and the mitochondria. They have been shown to regulate apoptosis and necrosis and are involved in specific cardiopathologies [24, 25]. PLA_2s represent one of the largest groups of lipid modifying enzymes. Phospholipase C is also involved in many lipid modifying activities. Both phospholipases have calcium activated and independent isoforms and may be directly involved in the cellular responses to PBOO [26, 27].

Thus our hypotheses were: **(1)** The shift from compensated bladder function to mild decompensation is primarily due to intracellular calcium overload of the smooth muscle cells and the activation of proteolytic and lipolytic enzymes (side A of the above schematic). **(2)** The shift from mild obstructive bladder dysfunction through severe dysfunction is primarily due to oxidative stress and the generation of oxidative free radicals resulting in oxidative damage to the same cellular and subcellular membrane systems as described above (side B of the above schematic).

4.1) Evidence that calcium channel blockers and modifying intracellular calcium alters the response of the rabbit bladder to ischemia /reperfusion; PBOO, and contractile responses:

There are numerous published studies which clearly demonstrated that calcium channel blockers can significantly protect the rabbit urinary bladder from several forms of in- vitro ischemic and hyperflexic dysfunctions [28, 29]. Calcium channel blockers have also been shown to be effective in the treatment of myocardial ischemia and other forms of ischemic damage [30-32] In a separate relevant study we evaluated ryanodine, which blocks the release of calcium from the SR, and thapsigargin, which blocks the ability of the SR to pump cytosolic calcium back into the storage sites. Rabbit bladders were obstructed for different periods of time, after which detrusor muscle strips were harvested and contractile performance was evaluated in the presence and absence of ryanodine and thapsigargin. The results demonstrated that the release of intracellular calcium increased significantly in the early phases of outlet obstruction. With prolonged obstruction and detrusor decompensation, the intracellular storage sites significantly reduced the ability to contribute to the generation of contractile force. The conclusions from this study clearly showed that alterations in the calcium handling ability of the bladder smooth muscle cell appear to have an important role in the progression of decompensation of bladder function in PBOO. Blocking the increase of intracellular calcium during the early stages of PBOO (compensated and mild decompensation) would be most responsive to calcium channel blockers [7].

The relationship between calcium channel blockers and ischemia / reperfusion relates to blood flow and vasodilation. Bladder outlet obstruction mediates cyclical ischemia / reperfusion by reducing blood flow to the bladder smooth muscle during contraction and rapidly increasing blood flow during bladder filling (relaxation) [33, 34]. Since a side effect of calcium channel blockers is vasodilation, it may well reduce the level of ischemia / reperfusion mediated by outlet obstruction thereby reducing the progression of the dysfunction.

4.2) Relevance of the rabbit model of PBOO to the study of human obstructive bladder dysfunction secondary to BPH [35-37]:

In humans, it is difficult to investigate the cellular mechanisms by which progressive bladder dysfunction occurs secondary to BPH. However, many of the functional changes associated with human bladder pathology can be induced in experimental animal models including the rabbit (see reviews [5, 35]). Rabbit bladder capacity is between 50 and 100 ml and compliance can be evaluated cystometrically using an 8 Fr. Foley catheter inserted into the bladder. The cystometric curve of the rabbit is similar in shape to that of humans. Also, bladder emptying occurs during the tonic phase of contraction as seen in humans. The bladder's ability to sustain increased pressure in response to stimulation is significantly reduced by PBOO before any change in maximal pressure generation occurs.

Major characteristics of the rabbit's response to PBOO include: **1)** an increase in bladder mass to a stable level, **2)** reduced compliance during bladder filling, **3)** increased micturition frequency and decreased volume per micturition (urgency and frequency) and **4)** development of overactive bladder dysfunction. These dysfunctions are very similar to symptoms secondary to BPH observed in men.

Ultrasound studies have confirmed that not only do men with obstructive uropathies exhibit an increase in bladder mass [38, 39], bladder wall thickness has been shown to be the most accurate non- invasive way to identify men with OBD. Another common feature of obstruction in both rabbits and man is denervation which has been demonstrated immunohistochemically and biochemically in both species [35, 40]. In addition, obstructed rabbits and men both show an increase in the density and distribution of connective tissue (CT) within the bladder wall resulting, functionally, in decreased compliance and higher pressures during filling [41, 42].

In two major studies performed with Dr. John Gosling (expert in electron microscopy of the lower urinary tract), we clearly demonstrated that the level of contractile dysfunction in rabbits subjected to PBOO correlated with the degree of ultrastructural damage to nerve, synaptic, mitochondrial, and SR membranes [40, 43] which in turn correlated with similar findings in men with obstructive bladder dysfunction.

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