The Role of Pharmacology in Ureteral Physiology and Expulsive Therapy

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Abstract. Research in the field of ureteral physiology and pharmacology has traditionally been directed toward relaxation of ureteral spasm as a mechanism of analgesia during painful ureteral obstruction, most often stone-induced episodes. However, interest in this field has expanded greatly in recent years with the expanded use of alpha-blocker therapy for inducing stone passage, a usage now termed "medical expulsive therapy". While most clinical reports involving expulsive therapy have focused on alpha receptor or calcium channel blockade, there are diverse studies investigating pharmacological ureteral relaxation with novel agents including cyclooxygenase inhibitors, small molecule beta receptor agonists, neurokinin antagonists, and phosphodiesterase inhibitors. In addition, cutting edge molecular biology research is revealing promising potential therapeutic targets aimed at specific molecular changes that occur during the acute obstruction that accompanies stone disease. The purpose of this report is to review the use of pharmacological agents as ureteral smooth muscle relaxants clinically, and to look into the future of expulsive therapy by reviewing the available literature of ureteral physiology and pharmacology research.

Keywords: ureter, expulsive therapy, pharmacology, smooth muscle, relaxation.
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INTRODUCTION

The mechanistic effects of pharmacologic agents on ureteral physiology have been studied for nearly four decades. The focus of this field of research has traditionally been toward identifying pharmacological agents useful in treating the painful symptomology associated with acute ureteral obstructions, most notably urinary stone disease. Many studies have evaluated compounds that have relaxed ureteral smooth muscle tone or spasm, a process that is known to potentiate the pain associated with acute obstructions. In 2003, Dellabella and associates found that stone passage rate improved significantly in response to tamsulosin, a selective alpha receptor antagonist usually prescribed for the treatment of benign prostatic hypertrophy [1]. This observation has now been repeated by several investigators. These findings have added substantial relevance to research in ureteral physiology and pharmacology, and dramatically shifted the focus of this research from pain management to expulsive therapy.

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While alpha receptor blockers remain the focus of clinical studies in ureteral pharmacology and expulsive therapy, numerous compounds have been investigated in basic ureteral pharmacology studies including cyclooxygenase inhibitors, neurokinin antagonists, beta receptor agonists, and phosphodiesterase inhibitors [2-8]. Given the efficacy of these novel compounds in vitro, they may ultimately prove to be particularly effective in expulsive therapy when translated to clinical studies. This report focuses on the current knowledge of ureteral pharmacology as it relates to ureteral smooth muscle relaxation, the proposed mechanism of expulsive therapy, with the hope of generating ideas for future clinical studies using these therapeutics toward improved outcomes in inducing ureteral stone passage and treating pain.

In order to put this discussion in proper context, this report begins with a discussion on basic ureteral physiology and a review of ureteral coordinated ureteral contraction (peristalsis) and hypercontractility during the obstructive condition. Next, literature on clinical studies of expulsive therapy will be reviewed, followed by a discussion of basic science in vitro and animal studies of ureteral pharmacology. It is likely that the future of expulsive therapy will arise from these studies.

BASIC URETERAL PHYSIOLOGY

Visceral urinary tissues, including the urethra, bladder, and ureter, are luminal structures consisting of an epithelial layer (urothelium), a mucosal layer containing capillaries and sensory and motor nerve terminals, the functional smooth muscle layer, and a surrounding layer of adventitia and serosa [9]. The urothelium is comprised of seven layers of urothelial cells that are oriented to basal-lateral and apical surfaces similar to other epithelial layers. While the urothelium has long been thought of as functioning as a protective surface of the urinary tract, it is now known that urothelial cells are critical to ureteral function [10]. Urothelial cells sense chemical signals in the urinary lumen, perceive pressure and distension-related events, and are critical in maintaining the gnotobiotic condition of intraluminal urinary organs. In response to changes in the luminal environment, urothelial cells signal to the smooth muscle, neurons, and capillaries via release of prostanoids, catecholamines, and cytokines, causing alterations in the function of the organ [10]. While the study of this cross-layer interactive environment is novel in the ureter and bladder, it is well established in epithelial-stromal interactions of the prostate and many analogous relationships are present throughout the urinary tract [11].

