British Journal of Pharmacology

DOI:10.1111/bph.12502
www.brjpharmacol.org

Themed Section: The pharmacology of TRP channels

REVIEW

TRP channels in lower urinary tract dysfunction

J Franken[1], P Uvin[1], D De Ridder[1] and T Voets[2]

[1]*Laboratory of Experimental Urology, KU Leuven, Leuven, Belgium, and* [2]*Laboratory of Ion Channel Research, KU Leuven, Leuven, Belgium*

Correspondence
Dr Thomas Voets, Laboratory of Ion Channel Research, KU Leuven, 3000 Leuven, Belgium. E-mail: thomas.voets@med.kuleuven.be

Keywords
TRP channel; lower urinary tract; urology

Received
17 September 2013
Revised
17 October 2013
Accepted
20 October 2013

Lower urinary tract dysfunction (LUTd) represents a major healthcare problem. Although it is mostly not lethal, associated social disturbance, medical costs, loss of productivity and especially diminished quality of life should not be underestimated. Although more than 15% of people suffer from a form of LUTd to some extent, pathophysiology often remains obscure. In the past 20 years, transient receptor potential (TRP) channels have become increasingly important in this field of research. These intriguing ion channels are believed to be the main molecular sensors that generate bladder sensation. Therefore, they are intensely pursued as new drug targets for both curative and symptomatic treatment of different forms of LUTd. TRPV1 was the first of its class to be investigated. Actually, even before this channel was cloned, it had already been targeted in the bladder, with clinical trials of intravesical capsaicin instillations. Several other polymodally gated TRP channels, particularly TRPM8, TRPA1 and TRPV4, also appear to play a prominent role in bladder (patho)physiology. With this review, we provide a brief overview of current knowledge on the role of these TRP channels in LUTd and their potential as molecular targets for treatment.

LINKED ARTICLES
This article is part of a themed section on the pharmacology of TRP channels. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2014.171.issue-10

Abbreviations
ACh, acetyl choline; BCR, bladder-cooling reflex; BOO, bladder outlet obstruction; BPS, bladder pain syndrome; CYP, cyclophosphamide; DRG, dorsal root ganglion; IC, interstitial cystitis; IDO, idiopathic detrusor overactivity; LUTd, lower urinary tract dysfunction; NDO, neurogenic detrusor overactivity; NGF, nerve growth factor; OAB, overactive bladder syndrome; PBS, painful bladder syndrome; RTX, resiniferatoxin

Introduction

Lower urinary tract dysfunction (LUTd)

While reading this text, most of you are probably unaware of the fact that your bladder is constantly filling. At a certain moment, you will suddenly sense that urine is accumulating, and if you are in an unfamiliar location, you will probably start looking around for a toilet. Still, you can postpone the actual voiding for an extended period of time, until an urgent desire to void will make further delay impossible. Or perhaps you may decide to go earlier, before the bladder is completely full, just because it is a convenient time for you. In the majority of the population, this process repeats itself a couple of times per day, seemingly automatically and probably without problems.

The situation is much less obvious when you are one of roughly 1 billion people worldwide suffering a form of LUTd. Achieving the task to store urine until it is socially acceptable and desirable to expel it requires perfect synchronization and integration of the bladder, the urethral sphincter, the afferent and efferent innervation, the spinal cord and the brain, and dysfunction at any level can lead to LUTd, with symptoms depending on the aetiology of the problem. A never-diminishing urge to run for the bathroom, having to void more than 10 times per day or pain with every void are only a few examples of how this simple physiological process can turn into a real handicap.

Severe dysfunction of the bladder or urethra indeed has a significant impact on the quality of life. Disability can range from slight social disturbances to being almost unbearable. In addition, these conditions are costly to treat, both for patients and for society. As an example, the direct cost of urinary incontinence in only the United States amounts yearly to $16.3 billion. Often, patients are being treated for

prolonged periods of time, mostly only symptomatically, because the underlying pathophysiology is still obscure. It is evident that better insight and new treatments are needed to tackle LUTd-related problems at the root.

Targeting transient receptor potential (TRP) channels to treat LUTd

Only late in the 20th century, urological research started to pay attention to the concept of sensation in the lower urinary tract. Before that, the bladder was widely depicted as a muscular bag, merely able to contract and to expel urine when the brain would command so. However, today it is generally accepted that correct bladder function critically depends on correct sensory information about the filling state and the content of the bladder, and that many pathologies arise from a dysfunctional afferent pathway.

Together with this change of view emerged the interest in TRP channels. These channels are ubiquitously expressed throughout the body and exert a plethora of functions, many of which are probably still to be discovered. Several TRP channels have been attributed a role in stimulus detection and sensory signalling, for which they have been put forward as the prime cellular sensors. Indeed, all five senses are – to a certain extent – regulated by TRPs. However, classifying TRP channels as mere sensors would understate the diversity of their functions because also diverse functions such as bowel movement, regulation of core body temperature and cardiac contractility depend on these channels.

At this point, it has been reported that genetic deletion of two TRP channels in mice, TRPV1 and TRPV4, leads to specific alterations in the functional parameters of the bladder, suggesting that they are crucial for normal bladder physiology. In this review, we only discuss those TRP channels (of a total 28 mammalian TRP channels) whose relevance to the field of urology has been described, and focus on the current knowledge about function and therapeutic potential of TRP channels related to LUTd.

LUTd with bladder overactivity

The terminology to describe LUTd associated with an increase in real or perceived bladder activity is not always used in an unambiguous manner. Detrusor overactivity (DO) can be objectively identified based on urodynamic observations of increased activity of the detrusor muscle (Figure 1). In contrast, overactive bladder syndrome (OAB) is a symptom complex, where the defining symptom is the subjective sensation of urgency in a patient (Abrams *et al.*, 2003). There is a good reason to distinguish both terms in the context of this review. One can find many reports that extrapolate conclusions from animal models directly to human OAB. However, one should be cautious with such interpretations as urgency, the key symptom of OAB, cannot be assessed in current animal models (Parsons and Drake, 2011). Although OAB patients often have objectively identified urodynamic patterns of DO, urgency can also be present without any objective DO. Depending on the underlying cause, a further distinction can be made between idiopathic DO (IDO) and neurogenic DO (NDO).

NDO

This specific form of DO encompasses all pathologies in which a neurological problem causes measurable overactivity of the detrusor muscle. Surprisingly, little data exist on its overall prevalence, but estimations can be made from the prevalence of underlying conditions and their relative risk to develop NDO (Table 1). Suprasacral neurological lesions almost invariably cause NDO (Madersbacher, 1990). There is a general consensus that this is due to the emergence of a spinal reflex arc caused by disinhibition of otherwise silent C-fibre afferents in the suburothelial layers (de Groat, 1997; Fowler, 2002). More recently, it has been suggested that the urothelial layer itself could also be involved in this reflex mechanism. There is indeed growing evidence that a large portion of the afferent signal in the bladder is generated by urothelial cells, and that deregulation of this signalling could contribute to the development of NDO (Birder and Andersson, 2013).

Although the mechanisms causing NDO are not fully understood, a variety of therapeutic strategies have already been employed. The current gold standard in first-line treatment is antimuscarinic drugs (De Ridder *et al.*, 2005), which primarily aim to inhibit the activity of the detrusor muscle by blocking muscarinic ACh receptors. Lately, reports have been emerging about its potential effect on afferent signalling as well (Andersson, 2011). However, these drugs have only limited effectivity and, especially in NDO patients, some bothersome side effects, including constipation, dryness of the mucosae and cognitive impairment. Therefore, instead of inhibiting the efferent signal to the detrusor muscle, interest has increased in interrupting the afferent limb of the C-fibre reflex. In this respect, significant interest has gone to the potential involvement of TRP channels.

TRPV1 – a heat- and vanilloid-sensitive TRP. Several years before the cloning of the first TRP channel gene, it was already known that capsaicin, the pungent compound in hot chilli peppers, modulates the micturition reflex in both rats and humans (Maggi *et al.*, 1986; 1989). In particular, intravesical administration of capsaicin had led to a disappearance or marked reduction of symptoms in NDO patients (Maggi *et al.*, 1989). After the cloning of TRPV1, originally described as the vanilloid receptor, and its characterization as a heat- and capsaicin-activated non-selective cation channel in sensory neurons in 1997, this channel has been a logical choice in the quest for a new NDO treatment.

Many groups have investigated the site of expression of TRPV1 in the normal bladder. Whereas there is general consensus that the channel is expressed in C-fibres innervating the bladder, there is ongoing debate whether there is also (physiologically relevant) expression in urothelial cells. Birder *et al.* (2001) were the first to describe the formerly called vanilloid type 1 receptor (VR-1) in rat and mouse urothelial cells, using reverse transcription PCR, calcium imaging and immunofluorescence. In 2004, immunofluorescence performed on human urothelium revealed TRPV1 expression as well (Lazzeri *et al.*, 2004). However, Yiangou *et al.* (2001) had performed similar experiments in human bladder before, using Western blot and immunocytochemistry, but they only described the suburothelium to be positive, while urothelium was not mentioned. Next, Avelino *et al.*

