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Which molecular targets do we need to focus on to restore voiding function?

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Abstract

The number of drugs approved for treatment of lower urinary tract symptoms (LUTS), including the overactive bladder (OAB) syndrome is limited to antimuscarinic drugs, the β3-adrenoceptor agonist, mirabegron, the phosphodiesterase-5 inhibitor, tadalafil (for male lower urinary tract symptoms), and the blocker of neuromodulator release, botulinum toxin. However, new alternatives are continually being explored. Molecular targets for new drugs may be found at different levels along the micturition reflex: the bladder/urethra themselves, their peripheral nervous control and the central nervous system. In a normal bladder, ATP contributes little to detrusor contraction. However, in the diseased bladder this is not the case and may contribute to OAB. Selective decrease of ATP release via adenosine A1 receptor stimulation may be possible and offers a potential treatment possibility. The urethra can be the starting point in the pathogenesis of OAB, and exploring structures in the urethra as treatment targets may be worthwhile. There is a medical need for relaxation of the outflow region in e.g., the neurogenic bladder, underactive bladder and in Fowler's syndrome. Candidates for relaxation of the smooth muscle may be found among e.g., the receptor subtypes of PGE_2 and PGD_2. Drugs for relaxation of the striated sphincter may target either the muscle directly or the spinal control of sphincter activity. The striated muscle of the human urethra consists mainly of type 1 fibres and drugs acting selectively on this type of fibre may be desirable. Fibrosis is a major problem in LUT dysfunction and the TGFβ pathway has been identified as an important initiator. Agents with an inhibitory effect on the TGFβ pathway, e.g, relaxin and BMP7, may be promising avenues. The central nervous system contains many possible targets for control of micturition. However, available drugs are often limited by low efficacy or adverse effects. Inhibitors of the glycine receptor Gly-T2 or adenosine A2 receptor antagonists for treatment of urinary dysfunction in Parkinson's disease await further development.

Introduction

For pharmacological treatment of lower urinary tract dysfunction (LUTS), three new drug principles have recently been approved for clinical use, represented by the β3-adrenoceptor agonist, mirabegron, the phosphodiesterase (PDE)-5 inhibitor, tadalafil (for treatment of male LUTS), and the blocker of neuromodulator release, botulinum toxin (1). These drugs have good initial response rates, but are not effective in all patients, and new alternatives are needed. Many new drug candidates were recently reviewed by the International Consultation of Incontinence (1). Based on a review of the literature on what is described as "emerging" or "innovative", drugs/targets for treatment of LUTS/overactive bladder (OAB), a long list of "possible" or "promising" alternatives was constructed (Table 1). However, a critical review of these alternatives revealed that although many agents may have theoretically interesting profiles, they do not seem to be in active development for different reasons, including insufficient efficacy or disturbing side effects.

New alternatives for treatment of LUT dysfunction are continuously being explored (1, 2). Molecular targets for new drugs may be found at different level along the micturition reflex: the bladder/urethra and their nervous control, and the central nervous system (Table 2), and the numbers of receptors potentially involved in the functional control are extensive (Table 3).

New molecular targets in the bladder

In the human bladder, both acetylcholine and ATP serve as contractile transmitters. In the normal bladder, acetylcholine is responsible for more than 90% of the contraction (3) whereas in the diseased bladder the contribution of ATP is up to 50% (4). Since increased ATP release, or decreased breakdown be ectoATPases in the nerve-muscle junction, may be

one of the factors involved in OAB symptoms and detrusor overactivity (DO), selective decrease of ATP release would theoretically be a means of decreasing OAB/DO.

Adenosine receptors and ATP release. It is well established that in most species, exocytotic vesicular release of ATP from parasympathetic neurons contributes to contraction of the bladder, particularly in the diseased bladder (5). However, ATP is released not only from parasympathetic nerves, but also from the urothelium. During bladder filling, the urothelium is stretched and ATP is released from these epithelial cells thereby activating mechano-transduction pathways. ATP release can also be induced by various mediators present in the urine and and/or released from nerves or other components of the lamina propria (2). Adenosine is a breakdown product of ATP via endonucleotidases, and stimulates different types of the P1 class of adenosine receptors (A_1, A_{2A}, A_{2B} and A_3). In the human bladder, adenosine relaxes contractions induced by agonists and electrical stimulation of nerves (6-9) and attenuates stretch-activated urothelial ATP release (2, 10). Pakzad et al. (9) demonstrated that adenosine reduced the magnitude of nerve-mediated contractions in human detrusor and suggested that adenosine preferentially reduced release of ATP rather than acetylcholine from the motor nerve terminal (Figure 1).