The mucosal layer of urinary tissues contains microvasculature and neuronal tissue. As such, it is a major communication point between the ureter or bladder and the rest of the body, particularly the cardiovascular and nervous systems. Pain mediators released from the urothelium act on sensory nerve terminals in this layer sending pain signals to the brain, and motor nerve axons extending from their dorsal root cell bodies communicate with the smooth muscle from this layer [10]. While they are extremely uncommon during stretch and distension, edema, hemorrhage, and inflammatory cell infiltrate present in this layer in response to infection or chemical stimuli, and are commonly present during urinary tract infections and interstitial cystitis [12].

The smooth muscle layer of the urinary tract is responsible for contraction, peristalsis (in the ureter), and structural support [13]. The smooth muscle is divided into two sub-layers: an inner layer of helical cellular arrangement and an outer layer of mesh-like cellular arrangement [13]. In the ureter, the inner helical layer is responsible for contractile function of the tissue, while the outer mesh-like layer provides structural support. Normal contraction of smooth muscle is the result of electrical activity of the smooth muscle cell membrane. In a resting smooth muscle cell, the membrane potential is approximately 80 mV [9]. When stimulated by chemical signal or cell-to-cell electrical conduction, Na^+ and K^+ ion conductance increases the membrane potential to 50 mV, at which point an action potential is generated. At this point, smooth muscle cells lose their preferential permeability to K^+ and become more permeable to Ca^{++} ions moving into the cell, primarily via L-type Ca^{++} channels [9]. This increase in intracytosolic calcium concentration ($[Ca^{++}]_i$) results in activation of calcium-dependent calmodulin (CaM). CaM activates myosin light chain kinase, which phosphorylates myosin, activating it as a motor protein to migrate up actin filaments thereby contracting the cell. In the case of normally functioning tissue, the increase in $[Ca^{++}]_i$ during upstroke of the action potential eventually activates outward Ca^{++}-dependent K^+ currents such that repolarization occurs and membrane potential is returned to its resting level [9].

In the ureter, this depolarization-repolarization cycle is coordinated in a peristaltic wave, where electrical potentials are generated in pacemaker tissue within the renal pelvis and propagated distally from cell to cell membrane conduction at intermediate junctions [9]. Smooth muscle cells possess resistive and capacitative membrane properties conducive to propagation of electrical currents. Such currents are generated in interstitial cells of Cajal in a rhythmic fashion [9]. Urine propulsion is performed in a bolus fashion, where small local distensions induce mediator release in concert with electrical wave potentials [9]. The result is coordinated peristalsis of urine from the renal pelvis to the bladder.

Hormones that elevate $[Ca^{++}]_i$ including prostanoids, neuropeptides, and cytokines, as well as integrin signaling, can induce smooth muscle cell contractility and increase ureteral contractility [14]. In the case of kidney-stone-induced obstruction, the activation of these cascades occurs, potentiating contraction. Contractility of urinary tract smooth muscle increases luminal pressure and contact (in the case of calculi) with the noxious stimulus, intensifying the pain cascade. Prostanoids, neuropeptides, and catecholamines activate $G_\alpha q$-protein coupled receptors (GPCRs) of the seven-transmembrane domain superfamily [15]. These ligand-receptor interactions lead to the activation of phospholipase C-β (PLCβ), which catalyzes the synthesis of inositol triphosphate (IP_3) and diacylglycerol (DAG) from phosphoinositol bisphosphate (PIP_2). IP_3 opens IP_3-sensitive calcium channels on the sarcoplasmic reticulum and mitochondria, causing Ca^{++} flux into the cytosol where it can interact with CaM and induce the actin-myosin contractile cascade [15]. It is likely that pharmacologic inhibition of prostanoid synthesis, neuropeptide receptors, alpha-adrenergic receptors, or phosphodiesterases ultimately works by preventing the activation of the above-

mentioned signal transduction mechanisms, leading to smooth muscle relaxation, spasm-induced pain relief, and stone passage.

CLINICAL STUDIES OF EXPULSIVE THERAPY

Tamsulosin

Prior to 2003, most ureteral physiology studies were conducted seeking novel ureteral smooth muscle relaxants to relieve the pain associated with ureteral spasm. In 2003, Dellabella and associates showed that the selective alpha adrenoreceptor antagonist tamsulosin increases stone passage rate from 70% to 100%, and decreased passage time from 111 hrs to 66 hrs, with 30 patients in each arm [1]. This same research group reported two years later that tamsulosin given alone produced a 90% stone free rate at a median time of 120 hours. Cervenakov and associates reported similar efficacy increases by tamsulosin [16]. In similar studies, DeSio et al reported that tamsulosin increased stone passage rate from 58% with diclofinac and aescin (anti-inflammatory drugs) alone to 90% with tamsulosin treatment [17]. Median passage time improved from 180 hours with anti-inflammatories alone to 116 hours with tamsulosin [17].