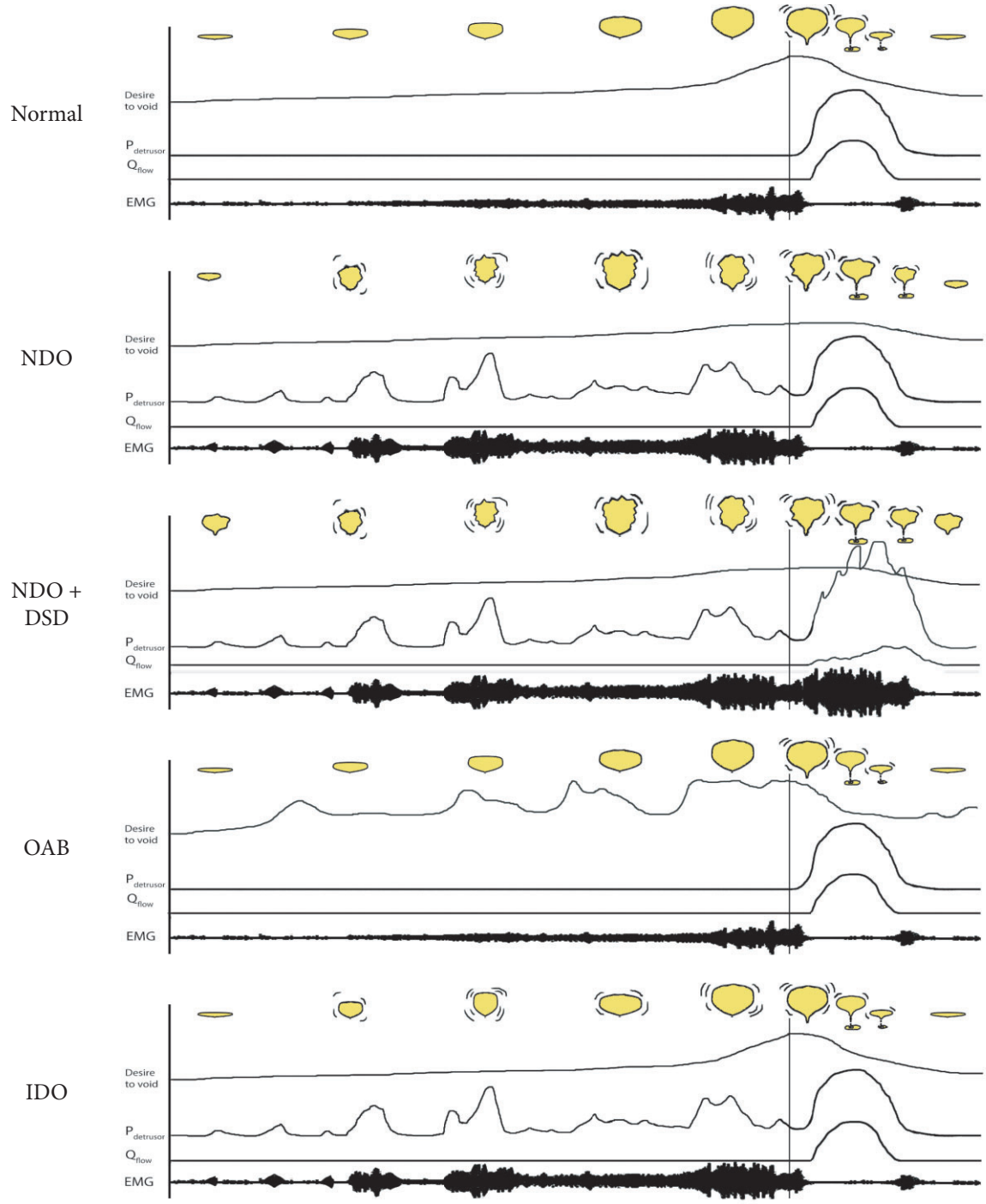

Figure 1

Schematic traces of video-urodynamic recordings, as seen in patients with different types of LUTd. LUTd can often be objectively measured by video-urodynamic recording. Although $P_{detrusor}$, a measure of the force generated by the bladder muscle, cannot be measured directly, it can be calculated by subtracting abdominal pressure (measured by a rectal catheter) from intravesical pressure (measured by an intravesical catheter). Q_{flow} visualizes the flow of urine through the urethra. In the EMG trace, activity of the urethral sphincter is measured. *Top figure:* Normal filling and voiding, as one would expect to see in a healthy person. *Second:* In patients with neurogenic detrusor overactivity (NDO), the bladder often has an altered shape, desire to void is less prominent due to the loss of afferent input and the detrusor muscle is overactive. *Third:* NDO is often accompanied by detrusor sphincter dyssynergia (DSD). The same features of NDO are present, but during voiding, the sphincter fails to relax, causing high intravesical pressure and reduced emptying of the bladder. *Fourth:* In patients with overactive bladder syndrome (OAB), bladder pressure, urine output, bladder shape and EMG recording are all normal, but patients report a strong desire to void at much lower volumes than normal controls. Maximal bladder volume is often also lower. *Last:* In contrast to OAB, idiopathic detrusor overactivity (IDO) does not cause earlier desire to void, but the detrusor is more active during the filling phase. OAB and IDO are often present simultaneously in patients.

Table 1

Most prevalent aetiologies of neurogenic lower urinary tract dysfunction (NLUTd), with their incidences and their relative risk to develop NLUTd

Cause	Incidence	RR (%)	References
Pontine glioma	5–10/1 000 000	71	Renier and Gabreels (1980); Freeman and Farmer (1998)
Alzheimer dementia	5–8/1000	23–48	Cacabelos *et al.* (1996); Di Carlo *et al.* (2002)
Mental retardation	1/1000	12–65	Mitchell and Woodthorpe (1981); Katusic *et al.* (1996)
Parkinson	13/100 000	38–70	Campos-Sousa *et al.* (2003); Van Den Eeden (2003)
Multiple sclerosis	2.8/100 000	50–90	Giannantoni *et al.* (1999); Dua *et al.* (2008)
Spinal cord lesions	30–40/1 000 000	99	Burns *et al.* (2001)
Spinal stenosis	90/100 000	61–62	Kawaguchi *et al.* (2001); Steurer *et al.* (2010)
Diabetes	10–16/1000	50	Ellenberg (1980); Diabetes Prevention Program Research Group *et al.* (2009)

(2002) provided a full description of VR-1 in the rat urinary tract, but again, only suburothelial staining of the nerve fibres was described. In addition, three other papers casted doubt on the expression of TRPV1 in the urothelium. In these articles, mouse and guinea pig urothelium was investigated for functional expression of TRPV1 using calcium imaging, but no responses were recorded (Xu *et al.*, 2009; Yamada *et al.*, 2009). Furthermore, commercially available antibodies used for immunohistochemistry proved to be unreliable for detection of TRPV1 as they produced the same staining in TRPV1 knockout (KO) mice (Everaerts *et al.*, 2009). One recent and very interesting paper seems to tip the balance in favour of the latter findings. Cavanaugh *et al.* (2011) investigated TRPV1 expression in TRPV1 reporter mice, which revealed a highly restricted brain distribution and expression in arteriolar smooth muscle cells, but no expression in urothelium. Analysing these discrepancies in the literature, they may reflect methodological approaches, antibody specificity issues and probably also species differences. We believe more and stronger evidence of TRPV1 expression in human urothelium is needed before we can make a firm statement that the channel is functionally present in these cells.

Despite the fact that the sites of TRPV1 expression in normal human bladder are not unambiguously known, a multitude of studies have further investigated the therapeutic potential of TRPV1 agonists to treat NDO. The leading theory is that strong activation of TRPV1 with agonists such as capsaicin is followed by prolonged desensitization of the channel and of the C-fibres, including depletion of neuropeptide stores in the nerve endings (Maggi, 1990). This way, the afferent limb of the spinal reflex arc would be impaired and thereby reduce symptoms in NDO. In addition, it has been suggested that capsaicin might also work via TRPV1 on urothelial cells, although the mechanism whereby activation/desensitization of urothelial cells would affect NDO symptoms is unclear. Moreover, capsaicin may induce cell death independently of TRPV1 (Bley, 2012) by interfering with mitochondrial electron transport (Athanasiou *et al.*, 2007).

Clinically, the first results of intravesical capsaicin treatment in NDO patients were quite spectacular, resulting in increased bladder capacity, reduced urinary frequency and less urge incontinence (Maggi *et al.*, 1989; Fowler *et al.*, 1992; Barbanti *et al.*, 1993; Geirsson *et al.*, 1995; Chandiramani *et al.*, 1996; Cruz *et al.*, 1997; De Ridder *et al.*, 1997; de Sèze *et al.*, 1998; Wiart *et al.*, 1998). In fact, 84% experienced significant improvement of their complaints (de Sèze *et al.*, 1999). Unfortunately, patients invariably suffered from severe side effects, including bladder pain, suprapubic burning and strong overactivity, which in many cases made the therapy hardly bearable and raised questions about potentially irreversible neurotoxicity. In addition, due to its low aqueous solubility capsaicin was difficult to apply, requiring high concentrations of ethanol (up to 30%), which is not an inert vehicle (Ost *et al.*, 2003).

To overcome these side effects, other TRPV1 agonists have been investigated. At this point, resiniferatoxin (RTX), a natural compound from the *Euphorbia resinifera* plant, is the most promising alternative to capsaicin. In comparison with capsaicin, RTX can activate and desensitize TRPV1 at 1000-fold lower concentrations, which results in less side effects (Maggi *et al.*, 1990; Cruz, 1998). In a multicentre, placebo-controlled randomized controlled trial (RCT) including 36 NDO patients, RTX was administered intravesically in doses ranging from 0.005 to 1 μM. Although results did not reach significance due to small sample size, bladder capacity increased on average by 50%. Patients with a capacity lower than 300 mL at baseline showed the greatest improvement, even up to 500% in some cases. Only modest discomfort was observed (Kim *et al.*, 2003). In comparison with capsaicin, RTX provided a larger improvement of NDO symptoms in spinal cord injury patients, whereas side effects were indeed less apparent (Giannantoni *et al.*, 2004). Several more trials were performed, investigating a total number of 196 patients. Although trial design, RTX concentration, vehicles and outcome parameters differed significantly, all studies reported symptom improvement after RTX instillation (Lazzeri *et al.*, 2000; Kuo, 2003a,b; Watanabe *et al.*, 2004; Silva *et al.*, 2005; Kuo *et al.*, 2006). Even the least optimistic study still reported that 30% of NDO patients responded to treatment with RTX (Kuo, 2005). Interestingly, it was recently shown that botulinum toxin A, which is used as a second-line treatment for NDO, reduced TRPV1 expression in bladder biopts of patients (Giannantoni *et al.*, 2013). This might indicate that, in addition to its known effects on the release of ACh by efferent neurons, reduction of TRPV1-mediated afferent signals could

contribute to the beneficial effects of botulinum toxin A in NDO patients.

In addition to the use of TRPV1 agonists to treat NDO, several studies have addressed the question whether TRPV1 expression in the bladder is altered in NDO patients. In a small series, immunoreactivity in the suburothelium and in the basal urothelial layers was increased in comparison with control patients (Brady *et al*., 2004), and a significant reduction in immunoreactivity was observed in NDO patients that responded positively to RTX treatment (Apostolidis *et al*., 2005). However, some caution may be warranted when interpreting these data, given the questionable specificity of several commercially available TRPV1 antibodies (Everaerts *et al*., 2009).

As an alternative to TRPV1 agonists, which cause desensitization of TRPV1-expressing (neuronal) cells, several preclinical studies have also evaluated the potential of TRPV1 antagonists. Such drugs may be expected to reduce TRPV1-dependent afferent activity, without causing pain and burning associated with the use of TRPV1 agonists. In a rat model of NDO, the TRPV1 antagonist GRC-6211 was indeed effective in reducing detrusor muscle contraction frequency (Santos-Silva *et al*., 2012). However, clinical trials in humans have not yet been performed because the first generations of TRPV1 antagonists caused hyperthermia and impaired noxious heat sensation, which have hampered their further development. A comprehensive review on pharmacology and clinical trials with TRPV1 antagonists can be found in Brederson *et al*. (2013).

In conclusion, promising results in treatment of NDO were obtained with intravesical capsaicin and RTX. However, their mechanism of action is not yet fully elucidated, and controversy remains about their general usefulness and long-term safety in treatment of NDO. An important reason for this lack of general adaption may have been the surging popularity of antimuscarinics as a relatively tolerable and harmless first-line treatment. However, given that the long-term efficacy of antimuscarinics is a questionable one, further research into TRPV1 as a molecular target is certainly warranted (Uvin *et al*., 2013b).

