Radboud Repository

Radboud University Nijmegen

PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.
http://repository.ubn.ru.nl/handle/2066/127322

Please be advised that this information was generated on 2017-03-09 and may be subject to change.

Disentanglement of myogenic related structures in the bladder wall of humans with and without overactive bladder syndrome

-Search for the urge-

Kamiel Alphons Joan Kuijpers

Kamiel A.J. Kuijpers was financially supported by a personal grant from
the Astellas European Foundation Fund.

Copyright © K.A.J. Kuijpers, 2014

Coverdesign by Kamiel Kuijpers and Theo Hafmans
Layout by Kamiel Kuijpers and Theo Hafmans
Printed by Ipskamp Drukkers Enschede

The research presented in this thesis was performed at the Department of Urology and the Department of Biochemistry of the Radboud University Nijmegen Medical Centre, The Netherlands.

Financial support for the publication of this thesis was provided by the Radboud University Medical Center, Ipsen Farmaceutica B.V. and Pohl Boskamp B.V.

Disentanglement of myogenic related structures in the bladder wall of humans with and without overactive bladder syndrome

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van rector magnificus prof. mr. S.C.J.J. Kortmann,
volgens besluit van het college van decanen
in het openbaar te verdedigen op vrijdag 11 juli 2014
om 12:30 uur precies

door
Kamiel Alphons Joan Kuijpers
geboren op 22 juni 1978
te Nijmegen

Promotor
Prof. dr. J.A. Schalken

Copromotor:
Dr. J.P.F.A. Heesakkers

Manuscriptcommissie:
Prof. dr. J.G.J. Hoenderop
Prof. dr. A.C.H. Geurts
Dr. G.A. van Koeveringe *(Maastricht Universitair Medisch Centrum)*

Table of contents

Chapter 1.	**General introduction and outline of this thesis**	7
Chapter 2.	**The complexities of diagnosing and treating OAB in men** (European Genito-urinary Disease, 2007; Issue I: 61-63)	25
Chapter 3.	**Pathophysiological aspects of the musculus detrusor in the human overactive bladder: an overview**	35
Chapter 4.	**Cadherin-11 is expressed in detrusor smooth muscle cells and myofibroblasts of normal human bladder** (European Urology 2007 Oct; 52(4): 1213-1221)	51
Chapter 5.	**Adherence junctions and cadherin-11 in normal and overactive human detrusor smooth muscle cells** (The Journal of Urology, 2011 May;185(5):1946-1951)	67
Chapter 6.	**An update of the interstitial cell compartment in the normal human bladder** (BioMed Research International, Volume 2014, Epub 2014 Feb 27)	81
Chapter 7.	**Alterations of the myovesical plexus of the human overactive detrusor** (BioMed Research International, Volume 2014, Epub 2014 April 15)	97
Chapter 8.	**Summary and conclusions**	113
Chapter 9.	**Future perspectives**	119
Chapter 10.	**Nederlandse samenvatting**	125
Acknowledgements / Dankwoord		135
List of abbreviations		137
List of publications and abstracts		138
Curriculum Vitae		141

1

General introduction and outline of this thesis

1. Anatomy of the lower urinary tract

The function of the lower urinary tract is storage of urine and emptying of the stored urine at an appropriate moment and place. This requires an interplay of relaxation and contraction of the bladder and bladder outlet.

1.1. The bladder

Traditionally, is was thought that the human urinary bladder is a hollow musculous organ that is primarily formed by smooth muscle. In more detail, the bladder wall was thought to consist of four layers.
The interior side of the bladder is lined by a protective boarder of mucosa, also called the urothelium. This layer consists of five to seven strata of transitional epithelial cells, also called urothelial cells. Urothelial cells prevent substances in the urine from crossing into the underlying layers of the bladder wall, thus serving a protective function. The submucosa, also known as the lamina propria, is a layer of elastic connective tissue that allows the bladder to stretch as it fills. It consists of blood and lympathic vessels and nerves within a stroma of fibrous connective tissue that join the urothelium to the inner layers. Adjacent to and outward of the submucosa is the muscle layer of the bladder, also known as the tunica muscularis or the detrusor muscle. It consists of three layers of muscle bundles. The inner and outer layer muscle bundles course mainly longitudinally. The layer in between has a circulatory orientation. All bundles branch off and meet elsewhere, hereby producing a dense wickerwork of muscle bundles that is heavily innervated. The outermost layer, known as the serosa is derived from the peritoneum and covers the bladder.
However, in the last decade is has become more and more clear that the human urinary bladder cannot merely be seen as a passive hollow musculous 'black box', solely controlled by neuronal input. Local bladder regulatory factors play a major part. This will be discussed further on in this thesis.

1.2. The bladder outlet

The male urethra is a fibromuscular tube of approximately 20cm long. At the male bladder neck, the internal urinary sphincter forms a complete circular collar. It is made of smooth muscle. It therefore is under involuntary or autonomic control. It is the primary muscle for prohibiting the release of urine, but also plays a major role in preventing semen influx at ejaculation. Distally, the bladder neck muscle merges with, and becomes indistinguishable from, the musculature in the stroma and capsule of the prostate gland.
In females, there is no anatomical internal smooth muscle sphincter at the bladder neck, as the muscle bundles in the female bladder neck extend obliquely or longitudinally into the urethral wall.
The external urinary sphincter is made up from a intrinsic and extrinsic component. The rhabdosphincter is the internal component and located in the walls of the male and female

urethra. It is made of striated muscle and therefore under voluntary or somatic control. This muscle is also called the intrinsic sphincter externus. Besides the urethra wall, the levator ani pelvic floor muscle forms an important effective contractile support structure. It is also known as the extrinsic sphincter externus. Contraction of the musculus levator ani is responsible for the compression of the urethra. In doing so, this muscle assists the rhabdosphincter to ensure continence.

2. Neuronal bladder control and physiology

The neural control of the lower urinary tract is distinct from other visceral organs such as the heart, blood vessels and the intestine. These are regulated exclusively by involuntary (autonomic) reflex mechanisms. Bladder storage and emptying, as well as coordinated contraction or relaxation of the urinary sphincter can be seen as a peripheral urinary reflex system that is mediated by neural circuits from the brain and spinal cord.

2.1. The peripheral urinary reflex system

The peripheral system consists of three sets of peripheral nerves: the pelvic nerves, the hypogastric nerves and the pudendal nerves. They contain both afferent (sensory) and efferent (motor) nerve fibres. Under additional sympathetic and parasympathetic control, the central and peripheral nervous system control the bladder and urethra as a functional unit. In general, urinary storage is a function of the sympathetic nervous system, whereas micturition is a function of the parasympathetic nervous system. Both are autonomic functions in nature. The somatic nervous system is responsible for the control of the external urinary sphincter, allowing for volitional continence.

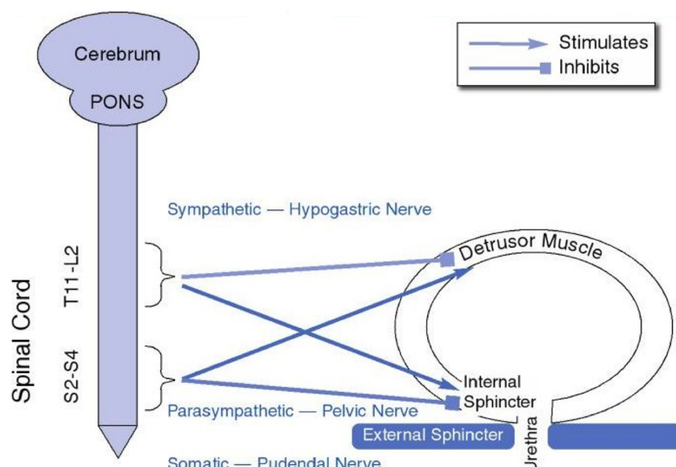


Figure 1 - Diagram illustrating the functional characteristics of the efferent pathways of the hypogastric, pelvic and pudendal nerves (bron Urol N&A).

Sympathetic afferent neurons are conducted via the hypogastric plexus and projects to the spinal levels Th11-L2 [1, 2]. They contain A-delta fibres (for sensation of bladder filling) and C-fibres (for sensations of noxious stimuli). The afferent information is also projected to the brain [3]. These neurons are active during the filling phase.

Sympathetic efferent neurons of the hypogastric plexus originate from the spinal level Th11-L2, pass the paravertebral sympathetic chain ganglia or the inferior mesenteric ganglia and connect with the pelvic and intramural ganglia of the bladder wall [4]. These neurons are active during the filling phase, which results in relaxation of the bladder dome and contraction of the bladder outlet [5,6].

Parasympathetic afferent neurons are conducted via the pelvic plexus and pelvic nerve to the sacral intermediate gray in the spinal cord S2-4. Here, they connect to efferent parasympathetic fibres to form a reflex arc. The afferent information is also projected to the brain. Most of the information of the bladder and urethra reaches the spinal cord via the pelvic nerve. This is in accordance to the finding that the bladder base contains a much higher level of afferent nerve endings than the bladder dome.

Parasympathic efferent neurons of the pelvic nerve originate from the sacral parasympathetic nucleus at spinal cord level S2-4. They synapse with the postganglionic nerve in the pelvic plexus or ganglia in the bladder wall. These neurons are active during the micturition phase, resulting in contraction of the bladder dome and relaxation of the bladder outlet [7].

Somatic neurons originate from the nucleus of Onuf at the spinal levels S2-4, pass through the pudendal nerves and innervate the rhabdosphincter [8]. Activation of these fibres results in relaxation of the sphincter [9].

2.2. Bladder control: mediation by the brain and spinal cord

The bladder has a continuous biphasic cycle consisting of a storage and micturition phase. As mentioned previously, the peripheral urinary reflex system is mediated by neural circuits from the brain and spinal cord. In infants these mechanisms function purely in a reflex manner to produce involuntary voiding. However, in adults urine storage and release are under voluntary control.

2.2.1 Storage phase

During urine storage, a low level of afferent (sensory) activity from the bladder is transported via the pelvic plexus and pelvic nerve to the spinal cord. These fibres are connected to efferent (motory) fibres to form a reflex arc. They activate efferent input to the urethral sphincter, resulting in contraction and hereby preserving continence. By bladder distension, the afferent activity from the bladder rises until a certain threshold tension is achieved.

2.2.2 Micturition phase

Afferent information from the bladder is also projected to the brain. This information is relayed in the periaqueductal gray (PAG). The PAG receives input from multiple brain regions, such as the hypothalamus, amygdala and the prefrontal and orbital cortex [10]. The PAG plays a major role in the micturition cycle. The decision to void is taken in the PAG. The PAG conveys the 'safe signal to void' based upon its incoming information from the bladder, cortical system and limbic system associated structures. The PAG communicates with the pontine micturition center (PMC), also located in the brain stem. The PMC can be seen as a regulator of a complex urinary reflex system, resulting in an accurate interplay of the bladder and bladder outlet. It functions as a 'ON/OFF' switch and consists of a L- and M-region. The L-region can be seen as a pontine storage center. The M-region can be seen as a pontine micturition center. Activating the M-region produces firing in the efferent pathways to the bladder and urethral outlet. The urethral sphincter and pelvic floor relax and the detrusor muscle contracts. Once the bladder is emptied, the pontine storage center is activated, the PMC returns into 'OFF' mode and the micturition cycle continues.

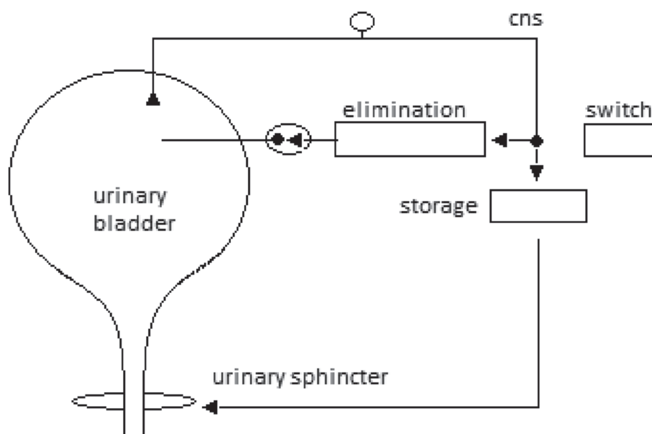


Figure 2 - Diagram illustrating the anatomy of the lower urinary tract and the switchlike function of the micturition reflex pathway. A high level of afferent activity induced by bladder distention activates the switching circuit in the central nervous system (CNS), producing firing in the efferent pathways to the bladder, inhibition of the efferent outflow to the urethral sphincter, and urine elimination.

3. Receptors at the bladder neuromuscular junction

3.1. Urinary storage; the adrenergic system

In general, urinary storage is coordinated by the sympathetic nervous system. Adrenergic receptors are stimulated by the sympathetic nervous system. Different types of adrenergic muscle receptors are found within the bladder and bladder outlet. The bladder dome houses mainly β-adrenergic receptors (β3 subtype) [11]. In the trigonum (base of bladder) and bladder neck a majority of α-adrenergic receptors is found (α1d subtype) [12]. Stimulation of β-adrenergic receptors results in muscle relaxation. Stimulation of α-adrenergic receptors results in muscle contraction. In theory, sympathetic stimulation contracts the bladder neck while the rest of the bladder is relaxated. In practice however,

the true role for sympathetic nerve system is probably overestimated, because bladder dysfunction has never been found after lumbal sympathectomy. Retrograde ejaculation however, is found. Which suggests a role for the sympathetic nervous system in the regulation of the internal urethral sphincter. The rabdosphincter is under somatic control by cholinergic receptors.

3.2. Voiding; the cholinergic system

Parasympathetic impulses travel to the bladder along the efferent fibres of the pelvic nerves resulting in stimulation of muscarinic receptors leading to contraction of the bladder dome [13]. These cholinergic receptors are widely distributed throughout the human body, explaining the associated side effects of antimuscarinic agents. The muscarinic receptor subtype 3 (M3) is responsible for the emptying contraction during voiding [14]. In the neurogenic dysfunctional bladder, an increased expression of M2 receptors is seen. M2 receptors prevent formation of cyclic adenosine monophosphate (cAMP), which mediates detrusor relaxation. However, the effect of parasympathetic nerve stimulation cannot totally be blocked by the parasympathicolyticum atropine, suggesting attributive pathways in bladder innervations.

4. Lower urinary tract dysfunction and terminology

The urinary bladder and urethra form a functional unit which is controlled by a complex interplay between the central and peripheral nervous systems accompanied by local bladder regulatory factors. Malfunction at these various levels may result in lower urinary tract symptoms (LUTS), which can roughly be classified as disturbances of filling/storage or disturbances of emptying.

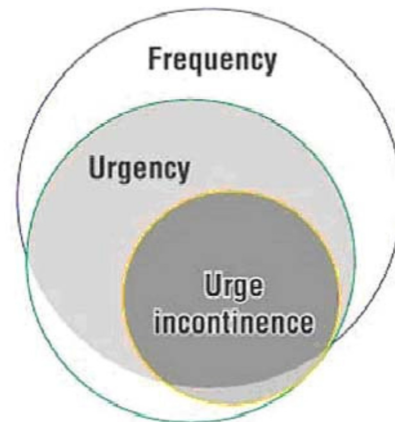
Failure to store urine may lead to various forms of incontinence (urge and stress incontinence), and failure to empty can lead to urinary retention, which may result in overflow incontinence. The most common problem with urine storage arises when the bladder fails to remain relaxed until an appropriate time for urination. This syndrome is called overactive bladder (OAB).

The first involuntary detrusor contractions probably were recorded towards the end of the 19th century during the early recordings of human bladder pressures (Dubois, 1876; Genouville, 1894). Unstable bladder contractions associated with bladder dysfunction were seen during World War 1 in soldiers suffering from spinal cord injuries or exposure to severe cold. Complaints of urge incontinence were accompanied by phasic pressure rises which were seen using urodynamics (Schwarz, 1915).

The terms unstable bladder and detrusor hyperreflexia were introduced 30 years ago. The Englishman Bates used the term unstable bladder to describe unvoluntary detrusor contractions seen during urodynamic studies as the bladder was filled. At the same time the Scandinavians, the other major innovators in urodynamics, were using the term detrusor hyperreflexia. To resolve this conflict the International Continence Society (ICS)

decided to use the term unstable bladder for involuntary contractions seen in patients with no obvious cause for the contractions. The term detrusor hyperreflexia however was used to describe patients whose involuntary contractions had a neurologic cause. In addition, the term overactive detrusor was used as the generic, overarching name.
The terms overactive bladder (OAB) and detrusor overactivity (DO) were introduced in 1999 by the International Continence Society (ICS). OAB is defined as urgency, with or without incontinence, usually with frequency and nocturia. The key symptom of OAB is urgency. Detrusor overactivity is a urodynamic observation (UDO) characterized by unvoluntary detrusor contractions during the filling phase, which may be spontaneous or provoked.

Figure 3 - Overactive bladder disease is a symptomatic diagnosis. Ring diagram showing that approximately half of individuals with urgency have coexisting urge incontinence, and almost all have coexisting daytime and nighttime frequency.



5. Correlation between symptomatic diagnosis of OAB and detrusor overactivity

OAB symptoms are suggestive of urodynamically demonstrable detrusor overactivity (involuntary detrusor contraction) during the filling phase which may be spontaneous provoked. However, involuntary contractions do not always cause urgency and urgency is not always caused by involuntary contractions. Indeed, involuntary contractions are found in asymptomatic subjects. They were found in 25% of 13 asymptomatic middle aged men [15].
Urgency occurs in 70% of patients with normal urodynamics and in 85% of those with instability, suggesting that it cannot distinguish between the two groups. Futhermore, the success of predicting instability from the symptoms was only 31-48% in women and 53% in men [16]. This suggests that the filling symptoms of unstable patients may not necessarily be caused by unstable contractions. This is important when considering the effects of treatment, because abolishing unstable contractions may not resolve all filling symptoms.
It is important to know that the diagnosis of OAB can only be made when other known pathologies, such as a proven infection, a bladder stone or bladder cancer, can be excluded.

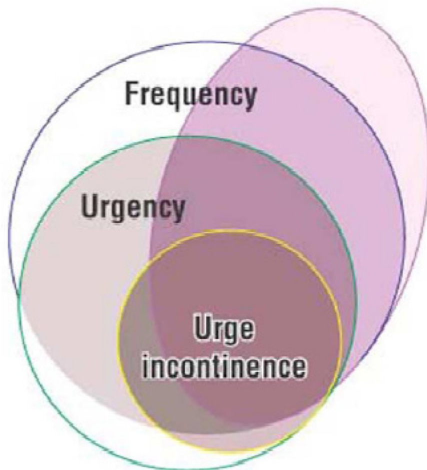


Figure 4. - Diagram demonstrating correlation between symptomatic diagnosis of overactive bladder disease (OAB) and urodynamic diagnosis of detrusor overactivity (pink ellipse). The correlation is greatest for urge incontinence and poorest for frequency (day and/or night).

6. Socioeconomic relevance of OAB

The OAB is a common disorder affecting the quality of life in huge numbers of people worldwide [17]. It is associated with significant problems for patients, not just physical limitations, but socially, physiologically and sexually as well. In addition, there are large economic costs involved.

OAB is often confused with incontinence, making epidemiologic incidence data vary widely. It is difficult to estimate the true prevalence of bladder overactivity for two reasons: (1) relatively few patients with the disease seek treatment, despite the considerable impact of bladder storage symptoms on quality of life. These patients remain uncounted. (2) Few epidemiologic surveys have been carried out on the symptoms of urgency and frequency alone without incontinence. Surveys on the prevalence of urinary incontinence have been done, and estimates of the prevalence of bladder overactivity have been obtained by adding together the fraction of urge incontinent patients to the number of those with mixed incontinence. Thus, the most prevalent figures that are available significantly underestimate the extent of the problem. In reality, OAB is probably more prevalent than asthma and heart disease and nearly as prevalent as arthritis and chronic sinusitis.

The majority of people with chronic symptoms of bladder overactivity complains of frequency and urgency, while only a third complains of urge incontinence. The total prevalence of OAB within the general population aged 40 years and over ranges from 12% to 22% in a group of 17 000 people in 6 European countries [18]. The risk of developing OAB increases with age. In the group of people 75 years and older, the prevalence ranges between 30% and 40%. In the USA, the costs of OAB are on the same level with those for depression (44 billion US dollars) and Alzheimer disease (100 billion US dollars). In US women, urinary incontinence accounts for more direct costs than breast cancer, the number one type of cancer in women, and similar to osteoporosis.

It is certain that the clinical impact of this syndrome will be tremendous as the world population ages. By 2020, 44% of the world population will be older than 65 years of age, and the already huge financial burden will become critical in the future.

7. Causes of the overactive bladder and detrusor overactivity

Because of the complexity of the nervous control of the lower urinary tract, there still is no consensus about the reason for developing an overactive bladder (OAB) and detrusor overactivity (DO). Detrusor overactivity can occur as a result of a variety of neurological disorders resulting in changes in the innervation and muscle components of the bladder. Injuries or diseases of the nervous system in adults can disrupt the voluntary control of micturition causing the reappearance of reflex micturition, resulting in detrusor overactivity and possible incontinence. Overactive bladder may occur in patients due to changes in the brain or nerves (Multiple Sclerosis, Cerebrovasculair accident, Parkinson's disease, brain tumors, Diabetes Mellitus). The aging bladder as well as patients with urethral outlet obstruction (e.g. prostate enlargement) or in those who are taking drugs that depress the neural control of the bladder are possible victims.
Experimental studies indicate that detusor overactivity occurs after: (1) interruption of cortical inhibitory circuits, (2) disruption of basal ganglia function in models of Parkinson's disease, (3) damage to pathways from the brain to the spinal cord (multiple sclerosis, spinal cord injury) and (4) sensitization of bladder afferents [19].
Theoretically, there may be 1) decreased inhibitory control in the central nervous system (CNS), 2) decreased inhibitory control in the peripheral ganglia, 3) increased afferent activity (sensitization), 4) increased sensitivity to efferent stimulation in the detrusor muscle or 5) a combination of these factors.

8. Treatment of the OAB

8.1. Behavioural therapy

Conservative management forms the first line of treatment and includes lifestyle modifications, bladder training and pelvic floor exercises. If this fails, pharmacotherapy, in the form of anticholinergic drugs, is initiated.

8.2. Pharmacotherapy

Pharmacotherapy and behavioral therapy are the mainstays of treatment of OAB. Although there are multiple central and peripheral sites and mechanisms that can influence bladder function, few are clinically useful. The problems are: (1) how to affect bladder function without interfering with the function of other organ systems (uroselectivity) and (2) how to eliminate overactivity without disturbing normal micturition. Multiple types of drug therapies are potentially useful for decreasing bladder contractility or decreasing sensation, but antimuscarinic medication (anticholinergica) have represented the current mainstay of pharmacotherapy for improving OAB symptoms. Reductions in OAB incontinence episodes with anticholinergic therapy range from 40% to 70% [20]. However, not all patients show adequate respons and the problem with current anticholinergics is the incidence of side effects such as dry mouth, blurred vision, constipation and voiding

difficulties with significant amounts of residual urine. These effects are significant enough to cause the patient to discontinue taking the medication [21]. In one study, it was estimated that only approximately 18% of patients remained on anticholinergic therapy for over 6 months [22]. Interestingly, a group of patients with OAB seems to respond to antimuscarinic treatment irrespective of the presence of DO [23]. This is in accordance with the increasing body of evidence that the mechanism of antimuscarinics on OAB symptoms could be on bladder sensory pathways rather than on motor pathways [24,25,26]. There are many antimuscarinic drugs, for example oxybutynin, tolterodine and trospium chloride. Each has a different specificity to bladder muscarinic receptors, thus producing different adverse effect profiles. New anticholinergic drugs, that have undergone phase III trials and are more specific to the muscarinic M3 bladder receptor, are being developed (e.g. darifenacin and varicamide). Imidafenacin is the latest antimuscarinic, but is marketed only in Japan and has been evaluated just in Assian populations [27]. Future studies with these antagonists will reveal whether or not the principle of selective M3 receptor antagonism offers therapeutic advantages. Since M3 receptors are not only located in the bladder, but also in the salivary glands and the intestine, this could mean that two of the most common side effects (dry mouth and constipation) will not be avoided.
Since there is a lack of alternatives to antimuscarinics in the treatment of OAB symptoms, there has been an intensive search for new drug targets. Discovery of the β3-adrenoceptor with high expression in the bladder suggested that this receptor, which mediates detrusor relaxation, could be a target for overactive bladder symptom [28,29,30]. Several β3-adrenoceptor agonists have been developed (e.g. isoprenaline,mirabegron). Available information suggests that β3-adrenoceptor agonists can be considered an attractive alternative to antimuscarinic for OAB treatment [31,32,33].

8.3. Non pharmacological options

Activity in urinary reflex pathways can be influenced by activating nerves that synapse with this reflex system. This physiological process is called neuromodulation. Neuromodulation has been used by some investigators with success [34]. Peripheral electrical stimulation is thought to act through a mechanism involving reflex inhibition of motor output to the bladder and by activating inhibitory sympathetic fibres. Sacral, vaginal, anal, and lower extremity electrodes have produced ‘cure’ rates of as high as 20% and improvement rates as high as 50% to 60% [35].
Other therapies as bladder overdistention, transvaginal alcohol or phenol injections, and transvaginal dissection, are forms of peripheral denervation or neurologic decentralization. Denervation is a misleading term because it is not a true denervation process. Denervation would be a technically difficult procedure because it is hard to destroy the parasympathetic fibres as they lie near or within the organs they innervate. Denervation therapies are associated with high relapse rates, up to 100% after 18 months [36].
Botulinum A toxin (Botox or Dysport), another new agent, blocks both afferent and efferent nerves when injected into the bladder wall. This causes a paralysis of the detrusor

that persists for several months. Botulinum A toxin is very well tolerated by patients and is a safe and impressive alternative [37]. No adverse events have been reported up till now. About 5% of the patients do not respond. Up to 90% of the responders will be completely continent and are able to stop or reduce the use of anticholinergics. However, the invasive procedure has to be repeated periodically.
Auto-augmentation refers to a type of cystoplasty that involves excision of a large portion of detrusor muscle with preservation of the underlying mucosa (detrusor myomectomy). This procedure allows a bladder diverticulum to form with consequent increase in capacity. However, such techniques are major surgical procedures with potential complications and it carries with it the risk of urinary retention.

8.4. Search for new pharmacological targets

Theoretically, OAB can be treated by pharmacological agents that decrease DO, decrease urgency and increase bladder capacity. Many drugs have been tried, but the results are often disappointing. Antimuscarinic agents, specifically M3 antagonists, were considered the 'gold standard' of drug therapy in OAB. However, their associated side effects, particularly dry mouth, constipation and blurred vision can be prohibitive. β3-adrenoceptor agonists can be considered an attractive alternative for the treatment of OAB. However, adverse side affects such as dry mouth and gastrointestinal disturbances were also recorded using these selective therapeutic agents [31]. It seems that identification of new suitable targets for pharmacological intervention is a prerequisite to effectively treat OAB.

9. A shifted paradigm: focus on the bladder

It appears that attempts to explain the cause of the OAB have been primarily focused on abnormal expression of the micturition reflex, the so-called "neurogenic hypothesis". However, evidence is accumulating that changes in the bladder occur, which may influence the outcome of nervous bladder control. For example, bladder outlet obstruction (BOO) due to benign prostatic enlargement (BPE) and OAB often occur together. Studies reported that nearly 50% of men with LUTS and urodynamically confirmed BOO had DO [38,39]. Interventions that target the prostate often leave a subset of patients with inadequate symptom control. It is shown that urgency, frequency and nocturia persist in 19% of men after TURP [40] and 83% of these patients experienced return of these symptoms at long-term follow-up [41]. Interestingly, these complaints comprise LUTS during storage of urine in the bladder, which are the same symptoms as seen in the OAB.
Furthermore, it has been shown that isolated strips of normal detrusor muscle contract spontaneously and widespread activity of the bladder wall has been found in bladders that have been disconnected from the nervous regulatory systems [42-46]. This activity is highly modified in specimens dissected from overactive bladder [47-49]. It is therefore plausible that the human bladder has an intrinsic substrate for developing DO which is in fact non neurogenic.

10. Thesis outline and study rationale

The etiology of the overactive bladder (OAB) is still poorly understood. In **chapter 2** we demonstrate the complexity of the syndrome by means of an example of the complex interplay between OAB and benign prostatic enlargement (BPE) in male Lower Urinary Tract Symptoms (LUTS). It seems that bladder outlet obstruction (BOO) plays a role in developing OAB. Focussing on nervous control of the lower urinary tract may therefore bring the false interpretation of considering the bladder as being a passive 'black box'.

Our study group started this project to disentangle the biochemical and structural changes occurring in the overactive detrusor. Our starting hypothesis is that the spontaneous pressure rises that occur in the overactive bladder are not neurogenic, but originate from non neuronal structures within the bladder wall itself.

Chapter 3 presents an overview of the current hypotheses trying to explain detrusor overactivity (DO), in which the non-neurogenic theories will be emphasized. While doing so, the molecular basis for cell-cell crosstalk between diverse cell types within the bladder will be discussed.

It seems that the human urinary bladder is a highly sophisticated organ, and much progress is made during the last decade in elucidating its cell biological and physiological characteristics. However, many questions remain and the molecular basis for cell-cell crosstalk between various cell types is yet an unelucidated mechanism. For instance, detrusor smooth muscle cells need to be mechanically coupled in order to obtain a functional syncytium. The physical interaction between cells in general is mediated by the filamentous cytoskeleton that is bridged from one cell to the other via membrane spanning cell adhesion molecules. The class of adhesion molecules that mediates physical interaction between cells of the same type are the cadherins. We hypothesize that a yet unknown member of the cadherin superfamily mediates the critical recognition signal and physical interaction between detrusor smooth muscle cells. We investigated the expression of multiple subtypes of the cadherin family in the human bladder. **Chapter 4** shows our results.

As mentioned previously, it seems that alteration in detrusor smooth muscle properties is a requisite for the production of the unstable pressure rises seen in OAB. During ultrastructural studies using Transmission Electron Microscopy (TEM), observations on detrusor smooth muscle cell arrangement, morphology and patterns revealed that the overactive detrusor has a distinctive 'disjunction pattern' compared to the normal bladder. It is characterized by widening of spaces between individual smooth muscle cells and reduction or loss of adhesive intermediate cell junctions. These changes are a common feature in all types of OAB, regardless of etiology. In **chapter 5** we investigate the biochemical composition of intermediate junctions in normal and overactive human detrusor using immunohistochemical and electron microscopic techniques. We

hypothesize that cadherin-11 is downregulated in the disjunction pattern of the overactive detrusor.

During our former studies, expression of the subtype N-cadherin was found by coincidence. Its morphological appearance was highly interesting. It showed remarkable resemblance with expression profile of generally accepted histochemical markers for interstitial cells (IC). ICs are a recently discovered new type of cell within the bladder wall. In **chapter 6** we used additional cell markers and electron microscopic techniques in an attempt to analyze our finding. We hypothesize that N-cadherin is a specific marker for interstitial cells in the human bladder.

The isolated bladder shows spontaneous non-neuronal contraction during the filling phase, also known as autonomous activity of the bladder. As frequency and urgency occur during this filling phase, detrusor overactivity can be seen as a consequence of exaggerated autonomous activity during the storage of urine. The interstitial cell is believed to play a potential role in coordination of this activity. In **chapter 7** we investigate if N-cadherin positive cells could play a role in the etiology of the OAB. We hypothesize that the expression profile of the population of N-cadherin positive cells is altered in the overactive detrusor.

In summary, our hypotheses are the following:

1) A yet unknown member of the cadherin superfamily mediates the critical recognition signal and physical interaction between detrusor smooth muscle cells.
2) Cadherin-11 is downregulated in the disjunction pattern of the overactive detrusor.
3) N-cadherin is a specific marker for interstitial cells in the human bladder.
4) The expression profile of the population of N-cadherin positive cells is altered in the overactive detrusor.

