Basic Research in Bladder Outlet Obstruction

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ABSTRACT

Partial bladder outlet obstruction (PBOO) in males is mostly caused by benign prostatic hyperplasia. PBOO induces significant alterations in the morphology and physiology of the urinary bladder wall and also results in an impaired ability of the urinary bladder to store and empty urine. Much of our current knowledge is based on animal experiments, and species differences may be a problem when extrapolating these animal findings to the human situation. The primary bladder dysfunction associated with PBOO is a decreased ability to sustain bladder contractions during the voiding phase of the micturation cycle. In animal model, bladder dysfunction and alterations of the mechanistic steps involve detrusor smooth muscle (DSM) contractility. Generally, in response to PBOO, the DSM undergoes compensatory hypertrophy to produce the increased force necessary to expel urine against an obstruction. Besides altering contractile proteins in DSM, PBOO also induces a change of regulatory proteins such as Rho-kinase and protein kinase C. In addition to the impaired ability to empty urine, PBOO is frequently accompanied by non-voiding contractions which can lead to clinical symptoms such as urinary frequency, urgency, and nocturia. Non-voiding contractions could result from a hypersensitivity of the contractile system and/or a reduced sensitivity of the relaxant system. The impairment of β-adrenergic relaxation of bladder smooth muscle and the enhancement of α-adrenergic contraction in PBOO are addressed in this review. Cyclic ischemia and reperfusion (I/R) changes are major etiologic factors in the progression of bladder dysfunction in PBOO. These changes are directly associated with a decrease in energy produced by oxidative phosphorylation in mitochondrial electron transport chain. Furthermore, defects in this chain lead to the generation of a significant quantity of reactive oxygen species in addition to cell apoptosis. Antioxidant agents and mitochondrial respiratory chain coenzyme have been shown to decrease PBOO-induced I/R damage to the bladder.

Key words: bladder, obstruction, ischemia, overactivity

INTRODUCTION

Partial bladder outlet obstruction (PBOO) is a common urological clinical problem, which mainly results from prostatic hyperplasia, ure-

thral stricture disease or other congenital anomalies such as posterior urethral valve. Most of our current knowledge is based on animal studies which suggest that several pathophysiologic mechanisms might underlie the pathogenesis of PBOO. This then results in an instant increase in urethral resistance, followed by an increase in bladder wall tension and micturition pressure [1]. These effects eventually lead to an increase in bladder wall thickness, changes in detrusor smooth muscle (DSM) morphology, progressive bladder wall denervation, alteration in detrusor receptor density and a decrease of blood flow to the bladder wall [2-4].

The present view focuses on the recent advances in basic research involving areas ranging from cellular receptors to the contractile machinery, including pathophysiological contraction and relaxation of bladder DSM associated with PBOO. It is known that the size of the bladder, the micturition patterns, the contractile property, and the contractile regulation vary between different species (animal and human). Moreover, results obtained with the different time point and the severity of obstruction may also vary in different animal models. Thus, it is important to address species differences when pathophysiological findings in bladders are examined in humans and animals with PBOO.