Theoretically, if an A_1 receptor modulator exerted no direct effect on detrusor muscle, but attenuated the release of ATP from nerve-terminals, it might be a drug target for human OAB (Figure 1). Adenosine has been used in humans, e.g., as an antiarrhymic agent (11), but is not without, sometimes serious, adverse effects. In addition, adenosine, via stimulation of A_1 and A_{2A} receptors can modulate nervous control of the micturition reflex at spinal and supraspinal sites (12, 13).

Whether adenosine can be developed as a treatment for bladder dysfunction requires further study.

Several other new targets have been suggested to be explored for potential development as treatment options for bladder dysfunction, e.g., bitter taste receptors for OAB (14), neurotensin receptors for modulation of micturition reflex and detrusor myocyte excitation-contraction coupling (15), and bombesin receptors for underactive bladder (16, 17). Further studies will reveal whether these targets hold any promise for the future.

New molecular targets in the urethra

Urethra as an initiator of OAB/DO. Afferent signaling is important in the pathophysiology of OAB/DO, and it has been speculated that the primary site may be in the urethra (18), where the greatest density of afferent nerves can be demonstrated. It has been reported that a rapid pattern of urethral pressure variation ("unstable urethra") found in women is closely associated with DO (19-22). Little is known of the signaling pathways conveying afferent activity associated with these urethral pressure changes and if they can be linked to OAB/DO. There is some evidence that the mucosal pathways within the proximal urethra, may play a role in continence and sensation (23,24), but what factors are involved have not been established. The presence of TRPV1-immunoreactive nerves in the human urethra, and the effects of capsaicin on both urethral and striated muscles (25) have raised the question whether this channel is involved in urethral functions that can be linked to OAB/DO. It was speculated that urothelial TRP receptors in the proximal urethra, activated by urine flow, may stimulate detrusor contraction. If such an effect on urethral muscle is involved in the "unstable urethra", and if it is linked to DO, remains to be established.

Need of drugs targeting urethra. Drugs are available that both increase and decrease outflow resistance. An increase of outflow resistance would be desirable in e.g., stress incontinence, but none of the drugs used has been very successful (1). Drugs for relaxation of the outflow region may be useful in neurogenic bladder, underactive bladder and in rare conditions such

as Fowler's syndrome. Targets for drug action may be either the smooth (internal sphincter) or the striated muscle (external sphincter).

Components of the urethra. In the human female, the smooth muscle of the whole length of the urethra contributes to outflow resistance. In the human male, the smooth muscle is concentrated to the proximal urethra, and this structure is believed to serve as a sexual sphincter with no role in the maintenance of continence. The external striated sphincter surrounds the urethra in both genders, but the anatomy differs (Figure 2). Human striated muscle fibres can be divided into two major types, slow twitch or fast twitch muscle fibres, depending on their speed of contraction and their susceptibility to fatigue (26-28). Slow twitch muscle fibres, also called type-1 muscle fibres, have high levels of oxidative enzymes, tend to produce small forces, and are resistant to fatigue due to their slow speed of contraction. Even if fast twitch muscle fibres (type-A and -2B) can be demonstrated, the type-1 fibres constitute the main fibre type in both the female and male striated sphincter (26).

Molecular targets for decreasing outflow resistance. Many transmitters that can relax the outflow region have been described (29). Nitric oxide (NO) seems to be an important mediator of urethral smooth muscle relaxation, but also acetylcholine, noradrenaline, neuropeptide Y (NPY), galanin, vasoactive intestinal polypeptide (VIP) and calcitonin gene-related peptide (CGRP) have been demonstrated within the urethral sphincter in patients and have relaxant effects on isolated urethral muscle (30, 31).