Non-selective Alpha Antagonists

Tamsulosin is considered a selective alpha-receptor antagonist [18], meaning it is selective for alpha-1A and 1D receptors (for review, please see Michelotti et al, 2000 [19]). However, non-selective alpha blockers have been shown to produce similar stone passage success rates as tamsulosin [20]. Yilmaz and associates report that tamsulosin, terazosin, and doxazosin all similarly increased stone passage rate from controls, from 52% to 79%, 78%, 75%, respectively [20]. In addition, Mohseni and associates have confirmed these findings with terazosin [21]. While it is clear that more studies need to be conducted to carefully compare the efficacy and safety of various alpha-receptor antagonists in stone passage, these early reports suggest that non-selective alpha blockade might be a viable treatment option in patients in which non-selective alpha-blockers are not contraindicated for other health concerns.

Calcium Channel Blockers

While alpha receptor antagonists have received the most attention as pharmacologic agents inducing stone passage, calcium channel blockers have performed equally well in clinical studies to date. Borghi and associates found that nifedipine increased stone passage rate from 65% to 87% [22]. Similar efficacy was reported by Saita and associates [23], while Porpiglia and associates report that nifedipine increased stone passage from 33% to 71%, and median passage time from 20 days to 7 [24]. Though no conclusive, large randomized study comparing alpha blockers to calcium channel blockers has been reported, an extensive meta-analysis by Hollingsworth et al.

calculates that calcium channel blockers' expulsive rate relative to control is 1.90 compared to 1.54 for tamsulosin [25]. The authors of this article conclude that both therapies are significantly improved over non-treated subjects, but there is no significant difference to date between alpha receptor antagonism and calcium channel blockade. This conclusion is supported by small comparison studies [26-27]. It is important to note that the large range of values and inconsistencies in the literature reports (inclusion criteria, stone size, location, etc.) make concrete comparisons difficult.

Corticosteroids

Numerous studies have looked at corticosteroids in combination with either alpha antagonists or channel blockers, but few have compared these compounds to other drug classes. To date, steroids appear to have additive effect with other drugs, but little efficacy alone [28]. When administered with deflazacort (a corticosteroid) tamsulosin increased stone passage rate increased to 97% (from 90%) with a median time of 72 hours [29]. At present, it appears corticosteroids may provide a modest improvement in stone passage when used in combination with either alpha antagonist or calcium blocker, but appear to have no significant effect alone.

Expulsive Therapy with SWL

Pharmacologic expulsive therapy also appears to have efficacy when used in combination with lithotripsy. Three studies to date have evaluated shockwave lithotripsy in combination with tamsulosin treatment, and all three report significant improvements in pain scores and number of renal/ureteral colic episodes [30-32]. In addition, studies by Gravina et al. and Kupeli et al. demonstrate that stone-free rates were significantly higher in patients on tamsulosin therapy relative to controls [31-32]. While further study is needed to fully characterize pharmacologic intervention in combination with SWL and comparisons between drug classes in this indication have yet to be performed, early studies in this area suggest that ureteral relaxant therapy may improve the efficacy of conventional stone treatments.

Summary

While the clinical treatment of pharmacologic expulsive therapy for kidney stones continues to evolve, several important concepts arise from clinical studies to date. Alpha adrenergic antagonism and calcium channel blockade significantly enhance stone passage rate relative to controls. At present, there appears to be no significant difference between these two drug classes with regard to clinical efficacy. In addition, most evidence suggests that non-selective alpha receptor antagonists, including doxazosin, have similar efficacy to tamsulosin at improving stone passage rates and median passage time. Corticosteroids may slightly enhance the expulsive efficacy of other therapeutics, but appear to have limited or no effect alone. Finally, early reports

suggest that expulsive therapy enhances the effectiveness of conventional stone therapy.