DETRUSOR OVERACTIVITY AFTER PARTIAL BLADDER OUTLET OBSTRUCTION

PBOO is associated with bladder dysfunction which involves not only obstructive symptoms such as weak stream but also irritative symptoms such as frequency, nocturia and urgency. The occurrence of irritative symptoms implies that the obstruction also impairs the storage function of the bladder. Similar to the neurogenic and aged bladder, several studies have reported that the obstructed bladder is partially denervated in several animal species including humans [5-8]. Immunological investigations demonstrated that nerve distributions were significantly changed in the obstructed rat bladder [3]. The hypertrophied smooth muscle bundles showed a complete absence of cholinergic nerve terminals and the density of the nerve structures was also significantly decreased [9]. Sibley et al showed that the response to intramural nerve stimulation was decreased, but there was super-sensitivity to acetylcholine in obstructed pig bladder strips [10]. A relevant explanation for altered responses to muscarinic receptor (MR) agonists in PBOO is a change of MR expression, including the total density or/and the relative functional role of MR subtypes [11]. A pig radioligand binding study showed no variation of overall MR density or the relative contribution of the M2 subtype after PBOO [12]. In contrast, an investigation in rats reported an increase in mRNA expression of M2 and M3 receptor subtypes in the hypertrophied bladder; while the former was
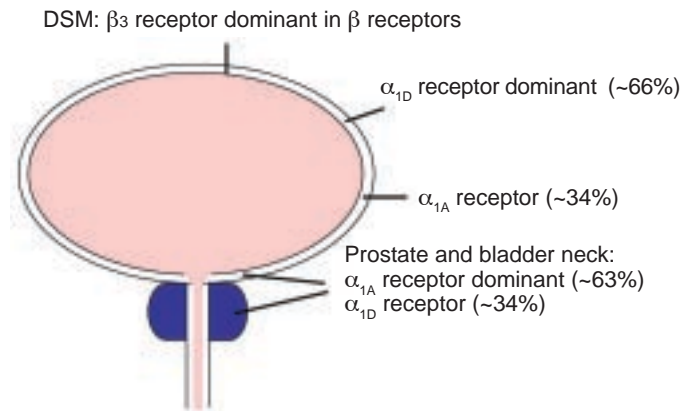
accompanied by an enhancement in receptor density, the latter was not affected in the protein level [13]. Accordingly, the currently available data are insufficient to draw a definitive conclusion regarding possible alterations of the density of MRs and their subtypes.

Besides possible alterations in MRs, variations in α and/or β-adrenergic receptor (AR) systems might serve as an alternative explanation in the etiology of irritative symptoms during PBOO. In a normal bladder, there is a predominance of α1A-AR mRNA expression, not only in the human trigone and bladder bases, but also in the bladder dome [14]. The distribution of adrenergic α and β receptors in the human bladder are shown in Fig. 1. Moreover, while the functional importance of α-ARs in the normal human bladder remains questionable, there is a possibility that α-ARs may change in detrusor over-activity associated with PBOO. Hampel et al reported that the total number of α1-ARs mRNA expression did not change after PBOO. Instead, there was a shift from mainly α1A- to α1D-ARs in a rat model [15]. In control animals, 70% of α1-AR mRNA were α1A-subtype, 5% were α1B, and 25% were α1D, whereas in the obstructed bladder, α1-AR expression was 23% for α1A, 2% for α1B and 75% for α1D [15]. Another rat model study using an early time point with relatively small bladder enlargement also confirmed an increase in α1D-adrenergic mRNA expression [16].

Under physiological conditions, β-ARs play a key role in smooth muscle relaxation and cause increases in bladder compliance during the micturition cycle filling phase. In addition, the balance between contraction-mediated α1-ARs and relaxation-mediated β-ARs may be changed in bladder outflow obstruction. In normal human bladders, α1A, α1B, α1D, β1, and β2-AR mRNAs were expressed at very low levels, while the β3-AR was the most predominantly expressed AR subtype [17]. However, in obstructed human bladders, the expressions of α1A, α1D, β2, and β3-AR mRNAs were increased, whereas the expressions of α1B and β1 mRNAs were decreased. In contrast, studies by Chapple et al could not confirm the occurrence of an increase in α-AR function in the over active, obstructed bladder [18]. Another investigation of normal and obstructed human bladders reported that the copy number of total mRNA was increased in obstructed bladders, but the distribution of the β-ARs between groups did not change, and β3 subtype accounted for over 95% of the total mRNA in both groups [19]. In rat, severely obstructed bladders were found to be associated with a decreased relax ability in response to the β-agonist norepinephrine, whereas mildly obstructed bladders were comparable to the control group [16]. The factors such as the duration and the severity of obstruction may have an important influence on ARs-mediated responses in the bladder.