References

1. Bahns E, Nelke A etal. Functional characteristics of lumbar visceral afferent fibres from the urinary bladder and the urethra in the cat. Pflugers Arch 1986;407:510-518
2. Habler H, Koltzenburg M et al. Activation of unmyelinated afferent fibres by mechanical stimuli and inflammation of the urinary bladder in the cat. J Physiol 1990;425:545-562
3. Blok B, Holstege G. The central nervous system control of micturition in cats and humans. Behav Brain Res 1998;92:119-125
4. Chai T, Steers W. Neurophysiology of micturition and continence. Urol Clin North Am 1966;23:221-236
5. De Groat W, Booth A. Physiology of the urinary bladder and urethra. Ann Intern Med 1980;92:312-315

6. Gosling J, Lendon R et al. The autonomic innervation of the human male and female bladder neck and proximal urthra. J Urol 1977;118:302-305
7. Dalmose A, Andersen I et al. Bladder and urethral responses to pelvic nerve stimulation in the pig. Scand J UrolNephrol Suppl 2002;210:34-45
8. Thor K, Morgan C et al. Organization of afferent and efferent pathways in the pudendal nerve of the female cat. J Comp Neurol 1981;201:415-440
9. Angel M, Fyda D et al. Primary afferent depolarization of cat pudendal afferents during micturition and segmental afferent stimulation. J Physiol 1994;479:451-461
10. Loewy A, Baker R et al. Descending projections from pontine micturition center. Brain res 1979;172:533-538
11. Yamagichi O. Beta3-adrenoreceptors in human detrusor muscle. Urology 2002;59(5):25-29
12. Malloy B, Price D et al. Alpha-1 adrenergic receptor subtypes in human detrusor. J Urol 1998;160(3):937-943
13. Sigala S, Spano P et al. Differential gene expression of cholinergic muscarinic receptor subtypes in male and female normal human urinary bladder. Urology 2002;60(4):719-725
14. Andersson K. Detrsuor contraction. Focus on muscarinic receptors. Scand J Urol Nephrol Suppl 2004;215:54-57
15. Jensen K, Madsen P et al. Urodynamic findings in elderly males without prostatic complaints. Urology 1984;24(2):211-213
16. Abrams P, Skidmore R et al. The concept and measurement of bladder work. J Urol. 1983;55(2):199
17. Rovner E, Wein A. Incidence and prevalence of overactive bladder. Curr Urol Rep. 2002;3(6):434-438
18. Debruyne F and Heesakkers JP. Clinical and socioeconomic relevance of overactive bladder. Urology 2004;63(3a):42-44
19. De Groat W. A neurological basis for the overactive bladder. Urology 1997;50(6a)36-52
20. Abrams P and Andersson KE. Muscarinic receptor antagonists for overactive bladder. BJU Int 2007;100:987–1006
21. Buser N, Ivic S, Bachmann L et al. Efficacy and adverse events of antimuscarinics for treating overactive bladder: network meta-analyses. Eur Urol 2012;62:1040–1060
22. Andersson KE, Appell R, Awad S et al: Pharmacological treatment of urinary incontinence BJU 1999;84(9):923-947
23. Lips K, Wunsch J, Zarghooni S, et al. Acetylcholine and molecular components of its synthesis and release machinery in the urothelium. Eur Urol 2007;51:1042-1053
24. Andersson KE. Antimuscarinic mechanisms and the overactivedetrusor: an update. Eur Urol 2011;59:377-386
25. Yoshimura N. Lower urinary tract symptoms (LUTS) and bladder afferent activity. Neurourol Urodyn 2007;26(6):908-913
26. Mehnert U,Reitz A, Schurch B et al. Proof of principle: the effect of antimuscarinics on bladder filling sensations in healthy subjects-a placebo controlled double blind investigation using 4 and 8mg tolterodine extended release. Neurourol Urodyn 2010;29:464-469

27. Maggiore L, Ferrero S et al. Imidafenacin for the treatment of overactive bladder. Expert Opin Pharmacother 2013;14(10):1383-1397
28. Chapple CR. beta3-Agonist therapy: a new advance in the management of overactive bladder? Eur Urol 2012;62:841–842
29. Ohlstein EH, von Keitz A, Michel MC. A multicenter, double-blind, randomized, placebo-controlled trial of the b3-adrenoceptor agonist solabegron for overactive bladder. Eur Urol 2012;62: 834-840
30. Gras J. Mirabegron for the treatment of overactive bladder. Drugs Today (Barc) 2012;48:25-32
31. Andersson KE, Nitti V et al. Selective β_3-adrenoceptor agonists for the treatment of overactive bladder. J Urol 2013 Oct;190(4):1173-1180
32. Bridgeman MB, Shah M et al. Mirabegron: β3-adrenergic receptor agonist for the treatment of overactive bladder. Ann Pharmacother. 2013;47(7-8):1029-1038
33. Chapple C, Martin C et al. Randomized Double-blind, Active-controlled Phase 3 Study to Assess 12-Month Safety and Efficacy of Mirabegron, a beta3-adrenoceptor agonist, in Overactive Bladder. Eur Urol. 2013 Feb;63(2):296-305
34. Levin P, Amundsen C et al. The efficacy of posterior tibial nerve stimulation for the treatment of overactive bladder in women: a systematic review. Int Urogyn J 2012;23(11):1591-1597
35. Balken van M, Vergunst H et al. The use of electric devices for the treatment of bladder dysfunction: a review of methods. J Urol 2004;172:846-851
36. Stephenson T and Mundy A. The urge syndrome. Urodynamics: principles, practice and applications. 1994;263-275
37. Duthie JB, Wilson D et al. Botulinum toxin injections for adults with overactive bladder syndrome. Cochrane Database Syst Rev 2011;7(12)
38. Fusco F, Groutz A, Blaivas JG et al., Videourodynamic studies in men with lower urinary tract symptoms: a comparison of community based versus referral urological practices. J Urol 2001;166:910-913
39. Lee JY, Kim HW, Lee SJ et al. Comparison of doxazosin with or without tolterodine in men with symptomatic bladder outlet obstruction and an overactive bladder. BJU Int 2004;94:817
40. Dmochowski RR, Staskin D, Overactive bladder in men: special considerations for evaluation and management. Urology 2002;60:56-62
41. Thomas AW, Cannon A et al. The natural history of lower urinary tract dysfunction in men: minimum 10-year urodynamic followup of transurethral resection of prostate for bladder outlet obstruction. J Urol 2005;174:1887-1891
42. Coolsaet BL, Van Duyl WA et al. New concepts in relation to urge and detrusor activity. Neurourol Urodyn 1993;12:463
43. Mills IW, Brading AF et al. Are unstable detrusor contractions dependent on efferent excitatory innervation? Neurourol Urodyn 1998;17:352-354
44. Skehan AM, Awad SA et al. The pathophysiology of contractile activity in the chronic decentralized feline bladder. J Urol 1993;149:1156-1164
45. Sethia KK, Brading AF et al. An animal model of non-obstructed bladder instability. J Urol 1990;143:1243-1246

46. Wat CY, Word RA et al. Effect of periurethral denervation on smooth muscles of the lower urinary tract. A J of Obst and Gyn 2004;119:1950-1960
47. German K, Brading AF et al. Physiological and morphometric studies into the pathophysiology of detrusor hyperreflexia in neuropathic patients. J Urol 1994;153:1678-1683
48. Turner WH. An experimental urodynamic model of lower urinary tract function and dysfunction. DM Thesis 1997
49. Mills IW, Brading AF et al. An investigation of the pathophysiology of idiopathic detrusor instability. Proceedings of the ICS 4th annual meeting 1997

2

The complexities of diagnosing and treating OAB in men

-New patients, new data, new treatment options-

Kamiel A.J. Kuijpers[1] and John P.F.A. Heesakkers[1]

[1]Department of Urology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

European Genito-urinary Disease, 2007; Issue I: 61-63

Summary

We emphasize that male overactive bladder (OAB) symptoms are storage lower urinary tract symptoms (LUTS). They may occur with or without bladder outlet obstruction (BOO), and are often found persistent after interventions targeted to the prostate. Because of the complex interplay between OAB and benign prostate enlargement (BPE) in male LUTS, we stress the necessity for measured evaluation and treatment regarding this interaction. In spite of concern about possible development of acute urinary retention (AUR) while using antimuscarinics, the first results of well-designed RCTs reveal that these agents (with or without an alpha-blocker) do not adversely affect urinary function and can be safely administered in men with LUTS.

1. Voiding and storage LUTS

The prevalence of lower urinary tract symptoms (LUTS) in men is high. A large-scale multi national study revealed that 62,5% percent of men in the general population (n=5460) reported at least 1 lower urinary tract symptom [1]. LUTS include storage symptoms (daytime urinary frequency, nocturia, urgency, urinary incontinence) and voiding symptoms (slow stream, splitting or spraying, intermittency, hesitancy, straining, terminal dribble) [2]. Storage LUTS are reported nearly twice as often (51.4%) as voiding LUTS (25.7%). LUTS can be associated with pathological conditions of the prostate, the bladder, or both [3], in which benign prostatic hyperplasia (BPH) and the overactive bladder (OAB) play a major role.

2. LUTS terminology: OAB and BPE

The overactive bladder (OAB) is characterized by urgency, frequency and nocturia with or without urge incontinence. In fact, it comprises the same symptoms as storage LUTS, but excludes types of incontinence other than urge incontinence. It therefore forms a comparable subset of storage LUTS as found being persistent after transurethral resection of the prostate. OAB is reasonably well correlated with detrusor overactivity (DO), which is defined as involuntary detrusor contractions during the filling phase, which the patient cannot inhibit.
The term benign prostatic hyperplasia (BPH) is a term used and reserved for the typical histological pattern that defines the disease. Benign prostatic enlargement (BPE) is caused by BPH and may cause bladder outlet obstruction (BOO), a functional diagnosis.

3. The complex interplay of OAB and BPE

Because DO may be primary or develop secondary to BOO, as shown in obstructive animal models [4], a complex interplay of prostatic pathology and bladder dysfunction eventually leads to storage LUTS in men. However, one must keep in mind that existence of BOO and DO does not always have a causal relationship.
The precise cause of DO in men with BOO has not been identified. It is suggested that BOO may cause DO by cholinergic denervation of the detrusor and consequent supersensitivity of muscarinic receptors [5]. BOO may also result in ischaemia, patchy denervation, altered electrical properties of smooth muscle and hypertrophied neurons. These changes lead to a re-organisation of the spinal micturition reflex, resulting in increased excitability and thereby triggering the sense of urgency and culminating in an involuntary detrusor contraction. This contraction clinically evokes storage LUTS.

4. Evaluation of male LUTS

Initial evaluation of men with LUTS starts with completing a voiding diary, which provides accurate data of normal urinary habits. LUTS questionnaires, like the ICS male questionnaire or the IPSS questionnaire, estimates the patients subjective bother. Further analysis of male LUTS is primary targeted to the prostate. This is not surprising, as historically used terms to describe male LUTS were all focused on the prostate as main perpetrator (e.g. prostatism or clinical BPH). The urinary flow test, which measures peak urinary flow and residual volume, is used to evaluate bladder emptying. This uroflowmetry provides relatively reasonable correlation with bladder outlet obstruction (BOO), but may be misinterpreted by inaccurate detrusor contractility. In these cases, pressure-flow analysis is used to distinguish BOO from detrusor underactivity during voiding.
However, many men with LUTS do not have BOO. Therefore, not all male LUTS can be screened in such a straightforward manner as previously described. Laniado et al. reported that urodynamically confirmed BOO occurred in only 48% of referred men with LUTS [6]. Additionally, BOO and OAB often occur together. Studies reported that nearly 50% of men with LUTS and urodynamically confirmed BOO had DO [7,8]. This illustrates the core of complexity surrounding the diagnosis and treatment of male LUTS. Therefore, the ideal approach for evaluating men with LUTS, in whom presumptive therapy fails, is to always use full urodynamic analysis (cystometry for filling and pressure-flow for voiding) to identify possible underlying bladder pathology, like DO.

5. Rationale for pharmacological treatment

Contraction of the bladder involves stimulation of muscarinic receptors by acetylcholine, released from activated cholinergic nerves. However, muscarinic receptors mediate not only normal bladder contraction, but also the abnormal contractions in DO [9]. Muscarinic receptor antagonists (antimuscarinics) therefore serve as the cornerstone in pharmacological treatment of OAB.
BPE has both static (increased tissue mass) and dynamic (increased smooth muscle tone) components in the prostate [10]. Medical management using 5-alpha-reductase inhibitors (5-ARI's) inhibits prostatic growth, whereas alpha-1-adrenergic receptor antagonists (alpha-blockers) reduce the tone of prostatic smooth muscle.

6. Treatment response in male LUTS varies

Practitioners should consider the possible involvement of bladder dysfunction in male LUTS, as interventions that target the prostate often leave a subset of patients with inadequate symptom control. It is shown that urgency, frequency and nocturia persist in 19% of men after TURP [11]. Interestingly, this subset of storage LUTS comprises the same symptoms as OAB. Also, Thomas et al [12] have recently shown that even in men who have had resolution of OAB symptoms after TURP, 48 (83%) of 58 experienced return of

their storage symptoms at long-term follow-up (mean, 12.6 years after surgery).
Lee et al [8] found that treatment response may vary in men with LUTS due to different conditions. After three months of treatment with doxazosin (alpha-blocker), 60 (79%) with BOO and 24 (35%) with BOO + DO reported symptomatic improvement. In those patients with no improvement, 32 of 44 (73%) with BOO + DO improved after combined therapy with tolterodine (antimuscarinic).

7. Undertreatment of OAB in male LUTS

Despite equal prevalence of OAB, fewer men than women are treated with antimuscarinics. This can be explained by the alternatives in potential pathophysiology: male LUTS can be caused solely by bladder dysfunction or mixed with prostatic pathology. This results in a complex clinical presentation of OAB symptoms in combination with other LUTS (voiding symptoms) [13]. Also, significant regional and country differences in the definitions of LUTS and OAB often lead to confusing terminology.
However, concerns about safety of treatment are most likely the major barrier to recognition of OAB symptoms in men with LUTS. Increased risk of acute urinary retention (AUR) after OAB treatment is still widely accepted.

8. Acute urinary retention

Acute urinary retention (AUR) is a condition characterized by a sudden inability to urinate, which is usually extremely painful and requires catheterisation [14]. Although relatively uncommon, AUR can be a dramatic event for the patient. Population-based studies estimate the incidence of AUR in men to be approximately 0.2% to 0.7% per year [15,16,17]. Although the incidence of AUR is relatively low in the general male population, the risk for AUR increases with age. The incidence of AUR was 3 times higher in men aged 60 to 69 and 8 times higher than in men aged 70 to 79 years, compared with younger men. The causes developing AUR can be classified into three categories. The first relates to any event that increases resistance to the urinary flow [16]. Secondly, weakness of detrusor muscle or interruption of sensory innervations of the bladder wall may results in AUR. The third category relates to any situation that permits the bladder to overdistend (e.g. post-surgery or drugs) [17].
Placebo arms of ‘BPH- trials’ may provide potential predictors of AUR. Several baseline characteristics correlate with the risk for spontaneous AUR and AUR-related surgery, including PSA, prostate volume and voiding International Prostate Symptom Score (IPSS). In the absence of AUR, baseline filling and storage subscores can be used as predictors of BPH-related surgeries [18-20]. The cumulative incidence of AUR in the placebo arms of the specified controlled ‘BPH’ trials was 0.6% to 2.1% per year [21-26]. Men in the placebo arms of these controlled trials were followed up for 2-4 years.
Post-void residual (PVR) volume may be used as a potential predictor of treatment outcomes. Results from the Medical Therapy of Prostatic Symptoms (MTOPS) trial demonstrated that higher baseline PVR was associated with slightly greater risk for clinical

progression [20]. According to clinical evidence, AUR develops in patients with steady increases in PVR but not with stable PVR throughout. Therefore, dynamic changes in PVR may be important [20,27,28].

9. Antimuscarinics and AUR

As antimuscarinics decrease detrusor contractility, administration of it theoretically elevates the risk for developing AUR. However, it is postulated that during voiding, massive release of acetylcholine occurs from parasympathetic nerves, displacing antimuscarinics via competitive binding, thus resulting in normal voiding [29]. It is suggested that, at therapeutic doses of antimuscarinics, there is no effect on normal voiding and no increased incidence of AUR [30].

10. Evidence on safety of antimuscarinics

Abrams et al. performed a multi-national, 12-weeks during, double-blind study, investigating the safety and tolerability of tolterodine instand release (IR) in men (n=221) with BOO and DO proven by urodynamics [31]. Patients were given tolterodine IR 2mg or placebo. Tolterodine IR improved volume to first detrusor contraction (VFC) (+59 ml, 95%) and maximum cystometric capacity (CMC) (+67 ml, 95%) significantly. Tolterodine did not adversely affect urinary function in men with OAB and BOO. Urinary flow rate was unaltered, and there was no evidence of clinically meaningful changes in voiding pressure. Tolterdine was well tolerated, suggesting it can safely be administered in men with BOO.
In other studies, a subanalysis of male patients with OAB in tolterodine extended release (ER) proved that treatment with tolterodine was not associated with increased incidence of AUR [32,34]. The authors concluded that the available data might be considered promising and the use of anticholinergic drugs was demonstrated to be “quite safe”. However, considering therapy with an alpha-blocker in combination with an antimuscarinic, studies were methodological not sufficient to support the clinical use of this combination therapy. Therefore, well-designed, large, double blind, placebo-controlled, long-term, randomized-controlled trials (RCT’s) were needed [34].

11. Well-designed RCTs

The TIMES study was performed to evaluate the efficacy and safety of an antimuscarinic, an alpha-blocker, or both in men with LUTS that include OAB symptoms [35]. In this 4-arm study, patients (n=879) were randomly assigned to receive placebo, tolterodine, tamsulosin, or both tolterodine and tamsulosin for 12 weeks. Tolterodine, administered with tamsulosin, was an efficacious treatment. Compared with placebo, patients experienced significant

reductions in urgency and frequency episodes. This led to significant improvements on the total international prostate symptom score. The incidence of AUR was low and in patients with PVR<200 ml and Qmax>5 ml/s, no increase of PVR or decrease of Qmax was found. Tolterodin or tamsulosin alone however, was not sufficient to show significant clinical efficacy.

References

1. Irwin DE, Milsom I, Hunskaar S et al. Population-based survey of urinary incontinence, overactive bladder, and other lower urinary tract symptoms in five countries: results of the EPIC study. Eur Urol 2006;50:1306-1315
2. Abrams P, Cardozo L, Fall M et al. The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Neurol Urodyn 2002;21:167-178
3. Abrams P, Cardozo L, Fall M et al. The standardisation of terminology in lower urinary tract function: report from the standardisation sub-committee of the International Continence Society. Urolog 2003;61:37-49
4. Mostwin JL, van Koeveringe G, Brooks EL et al. The guinea pig as a model of gradual urethral obstruction. J Urol 1991;145:854-858
5. Steers WD. Pathophysiology of overactive bladder and urge urinary incontinence, Rev Urol 2002;4:S7–S18
6. Laniado ME, Ockrim JL, Marronaro A et al. Serum prostatespecific antigen to predict the presence of bladder outlet obstruction in men with urinary symptoms. BJU 2004;94: 1283–1286
7. Fusco F, Groutz A, Blaivas JG et al. Videourodynamic studies in men with lower urinary tract symptoms: a comparison of community based versus referral urological practices. J Urol 2001;166:910–913
8. Lee JY, Kim HW, Lee SJ et al. Comparison of doxazosin with or without tolterodine in men with symptomatic bladder outlet obstruction and an overactive bladder. BJU Int 2004;94:817
9. De Groat WC. A neurologic basis for the overactive bladder. Urology 1997;50:36–52
10. Ziada A, Rosenblum M and Crawford ED Benign prostatic hyperplasia: an overview. Urology 1999;53:1–6
11. Dmochowski RR and Staskin D. Overactive bladder in men: special considerations for evaluation and management. Urology 2002;60(A):56–62
12. Thomas AW, Cannon A, Bartlett E et al. The natural history of lower urinary tract dysfunction in men: minimum 10-year urodynamic follow up of transurethral resection of prostate for bladder outlet obstruction. J Urol 2005;174:1887–1891
13. Chapple CR and Roehrborn CG. A shifted paradigm for the further understanding, evaluation, and treatment of lower urinary tract symptoms in men: focus on the bladder. Eur Urol 2006;49: 651–659

14. Dawson C and Whitfield H. ABC of Urology. Urological emergencies in general practice. BMJ 1996;312:838–840
15. Jacobsen SJ, Girman CJ, Guess HA et al., Natural history of prostatism: longitudinal changes in voiding symptoms in community dwelling men. J Urol 1996;155:595–600
16. Jacobsen SJ, Jacobson DJ, Girman CJ et al. Natural history of prostatism: risk factors for acute urinary retention. J Urol 1997;158:481–487
17. Verhamme KM, Dieleman JP, van Wijk MA et al. Low incidence of acute urinary retention in the general male population: the triumph project. Eur Urol 2005;47:494–498
18. Roehrborn CG, McConnell JD, Saltzman B et al. Storage (irritative) and voiding (obstructive) symptoms as predictors of benign prostatic hyperplasia progression and related outcomes. Eur Urol 2002;42:1–6
19. Roehrborn CG, Malice M, Cook TJ et al. Clinical predictors of spontaneous acute urinary retention in men with LUTS and clinical BPH: a comprehensive analysis of the pooled placebo groups of several large clinical trials. Urology 2001;58:210–216
20. McConnell JD, Bruskewitz R, Walsh P et al. The effect of finasteride on the risk of acute urinary retention and the need for surgical treatment among men with benign prostatic hyperplasia. Finasteride Long-Term Efficacy and Safety Study Group. N Engl J Med 1998;338:557–563
21. Placebo therapy of benign prostatic hyperplasia: a 25-month study. Canadian PROSPECT Study Group. Nickel CJ. Br J Urol, 1998;81:383–387
22. Efficacy and safety of finasteride therapy for benign prostatic hyperplasia: results of a 2-year randomized controlled trial (the PROSPECT study). PROscar Safety Plus Efficacy Canadian Two year Study. Nickel CJ et al. CMAJ,1996;155:1251–1259
23. Marberger MJ. Long-term effects of finasteride in patients with benign prostatic hyperplasia: a double-blind, placebocontrolled, multicenter study. PROWESS Study Group. Urology 1998;51:677–686
24. DeBruyne F, Barkin J, van Erps P et al. Efficacy and safety of long-term treatment with the dual 5 alpha-reductase inhibitor dutasteride in men with symptomatic benign prostatic hyperplasia. Eur Urol 2004;46:488–494
25. Roehrborn CG, Bruskewitz R, Nickel GC et al. Urinary retention in patients with BPH treated with finasteride or placebo over 4 years. Characterization of patients and ultimate outcomes. The PLESS Study Group. Eur Urol 2000;37:528–536
26. McConnell JD, Roehrborn CG, Bautista OM et al. The longterm effect of doxazosin, finasteride, and combination therapy on the clinical progression of benign prostatic hyperplasia. N Engl J Med 2003;349:2387–2398
27. Roehrborn CG. Alfuzosin 10 mg once daily prevents overall clinical progression of benign prostatic hyperplasia but not acute urinary retention: results of a 2-year placebo-controlled study. BJU Int 2006;97:734–741
28. Emberton M. Definition of at-risk patients: dynamic variables. BJU Int 2006;97(2):12–15
29. Andersson KE and Yoshida M. Antimuscarinics and the overactive detrusor-which is the main mechanism of action? Eur Urol 2003;43:1–5

30. Reynard JM. Does anticholinergic medication have a role for men with lower urinary tract symptoms/benign prostatic hyperplasia either alone or in combination with other agents? Curr Opin Urol 2004,14:13–16
31. Abrams P, Kaplan S, De Koning Gans HJ et al. Safety and tolerability of tolterodine for the treatment of overactive bladder in men with bladder outlet obstruction. J Urol 2006;175:999–1004
32. Roehrborn CG, Abrams P, Rovner ES et al. Efficacy and tolerability of tolterodine extended-release in men with overactive bladder and urgency urinary incontinence. BJU Int 2006;97:1003–1006
33. Elinoff V, Bavendam T, Glasser DB et al. Symptom-specific efficacy of tolterodine extended release in patients with overactive bladder: the IMPACT trial. Intl J Clin Pract 2006;60:745–751
34. Novara G, Galfano A, Ficarra V et al. Anticholinergic drugs in patients with bladder outlet obstruction and lower urinary tract symptoms: A systematic review. Eur Urol 2006;50:675–683
35. Kaplan SA, Roehrborn CG, Rovner ES et al. Tolterodine and tamsulosin for treatment of men with lower urinary tract symptoms and overactive bladder: a randomized controlled trial. JAMA 2006;296:2319–2328

3

Pathophysiological aspects of the musculus detrusor in the human overactive bladder: an overview

Kamiel A.J. Kuijpers[1], John P.F.A. Heesakkers[1] and Jack A. Schalken[1]

[1]Department of Urology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

Summary

The neurogenic theory suggests that detrusor overactivity occurs as a result of changes in the peripheral and central nervous system of the bladder. However, focussing on nervous control of the lower urinary tract may bring the false interpretation of considering the muscular end organ as being a passive ’black box’. It seems that alteration in the musculus detrusor is a requisite for the production of detrusor overactivity: bladders that have been disconnected from central nervous system show spontaneous activity. The origin of this pacemaking activity remains unelucidated. But there is a growing body of evidence that detrusor overactivity can be seen as a consequence of exaggerated autonomous activity.

Although the smooth muscle cells constitute the majority of cells within the human bladder wall, other type cells may have important functions in coordinating spontaneous activity. The human bladder could be arranged into modules of detrusor muscle, mediated by an intramural myovesical plexus that consists of neurons and interstitial cells. If the myovesical plexus could communicate with a suburothelial layer of interstitial cells that act as a sensing layer for bladder volume, we might have a proper model for integrating urge bladder sensation and detrusor contractions.

It is proposed that synchronization of the modules would be imposed by the central nervous system during normal voiding. However, detrusor overactivity might be the consequence of synchronization of the modules arising during the filling phase. As alterations to the intramural myovesical plexus are found in detrusor overactivity of any origin, this cellular system could play a main role in the disentanglement of the OAB syndrome.

This is an exciting time for basic research considering the overactive human detrusor. Although the pathophysiological aspects of the human detrusor are becoming more and more clear, the interaction of detrusor smooth muscle cells, interstitial cells, neurones and the urothelium in the human bladder remains a highly interesting field for further research.

1. Introduction

Lower urinary tract symptoms can be categorized as storage, voiding and postmicturition symptoms. The most common problem with urine storage arises when the bladder fails to remain relaxed until an appropriate time for urination. This syndrome is called the overactive bladder (OAB). The OAB syndrome is defined as urgency, with or without incontinence, usually with frequency and nocturia [1]. The key symptom of the OAB is urgency. The disorder can arise in the presence of bladder outflow obstruction (BOO) and in people with neurological disease, but is idiopathic in many cases [2]. OAB symptoms are suggestive of urodynamically demonstrable detrusor overactivity (DO) during the filling phase. DO is a generic designation of involuntary detrusor contractions during bladder filling, which the patient cannot inhibit, commonly resulting in urge to void, leakage of urine (incontinence) or both [3].

The etiology of the OAB and DO is still poorly understood and pharmacological tools to control it remain relatively ineffective. This is due to the fact that the mechanism of bladder contractility is not elucidated. Attempts to explain the cause of DO have been primarily focused on abnormal expression of the micturition reflex, the so-called “neurogenic hypothesis” [4]. However, evidence is accumulating that the spontaneous rises in pressure occurring in the OAB, regardless of etiology, are in fact non-neurogenic. Other theories have therefore been proposed regarding the cause of DO.

The objective of this review is to give an overview of the current hypotheses trying to explain DO, in which the non-neurogenic theories will be emphasized. While doing so, the molecular basis for cell-cell crosstalk between diverse cell types within the bladder will be discussed.

2. Neurogenic bladder hypothesis

Normal voiding and storage is controlled by central and peripheral nervous systems [4]. The central pathways in the brain and spinal cord are organized as simple on-off switching circuits. The peripheral system consists of three sets of peripheral nerves: the pelvic nerves, the lumbar nerves and the pudendal nerves. These nerves contain both afferent (sensory) axons and efferent (motory) pathways. Under additional sympathetic and parasympathetic control, the central and peripheral nervous system control the bladder and urethra as a functional unit.

The neurogenic theory suggests that DO can occur as a result of changes in peripheral and central nervous system of the bladder, hereby disrupting the voluntary control of the micturition pathway and causing an abnormal expression of the micturition reflex. These reflexes closely resemble the so-called primitive voiding reflexes. They can be induced by damage to the inhibitory neuronal pathways or sensitization of peripheral afferent terminals in the bladder. Neurogenic DO is often found in patients who suffer from cerebrovascular events, brain tumors, multiple sclerosis, spinal cord lesion, diabetes mellitus and Parkinson’s disease. However, focussing on nervous control of the lower urinary tract may bring the false interpretation of considering the muscular end organ as being a passive “black box”.

3. Myogenic bladder hypothesis

The human bladder is all but a passive “black box”. Isolated strips of normal detrusor muscle contract spontaneously and widespread activity of the bladder wall has been shown in bladders that have been disconnected from central nervous system control [5-9]. It seems that the human bladder has an intrinsic substrate within the bladder wall for developing this spontaneous activity, which is non-dependent of the nervous system. This activity is highly modified in detrusor strips dissected from overactive bladder, regardless the etiology [10-12]. The myogenic basis for detrusor overactivity suggests that denervation of the bladder wall alters the properties and structure of detrusor smooth muscle, leading to increased excitability and increased ability of activity to spread between detrusor smooth muscle cells, resulting in coordinated myogenic contractions of the entire detrusor muscle and highly modified spontaneous activity [13]. It seems that alteration in the musculus detrusor is a requisite for the production of DO.

4. Tissue remodeling of the detrusor muscle

Neurogenic, obstructed and idiopathic overactive detrusors all show patchy changes in detrusor smooth muscle fascicle structure [14-18]. Bundles of smooth muscle loose their intrinsic nerves and become infiltrated by elastin (elastosis) and collagen (collagenosis). Such remodeled bundles can exist alongside normal bundles. It must be noted that as defects in the overactive detrusor are punctuate and normal and modified areas are present within the same analyzed specimen, this has important implications for experimental studies on the nature of DO.

During ultrastructural studies using electron microscopy (EM), it was found that the overactive detrusor has a distinctive ‘disjunction pattern’ compared to the normal bladder [19-26]. Next to denervation, this pattern is characterized by (1) marked reduction or loss of adherens junctions (AJs) between detrusor smooth muscle cells, (2) widening of spaces between individual smooth muscle cells coexisting with collagenosis and elastosis, and (3) appearance of abnormal ultraclose abutments and protrusion junctions which are alien to the normal detrusor. The authors claimed that these changes are a common feature in all types of the overactive detrusor, regardless of etiology. Therefore, they proposed that EM could be used as a diagnostic tool for DO.

5. Ultrastructural studies and diagnostics

Elbadawi et al. concluded that EM examination of detrusor biopsies could be used in diagnosis of DO, replacing time-consuming and costly urodynamic studies [27]. Tse et al. found that adherens junctions, protrusion junctions and ultraclose abutments were all present in both stable and overactive specimens. However, the authors concluded that EM could still be used as an adjunctive technique to diagnose DO by calculating the ratio of abnormal-to-normal junctions [28]. This finding is in contrast to other studies in which a specific relationship between ultrastructural detrusor smooth muscle features and DO

was not confirmed [29,30]. Holm et al. investigated 25 patients with BOO-DO using the protocol of Elbadawi's group. They found that the only significant parameter in bladder function was elastosis.
The characterization of smooth muscle cell junctions by EM has important limitations, as the difficulty remains in classifying and interpreting more rudimentary forms of various types of cell junctions. To overcome this problem, Carey et al. used vinculin as a structural protein associated with AJ's to study the disjunction pattern in normal and overactive detrusor [31]. The authors found expression of vinculin virtually occupying the entire detrusor smooth muscle cell membrane. However, their analysis relied on separate standard transmission EM and immunohistochemistry techniques: immuno-gold labeling of vinculin was not included. Also, vinculin is known as a classical attachment protein involved in the indirect binding of intracellular actin filaments to fibronectin of the extracellular matrix. Kuijpers et al. showed that cadherin-11 is an integral and structural protein of the AJ, using immunogold EM techniques [32]. Cadherins are a superfamily of transmembrane glycoproteins, which participate in cell-cell recognition and adhesion [33,36]. Cadherin-11 mediated adherens junctions form the basis for physical coupling between individual smooth muscle cells and suburothelial myofibroblasts by anchoring cytoskeletal myofilaments to the cell membrane in two opposing cell surfaces [37]. Expression of cadherin-11 in normal detrusor did not differ from overactive detrusor. Also, regional variation of cadherin-11 was found, contradicting the homogenous nature of the disjunction pattern. Although the role for cadherin-11 in tissue remodeling of the overactive detrusor remains unelucidated, the authors hypothesized that loss of cadherin-11 is a potential trigger for widening of spaces between individual smooth muscle cells and the appearance of collagenosis. Brierly et al. also could not reproduce the ultrastructural changes in the pathological bladder as described by Elbadawi et al [38,39]. It is therefore surprising that Elbadawi et al. were able to report perfect agreement between urodynamics and ultrastructure. In conclusion, it seems that the application of ultrastructural analysis as a diagnostic tool for DO cannot replace conventional urodynamics.

6. Electrical coupling in detrusor smooth muscle

Although the EM analysis as described by Elbadawi et al. does not seem reproducible, it shows interesting features such as the appearance of abnormal ultraclose abutments and protrusion junctions. This appearance of abnormal junctions seems to co-exist with an increased expression of connexin-45, a gap-junction associated protein [40-42]. Gap junctions are specialized regions of plasma membranes formed by unique channels, which provide sites of low resistance. By mediating calcium transport they are likely to play an important role in the transduction of electrical stimuli necessary for multicellular smooth muscle contraction [43]. Detrusor smooth muscle cells of the normal bladder are poorly electrically coupled compared to other smooth muscles [44,45]. However, the newly formed abnormal junctions might be identified as family like gap junctions, thereby increasing the level of functional connectivity between smooth muscle cells. According to the myogenic hypothesis, a local contraction occurring at a particular region within

the detrusor wall may easier spread to adjacent cells and hence generate multicellular contractile responses resulting in DO. Since loss of AJ's is believed to restrict the main vehicle for mechanical syncytium in detrusor smooth muscle cells, it might be possible that the overactive detrusor tries to restore this syncytium by forming a new and highly developed electrical coupled multicellular network. However, gap junctions expressing connexins have never been found in a convincing matter between detrusor smooth muscle cells during immunogold EM studies. It therefore remains unknown whether the abnormal ultraclose abutments and protrusion junctions are in fact true functional gap junctions. In conclusion, neither the neurogenic nor the myogenic hypothesis fully explains the recognized clinical and experimental findings. Therefore, other ways of approaching the pathophysiology of DO must be sought.