IN VITRO STUDIES OF URETERAL RELAXATION

In depth pharmacological analysis of the ureter began in the early 1970's. The goal of this research was to further the basic knowledge of this understudied organ with the hope that ureteral smooth muscle relaxation might benefit renal hydronephrosis proximal to obstruction and pain associated with urinary obstructions, including stones. Stone expulsion was not an objective. While anecdotal evidence suggested ureteral smooth muscle relaxation would relieve pain associated with acute ureteral obstruction, Laird and associates provided direct evidence that spasm and hypercontractility significantly potentiate the pain response in 1997 [33]. Many antagonists/enzyme inhibitors were studied for pharmacological activity, working toward developing non-narcotic therapeutics for analgesia using ureteral smooth muscle relaxation as a model. Regardless, the belief was that inhibiting contraction of the ureter, and therefore peristalsis, would prevent stone passage rather than promote it. Today, there is substantial evidence that pharmacologic relaxation of the ureter enhances stone passage rate. As such, the clinical studies previously reviewed have added substantial gravity and energy to the field of ureteral physiology and pharmacology, with the hope that drugs originally investigated to relieve pain might be candidates for expulsive therapy. Because alpha receptor antagonists and calcium channel blockers may not be suitable for all patients, it is critical to look to the currently available knowledge of pharmacologic agents in vitro as a source of therapeutic candidates in the future expulsive therapy.

Cyclooxygenase Inhibitors

Cyclooxygenase (COX) inhibitors have been studied in ureter since 1986, and have proven successful at inhibiting ureteral contractility in numerous studies [2-4,34-36]. COX catalyzes the synthesis of prostanoids from arachidonic acid and exists in two isoforms: COX-1 and COX-2 [37]. Though COX-1 can be regulated, it is usually considered to be expressed constitutively. In contrast, COX-2 is highly inducible by inflammatory and mechanical stimuli, and is highly induced during ureteral obstruction [38-40]. Because prostanoids are highly potent smooth muscle contractants, inhibition of their synthesis expectedly causes smooth muscle relaxation. Nonselective COX inhibitors have been successful in treating the pain associated with urinary calculi [41-43], and these compounds produce complete ureteral relaxation overtime, unlike other compounds. Interestingly, ureteral contractility is inhibited by nonselective and COX-2 selective inhibitors (including celecoxib) equally well, suggesting a primary role for COX-2-derived prostanoids in this system [4,34,36].

Despite promising in vitro data, use of COX inhibitors in expulsive therapy may be limited. Prostacyclin-driven contralateral renal vasodilation is COX-2 dependent, explaining the high rate of renal insufficiency with these compounds, particularly in

the face of obstruction [44]. In addition, there are conflicting reports on the effect of COX inhibitors on stone passage rates, with most reporting minimal or no effect despite clear efficacy in ureteral relaxation relative to alpha receptor antagonists [45-47]. It has been proposed that this may be due to a complete inhibition of contraction of the ureter by COX inhibitors, whereas alpha antagonists may block spasm but not coordinated peristalsis [48]. However, this assertion is yet to be supported by clear scientific evidence. Future related research might be directed toward specific prostanoid receptor antagonism, as it has been shown that the EP-3 prostanoid receptor plays a role ureteral contractility [49-50]. However, studies in this area are limited by lack of quality specific antagonists at present.

Phosphodiesterase Inhibitors

Phosphodiesterase (PDE) inhibitors have been studied in the ureter since the late 1980's. PDE inhibitors produce substantial effects on ureteral contractility, and appear to be nearly as efficacious as COX inhibitors [51-53]. PDE enzymes degrade the cyclic nucleotides, cAMP and cGMP, and the mechanism of these compounds is inhibition of this process. The resulting elevation of cyclic nucleotide concentrations leads to smooth muscle relaxation via activation of protein kinase A and subsequent phosphorylation and inhibition of myosin light chain kinase. PDE exist in seven isoforms, each with varying specificities for cAMP and cGMP [37]. Specific inhibition of the PDE-IV isoform has repeatedly shown greater efficacy than inhibition of the other isoforms [51], though it appears that PDE-V isoform inhibition does cause ureteral relaxation [53]. Of the clinically available drugs studied to date, rolipram, a selective PDE-IV inhibitor, has been shown to be the most potent PDE inhibitor in vitro at inhibiting ureteral contractility [52]. However, PDE-IV inhibitors are notoriously high in systemic effects, and this would likely limit their clinical utility particularly in a market pre-possessing alpha receptor and calcium channel inhibitors [54].

Neurokinin Receptor Antagonists

Among the most efficacious ureteral contractility inhibitors are the neurokinin receptor antagonists. There are three known neurokinin receptors in mammals: NK-1, NK-2, and NK-3; each receptor has preferential affinity for the ligands substance P, neurokinin A, and neurokinin B, respectively [55]. Antagonism of these receptors prevents activation of G-protein coupled receptor signaling cascades, phospholipase C synthesis and ultimately, calcium flux, thereby causing relaxation [55]. The NK-2 receptor is clearly the most dominant NK receptor in the ureter, and inhibition of this receptor inhibits both spontaneous and inducible contractility [5-6]. Though these compounds have not been evaluated clinically, NK-2 antagonism would likely offer analgesia and possibly, enhanced stone passage via ureteral relaxation. Systemic effects of these compounds have not been evaluated to date, as none of these compounds are as of yet clinically available. Future research in this field should include development of clinically-available NK antagonists.