DETRUSOR MUSCLE CONTRACTILITY CHANGE AFTER PARTIAL BLADDER OUTLET OBSTRUCTION

In both human and experimental animals with PBOO, the DSM undergoes hypertrophy/hyperplasia to compensate for the increased force required to expel urine against the augmented urethra resistance. DSM hypertrophy develops initially following PBOO to compensate against the increased muscle contractility (compensation phase), whereas prolonged obstruction leads to bladder dysfunction (decompensation phase). The muscle bundles become larger and longer and the transverse area of the smooth muscle cells is increased, suggesting hypertrophy of the detrusor muscle cells in the compensation phase.

DSM: β3 receptor dominant in β receptors

α_{1D} receptor dominant (~66%)

α_{1A} receptor (~34%)

Prostate and bladder neck:
α_{1A} receptor dominant (~63%)
α_{1D} receptor (~34%)

Fig. 1. The illustration demonstrates the distribution of adrenergic α and β receptors in the normal human bladder. DSM=detrusor smooth muscle.

In addition to smooth muscle hypertrophy, the increased bladder mass can also be associated with alterations in extracellular matrix. Gosling et al found an increase in extracellular material and collagen in trabeculated bladder after PBOO [20]. In the hypertrophic rat urinary bladder, total collagen increases, although the concentration of collagen appears to decrease.

Physiological investigation using muscle strips from hypertrophied DSM showed a decrease in contraction force in response to carbachol and electrical field stimulation. Furthermore, in vitro isometric cystometry studies have demonstrated that the rate of emptying is significantly reduced and the time required is appreciably increased [21]. Several reports have described the alterations in DSM contractile and regulatory proteins after PBOO. Myosin II is the molecular motor unit for contraction in all types of smooth muscles. The velocity of force generated by the smooth muscle is determined by the myosin II ATPase activity while interacting with actin [22]. DSM myosin is a type II myosin that is composed of a pair of myosin heavy chains (MHCs) and two pairs of myosin light chains (MLCs). Two MHC isoforms, SM1 (204 kDa) and SM2 (200 kDa) have been identified in DSM. Previous studies have shown that the ratio of SM2 to SM1 in the normal rabbit bladder is about 1.7:1, whereas the ratio is shifted to 1:1 in decompensated hypertrophied PBOO bladder in rabbit [23]. However, in human hypertrophied bladder sample, an increase of the ratio of SM2 to SM1 has been reported [24]. Although the functional consequences of these changes in the SM1 to SM2 ratio remain unclear, a decrease in SM2 to SM1 ratio may stabilize the thick filament and exhibit a profound effect on contractile function.

The concentration of intracellular Ca^{2+} ($[Ca^{2+}]i$) is the major determinant of smooth muscle contraction. In the DSM, when cholinergic agonists bind to MRs, $[Ca^{2+}]i$ increases, which induces calmodulin-mediated activation of MLC kinase. MLC kinase, in turn, phosphorylates MLC and thereafter induces smooth muscle contraction [25]. After $[Ca^{2+}]i$ returns to its baseline level, the DSM maintains contraction through Ca^{2+} sensitization mechanism independent of $[Ca^{2+}]i$. There are two main pathways that regulate DSM Ca^{2+} sensitization. One is known to involve Rho kinase (ROK), which is activated via G-protein-coupled receptors by RhoA protein [25]. The other pathway involves protein kinase C (PKC) activation [25-28]. The main pathways of DSM contractile activation are shown in Fig. 2 in context of MR signaling. With regard to the role of ROK in the obstructed bladder, Bing et al reported that the ROK pathway is partly responsible for the force main-