7. Autonomous bladder hypothesis

A third and recently proposed hypothesis underlines the coordinated waves of contractions of the musculus detrusor that has been disconnected from central nervous control. It shows that there are two distinct types of contractile activity in the bladder: micturition contraction and autonomous activity [46,47]. Micturition contraction is associated with voiding. However, the human bladder also reveals contractile activity during the filling phase of the micturition cycle. This is shown in detrusor preparations, in isolated denervated bladders of animals and in human bladders during ambulatory urodynamics [48-52]. This non-neurogenic spread of excitation is generated within the bladder wall and is called autonomous activity. It has first been described for over 120 years now, but has regained new interest [49]. Autonomous activity can be described as localized propagating waves of contraction traveling through the bladder wall. The number of these localized propagating waves increases as bladder filling proceeds. As OAB symptoms occur during the filling phase, urge to void and DO could be seen as a consequence of exaggerated autonomous activity during the storage of urine [48,53].

8. Modulation of autonomous activity

Isolated detrusor strips and whole bladder models are used to further explore the physiological mechanism of autonomous contraction [53-58]. In the resting isolated guinea pig bladder, autonomous activity involves waves of contraction and localized stretches of the bladder wall at multiple locations, resulting in minimal amplitude of intravesical pressure changes. One should note the resemblance with non-micturition activity as found in human ambulatory urodynamics [59]. This activity was found in approximately 70% of patient who did not suffer from OAB complaints.) These waves of contraction travel considerate distances. Therefore, like the myogenic hypothesis presumes, synchronous contraction of a large number of detrusor smooth muscle cells must be a prerequisite for the occurrence of these large waves.

Intramural neurons and detrusor smooth muscle cells seem to be potential cellular candidates in explaining the nature of coordination of autonomous activity. The

bladder contains a highly developed intramural network of neuronal elements which could coordinate synchronous contraction. However, alterations to this system fail to explain autonomous contraction waves as denervated bladder strips show exaggerated autonomous activity [60,61]. Therefore, autonomous contraction waves are most likely mediated by non-neuronal structures.

Detrusor smooth muscle cells could play a role in coordination of autonomous activity: gap junctions activate adjacent smooth muscle cells to eventually generate a large-scale multicellular contraction [62]. However, detrusor smooth muscle cells are relatively poorly electrically coupled in normal bladder [44]. It seems that myogenic transmission can only travel relatively short distances. Furthermore, quantitative impedance measurements showed an increased intercellular resistance in detrusor smooth muscle cells of the OAB [62,63]. It is therefore highly unlikely that smooth muscle cells account for a pathway by which spontaneous activity might travel throughout the bladder wall. Additionally, the anatomical organization of detrusor smooth muscle cells does not seem to allow a direct coupling between adjacent areas of muscle: detrusor smooth muscle cells are typically arranged in bundles that form an interlocking basket-weave pattern [64]. An arrangement such as this does not give rise to many close connections between cells. Thus, within the attempt to elucidate the physiological mechanism of autonomous contraction, there must be another cellular player.

Gillespie et al. further analyzed the distinct types of bladder contraction. Nerve stimulation, direct application of muscarinic agonists and increase of intravesical volume all augmented autonomous activity [46,53]. However, the phasic contractions of autonomous activity induced by muscarinic agonists could not be inhibited by the neurotoxin tetrodotoxin, suggesting that these phasic contractions do not involve the classical neuromuscular junction. Muscarinic receptors are expressed by detrusor smooth muscle cells, but also by another class of suburothelial cells [65,66]. These suburothelial cells show structural similarities to smooth muscle cells and fibroblasts and are closely associated with afferent and efferent nerve endings. They express the adherence junctional protein cadherin-11 [37] and the gap junctional protein connexin-43, probably forming a tight physical and electrical syncytium [67,68]. It has been is proposed that these suburothelial cells could function as a sensing network for bladder filling, firmly held together by adherence junctions and communicating via gap junctions. Possibly, they receive input from the urothelium and efferent nerve endings, modulating afferent bladder function.

These suburothelial cells have recently been identified as myofibroblasts and are also called interstitial cells (ICs). Initially, ICs were thought to represent a specialized type of neurons, but it is now concluded that ICs are a unique class of cells, with a clear structural definition, functional role and pathological potential [69]. ICs are also housed in the detrusor layer, located on the boundary of muscle bundles. Like in the suburothelial layer, they are closely associated with intramural neurons and ganglia [70,71,72]. The detrusor ICs seem to form an intramural network, in which possibly information is relayed from the neuronal plexus. Considering their morphological appearance, they could play a major role in the coordination of autonomous activity of the human bladder.

9. Markers to characterize bladder interstitial cells

ICs are often referred to as myofibroblasts. However, there is no consensus about whether these are the same cells. Myofibroblasts are considered to be smooth-muscle-like fibroblasts. They are contractile and express cytoskeletal filaments as vimentin, smooth muscle actin, desmin and heavy chain myosin [73]. They are physically coupled to each other by cadherin-11 mediated adherence junctions [74]. They have a spindle like shaped morphology and abundant pericellular matrix. Ultrastructurally, the myofibroblast has a prominent rough endoplasmatic reticulum (RER), a golgi apparatus producing collagen granules, multiple mitochondria, myofilaments with dense plaques, adherence junctions, gap junctions and fibronexus junctions. The human bladder ICs differ from myofibroblasts as they lack expression of desmin and heavy chain myosin [75,76].
Recent studies have identified various surrogate markers for bladder ICs, such as expression of the stem cell receptor C-kit and cyclic guanosine mono-phosphate (cGMP) [77,78]. C-kit is not expressed by all types of ICs and it can be detected in other cell types [79,80]. cGMP is induced by ICs in the bladder but is also expressed by urothelial cells [78]. Recently, it was found that N-cadherin was specifically expressed at cells with branched cell bodies and multiple processes in the suburothelial lamina propria and detrusor layer [81]. According to the authors, further immunohistochemical phenotyping and ultrastructural characterization using Transmission Electron Microscopy (TEM) indicated that this N-cadherin+ cell population most probably embodies ICs and thus could play a role as a discriminatory marker for ICs in the human bladder. This new marker for ICs in the human bladder was a welcome finding, as neither C-kit, cGMP nor vimentin labelling seem to be ideal in all tissues of the urinary tract.
Nonetheless, the specific immunophenotype of human ICs is still controversial as it seems that these cells represent a heterogeneous population. Although it is tempting to speculate that bladder ICs are indeed myofibroblasts, caution is needed before a more complete understanding of their morphological, ultrastructural and physiological characterisation is realized. In this review we have adopted the term interstitial cells for all myofibroblastic cells (non fibroblasts) in the human bladder wall.

10. Bladder interstitial cells and autonomous activity

The exact function of the network of bladder ICs has not been described, but can be hypothesized from analogous cells located in the gut. In the human gastrointestinal tract, a highly developed network of specialized ICs of Cajal (ICCs) is found in which ICCs interconnect through gap junctions [82]. ICCs have been placed in a central position in the nerve to muscle signal transmission, receiving direct neuronal input and transferring it to the smooth muscle cells [83,84]. They hereby coordinate gut peristalsis.
ICs of the human bladder share properties with the ICCs. However, they embody a different subtype than the ICCs [85,86,87]. As ICs are closely associated with intramural nerves they might, like the gut, form an integrative plexus with neuronal structures within the bladder wall. It is hypothesized that the bladder, like the gut, is arranged into modules [88].

Modules of the bladder are circumscribed areas of detrusor muscle that are active during the filling phase. They are controlled by an intramural network of neuronal structures and ICs, also called the myovesical plexus. In this plexus, intramural ganglia play an important role. Ganglia can be considered as neuronal stations that receive diverse inputs from their environment. They are thought to relay the incoming information to an excitatory or inhibitory output signal. Thus, the myovesical plexus could form an integrative functional module that receives excitatory and inhibitory neuronal inputs and is able to realize local contractions [89]. The network of ICs in the detrusor layer could therefore not only play a major role in coordination of autonomous activity, but in voiding contractions as well.

11. Bladder pacemaking

In order to develop autonomous activity in the denervated bladder, the bladder also requires an intramural site for pacemaking. Several structures such as the urothelium, intramural neuronal structures, ICs and smooth muscle cells have been investigated for this feature.

The urothelium is no longer considered to be a passive membrane. It is regarded as an integrated element in bladder signaling, receiving and expressing neurotransmitters. Vanilloid and purinergic receptors, adenosine triphosphate (ATP), nitric oxide (NO) and acetylcholine play an important role in urothelial signaling mechanisms [90]. Although the urothelium seems to be an important player in the system of bladder function, the urothelium is not considered to play a pacemaking role [91].

In experiments using denervated detrusor strips and whole guinea pig bladders, the peripheral nervous system could still play a pacemaking role. As denervation in DO is patchy, intrinsic nerves running through the human bladder wall could partly be maintained. However, localized contractions were not affected by atropine and the neurotoxin tetrodotoxin, which block nerve conduction [46]. Therefore, it is highly unlikely that autonomous activity is generated by neural pacemaking.

Recordings of spontaneous electrical activity in detrusor strips using intracellular microelectrodes demonstrated that action potentials often occur in bursts that trigger multicellular contractions [92,93,94]. ICs in the guinea pig are spontaneously active and react to muscarinic receptor stimulation with firing of Ca+ mediated bursts [95]. In the detrusor layer, ICs are also located at the boundary of smooth muscle bundles. From here, spontaneous calcium transients originate, suggesting that ICs may be crucial in generating spontaneous activity [77,95]. However, others found that these spontaneous calcium bursts occurred independently from calcium transients originating from smooth muscle cells, even when synchronous calcium waves swept across the muscle bundles [85,87]. Therefore, it was proposed that ICs in the human bladder are not pacemakers for autonomous activity and thus are not a simple analogy of ICCs as found in the gut.

Other studies using microelectrodes and intercellular calcium imaging have demonstrated that isolated detrusor smooth muscle cells are capable of generating spontaneous action potentials which are almost identical to those recorded from intact preparations [96,97,98]. It was suggested that autonomous contractions in the human bladder are

initiated by specialized detrusor smooth muscle cells and transmitted by ICs throughout the rest of the bladder.
In conclusion, the search for the cellular mechanism triggering autonomous activity is characterized by conflicting findings. It seems that the intramural cellular pacemaker that triggers autonomous activity remains unelucidated.

12. Interstitial cells and detrusor overactivity

Synchronisation of activity between different modules, mediated by increased coupling of ICs, could lead to an inappropriate augmentation of autonomous activity and predispose for DO. It seems that the network of ICs in the obstructed bladder is highly proliferated, resulting in intense bridging of the suburothelial ICs and the ICs located within the detrusor layer [99,100,101]. If these ICs form a functionally linked network, signals generated in the urothelium could be transported through this system towards the detrusor smooth muscle cells, where they could result into exaggerated autonomous contraction. Evidence for such an urothelium derived excitation has been postulated in the bladder of the guinea pig and the rat [102,103]. It must be mentioned that the urothelium not only forms a high-resistance barrier to ion, solute and water flux, and pathogens, but also functions as an integral part of a sensory web which receives, amplifies, and transmits information about its external milieu [104].
Others have also found a correlation between upregulation of IC-like cells and exaggerated autonomous activity. The authors investigated idiopathic and neurogenic detrusor overactivity specimens [105]. Imatinib mesylate (Glivec; a specific C-kit receptor inhibitor) had an inhibitory effect on the overactive detrusor. As C-kit labeling showed significantly more IC-like cells in overactive human detrusor layer than in normal specimens, it seems highly likely that this inhibitory effect is due to the upregulation of detrusor ICs. Additionally, reduction of ICCs is found in syndromes with reduced autonomous activity of the gut, such as Hirschsprung's disease and functional intestinal obstruction [106].
Although the exact role for ICs in bladder function has not yet been described, structural changes to the interstitial network might not only result in alterations to the distribution of this activity, but also to the level of intrinsic activity and its sensitivity to excitatory and inhibitory mediators.

References

1. Wein AJ. Diagnosis and treatment of the overactive bladder. J Urol 2003;5B:20-27
2. Andersson K. Storage and voiding symptoms: pathophysiologic aspects. Urology 2003;5B:2-10
3. Abrams P. Describing bladder storage function: overactive bladder syndrome and detrusor overactivity. J Urol 2003;62(5B):28-37
4. De Groat W. A neurological basis for the overactive bladder. Urology 1997;50(5A):36-52
5. Coolsaet BL, Van Duyl WA et al. New concepts in relation to urge and detrusor activity. Neurourol Urodyn 1993;12:463-471

6. Mills IM and Brading AF. Are unstable detrusor contractions dependent on efferent excitatory innervation? Neurourol Urodyn 1998;17:352-354
7. Skehan AM, Downie JW and Awad SA. The pathophysiology of contractile activity in the chronic decentralized feline bladder. J Urol 1993;149:1156-1164
8. Sethia KK, Brading AF et al. An animal model of non-obstructed bladder instability. J Urol 1990;143:1243-1246
9. Wai CY, Liehr P, Word RA et al. Effect of periurethral denervation on smooth muscles of the lower urinary tract. A J of Obst and Gyn 2004;119:1950-1960
10. German K, Brading AF, Stephenson TP et al. Physiological and morphometric studies into the pathophysiology of detrusor hyperreflexia in neuropathic patients. J Urol 1994;153:1678-1683
11. Turner WH. An experimental urodynamic model of lower urinary tract function and dysfunction. DM Thesis 1997
12. Mills IW and Brading AF. An investigation of the pathophysiology of idiopathic detrusor instability. Proceedings of the ICS 4th annual meeting 1997
13. Brading AF. A myogenic basis for the overactive bladder. Urology 1997;50(6A):57-67
14. Charlton RA, Morley AR, Gillespie JI et al. Focal changes in nerve, muscle and connective tissue in normal and unstable human bladder. BJU International 1999;84:953–960
15. Brierly RD, Hindley RG, Thomas PJ et al. A prospective evaluation of detrusor ultrastructural changes in bladder outlet obstruction. BJU International 2003;91:360-364
16. Brierly RD, Hindley RG and Thomas PJ. Comment on: Focal changes in nerve, muscle and connective tissue in normal and unstable human bladder. BJU 2000;86(3):407
17. Drake MJ, Gardner BP and Brading AF. Innervation of the detrusor muscle bundle in neurogenic detrusor overactivity. BJU International 2003;91:701-710
18. Mills IW, Greenland JE, Brading AF et al. Studies of the pathophysiology of idiopathic detrusor instability: the physiological properties of the detrusor smooth muscle and its pattern of innervation. The Journal of Urology 2000;163:646-651
19. Elbadawi A, Yalla SV and Resnick NM. Structural basis of geriatric voiding dysfunction. I. Methods of a prospective ultrastructural/urodynamic study and an overview of the findings. J Urol 1993;150(5):1650-1656
20. Elbadawi A, Yalla SV and Resnick NM. Structural basis of geriatric voiding dysfunction. II. Aging detrusor: normal versus impaired contractility. J Urol 1993;150(5):1657-1667
21. Elbadawi A, Yalla SV and Resnick NM. Structural basis of geriatric voiding dysfunction. III. Detrusor overactivity. J Urol 1993;150(5):1668-1680
22. Elbadawi A, Yalla SV and Resnick NM. Structural basis of geriatric voiding dysfunction. IV. Bladder outlet obstruction. J Urol 1993;150(5):1681-1695
23. Elbadawi A, Yalla SV and Resnick NM. Structural basis of neurogenic bladder dysfunction. I. Methods of prospective ultrastructural study and overview of the findings. J Urol 2003;169(2):540-546
24. Elbadawi A, Yalla SV and Resnick NM. Structural basis of neurogenic bladder dysfunction. II. Myogenic basis of detrusor hyperreflexia. J Urol 2003;169(2):547-554
25. Elbadawi A, Yalla SV and Resnick NM. Structural basis of geriatric voiding dysfunction. III. Detrusor overactivity. J Urol 1993;150(5):1668-1680

20. Elbadawi A, Yalla SV and Resnick NM. Structural basis of geriatric voiding dysfunction. V. Standardized protocols for routine ultrastructural study and diagnosis of endoscopic edtrsuor biopsies. J Urol 1997;157:1783-1801
27. Elbadawi A, Yalla SV and Resnick NM. Functional pathology of urinary bladder muscularis: the new frontier in diagnostic uropathology. Semin Diagn Pathol 1993;10(4):314-354
28. Tse V, Khadra MH et al. The application of ultrastructural studies in the diagnosis of bladder dysfunction in a clinical setting. J Urol 2000 ;163:535-539
29. Holm NR, Rossette de la JN et al. The detrusor muscle cell in bladder outlet obstruction. Scand J Urol Nephrol 2003;37:309-315
30. Brierly RD. Thomas PJ et al. A prospective evaluation of detrusor ultrastructural changes in bladder outlet obstruction. BJU International 2003;91:360-364
31. Carey MP, Friedhuber A et al. A prospective evaluation of the pathogenesis of detrusor instability in women, using electron microscopy and immunohistochemistry. BJU Int 2000;86: 970-978
32. Kuijpers KA, Heesakkers JP, Schalken JA et al. Adherence junctions and cadherin-11 in stable and overactive human detrusor smooth muscle cells. J Urol 2011;185(5):1946-1951
33. Bryant PJ. Junction genetics. Dev Genet 1997;20:75-90
34. Nagafuchi A. Molecular architecture of adherens junctions. Curr Op Cell Biol 2001;13:600-603
34. Yap AS and Goodwin M. Classical cadherin adhesion molecules: coordinating cell adhesion, signaling and the cytoskeleton. J Mol Hist 2004,35:839-844
36. Wheelock MJ and Johnson KR. Cadherins as modulators of cellular phenotype. Annu Rev Cell Dev Biol 2003,19:207-235
37. Kuijpers KA, Heesakkers JP, Schalken JA et al. Cadherin-11 is expressed in detrusor smooth muscle cells and myofibroblasts of normal human bladder. Eur Urol 2007;52(4):1213-1221
38. Brierly RD, Thomas PJ et al. A prospective evaluation of detrusor ultrastructural changes in bladder outlet obstruction. BJU International 2003;91:360-364
39. Brierly RD, Thomas PJ Et al. Comment on: Focal changes in nerve, muscle and connective tissue in normal and unstable human bladder. BJU 2000 Aug;86(3):407
40. Haferkamp A et al. Increased expression of connexin 43 in the overactive neurogenic detrusor. Eur Urol 2004;46(6):799-805
41. Yin JL et al. Gap junction protein expression in the unstable human detrusor. J Urol 2003;169: 317
42. Haefliger J.A. et al. Connexins 43 and 26 are differentially increased after rat bladder outlet obstruction. Exp Cell Res. 2002 Apr 1;274(2):216-225
43. Bryant PJ et al. Junction genetics. Dev Genet 1997;20(2):75-90
44. Bramich NJ and Brading AF. Electrical properties of smooth muscle in guinea pig urinary bladder. J Physiol 1996,492:185-198
45. Hanani M and Brading AF. Electrical coupling in smooth muscles. Is it universal? J Basic Clin Physiol Pharmacol 2000,11:321-330
46. Gillespie JI, Drake MJ et al. Agonist- and nerve- induced phasic activity in the isolated whole bladder of the guinea pig: evidence for two types of bladder activity. Exp Physiol 2003:342-357

47. Sanders KM. A case for the interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. Gastroenterology 1996;111:492-515
48. Gillespie JI. The autonomous bladder: a view of the origin of bladder overactivity and sensory urge. BJU International 2004;93:478-483
49. Sherrington. Notes of the arrangement of some motor fibres in the lumbo-sacral plexus. J Physiol 1882;13:621-772
50. Klevmark B. Motility of the urinary bladder in cats during filling at physiological rates. II. Effects of extrinsic bladder denervation on intramural tension and on intravesical pressure patterns. Acta Physiol Scand 1977;101(2):176-184
51. Vaughan CW and Satchell PM. Role of the sympathetic innervation in the feline continence process under natural filling conditions. J Neurophysiol 1992;68(5):1842-1849
52. Gillespie JI. A developing view of the origin of urgency: the importance of animal models. BJU Int 2005;96(1):22-28
53. Drake MJ and Gillespie JI. Autonomous activity in the isolated guinea pig bladder Exp Physiol 2003;88:19-30
54. Sibley GN. A comparison of spontaneous and nerve mediated activity in bladder muscle from man, pig and rabbit. J Physiol 1984;354:431-434
55. Levin RM, Wein AJ et al. Relevance of spontaneous activity to urinary bladder function: an in vitro and in vivo study. J Urol 1986;136:517-521
56. Kinder RB, Mundy AR et al. Pathophysiology of idiopathic detrusor instability and detrusor hyper-reflexia. An in vitro study of human detrusor muscle. Br J Urol 1987;60:509-515
57. Potjer RM, Constantinou CE et al. Frequency of spontaneous contractions in longitudinal and transverse bladder strips. Am Physiol 1989;257:781-787
58. Foster CD, Brading AF et al. The effect of cromakalim on the smooth muscle of the guinea-pig urinary bladder. Br J Pharmacol 1989;97:281-291
59. Bristow SE, Neal DE et al. Ambulatory urodynamics. Br J Urol 1996;77(3):333-338
60. Mills IW, Brading AF et al. Studies of the pathophysiology of idiopathic detrusor instability: the physiological properties of the detrusor smooth muscle and its pattern of innervation. J Urol 2000;163:646-651
61. Drake MJ, Brading AF et al. Innervation of the detrusor muscle bundle in neurogenic detrusor overactivity. BJU International 2003;91:701-710
62. Fry CH, Wu C et al. Spontaneous activity and electrical coupling in human detrusor smooth muscle: implications for detrusor overactivity? Urology 2004;63(3A): 3-10
63. Sui GP, Fry CH et al. Impedance measurements and connexin expression in human detrusor muscle from stable and unstable bladders. BJU Int 2003;92:297-305
64. Daniel EE et al. Structural bases for neural and myogenic control of human detrusor muscle. Can J Physiol Pharmacol 1983;61:1247-1273
65. Grol S, Gillespie JI et al. M3 muscarinic receptor expression on suburothelial interstitial cells. BJU Int 2009;104(3):398-405
66. Chapple CR, Chess-Williams C et al. Muscarinic receptor subtypes and management of the overactive bladder. Urology 2002;60(5A):82-89
67. Sui GP, Rothery S, Dupont E et al. Gap junctions and connexin expression in human suburothelial interstitial cells. BJU Int 2002;90:118-129

68. John H, Wang X, Wehrli E et al. Evidence of gap junctions in the stable nonobstructed human bladder. J Urol 2003;169:745-749
69. Eyden B. The myofibroblast: an assessment of controversial issues and a definition useful in diagnosis and research. Ultrastruct Pathol 2001;25:39-50
70. Smet PJ, Vente J et al. Distribution of nitric oxide synthase-immunoreactive nerves and identificatuion of the cellular targets of nitruc oxide in guinea-pig and human urinary bladder by cGMP immunohiostochemistry. Neuroscience 1996;71:337-348
71. McCloskey KD and Davidson RA. Morpholgy and localization of interstitial cells in the Guinea pig bladder: structural relationships with smooth muscle and neurons. Journal of Urology 2005;173:1385-1390
72. De Vente J, Gillepsie JI et al. Interstitial cells and cholinergic signaling in the outer muscle layers of the guinea-pig bladder. BJU Int 2006;97:379-385
73. Eyden B. The myofibroblast: phenotypic characterization as a prerequisite to understanding its functions in translational medicine. J Cell Mol Med 2008;12(1):22-37
74. Eyden B, Drake MJ et al. Structural characterization of myofibroblasts in the bladder. BJU Int 2006 ;97:29-32
75. Hinz B, Smith-Clerc J et al. Myofibroblast development is characterized by specific cell–cell adherens junctions. Mol Biol Cell 2004;15:4310-4320
76. Blyweert W et al. Interstitial cells of the bladder: the missing link? BJOG 2004,111(1): 57-60
77. McCloskey KD and Gurney AM Kit positive cells in the guinea pig bladder. J Urol. 2002;168:842-836
78. Gillespie JI and De Vente J. cGMP-generating cells in the bladder wall: identification of distinct networks of interstitial cells. BJU Int. 2004; 94:1114-1124
79. Moll R, Bath PJ et al. Fibrocytes, alpha-smooth muscle antigenpositive myofibroblasts, and CD117 expression in the stroma of invasive squamous cell carcinomas of the oral cavity, pharynx and larynx. Virchows Arch 2004; 44: 231-234
80. Drake MJ, Andersson KK et al. Morphology, phenotype and ultrastructure of fibroblastic cells from normal and neuropathic human detrusor: absence of myofibroblast characteristics. J Urol 2003;169:1573-1976
81. Kuijpers KA, Heesakkers JP, Schalken JA et al. An update of the interstitial cell compartment in the normal human bladder. BioMed Research International 2014;Feb
82. Sanders KM. A case for the interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. Gastroenterology 1996;111:492-515
83. Sanders KM,Ward SM et al. Development and plasticity of interstitial cells of Cajal. Neurogastroenterol Motil 1999;11:311-338
84. Ward SM, Sanders KM et al. Interstitial cells of Cajal mediatecholinergic neurotransmission from enteric motor neurons. J Neurosci 2000;20:1393-1403
85. McCloskey KD and Davidson RA. Morpholgy and localization of interstitial cells in the Guinea pig bladder: structural relationships with smooth muscle and neurons. J Urol 2005;173:1385-1390
86. De Ridder D, van der Aa F et al. Identification of kit positive cells in the human urinary tract. Journal of Urology 2004;171:2492-2496

87. McCloskey KD, Gurney AM Kit Positive Cells In The Guinea Pig Bladder. Journal of Urology 2002;16(2):832-836
88. Gillepsie JI, Drake MJ et al. Model of peripheral autonomous modules and a myovesical plexus in normal and overactive bladder function. The Lancet 2001;358:401-403
89. Hashitani H. Interaction between interstitial cells and smooth muscles in the lower urinary tract and penis. J Physiol 2006;707-714
90. Birder LA. Role of the urothelium in urinary bladder dysfunction following spinal cord injury. Prog Brain Res 2006;152:135-146
91. Andersson K and Hedlund P. Pharmacological perspective on the physiology of the lower urinary tract. Urology 2002;60(5A):13–21
92. Hashitani H and Suzuki H. Identification of interstitial cells of Cajal in corporal tissues of the guinea pig penis. Br J Pharmacol 2004;141:199-204
93. Hashitani H and Brading AF. Electrical properties of detrusor smooth muscles from the pig and human urinary bladder. Br J Pharmacol 2003,140:146-158
94. Hashitani H and Brading AF. Ionic basis for the regulation of spontaneous excitation in detrusor smooth muscle cells of the guinea pig urinary bladder. Br J Pharmacol 2003;140:159-169
95. Hashitani H, Suzuki H et al. Origin and propagation of spontaneous excitation in smooth muscle of the guinea pig urinary bladder. J Physiol 2001;530:273-286
96. Sui G, Wu C et al. Aberrant Ca2+ oscillations in smooth muscle cells from overactive human bladders. Cell Calcium 2009;45(5):456-464
97. Sui GP, Fry CH et al. The association between T-type Ca2+ current and outward current in isolated human detrusor cells from stable and overactive bladders. BJU Int 2007;99(2):436-441
98. Yanai Y, Suzuki H et al. The role of Ni(2+)-sensitive T-type Ca(2+) channels in the regulation of spontaneous excitation in detrusor smooth muscles of the guinea-pig bladder. BJU Int 2006 ;97(1):182-189
99. Gillespie JI, De Vente J et al. Endogenous nitric oxide/cGMP signalling in the guinea pig bladder: evidence for distinct populations of sub-urothelial interstitial cells. Cell Tissue Res 2006;325-332
100. Kubota Y and Kohri K. Altered distribution of interstitial cells in the guinea pig bladder following bladder outlet obstruction. Neurourology and urodynamics 2008;27:330-340
101. Kuijpers KA, Heesakkers JP, Schalken JA et al. Alterations of the myovesical plexus of the human overactive detrusor. BioMed Research International 2014, April
102. Lagou M, Drake MJ et al. Location of interstitial cells and neurotransmitters in the mouse bladder. BJU Int 2006;97(6):1332-71337
103. Ikeda Y, Kanai A et al. The Role of Gap Junctions in Spontaneous Activity of the Rat Bladder. Am J Physiol Renal Physiol 2007;203(4):1018-1025
104. Birder L and Andersson K. Urothelial signaling. Physiol Rev 2013;93(2):653-80
105. Biers SM, Brading AF et al. The functional effects of a C-kit tyrosine inhibitor on guinea pig and human detrusor. BJU Int 2006;97:612-616
106. Piotrowska AP, Puri P et al. Interstial cells of Cajal in the human normal urinary bladder and in the bladder of patients with megacystis-microcolon intestinal hypoperistalsis syndrome. BJU Int 2004;94:143-146

4

Cadherin-11 is expressed in detrusor smooth muscle cells and myofibroblasts of normal human bladder

Kamiel. A.J. Kuijpers[1], John P.F.A. Heesakkers[1], Cornelius F.J. Jansen[1] and Jack A. Schalken[1]

[1]Department of Urology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

European Urology 2007 Oct; 52(4): 1213-1221

ABSTRACT

Objectives:
It has recently been found that detrusor smooth muscle cells and myofibroblasts are coupled via gap junctions. However, gap junctions cannot account for strong physical interaction between cells, which has prompted the search for intercellular adhesion molecules. Cadherin-11 is a candidate for such a molecule, since it mediates the interaction of dermal myofibroblasts in contractile wound granulation tissue. We therefore hypothesised that the physical adhesion between detrusor smooth muscle cells and myofibroblasts is mediated by cadherin-11. The aim of this study was to test this hypothesis.
Methods:
Bladder biopsies from eight radical cystectomy specimens were snap-frozen, sectioned, and stained for E-cadherin; cadherin-11; α-catenin; β-catenin; γ-catenin and smooth muscle cell/myofibroblast markers connexin-43, vimentin, desmin, smooth muscle actin, and smoothelin. Specimens were analysed by using binocular epifluorescent and confocal laser–scanning microscopy.
Results:
Specific positive membranous expression of all adhesion complex molecules except E-cadherin was detected in detrusor suburothelial tissue. All biopsies showed a similar punctate pattern of expression for cadherin-11 within bundles of smooth muscle cells and a suburothelial layer of cells. Cadherin-11 was specifically located at the cell membrane, in distinct linear domains.
Conclusions:
To our knowledge this is the first time evidence has been provided for cadherin-mediated smooth muscle and suburothelial myofibroblast cell-cell interaction in the human bladder. Cadherin-11 most probably plays an important role in the intercellular physical coupling of detrusor smooth muscle cells and also of myofibroblasts.

1. INTRODUCTION

In the last few years, considerable progress has been made in understanding the cellular basis of contraction of the detrusor muscle. In contrast to the thought that the detrusor muscle is solely a collection of independent contractile cells, each activated by separate neural inputs, evidence is now accumulating that the detrusor muscle should be regarded as a set of functional units of smooth muscle cells that act cooperatively. The recent finding that detrusor smooth muscle cells and myofibroblasts (a recently identified suburothelial layer of cells) are coupled via so-called gap junctions supports this theory [1,2]. Gap junctions are specialized regions of plasma membranes formed by unique channels, which provide sites of low resistance. By transmitting small molecules and electric currents between the intracellular compartments, they can be instrumental in activating adjacent smooth muscle cells. These functional connections are, therefore, thought to regulate coordinated and synchronised detrusor smooth muscle contraction [2].

However, muscle cells also need intact intercellular mechanical adhesion. Without this adhesion, muscle bundles will break into pieces if simultaneous excitation occurs. This phenomenon is shown in plakoglobin knockout experiments in which disintegration of adhesion junctions between cardiac myocytes results in premature death of the animal because the heart literally breaks when it starts pumping [3]. Gap junctions cannot account for the required adhesion between muscle cells. This finding has prompted the search for adhesion complexes between detrusor smooth muscle cells.

Recently, evidence was provided for the presence of cadherins in adhesion junctions in cardiac myocytes [4,5]. Cadherins seemed to be a prerequisite for the formation of gap junctions. Cadherins are a superfamily of transmembrane glycoproteins, which participate in cell-cell recognition and cell-cell adhesion [6]. They play a crucial role in embryogenesis, organogenesis, and differentiation of cells in general. Cell type–specific expression has been demonstrated in various organs. So far, evidence for expression of cadherins in the human detrusor is still lacking. The best-characterised member of the cadherins is E-cadherin [6,7]. It mechanically connects epithelial cells via their extracellular domains, forming membrane-bound adherens junctions (AJs). Intracellularly, the cytoplasmic tail is coupled to the actin filamental network via catenins. AJs are in fact anchoring points for the actin (microfilament) bundles of the cytoskeleton. The AJ typically mediates homotypic cell-cell interaction. A candidate cadherin for expression in smooth muscle cells is cadherin-11, a type II cadherin, since it was identified in dermal myofibroblasts in contractile wound granulation tissue. Moreover, Hinz and colleagues [8] have shown that myofibroblast contraction can be attenuated by cadherin-11 antibodies.

We therefore consider cadherin-11 a suitable candidate for mediation of homophilic cell-cell adhesion between detrusor smooth muscle cells and myofibroblasts. In this study we report our results about the expression of several cadherins, catenins and cytoskeletal proteins in the normal human bladder. To our knowledge this is the first time that cadherins and catenins have been identified in suburothelial myofibroblasts and detrusor smooth muscle of the normal human bladder.