TRPA1 and TRPM8 – cold- and menthol-sensitive TRP channels. TRPA1 was first characterized as a cold-activated cation channel activated at noxiously cold temperatures (Story *et al*., 2003). Although the overall evidence is currently much weaker than in the case of TRPV1, recent research has started to focus on the potential implications of TRPA1 in NDO. Besides cold, TRPA1 can be activated by a huge variation of irritant/noxious chemicals, including plant-derived compounds such as mustard oil, cinnamaldehyde, nicotine, menthol or allicin, and by various electrophiles such as formaldehyde, hydrogen peroxide, acrolein, 4-hydroxynonenal, hydrogen sulphide, tear gasses and more (Fernandes *et al*., 2012; Vay *et al*., 2012). In the bladder, TRPA1 has been located in sensory nerve terminals (Nagata *et al*., 2005) and in the urothelium in rats, but not in mice (Streng *et al*., 2008; Everaerts *et al*., 2010a). Retrograde tracing studies confirmed that about 50% of dorsal root ganglion (DRG) neurons that innervate the bladder express TRPA1 (Madersbacher, 1990; Du *et al*., 2008). The findings that many sensory neurons coexpress TRPA1 and TRPV1 (Du *et al*., 2008), and that TRPA1 and TRPV1 can be functionally coupled (Akopian *et al*., 2008), made TRPA1 a logical target for NDO research.

However, in contrast to TRPV1, where capsaicin and RTX are highly selective agonists that can be used *in vivo* and even in patients, tools for studying TRPA1 are much less potent and versatile. Whereas bladder instillation of TRPA1 agonists contracts isolated rat urinary bladder strips (Andrade *et al*., 2006) and causes DO (Du *et al*., 2007; Streng *et al*., 2008), it is not always clear that this represents a TRPA1-dependent effect. For instance, the frequently used TRPA1 agonist mustard oil (allyl isothiocyanate) also activates TRPV1, thereby contributing to the observed DO (Everaerts *et al*., 2011), and also has a direct effect on L-type calcium channels (Capasso *et al*., 2012). Data concerning the functional role of TRPA1 should thus be interpreted with care, and TRPA1 KO animals should be used for validation whenever possible. Interestingly, pharmacological inhibition of TRPA1 using HC030031, a widely used TRPA1 antagonist with high specificity versus other sensory TRP channels (McNamara *et al*., 2007), ameliorates bladder overactivity in a rat model of spinal cord injury (Andrade *et al*., 2011). The mechanisms by which TRPA1 may affect NDO remain largely unknown but may be related to its role in initiating the afferent signal from the bladder, similar to what was described earlier for TRPV1.

The bladder-cooling reflex (BCR) represents another potential but poorly understood link between NDO and cold-activated TRP channels such as TRPA1 and TRPM8. In 1957, Bors and Blinn first described that injection of ice-cold water in the bladder (the so-called ice-water test) elicited a bladder contraction in patients with upper motor neuron lesions, but not healthy subjects or patients with lower motor neuron lesions. In the era before MRI and CT scans were available, the ice-water test served as a diagnostic tool to identify neurologic lesions as the cause of DO.

Today, MRI has largely abolished the diagnostic value of the BCR, and BCRs have also been found in patients lacking obvious neurological lesions. However, understanding its mechanisms may provide important clues about bladder physiology and NDO. Multiple studies have investigated the ice-water test in animals. A BCR could successfully be elicited in anaesthetized cats, but not in awake animals (Fall *et al*., 1990; Jiang *et al*., 2002; Lindström *et al*., 2004; Maziéres *et al*., 2006). This strengthened the hypothesis that C-fibres, which are suppressed by CNS control in healthy awake animals, are responsible for the effect. Given that TRPA1 and TRPM8 have been put forward as the main cold sensors in our somatosensory system and are expressed in sensory neurons innervating internal organs including the bladder, they are prime molecular candidates for initiating the BCR response. Tsukimi *et al*. were the first to suggest that TRPM8 could be the receptor activating the reflex. They found that the BCR is present in guinea pigs, but only after intravesical pretreatment with the archetypical TRPM8 agonist menthol (Tsukimi *et al*., 2005). Similarly, infusion of ice-cold saline in the bladder of anaesthetized guinea pigs (but not awake animals) lowered threshold volume, an effect that was enhanced by menthol exposure (Jiang *et al*., 2008). Nomoto *et al*. (2008) also found that the intravesical infusion of menthol facilitated the micturition reflex in rats, whereas capsaicin pretreatment had no effect on this response.

Although these studies all concluded that TRPM8 is a key sensor for triggering the BCR, some caution is certainly warranted. First, it is now well established that cold and menthol activate both TRPM8 and TRPA1, so the latter channel should not be disregarded (Karashima *et al.*, 2007) in this context. Second, although the hypothesis that the BCR results from a C-fibre reflex arc has almost been considered a fact, there are reports showing that cooling induces contraction of isolated rat detrusor muscle (Mustafa and Thulesius, 1999; Atalik *et al.*, 2010). Obviously, in this experimental setting all communication with the spinal cord is interrupted, which seems to argue against a purely C-fibre reflex arc-mediated phenomenon. The question then remains why the BCR is absent in healthy awake subjects. Preliminary results from our laboratory indicate that stimulation of β-adrenergic receptors can effectively reduce cold-induced contractility of bladder strips. These findings may indicate that constant orthosympatic stimulation of the bladder may be the mechanism whereby the BCR is suppressed in healthy awake subjects (Uvin *et al.*, 2013a). Clearly, further research is needed to understand the mechanisms that trigger the BCR and the role of TRP channels therein.

Overactive bladder and IDO

OAB exists in different forms and severities but always includes the defining symptom of urgency. Although sometimes an objective cause (e.g. a neurological lesion or cystitis) can be identified, the aetiology is mostly unknown. Because OAB is often associated with DO, OAB and IDO are closely related. Until now, first-line treatment has been limited to anticholinergic drugs. Recently, β_3-receptor agonists have come to strengthen the urologist's armoury. These treatments are mainly aimed at relaxing the detrusor muscle. However, there is growing evidence that TRP channels may also have therapeutic value in IDO and/or OAB. The underlying rationale is to target the afferent signal generation in the bladder, in which TRPs are believed to be highly involved. Unfortunately, there are currently no established animal models for OAB, which is due to the fact that the feeling of urgency cannot (yet) be measured in animals. Unravelling the expression pattern, function and role of these channels will not only provide us with new therapeutic options, but it will also help us in our understanding of IDO and OAB pathogenesis.

TRPV1, jack of all trades? Hypothesizing that TRPV1 may be involved in abnormal bladder sensation in response to otherwise harmless stimuli, Liu *et al.* (2007) investigated an alteration of TRPV1 expression in bladder samples of both OAB and IDO patients. They found that TRPV1 mRNA expression was up-regulated in OAB patients, and found a correlation between a low volume at first sensation of filling and TRPV1 expression levels. The same authors later showed that also a small portion of IDO patients had higher TRPV1 mRNA levels, and these were more likely to benefit from RTX treatment than IDO patients without TRPV1 up-regulation (Liu and Kuo, 2007). In addition to these reported changes in TRPV1 expression, there are also indications for altered TRPV1 function in OAB. Li *et al.* (2011) reported increased calcium influx and larger whole-cell currents in response to 6 μM capsaicin in primary urothelial cell cultures from two OAB patients, and these responses were blocked by the TRPV1 antagonist capsazepine. Similarly, Birder *et al.* (2013) showed significantly higher TRPV1 protein expression and capsaicin-induced release of ATP in urothelial cells from OAB patients compared with control samples.

Although these data are indicative of a potential role of TRPV1 as a target to treat OAB and selected IDO patients, to our knowledge only one clinical trial with RTX instillations has been conducted to address this possibility (Silva *et al.*, 2007). In this study, 23 OAB patients with refractory urgency were included, although, unfortunately, a clear subdivision between OAB, IDO and NDO was not made. First, all patients received an instillation of the vehicle (10% ethanol in saline), and after 30 days, RTX 50 nM was administered. Data were based on self-made voiding charts before and after RTX instillation, and on the subjective impression of patients after treatment. The authors reported a significant improvement of voiding symptoms, and two-thirds of patients were willing to repeat the RTX instillation. These data are promising, but clearly further investigation is needed.

In summary, given the lack of high-level evidence, vanilloids currently have very limited use in the treatment of OAB and IDO. Again, development of selective antagonists with reduced side effects could provide us with new options to treat selected OAB or IDO patients in the future.

TRPM8. The expression pattern of the cold- and menthol-sensitive TRPM8 in the normal human bladder is not yet fully established, whereas its expression in a subset of DRG neurons is widely acknowledged. TRPM8 expression has also been reported in the urothelium of both humans and rats (Stein *et al.*, 2004; Kullmann *et al.*, 2009), but we were unable to confirm molecular or functional TRPM8 expression in mouse urothelium (Everaerts *et al.*, 2010a). Although Du *et al.* (2008) found TRPM8 mRNA expression in the human urothelium, they also questioned its physiological relevance, noting that expression was more than 3000-fold lower than in prostate tissue. Again, methodological variances, lack of antibody specificity and species differences have been teaming up to cause confusion.

TRPM8 is known as the predominant thermoceptor for cellular and behavioural responses to cold temperatures (Knowlton and McKemy, 2011) and it has been implicated in pain sensation (Colburn *et al.*, 2007). Mukerji *et al.* investigated changes in TRPM8 expression in bladder biopsies from patients having painful bladder syndrome (see below) or IDO compared with healthy controls. They not only found that patients with bladder pain syndrome (BPS) showed a marked increase in expression, but also noted that both density and intensity of immunoreactivity were increased in IDO patients. Immunoreactivity was linearly correlated with frequency and pain, but not with urgency symptoms, as reported by the patient through validated scores (Mukerji *et al.*, 2006c).

However, at this point, the consequences of increased TRPM8 expression and/or function in the bladder remain unclear. Menthol and the more potent agonist icilin have been used to probe TRPM8, either in isolated bladder strips or upon intravesical instillation (Nomoto *et al.*, 2008; Vahabi *et al.*, 2013), leading to altered carbachol-induced contractions, but also lower micturition thresholds and increased

frequency. However, given the lack of specificity of menthol and icilin, interpretation of these results is ambiguous (Karashima *et al*., 2007; Story *et al*., 2003). Overall, evidence for a specific role for TRPM8, both in healthy bladder and in OAB or IDO, remains sparse.