It is well established that prostanoids have a dual effect on lower urinary tract smooth muscle. PGE_1, PGE_2, and $PGF_{2\alpha}$ contract detrusor muscle. In the urethra, PGE_1 and PGE_2 produces relaxation, but $PGF_{2\alpha}$ causes contraction (32). In women receiving PGE_2 intravesically or intraurethrally, a decrease in the maximum urethral pressure and a reduction of the closure

pressure were found (33, 34). PGE_2 is considered to contributes to the pathophysiology of OAB and DO, and though being an agonist of EP receptors 1 to 4, the effects of PGE2 on bladder functions are via EP1 receptors (35). PGE2 results in stimulation of bladder contractile activity by sensitization of afferent nerves; levels are increased in urine from patients with LUTS (32). Additionally, PGE_2 relaxes the urethral smooth muscle and the dual direct effects on bladder and urethra may seem appropriate for bladder emptying. It would thus seem reasonable to assume that inhibition of the EP1 receptor would have a beneficial effect on OAB/DO. Despite promising results in animal experiments, a double-blind, placebo-controlled phase II study in OAB patients concluded that the role of an EP1 receptor antagonist in the management of OAB syndrome is minimal (36). This may be vary in different types of DO. In spinal cord injured (SCI) rats, Wada et al. (37) found that the PGE2-induced activation of EP1 receptors in the spinal cord contributes to the initiation of DO, and concluded that the EP1 receptor could be a therapeutic target for the treatment of neurogenic DO due to SCI. The selective EP2 receptor agonist, CP-533,536, was tested on voiding efficiency (VE) in anesthetised rats with functional urethral obstruction, induced by midodrine (a prodrug of the active metabolite desglymidodrine, an α_1-receptor agonist; Kurihara et al., 2016). CP-533,536 dose-dependently decreased perfusion pressure elevated by midodrine. However, it had no effect on maximum voiding pressure, intercontraction interval, or intravesical threshold pressure. In conscious rats, midodrine markedly increased residual volume and reduced VE and CP-533,536 dose-dependently counteracted these effects. EP3 receptors in the central nervous system exert an excitatory effect on the bladder through modulation of bladder afferents, and centrally acting EP3 receptor antagonists have been shown to inhibit bladder rhythmic contractions (39). Uniquely, EP3 receptor antagonism also has an antidiuretic effect and both effects may be useful for treating OAB (40).

Guan et al. (41) studied the effects of PGD2 on contractility of guinea pig urothelium-intact trigone and proximal urethra in organ bath experiments. They found by Western blot analysis that DP1/DP2 were expressed in the trigone and proximal urethra and that PGD2 in a dose-dependent manner inhibited trigone contractions induced by electrical field stimulation (EFS) and inhibited spontaneous contractions of the proximal urethra. PGD2 was equally (trigone) or slightly less (urethra) potent compared with PGE2. The authors concluded that PGD2 may be a modulator of the bladder out-flow region, possibly having a function in regulation of micturition and a role in the OAB syndrome.

In a rat lumbar spinal canal stenosis model of neurogenic bladder underactivity, the novel highly potent and selective agonist for EP_2 and EP_3 receptors, ONO-8055, relaxed urethral strips (42). Awake cystometry showed that ONO-8055 significantly decreased bladder capacity, post-void residual urine and voiding pressure, and significantly decreased urethral pressure. The same group evaluated the drug in an underactive bladder (UAB) model (induced by radical hysterectomy) in non-human primates (43). They found that in vitro responses to carbachol, K^+, and electrical stimulation decreased significantly, whereas in vivo voided volume, maximum and average flow rates, decreased and voiding time (VT) increased. ONO-8055 significantly improved these parameters, and the authors concluded that the drug has the potential to be a candidate for neurogenic UAB pharmacotherapy. If these results have translational impact, they would be useful in some cases of UAB.

Molecular targets for increasing outflow resistance – female stress incontinence

Among the many factors involved in the pathogenesis of stress urinary incontinence (SUI) in women are: urethral support and function; bladder neck support; and function of the nerves and musculature of the bladder, urethra, and pelvic floor (44, 45), it could be assumed the molecular targets suitable as treatment targets would be easy to identify. This does not seem

to be the case, based on the limited efficacy of current treatment options recently reviewed by the ICI (1; Table 4). It is obvious that structural factors cannot be treated pharmacologically, and drug treatment is not even mentioned as a treatment option in a recent review of SUI treatment in women (46). Stem cell treatment of SUI is an interesting avenue, but treatment efficacy seems limited (46, 47). More interesting are the findings in a non-human primate model that the cytokine CXCL12 (sometimes referred to as SDF-1) can improve intrinsic sphincter deficiency. CXCL12 plays a major role in cell trafficking and homing of progenitor cells to sites of injury. This is produced through a receptor (CXCR4) mechanism and enhances cell survival once at the injury site (48, 49). During injury, cells from the injured organ highly express CXCL12, which causes an increase of localized CXCL12 levels and peripheral and bone marrow progenitor cells follow the chemical gradient to the organ. Local injection of CXCL12 had better effect than injected skeletal muscle precursor cells in restoring sphincter structure and function (47). This raises interesting possibilities of CXCL12 therapy (alone or in combination with cells or other growth factors) for cohorts of patients resistant to cell therapy.