2. METHODS

2.1. Patients

Bladder tissue was obtained from eight individuals in whom radical cystectomy was performed because of localized bladder cancer. Samples were dissected from tumour-free bladder areas at least 3 cm distant from tumour zones. Mean patient age was 57 yr (range: 51–72), four male and four female. Biopsies were all taken from the vesical dome at tumour-free bladder regions from functionally normal bladders. The local ethics committee approved the study and informed consent was obtained from all patients.
Full-thickness tissue pieces were collected and placed for cryosectioning in a mould containing Tissue-Tek (4583; Sakura, Zoeterwoude, The Netherlands) and snap-frozen immediately in isopentane at -80˚C. Using haematoxylineosin staining techniques, tissue was analysed under a binocular microscope for the presence of intact urothelium and smooth muscle.

2.2. Immunofluorescence

Sections of 4 mm of directly frozen specimens were prepared with the use of a cryostat and mounted on Super Frost Plus (Menzel-Gläser) slides. For cadherin and catenin staining (Table 1), the unfixed sections were immersed in 3% paraformaldehyde for 10 min. Cell membranes were permeabilised in 0.2% Triton X-100 for 5 min. For cytoskeletal protein, staining samples were fixed in acetone for 10 min and air dried at room temperature for 2 h. Each step was separated by washing samples three times for 2 min in magnesium and calcium-containing phosphate-buffered saline (PBSExtra: 40 ml PBS, 960 ml demiwater, 100 ml 1 mol/l MgCl2, 100 ml 1 mol/l CaCl2). After washing, sections were incubated for 1 h with the use of primary antibodies diluted in PBS 1% bovine serum albumin. The optimal antibody dilution was chosen empirically. Sections again were washed three times in PBS-Extra. Next, the sections were incubated with Alexa Fluor 488 goat antimouse antibody (A-11017; Molecular Probes, Eugene OR, USA) and Alexa Fluor 488 goat anti rabbit antibody (A-11070; Molecular Probes). The range of different fluorochromes was used to optimise clarity of visualisation against natural background fluorescence of the bladder tissue, which was mainly due to autofluorescent elastic fibres. Finally, treatment with DAPI (24653; Merck, Darmstadt, Germany) or propidiumiodide (P4170; Sigma-Aldrich, St Louis, MO, USA) was performed for staining of the nucleus. All sections were mounted in Dako Cytomation Fluorescent Mounting Medium (S3023; Dako Cytomation, Glostrup, Denmark) for analysis. Negative controls included omission of primary antibodies and incubation with PBS-Extra instead. Positive controls included urothelial layer (internal control) in all sections and prostate cancer specimens for cadherin-11 (external control).

Table 1 - Primary antibodies used

No.	Protein	Clone	Provided by
Cadherins			
(1)	E-cadherin	Mouse mAb clone HECD-1	M106 TBI
(2)	Cadherin-11	Mouse mAb clone 16a	Bussemakers et al [21]
Catenins			
(3)	α-Catenin	Rabbit pAb	C2081 SA
(4)	β-Catenin	Mouse mAb αABC	Van Noort et al [22]
(5)	γ-Catenin	Mouse mAb clone PG 5.1	61005 PN
Cytoskeletal filaments			
(6)	Smoothelin	Mouse mAb R4A	Van Der Loop et al [23]
(7)	Phalloidin	—	T7471 MP
(8)	Smooth muscle actin	Mouse mAb 1A4	A2547 SA
(9)	Desmin	Mouse mAb RD301	Verhagen et al [24]
(10)	Vimentin	Mouse mAb RV203	Schaart et al [25]
(11)	Connexin-43	Rabbit pAb	71-0700 ZL

TBI = Takara Bio Inc, Otsu, Shiga, Japan; SA = Sigma-Aldrich, St Louis, MO, USA; PN = Progen, Heidelberg, Germany; MP = Molecular Probes, Eugene, OR, USA; ZL = Zymed Laboratories, San Francisco, CA, USA.
Note that γ-catenin is homologous to desmoplakin (see Introduction). Phalloidin was used because it selectively binds to polymeric filamentous actin. Staining for connexin-43 was performed because double staining with vimentin is considered a marker for suburothelial myofibroblasts in human detrusor.

2.3. Analysis

Immunostained sections were examined by binocular epifluorescent (DFC FX; Leica) and confocal laser–scanning microscopy (TCS NT; Leica). Series of images were sequentially recorded for each channel to avoid signal crossover. To help interpret the fluorescent images, we also stained cryosections with haematoxylin-eosin for examination using standard bright field optics. Cadherin and catenin expression profiles were related to tissue morphology and cytoskeletal markers.

3. RESULTS

3.1. Suburothelial myofibroblasts express adherens junction molecules and contain contractile stress fibres

In all bladder specimens four distinct layers could be discriminated: the urothelium, the suburothelium, the detrusor muscle, and the serosa. There was a clear demarcation between the detrusor muscle and the urothelium and suburothelium. The urothelium was four to five cell layers thick, whereas the suburothelium contained connective tissue, blood vessels, and nerves.
In all eight biopsies, the suburothelial layer of cells showed similar positive expression of several adherens junction molecules (cadherin-11, α-catenin, β-catenin) and cytoskeletal proteins (actin, vimentin). The urothelium served as an internal positive control for the E-cadherin and catenin antibodies. For cadherin-11 however, which is not expressed in bladder urothelium, prostate cancer specimens were used as a positive control instead. No immunoreactions were detected in the negative controls in any bladder specimen, but background signal was caused by autofluorescent elastic tissue fibres (Fig. 1). Specific punctate expression of cadherin-11 protein was found in a band of interstitial cells located

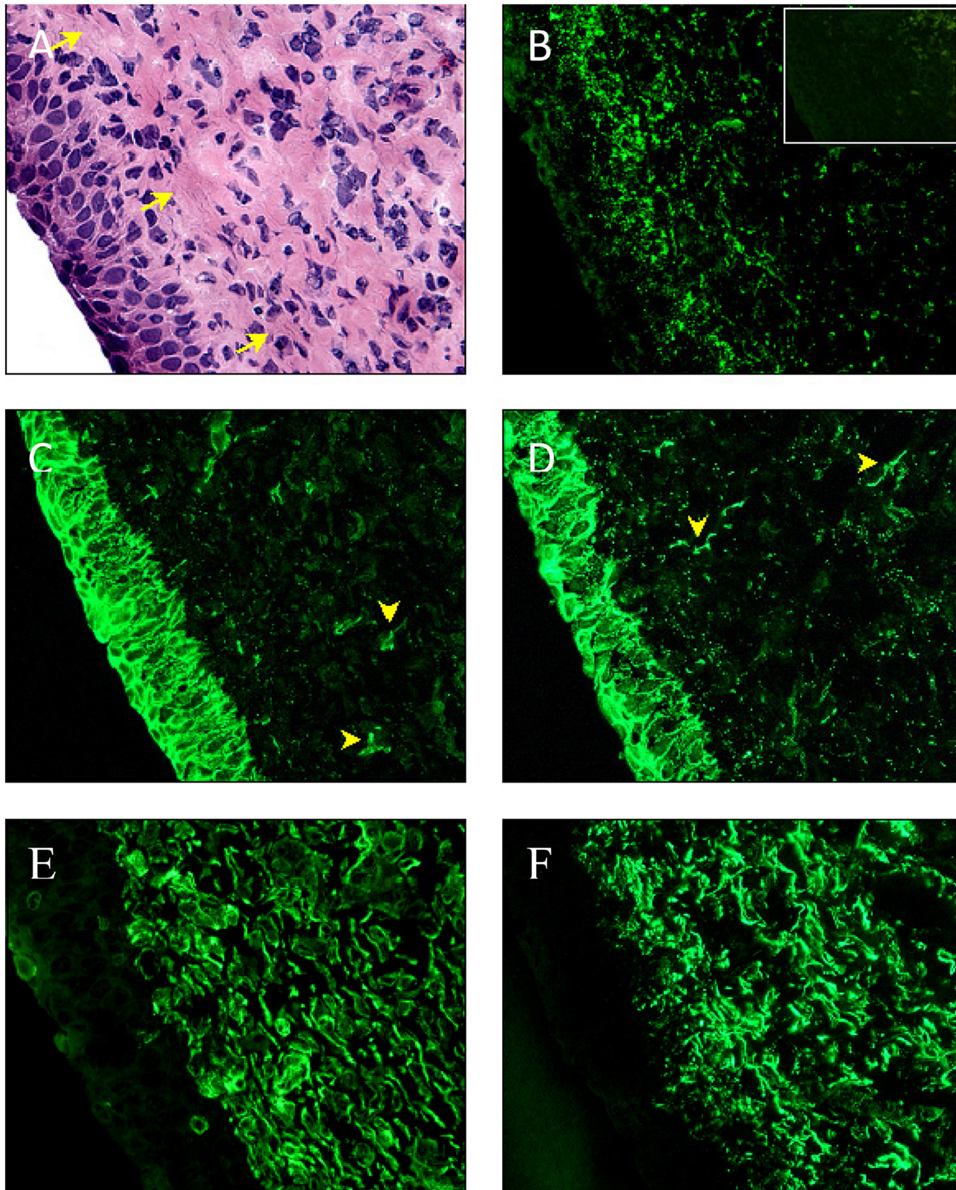


Figure 1 – Expression of adherens junction molecules and cytoskeletal proteins in the urothelial and suburothelial layer. Specimen overview by haematoxylin-eosin stain showing multiple layers of flattened cells just below the urothelial lining (A). Note urothelium and underlying layer of flat myofibroblasts (arrows A). Note autofluorescent collagen fibres in negative control (insert B). Clear punctate expression of cadherin-11 (B), α-catenin (C), and β-catenin (D) was found in the flattened suburothelial cells. These cells also expressed smooth muscle actin (E), vimentin (F), and connexin-43 (data not shown). Arrow heads (C and D) show linear, nonpunctate structures, most probably embodying small neurones and vessels. Binocular epifluorescent microscopy, magnification x100.

in the suburothelial zone running parallel with and adjacent to the urothelium (Fig. 1). Several layers of flattened cells could be distinguished within this area by haematoxylin-eosin staining. Double labeling with and expression of connexin-43 and vimentin, which are considered markers for myofibroblasts [9] (Table 2), identified these flattened cells as suburothelial myofibroblasts. At the bladder wall side facing the intravesical lumen, cadherin-11 immunoreactivity in these cells was limited to the urothelial basement membrane. Urothelial cells showed no immunoreactivity for cadherin-11. On the submucosal side, the density of cadherin-11 labelling reduced progressively as the depth increased and eventually disappeared at the deeper located layer of collagenous tissue (Fig. 2). Similar to the pattern of expression of cadherin-11, prominent punctate immunoreactivity for β-catenin was found just proximal to the basement membrane that lines the urothelium. However, punctate α-catenin was less intensively expressed, and staining for E-cadherin and γ-catenin was negative (data not shown). The suburothelial myofibroblasts expressed cytoplasmic filaments containing smooth muscle actin (Fig. 1), but appeared to be negative for desmin and smoothelin (data not shown). Actin filament bundles showed close association with the punctate cadherin-11 staining (Fig. 2).

In addition to the above-described punctate expression of cadherin-11 and catenins, positive staining of these proteins was found as non punctate linear structures within the suburothelial layer (Fig. 1). These suburothelial structures most probably reflect small vessels and neurones situated within and just below the layer of suburothelial myofibroblasts, since they showed remarkable resemblance in immunoreactivity with larger neurons and vessels situated in the serosa.

Table 2 – Twelve fields per specimen were examined independently of clinical data using a semi quantitative scale: S, no staining; +/S, low staining; +, intermediate staining; ++, high staining (average score)

No.		Urothelium	Myofibroblasts	Smooth muscle
Cadherins				
(1)	E-cadherin	++	–	–
(2)	Cadherin-11	–	++	++
Catenins				
(3)	α-Catenin	++	+	–
(4)	β-Catenin	++	++	++
(5)	γ-Catenin	++	–	–
Myofibroblast & smooth muscle markers				
(6)	Smoothelin	–	–	++
(7)	Phalloidin	+	+	++
(8)	Smooth muscle actin	–	++	++
(9)	Desmin	–	–	++
(10)	Vimentin	–	++	–
(11)	Connexin-43	–	++	–

No staining was performed to confirm presence of myosin heavy chain filaments, but smoothelin was used as a smooth muscle specific marker instead. Smoothelin is a recently discovered cytoskeletal protein which is solely found in contractile smooth muscle cells and does not belong to one of the classes of structural proteins presently known [13].

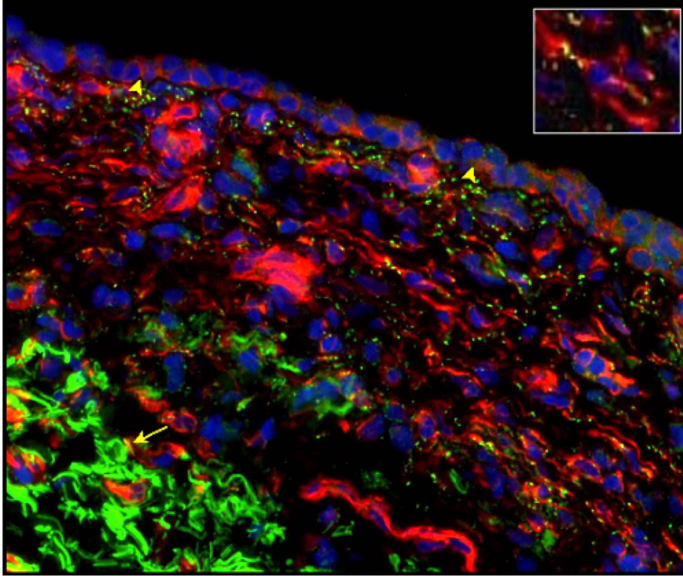


Figure 2 – Costaining of cadherin-11 (green), smooth muscle actin (red), and nuclei (blue) in the urothelium and suburothelial layer. Arrow heads mark thin urothelial layer. Suburothelial myofibroblasts revealed punctuate staining for cadherin-11. Punctate labelling disappeared at the layer of collagenous tissue, which was detected as green fibres due to autofluorescence (arrow bottom left). Insert shows enlargement of a few suburothelial myofibroblasts. Erratic expression of actin filaments seemed to associate with membrane-located cadherin-11. Note the nucleus staining centrally in the cell. Binocular epifluorescent microscopy, magnification x400.

3.2. Detrusor smooth muscle cells and suburothelial myofibroblasts show similar expression of cadherin-11

In all eight specimens the overall appearance of smooth muscle bundles was uniform, showing no loosened fascicular structure or widening of intercellular spaces between individual smooth muscle cells (Fig. 3). Muscle bundles were embedded in a band of connective tissue with a relatively sparse vasculature. Smooth muscle cells showed sharply defined fluorescent spots of immunoreactivity for cadherin-11 and β-catenin (Fig. 3) in all biopsies, leaving no gender-related differences. This staining pattern showed great similarity with the punctate immunofluorescent signals as found in the suburothelial myofibroblasts. Costaining for smooth muscle actin (using phalloidin), cadherin-11, and nuclei showed that cadherin-11 expression seemed to be localised at the boundaries of closely adjacent smooth muscle cells (Fig. 4). In addition, no cadherin-11 expression was found at smooth muscle bundle boundaries, their component fascicles and nonadjacent smooth muscle cells. Thus, expression of cadherin-11 seemed highly specific for homotypic detrusor smooth muscle cell-cell interaction. Detrusor smooth muscle cells did not show expression of E-cadherin or γ-catenin (data not shown) and α-catenin (Fig. 3).
Smoothelin was used as a smooth muscle-specific marker. All smooth muscle cells showed positive staining for cytoplasmic smoothelin, desmin (data not shown), and smooth muscle actin (Fig. 3). Smooth muscle cells showed no expression of vimentin. However, vimentin-positive cells were present between smooth muscle bundles, as well as in the

microseptae surrounding their component fascicles (data not shown), but not between individual smooth muscle cells. They most probably reflect fibroblasts delineating the boundary of muscle fascicles.

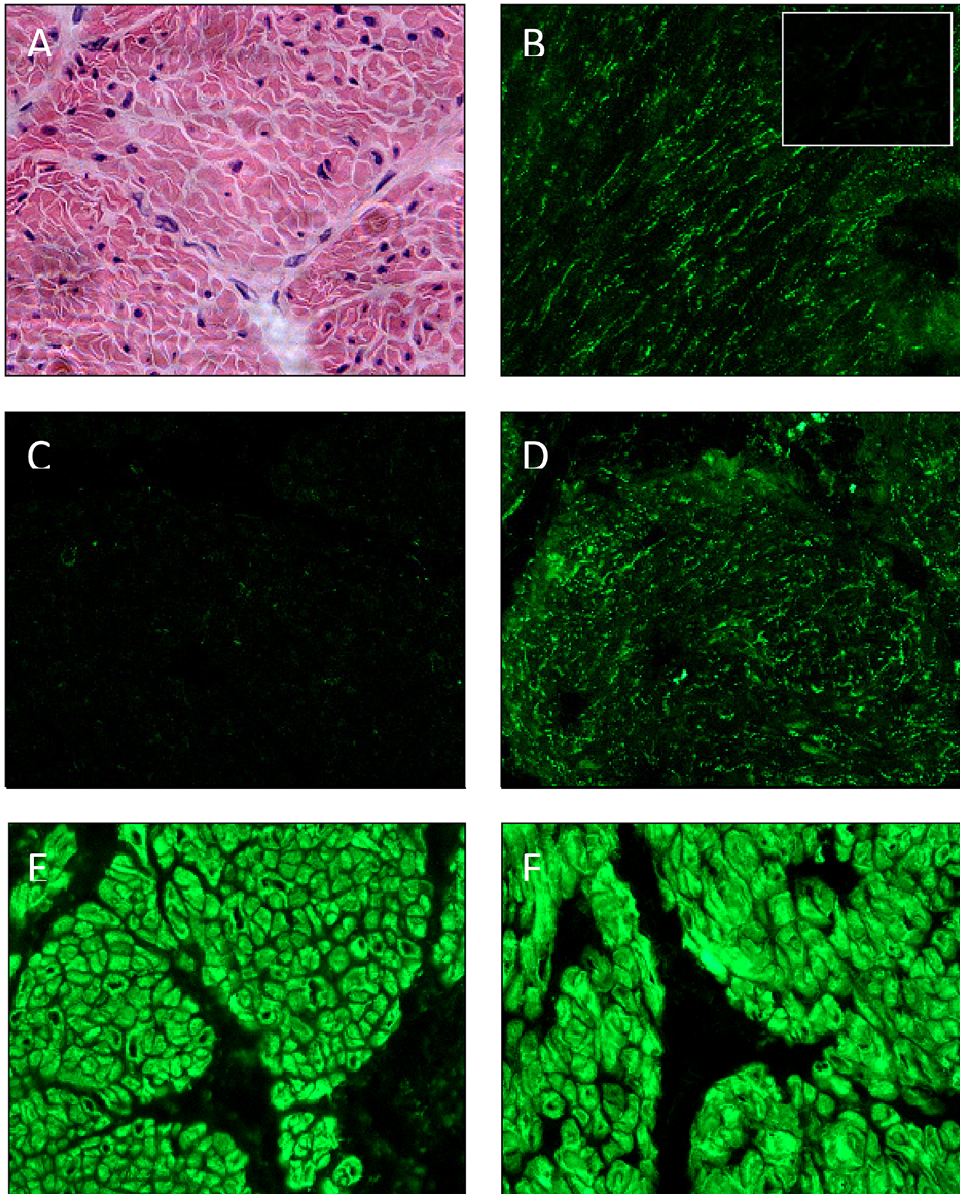


Figure 3 – Expression of adherens junction molecules and cytoskeletal proteins in detrusor smooth muscle cells. Specimen overview by haematoxylin-eosin stain showing no loosened fascicular structure or widening of intercellular spaces between individual smooth muscle cells (A). No immunoreactions were detected in negative controls (insert B). Detrusor smooth muscle cells expressed clear punctate staining of cadherin-11 (B) and β-catenin (D). 'In-line' expression reveals the spindle-like shape of smooth muscle cells. No expression of α-catenin was found (C). Smooth muscle cells expressed smoothelin (E) and smooth muscle actin (F). Binocular epifluorescent microscopy, magnification x100.

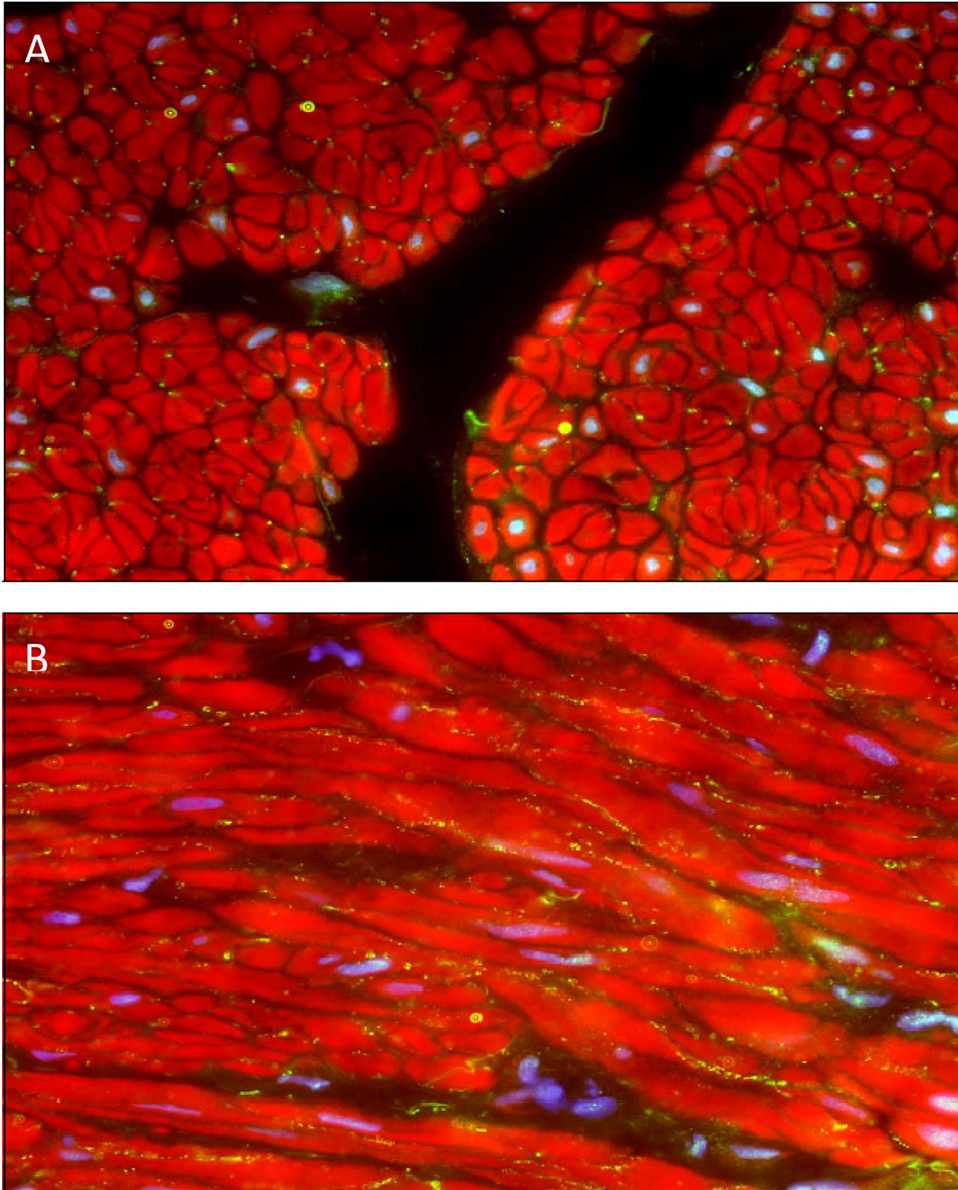
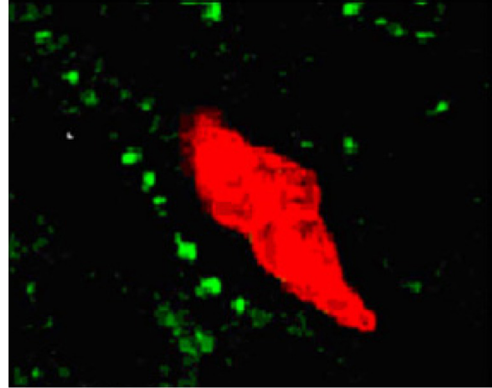


Figure 4 – Costaining for smooth muscle actin with phalloidin (red), cadherin-11 (green), and nuclei (blue) in transverse sections (A) of detrusor smooth muscle bundles. Cadherin-11 expression seemed localised at the edges of cytoplasmatic phalloidin-positive protrusions, leaving the flat sides in between unoccupied. However, 'in-line' expression of cadherin-11, as found using a longitudinal approach (B), showed a punctate pattern that occupied virtually the entire cell membrane. Like in the suburothelial myofibroblasts, cadherin-11 and actin stainings were closely associated. Binocular epifluorescent microscopy, magnification x400.

Figure 5 – Confocal image, showing double labelling of cadherin-11 (green) and propidiumjodide (red) for nuclei in a few detrusor smooth muscle cells. Cadherin-11 protein was expressed as large dots. Note lack of expression of cadherin-11 within smooth muscle cell cytoplasm and nucleus. Cadherin-11 signal seemed membranously located, describing an 'in-line' configuration. Neighbouring cells lack nucleus staining, most likely because of decentral transection of these cells. Confocal epifluorescent microscopy, magnification x10,000.



3.3. Punctate expression of cadherin-11 demarcates detrusor smooth muscle cell membrane

The dotted cadherin-11 expression pattern was characterised by several dots 'in line' and seemed to be confined to the membrane. However, using epifluorescent microscopy we could not unambiguously show that cadherin-11 was solely membrane bound (Fig. 4). Therefore, confocal laser–scanning microscopy was used to evaluate expression in one optical plane (Fig. 5). Costaining of cadherin-11 and popidiumiodide showed lack of expression for cadherin-11 within smooth muscle cell cytoplasm and nucleus. In fact, stainings constantly showed considerable gaps between immunoreactivity for cadherin-11 and propidiumiodide (Fig. 4). Lined up, point-like expression of cadherin-11 seemed to be located at the cell membrane of detrusor smooth muscle cells.

4. DISCUSSION

While the molecular basis for cell-cell communication between detrusor smooth muscle cells is getting more and more clear, the function of the suburothelial myofibroblasts remains unknown. Myofibroblasts are fibroblastic cells with certain characteristics of smooth muscle. They possibly act in a manner analogous to the interstitial cells of Cajal, which have a role in pacemaker activity and transmission of excitation in the gut [9–11]. Others have suggested that myofibroblasts have the capacity to form a functional syncytium and could act as a layer of mechanoreceptors [1,12]. However, no contacts with detrusor smooth muscle have been described [1,12].
It has recently been found that detrusor smooth muscle cells and suburothelial myofibroblasts show prominent expression of connexins [1,2], which are proteins that form so-called gap junctions. Gap junctions cannot account for the required strong adhesion between smooth muscle cells. This fact prompted us to search for a cell junction, responsible for strong physical interaction between detrusor smooth muscle cells. To molecularly characterise these junctional complexes, we used cadherin-11 as a lead molecule, since for many other cell types the cadherins play a pivotal role in these junctions that are critical for cell-cell interaction and coupling.

In this study detrusor smooth muscle cells and suburothelial myofibroblasts expressed cadherin-11 in a punctate pattern at their cell membrane, highly resembling expression of adherens junctions in contractile myofibroblasts as found by Hinz et al [8]. Cadherin-11 was not expressed at smooth muscle bundle boundaries, their component fascicles, and nonadjacent smooth muscle cells. This proves that the cadherin-11 signal is indeed specific for the smooth muscle and myofibroblast cell-cell interaction. To our knowledge this is the first time evidence has been provided for cadherin mediated smooth muscle and suburothelial myofibroblast cell-cell interaction in the human bladder. This finding is in contrast to a previous report in which the authors concluded that cadherins are not part of the detrusor smooth muscle cell-cell adhesion complex [14]. They found no expression for cadherins, using a pan-cadherin antibody. Also, α-catenin, β-catenin, and γ-catenin expression could not be detected in any detrusor smooth muscle compartment. Since the internal controls (i.e., urothelium) were positive, the authors concluded that cadherins were not expressed. In our study, we used a highly sensitive and specific antibody for one cadherin subtype (cadherin-11). We evaluated the specimens with high-resolution imaging techniques and were able to visualise a complex containing cadherin-11 and b-catenin within the detrusor smooth muscle cells and the suburothelial myofibroblasts.

Although dissection of our specimens was performed distant from tumour sites, an influence of cancer on cadherin and catenin expression in our biopsies cannot be ruled out. However, the similar constant level of cadherin-11 expression, as shown in all biopsies, may indicate that it was unaffected by tumour-related factors.

Three-colour imaging with cadherin-11, phalloidin, and DAPI showed that the dotted expression of cadherin-11 seemed to be confined to the membrane. This confinement, however, could not be concluded indisputably from this staining because some smooth muscle cells seemed to express some cadherin-11 in their cytoplasmic compartment as well. Confocal imaging was used to reject out-of focus fluorescence and evade this problem, which resulted in a clean image showing that cadherin-11 is located at the cell membrane of detrusor smooth muscle cells.

β-Catenin expression was localised to cadherin-11 expression. In contrast, α-catenin was not detectable in smooth muscle cells. Literature shows that membraneously bound β-catenin forms a complex with α-catenin [4]. The absence of α-catenin expression, as found in this study, suggests that physical interaction between β-catenin and cytoskeletal actin in detrusor smooth muscle cells is mediated by a yet unknown isoform of α-catenin or another molecule with homologous function. Remarkably, α-catenin was expressed in the suburothelial layer.

It is generally accepted that actin is the predominant form of filaments within contractile cells. Interestingly, the intermediate filament composition of the suburothelial- and smooth muscle cells was different. Smooth muscle cells were positive for desmin, whereas the suburothelial myofibroblasts also expressed the intermediate filament protein vimentin. The filament composition may be associated with functional differences of these cells. For example, evidence is provided that vimentin-based cadherin adhesion complexes cooperate with the traditional actin-based complex to promote strong cell-cell adhesion [15]. It remains to be further investigated which filament actually forms a physical association with the cadherin-11 complex within smooth muscle cells and myofibroblasts.

In this study, several components involved in chain-like cell-to-cell linkage were studied and were shown to be expressed within suburothelial myofibroblasts and smooth muscle cells, except α-catenin. Since cadherin-11 is considered to be an essential protein in cell-cell contact, we consider cadherin-11 an excellent candidate to further molecularly characterise the cell-cell junctions in myofibroblasts and detrusor smooth muscle cells. This finding confirms the hypothesis that both cell types (myofibroblasts as well as detrusor smooth muscle cells) have the capacity to form a physically functional network of numerous smooth muscle cells acting cooperatively.
In ultrastructural studies using electron microscopy, observations revealed that adherens junctions predominate in normal detrusor smooth muscle cells [16] and myofibroblasts [17]. Adherens junctions anchor convergent cytoskeletal myofilaments to the cell membrane in two opposing cell surfaces [18] and are considered to facilitate mechanical coupling [19].

5. CONCLUSIONS

To our knowledge this is the first report of prominent puntate cadherin-11 expression between detrusor smooth muscle cells and suburothelial myofibroblasts. Considering its cellular location we hypothesise that cadherin-11 can play an important role in the intercellular physical coupling of detrusor smooth muscle and suburothelial myofibroblasts. The linear punctate expression of cadherin-11 could in fact mark the predominating adherens junctions in normal detrusor smooth muscle and suburothelial myofibroblasts as identified during former ultrastructural studies. We have now met the basic requirement for evaluating the functional role of cadherin-11 in association with bladder function. Future research will focus on electron microscopic and immunochemical evaluation of possible changes in adherens junctions as seen in different bladder dysfunctions. This focus seems particularly important as evidence is accumulating that detrusor muscle alterations play a major role in determining lower urinary tract symptoms [25].