Recently, TRPM8 has also been put forward to play a role in a specific form of bladder overactivity, namely, cold urgency. OAB patients frequently present with uncontrollable desire to void when exposed to environmental cold (cold weather, air conditioning or bare feet on a cold floor). Although this is also sometimes observed in healthy subjects, it is more frequent in OAB patients, where 46% claim that cold weather aggravates their symptoms (Ghei and Malone-Lee, 2005). External cold can also elicit measurable DO in animals. When conscious rats were transferred to a lower temperature, voiding interval and bladder capacity significantly decreased (Imamura *et al*., 2008). Spraying menthol (50–99%) on the back or hindpaw also elicited DO (Chen *et al*., 2010). By retrograde labelling of DRG neurons, Shibata *et al*. found that many sensory afferents are dichotomizing, innervating both skin and bladder. *In situ* hybridization showed TRPM8 mRNA in 8% of these cells, which led the authors to suggest that these axons could be responsible for the urinary urgency evoked by cold sensation (Shibata *et al*., 2011). These promising results suggest that selective and safe TRPM8 antagonists may cause benefits in the large group of patients with cold-aggravated OAB.

TRPV4 – a stretch sensor in the bladder? TRPV4 is a ubiquitously expressed cation channel with multiple functions in different organ systems. It is activated by hypotonicity-induced cell swelling, moderate heat, several chemical substances and importantly also by stretch and shear stress, although it is highly debatable whether the channel itself is mechanosensitive (Liedtke *et al*., 2007b). In a comprehensive review, Nilius and Voets (2013) summarized the puzzling results obtained from previous research on TRPV4.

Interest in TRPV4 as a therapeutic target for LUTd grew with the discovery that mice lacking TRPV4 have a clearly altered LUT function (Gevaert *et al*., 2007). In urinary spotting experiments, TRPV4 KO mice tended to void more and bigger spots, suggesting higher bladder capacity. In addition, more spots were seen in the centre of the cage, suggestive of incontinence. Cystometry confirmed these findings, with more non-voiding contractions during storage and longer periods between two voids. Spontaneous activity of detrusor preparations was also altered: TRPV4 KO mice seemed to have a decrease in frequency, but an increase in amplitude of autonomous contractions. These findings led to the idea that TRPV4 functions as a stretch sensor in the bladder. As such, increased expression/sensitivity of the channel could thus contribute to DO and possibly OAB, whereas inhibition of the channel may provide symptomatic improvement of urinary frequency in various forms of LUTd. Everaerts *et al*. (2010b) provided proof of principle that pharmacological inhibition of TRPV4 can indeed reduce voiding frequency in healthy rats and mice, as well as in animals with increased frequency due to cyclophosphamide (CYP)-induced cystitis.

Another potential link between TRPV4 and OAB is provided by the recent discovery that gain of function mutations in TRPV4 are the direct cause of several hereditary human diseases, including various forms of skeletal dysplasias as well as motor/sensory neuropathies (Nilius and Voets, 2013). Although not studied in great depth, there is apparently a high incidence of LUTd in subsets of patients with such TRPV4 mutations. This was particularly the case in patients with Charcot–Marie–Tooth type 2c, which not only exhibit progressive muscle wasting and loss of sensory function, but also a high incidence of OAB – sometimes with incontinence (Landouré *et al*., 2010). It remains to be determined, however, whether these symptoms are a consequence of increased TRPV4 activity in the bladder wall or represent a mere consequence of the axonal damage in these patients.

LUTd with pain and irritation

According to the definition of the International Continence Society, painful bladder syndrome (PBS), also known as BPS, is 'the complaint of suprapubic pain related to bladder filling, accompanied by other symptoms such as increased day-time and night-time frequency, in the absence of proven urinary infection or other obvious pathology' (Abrams *et al*., 2003, p. 40). The term PBS thus describes a symptom complex, as subjectively experienced by patients. PBS is often equalled to interstitial cystitis (IC). However, IC is a specific diagnosis that requires confirmation based on typical cystoscopic and histological features, such as Hunner's ulcer, thickening of the bladder wall, urothelial denudation and intense subepithelial reaction. By far, the most prevalent cause of bladder-related pain is cystitis due to bacterial infection. In addition, bladder pain and irritation can also result from non-infectious aetiologies such as radiation cystitis, drug-induced cystitis and foreign body cystitis. Both infectious and non-infectious forms are difficult to treat and easily relapse.

Painful bladder syndrome, BPS and IC

These forms of LUTd are pain syndromes. Given the well-established role of several TRP channels in various forms of acute and chronic pain, a contribution of these channels to PBS or IC is not unexpected.

TRPM8. Defining a general role for TRPM8 in pain is not straightforward. On the one hand, TRPM8-activating stimuli such as cold and menthol are known for ages to provide analgesia, and there is indeed evidence that this represents a TRPM8-dependent effect (Proudfoot *et al*., 2006; Jordt and Ehrlich, 2007; Klein *et al*., 2010). On the other hand, TRPM8 is also (at least partially) responsible for cold-induced pain and cold hyperalgesia following nerve injury or inflammation (Colburn *et al*., 2007; Xing *et al*., 2007; Knowlton and McKemy, 2011).

In the bladder, TRPM8 immunostaining of suburothelial C-fibres was significantly enhanced in a group of PBS patients (Mukerji *et al*., 2006c). In addition, the density of positive nerve fibres showed a clear correlation with urinary frequency and pain scores in these patients. In a second study from the same researchers, 17 patients with PBS were subjected to an ice-water test (Mukerji *et al*., 2006a). Although the test did not elicit the same reflex contraction as it did in NDO and IDO patients, 13 out of 17 patients experienced

substantially more discomfort from filling with ice-cold saline than from normal temperature filling. Based on these results, the authors proposed that TRPM8 could be involved in the pain symptoms of PBS patients, and that pain during the ice-water test could represent a useful predictive marker for clinical trials with TRPM8 antagonists.

TRPV1. In patients with PBS, the number of suburothelial TRPV1-positive nerve fibres increases, and the relative TRPV1-positive nerve density in the urothelial layer correlates with pain scores in these patients (Mukerji *et al.*, 2006b). This may be a consequence of changes in inflammatory mediators in the bladder, as nerve growth factor (NGF), which has been shown to stimulate TRPV1 expression (Bonnington and McNaughton, 2003), is significantly increased in the urine and the urothelium of these patients. Taken together, it is likely that NGF contributes to hyperproliferation of TRPV1 nerve fibres in patients with PBS (Everaerts *et al.*, 2008). Lazzeri *et al.* (2000) performed a small RCT, treating PBS patients with RTX, with good results, but this was not corroborated in larger studies (Payne *et al.*, 2005; Ham *et al.*, 2012; Matsuoka *et al.*, 2012).

In a recent study, Homma *et al.* (2013) reported small but significant changes in mRNA levels for TRPA1, TRPM2, TRPM8, TRPV1, TRPV2 and TRPV4 in different subtypes of IC compared with normal tissue. However, because only one endogenous control was used in the quantitative PCR assays and the cellular composition of the tissue samples may vary strongly, further confirmation of these results is definitely needed.

Other forms of cystitis

Essential symptoms in all forms of cystitis are pollakisuria (frequent urination of small volumes), dysuria, lower abdominal pain and urgency, symptoms that closely resemble what we see in OAB. Again, only part of these symptoms can be objectively assessed, and therefore it is difficult to investigate cystitis in animal models. However, different models for various forms of cystitis have been developed, which are comprehensively reviewed elsewhere (Bjorling *et al.*, 2011).

TRPV1. As described earlier for NDO, activation of TRPV1 may contribute to C-fibre activity and DO, and inhibition of TRPV1 function may thus reduced cystitis symptoms. For instance, it has been shown that TRPV1-deficient mice do not develop bladder overactivity in response to LPS treatment (Charrua *et al.*, 2007). In addition, spinal c-fos expression was also lower in TRPV1-deficient mice than in wild-type (WT) controls. However, histological examination of the bladder showed similar inflammatory changes in both genotypes. This indeed suggests that TRPV1 is not involved in initiating cystitis but in initiating bladder overactivity in response to cystitis. Charrua *et al.* (2009) also demonstrated that GRC-6211, a specific TRPV1 antagonist, effectively decreases bladder overactivity and c-fos expression in the spinal cord of rats and mice with CYP- and LPS-induced cystitis. In addition, Wang *et al.* (2008) revealed that CYP-induced cystitis induces mechanical hypersensitivity in the hindpaws of mice, whereas TRPV1 KO mice seemed to lack this hypersensitivity. Histological examination again showed inflammation in both WT and KO animals. Adding to this information, patch clamp recordings revealed enhanced TRPV1-mediated currents in bladder-innervating DRG neurons from rats with CYP-induced cystitis (Dang *et al.*, 2013). In conclusion, cystitis seems to be yet another interesting field of research where TRPV1 antagonists could be tested in clinical trials.

TRPV4. There is evidence from animal models that inhibition of TRPV4 function may help in reducing the pollakisuria symptoms in cystitis (Everaerts *et al.*, 2010b). Mice lacking the TRPV4 receptor developed the same clear signs of haemorrhagic cystitis as their WT counterparts upon treatment with CYP, but failed to develop pollakisuria. Moreover, the selective TRPV4 antagonist HC-067047 was able to improve the symptoms in CYP-treated mice and rats (Everaerts *et al.*, 2010b). TRPV4 activation can mediate ATP release from urothelial cells in response to stretch (Gevaert *et al.*, 2007; Mochizuki *et al.*, 2009; Birder and Andersson, 2013), which may in turn activate purinergic receptors such as P2X3 on the afferent nerve fibres, paving the way for the afferent signal to travel up to the brain (Cockayne *et al.*, 2000). Knowing the exact effect of TRPV4 activation in the urothelium could greatly enhance our understanding of pathophysiology in multiple LUTd.

TRPA1. Sometimes, cystitis is due to neurogenic inflammation (Jasmin *et al.*, 1998; Schaeffer, 1999). It is described as an inflammatory response triggered by the activation of primary sensory neurons and the subsequent release of inflammatory neuropeptides, such as substance P and calcitonin gene-related peptide (Geppetti *et al.*, 2008). Likely, CYP-induced cystitis is partly caused by neurogenic inflammation by acrolein, the renaly excreted metabolite of CYP. We know that TRPA1 is a crucial mediator to induce this inflammatory reaction to acrolein (Bautista *et al.*, 2006). Indeed, blocking of TRPA1 by HC-030031 effectively lowered nociceptive responses in mice with CYP-induced cystitis. However, histological evaluation showed the same haemorrhagic oedema as in vehicle-injected animals (Pereira *et al.*, 2013). Furthermore, it seems that nocifensive behaviour in mice with CYP cystitis is not changed after treatment with TRPA1 antagonists (Matsunami *et al.*, 2012).

TRPC1 and TRPC4. Several TRPCs have been implicated in neuronal cell growth, remodelling, axon guidance and growth cone signalling (Liedtke *et al.*, 2007a). Both in patients and in animal models of cystitis a deep remodelling of bladder-innervating neurons has been observed (Vizzard *et al.*, 1996; Okragly *et al.*, 1999; Vizzard, 2001). To our knowledge, only one report has been published investigating the link between both phenomena. In this study, repeated CYP injections induced a specific increase in the expression of TRPC1 and TRPC4 in bladder- innervating sensory neurons and the sprouting of sensory fibres in the bladder mucosa. Interestingly, CYP- treated TRPC1/C4 double KO mice no longer exhibited increased bladder innervation, and concomitantly, the development of bladder overactivity was diminished.