Cannabinoids

The mechanism by which cannabinoids exert their action on LUT control is not fully understood. Cannabinoid receptors CB1 and CB2 are distributed widely, including the central nervous system and bladder (detrusor and urothelium). Several animal studies have suggested a modulatory role of CB2 in both afferent and cholinergic nerve activity (50). In vivo, selective CB2 receptor agonists increase micturition intervals and volumes, and increased sensory thresholds (51). Two randomised controlled trials and one open-label study have shown that cannabinoids decreased the number of incontinence episodes in neurological patients (52). Uncovering the mechanism of action of cannabinoids potentially

opens up new targets to restore bladder functions, as well as development of local delivery (intravesical or intrathecal) to minimise systemic side effects (50).

New molecular targets in the lower urinary tract

Fibrosis – antifibrosis factors. Fibrosis is a major player in LUT disease, both as a contributor to the development of disease, as well as a post-injury response that drives progression (53). Despite the identification of many mechanisms responsible for fibrosis in other tissues, to date no treatments have emerged that have effectively reduced the excess deposition of extracellular matrix associated with fibrotic conditions in the LUT. Novel treatments have recently been identified that hold promise as potential therapeutic agents for cardiovascular diseases associated with fibrosis, as well as other fibrotic conditions. Transforming growth factor-beta (TGF-β), has long been identified as a key factor in fibrosis (54, 55; Figure 3). TGF-β antibodies and inhibitors of the TGF-β1 receptor (ALK-5) reduce cardiac fibrosis in animal models, but they were associated with severe adverse cardiovascular effects (56, 57). Since TGF-β inhibits inflammation, broad targeting of TGF-β may be problematic. Another factor, bone morphogenetic protein-7 (BMP7), has beneficial effects in multiple models of fibrotic disease (53). An interesting agent is the polypeptide hormone, relaxin, long known for its extracellular remodeling properties in pregnancy. Relaxin is rapidly emerging as an effective antifibrotic agent in a number of organ systems (53; Figure 4). Recently, its ability to reverse fibrosis in the rat bladder exposed to ionising radiation has been demonstrated (58).

New molecular targets in the CNS

Many parts of the brain seem to be activated during storage and voiding (59), and there is increasing interest in drugs modulating the micturition reflex by a central action (60, 61). The

main excitatory CNS transmitter is glutamate, whereas glycine, GABA (gamma-amino butyric acid), and opioid peptides (e.g., enkephalins) are the most abundantly expressed inhibitory neurotransmitters (Figure 5). Several drugs used for pain treatment also affect micturition; morphine and some antiepileptic drugs being a few examples. However, even if many drugs with a CNS action have shown a positive proof of concept (Figure 6) central nervous mechanisms have so far not been preferred targets for drugs aimed to treat OAB, since selective actions may be difficult to obtain.

Glycine is well known to have a role in the control of spinal nociceptive pathways and lower urinary tract function in both physiological and pathological conditions. Yoshikawa et al (62) determined the effects of selective glycine inhibitors administered intrathecally to cyclophosphamide (CYP)-treated rats under urethane anaesthesia. A selective GlyT2 inhibitor (ALX-1393), but not a GlyT1 inhibitor (sarcosine), produced significant increases in intercontraction interval (ICI) and micturition pressure threshold. The authors concluded that inhibition of GlyT2, but not GlyT1, could be a novel therapeutic modality for the treatment of OAB and/or bladder hypersensitive disorders such as IC/BPS, without affecting the glycinergic mechanism in the brain.

Kitta et al. (63) performed a study of the effect of an adenosine A_{2A} receptor antagonist, ZM241385 (ZM) on the micturition reflex in a rat model of Parkinson's disease (PD). The results indicated that the adenosine A_{2A} receptor-mediated excitatory mechanism is enhanced at a supraspinal site to induce bladder overactivity that can be reduced by an antagonist of A_{2A} receptors. Whether GlyT2 inhibitors or adenosine A_{2A} receptor antagonists will reach the clinic requires further study.