Adrenergic Receptor Agonists/Antagonists

While the presence and activity of adrenergic receptors in the ureter has long been described, the use of specific receptor modulators in ureteral physiology studies has been recently buoyed by clinical reports of stone passage induced by these compounds. There are three groups of adrenergic receptors: alpha-1 receptors that induce contractility by phospholipase-C and calcium-dependent signaling; alpha-2 receptors that induce contractility by inhibition of cyclic nucleotide signaling; and beta receptors that inhibit contractility by induction of cyclic nucleotide signaling [19]. Despite the clinical efficacy of alpha-receptor antagonists at inducing stone passage, these compounds appear to have less efficacy in vitro than the above mentioned classes of inhibitors. However, Nakada and associates have recently reported that alpha receptor antagonism causes complete ureteral relaxation only in the presence of epinephrine, the endogenous ligand of both alpha and beta receptors [56]. This suggests a key role for beta receptors in alpha antagonist efficacy, such that beta activation by epinephrine is unopposed during alpha receptor antagonism. While this has yet to be proven experimentally, studies using selective beta agonists have shown remarkable relaxation of ureteral smooth muscle [7], further supporting a role for beta receptors in this system. Selective beta agonists are available clinically for the treatment of asthma, and future studies of expulsive therapy will likely include these compounds.

Future Studies in Ureteral Physiology and Pharmacology

Several other interesting pharmacologic compounds have been evaluated in ureteral smooth muscle relaxation. 5-hydroxytryptamine (5-HT, serotonin) receptor antagonists have shown efficacy in relaxing the ureter [57], and these compounds may become of greater interest upon further receptor characterization in the ureter. Similarly, many adenosine receptor subtypes exist in the ureter, and Hernandez and associates have reported that A2B adenosine receptors mediate ureteral relaxation [58]. Selective agonists of this receptor may ultimately prove useful in relaxing the ureter and promoting stone passage. Finally, the tricyclic antidepressant amitriptyline has been shown to relax ureteral smooth muscle, though a mechanism for this action has not been defined [59].

Novel experimental models hold the potential to dynamically shift the experimental direction of ureteral research in the future. An in vivo animal model developed by Venkatesh and associates is able to measure ureteral contractility rates in vivo [60]. Using this model, this group has evaluated the in vivo relaxation effects of calcium channel blockers, neurokinin receptor antagonists, and theophyline. Future studies with this model would likely include characterizing the effects of known relaxants in vivo, in vitro, and in clinical studies.

Novel molecular and cellular biology techniques will allow the systematic determination of new therapeutic targets using genetic and biochemical knockdown of specific molecules within ureteral cells. A model developed by Jerde et al has been used to characterize COX-2 induction in ureteral-derived primary epithelial cells in

culture [40]. Using this model, our group has found that mechanically-induced COX-2 expression in cell culture mimics that of the in vivo condition. Distension-induced COX-2 expression is regulated at transcriptional and post-transcriptional levels (via mRNA stability), and is dependent upon calcium and protein kinase C-zeta signaling [61]. Future studies springing from this work would be directed toward development of a novel protein kinase C zeta inhibitor and evaluation of this compound in vitro, in animals, and ultimately, in expulsive therapy.

Summary

Relaxation of the ureter in the lab has been studied for many years, primarily for developing improved analgesia. Experimental studies show the most effective compounds have been the NSAIDs, PDE-IV inhibitors, and NK antagonists. COX inhibitors are very successful at reducing ureteral contractility, but produce systemic effects that are pronounced during acute obstruction, limiting their clinical utility. Although alpha receptor antagonism had modest effects on ureteral contractility in vitro, the presence of epinephrine significantly enhances the effect, suggesting a likely role for the beta receptors. This hypothesis is supported by studies using selective beta receptor agonists. Cellular and molecular studies are identifying new therapeutic targets (such as PKCζ) that may minimize systemic effects while optimizing therapeutic effect. Future work in ureteral physiology and pharmacology will likely include translating in vitro and molecular studies to the clinics and improving drug development of novel therapeutic agents identified in vitro or in cell assays.

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