In conclusion, improvements seen from antagonizing TRPV1, V4 as well as A1 in animal models of cystitis reduced

accompanying bladder overactivity, but histologically, cystitis never improved and mice still showed the same nocifensive behaviour when treated with TRPA1 antagonists. Therefore, it is to be expected that these TRP channel antagonists will only be able to provide symptomatic relief in cystitis. However, simultaneous action of TRPC1 and TRPC4 could be important in pathogenesis of cystitis due to neural remodellation.

LUTds with bladder outlet dysfunction

In the literature on lower urinary tract function, focus is mostly on the bladder. However, already 30 years ago it was suggested that defective urethral function could be one of the primary causes of LUTd (Hindmarsh *et al.*, 1983). Under normal conditions, a complex combination of reflex mechanisms keeps the sphincter and the bladder synchronized. When a person voluntarily wants to empty his or her bladder, the spino-bulbo-spinal reflex and sympathetic outflow reflexes are inhibited, whereas parasympathetic outflow to the urethra and the bladder is activated. NO is released in the urethral smooth muscle and the urethra relaxes. Then, after initiation of voiding, the flow of urine through the urethra is thought to initiate a urethra-to-bladder reflex, which further promotes emptying. This reflex is thought to be under pontine control, which probably contributes to why NDO patients with a lesion between the sacral region and the pons often have detrusor–sphincter dyssynergia. Furthermore, bladder outlet dysfunction can have secondary effects on the bladder. For example, OAB or DO often develop after prolonged bladder outlet obstruction (BOO) and can remain even after the obstruction is removed. Pathophysiological mechanisms of how the urethra and the bladder outlet can influence storage and voiding, both directly and indirectly, remain unclear.

Detrusor–sphincter dyssynergia

In patients with detrusor–external sphincter dyssynergia, the urethra fails to relax during a voiding attempt. A limited number of studies have implicated TRP channels in functional defects of the urethra and the bladder outlet in a similar way they purportedly act in the bladder.

TRPV4. As outlined earlier, TRPV4 has been proposed to act as a stretch sensor in the bladder. However, as TRPV4 is also expressed in the urothelium lining the urethra (Birder *et al.*, 2007), it has been suggested to function as a flow and stretch sensor involved in the urethra-to-bladder reflex. In awake ewes, Combrisson *et al.* found that instillation of body warm saline into the urethra induced a bladder contraction. This effect linearly decreased when lowering temperature of the infusion (Combrisson *et al.*, 2007). Knowing that TRPV4 is activated by shear stress at body temperature, it was put forward as a good candidate to serve as the initiator of the urethra-to-bladder reflex. This represents an interesting topic of future research.

Other TRP channels. TRPV1 immunoreactivity has been shown in urethral nerve endings, and capsaicin acts on both urethral and striated muscles (Everaerts *et al.*, 2008). Therefore, it is conceivable that TRPV1 can play a role in the urethra-to-bladder reflex. In addition, TRPA1 agonists were shown to induce relaxation in precontracted human urethral preparations (Gratzke *et al.*, 2009; Weinhold *et al.*, 2010). This relaxation seemed to work in cooperation with TRPV1-mediated signals (Weinhold *et al.*, 2010). Both channels thus present as possible mediators of urethral function, but little evidence has been gathered.

BOO

Different pathologies can cause BOO. The most frequent aetiology is benign prostatic hyperplasia (BPH). Not only can BPH have bothersome effects due to direct mechanical obstruction (problems with voiding initiation, decreased force of urination and post-void leakage), but it can also cause secondary bladder overactivity. Especially long-lasting BOO can cause OAB symptoms and DO, but the molecular mechanisms behind this are unknown.

Currently, treatment options are limited to symptomatic treatment. 5α-Reductase inhibitors are used to reduce prostate size and α-adrenergic receptor antagonists to relax the bladder outlet. When medical therapy fails, the prostate volume can be surgically reduced. Clearly, new non-invasive therapies are needed for this extremely frequent condition.

TRP channels have been explored as molecular candidates that might play a role in the pathogenesis of overactivity symptoms in response to BOO. Du *et al.* (2008) reported that TRPA1 mRNA levels are higher in bladders of BOO patients. In a rat model of BOO, TRPM8 protein levels were increased, and menthol had stronger effect on urodynamic parameters than in control animals (Hayashi *et al.*, 2011; Jun *et al.*, 2012). Similarly, expression of TRPV1 and TRPV4 was found to increase in rats with BOO (Ha *et al.*, 2011). Cho *et al.* (2013) showed an increased TRPV4 expression in the urothelium and detrusor of rats with BOO. At this point, it is difficult to determine to what extent BOO-induced LUTd differs from other forms of DO. Involvement of TRP channels might play a role in this form of LUTd, but more well-designed studies are definitely needed before any conclusion can be drawn.

Conclusions and future perspectives

From the above it seems clear that TRP channels are involved in bladder function and dysfunction (Figure 2). However, our understanding is still very limited, and TRP channels have proven to be hard pharmacological nuts to crack. Despite tremendous efforts, no TRP channel modulator has yet been able to make it to the pharmacy around the corner. And even in highly specialized clinical settings, only a few applications target TRP channels. Still, they remain very appealing as therapeutic targets. Other ion channels have already been targeted successfully, even though subtype selectivity could not be attained.

Unfortunately, current literature on TRP channels in the lower urinary tract is replete with unclarity and inconsistency. So far, it seems that we have not been able to come to a full understanding of how any TRP channel is involved in bladder or urethral (patho)physiology.

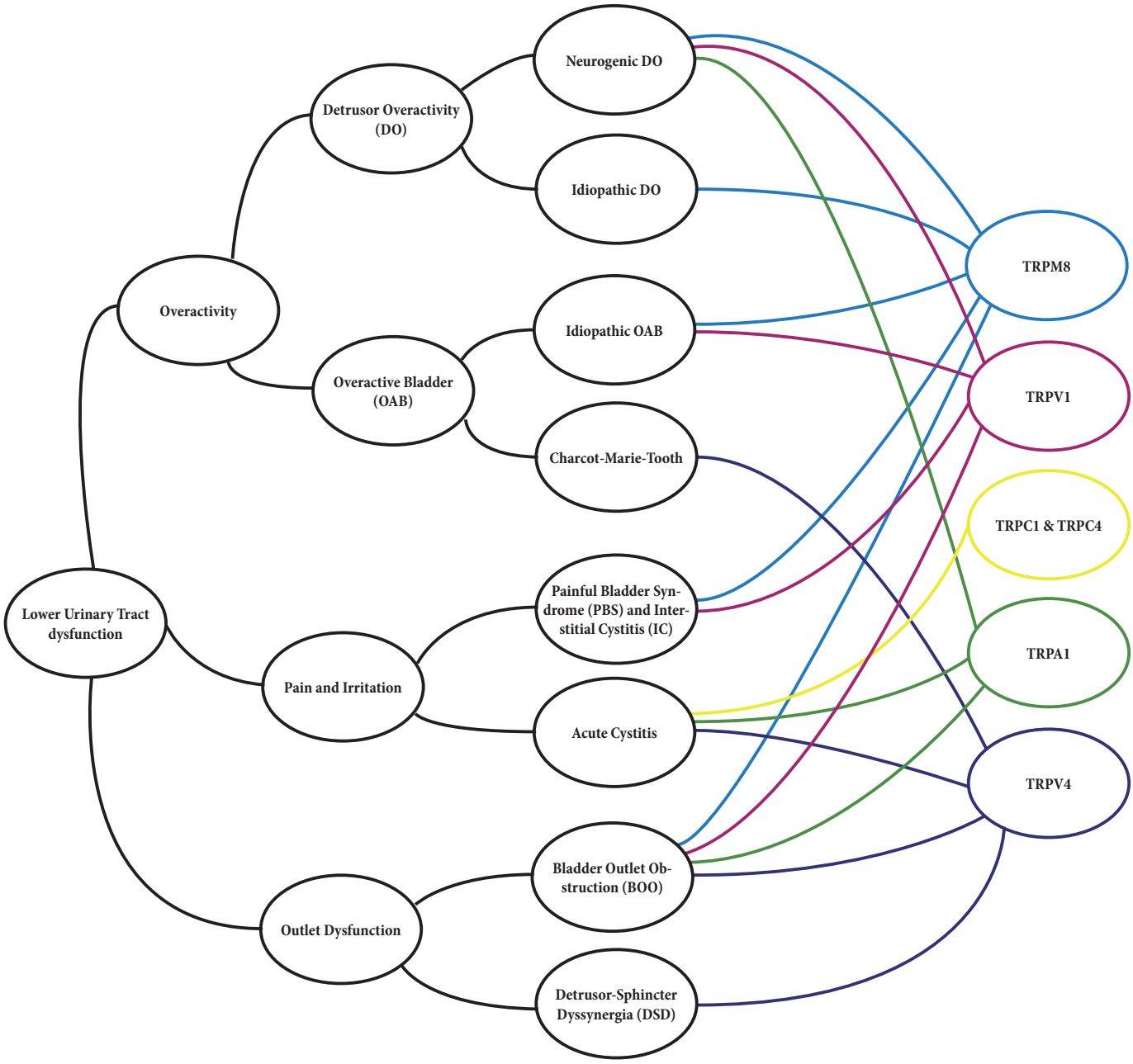

Figure 2

TRP channels in LUTd. Overview of all forms of LUTd that are discussed in this review, with their respective links to TRP channels that have been implicated in their treatment or pathogenesis.

Progress in our understanding about TRP channels in the lower urinary tract has been hampered by the lack of unambiguous experimental tools. Multiple TRP channel antibodies have been proven to be unspecific, and pharmacological agents that were once regarded as highly selective later appeared to be almost unusable. Basic knowledge about localization, structure and function is therefore still lacking and should be addressed. Moreover, most information we have comes from animal research, and confirmation of these findings in human samples is definitely needed in the future. Finally, current experimental approaches do not allow to reproduce and/or quantify aspects such as urgency, which is a hallmark of OAB.

In parallel with basic research, clinical trials have already been developed to explore the potential of TRP channels in practice. With the use of capsaicin to target TRPV1 for the treatment of NDO, the field of urological research has actually been one of the earliest – not to say the first – domains where TRPs were tested as drug targets. Currently, a major portion of clinical TRP channel research is focusing on (neu-

rogenic) pain treatment, so it may be expected that our knowledge on the involvement of TRPs in urological pain syndromes such as PBS and IC will see an upsurge in the coming years. Further development of selective antagonists will provide us with interesting tools to further explore the potential of these channels to relieve LUTd.

Disregarding the practical issues we are facing, TRP channels remain increasingly interesting drug targets, and their real breakthrough in LUTd is probably still to come.