REFERENCES

1. Sui GP, Rothery S, Dupont E et al. Gap junctions and connexin expression in human suburothelial interstitial cells. BJU Int 2002;90:118-129
2. John H, Wang X, Wehrli E et al. Evidence of gap junctions in the stable nonobstructed human bladder. J Urol 2003;169:745-749
3. GallicanoGI, BauerC and Fuchs E. Rescuing desmoplakin function in extra-embryonic ectoderm reveals the importance of this protein in embryonic heart, neuroepithelium, skin and vasculature. Development 2001;128:929-941
4. Wu JC, Tsai RY and Chung TH. Role of catenins in the development of gap junctions in rat cardiomyocytes. J Cell Biochem 2003;88:823-835
5. Barker RJ, Price RL and Gourdie RG. Increased association of ZO-1 with connexin43 during remodeling of cardiac gap junctions. Circ Res 2002;90:317-324
6. Bryant PJ. Junction genetics. Dev Genet 1997;20:75-90

7. Bussemakers MJ, van Bokhoven A, Voller M et al. The genes for the calcium-dependent cell adhesion molecules P- and E-cadherin are tandemly arranged in the human genome. Biochem Biophys Res Commun 1994;203:1291-1294
8. Hinz B, Pittet P, Smith-Clerc J et al. Myofibroblast development is characterized by specific cell-cell adherens junctions. Mol Biol Cell 2004;15:4310-4320
9. Drake MJ, Fry CH and Eyden B. Structural characterization of myofibroblasts in the bladder. BJU Int 2006;97:29-32
10. Brading AF and McCloskey KD. Mechanisms of disease: specialized interstitial cells of the urinary tract-an assessment of current knowledge. Nat Clin Pract Urol 2005;2:546-554
11. Hashitani H, Yanai Y and Suzuki H. Role of interstitial cells and gap junctions in the transmission of spontaneous Ca2+ signals in detrusor smooth muscles of the guineapig urinary bladder. J Physiol 2004;559(2):567–581
12. Wiseman OJ, Fowler CJ and Landon DN. The role of the human bladder lamina propria myofibroblast. BJU Int 2003;91:89-93
13. van der Loop FT, Schaart G, Timmer ED et al. Smoothelin, a novel cytoskeletal protein specific for smooth muscle cells. J Cell Biol 1996;134:401-411
14. Wagener N and Haferkamp A. Adherens junctions of the human detrusor. BJU Int 2005;96:843-847
15. Kim YJ, Sauer C, Testa K et al. Modulating the strength of cadherin adhesion: evidence for a novel adhesion complex. J Cell Sci 2005;118:3883-3894
16. Elbadawi A. Functional pathology of urinary bladder muscularis: the new frontier in diagnostic uropathology. Semin Diagn Pathol 1993;10:314-354
17. Wiseman OJ, Fowler CJ and Landon DN. The role of the human bladder lamina propria myofibroblast. BJU Int 2003;91:89-93
18. Ehler E and Perriard JC. Cardiomyocyte cytoskeleton and myofibrillogenesis in healthy and diseased heart. Heart Fail Rev 2000;5:259-569
19. Carey MP, De Jong S, Friedhuber A et al. A prospective evaluation of the pathogenesis of detrusor instability in women, using electron microscopy and immunohistochemistry. BJU Int 2000;86:970-976
20. Bussemakers MJG, Van Bokhoven A, Tomita K et al. Cadherin mediated molecules in human prostate cancer cell lines. Int J Cancer 2000;85:446-450
21. Van Noort M, Meeldijk J, Van Der Zee R et al. Wnt signaling controls the phophorylation status of b-catenin. J Biol Chem 2002;20:17901-17905
22. Van Der Loop FTL, Schaart G, Timmer EDJ et al. Smoothelin, a novel cytoskeletal protein specific for smooth mscle cells. J Cell Biol 1996;134:401-411
23. Verhagen AP, Aalders TW, Ramaekers FC et al. Differential expression of keratins in the basal and luminal compartments of rat prostatic epithelium during degeneration and regeneration. Prostate 1988;13:25-38
24. Schaart G, Pieper FR, Kuijpers HJ et al. Baby hamster kidney (BHK-21/C13) cells can express striated muscle type proteins. Differentiation 1991;46:105-115
25. Mirone V, Imbimbo C and Longo N. The detrusor muscle: an innocent victim of bladder outlet obstruction. Eur Urol 2007 ;51(1):57-66

5

Adherens junctions and Cadherin-11 in normal and overactive human detrusor smooth muscle cells

Kamiel A.J. Kuijpers[1], John P.F.A. Heesakkers[1], Theo G.M. Hafmans[2] and Jack A. Schalken[1]

[1]Department of Urology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands. [2]Department of Biochemistry, Radboud University Nijmegen Medical Centre, The Netherlands.

The Journal of Urology, 2011 May;185(5):1946-1951

ABSTRACT

Purpose:
We investigated whether analysis of adherens junctions in human detrusor could be used as a diagnostic tool to determine detrusor overactivity.
Materials and methods:
We characterized the protein composition of adherens junctions in the human bladder using cadherin-11 since our group previously found that cadherin-11 could be an integral structural protein of adherens junctions. We obtained a total of 46 biopsies from 23 patients categorized into 4 groups, including 5 who were normal, and 6 each with neurogenic disease with detrusor overactivity, bladder outlet obstruction with detrusor overactivity and idiopathic detrusor overactivity. Specimens were processed to study cadherin-11 expression using combined immunohistochemical and immunogold electron microscopy techniques. Cadherin-11 expression was semiquantitatively analyzed and correlated to with muscle fascicle structure and collagen in the extracellular spaces.
Results:
Immunogold labeling showed highly specific cadherin-11 expression at adherens junctions in detrusor smooth muscle cells. During immunohistochemical staining a wide variety of cadherin-11 expression and fascicle structure was found in the same specimen. No correlation was noted between detrusor overactivity and cadherin-11 expression. However, cadherin-11 seemed to be downregulated with intercellular space widening and collagenosis.
Conclusions:
Cadherin-11 is an integral structural protein of the adherens junction. Defects in the overactive detrusor are highly punctate. Quantitative analysis of adherens junctions using biopsy cannot replace urodynamic evaluation as a predictor of detrusor overactivity in the human bladder.

1. INTRODUCTION

The etiology of the overactive bladder (OAB) and detrusor overactivity (DO) is still poorly understood. DO can arise in the presence of bladder outlet obstruction (BOO) and in individuals with neurological disease but it is idiopathic in many patients [1]. Evidence is accumulating that structural changes in detrusor smooth muscle are associated with the unstable pressure increases seen in the OAB. In ultrastructural studies using electron microscopy (EM) it was found that the overactive detrusor has a disjunction pattern that is not found in the normal bladder [2–5]. It is characterized by adherens junction (AJ) loss, collagenosis and widening of spaces between individual smooth muscle cells. AJs predominate in the normal detrusor [6]. They are believed to facilitate mechanical coupling between adjacent smooth muscle cells by anchoring cytoskeletal myofilaments to cell membranes [7]. Cadherins are a super family of transmembrane glycoproteins that mediate homotypic adhesion between cells [8] thus, regulating embryogenesis, organogenesis and differentiation of cells [9]. Our group recently reported that the human bladder expresses cadherin-11 at the surface of adjacent detrusor smooth muscle cells [10]. We hypothesized that cadherin- 11 has an important role in the intercellular physical coupling of detrusor smooth muscle cells and in fact could be an essential, integral protein of the AJ. The aim of this study was to prove our hypothesis and investigate whether analysis of cadherin- 11 expression could be used as a diagnostic tool to determine detrusor overactivity.

2. METHODS

2.1. Patients

This study was done in biopsies from 12 female and 11 male patients 47 to 68 years old (mean age 60) with OAB complaints or genuine stress incontinence. Two cold cup bladder biopsies were obtained per patient from the posterior bladder wall during cystoscopic procedures. The local ethics committee approved the study and informed consent was obtained from all patients.

All patients underwent full urodynamic analysis and were categorized into 3 groups, including 2 males and 4 females with neurogenic disease and DO, 5 males and 1 female with BOO and DO, and 1 male and 5 females with idiopathic DO. Three females and 2 males 53 to 75 years old (mean age 65.6) with stress urinary incontinence and urodynamically proven, nonoveractive detrusor served as controls. These patients did not have neurogenic disease or BOO and were undergoing check cystoscopy. Filling cystometry at 50 ml per minute in patients with OAB revealed DO in all with a median CBC of 203 ml (range 28 to 450). Median CBC in control bladders was 400 ml (range 205 to 500). The CBC of 205 ml was caused by major stress incontinence. In all patients with OAB intravesical pressure increases during filling were due to DO since compliance was normal. Acontractile detrusors were not included in analysis.

2.2. Electron Microscopy

Three human bladder biopsies from cases urodynamically proven to be not OAB were processed for standard transmission EM and immunogold EM. Processing for transmission EM was done according to the standard protocol using Somogyi fixative [11]. For immunogold EM analysis of cadherin-11 a post-embedding immunohistochemical technique was applied on thin sections obtained from material embedded at low temperature in Lowicryl® HM20 resin according to a previously used protocol [12]. Ultrathin sections were photographed using a TEM 1010 electron microscope (JEOL, Peabody, Massachusetts). Immunogold labeling control studies were done in human heart and kidney tissue.
During ultrathin sectioning specimens were alternately stained with toluidine blue and analyzed for intact urothelium and detrusor smooth muscle tissue using a standard binocular microscope under low magnification. We subsequently selected areas of detrusor smooth muscle cells for further analysis.

2.3. Immunohistochemistry

Bladder specimens were collected and placed in a mold containing Tissue-Tek®. They were snap frozen immediately in isopentane at -80˚C. Sections (4 µm) were prepared using a cryostat and mounted on Superfrost Plus® slides. Using hematoxylin and eosin staining tissue was analyzed for intact urothelium and smooth muscle. Immunohistochemical staining for cadherin-11, smoothelin and 4,6-diamidino-2-phenylindole was done as previously described by our group [10].

2.4. Morphological Analysis

Obtaining bladder specimens by the cold cup biopsy method allows the smooth muscle detrusor layer to be penetrated to a maximum of 2 muscle bundles deep. Thus, a limited amount of detrusor tissue is available for analysis compared to that of full-thickness biopsy. Four muscle fascicles per biopsy were photographed. The elastic van Gieson stain was used in parallel to visualize smooth muscle cells and extracellular connective tissue. Immunohistochemical staining was semiquantitatively analyzed for smooth muscle fascicle structure, cadherin-11 expression and the presence of collagen in the extracellular spaces. Fascicle structure was divided into 3 types, including compact structure, intermediate segregation and severe segregation of smooth muscle cells. According to cadherin-11 expression and collagen fiber presence each fascicle was semiquantitatively graded as features not present in any photographs (-), present in fewer than 0 to a third (+), present in a third to two-thirds (++) and present in two-thirds to entire fascicle (+++).

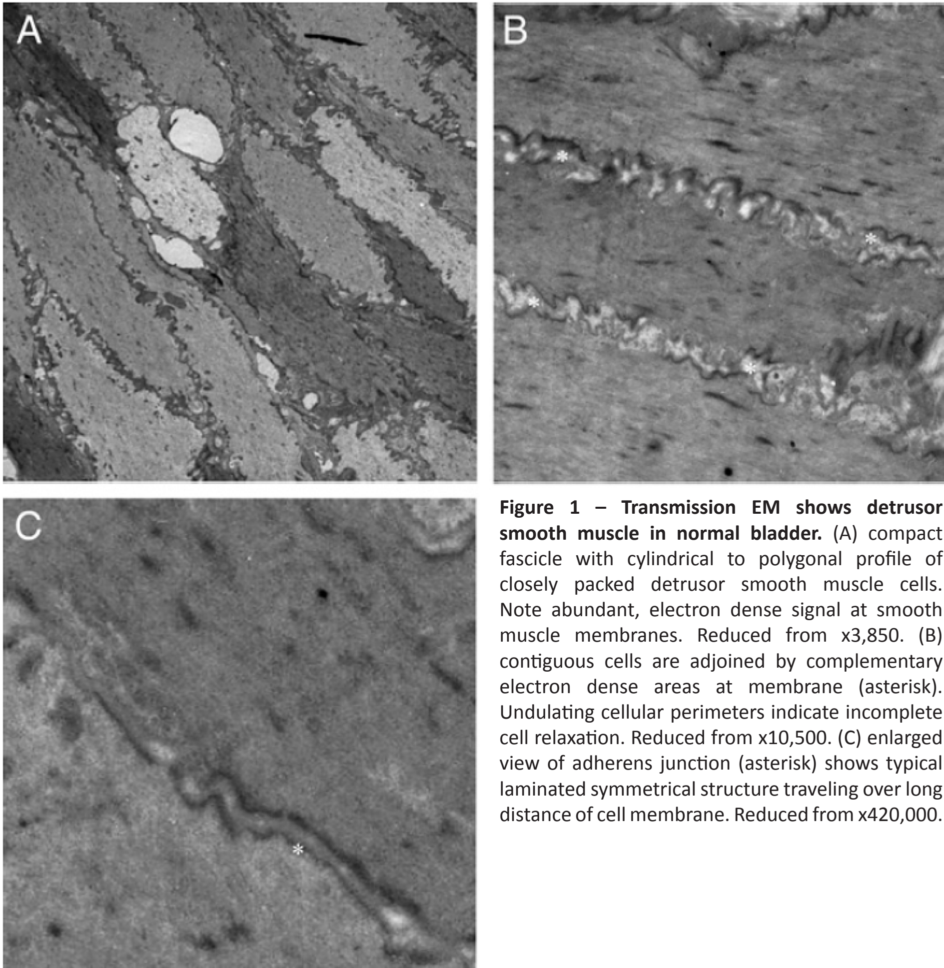


Figure 1 – Transmission EM shows detrusor smooth muscle in normal bladder. (A) compact fascicle with cylindrical to polygonal profile of closely packed detrusor smooth muscle cells. Note abundant, electron dense signal at smooth muscle membranes. Reduced from x3,850. (B) contiguous cells are adjoined by complementary electron dense areas at membrane (asterisk). Undulating cellular perimeters indicate incomplete cell relaxation. Reduced from x10,500. (C) enlarged view of adherens junction (asterisk) shows typical laminated symmetrical structure traveling over long distance of cell membrane. Reduced from x420,000.

3. RESULTS

3.1. Cadherin-11 localized to AJs

General smooth muscle fascicle structure was organized as compact bundles, interspersed by fascicles with moderate cell-cell separation (Fig. 1). Smooth muscle cells showed a cylindrical cell profile with crenellated cell contours. The sarcoplasm was packed with evenly distributed myofilaments of uniform orientation and alignment. It contained evenly dispersed dense bodies that appeared as uniform cigar-shaped densities.
In all specimens the cell membrane was almost completely covered with classic AJs and dense plaques to which myofilaments seemed attached. However, AJs were not found at all smooth muscle cells. Dense plaques were formed of a complementary pair of electron dense areas located at the membrane of adjacent smooth muscle cells. However, AJs consisted of a pair of electron dense structures with a much smaller intercellular distance.

Their membranous lining was much longer and more symmetrical.
In all immunogold EM samples classic AJs and dense plaques were found. Cadherin-11 was specifically expressed in AJs in a complementary manner (Fig. 2). This finding emphasized the homophilic fashion of cadherin mediated adhesion behavior, that is cells cohere with other cells that express the same cadherin subtype. However, due to the combination of oblique sectioning of AJs and ultrathin processing of specimens not all AJs showed cadherin-11 expression in a complementary manner. Also, cadherin-11 was expressed alternately at the intracellular and extracellular domains. This can be explained by the fact that cadherins are repetitive transmembrane macromolecules that contain intracellular and extracellular domains. Dense plaques did not express cadherin-11.

3.2. Cadherin-11 Heterogeneous Expression

In the suburothelial layer cadherin-11 expression was found in a band of myofibroblast-like cells running parallel with and adjacent to the urothelium (data not shown). Fluorescence signal intensity showed comparable results among samples but penetration depth into the deeper lamina propria varied considerably in each specimen.
In smooth muscle cells cadherin-11 was expressed as a dense punctate pattern that occupied virtually the entire cell membrane. No cadherin-11 expression was found at smooth muscle fascicle boundaries and nonadjacent smooth muscle cells (Fig. 3). In all specimens cadherin-11 expression showed wide variety between smooth muscle fascicles throughout all detrusor muscle layers. Variety of cadherin-11 expression was also found within smooth muscle fascicles since some fascicles expressed cadherin-11 homogeneously, others expressed it heterogeneously and some did not express it at all (Fig. 3). Duplicate analysis revealed identical expression patterns. Variety of cadherin-11 expression seemed less in normal specimens but generally cadherin-11 expression did not seem to differ between normal and overactive detrusors.

3.3. Normal and Overactive Detrusor Structure Regional Variation

In all biopsies overall appearance in fascicle structure was variable rather than uniform. Elastic von Gieson stain revealed that the area between widely separated cells consisted mostly of collagen and, thus, embodied actual extracellular matrix. Thus, in vitro separation of adjacent cells during semithin sectioning was kept to a minimum. Although severe segregation of smooth muscle cells was often found in obstructed specimens, we noted no significant difference between normal and overactive detrusor when considering fascicle structure.

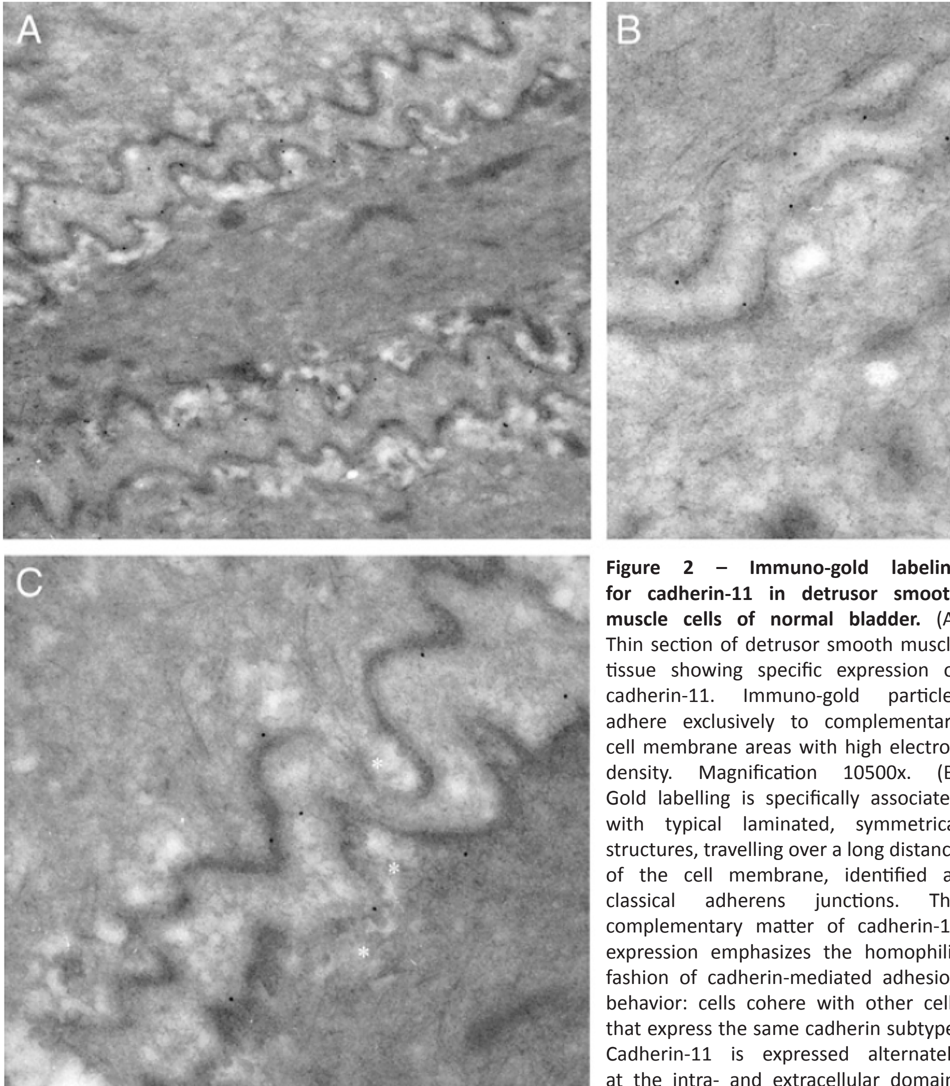


Figure 2 – Immuno-gold labeling for cadherin-11 in detrusor smooth muscle cells of normal bladder. (A) Thin section of detrusor smooth muscle tissue showing specific expression of cadherin-11. Immuno-gold particles adhere exclusively to complementary cell membrane areas with high electron density. Magnification 10500x. (B) Gold labelling is specifically associated with typical laminated, symmetrical structures, travelling over a long distance of the cell membrane, identified as classical adherens junctions. The complementary matter of cadherin-11 expression emphasizes the homophilic fashion of cadherin-mediated adhesion behavior: cells cohere with other cells that express the same cadherin subtype. Cadherin-11 is expressed alternately at the intra- and extracellular domain. Magnification 84000x. (C) Numerous submembranous caveolae are found at the cell membrane, which are the likely site for the entry of action potentials. In general, gold particles are expressed in pairs. Magnification x84000.

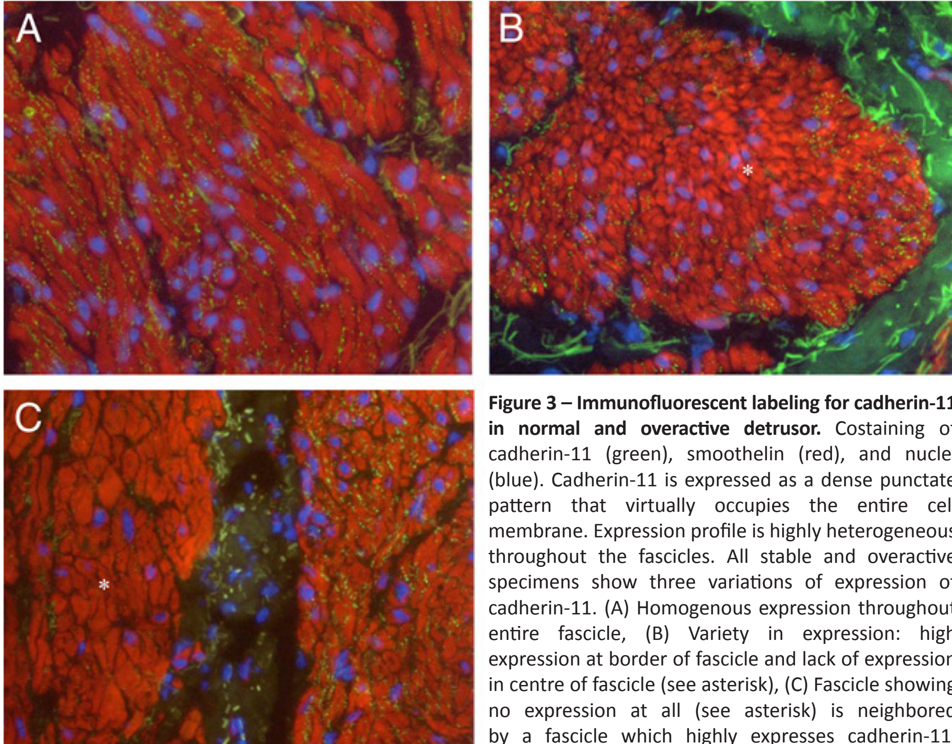


Figure 3 – Immunofluorescent labeling for cadherin-11 in normal and overactive detrusor. Costaining of cadherin-11 (green), smoothelin (red), and nuclei (blue). Cadherin-11 is expressed as a dense punctate pattern that virtually occupies the entire cell membrane. Expression profile is highly heterogeneous throughout the fascicles. All stable and overactive specimens show three variations of expression of cadherin-11. (A) Homogenous expression throughout entire fascicle, (B) Variety in expression: high expression at border of fascicle and lack of expression in centre of fascicle (see asterisk), (C) Fascicle showing no expression at all (see asterisk) is neighbored by a fascicle which highly expresses cadherin-11. Although cadherin-11 expression varied throughout the specimens, this did not always seem to coexist with cell-cell separation. Binocular epifluorescent microscopy, magnification x100.

3.4. Cadherin-11 Down-Regulated With Collagenosis

Abundant collagen fibers in the intercellular spaces were found in 5 specimens, including to a moderate degree in 2 neurogenic bladder specimens and to a severe degree in 3 obstructed bladder specimens (Fig. 4). However, collagenosis varied highly between fascicles. Some fascicles showed high collagenosis but adjacent fascicles showed no collagenosis. Generally cadherin-11 expression seemed to be down-regulated in areas with collagenosis since no cadherin-11 expression was found in fascicles with widely separated smooth muscle cells and abundant interstitial collagen fibers. However, although some fascicles lacked expression of cadherin-11, this did not always seem to coexist with collagenosis and cell-cell separation.

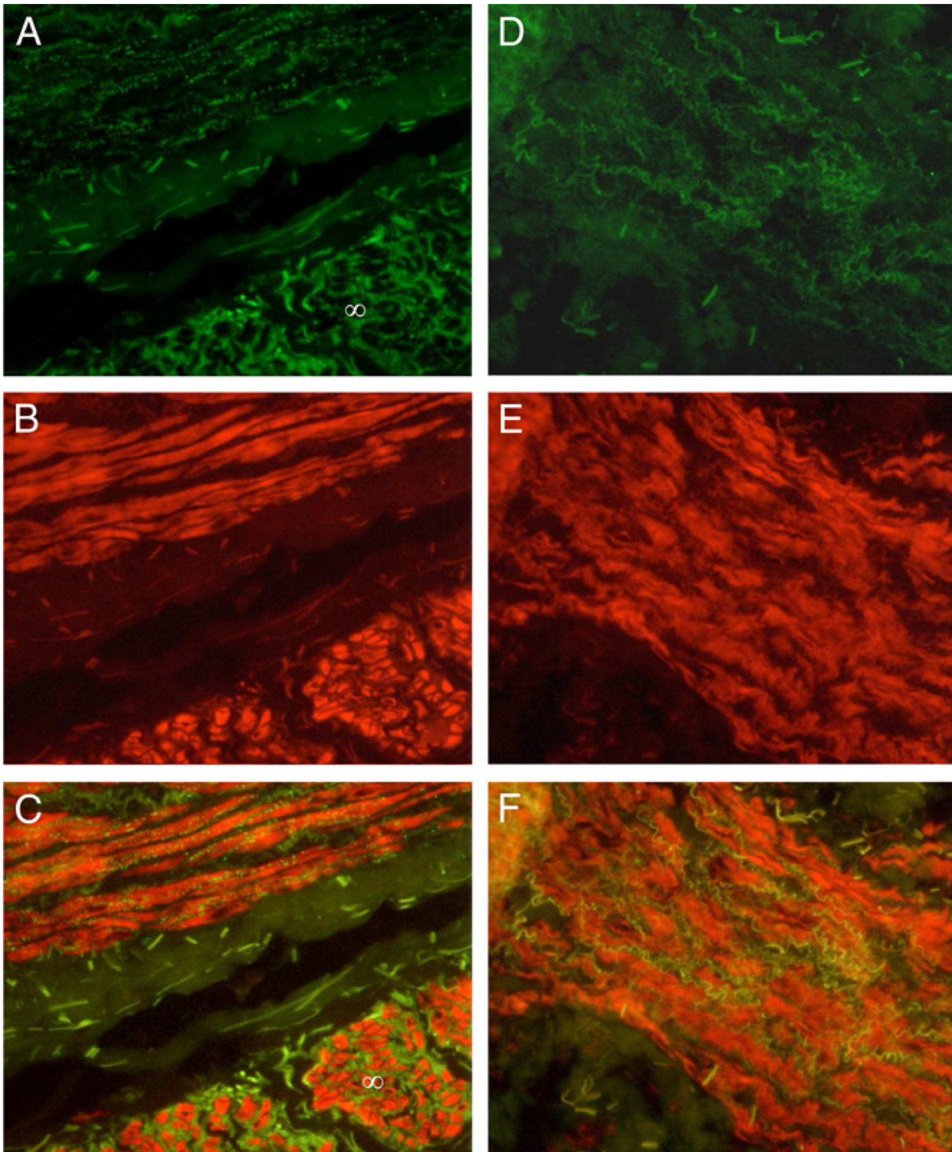


Figure 4 – Cadherin-11 and collagenosis in overactive detrusor. DO due to BOO. (A&D) cadherin-11 (green), (B&E) smoothelin (red), (C&F) merge. (A-C) Severe collagenosis (cigar shaped green background signal) and lack of cadherin-11 expression is found in the lower fascicle (∞). This is in contrast to the upper fascicle in which a lively expression of cadherin-11 in combination of nearly no collagenosis is found. Upper longitudinal bundle and lower transverse bundle represent first and second layer of detrusor bundles in bladder wall. (D-F) Again severe collagenosis with no expression of cadherin-11. Note the 'jigsaw puzzle' of smooth muscle cell contours. Contortion and twisting of smooth muscle cell branches seems to coexist with intermingling of collagen fibrils. (G) Suburothelium expression of cadherin-11 served as positive internal control of specimen in D. Binocular epifluorescent microscopy, magnification x100.

5

4. DISCUSSION

Evidence is accumulating that structural changes in detrusor smooth muscle are associated with the production of the unstable pressure increases seen in the overactive bladder (OAB). Elbadawi et al found that the overactive detrusor has a distinctive disjunction pattern in which loss of adherens junctions (AJs) and increased intercellular distance have a pivotal role [2–5] The studies of Elbadawi et al [2,4–6] led to the proposition that it is possible to diagnose detrusor overactivity (DO) by analyzing detrusor ultrastructural characteristics. However, numerous attempts to reproduce their results failed [13–15]. These conflicting results may be explained by the fact that electron microscopy (EM) only relies on ultrastructure to characterize junctions. Thus, classification is less certain if rudimentary forms of these AJs are present. Therefore, a highly sensitive, specific marker is needed for functional AJs.

The human bladder expresses cadherin-11 at the surface of adjacent smooth muscle cells [10]. Cadherin-11 is believed to have an important role in the physical coupling of detrusor smooth muscle cells and could in fact mark AJs. To test this hypothesis we integrated cadherin-11 expression and AJ ultrastructure. Immunogold labeling revealed that caderin-11 is an integral structural protein of AJs. Dense plaques did not express cadherin-11 and showed a much wider intercellular gap. They could represent separated AJs or other rudimentary forms of junctions.

Carey et al concluded that vinculin is a sensitive, specific marker for AJs in detrusor smooth muscle cells [14]. However, their study did not include the highly specific technique of immunogold labeling. Also, since vinculin is known as a classic attachment protein involved in the binding of intracellular actin filaments to fibronectin of the extracellular matrix, we question their conclusions. All specimens showed remarkable variation in cadherin-11 expression in the detrusor layer. Some fascicles did not express cadherin-11 at all. We believe that this heterogeneous expression profile is not based on processing artifacts since duplicate analysis showed identical results. The finding that AJs were not expressed by all smooth muscle cells in standard EM supports our finding.

This study was done in a relatively small patient cohort. However, analysis was performed in duplicate with 2 biopsies obtained per patient. Also, the complementary matter in which standard EM, immuno-EM and immunohistochemical techniques were used created a highly sensitive, specific analysis tool that merged protein characterization and cellular morphology.

Heterogeneous expression of cadherin-11 was also found in control specimens, which could be explained by the limitations of urodynamics. Alternatively it was suggested that sensory urgency is a precursor of DO and may simply be earlier in the spectrum of disease. However, no control patient complained of OAB symptoms.

Roosen et al described cadherin-11 expression in bladder biopsies of patients with DO [16]. They found no changed expression in the detrusor layer. However, they did not describe regional variations in cadherin-11 expression. Since we found major variety in cadherin-11 expression, we believe that it is unfeasible to quantitatively analyze immunohistochemical staining for cadherin-11 expression in the human bladder.

Morphological analysis does not determine tissue functionality. However, the cadherin family of proteins is not only considered an integral part of adhesion junctions but actually defines their presence [17]. Thus, cadherin-11 down-regulation most probably reflects a disintegration of the adhesion complex at the cell membrane, making cadherin-11 a functional marker of AJs in the human detrusor. Restriction of this main vehicle for mechanical coupling between smooth muscle cells would allow widening of the intercellular space, as found in this study.

Loss of the cadherin-11 complex could also account for the appearance of collagenosis. Fibroblasts are well-known producers of extracellular collagen. However, previous studies showed that the smooth muscle cell is also capable of producing collagen under specific conditions [18]. Cadherins are important morphogenetic regulators that confer cellular phenotypes by modulating the expression of their target genes located in the cell nucleus [9]. Therefore, loss of cadherin mediated signaling could not only explain loss of intercellular adhesion and widening of the intracellular space but also may change the phenotype of detrusor smooth muscle into collagen producing cells.

Highly modified areas were found adjacent to apparently normal areas. Areas that appeared normal in DO specimens were indistinguishable from control tissue. Such focal changes were also found by other investigators [15,19]. It was hypothesized that these areas match the topography of detrusor modules with each controlled by dedicated integrative circuits, as in the gut [20].

Furthermore, it seems that normal bladders can include highly modified modules without becoming overactive. If we consider that modified detrusor areas with cadherin-11 down-regulation and increased intercellular space behave pathologically, exceeding their number beyond a certain threshold may drive the bladder into becoming overactive. Loss of cadherin-11 could mark a point of no return in this pathological condition.

5. CONCLUSIONS

Cadherin-11 is an integral structural protein of adherens junctions (AJs). Therefore, it can be used to classify the uncertain rudimentary forms of cell junctions described by ultrastructural analysis. However, defects in the overactive detrusor seem to be highly punctate, making it unfeasible to obtain representative images of cadherin-11 expression. Thus, we conclude that quantitative analysis of AJs using immunostaining cannot replace urodynamic evaluation as a predictor of detrusor overactivity (DO). However, better understanding of cadherin-11 could shed more light on detrusor function. Although to our knowledge the progression of events that transform normal bladder behavior into overactivity remains unelucidated, we hypothesize that loss of the cadherin-11 adhesion complex could be a potential trigger for widening the spaces between individual smooth muscle cells and collagenosis.

ACKNOWLEDGMENTS

The authors would like to thank Paul H.K. Jap from the department of Biochemistry (Radboud University Nijmegen Medical Centre, The Netherlands) for his support and valued discussions.