Near future research needs

Basic LUT research is of upmost importance in development of functional urology and emerging areas and mechanisms of action should be explored to much greater extent. In general, multiple targets (such as cannabinoids, bombesin- neurotensin and bitter taste receptors) have been explored to only a limited extent with regard to mechanisms of action and deliverance in the lower urinary tract, and their translational value to the human situation is poorly understood. The near-future pharmacological targeting needs, as recommended by the ICI-RS panel, are presented below and divided by anatomical location:

- Bladder
    o Adenosine receptor modulators are effective in reducing nerve-mediated contractions of the bladder. However, due to the (systemic) side-effects profile adenosine receptor modulators in LUT function have not been explored to significant extent. Exploring the possibilities for local administration of adenosine receptor modulators to limit systemic effects (and to avoid spinal and supraspinal effects) may be of importance in determining the feasibility of adenosine modulator agents as potentially LUT compound.
    o Fibrosis is regarded a major player in LUT dysfunctions. Therefore, the role of antifibrotic factors (i.e. relaxin) is of interest, with specific attention to the role of antifibrotic factors in voiding dysfunction. One of the main questions still to be answered is to the extent to which these factors can reverse fibrosis in the lower urinary tract. In addition, specific drug targeting in the LUT is needed as targeting the anti-inflammation pathway, in which TGF-β is involved, could elicit a major side-effect profile.
- Urethra
    o Only little is known on signaling pathways of afferent activity in association with urethral anatomy and urethral pressure changes in normal bladder filling

and if they might be linked to OAB/DO. This basic knowledge seems essential in order to understand and further explore LUT compounds that affect urethral function and afferent activity.

- o Pharmacological treatment of sphincter function improvement in SUI appears difficult and major focus has been shifted towards stem cell therapy. However, as discussed in this article, the role of cytokines involved in cell trafficking and homing of progenitor cells to sites of injury are potentially superior compared to stem cell therapy. Therefore, research emphasis in this field should be targeted towards translation of non-human primate model studies to a human proof-of-principle study.

- CNS
  - o As a specific GlyT2 inhibitor has been suggested as a novel treatment modality, further exploration with regard to the role of local glycine altered regulation in OAB and/or bladder hypersensitive disorders would be of research interest.

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Table 1.

Current status of possible future drugs/targets

Negative proof of concept

- Potassium channel openers
- Prostaglandin receptor antagonists

Positive proof of concept

- Neurokinin receptor antagonists
- Vitamin D3 receptor agonists
- Monoamine reuptake inhibitors
- Opioid receptor agonists
- Cox inhibitors

Promising based on animal data

- Rho- kinase - inhibitors
- Drugs acting on GABA receptors
- Purinergic system – P2X3 receptor antagonists
- Cannabinoid system – exocannabinoids; FAAH inhibitors
- TRP channel family – TRP channel antagonists

Table 2.

Target levels

Bladder

Bladder wall: detrusor muscle, urothelium, lamina propria structures

Nervous regulation: afferents, CNS handling, efferents

Urethra

Urethral wall: smooth muscle (internal sphincter), striated muscle (external sphincter), urothelium, lamina propria structures, including vasculature

Nervous regulation: afferents, CNS handling, efferents

Central nervous system

Brain

Spinal cord

Table 3.

Transmitter involve in the regulation of micturition

Adrenergic System	β_2 receptor			↑			↓
	β_2 receptor			↑			↓
	β_3 receptor			↑			↓
Cholinergic System	Nicotinic receptors	↑					↑
	Muscarinic receptor M_1		↑				↑
	Muscarinic receptor M_2			↓			↑
	Muscarinic receptor M_3		↑				↑
	Muscarinic receptor M_4			↓			↑
	Muscarinic receptor M_5		↑				↑
Prostanoid System	EP_1 receptor			↓			↑
	EP_2 receptor			↑			↓
	EP_3 receptor			↓			↑
	EP_4 receptor			↓			↑
Purinergic System	$P2X_2$ receptor	↑					↑
	$P2X_3$ receptor	↑					↑
	$P2X_7$ receptor	↑					↑
	$P2Y_4$ receptor		↑				↑
Cyclic Nucleotides	cAMP			↑			↓
	cGMP				↑		↓
Cannabinoid System	CB1 receptor			↓		↑	↑
	CB2 receptor			↓		↑	↑
Bombesines	BB1 receptor		↑				↑
	BB2 receptor		↑				↑
	BB3 receptor		↑				↑

Table 4.