Conflict of interest

D. D. R. is a senior clinical researcher of the FWO Vlaanderen and is a group leader of the TRUST FP 7 programme. The work was supported by grants from the Belgian Federal Government (IUAP P7/13), the Research Foundation – Flanders (G.0825.11), the Research Council of the KU Leuven (PF-TRPLe) and by an unconditional grant from Astellas.

References

Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U *et al.* (2003). The standardisation of terminology in lower urinary tract function: report from the standardisation sub-committee of the International Continence Society. Urology 61: 37–49.

Akopian AN, Ruparel NB, Patwardhan A, Hargreaves KM (2008). Cannabinoids desensitize capsaicin and mustard oil responses in sensory neurons via TRPA1 activation. J Neurosci 28: 1064–1075.

Andersson K-E (2011). Antimuscarinic mechanisms and the overactive detrusor: an update. J Urol 59: 377–386.

Andrade EL, Ferreira J, André E, Calixto JB (2006). Contractile mechanisms coupled to TRPA1 receptor activation in rat urinary bladder. Biochem Pharmacol 72: 104–114.

Andrade EL, Forner S, Bento AF, Leite DFP, Dias MA, Leal PC *et al.* (2011). TRPA1 receptor modulation attenuates bladder overactivity induced by spinal cord injury. Am J Physiol Renal Physiol 300: F1223–F1234.

Apostolidis A, Brady CM, Yiangou Y, Davis J, Fowler CJ, Anand P (2005). Capsaicin receptor TRPV1 in urothelium of neurogenic human bladders and effect of intravesical resiniferatoxin. Urology 65: 400–405.

Atalik KE, Okudan N, Gokbel H, Kalkan S, Cuce G (2010). Diabetes mellitus- and cooling-induced bladder contraction: an *in vitro* study. J Smooth Muscle Res 46: 175–183.

Athanasiou A, Smith PA, Vakilpour S, Kumaran NM, Turner AE, Bagiokou D *et al.* (2007). Vanilloid receptor agonists and antagonists are mitochondrial inhibitors: how vanilloids cause non-vanilloid receptor mediated cell death. Biochem Biophys Res Commun 354: 50–55.

Avelino A, Cruz C, Nagy I, Cruz F (2002). Vanilloid receptor 1 expression in the rat urinary tract. Neuroscience 109: 787–798.

Barbanti GG, Maggi CAC, Beneforti PP, Baroldi PP, Turini DD (1993). Relief of pain following intravesical capsaicin in patients with hypersensitive disorders of the lower urinary tract. Br J Urol 71: 686–691.

Bautista DM, Jordt S-E, Nikai T, Tsuruda PR, Read AJ, Poblete J *et al.* (2006). TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell 124: 1269–1282.

Birder L, Andersson K-E (2013). Urothelial signaling. Physiol Rev 93: 653–680.

Birder LA, Kanai AJ, de Groat WC, Kiss S, Nealen ML, Burke NE *et al.* (2001). Vanilloid receptor expression suggests a sensory role for urinary bladder epithelial cells. Proc Natl Acad Sci USA 98: 13396–13401.

Birder LA, Kullmann FA, Lee H, Barrick S, de Groat W, Kanai A *et al.* (2007). Activation of urothelial transient receptor potential vanilloid 4 by 4alpha-phorbol 12,13-didecanoate contributes to altered bladder reflexes in the rat. J Pharmacol Exp Ther 323: 227–235.

Birder LA, Wolf-Johnston AS, Sun Y, Chai TC (2013). Alteration in TRPV1 and Muscarinic (M3) receptor expression and function in idiopathic overactive bladder urothelial cells. Acta Physiol (Oxf) 207: 123–129.

Bjorling DE, Wang Z-Y, Bushman W (2011). Models of inflammation of the lower urinary tract. Neurourol Urodyn 30: 673–682.

Bley K (2012). Effects of topical capsaicin on cutaneous innervation: implications for pain management. Open Pain J 6: 81–94.

Bonnington JK, McNaughton PA (2003). Signalling pathways involved in the sensitisation of mouse nociceptive neurones by nerve growth factor. J Physiol (Lond) 551: 433–446.

Bors EH, Blinn KA (1957). Spinal reflex activity from the vesical mucosa in paraplegic patients. AMA Arch Neurol Psychiatry 78: 339–354.

Brady CM, Apostolidis AN, Harper M, Yiangou Y, Beckett A, Jacques TS *et al.* (2004). Parallel changes in bladder suburothelial vanilloid receptor TRPV1 and pan-neuronal marker PGP9.5 immunoreactivity in patients with neurogenic detrusor overactivity after intravesical resiniferatoxin treatment. BJU Int 93: 770–776.

Brederson J-D, Kym PR, Szallasi A (2013). Targeting TRP channels for pain relief. Eur J Pharmacol 716: 61–76.

Burns AS, Rivas DA, Ditunno JF (2001). The management of neurogenic bladder and sexual dysfunction after spinal cord injury. Spine 26: S129–S136.

Cacabelos R, Rodríguez B, Carrera C, Caamaño J, Beyer K, Lao JI *et al.* (1996). APOE-related frequency of cognitive and noncognitive symptoms in dementia. Methods Find Exp Clin Pharmacol 18: 693–706.

Campos-Sousa RN, Quagliato E, da Silva BB, de Carvalho RM, Ribeiro SC, de Carvalho DFM (2003). Urinary symptoms in Parkinson's disease: prevalence and associated factors. Arq Neuropsiquiatr 61: 359–363.

Capasso R, Aviello G, Romano B, Borrelli F, De Petrocellis L, Di Marzo V *et al.* (2012). Modulation of mouse gastrointestinal motility by allyl isothiocyanate, a constituent of cruciferous vegetables (*Brassicaceae*): evidence for TRPA1-independent effects. Br J Pharmacol 165: 1966–1977.

Cavanaugh DJ, Chesler AT, Jackson AC, Sigal YM, Yamanaka H, Grant R *et al.* (2011). Trpv1 reporter mice reveal highly restricted brain distribution and functional expression in arteriolar smooth muscle cells. J Neurosci 31: 5067–5077.

Chandiramani VA, Peterson T, Duthie GS, Fowler CJ (1996). Urodynamic changes during therapeutic intravesical instillations of capsaicin. Br J Urol 77: 792–797.

Charrua A, Cruz CD, Cruz F, Avelino A (2007). Transient receptor potential vanilloid subfamily 1 is essential for the generation of noxious bladder input and bladder overactivity in cystitis. J Urol 177: 1537–1541.

Charrua A, Cruz CD, Narayanan S, Gharat L, Gullapalli S, Cruz F *et al.* (2009). GRC-6211, a new oral specific TRPV1 antagonist, decreases bladder overactivity and noxious bladder input in cystitis animal models. J Urol 181: 379–386.

Chen Z, Ishizuka O, Imamura T, Aizawa N, Kurizaki Y, Igawa Y *et al.* (2010). Stimulation of skin menthol receptors stimulates detrusor activity in conscious rats. Neurourol Urodyn 29: 506–511.

Cho KJ, Park EY, Kim HS, Koh JS, Kim JC (2013). Expression of transient receptor potential vanilloid 4 and effects of ruthenium red on detrusor overactivity associated with bladder outlet obstruction in rats. World J Urol (Epub ahead of print).

Cockayne DA, Hamilton SG, Zhu QM, Dunn PM, Zhong Y, Novakovic S *et al.* (2000). Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. Nature 407: 1011–1015.

Colburn RW, Lubin ML, Stone DJ Jr, Wang Y, Lawrence D, D'Andrea MR *et al.* (2007). Attenuated cold sensitivity in TRPM8 null mice. Neuron 54: 379–386.

Combrisson H, Allix S, Robain G (2007). Influence of temperature on urethra to bladder micturition reflex in the awake ewe. Neurourol Urodyn 26: 290–295.

Cruz F (1998). Desensitization of bladder sensory fibers by intravesical capsaicin or capsaicin analogs. A new strategy for treatment of urge incontinence in patients with spinal detrusor hyperreflexia or bladder hypersensitivity disorders. Int Urogynecol J Pelvic Floor Dysfunct 9: 214–220.

Cruz FF, Guimarães MM, Silva CC, Rio MEM, Coimbra AA, Reis MM (1997). Desensitization of bladder sensory fibers by intravesical capsaicin has long lasting clinical and urodynamic effects in patients with hyperactive or hypersensitive bladder dysfunction. J Urol 157: 585–589.

Dang K, Bielefeldt K, Gebhart GF (2013). Cyclophosphamide-induced cystitis reduces ASIC channel but enhances TRPV1 receptor function in rat bladder sensory neurons. J Neurophysiol 110: 408–417.

De Ridder D, Chandiramani V, Dasgupta P, Van Poppel H, Baert L, Fowler CJ (1997). Intravesical capsaicin as a treatment for refractory detrusor hyperreflexia: a dual center study with long-term followup. J Urol 158: 2087–2092.

De Ridder D, Ost D, Van der Aa F, Stagnaro M, Beneton C, Gross-Paju K *et al.* (2005). Conservative bladder management in advanced multiple sclerosis. Mult Scler 11: 694–699.

Di Carlo A, Baldereschi M, Amaducci L, Lepore V, Bracco L, Maggi S *et al.* (2002). Incidence of dementia, Alzheimer's disease, and vascular dementia in Italy. The ILSA Study. J Am Geriatr Soc 50: 41–48.

Diabetes Prevention Program Research Group, Knowler WC, Fowler SE, Hamman RF, Christophi CA, Hoffman HJ *et al.* (2009). 10-year follow-up of diabetes incidence and weight loss in the Diabetes Prevention Program Outcomes Study. Lancet 374: 1677–1686.

Du S, Araki I, Yoshiyama M, Nomura T, Takeda M (2007). Transient receptor potential channel A1 involved in sensory transduction of rat urinary bladder through C-fiber pathway. Urology 70: 826–831.

Du S, Araki I, Kobayashi H, Zakoji H, Sawada N, Takeda M (2008). Differential expression profile of cold (TRPA1) and cool (TRPM8) receptors in human urogenital organs. Urology 72: 450–455.

Dua T, Rompani P, World Health Organization (2008). Atlas (World Health Organization).

Ellenberg M (1980). Development of urinary bladder dysfunction in diabetes mellitus. Ann Intern Med 92: 321–323.

Everaerts W, Gevaert T, Nilius B, De Ridder D (2008). On the origin of bladder sensing: Tr(i)ps in urology. Neurourol Urodyn 27: 264–273.

Everaerts W, Sepúlveda MR, Gevaert T, Roskams T, Nilius B, de Ridder D (2009). Where is TRPV1 expressed in the bladder, do we see the real channel? Naunyn Schmiedebergs Arch Pharmacol 379: 421–425.

Everaerts W, Vriens J, Owsianik G, Appendino G, Voets T, De Ridder D *et al.* (2010a). Functional characterization of transient receptor potential channels in mouse urothelial cells. Am J Physiol Renal Physiol 298: F692–F701.