REFERENCES

1. Andersson KE. Storage and voiding symptoms: pathophysiologic aspects. Urology 2003;62:3-10
2. Elbadawi A, Yalla SV and Resnick NM. Structural basis of geriatric voiding dysfunction. I. Methods of a prospective ultrastructural/urodynamic study and an overview of the findings. J Urol 1993;150:1650-1656
3. Hailemariam S, Elbadawi A, Yalla SV et al. Structural basis of geriatric voiding dysfunction. V. Standardized protocols for routine ultrastructural study and diagnosis of endoscopic detrusor biopsies. J Urol 1997;157:1783-1803
4. Elbadawi A, Yalla SV and Resnick NM. Structural basis of geriatric voiding dysfunction. IV. Bladder outlet obstruction. J Urol 1993;150:1681-1695
5. Elbadawi A, Resnick NM, Dorsam J et al. Structural basis of neurogenic bladder ysfunction. I. Methods of a prospective ultrastructural study and overview of the findings. J Urol 2003;169:540-546
6. Elbadawi A. Functional pathology of urinary bladder muscularis: the new frontier in diagnostic uropathology. Semin Diagn Pathol 1993;10:314-354
7. Ehler E and Perriard JC. Cardiomyocyte cytoskeleton and myofibrillogenesis in healthy and diseased heart. Heart Fail Rev 2000;5:259-269
8. Goodwin M and Yap AS. Classical cadherin adhesion molecules: coordinating cell adhesion, signalling and the cytoskeleton. J Mol Histol 2004;35:839-844
9. Wheelock MJ and Johnson KR. Cadherins as modulators of cellular phenotype. Annu Rev Cell Dev Biol 2003;19:207-235
10. Kuijpers KA, Heesakkers JP, Schalken JA et al. Cadherin-11 is expressed in detrusor smooth muscle cells and myofibroblasts of normal human bladder. Eur Urol 2007; 52: 1213-1221
11. van Strien FJ, de Rijk EP, Hafmans TG et al. Demonstration of dopamine in electron-dense synaptic vesicles in the pars intermedia of Xenopus laevis, by freeze substitution and postembedding immunogold electron microscopy. Histochem 1991;96:505-510
12. Jansen EJ, Scheenen WJ, Hafmans TG et al. Accessory subunit Ac45 controls the V-ATPase in the regulated secretory pathway. Biochim Biophys Acta 2008;1783:2301-2010
13. Holm NR, Nordling J, de la Rosette J et al. The detrusor muscle cell in bladder outlet obstruction. Scand J Urol Nephrol 2003;37:309-315
14. Carey MP, De Jong S, Scurry J et al. A prospective evaluation of the pathogenesis of detrusor instability in women, using electron microscopy and immunohistochemistry. BJU Int 2000;86:970-976

15. Brierly RD, Hindley RG, Thomas PJ et al. A prospective evaluation of detrusor ultrastructural changes in bladder outlet obstruction. BJU Int 2003;91:360-364
16. Roosen A, Apostolidis A, Elneil S et al. Cadherin- 11 up-regulation in overactive bladder suburothelial myofibroblasts. J Urol 2009;182:190-195
17. Provost E and Rimm DL. Controversies at the cytoplasmic face of the cadherin-based adhesion complex. Curr Opin Cell Biol 1999;11:567-572
18. Aumuller G. Smooth muscle in the male genital tract. Ultrastructure of Smooth Muscle. Edited by PM Motta. Boston: Kluwer Acadamic Publishers 1990;9:171-195
19. Charlton RG, Morley AR, Gillespie JI et al. Focal changes in nerve, muscle and connective tissue in normal and unstable human bladder. BJU Int 1999;84:953-960
20. Kunze WA and Furness JB. The enteric nervous system and regulation of intestinal motility. Annu Rev Physiol 1999;61:117-142

6

An update of the interstitial cell compartment in the normal human bladder

Kamiel A.J. Kuijpers[1], John P.F.A. Heesakkers[1], Theo G.M. Hafmans[2], Cornelius F.J. Jansen[1] and Jack A. Schalken[1]

[1]Department of Urology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands. [2]Department of Biochemistry, Radboud University Nijmegen Medical Centre, The Netherlands.

BioMed Research International, Volume 2014, Epub 2014 Feb 27

ABSTRACT

Aims:
Interstitial cells, also called myofibroblasts, most probably play a major role in the pathogenesis of the overactive bladder. However, no specific phenotypic marker has been identified. We investigated whether N-cadherin could play a role as a discriminatory marker for interstitial cells in the human bladder.
Methods:
Bladder biopsies (n = 16) were collected from macroscopically nonpathological locations during cystectomy which was performed because of bladder cancer. Tissue was analyzed for expression of N-cadherin. N-cadherin+ cells were phenotyped using antibodies against PGP9.5, smoothelin, vimentin, and C-kit. Findings were related to bladder tissue histology and ultrastructure of myofibroblastic cells.
Results:
N-cadherin+/vimentin+ cells with branched cell bodies were found in the lamina propria and detrusor layer. They were closely associated with neurons and showed no colocalization of PGP9.5 or smoothelin. A second type of N-cadherin+ cells was found at the boundary of detrusor bundles and in the lamina propria. These cells colocalized C-kit. We assumed that N-cadherin+/vimentin+ cells are similar to the ultrastructurally defined myofibroblasts.
Conclusions:
N-cadherin can play a role as a discriminatory marker for interstitial cells in the human bladder, as the interstitial compartment of the human bladder houses a population of cells from mesenchymal origin, immunopositive for N-cadherin, vimentin and C-kit.

1. INTRODUCTION

The human bladder shows spontaneous contractile activity during the filling phase of the micturition cycle [1]. As this activity is also found in bladders that are isolated from the central nervous system, it seems that it is generated within the bladder wall. The overactive bladder (OAB) syndrome is associated with complaints of frequency and urgency that typically occur during the filling phase [2,3]. Spontaneous contractile activity of the bladder may share characteristics with peristaltic activity in the gastrointestinal tract [4]. Specialized pacemaker cells of the gut, also known as interstitial cells of Cajal (ICCs), are thought to behave as pacemaker cells that transmit their electrical activity to the smooth muscle [5,6]. Interstitial cells (ICs) are also found in the human bladder [7,8]. These cells are located throughout the lamina propria and detrusor layer. It has been suggested that they form a network integrating signals and responses in the bladder wall between various types of cells. However, ICs have several subtypes based on morphological appearance and differential expression of markers, like myofibroblasts, making the search for their exact functional role in the human bladder a contentious subject.
Recent studies have identified various surrogate histochemical markers for ICs, such as the stem cell receptor C-kit and cyclic guanosine monophosphate (cGMP) [8]. However, the specific immunophenotype of ICs is still controversial. C-kit is not expressed by all types of ICs and it can also be detected in other cell types [9]. Additionally, many C-kit antibodies fail to detect ICs in positive control tissues. cGMP is a marker for ICs in the bladder but it is also expressed by urothelial cells [8]. Thus, so far, no marker has been identified that can be considered as a specific phenotypic marker for ICs in the human bladder. Therefore, irrefutable confirmation of the interstitial phenotype still depends on application of transmission electron microscopy (TEM), which is highly time consuming.
Cadherins constitute a superfamily of glycoproteins that participate in cell-cell recognition by functioning as signaling centers [10,11]. We have previously shown that cadherin-11 is expressed by ICs in the lamina propria [12]. Cadherins may therefore play a role in regulating an intramural network of these cells. As subpopulations of bladder ICs exist, another subclass of cadherins might account for a specific discriminatory marker for interstitial cells in the interfascicular planes of the detrusor layer. N-cadherin is known to regulate mesenchymal cell development [13] and is the most commonly expressed cadherin in stromal cells [14,15]. We therefore investigated the expression of N-cadherin in the normal human bladder. We used additional immunohistochemical cell markers as well as transmission electron microscopy (TEM).

2. MATERIAL AND METHODS

2.1. Patients

Bladder biopsies (n=16) were collected from sixteen individuals in whom radical cystectomy was performed because of muscle invasive bladder cancer. Mean patient age was 62 years (52–75), nine males and seven females. Samples were dissected from tumour-free bladder

areas at least 3 cm distant from tumour zones. Biopsies were all taken from the vesical dome from functionally stable bladders. All of the patients underwent primary resection in terms of early cystectomy and none of the patients underwent intravesical installation. The local ethics committee approved the study and informed consent was obtained from all patients. Full thickness specimens were collected and placed in a mould containing Tissue-Tek (Sakura) for cryosectioning. Specimens were snap frozen in isopentane at –80°C. Tissue was checked for intact urothelium using a hematoxylin-eosin stain.

2.2. Immunohistochemistry

Sections of 4 µm specimens were prepared using a cryostat and mounted on Super Frost Plus slides (Menzel-Gläser). The unfixed sections were immersed in 3% paraformaldehyde for ten minutes and stained for N-cadherin (M142 Takara; C2542 Clone GC-4 Sigma). Cell membranes were permeabilized in 0.2% Triton X-100 for 5 minutes. For cytoskeletal protein staining, samples were fixed in acetone for ten minutes and air dried at room temperature for 2 hours. Each step was separated by wash in magnesium and calcium containing PBS (PBS-Extra: 40 mL 25x PBS, 960 mL demi-water, 100 µL 1 M $MgCl_2$, 100 µL 1 M $CaCl_2$). Sections were incubated for 1 hour using primary antibodies diluted in PBS 1% bovine serum albumin for blocking. Sections again were washed three times in PBS-Extra. Next, the sections were incubated with Alexa Fluor 488 (A-11017, A-11070 Molecular Probes) or Alexa Fluor 594 (A-20185, A-11032 Molecular Probes). Finally, treatment with DAPI (24653 Merck) was performed for staining the nuclei. All sections were mounted in Fluorescent Mounting Medium (S3023 Dako Cytomation). Negative controls included omission of primary antibodies. The following antibodies were used to further phenotype N-cadherin+ cells: PGP9.5 (a pan-neuronal marker) (7863-0504 AbD Serotec), smoothelin (specific marker for smooth muscle cells [16]) (R4A ab8969 Abcam), vimentin (marker for fibroblasts) (RV203 Eurogentec), and C-kit (CD117 DAKO). For the latter antibody, specimens of human jejunum were used as positive controls.

2.3. Transmission Electron Microscopy

Sixteen human normal bladder biopsies were also processed for standard transmission electron microscopy (TEM). Processing for TEM was done according to the standard protocol using Somogyi fixative [17]. Ultrathin sections were photographed using a TEM 1010 electron microscope (JEOL, Peabody, Massachusetts).

2.4. Analysis

Immunostained sections were examined by binocular epifluorescent microscopy (Leica DFC FX). Four times ten slides were analyzed per full-thickness specimen. Each set of ten slides was separated by approximately 5 mm of tissue. Cryosections were also stained with hematoxylin-eosin to interpret the fluorescent images. Morphology, phenotypic expression of above mentioned markers, and the ultrastructure of myofibroblastic cells were evaluated.

3. RESULTS

3.1. N-cadherin expression in normal human bladder

Throughout the entire bladder wall, N-cadherin positive structures were found. These structures were located immediately below the urothelium, throughout the lamina propria and in the detrusor layer (Fig. 1). Counterstaining with DAPI showed that the N-cadherin+ structures embodied branched cells provided with multiple processes (Fig. 2). N-cadherin expression showed a punctate pattern distributed throughout the entire cell body.

Suburothelial N-cadherin+ cells had branched morphology with multiple processes that seemed to form a network. In the detrusor, N-cadherin+ cells were found at different levels. N-cadherin+ cells with stellate morphology were also located at the boundaries of smooth muscle bundles. They seemed to interact with elongated N-cadherin+ cells running in the interfascicular planes, continuing as slender N-cadherin+ processes between smooth muscle cells.

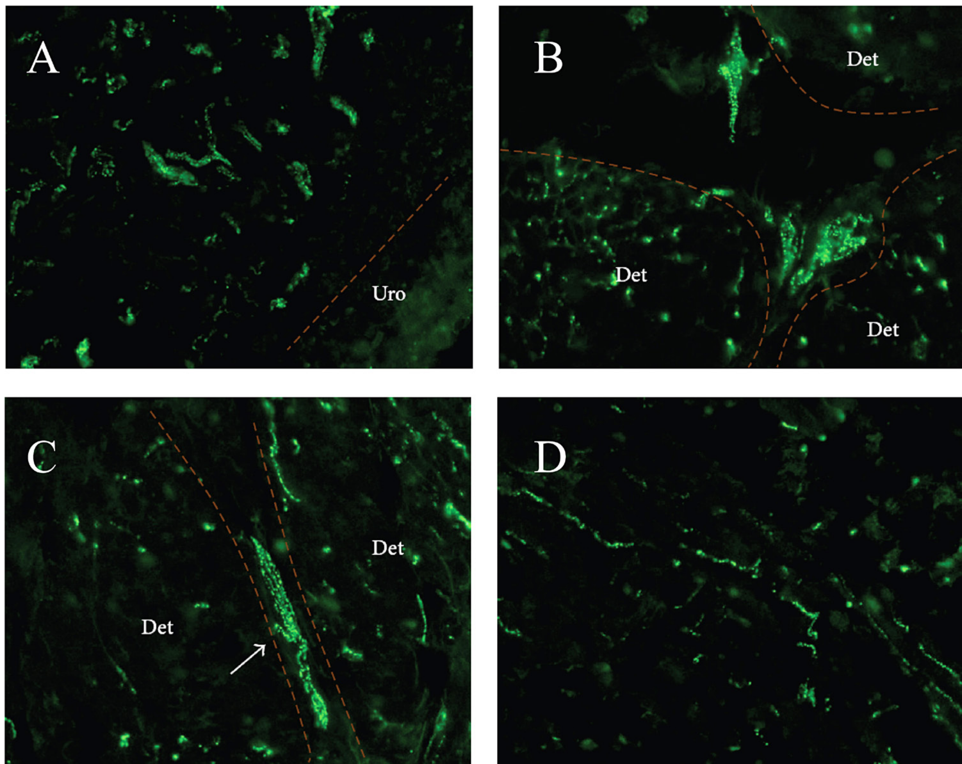


Figure 1 – N-cadherin+ structures in the normal bladder wall. (A–D) A punctate signal for N-cadherin (green) reveals numerous positive N-cadherin+ cells within the bladder wall. (A) N-cadherin+ cells with multiple processes in the lamina propria. (B) Closely associated N-cadherin+ cells at the boundary of smooth muscle bundles. Note large size and stellate morphology. (C) N-cadherin+ cells running between smooth muscle fascicles show elongated instead of stellate morphology and reveal lateral branches (arrow) running into the muscle fascicles. (D) Intrafascicular, slender elongated N-cadherin+ branches run in parallel with the smooth muscle bundles. Magnification ×400. Uro: urothelium; Det: detrusor muscles.

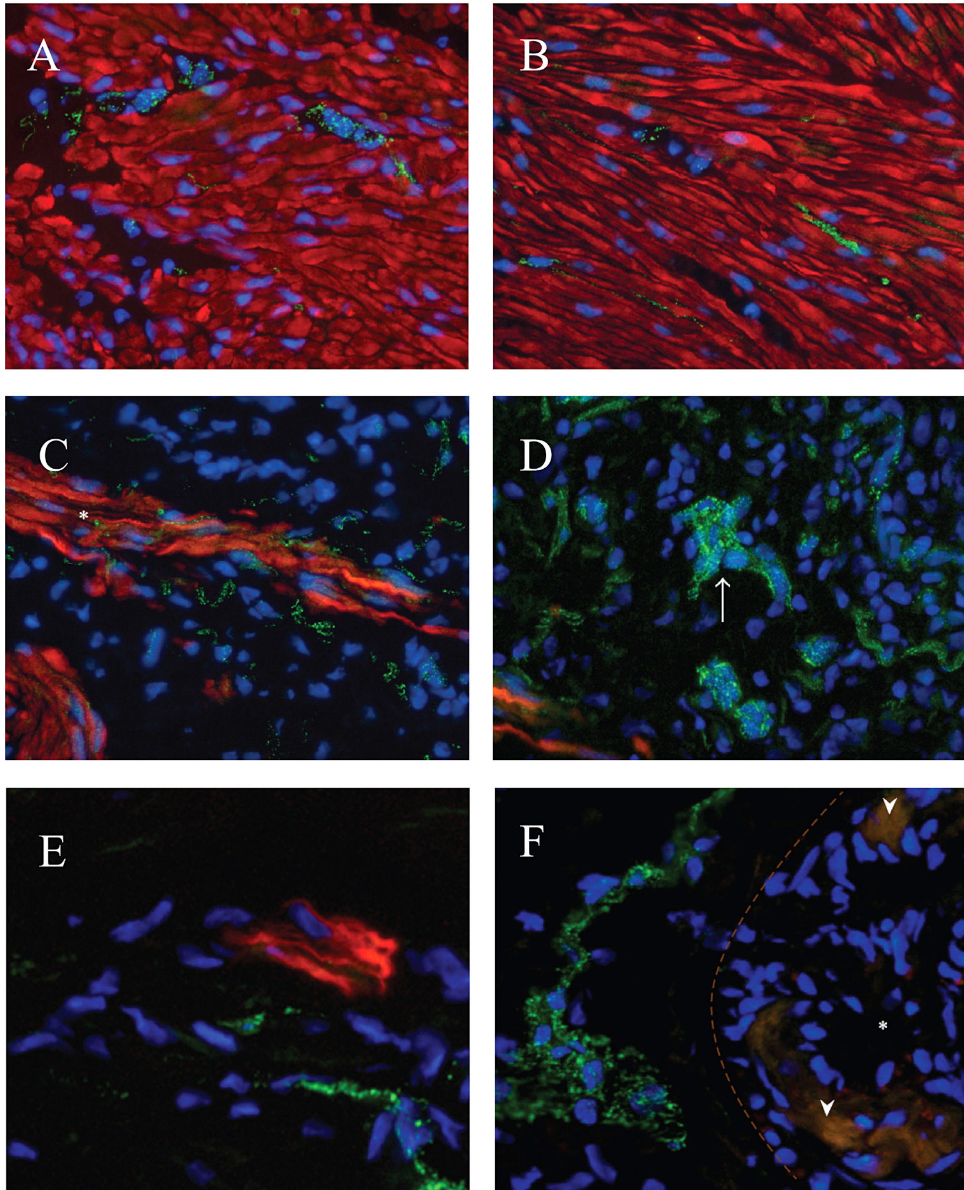


Figure 2 – Double staining of N-cadherin with smoothelin and PGP9.5. (A–D) Costaining of N-cadherin (green) and smoothelin (red) in the bladder wall shows no colocalization. (A) Transversal and (B) longitudinal sections. (C) N-cadherin+ structures intermingle with smooth muscle cells in the region of the muscularis mucosae (asterisk) (D) Note the aggregation of multiple N-cadherin+ cells in the lamina propria (arrow). (E) PGP9.5 is expressed by a nerve trunk (red). (F) Aggregated N-cadherin+/PGP9.5− cells are closely associated with a ganglion region (asterisk). Ganglion coexpresses background signal of N-cadherin, resulting in orange bodies (arrowheads). Note high contrast to the punctate expression of N-cadherin in the N-cadherin+/PGP9.5− cells. DAPI (blue) for nuclei. Magnification ×400.

3.2. Phenotyping of N-cadherin positive cells

Staining for smoothelin confirmed that N-cadherin+ but smoothelin- cells were housed at the border of smooth muscle fascicles (Fig. 2). Inside the fascicles, they continued as elongated processes running in parallel with smooth muscle orientation spanning numerous smooth muscle cells. Irregularly arranged bundles of cells expressing smoothelin were found midway between the urothelium and the detrusor smooth muscle bundles. Those so-called muscularis mucosae varied considerably in diameter and formed a discontinuous layer of cells, densely surrounded and traversed by N-cadherin+ structures. PGP9.5 immunoreactivity was found in both the lamina propria and detrusor layer. In the detrusor layer, primary nerve trunks run close to the detrusor bundles. Secondary neuronal structures were found in the connective tissue between smooth muscle fascicles, whereas smaller fibers run between small groups of smooth muscle cells. Double staining of N-cadherin and PGP9.5 showed no colocalization. However, close association between N-cadherin+ cells and PGP9.5+ neuronal structures was found (Fig. 2).
N-cadherin and vimentin were coexpressed by cells of ramified morphology in the suburothelial layer and deeper lamina propria (Fig. 3). These cells seemed to form a suburothelial network. However, many vimentin+ cells did not express N-cadherin. These N-cadherin−/vimentin+ cells showed different morphology. They were smaller and less elongated, had little perinuclear cytoplasm, and presumably embodied fibroblasts.
N-cadherin+/vimentin+ cells were also found at the border of detrusor smooth muscle bundles. Similar to the suburothelial region, these cells seemed to interconnect with each other, expanding into and throughout the smooth muscle fascicles like a network of N-cadherin+/vimentin+ processes.
The punctate pattern of N-cadherin expression was expressed throughout the entire cell body and at the cell membrane. In general, the small vimentin+ cells with little perinuclear cytoplasm did not express N-cadherin.
Most N-cadherin+ cells coexpressed vimentin. However, a second type of N-cadherin+ cells was found that did not coexpress vimentin. These cells were not housed between smooth muscle cells but were restricted to the edge of smooth muscle fascicles and were also found in the lamina propria (Fig. 3(f)). In contrast to the N-cadherin+/vimentin+ cells, these N-cadherin+/vimentin− cells showed different morphology: they had small appearance with little perinuclear cytoplasm sprouting into multiple cytoplasmatic processes.

3.3. N-cadherin positive cells and interstitial cell marker C-kit

C-kit+ cells were found throughout the entire bladder wall (Figure 4). A large number of these cells coexpressed N-cadherin and showed similar morphology to the previously described N-cadherin+/vimentin− cells (Fig. 4(b)). They were located on the boundary of smooth muscle fascicles and in the lamina propria. Specimens of the human jejunum were used as a positive control for C-kit. The gut showed a large population of cells coexpressing N-cadherin and C-kit. Although most cells showed coexpression of N-cadherin and C-kit, N-cadherin+/C-kit− and N-cadherin−/C-kit+ cells were also found.

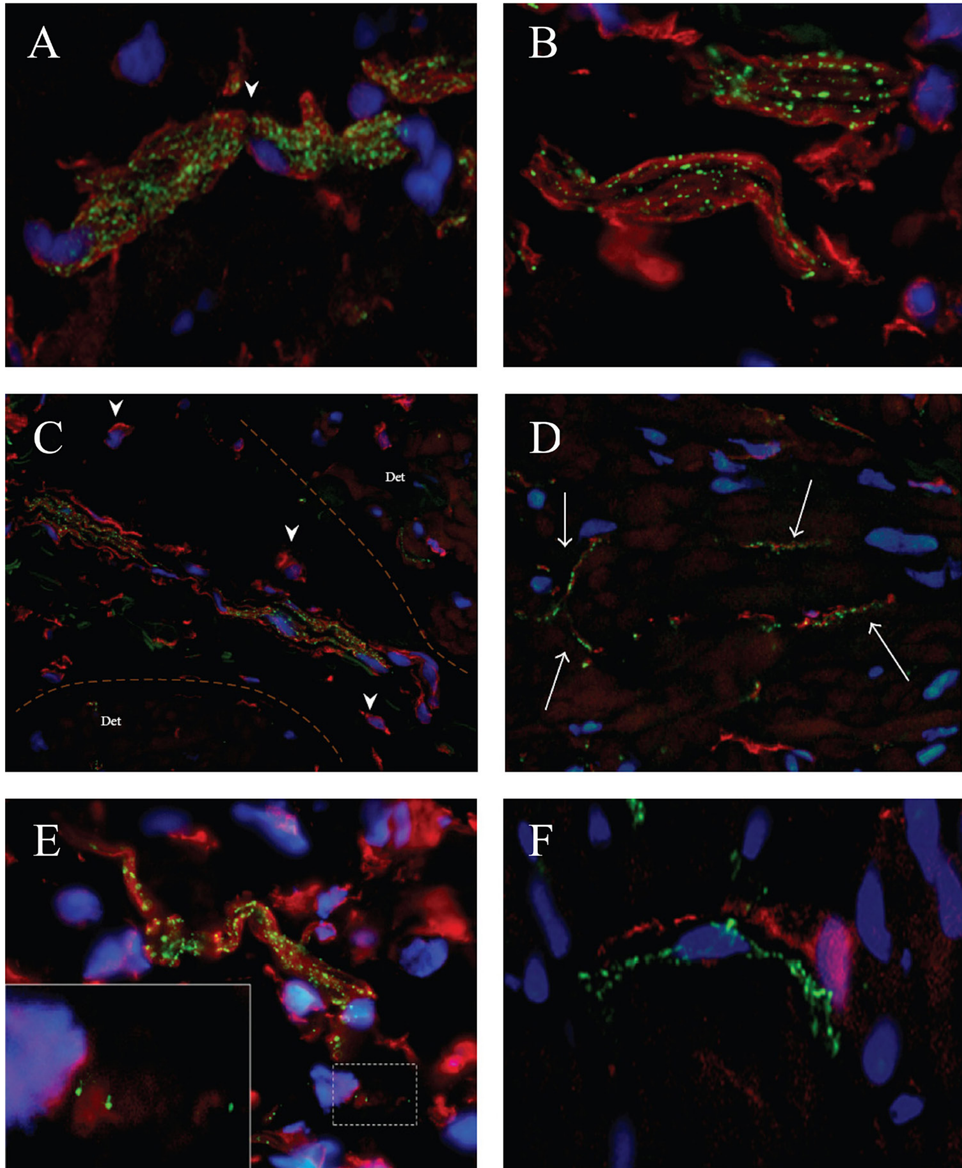


Figure 3 – Double staining of N-cadherin and vimentin. (A) Two suburothelial cells coexpressing N-cadherin (green) and vimentin (red) are closely associated with each other (arrowhead). (B) Note punctate pattern of N-cadherin in contrast to the vimentin+ filaments, which both are expressed within the same cell body. Magnification ×1000. (C) Multiple elongated N-cadherin+/vimentin+ containing cells are closely associated at the border of detrusor smooth muscle bundles. Arrowheads show small vimentin+ cells with little perinuclear cytoplasm, highly resembling regular fibroblasts. (D) Detrusor fascicles show numerous N-cadherin+/vimentin+ processes (arrows) running between muscle cells. Magnification ×630. (E) Higher magnification shows expression of N-cadherin that is located at the cell membrane of some vimentin+ cells. Magnification ×1000. (F) Two cells localized at the edge of a smooth muscle fascicle. N-cadherin+/vimentin− cell is neighboured by a N-cadherin−/ vimentin+ cell. Magnification ×1000.

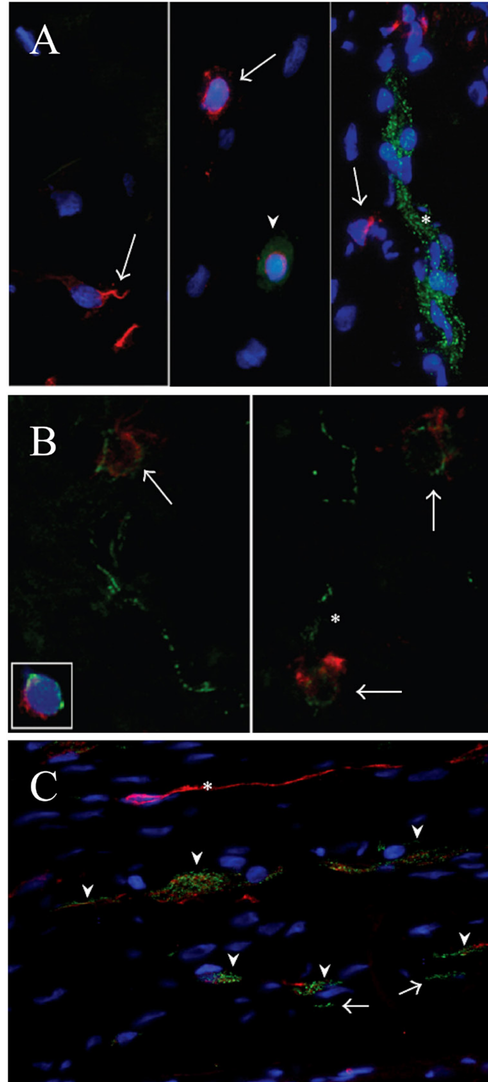


Figure 4 – Double staining of N-cadherin and C-kit. (A) Arrows show cells in the bladder expressing C-kit (red). They lack expression of N-cadherin (green). Arrowhead shows a round cell with perinuclear expression of C-kit and diffuse cytoplasmic background signal for N-cadherin, highly resembling a mast cell. Elongated clusters of punctate N-cadherin+ cells lack expression of C-kit (asterisk). Magnification ×630. (B) Staining lacking DAPI for better orientation. Arrows show cells in the bladder expressing C-kit and punctate N-cadherin. These cells seem to give rise to N-cadherin+ branches (asterisk). Magnification ×1000. (C) N-cadherin+/C-kit+ cells running parallely (arrowheads) are neighboured by a slender elongated N-cadherin−/C-kit+ cell body (asterisk) and several smaller N-cadherin+/C-kit− cells (arrows) in the jejunum. Magnification ×630.

3.4. Ultrastructure of ICs in lamina propria and detrusor muscle

In electron microscopy, interstitial cells with stellate morphology were found in the lamina propria and the musculus detrusor layer. They had characteristic features of myofibroblasts, such as cytoplasmatic filaments, focal densities and membranous attachment plaques, interrupted basal lamina of extracellular matrix, numerous mitochondria, and prominent rough endoplasmic reticulum (Fig. 5). From a morphological point of view, they appear to be similar to the N-cadherin+ cells as described in Figure 1.

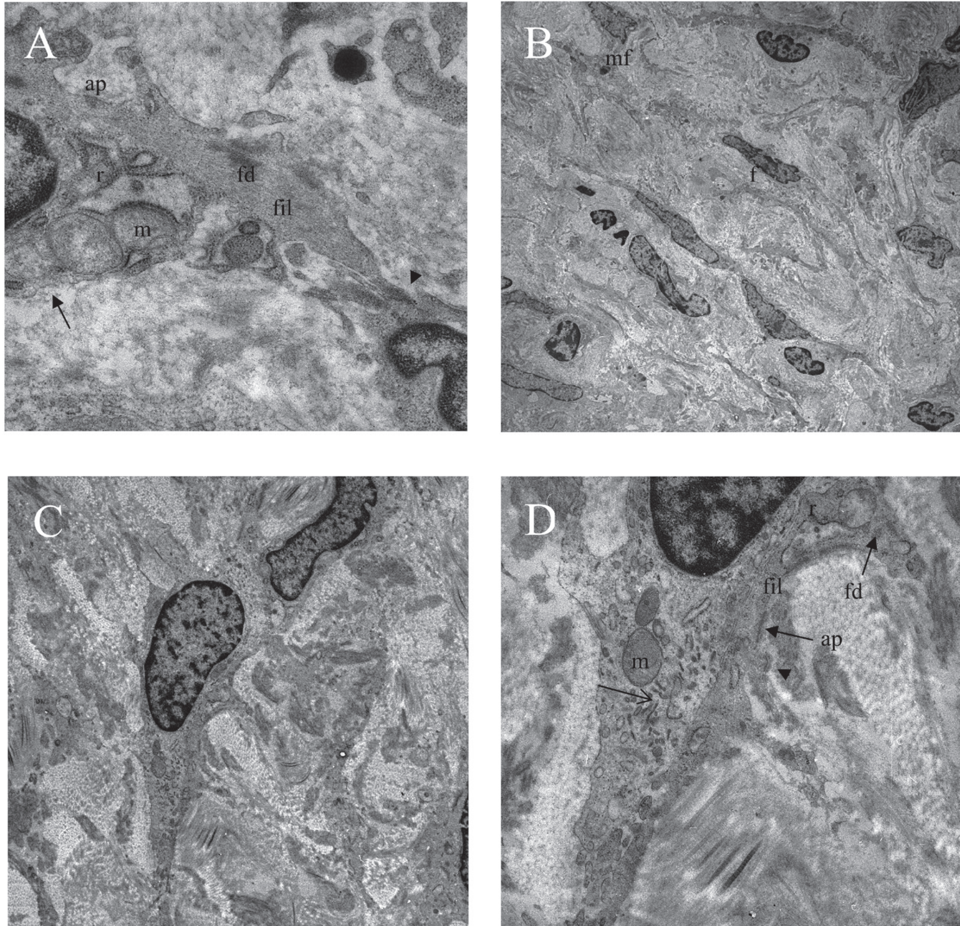


Figure 5 – Ultrastructure of interstitial cells. (A) A myofibroblast located in the lamina propria. The cell is identified by filaments (fil), focal densities (fd), membranous attachment plaques (ap), subsurface vacuoles (arrow), mitochondria (m), and prominent rough endoplasmic reticulum (r). Cytoplasmic filaments cohere to a membranous attachment plaque, showing intimate association with its neighboring cell (arrowhead). Magnification ×420,000. (B) Overview of cells in the lamina propria. The left upper corner shows a myofibroblast (mf). Note its stellate morphology with multiple branches. The cell is accompanied by fibroblasts (f). Magnification ×10,500. (C) Two closely associated interstitial cells in the detrusor layer. (D) Higher magnification of (C). Branched interstitial cell shows mitochondria (m), interrupted basal lamina (arrowhead), peripheral filaments (fil), membranous attachment plaques (ap), focal densities (fd), and prominent rough endoplasmic reticulum (r). Numerous tubulovesicular structures (open arrow) were found exclusively in this cell type.

4. DISCUSSION

During the filling phase, the human bladder shows contractile activity which is generated within the bladder wall and does not result in intravesical pressure rise. It is also called autonomous activity [1–3]. It has been proposed that this activity shares characteristics with electrical rhythmicity in gastrointestinal muscles. Throughout the gastrointestinal tract, a network of interstitial cells of Cajal (ICCs) acts as pacemakers and conductors of electrical activity along the gut wall [5]. Initially, interstitial cells (ICs) were thought to

represent a specialized type of neurons, but it is now concluded that they are a unique class of cells [6]. As ICs are also found in the human bladder, it has been proposed that they mediate autonomous bladder activity [2].

Currently, ICs are identified by various surrogate phenotypic markers, but no immunophenotype has been identified that is fully characteristic for ICs in the human bladder [9]. Irrefutable confirmation of the interstitial phenotype depends on application of electron microscopy, which is highly time consuming. This study was performed in search of a specific marker for interstitial cells in the human bladder and electron microscopic investigations of the same biopsies had to confirm the immunohistochemical findings.