tenance in response to depolarizing stimulus of KCl in the decompensated bladder of rabbit [28]. Another investigation showed an enhanced MR-coupled RhoA, and ROK pathway seems to induce adaptation of the DSM to facilitate force maintenance in an attempt to expel urine against the outlet obstruction [29]. ROK has two isoforms, namely ROKα and ROKβ. ROKα has 64% sequence identity with ROKβ and these two isoforms share 90% identity in their kinase domain. Although the exact functional difference between these two isoforms is not clearly defined, Takahashi et al found that the expression of both ROKα and ROKβ is increased in the rat obstructed bladder [29]. Guven et al examined the expression of ROK isoforms in rabbit DSM up to 8 weeks of PBOO, which demonstrated that ROKα was increased, whereas ROKβ was decreased in both protein and mRNA levels [30]. In contrast, Bing et al reported that ROKα was not changed, but ROKβ was over-expressed in the rabbit bladder in response to 2 weeks of PBOO [28]. These studies showed that PBOO alters the expression of ROK isoforms and increases either ROKα or ROKβ. The functional role of RhoA/ROK pathway may be involved in the maintenance of force generated in the obstructed bladder.

PKC is another important regulatory protein in maintaining DSM contractility [31-33]. The PKC agonist phorbol 12,13-dibutyrate (PDBu) has been shown to induce rabbit DSM contraction [33]. The activated PKC phosphorylate PKC potentiates inhibitory protein of protein phosphatase-1 (CPI-17) [34]. Phosphorylation of CPI-17 produces over a 1000-fold increase in the inhibitory potency of CPI-17. Thereafter, phosphorylated CPI-17 inhibits myosin phosphatase activity, leading to increased phosphorylation of MLC and maintained contraction of DSM [35-37]. A recent study reported that PKC induced contraction of rabbit DSM was abolished in PBOO [37]. However, another investigation

demonstrated that there was no significant change in PKC-induced contraction and PKC function between compensated bladder and the control [38]. Moreover, decompensated bladders were found to generate significantly less PKC-induced contraction accompanied by appreciable decreases in PKC expression, PKC activity and CPI-17 phosphorylation when compared with the control [38]. This finding suggested that an impaired PKC pathway might be correlated with severe bladder dysfunction observed in decompensated bladders.