Everaerts W, Zhen X, Ghosh D, Vriens J, Gevaert T, Gilbert JP *et al.* (2010b). Inhibition of the cation channel TRPV4 improves bladder function in mice and rats with cyclophosphamide-induced cystitis. Proc Natl Acad Sci USA 107: 19084–19089.

Everaerts W, Gees M, Alpizar YA, Farre R, Leten C, Apetrei A *et al.* (2011). The capsaicin receptor TRPV1 is a crucial mediator of the noxious effects of mustard oil. Curr Biol 21: 316–321.

Fall M, Lindström S, Maziéres L (1990). A bladder-to-bladder cooling reflex in the cat. J Physiol (Lond) 427: 281–300.

Fernandes ES, Fernandes MA, Keeble JE (2012). The functions of TRPA1 and TRPV1: moving away from sensory nerves. Br J Pharmacol 166: 510–521.

Fowler CJ (2002). Bladder afferents and their role in the overactive bladder. Urology 59: 37–42.

Fowler CJC, Jewkes DD, McDonald WIW, Lynn BB, de Groat WCW (1992). Intravesical capsaicin for neurogenic bladder dysfunction. Lancet 339: 1239–1239.

Freeman CR, Farmer JP (1998). Pediatric brain stem gliomas: a review. Int J Radiat Oncol Biol Phys 40: 265–271.

Geirsson GG, Fall MM, Sullivan LL (1995). Clinical and urodynamic effects of intravesical capsaicin treatment in patients with chronic traumatic spinal detrusor hyperreflexia. J Urol 154: 1825–1829.

Geppetti P, Nassini R, Materazzi S, Benemei S (2008). The concept of neurogenic inflammation. BJU Int 101 (Suppl. 3): 2–6.

Gevaert T, Vriens J, Segal A, Everaerts W, Roskams T, Talavera K *et al.* (2007). Deletion of the transient receptor potential cation channel TRPV4 impairs murine bladder voiding. J Clin Invest 117: 3453–3462.

Ghei M, Malone-Lee J (2005). Using the circumstances of symptom experience to assess the severity of urgency in the overactive bladder. J Urol 174: 972–976.

Giannantoni A, Scivoletto G, Di Stasi SM, Grasso MG, Finazzi Agrò E, Collura G *et al.* (1999). Lower urinary tract dysfunction and disability status in patients with multiple sclerosis. Arch Phys Med Rehabil 80: 437–441.

Giannantoni A, Mearini E, Di Stasi SM, Costantini E, Zucchi A, Mearini L *et al.* (2004). New therapeutic options for refractory neurogenic detrusor overactivity. Minerva Urol Nefrol 56: 79–87.

Giannantoni A, Conte A, Farfariello V, Proietti S, Vianello A, Nardicchi V *et al.* (2013). Onabotulinumtoxin-A intradetrusorial injections modulate bladder expression of NGF, TrkA, p75 and TRPV1 in patients with detrusor overactivity. Pharmacol Res 68: 118–124.

Gratzke C, Streng T, Waldkirch E, Sigl K, Stief C, Andersson K-E *et al*. (2009). Transient receptor potential A1 (TRPA1) activity in the human urethra-evidence for a functional role for TRPA1 in the outflow region. Eur Urol 55: 696–704.

de Groat WC (1997). A neurologic basis for the overactive bladder. Urology 50: 36–52; discussion 53–56.

Ha U-S, Park EY, Kim JC (2011). Effect of botulinum toxin on expression of nerve growth factor and transient receptor potential vanilloid 1 in urothelium and detrusor muscle of rats with bladder outlet obstruction-induced detrusor overactivity. Urology 78: 721.e1–721.e6.

Ham BK, Kim JH, Oh MM, Lee JG, Bae JH (2012). Effects of combination treatment of intravesical resiniferatoxin instillation and hydrodistention in patients with refractory painful bladder syndrome/interstitial cystitis: a pilot study. Int Neurourol J 16: 41–46.

Hayashi T, Kondo T, Ishimatsu M, Takeya M, Igata S, Nakamura K-I *et al*. (2011). Function and expression pattern of TRPM8 in bladder afferent neurons associated with bladder outlet obstruction in rats. Auton Neurosci 164: 27–33.

Hindmarsh JR, Gosling PT, Deane AM (1983). Bladder instability. Is the primary defect in the urethra? Br J Urol 55: 648–651.

Homma Y, Nomiya A, Tagaya M, Oyama T, Takagaki K, Nishimatsu H *et al*. (2013). Increased mRNA expression of genes involved in pronociceptive inflammatory reactions in bladder tissue of interstitial cystitis. J Urol 190: 1925–1931.

Imamura T, Ishizuka O, Aizawa N, Zhong C, Ogawa T, Nakayama T *et al*. (2008). Cold environmental stress induces detrusor overactivity via resiniferatoxin-sensitive nerves in conscious rats. Neurourol Urodyn 27: 348–352.

Jasmin L, Janni G, Manz HJ, Rabkin SD (1998). Activation of CNS circuits producing a neurogenic cystitis: evidence for centrally induced peripheral inflammation. J Neurosci 18: 10016–10029.

Jiang C, Yang H, Fu X, Qu S, Lindström S (2008). Bladder cooling reflex and external urethral sphincter activity in the anesthetized and awake guinea pig. Pflugers Arch 457: 61–66.

Jiang CH, Maziéres L, Lindström S (2002). Cold- and menthol-sensitive C afferents of cat urinary bladder. J Physiol (Lond) 543: 211–220.

Jordt SE, Ehrlich BE (2007). TRP channels in disease. Subcell Biochem 45: 253–271.

Jun JH, Kang HJ, Jin MH, Lee HY, Im YJ, Jung HJ *et al*. (2012). Function of the cold receptor (TRPM8) associated with voiding dysfunction in bladder outlet obstruction in rats. Int Neurourol J 16: 69–76.

Karashima Y, Damann N, Prenen J, Talavera K, Segal A, Voets T *et al*. (2007). Bimodal action of menthol on the transient receptor potential channel TRPA1. J Neurosci 27: 9874–9884.

Katusic SK, Colligan RC, Beard CM, O'Fallon WM, Bergstralh EJ, Jacobsen SJ *et al*. (1996). Mental retardation in a birth cohort, 1976–1980, Rochester, Minnesota. Am J Ment Retard 100: 335–344.

Kawaguchi Y, Kanamori M, Ishihara H, Ohmori K, Fujiuchi Y, Matsui H *et al*. (2001). Clinical symptoms and surgical outcome in lumbar spinal stenosis patients with neuropathic bladder. J Spinal Disord 14: 404–410.

Kim JH, Rivas DA, Shenot PJ, Green B, Kennelly M, Erickson JR *et al*. (2003). Intravesical resiniferatoxin for refractory detrusor hyperreflexia: a multicenter, blinded, randomized, placebo-controlled trial. J Spinal Cord Med 26: 358–363.

Klein AH, Sawyer CM, Carstens MI, Tsagareli MG, Tsiklauri N, Carstens E (2010). Topical application of L-menthol induces heat analgesia, mechanical allodynia, and a biphasic effect on cold sensitivity in rats. Behav Brain Res 212: 179–186.

Knowlton WM, McKemy DD (2011). TRPM8: from cold to cancer, peppermint to pain. Curr Pharm Biotechnol 12: 68–77.

Kullmann FA, Shah MA, Birder LA, de Groat WC (2009). Functional TRP and ASIC-like channels in cultured urothelial cells from the rat. Am J Physiol Renal Physiol 296: F892–F901.

Kuo H-C (2003a). Effectiveness of intravesical resiniferatoxin for anticholinergic treatment refractory detrusor overactivity due to nonspinal cord lesions. J Urol 170: 835–839.

Kuo H-C (2003b). Effectiveness of intravesical resiniferatoxin in treating detrusor hyper-reflexia and external sphincter dyssynergia in patients with chronic spinal cord lesions. BJU Int 92: 597–601.

Kuo H-C (2005). Multiple intravesical instillation of low-dose resiniferatoxin is effective in the treatment of detrusor overactivity refractory to anticholinergics. BJU Int 95: 1023–1027.

Kuo H-C, Liu H-T, Yang W-C (2006). Therapeutic effect of multiple resiniferatoxin intravesical instillations in patients with refractory detrusor overactivity: a randomized, double-blind, placebo controlled study. J Urol 176: 641–645.

Landouré G, Zdebik AA, Martinez TL, Burnett BG, Stanescu HC, Inada H *et al*. (2010). Mutations in TRPV4 cause Charcot–Marie–Tooth disease type 2C. Nat Genet 42: 170–174.

Lazzeri M, Beneforti P, Spinelli M, Zanollo A, Barbagli G, Turini D (2000). Intravesical resiniferatoxin for the treatment of hypersensitive disorder: a randomized placebo controlled study. J Urol 164: 676–679.

Lazzeri M, Vannucchi MG, Zardo C, Spinelli M, Beneforti P, Turini D *et al*. (2004). Immunohistochemical evidence of vanilloid receptor 1 in normal human urinary bladder. Eur Urol 46: 792–798.

Li M, Sun Y, Simard JM, Chai TC (2011). Increased transient receptor potential vanilloid type 1 (TRPV1) signaling in idiopathic overactive bladder urothelial cells. Neurourol Urodyn 30: 606–611.

Liedtke WB, Heller S, Cui K, Yuan X-B (2007a). TRP Channels and Axon Pathfinding. CRC Press: Boca Raton, FL.

Liedtke WB, Heller S, Plant TD, Strotmann R (2007b). TRPV4: A Multifunctional Nonselective Cation Channel with Complex Regulation. CRC Press: Boca Raton, FL.

Lindström S, Mazières L, Jiang C-H (2004). Inhibition of the bladder cooling reflex in the awake state: an experimental study in the cat. J Urol 172: 2051–2053.

Liu H-T, Kuo H-C (2007). Increased expression of transient receptor potential vanilloid subfamily 1 in the bladder predicts the response to intravesical instillations of resiniferatoxin in patients with refractory idiopathic detrusor overactivity. BJU Int 100: 1086–1090.

Liu L, Mansfield KJ, Kristiana I, Vaux KJ, Millard RJ, Burcher E (2007). The molecular basis of urgency: regional difference of vanilloid receptor expression in the human urinary bladder. Neurourol Urodyn 26: 433–438; discussion 439; discussion 451–453.

McNamara CR, Mandel-Brehm J, Bautista DM, Siemens J, Deranian KL, Zhao M *et al*. (2007). TRPA1 mediates formalin-induced pain. Proc Natl Acad Sci USA 104: 13525–13530.

Madersbacher H (1990). The various types of neurogenic bladder dysfunction: an update of current therapeutic concepts. Paraplegia 28: 217–229.

Maggi CA (1990). The dual function of capsaicin-sensitive sensory nerves in the bladder and urethra. Ciba Found Symp 151: 77–83; discussion 83–90.