It has been suggested that ICs of the human bladder form a network of interacting cells [7,8]. Cadherin complexes participate in cell-cell recognition by functioning as signaling centers [10,11]. The subtype N-cadherin is known to regulate mesenchymal cell development [13] and is the most commonly expressed cadherin in the interstitial compartment [14,15]. We therefore investigated the expression of N-cadherin in the human normal bladder.

N-cadherin+ cells were found in the lamina propria and the detrusor layer. They showed abundant punctuate expression of N-cadherin at their cell membrane and throughout their cell-body. We cannot fully explain why N-cadherin was not exclusively expressed at the plasma membrane. However, other investigators also found that cadherins can be localized intracellular, rather than being characteristically concentrated at regions of cell-cell contact [18].

Additional cell markers were used to further analyze our findings. Smoothelin is a smooth muscle cell specific marker [16]. As no colocalization of N-cadherin and smoothelin was found, we believe that the N-cadherin+ cells do not represent smooth muscle cells.

Vimentin is expressed by fibroblastic cells [9]. A large population of N-cadherin+ cells coexpressed vimentin. They showed elongated or stellate morphology with multiple processes that seemed to form a network. However, many vimentin+ cells did not express N-cadherin. These cells were smaller and less elongated, had little perinuclear cytoplasm compared to the N-cadherin+/vimentin+ cell, and appeared to be regular fibroblasts.

PGP9.5 was chosen as a pan-neuronal marker as it is generally accepted that this protein is expressed by all neuronal structures of the bladder wall [19]. No colocalization of N-cadherin+/vimentin+ cells and PGP9.5 was found. However, N-cadherin+ cells and PGP9.5+ neurons were closely associated. Furthermore, as mature neurons lack expression of vimentin [20], we believe that the N-cadherin+/vimentin+ cells do not represent neuronal structures.

C-kit is a widely used marker for ICCs of the gut [21]. We used specimens of the human gut as a positive control. ICCs of the gut coexpressed N-cadherin and C-kit. In the bladder, N-cadherin and C-kit were coexpressed by cells with little perinuclear cytoplasm seeming to sprout into N-cadherin+ processes. As these cells seemed highly similar to C-kit+ cells in human detrusor as found by others [22], we believe that they embody interstitial cells.

Interstitial cells are found in both the lamina propria and the detrusor layer of the human bladder. Myofibroblasts are a recently documented interstitial cell type housed in the interstitial compartment. They share characteristics with smooth muscle cells and fibroblasts. In a previous study, no ultrastructural evidence for myofibroblast

differentiation in the detrusor layer was discerned [23]. Interstitial cells within this layer were identified as fibroblasts. It is therefore generally believed that myofibroblasts in the human bladder solely refer to a specific group of interstitial cells within the suburothelial layer [24]. However, during our study, TEM revealed interstitial cells with stellate morphology in the lamina propria and the musculus detrusor layer. They had characteristic features of myofibroblasts. We previously showed that a suburothelial layer of cells expresses alpha smooth muscle actin myofilaments [12], most probably embodying the cytoplasmic filaments as shown during TEM in this study. Furthermore, fully differentiated myofibroblasts express alpha smooth muscle actin [25]. We therefore conclude that both the lamina propria and the detrusor layer house myofibroblasts, a unique class of interstitial cells.

From a morphological point of view, ultrastructurally defined myofibroblasts in the lamina propria and detrusor layer appear to be similar to the N-cadherin+/vimentin+ cells. Both techniques identified specified cells of mesenchymal origin with highly branched morphology and multiple processes that were closely associated with neighbouring homotypic cells.

This study shows a population of cells from mesenchymal origin with multiple phenotypes, immunopositive for N-cadherin, vimentin, and C-kit. These cells are housed in the interstitial compartment throughout the entire human bladder wall. The findings are in accordance with a recent study of Monaghan et al. in which multiple subgroups of vimentin+ cells with distinctive morphology were found in all layers of the bladder wall [26]. It is therefore likely that not all IC's may be labeled with the same markers.

Heterogeneity of the interstitial compartment could be explained by a model in which C-kit and N-cadherin regulate mesenchymal cell differentiation. C-kit is an important stem cell marker used to identify certain types of progenitor cells [27]. Signaling through C-kit plays a role in cell differentiation, proliferation, and survival. The cadherins constitute a superfamily of glycoproteins that participate in cell-cell recognition [10]. Like C-kit, they play a crucial role in cellular differentiation and embryogenesis.

From a functional point of view, one should consider the following characteristics associated with the markers used. Similar to the gut, C-kit+ cells in the human bladder possibly act as pacemaker cells from which spontaneous calcium transients originate [28]. Cadherin complexes play a major role in cell-cell recognition and function as signaling centers [11]. Therefore, the population of N-cadherin+/vimentin+ cells may participate in specialized events such as spread of pacemaking activity. Although our results are promising, this study relies on morphological evidence. In order to illuminate functional properties of these cells, future research is needed using functional cell analyses, such as Ca-imaging and patch clamp techniques.

Although dissection of our specimens was performed distant from tumour sites, an influence of cancer cannot be ruled out. However, no thickening of the urothelial layer or abnormal urothelial morphology was found (Figure 6). Also, urothelium did not express N-cadherin, which is often seen in urothelial bladder cancer [29]. We therefore believe that our findings are unaffected by tumour-related factors.

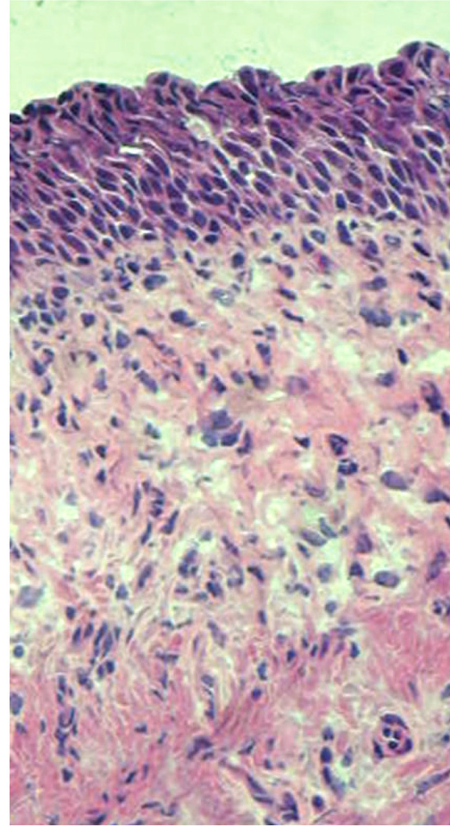


Figure 6 – Representative hematoxylin and eosin stain of urothelial area of the specimens used. Normal urothelium consists of approximately 3–5 cell layers. Note that no abnormal thickening of the urothelial layer or abnormal suburothelial morphology is found

5. CONCLUSIONS

This study shows that the interstitial compartment of the human bladder houses a heterogenous population of cells from mesenchymal origin, immunopositive for N-cadherin, vimentin, and C-kit. Due to characteristics associated with these proteins, this population of cells may participate in specialized events of the human bladder. We assume that N-cadherin/vimentin is a specific marker for a subpopulation of interstitial cells in the human bladder, that is, the ultrastructurally defined myofibroblasts. These cells may participate in spread of pacemaking activity. Although further insight is needed in the correlation between morphology and function of these cells, these findings could be promising in understanding normal and overactive bladder behaviour. Furthermore, we question the possible existence of one specific marker which defines the entire group of ICs in the human bladder.

ACKNOWLEDGEMENTS

The authors would like to thank P. H. K. Jap from the Department of Biochemistry and Professor A. C. H. Geurts from the Department of Rehabilitation Medicine (Radboud University Nijmegen Medical Centre, The Netherlands) for their support and valued discussions. We also acknowledge J. I. Gillespie and G.A. van Koeveringe for kindly providing us the pan-neuronal marker PGP9.5.

REFERENCES

1. Kumar V, Chess-Williams R, Chapple CR et al. Recent advances in basic science for the overactive bladder. Current Opinion in Urology 2005;15:222-226
2. Gillespie JI. The autonomous bladder: a view of the origin of bladder overactivity and sensory urge. BJU International 2004;93:478-483
3. Drake MJ, Harvey IJ and Gillespie JI. Autonomous activity in the isolated guinea pig bladder. Exp. Physiol 2003;88:19-30
4. Brading AF and McCloske KD. Mechanisms of Disease: specialized interstitial cells of the urinary tract-an assessment of current knowledge. Nature 2005;2:546-554
5. Sanders KM. A case for the interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. Gastroenterology 1996;111:492-515
6. Faussone-Pellegrini MS. Histogenesis, structure and relationships of interstitial cells of Cajal (ICC): from morphology to functional interpretation. European Journal of Morphology 1992;30(2):137-148
7. Davidson RA and McCloskey KD. Morphology and localization of interstitial cells in the Guinea pig bladder: structural relationships with smooth muscle and neurons. Journal of Urology 2005;173:1385-1390
8. Gillespie JI, Markerink-van Ittersum M and de Vente J. cGMP-generating cells in the bladder wall: identification of distinct networks of interstitial cells. BJU Int 2004;94(7):1114-1124
9. Drake MJ, Fry CH and Eyden B. Structural characterization of myofibroblasts in the bladder. BJU International 2006;97:29-32
10. Marss JA and Nelson WJ. Cadherin cell adhesion molecules in differentiation and embryogenesis. Int Rev Cytol. 1996;165:159-205.
11. Cavallaro U and Christofori G. Cell adhesion an signalling by cadherins and Ig-CAMS in cancer. Nature reviews 2004;4:118-132
12. Kuijpers KA, Heesakkers JP, Schalken JA et al. Cadherin-11 is expressed in detrusor smooth muscle cells and myofibroblasts of normal human bladder. Eur Urol 2007;52(4):1213-1221
13. Bhowmick NA, Ghiassi M, Bakin A et al. Transforming growth factor beta1 mediates epithelial to mesenchymal transdifferentiation through a RhoA-dependent mechanism. Mol Biol Cell 2001;12:27-36
14. Geiger B, Hynes RO, I Sabany et al. Broad spectrum pan-cadherin antibodies, reactive with the C-terminal 24 amino acids residues of N-cadherin. J. Cell Sci 1990;97:607-614
15. Hinz B, Pittet P, Meister JJ et al. Myofibroblast development is characterized by specific cell-cell adherens junctions. Mol Biol Cell 2004;15:4310-4320
16. van der Loop FT, Ramaekers FC, van Eys GJ et al. Smoothelin, a novel cytoskeletal protein specific for smooth muscle cells. The Journal of Cell Biology 1996;134(2):401-411
17. van Strein F, de Rijk EP, Hafmans T et al. Demonstration of dopamine in electron-dense synaptic vesicles in the pars intermedia of Xenopus laevis, by freeze substitution and postembedding immunogold electron microscopy. Histochemistry 1991;96(6):505-510
18. Islam S, Johnson J, Trendel J et al. Vimentin expression in human squamous carcinoma cells: relationship with phenotypic changes and cadherin-based cell adhesion. Journal of Cellular Biochemistry 2000;78:141-150

19. Gillespie J, Markerink-van Ittersum M and de Vente J. Interstitial cells and cholinergic signalling in the outer muscle layers of the guinea-pig bladder. BJU International 2006;97(2);379-385
20. Fliegner K, Kaplan M, Liem et al. Expression of the gene for the neuronal intermediate filament protein α-internexin coincides with the onset of neuronal differentiation in the developing rat nervous system. Journal of Comparative Neurology 1994;342(2):161-173
21. Sanders K. A case for interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. Gastroenterology 1996;111(2): 492-515
22. Shafik A, El-Sibai O, Shafik I et al. Identification of interstitial cells of Cajal in human urinary bladder: concept of vesical pacemaker. Urology 2004;64(4):809-813
23. Drake M, Hedlund P, Landon DN et al. Morphology, phenotype and ultrastructure of fibroblastic cells from normal and neuropathic human detrusor: absence of myofibroblast characteristics. The Journal of Urology, vol. 169, no. 4, pp. 1573-1576, 2003
24. Wiseman O, Fowler C and Landon DN. The role of the human bladder lamina propria myofibroblast. BJU International 2003;91(1):89-93
25. Hinz B, Phan SH, Gabianni G et al. The myofibroblast: one function, multiple origins. Am J Pathol 2007;170(6):1807-1816
26. Monaghan KP, McCloskey K and Johnston L. Identification of PDGFR α positive populations of interstitial cells in human and guinea pig bladders. J Urol 2012;188:639-647
27. Eaves C, Kent D, Bowie M et al. Regulation of hematopoietic stem cells by the steel factor/KIT signaling pathway. Clin Cancer Res. 2008;14(7):1926-30
28. McCloskey KD and Gurney AM. Kit Positive Cells In The Guinea Pig Bladder. Journal of Urology 2002;168(2):832-836
29. Lascombe I., Bittard H. et al. N-Cadherin as a Novel Prognostic Marker of Progression in Superficial Urothelial Tumors. Clin Cancer Res 2006;12(9): 2780-2787

7

Alterations of the myovesical plexus of the human overactive detrusor

Kamiel A.J. Kuijpers[1], John P.F.A. Heesakkers[1] and Jack A. Schalken[1]

[1]Department of Urology, Radboud University Nijmegen Medical Centre,
The Netherlands.

BioMed Research International, Volume 2014, Epub 2014 April 15

ABSTRACT

Objectives:
The human bladder shows spontaneous autonomous activity. Detrusor overactivity could be seen as a consequence of exaggerated autonomous activity. Interstitial cells (ICs) play a potential role in coordination of autonomous activity. As it is suggested that changes in ICs coexist with detrusor overactivity (DO), we investigated possible alterations to human bladder ICs.

Methods:
Biopsies were obtained from 23 patients and were categorized into four groups: genuine stress incontinence (without DO) (n=5), neurogenic disease with DO (n=6), bladder outlet obstruction with DO (n=6), or idiopathic DO (n=6). Specimens were processed to investigate expression of N-cadherin and PGP9.5. N-cadherin expression was semiquantitatively analyzed and correlated to PG9.5 expression and bladder wall morphology.

Results:
The population of cells expressing N-cadherin is altered in the overactive detrusor, making no difference between the sources of DO. Punctate distribution of morphological changes was found and downregulation of PGP9.5 expression seemed to coexist with upregulation of N-cadherin expression in the detrusor layer.

Conclusions:
The population of N-cadherin+ cells of the interstitial compartment of the human bladder has the ability to proliferate. As this proliferation seems to coexist with denervation, it could be possible that a highly developed network of interstitial cells replaces the loss of innervation in overactive detrusor.

1. INTRODUCTION

The overactive bladder (OAB) is a symptomatic diagnosis based on the presence of urgency, with or without incontinence, and is usually accompanied by frequency and nocturia [1]. Its presence imposes a huge burden on the healthcare system, society, and affected individuals [2]. Patients with OAB symptoms and detrusor overactivity (DO) can be divided into three groups; those with neuropathic laesions, those with bladder outlet obstruction (BOO), and those with neither (idiopathic DO) [3].
Although relatively little is known about the aetiology of DO, it is now clear that the human urinary bladder cannot merely be seen as a passive 'black box,' solely controlled by neuronal input. Recently, it was found that the isolated bladder shows spontaneous nonneuronal contraction during the filling phase, also known as autonomous activity of the bladder [4]. As frequency and urgency occur during this filling phase, DO can be seen as a consequence of exaggerated autonomous activity during the storage of urine [5].
Two cell types play a potential role in coordination of autonomous activity: interstitial cells (ICs) and intramural neurons of the bladder wall. In the human gastrointestinal tract, specialized ICs of Cajal (ICCs) interconnect through gap junctions and function as pacemakers and conductors of electrical activity between enteric neurons and smooth muscle cells [6]. They hereby coordinate gut peristalsis. ICs are also found in the human bladder, albeit of a different subtypes than the ICs of Cajal [7,8]. In the bladder, ICs are immunoreactive for the stem cell receptor C-kit, the cytoskeletal filament vimentin, the gap junctional protein connexin-43, the second messenger cyclic guanosine monophosphate (cGMP), and N-cadherin [9–12]. They form a network in the suburothelial area and between the detrusor smooth muscle fascicles. Double labelling confocal microscopy experiments revealed that the ICs are positioned in proximity to nerves [9]. Recent reports have shown that the bladder ICs respond to application of neurotransmitters, firing calcium waves when stimulated by carbachol or ATP [9,13]. It can therefore be hypothesized that by consecutive interaction of ICs with smooth muscle cells, neuronal firing could consequently result in detrusor muscle activation. Although the exact role for ICs in bladder function has not yet been elucidated, it is highly likely that either quantitative or qualitative changes in bladder ICs coexist with DO. We therefore investigated possible alterations to the network of human bladder ICs in the overactive detrusor using N-cadherin. Additionally, we used PGP9.5 (Protein Gene Product 9.5) as a pan-neuronal marker as it is generally accepted that this protein is expressed by all neuronal structures of the bladder wall [14].

2. METHODS

2.1. Patients

This study was conducted on biopsies from 12 female and 11 male patients, aged 47 to 68 years (mean 60 years), suffering from OAB complaints or genuine stress incontinence. Two cold cup bladder biopsies were obtained from each patient from the posterior bladder wall during cystoscopic procedures. The local ethics committee approved the

study and informed consent was obtained from all patients. All patients underwent full urodynamic analysis and were categorized into three groups: neurogenic disease with detrusor overactivity (DO) (n=6; 2 male, 4 female), bladder outlet obstruction (BOO) with DO (n=6; 5 male, 1 female), or idiopathic DO (n=6; 1 male, 5 female). Three females and two males aged 53 to 75 years (mean age 65.6 years) with stress urinary incontinence and urodynamically proven nonoveractive detrusor served as controls. These patients did not suffer from neurogenic disease or bladder outlet obstruction and were all undergoing check cystoscopy. Filling cystometry (50 mL/min) in patients with OAB all revealed DO with a median cystometric bladder capacity (CBC) of 203 mL (range from 28 to 450). The median CMC in control bladders was 400 mL (range from 205 to 500). The CMC of 205 mL was caused by major stress incontinence. In all patients suffering from OAB, intravesical pressure rises during filling were due to DO, as compliance was normal. Acontractile detrusors were not included.

2.2. Immunohistochemistry

Bladder specimens were collected and placed in a mould containing Tissue-Tek (Sakura). They were snap-frozen immediately in isopentane at −80°C. Sections of 4 µm were prepared using a cryostat and mounted on Super Frost Plus (Menzel-Gläser) slides. Using haematoxylin-eosin staining techniques, tissue was analyzed for presence of intact urothelium and smooth muscle.

Immunohistochemical staining was performed as previously described by our group [12]. Antibodies against the following markers were used: N-cadherin (rabbit polyclonal antibody); (M142 Takara, mouse monoclonal antibody; C2542 Sigma Clone GC-4), smoothelin [15], and PGP9.5 (mouse monoclonal antibody 7863-0504; AbD Serotec). Antibodies against PGP9.5 were used as it is generally accepted that PGP9.5 is expressed by all neuronal structures of the bladder wall [14,16,17]. Negative controls included omission of primary antibodies and incubation with PBS-Extra instead. Positive controls included human prostate cancer specimens [18].

2.3. Morphologic analysis

Immunohistochemical photomicrographs were analyzed semiquantitatively for PGP9.5+ nerve profiles and N-cadherin+ structures. As cold cup biopsies were used, a limited amount of detrusor smooth muscle was available for analysis compared to transmural biopsies. A maximum number of four smooth muscle fascicles per biopsy were photographed. Three slides per specimen were analyzed in duplo. Each set of ten slides was separated by approximately 1 mm of tissue. According to expression of N-cadherin and PGP9.5, each fascicle was semiquantitatively graded as follows: features not present in any photographs, 0; present 0–1/3, +; present in 1/3–2/3, ++; present in 2/3-entire fascicle, +++.

3. RESULTS

3.1. N-cadherin+ ICs in control bladder

All bladder specimens contained the following three layers of tissue: urothelium, suburothelium, and detrusor. Because of the used cold cup biopsy method, the detrusor smooth muscle layer was penetrated to a maximum of two muscle bundles in depth. Full thickness biopsies of human bladder consist of three layers of smooth muscle bundles.
According to our previous study [12], N-cadherin positive structures were found throughout the entire bladder wall. They coexpressed vimentin (Fig. 1 and 2), but showed no coexpression of the pan-neuronal marker PGP9.5 (Fig. 3) or the smooth muscle specific marker smoothelin (data not shown). No immunoreactivity for N-cadherin was found in the urothelial layer (data not shown). N-Cadherin expression profile highly resembled punctate C-kit expression as found by others [16].
N-Cadherin+ cells were located immediately below the urothelium and extended throughout the suburothelial lamina propria into the detrusor layer. They showed a branched morphology with multiple processes that were closely associated (Fig. 1). In the detrusor layer, N-cadherin was expressed by cells housed at the border of smooth muscle bundles, perifascicular, and within smooth muscle fascicles (Fig. 2).
PGP9.5 was expressed in all control specimens. PGP9.5+ nerves were often closely associated with N-cadherin+ cells. In the detrusor layer; muscle fascicles were neighboured by primary nerve trunks housed in planes of connective tissue. Smaller nerve branches were found between detrusor fascicles, continuing as small fibers penetrating the fascicles.

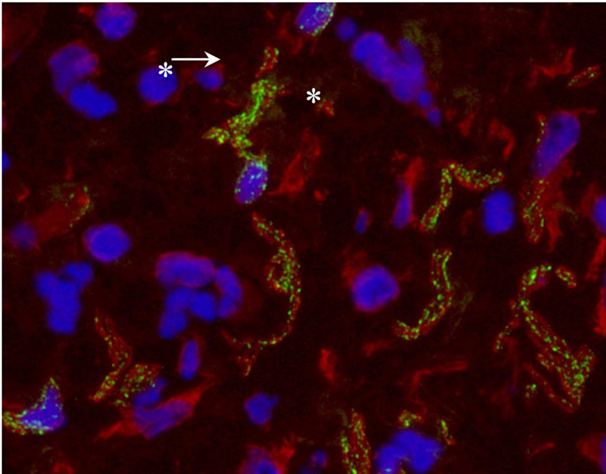


Figure 1 - Suburothelial interstitial cells in control bladder. Multiple suburothelial cells with bizarre morphology and multiple processes coexpress a punctate signal for N-cadherin (green) and a filamentous signal for vimentin (red). Nuclei stained with Dapi (blue). Two cells are closely associated with each other (arrow). Cells expressing vimentin but lacking expression of N-cadherin embody fibroblasts (asterisk). Magnification X630. Binocular epifluorescent microscopy.

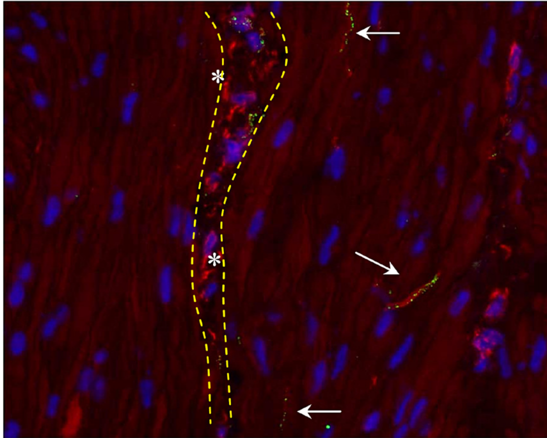


Figure 2 – Interstitial cells in the detrusor layer of control bladder. Cells coexpressing N-cadherin (green) and vimentin (red) as found in the detrusor layer. Nuclei stained with Dapi (blue). Note red background signal in smooth muscle cells that was upgraded to facilitate tissue orientation. Arrowheads show slender punctate N-cadherin expression embodying ICs located in the interfascicular clefts (indicated by dotted orange lines). Slender intrafascicular IC-structures (arrows) run in parallel with and between individual smooth muscle cells. Cells expressing vimentin but lacking expression of N-cadherin (asterisk) embody fibroblasts. Magnification X400. Binocular epifluorescent microscopy.

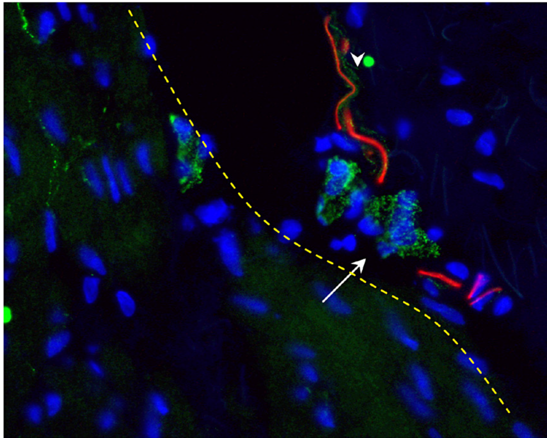
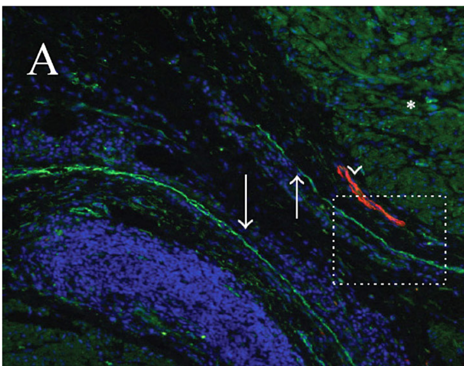


Figure 3 – N-Cadherin and PGP9.5. N-Cadherin (green) was double stained with panneuronal PGP9.5 (red) and counterstained with Dapi (blue). N-Cadherin positive interstitial cell (arrow) is neighboured by a PGP9.5 positive nerve ending (arrow head). Both cell types do not show coexpression of both markers. Cigar shaped background signal embody collagenous fibres. Enlarged from magnification X400. Binocular epifluorescent microscopy.



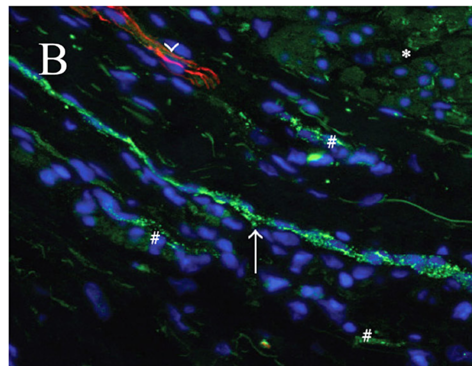


Figure 4 – N-Cadherin positive strings at the border of detrusor bundles in overactive detrusor. N-Cadherin (green) double labeled with PGP9.5 (red) and counterstained with Dapi (blue). (A) Two fascicles are almost entirely surrounded by planes of N-cadherin cells (arrows). A large nerve trunk (arrowhead in A and B) is found in the perifascicular connective tissue. However, note lack of PGP9.5 expression in the upper fascicle. Instead, this fascicle shows a relatively high expression of N-cadherin (asterisk in A and B). Note green background signal in smooth muscle cells that was upgraded to facilitate tissue orientation. Magnification X100. (B) Magnification of white rectangle in (A). Slender strings consisting of numerous N-cadherin+ cells (arrow) are accompanied by multiple N-cadherin+ cells (#) in their connective tissue coat. Magnification X400. Binocular epifluorescent microscopy.

3.2. Changes to the population of N-cadherin+ cells in the OAB

In all specimens, N-cadherin and PGP9.5 expression showed wide variety between smooth muscle fascicles throughout all detrusor muscle layers. In general, N-cadherin expression seemed upregulated in overactive detrusor specimens compared to control specimens, making no difference between the sources of DO (Table 1). In overactive detrusor specimens, changes to the network of N-cadherin+ cells were found at intrafascicular level, perifascicular level, and at the border of smooth muscle bundles. Detrusor smooth muscle bundles with upregulated expression of N-cadherin seemed surrounded by long N-cadherin positive planes (Fig. 4). These planes were built up from numerous cells expressing N-cadherin, had a slender morphology, and were housed in a thick layer of connective tissue. At the area in which the interfascicular cleft met the outside of the muscle bundle, the strings were thickened (Fig. 5) and seemed to give rise to N-cadherin+ processes running in the interfascicular connective tissue planes. Interfascicular N-cadherin+ processes seemed to junction to nodes (Fig. 6). These nodes were not only housed between fascicles, but also between smaller groups of smooth muscle cells. In control specimens, higher expression level of intrafascicular N-cadherin was found in some fascicles as well. However, large strings, interfascicular penetrating structures, and large intrafascicular nodes expressing N-cadherin were never found in control bladders.

The invasion of N-cadherin+ structures did not occur throughout the entire bladder wall but was solely found in smooth muscle bundles that were surrounded by long N-cadherin+ strings. Changes to the IC network were found at each level of depth of the bladder wall and were not restricted to the urothelial side or outer layer of detrusor muscle. However, cold cup biopsies do not enable analysis of full thickness bladder wall morphology. No clear differences between overactive and control specimens were found in the suburothelial N-cadherin+ network.

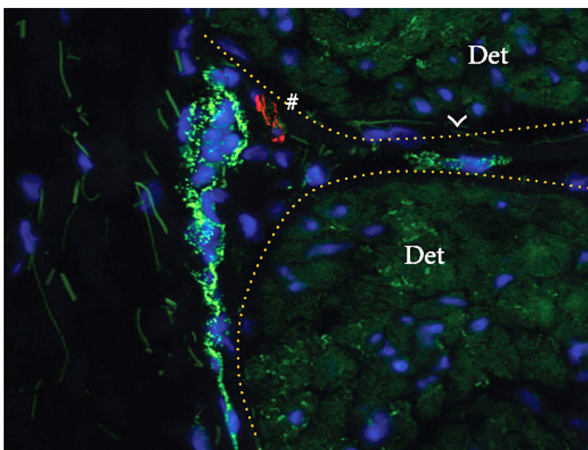


Figure 5 – N-Cadherin positive strings and interfascicular cells. N-Cadherin (green) double labeled with PGP9.5 (red) and counterstained with Dapi (blue). Detrusor smooth muscle fascicles (Det) are demarcated by the dotted orange line. Note green background signal in smooth muscle cells that was upgraded to facilitate tissue orientation. N-Cadherin+ string closely associated to an adjacent detrusor smooth muscle fascicle. A cell expressing N-cadherin is housed within the interfascicular connective tissue plane (arrowhead). Note the close association of a PGP9.5+ nerve profile (#) with N-cadherin+ cells. PGP9.5 is not intrafascicularly expressed. Magnification X400. Binocular epifluorescent microscopy.

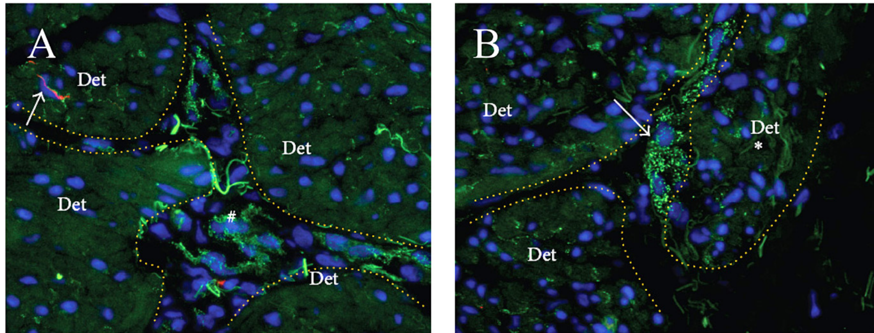


Figure 6 – Intrafascicular N-cadherin positive nodes in the overactive detrusor. N-Cadherin (green) double labeled with PGP9.5 (red) and counterstained with Dapi (blue). Note green background signal in smooth muscle cells that was upgraded to facilitate tissue orientation. Detrusor smooth muscle fascicles (Det) are demarcated by the dotted orange line. (A) At the region where interfascicular connective planes meet, N-cadherin+ cells seem to accumulate and form interfascicular nodes (#). Note high level of intrafascicular N-cadherin in combination with low level of PGP9.5 expression (arrow). Filamentous shaped autofluorescent signal embody collagenous fibres. (B) A small group of muscle cells (asterisk) seems to be separated from the main fascicle by a penetrating N-cadherin positive structure (arrow). Magnification X400. Binocular epifluorescent microscopy.