Drugs used in the treatment of stress incontinence

Drug	Level of evidence	Grade of recommendation	
Clenbuterol	3	C	
Duloxetine	1	B	
Ephedrine	3	D	
Estrogen	2	D	
Imipramine	3	D	
Methoxamine	2	D	
Midodrine	2	C	
Norephedrine	3	D	(phenylpropanolamine)
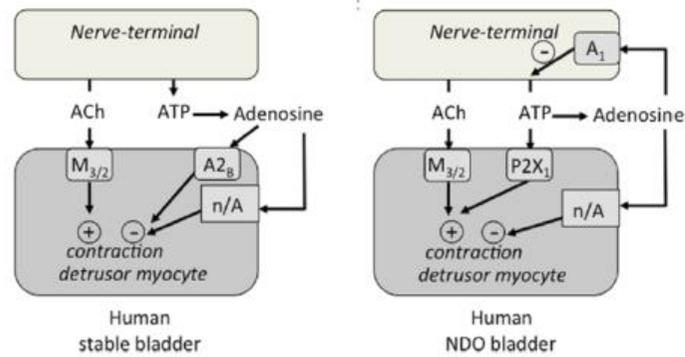
Figure 1. A model of the effects of adenosine on nerve-endings and smooth muscle to modulate contractile function in human detrusor (from Pakzad et al., 2016)