ISCHEMIA/REPERFUSION DAMAGE AFTER PARTIAL BLADDER OUTLET OBSTRUCTION

Several investigations have demonstrated that ischemia and reperfusion (I/R), with the accompanied generation of free radicals, are major etiologic factors in obstructive bladder dysfunction [39,40]. It has been shown that during bladder emptying, the increased intrawall tension results in blood vessel compression, decreased blood flow and tissue hypoxia. Although these changes also occur in normal bladders, the extent of effects is significantly exaggerated in the obstructed hypertrophied bladder. Both reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated following the cycles of I/R [41]. Superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) are examples of ROS that are generated. Nitric oxide (NO) can also act as a source of RNS. Elevated levels of nitrotyrosine are detected in bladder proteins subjected to BPOO [42]. Other oxidative pathways involving ROS and RNS also give rise to carbonyl products with amino acid residues [43]. Under conditions of increased oxidative stress, cellular and subcellular membranes are subjected to attack when the generation of free radicals outweighs the system's
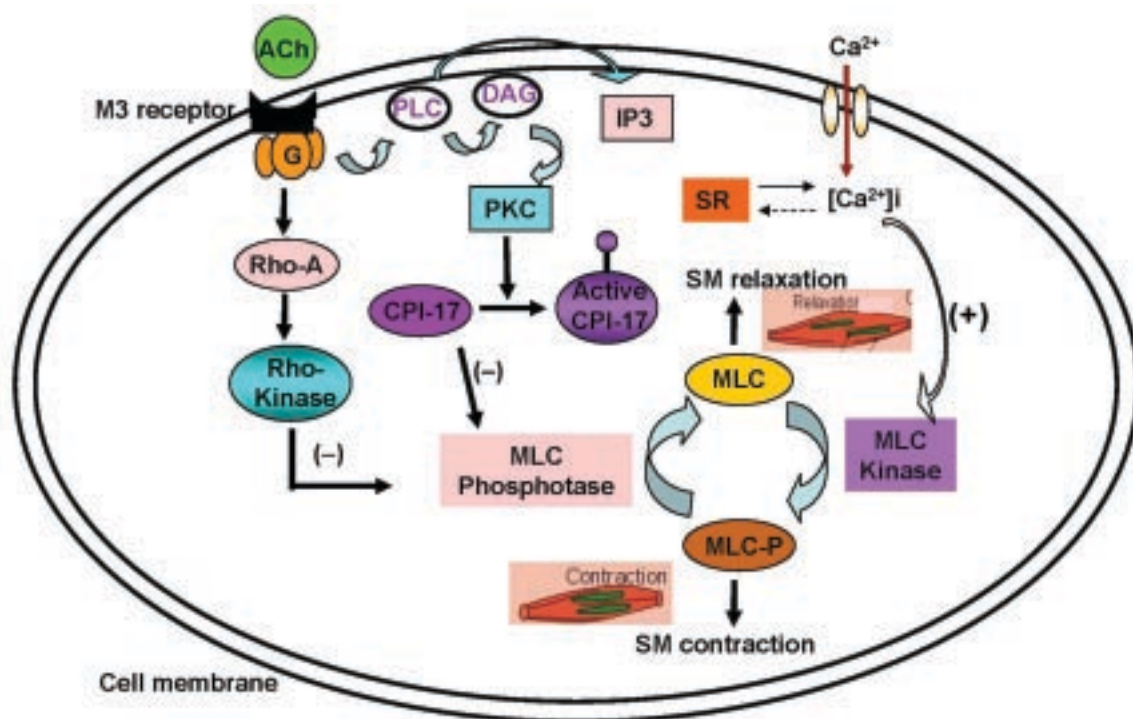
Fig. 2. Intracellular signal pathways involved in activation of detrusor smooth muscle contraction and relaxation via muscarinic M3 receptors. Ach=acetycholine; PLC=phospholipase; DAG=diacylglycerol; PKC=protein kinase C; SM=smooth muscle; MLC=myosin light chain; IP3=inositol trisphosphate; SR=sarcoplasmic reticulum; [Ca^{2+}]i=intracellular Ca^{2+}; CPI-17=PKC potentiates inhibitory protein of protein phosphatase-1.

ability to eliminate them. The physiological consequences of cellular and subcellular membrane oxidation include decreased mitochondrial function, nerve damage, and contractile dysfunction [44]. Fig. 3 demonstrates the progression of cyclic I/R injuries of bladder with regard to PBOO.

Conners et al showed the oxidative stress marker, nitrotyrosine level, was significantly increased as early as 3 days following PBOO surgery in rabbit bladder [42]. Pretreatment of rabbits with nitric oxide synthase inhibitor, N-nitro-L-arginine methyl ester (L-NAME), enhanced ischemic damage one day after obstruction, but protected the bladder from nitric oxide-generated free radical damage at the later time periods by inhibiting the generation of nitrotyrosine. Our previous studies demonstrated that protein carbonylation was increased significantly in obstructed bladders when compared with the controls (both in mucosa and muscles) and reached the highest level after 4 weeks of obstruction [5]. There was a 2-fold increase for nitrotyrosine in mucosa after 8 weeks of obstruction. The increased expression of nitrotyrosine in muscle was maximized at 4 weeks of obstruction. Using a cumene hydroperoxide organ bath to mimic oxidative stress, Jongh et al reported that oxidative stress reduced the optimal effect to the MR and lowered the affect on contraction induced by potassium chloride and adenosine triphosphate (ATP) [45]. It is likely that oxidation and nitration damages the membrane function (L-type calcium channels), dysregulate Ca^{2+} homeostasis, increase the activities of Ca^{2+}-dependent enzymes (phospholipase and calpain), mediates the observed progressive denervation of bladder wall and mucosa thinning, and increase the mucosa permeability [46,47]. The functional significance of protein carbonylation and nitration may be correlated with these changes. Clinically, during the recovery of bladder function after transurethral resection of prostate surgery, several patients continued to have irritative storage symptoms. The increased level of protein oxidation and nitration of the bladder mucosa and wall might be involved in the increased frequency of hyper-reflexia in obstructed rabbits. Lin et al imposed PBOO in rabbits and released the obstruction after 4 weeks of surgery. The hypertrophied smooth muscle bundles of the obstructed bladders were found to regress to near-normal size. There was a significant increase in the level of carbonylation and nitrotyrosination after PBOO, and a progressive decrease in the 4-week reversal groups, which reached nearing control values by 8 weeks. The nitrotyrosine in bladder mucosa was reported to regress earlier than bladder wall, implying that mucosa has more blood supply than bladder wall.