Maggi CA, Santicioli P, Giuliani S, Furio M, Meli A (1986). The capsaicin-sensitive innervation of the rat urinary bladder: further studies on mechanisms regulating micturition threshold. J Urol 136: 696–700.

Maggi CA, Barbanti G, Santicioli P, Beneforti P, Misuri D, Meli A *et al.* (1989). Cystometric evidence that capsaicin-sensitive nerves modulate the afferent branch of micturition reflex in humans. J Urol 142: 150–154.

Maggi CA, Patacchini R, Tramontana M, Amann R, Giuliani S, Santicioli P (1990). Similarities and differences in the action of resiniferatoxin and capsaicin on central and peripheral endings of primary sensory neurons. Neuroscience 37: 531–539.

Matsunami M, Miki T, Nishiura K, Hayashi Y, Okawa Y, Nishikawa H *et al.* (2012). Involvement of the endogenous hydrogen sulfide/Ca(v) 3.2 T-type Ca2+ channel pathway in cystitis-related bladder pain in mice. Br J Pharmacol 167: 917–928.

Matsuoka PK, Haddad JM, Pacetta AM, Baracat EC (2012). Intravesical treatment of painful bladder syndrome: a systematic review and meta-analysis. Int Urogynecol J 23: 1147–1153.

Maziéres L, Jiang CH, Lindström S (2006). Recurrent inhibition of the bladder C fibre reflex in the cat and its response to naloxone. J Physiol (Lond) 575: 603–615.

Mitchell SJ, Woodthorpe J (1981). Young mentally handicapped adults in three London boroughs: prevalence and degree of disability. J Epidemiol Community Health 35: 59–64.

Mochizuki T, Sokabe T, Araki I, Fujishita K, Shibasaki K, Uchida K *et al.* (2009). The TRPV4 cation channel mediates stretch-evoked Ca2+ influx and ATP release in primary urothelial cell cultures. J Biol Chem 284: 21257–21264.

Mukerji G, Waters J, Chessell IP, Bountra C, Agarwal SK, Anand P (2006a). Pain during ice water test distinguishes clinical bladder hypersensitivity from overactivity disorders. BMC Urol 6: 31.

Mukerji G, Yiangou Y, Agarwal SK, Anand P (2006b). Transient receptor potential vanilloid receptor subtype 1 in painful bladder syndrome and its correlation with pain. J Urol 176: 797–801.

Mukerji G, Yiangou Y, Corcoran SL, Selmer IS, Smith GD, Benham CD *et al.* (2006c). Cool and menthol receptor TRPM8 in human urinary bladder disorders and clinical correlations. BMC Urol 6: 6.

Mustafa SM, Thulesius O (1999). Cooling-induced bladder contraction: studies on isolated detrusor muscle preparations in the rat. Urology 53: 653–657.

Nagata K, Duggan A, Kumar G, García-Añoveros J (2005). Nociceptor and hair cell transducer properties of TRPA1, a channel for pain and hearing. J Neurosci 25: 4052–4061.

Nilius B, Voets T (2013). The puzzle of TRPV4 channelopathies. EMBO Rep 14: 152–163.

Nomoto Y, Yoshida A, Ikeda S, Kamikawa Y, Harada K, Ohwatashi A *et al.* (2008). Effect of menthol on detrusor smooth-muscle contraction and the micturition reflex in rats. Urology 72: 701–705.

Okragly AJ, Niles AL, Saban R, Schmidt D, Hoffman RL, Warner TF *et al.* (1999). Elevated tryptase, nerve growth factor, neurotrophin-3 and glial cell line-derived neurotrophic factor levels in the urine of interstitial cystitis and bladder cancer patients. J Urol 161: 438–441; discussion 441–442.

Ost D, Van Der Aa F, de Ridder D (2003). Intravesical ethanol 10% in saline is not an inert vehicle. Neurourol Urodyn 22: 353–355.

Parsons BA, Drake MJ (2011). Handbook of Experimental Pharmacology. Springer Berlin Heidelberg: Berlin, Heidelberg.

Payne CK, Mosbaugh PG, Forrest JB, Evans RJ, Whitmore KE, Antoci JP *et al.* (2005). Intravesical resiniferatoxin for the treatment of interstitial cystitis: a randomized, double-blind, placebo controlled trial. J Urol 173: 1590–1594.

Pereira LMS, Lima-Júnior RCP, Bem AXC, Teixeira CG, Grassi LS, Medeiros RP *et al.* (2013). Blockade of TRPA1 with HC-030031 attenuates visceral nociception by a mechanism independent of inflammatory resident cells, nitric oxide and the opioid system. Eur J Pain 17: 223–233.

Proudfoot CJ, Garry EM, Cottrell DF, Rosie R, Anderson H, Robertson DC *et al.* (2006). Analgesia mediated by the TRPM8 cold receptor in chronic neuropathic pain. Curr Biol 16: 1591–1605.

Renier WO, Gabreels FJ (1980). Evaluation of diagnosis and non-surgical therapy in 24 children with a pontine tumour. Neuropediatrics 11: 262–273.

Santos-Silva A, Charrua A, Cruz CD, Gharat L, Avelino A, Cruz F (2012). Rat detrusor overactivity induced by chronic spinalization can be abolished by a transient receptor potential vanilloid 1 (TRPV1) antagonist. Auton Neurosci 166: 35–38.

Schaeffer AJ (1999). Activation for CNS circuits producing a neurogenic cystitis: evidence for centrally induced peripheral inflammation. J Urol 162: 282.

de Sèze M, Wiart L, Joseph PA, Dosque JP, Mazaux JM, Barat M (1998). Capsaicin and neurogenic detrusor hyperreflexia: a double-blind placebo-controlled study in 20 patients with spinal cord lesions. Neurourol Urodyn 17: 513–523.

de Sèze M, Wiart L, Ferrière J, de Sèze MP, Joseph P, Barat M (1999). Intravesical instillation of capsaicin in urology: a review of the literature. Eur Urol 36: 267–277.

Shibata Y, Ugawa S, Imura M, Kubota Y, Ueda T, Kojima Y *et al.* (2011). TRPM8-expressing dorsal root ganglion neurons project dichotomizing axons to both skin and bladder in rats. Neuroreport 22: 61–67.

Silva C, Silva J, Ribeiro M-J, Avelino A, Cruz F (2005). Urodynamic effect of intravesical resiniferatoxin in patients with neurogenic detrusor overactivity of spinal origin: results of a double-blind randomized placebo-controlled trial. Eur Urol 48: 650–655.

Silva C, Silva J, Castro H, Reis F, Dinis P, Avelino A *et al.* (2007). Bladder sensory desensitization decreases urinary urgency. BMC Urol 7: 9.

Stein RJ, Santos S, Nagatomi J, Hayashi Y, Minnery BS, Xavier M *et al.* (2004). Cool (TRPM8) and hot (TRPV1) receptors in the bladder and male genital tract. J Urol 172: 1175–1178.

Steurer J, Nydegger A, Held U, Brunner F, Hodler J, Porchet F *et al.* (2010). Study protocol LumbSten: the lumbar spinal stenosis outcome study. BMC Musculoskelet Disord 11: 254.

Story GM, Peier AM, Reeve AJ, Eid SR, Mosbacher J, Hricik TR *et al.* (2003). ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell 112: 819–829.

Streng T, Axelsson HE, Hedlund P, Andersson DA, Jordt S-E, Bevan S *et al.* (2008). Distribution and function of the hydrogen sulfide-sensitive TRPA1 ion channel in rat urinary bladder. Eur Urol 53: 391–399.

Tsukimi Y, Mizuyachi K, Yamasaki T, Niki T, Hayashi F (2005). Cold response of the bladder in guinea pig: involvement of transient receptor potential channel, TRPM8. Urology 65: 406–410.

Uvin P, Boudes M, Franken J, Pinto S (2013a). The role of TRPA1 in the bladder cooling reflex; a possible new therapeutic target. J Urol 189: e12.

Uvin P, Boudes M, Menigoz A, Franken J, Pinto S, Gevaert T *et al.* (2013b). Chronic administration of anticholinergics in rats induces a shift from muscarinic to purinergic transmission in the bladder wall. Eur Urol 64: 502–510.

Vahabi B, Parsons BA, Doran O, Rhodes A, Dean S, Drake MJ (2013). TRPM8 agonists modulate contraction of the pig urinary bladder. Can J Physiol Pharmacol 91: 503–509.

Van Den Eeden SK (2003). Incidence of Parkinson's disease: variation by age, gender, and race/ethnicity. Am J Epidemiol 157: 1015–1022.

Vay L, Gu C, McNaughton PA (2012). The thermo-TRP ion channel family: properties and therapeutic implications. Br J Pharmacol 165: 787–801.

Vizzard MA (2001). Alterations in neuropeptide expression in lumbosacral bladder pathways following chronic cystitis. J Chem Neuroanat 21: 125–138.

Vizzard MA, Erdman SL, de Groat WC (1996). Increased expression of neuronal nitric oxide synthase in bladder afferent pathways following chronic bladder irritation. J Comp Neurol 370: 191–202.

Wang Z-Y, Wang P, Merriam FV, Bjorling DE (2008). Lack of TRPV1 inhibits cystitis-induced increased mechanical sensitivity in mice. Pain 139: 158–167.

Watanabe T, Yokoyama T, Sasaki K, Nozaki K, Ozawa H, Kumon H (2004). Intravesical resiniferatoxin for patients with neurogenic detrusor overactivity. Int J Urol 11: 200–205.

Weinhold P, Gratzke C, Streng T, Stief C, Andersson K-E, Hedlund P (2010). TRPA1 receptor induced relaxation of the human urethra involves TRPV1 and cannabinoid receptor mediated signals, and cyclooxygenase activation. J Urol 183: 2070–2076.

Wiart LL, Joseph PAP, Petit HH, Dosque JPJ, de Sèze MM, Brochet BB *et al.* (1998). The effects of capsaicin on the neurogenic hyperreflexic detrusor. A double blind placebo controlled study in patients with spinal cord disease. Preliminary results. Spinal Cord 36: 95–99.

Xing H, Chen M, Ling J, Tan W, Gu JG (2007). TRPM8 mechanism of cold allodynia after chronic nerve injury. J Neurosci 27: 13680–13690.

Xu X, Gordon E, Lin Z, Lozinskaya IM, Chen Y, Thorneloe KS (2009). Functional TRPV4 channels and an absence of capsaicin-evoked currents in freshly-isolated, guinea-pig urothelial cells. Channels (Austin) 3: 156–160.

Yamada T, Ugawa S, Ueda T, Ishida Y, Kajita K, Shimada S (2009). Differential localizations of the transient receptor potential channels TRPV4 and TRPV1 in the mouse urinary bladder. J Histochem Cytochem 57: 277–287.

Yiangou Y, Facer P, Ford A, Brady C, Wiseman O, Fowler CJ *et al.* (2001). Capsaicin receptor VR1 and ATP-gated ion channel P2X3 in human urinary bladder. BJU Int 87: 774–779.