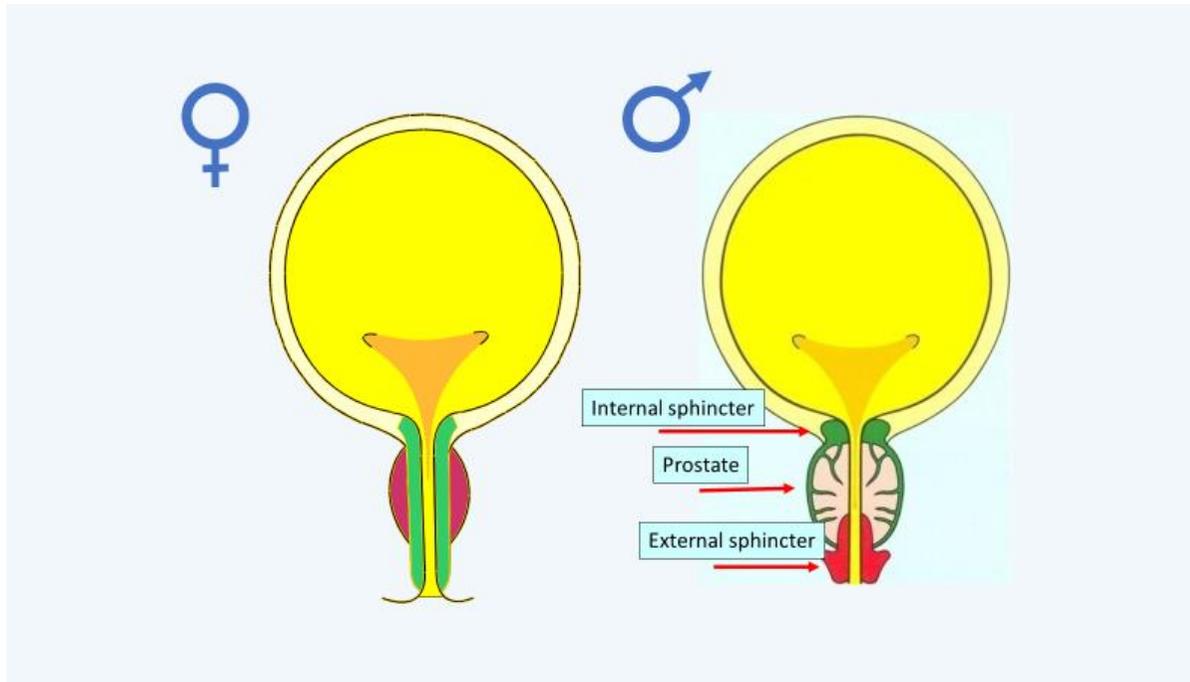
Figure 2. Gender differences in the anatomy of the urethra
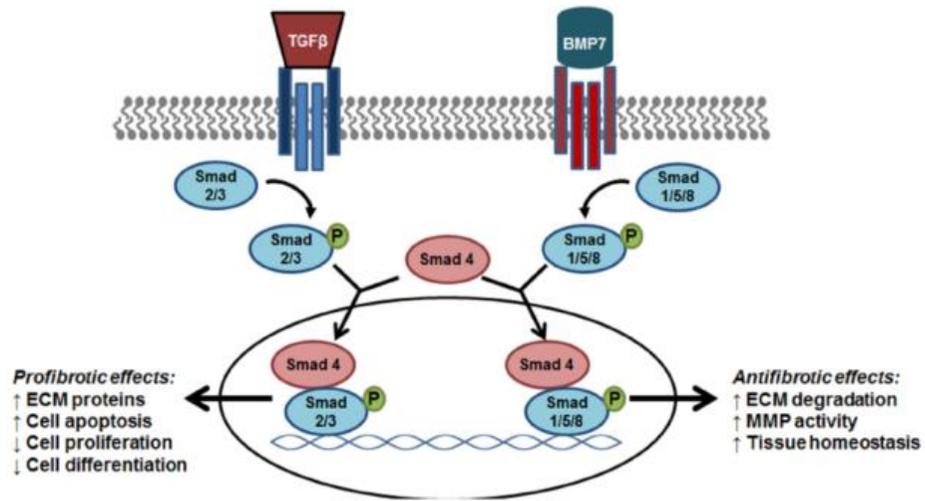
Figure 3. Profibrotic and antifibrotic effects of TGF-β and BMP7 (from McVicker and Bennett, 2017)
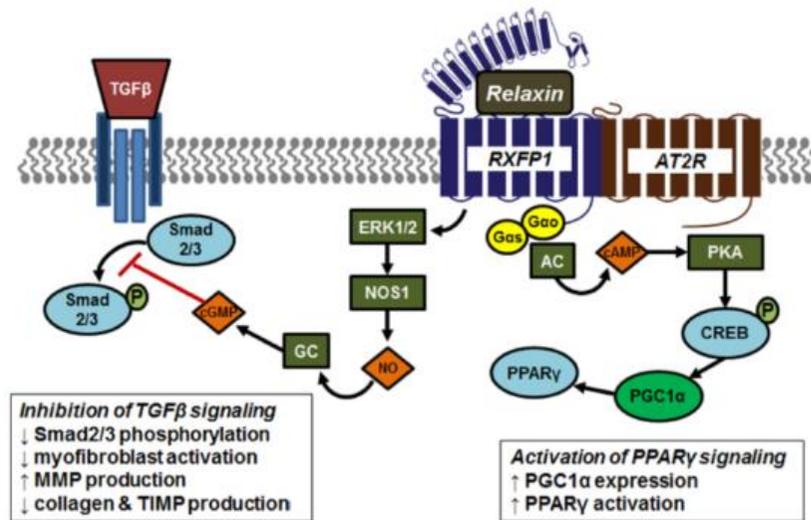
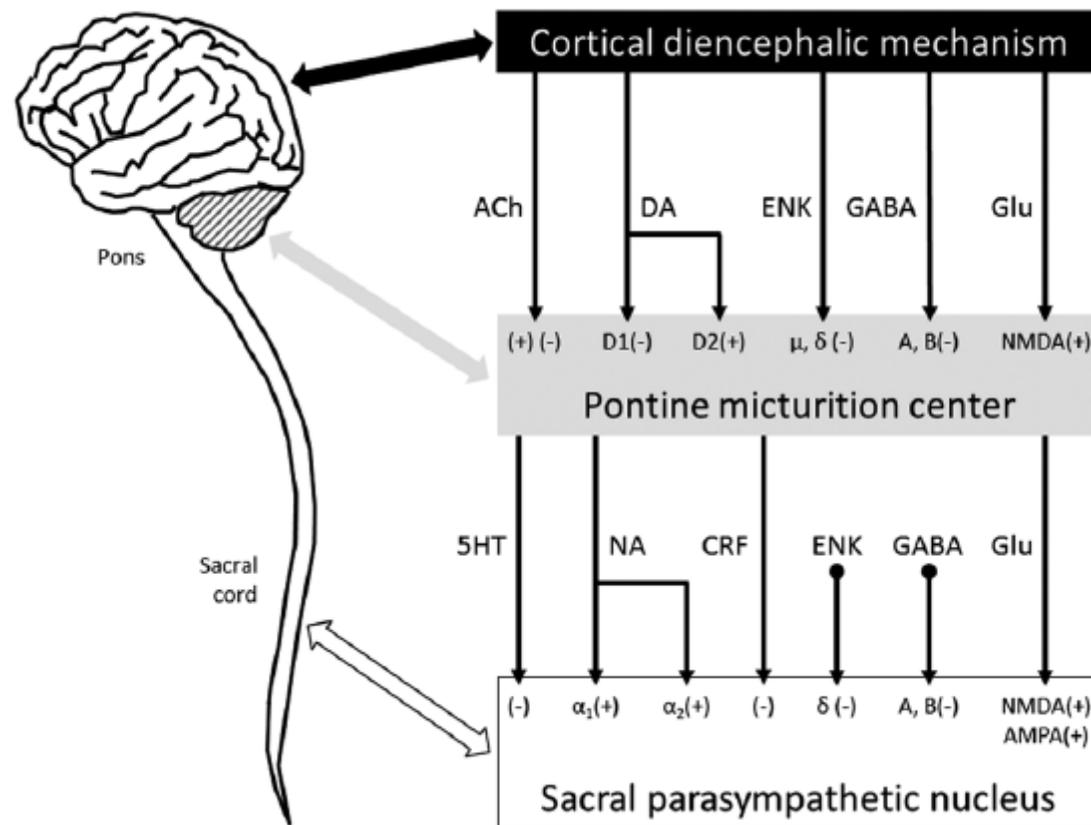**Figure 4.** Antifibrotic effect of of relaxin (from McVicker and Bennett, 2017)