CONCLUSION

The storage symptoms typically observed upon PBOO in patients or experimental animals could theoretically be explained by an increased muscarinic or α-adrenergic input or a decreased β-adrenergic input. Moreover, the partial bladder denervation during bladder outlet obstruction should have larger effects on the cholinergic than on the sympathetic side of the system, since the former is more dominant in the bladder than the latter. PBOO has been reported to reduce detrusor contractility despite displaying signs of bladder over-activity. Besides the changes of contractile proteins in DSM, PBOO also induces an alteration of regulatory proteins such as ROK and PKC. Cyclic I/R changes are major etiologic factors in the progression of bladder dysfunction in PBOO. These changes are directly related to the decreased energy produced by mitochondrial electron transport chain and oxidative phosphorylation. Although our understanding of bladder dysfunction following PBOO has advanced during recent years, the detrusor muscle still offers a considerable challenge for future basic, clinical, and translational research. Accordingly, bladder dysfunction following PBOO remains an interesting and fruitful field for further research.
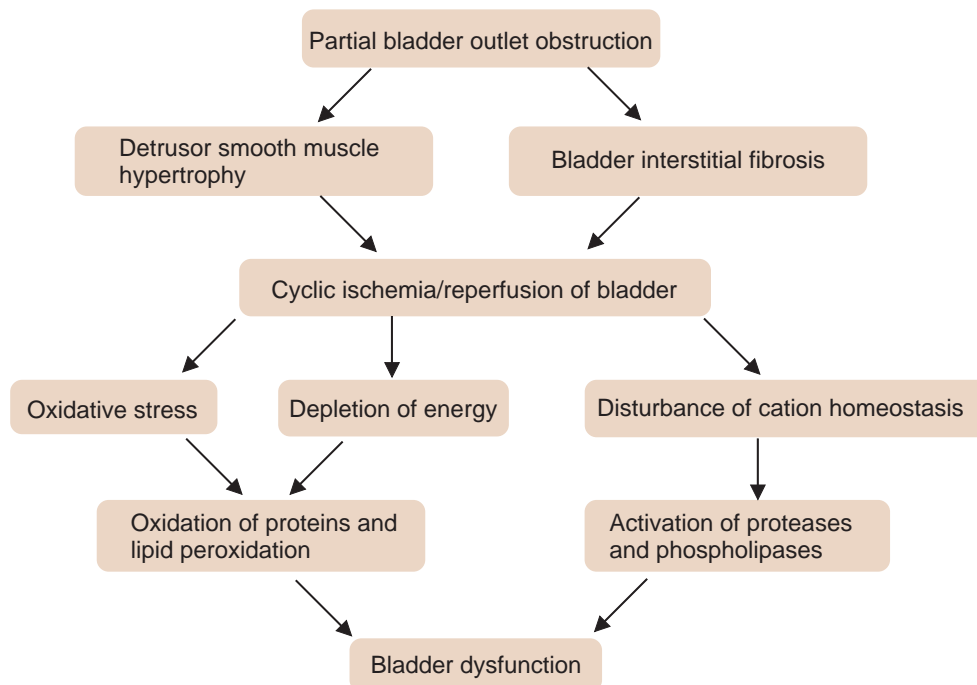
Fig. 3. The flow chart shows the progression of cyclic ischemia/reperfusion injuries of bladder with regard to partial bladder outlet obstruction.

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