Figure 5. Neurotransmitters at spinal and supraspinal sites (from Yoshimura et al., 2014)
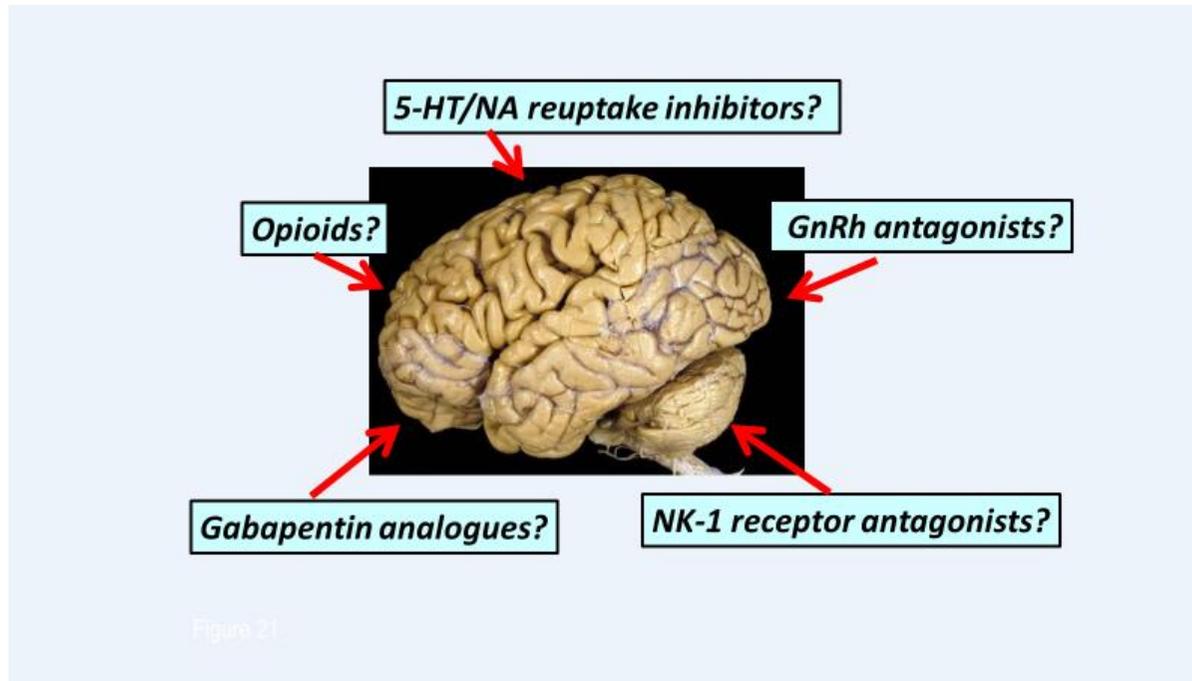
Figure 6. Drugs with a CNS mode of action with positive proof of concept. Clinical use limited by low efficacy or adverse effects