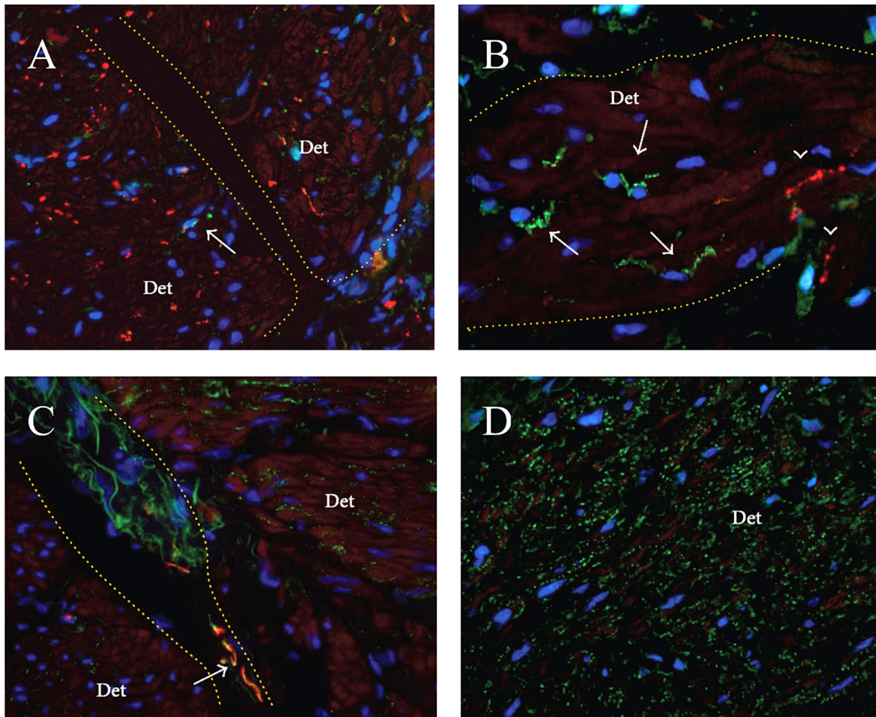


Figure 7 - N-Cadherin upregulation and PGP9.5 downregulation in smooth muscle bundles. N-Cadherin (green) was double labeled with PGP9.5 (red) and counterstained with Dapi. Note red background signal in smooth muscle cells that was upgraded to facilitate tissue orientation. Detrusor smooth muscle fascicles (Det) are demarcated by the dotted orange line. (A) Detrusor smooth muscle bundles in normal bladder show a dense PGP9.5 nerve profile in combination with a low level of N-cadherin expression. Arrow shows N-cadherin positive cell. (B) A muscle fascicle in NDO specimen with relatively low PGP9.5 expression (arrowheads) and a relatively high level of N-cadherin+ ICs (arrows). Magnification X630. (C) Cryosection of NDO specimen showing PGP9.5 negative muscle fascicles with high level of punctate N-cadherin expression. Note that PGP9.5+ nerve profiles in the interfascicular cleft closely associate with N-cadherin+ structures (arrow). (D) Smooth muscle fascicle with severely upregulated expression of N-cadherin. Note that the intensity of N-cadherin expressions seems higher than PGP9.5 expression in normal bladder as shown in (A). Magnification X400. Binocular epifluorescent microscopy.

Table 1 - Semiquantitative analysis of intrafascicular N-cadherin and PGP9.5 expression in control and overactive detrusor.

	n	Fascicle 1		Fascicle 2		Fascicle 3		Fascicle 4	
		N-cad	PGP9.5	N-cad	PGP9.5	N-cad	PGP9.5	N-cad	PGP9.5
GSI	1	+	++	+	+	+	++	+	++
		+	++	+	++	+	++	+	+++
	2	++	++	+	++	++	−	Error	Error
		+	−	+	++	+	+	+	++
	3	+	++	+	++	+	+	+	+
		+	++	+	+	+	++	+	+++
	4	+	+	++	++	++	−	+	+
		++	++	+	++	+	+	+	++
	5	+	++	+	+	+	++	+	++
		+	++	+	++	+	++	+	+
NDO	1	++	+	++	+	++	+	Error	Error
		+	+	++	++	+++	−	++	−
	2	+	+	++	+	+	+	++	+
		+	−	+	−	++	+	++	−
	3	+	+	++	++	+	+	++	+
		++	+	+	+	+	−	++	−
	4	++	+	Error	Error	+	+	Error	Error
		+	−	++	+	++	−	+++	−
	5	++	+	++	+	+	++	+	++
		+	−	+++	−	+	+	++	+
	6	+	+	Error	Error	++	+	+	+
		+	−	++	+	+	−	+++	−
BDO	1	+	+	++	+	+	+	++	+
		++	+	+	−	+++	−	++	−
	2	+	+	+	+++	+	++	++	+
		++	−	++	+	++	+	++	+
	3	++	+	++	+	++	+	Error	Error
		++	−	+	+	++	−	++	−
	4	+	+++	Error	Error	++	+	+	+
		+++	−	+++	−	++	−	++	−
	5	+	+	+	++	+	+	+	++
		+	++	+++	−	+++	−	+	++
	6	+	+	++	++	+	+	+++	+
		++	+	++	++	+	+++	+	++
IDO	1	++	+	+	+	+++	+	+	+
		+++	−	++	−	+++	−	++	++
	2	Error	Error	++	+	Error	Error	++	+
		++	+	+	+	+++	−	++	−
	3	+	++	+	++	++	+	++	+
		+	−	++	+	+	++	++	−
	4	+++	+	++	+	+++	+	Error	Error
		+	+	+++	−	++	−	++	−
	5	++	+	++	+	Error	Error	+	+
		++	++	++	+	++	−	+	++
	6	++	+	+	+++	+	+++	+++	−
		+	++	+++	−	+++	−	+	++

3.3. Smooth muscle denervation co-exists with upgrade of IC network

Intrafascicular expression of N-cadherin and PGP9.5 both were heterogeneously expressed and varied from low, to intermediate, and high level of expression (Fig. 7). In general, the degree of immunoreactivity for N-cadherin seemed correlated with the level of PGP9.5+ innervation profile. Low expression level of N-cadherin was found to coexist with a high level of PGP9.5+ innervation grade (Table 1). In other fascicles, intermediate level of N-cadherin+ structures coexisted with an intermediate level of PGP9.5 expression.

Smooth muscle fascicles with limited or no expression of PGP9.5+ nerve profiles showed intense expression of N-cadherin.
We observed that although PGP9.5+ denervation was found, PGP9.5+ structures were still found in the interfascicular cleft (Fig. 7(C)). Large PGP9.5+ nerve trunks located in connective tissue planes outside smooth muscle bundles seemed not downregulated in overactive detrusor specimens.

4. DISCUSSION

Understanding the pathophysiological mechanism behind detrusor overactivity (DO) is a challenge, as the bladder wall has a complicated structure. While the molecular basis for cell-cell communication between urothelium, neurons, and detrusor smooth muscle cells is getting more and more clear, the exact function of interstitial cells in the human bladder remains unelucidated.
The human urinary bladder shows spontaneous localized and propagating contractions during the storage phase [5]. The bladder may share characteristics with peristaltic activity in the gastrointestinal tract [19,20]. In the gut, the myenteric plexus controls peristalsis. This plexus is embodied by the intramural neurons and interstitial cells of Cajal (ICCs) [6]. It has recently been proposed that the human bladder, like the gut, consists of modules that contract independently or synchronously with neighbouring modules [21]. Furthermore, the modules would be abnormally active and better coordinated in DO, compared to the normal bladder.
According to this hypothesis, two cell types play a potential role in coordination of autonomous activity in the bladder: interstitial cells (ICs) and intramural neurons. ICs of the bladder share properties of the ICCs [7,8]. However, they embody a different subtype than the ICCs [7,8]. In the human bladder, ICs are immunoreactive for the stem cell receptor C-kit, the cytoskeletal filament vimentin, the gap junctional protein connexin-43, the second messenger cyclic guanosine monophosphate (cGMP), and N-cadherin [9–12]. It is highly likely that they form a network in the suburothelial area and between the detrusor smooth muscle fascicles. Although the exact role for ICs in bladder function has not yet been described, it seem that either quantitative or qualitative changes in bladder ICs coexist with increased excitability in the OAB. We therefore investigated possible alterations to the network of human bladder ICs using N-cadherin.
In our study, all biopsies possessed a large population of N-cadherin+ cells. During previous studies we found that N-cadherin most probably can be used as a marker for a subpopulation of bladder ICs [12]. N-Cadherin+ ICs were located in the suburothelial lamina propria and the detrusor layer. In the lamina propria, they showed a bizarre morphology with multiple processes that seemed to form a network. In the detrusor, N-cadherin+ ICs were housed at the border of smooth muscle bundles, perifascicular, and within smooth muscle fascicles. Throughout the bladder wall, ICs expressing N-cadherin were closely associated with PGP9.5 positive neurons. Therefore, these cells could form a myovesical plexus as found in the human gut. Possibly, neuronal information is received in the larger ICs at the border of bundles and passed through the intermediate N-cadherin

positive structures to small groups of detrusor smooth muscle cells.
We found changes to the network of N-cadherin+ ICs in most overactive detrusor specimens. These specimens revealed a remarkable variation in N-cadherin expression between and even within detrusor fascicles. This heterogeneous expression profile of N-cadherin is not based on processing artefacts, as in duplo analysis showed exact corresponding pattern of expression.
In the overactive detrusor specimens, upregulation of N-cadherin expression was found at three levels according to anatomical degree, in terms of smooth muscle bundles (level 1), smooth muscle fascicles (level 2), and smooth muscle cells (level 3). It seemed as if large N-cadherin positive structures surrounding smooth muscle bundles were newly formed (level 1), continuing as interfascicular N-cadherin+ processes junctioning to nodes (level 2) and slender intrafascicular N-cadherin+ branches possibly interacting with smooth muscle cells forming small modules (level 3).
In fascicles with high expression of N-cadherin, PGP9.5 expression seemed severely downregulated. The intensity of integration of N-cadherin+ structures between smooth muscle cells shows remarkable resemblance with morphological profile of intrafascicular PGP9.5+ innervation in normal bladder, albeit that the N-cadherin+ network seems much more developed. It is generally accepted that PGP9.5 is a nonspecific neuronal marker as it is expressed by all neuronal structures of the human bladder wall [14,16,17]. Therefore, it seems that the smooth muscle fascicles lacking PGP9.5 expression are actually denervated. Other investigators also found denervation in overactive detrusor and it is believed to be a general feature of pathological fascicles in the overactive bladder [22–24]. It could therefore be proposed that a highly developed network of ICs replaces the loss of innervation of detrusor smooth muscle fascicles in the overactive detrusor.
Kubota et al. claimed to have found an increased population of suburothelial ICs coexpressing C-kit and vimentin in the BOO-guinea-pig model [25]. However, a network of fibroblasts weakly expressing C-kit and vimentin is also present in the human detrusor layer. These cells do not have IC-like ultrastructure [26]. Therefore, the suburothelial C-kit positive cells as found by Kubota et al. might possibly embody fibroblasts instead of ICs.
De Jongh et al. found differences in the number and distribution of cGMP+ ICs in the bladders of guinea pigs with surgically induced bladder outflow obstruction [17]. Unlike us, they found alterations occurring in the suburothelial area. It is known that suburothelial ICs consist of distinct populations of cells [27]. It might very well be possible that N-cadherin+ ICs embody a subpopulation of ICs as identified by cGMP expression which is not upregulated in the overactive detrusor. Also, as their study was performed in guinea pigs, upregulation of N-cadherin+ ICs in the human bladder could be reserved to the detrusor layer, while the population of suburothelial N-cadherin+ ICs remains unaffected.
Others also found a correlation between upregulation of IC-like cells and exaggerated autonomous activity. Imatinib mesylate (Glivec; a specific C-kit receptor inhibitor) had an inhibitory effect on the overactive detrusor [28]. As C-kit labeling showed significantly more IC-like cells in overactive human detrusor than in normal specimens, it seems likely that this inhibitory effect is due to the upregulation of detrusor ICs. Also, reduction of ICCs

is seen in syndromes with reduced autonomous activity of the gut, such as Hirschsprung's disease and functional intestinal obstruction [29].
Normal detrusor smooth muscle cells have poor electrical coupling [30]. Local contractions of groups of smooth muscle cells have been shown to occur in the normal bladder, but these did not lead to intravesical pressure rise [31]. Studies of the overactive detrusor ultrastructure demonstrated the existence of ultraclose abutments and protrusion junctions [32]. It was proposed that these might be the routes of spread of electrical activity in the overactive bladder. However, abnormally wide spread propagation of spontaneous activity could not only result from increased coupling between smooth muscle cells, but also from altered properties of the IC network. If we consider denervated fascicles with an upregulation of N-cadherin expression to be overactive, exceeding the number of overactive fascicles beyond a certain threshold might drive the bladder into behaving overactive. Also, as detrusor smooth muscle bundles house pacemaker cells [9], DO might not need the synchronized nerve-mediated smooth muscle excitation in order to develop.

5. CONCLUSIONS

It seems that the network of N-cadherin positive ICs in human urinary bladder has the ability to proliferate. As upregulation of N-cadherin+ ICs was found to coexist with denervation, it could be proposed that a developed network of interstitial cells replaces the loss of innervation of detrusor smooth muscle fascicles in overactive detrusor. However, further study is needed to gain more insight into the role of this cellular mechanism and its possible role in exaggerated autonomous activity in the pathological bladder.

REFERENCES

1. Wein AJ. Diagnosis and treatment of the overactive bladder. Urology 2003;62(5):20–27
2. Serels S. The wet patient: understanding patients with overactive bladder and incontinence. Current Medical Research and Opinion 2004;20(6):791-801
3. Andersson KE. Storage and voiding symptoms: pathophysiologic aspects. Urology 2003;62(5):3-10
4. Gillespie JI, Harvey IJ and Drake MJ. Agonist- and nerve-induced phasic activity in the isolated whole bladder of the guinea pig: evidence for two types of bladder activity. Experimental Physiology 2003;88(3):343-357
5. Drake MJ, Harvey IJ and Gillespie JI. Autonomous activity in the isolated guinea pig bladder. Experimental Physiology 2003;88(1):19-30
6. Sanders KM. A case for interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. Gastroenterology 1996;111(2):492-515
7. Gillespie JI. The autonomous bladder: a view of the origin of bladder overactivity and sensory urge. BJU International 2004;93(4):478-483
8. McCloskey KD. Interstitial cells in the urinary bladder—localization and function. Neurourology and Urodynamics 2010;29(1):82-87

9. McCloskey KD and Gurney AM. Kit positive cells in the guinea pig bladder. The Journal of Urology 2002;168(2):832-836
10. Sui GP, Rothery S, Severs NJ et al. Gap junctions and connexin expression in human suburothelial interstitial cells. BJU International 2002; 90(1):118-129
11. Gillespie JI, Markerink-Van Ittersum M and de Vente J. cGMP-generating cells in the bladder wall: identification of distinct networks of interstitial cells. BJU International 2004;94(7):1114-1124
12. Kuijpers KA, Heesakkers JP, Schalken JA et al. An update of the interstitial cell compartment in the normal human bladder. BioMed Research International 2014; Epub April 15
13. Fry C and Sui G. Cellular properties of human suburothelial myofibroblasts, their role in bladder sensation. Neurourology and Urodynamics 2003;22:374-375
14. Gillespie JI, Markerink-Van Ittersum M and de Vente J. Interstitial cells and cholinergic signalling in the outer muscle layers of the guinea-pig bladder. BJU International 2006;97(2):379-385
15. van der Loop FT, Schaart G, van Eys GJ et al. Smoothelin, a novel cytoskeletal protein specific for smooth muscle cells. Journal of Cell Biology 1996;134(2):401-411
16. Davidson RA and McCloskey KD. Morphology and localization of interstitial cells in the guinea pig bladder: structural relationships with smooth muscle and neurons. The Journal of Urology 2005;173(4):1385-1390
17. de Jongh R, van Koeveringe GA, Gillespie JI et al. Alterations to network of NO/cGMP-responsive interstitial cells induced by outlet obstruction in guinea-pig bladder. Cell and Tissue Research 2007;330(1):147-160
18. Tomita K, van Bokhoven A, van Leenders GJ et al. Cadherin switching in human prostate cancer progression. Cancer Research 2000;60(13):3650-3654
19. Brading AF and McCloskey KD. Mechanisms of disease: specialized interstitial cells of the urinary tract—an assessment of current knowledge. Nature Clinical Practice Urology 2005;11(20):546-554
20. Hashitani H, Yanai Y and Suzuki H. Role of interstitial cells and gap junctions in the transmission of spontaneous Ca^{2+} signals in detrusor smooth muscles of the guinea-pig urinary bladder. Journal of Physiology 2004;559(2):567-581
21. Drake MJ, Mills IW and Gillespie JI. Model of peripheral autonomous modules and a myovesical plexus in normal and overactive bladder function. The Lancet 2001;358:401-403
22. Charlton RG, Morley AR, Gillespie JI et al. Focal changes in nerve, muscle and connective tissue in normal and unstable human bladder. BJU International 1999;84(9):953-960
23. Drake MJ, Gardner BP and Brading AF. Innervation of the detrusor muscle bundle in neurogenic detrusor overactivity. BJU International 2003;91(7):702-710
24. Mills IW, Greenland JE, Mcmurray G et al. Studies of the pathophysiology of idiopathic detrusor instability: the physiological properties of the detrusor smooth muscle and its pattern of innervations. The Journal of Urology 2000;163(2):646-651
25. Kubota Y, Hashitani H, Shirasawa H et al. Altered distribution of interstitial cells in the guinea pig bladder following bladder outlet obstruction. Neurourology and Urodynamics 2008;27(4):330-340

26. Drake MJ, Hedlund P, Andersson KE et al. Morphology, phenotype and ultrastructure of fibroblastic cells from normal and neuropathic human detrusor: absence of myofibroblast characteristics. The Journal of Urology 2003;169(4):1573-1576
27. Gillespie JI, Markerink-Van Ittersum M and de Vente J. Endogenous nitric oxide/cGMP signalling in the guinea pig bladder: evidence for distinct populations of sub-urothelial interstitial cells. Cell and Tissue Research 2006;325(2):325-332
28. Biers SM, Reynard JM, Brading AF et al. The functional effects of a c-kit tyrosine inhibitor on guinea-pig and human detrusor. BJU International 2006;97(3):612-616
29. Piotrowska AP, Rolle U, Puri P et al. Interstitial cells of Cajal in the human normal urinary bladder and in the bladder of patients with megacystis-microcolon intestinal hypoperistalsis syndrome. BJU International 2004;94(1):143-146
30. Brading AF. A myogenic basis for the overactive bladder. Urology 1997;50(6):57-73
31. Coolsaet BL, van Duyl WA, de Bakker HV et al. New concepts in relation to urge and detrusor activity. Neurourology and Urodynamics 1993;12(5):463-471
32. Elbadawi A, Yalla SV and Resnick NM. Structural basis of geriatric voiding dysfunction. III. Detrusor overactivity. The Journal of Urology 1993;150(5):1668-1680

8

Summary and conclusions

The overactive bladder (OAB) syndrome is defined as a symptom complex that includes urinary urgency, with or without incontinence, usually with frequency and nocturia. It is a common disorder affecting the quality of life in huge numbers of people worldwide. OAB symptoms are suggestive of urodynamically demonstrable detrusor overactivity (DO). The 'golden standard' urodynamic investigation has its limitations in diagnosing OAB symptoms and more sensitive and specific diagnostic tools are desired. Also, new suitable targets for pharmacological intervention are necessary. In search of potential pharmacological targets one needs to understand more of the etiology of the overactive bladder. Attempts to explain the cause of OAB have been primarily focused on abnormal expression of the neuronal micturition reflex system (the neurogenic hypothesis). However, we hypothesize that focussing on nervous control of the lower urinary tract may bring the false interpretation of considering the muscular end organ as being a passive 'black box'. Evidence is accumulating that DO originates from within the bladder wall itself, due to structural changes of the detrusor smooth muscle. Our study group started this project to disentangle the biochemical and structural changes occurring in the overactive detrusor.

In **chapter 1** an introduction, the study rationale and outline of this thesis is given. It describes the physiological aspects of the lower urinary tract and the traditional view of bladder wall anatomy. Terminology and definitions of normal lower urinary tract function and dysfunction are presented. We demonstrate the size of the problem called the OAB and discuss limitations in current diagnostic tools and therapies.

Chapter 2 describes the complexity of terminology, evaluation and treatment of lower urinary tract symptoms (LUTS). LUTS include storage and voiding symptoms. The OAB syndrome comprises the same symptoms as storage LUTS, but excludes types of incontinence other than urge incontinence. Storage LUTS often persist after transurethral resection of the prostate (TURP) in benign prostatic enlargement (BPE). This finding suggests that non neurogenic factors, such as bladder outlet obstruction (BOO), play a role in development of the OAB syndrome. However, the precise cuase of DO in BOO has not been identified. Also, it raises the question whether an intrinsic substrate within the bladder wall exists that is responsible for developing DO.

In **chapter 3** an overview is given of the current theories trying to explain DO. The neurogenic, myogenic and autonomous hypotheses are discussed. The neurogenic theory suggests that DO can occur as a result of changes in the peripheral and central nervous system of the bladder, hereby disrupting the voluntary control of the micturition pathway and causing an abnormal expression of the micturition reflex. The myogenic theory suggests that changes in properties and structure of detrusor smooth muscle occur. This would lead to increased excitability and increased ability of activity to spread between detrusor smooth muscle cells, resulting in highly modified spontaneous activity. The autonomous theory suggests that DO can be seen as a consequence of exaggerated autonomous activity during the storage of urine. To test these hypotheses, one needs to assess the integrative processes of the entire organ, starting with detailed microscopic investigation of the human bladder.

In **chapter 4** adhesion complexes between detrusor smooth muscle cells are described. In most smooth muscle tissue, smooth muscle cells are extensively connected both electrically and structurally to produce coordinated contractions. Connexins are expressed by smooth muscle cells. They are the subunits that form the so called gap junctions that are in fact responsible for the transport of small molecules, as well as, charged molecules, from one cell to the other. Gap junctions are likely to be crucial for the transduction of electric stimuli, hereby mediating cell-cell communication and cell-cell contraction. However, muscle cells also need intact intercellular mechanical adhesion. Without this adhesion, muscle bundles will break into pieces if simultaneous excitation occurs. Gap junctions cannot account for the required tensile strength between muscle cells. The class of molecules that mediates homophilic adhesion are the cadherins. We have therefore investigated the expression of multiple subtypes of the cadherin family in the normal human bladder. Bladder tissue was obtained from eight individuals in whom radical cystectomy was performed because of localized bladder cancer. The study showed punctate expression of cadherin-11 in the membranes of detrusor smooth muscle cells, but also in suburothelial myofibroblasts. Myofibroblasts are a recently documented interstitial cell (IC) type localized in the interstitial compartment. They share characteristics with smooth muscle cells and fibroblasts. In both cell types cadherin-11 expression was closely associated with intracellular actin filaments of the cytoskeleton. We have concluded that cadherin-11 mediates the critical recognition signal and physical interaction between detrusor smooth muscle cells within a bundle but also between suburothelial myofibroblasts. The suburothelial myofibroblasts may need strong intercellular adhesion as it is suggested that they could act as a stretch sensor during bladder filling, resulting in the sensation of urge to void.

In **chapter 5** we have investigated whether analysis of adherens junctions (AJs), or adhesive junctions, in human detrusor could be used as a diagnostic tool to determine detrusor overactivity. During former ultrastructural studies using electron microscopy (EM), it was found that the overactive detrusor has a distinctive 'disjunction pattern' compared to the normal bladder. This pattern is characterized by reduction or loss of AJs between detrusor smooth muscle cells and widening of spaces between individual smooth muscle cells coexisting with collagenosis. Specimens from 23 patients were investigated, categorized into 4 groups, including 5 who were normal, and 6 each with neurogenic disease with DO, bladder outlet obstruction with DO and idiopathic DO. Using an interplay of immunohistochemical and immunogold electron microscopic techniques, we showed that cadherin-11 is an integral structural protein of AJs between detrusor smooth muscle cells in the normal and overactive detrusor. In fact, the punctate expression of cadherin-11 marks AJs as identified during ultrastructural studies for the disjunction pattern. Cadherin-11 seemed to be down regulated with intercellular space widening and collagenosis. However, a heterogeneous expression pattern of cadherin-11 expression and fascicle structure was found in the same specimen. No correlation was noted between DO and cadherin-11 expression. We have concluded that quantitative analysis of AJs using immunostaining cannot replace urodynamic evaluation as a predictor of DO.

In **chapter 6** we have investigated whether N-cadherin could play a role as a discriminatory marker for interstitial cells (ICs) in the human bladder. ICs, also referred to as myofibroblasts, most probably play a major role in the pathogenesis of the overactive bladder by forming a network integrating signals in the bladder wall between various types of cells. However, no marker has been identified that can be considered as a specific phenotypic marker for ICs in the human bladder. Cadherins function as signaling centers between cells. N-cadherin is the most commonly expressed cadherin in stromal cells. Sixteen bladder biopsies were collected from sixteen individuals in whom radical cystectomy was performed because of muscle invasive bladder cancer. Bladder specimens were processed using combined immunohistochemical and transmission electron microscopy techniques. The study showed N-cadherin positive cells with branched cell bodies and multiple processes which were localized in the suburothelial lamina propria and detrusor layer of the normal human bladder. They showed no co-localisation of pan-neuronal nor pan-smooth muscle markers. Transmission electron microscopy revealed ICs with stellate morphology in both the lamina propria and the musculus detrusor layer. They had characteristic features of myofibroblasts and appeared to be similar to the N-cadherin positive cells. It seemed that N-cadherin was not a specific marker for the entire population of ICs. But we have concluded that N-cadherin can be used as a specific marker for a subpopulation of ICs in the human bladder, i.e. the ultrastructurally defined myofibroblasts.

In **chapter 7** possible alterations to the population of N-cadherin positive cells are discussed. The autonomous hypothesis suggests that DO can be seen as a consequence of exaggerated autonomous activity. Autonomous activity of the bladder is defined as spontaneous non micturition contractions of the bladder during normal filling. Localized propagating waves of contraction travel through the bladder wall. This activity is intrinsic to the bladder wall. Abnormally wide spreaded propagation of spontaneous activity could not only result from increased coupling between smooth muscle cells (the myogenic hypothesis), but also from altered properties of the IC network. Specimen from 23 patients were analyzed, categorized into 4 groups, including 5 who were normal, and 6 each with neurogenic disease with DO, bladder outlet obstruction with DO and idiopathic DO. The study showed that the expression profile of the population of cells expressing N-cadherin was altered in the overactive detrusor, making no difference between the source of DO. Also downregulation of PGP9.5 (a pan-neuronal marker) expression seemed to coexist with upregulation of N-cadherin expression in the detrusor layer. We have concluded that the population of N-cadherin positive cells in the human bladder has the ability to proliferate. Furthermore, we suggested that a highly developed network of ICs replaces the loss of innervation of detrusor smooth muscle cells in the overactive detrusor.

9

Future perspectives

Animal models

Analysis of bladder biopsies to study the etiology of the overactive bladder has its limitations. Availability of bladder specimens from healthy individuals with normal bladder function and without comorbidity is low. For this reason, a number of specimens in this thesis were collected from individuals in whom radical cystectomy was performed because of localized bladder cancer. Dissection was performed distant from tumour sites. We believe that our findings are unaffected by tumour-related factors as no thickening of the urothelial layer or abnormal urothelial morphology was found. However, influence of cancer cannot completely be ruled out. Furthermore, the overactive bladder shows focal morphological changes, resulting in highly modified areas adjacent to apparently normal regions. These issues may have important implications for experiments on the etiology of overactivity that use bladder biopsies, isolated detrusor muscle strips or dissociated cells. Research focussing on animal models using whole bladder preparations could overcome these problems. However, one must keep in mind that the overactive bladder (OAB) syndrome is a clinical and symptomatic diagnosis, and animals cannot report symptoms. Also, there are differences in the regulation of bladder function between humans and animal species.

Cadherin-11 as morphogenetic regulator

A-δ nerve fibers are the ones that have control in normal situations. Bladder contractions occur by activation of M2- and M3- muscarinic receptors by acetylcholine. In normal subjects the M3 receptor is most active, whereas in denervated bladders an increased expression of M2 receptors is seen. In pathological situations C-fibers appear to play a more pronounced role. Herein, the purinergic and vanilloid receptors are considered as suitable drugable targets. Cadherins are important morphogenetic regulators that confer cellular phenotypes by modulating the expression of their target genes located in the cell nucleus. It is now well established that loss and gain of cadherin function has profound cell biological consequences, such as change of cellular phenotype. Therefore, impaired function of the critical intercellular cadherin-11 mediated adhesion mechanism, as found in chapter 5, could account for dedifferentiation of the smooth muscle cell and explain switch in subtype expression and expression pattern of adrenergic/muscarinic/purigenergic and or vanilloid receptors.

Highly modified detrusor fascicles were found adjacent to apparently normal areas. Other investigators hypothesized that these areas match the topography of detrusor modules with each controlled by dedicated integrative circuits, as in the gut. It seems that normal bladders can include highly modified modules without becoming overactive. If we consider that modified detrusor areas with cadherin-11 down-regulation behave pathologically, exceeding their number beyond a certain threshold may drive the bladder into becoming overactive. Loss of cadherin-11 could mark a point of no return in this pathological condition. Further research is needed to gain further insight in this matter.

Identification of interstitial cells

Interstitial cells (ICs) have several populations that can be discriminated by morphological appearance and differential expression of markers. This makes the search for their exact functional role in the human bladder a contentious subject. Various surrogate histochemical markers are available for ICs, such as the stem cell receptor C-kit and cyclic guanosine mono-phosphate (cGMP). In this thesis, we have concluded that N-cadherin could be used as a specific marker for a subpopulation of ICs in the human bladder. However, the specific immunophenotype of ICs is still controversial. Future research should focus on identification of the interstitial phenotype using above mentioned markers in combined electron microscopy and immunostaining techniques, e.g. immunogold electron microscopy. This would create a highly sensitive and specific analysis tool that merges protein characterization and cellular ultrastructure.

Cell-cell communication in interstitial cells

Denervation of the bladder wall is believed to be a general feature of the overactive bladder. In chapter 7 we showed that the interstitial population of cells has the ability to proliferate. If a highly developed network of interstitial cells replaces the loss of innervation, this could lead to increased ability of activity to spread between smooth muscle cells and increased excitability of the detrusor muscle. However, it remains to be investigated whether the interstitial cells function as an intercellular communicating network. In order to elucidate functional properties of these cells, more research is needed using functional cell analyses, such as Ca-imaging and patch clamp techniques.

Final remarks

The relatively new field of translational research of the overactive bladder is exciting and much progress is made during the last decade in elucidating its cell biological and physiological characteristics. However, many questions remain. Several hypotheses have been proposed trying to explain the cause of detrusor overactivity. In conclusion, neither the neurogenic nor the myogenic hypothesis fully explains the recognized clinical and experimental findings. The recently proposed autonomous theory however, is very promising. DO could be seen as a consequence of exaggerated autonomous activity during the storage of urine.

Although our group mainly focused on the detrusor layer, other areas in the human bladder could also play an important role in modulating urgency and detrusor overactivity. It is becoming more and more clear that the urothelium is more than a barrier that seperates urine from extracellular fluid. It also has sensory properties and is thought to serve as a mechano-sensor [1]. Urothelium releases several substances in response to physical and chemical stimulation [2]. Furthermore, it is has been shown that the lamina propria is heavily innervated [3,4]. It is suggested that the urothelium can control activity in afferent

nerves [5]. Therefore, the urothelium and lamina propria are considered as important regulatory parts of the bladder wall. This so-called urothelium based hypothesis may account for possible targets for therapeutic agents.

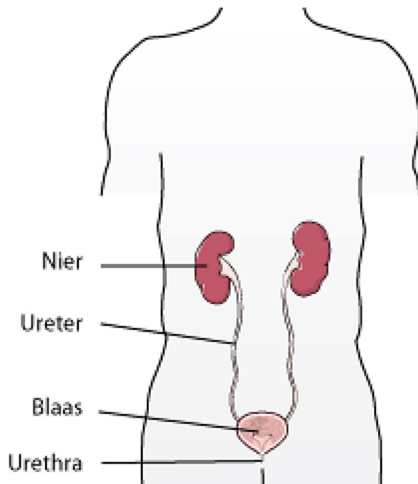
References

1. Birder L, Ruan H, Burnstock G et al. Alterations in P2X and P2Y purinergic receptor expression in urinary bladder from normal cats and cats with interstitial cystitis. American Journal of Physiology 287,1084-1091
2. Yoshida M, Inadome A, Murakami S et al. Non-neuronal cholinergic system in human bladder urothelium. Urology 2006;67:425-430
3. Gillespie J, Markerink-van Ittersum M and de Vente J. Expression of neuronal nitric oxide synthase (nNOS) and nitric-oxide-induced changes in cGMP in the urothelial layer of the guimea pig bladder. Cell and Tissue Research 2005;321:341-351
4. Chopra B, Barrick SR, Birder L et al. Expression and function of bradykinin B1 and B2 receptors in normal and inflamed rat urinary bladder urothelium. Journal of Physiology 2005;562:859-871
5. Birder L, Kanai AJ, Fry C et al. Is the urothelium intelligent? Neurourology and Urodynamics 2010;51:29-32

10

Nederlandse samenvatting

De nieren zijn twee boonvormige organen die hoog in de buikholte aan weerszijden van de wervelkolom liggen. Ze filteren afvalstoffen uit het bloed en produceren hierbij urine. Via de urineleiders (ureters) wordt de urine getransporteerd naar de blaas. De blaas is een holle spier die vooraan in het kleine bekken, op de bekkenbodem achter het schaambeen ligt. De blaas heeft een cyclus van opslag en lediging. Via de urinebuis (urethra) verlaat de urine het lichaam.



Figuur 1 - Schematisch overzicht van de urinewegen met de nieren, ureters, blaas en urethra (bron: blaasproblemen.nl).

Het overactieve blaassyndroom is een symptomencomplex dat bestaat uit onbedwingbare aandrang voor urine, met of zonder urineverlies, meestal gepaard gaande met vaak plassen (meer dan 8x per dag) en 's nachts plassen (minimaal 1x per nacht). Het is een veelvoorkomende aandoening die een grote negatieve impact heeft op de kwaliteit van leven van de patiënt. Wanneer de holle blaasspier (musculus detrusor) tijdens de opslagfase niet ontspannen blijft, maar willekeurig samenknijpt (detrusor overactiviteit), kan de patiënt ongewild urine verliezen. Deze onwillekeurige samentrekking wordt meestal ervaren als aandrang om te plassen. Een blaasdrukmeting (urodynamisch onderzoek) is de huidige gouden standaard om vast te stellen of iemand lijdt aan een overactieve blaasspier. Door de blaas langzaam met water te vullen wordt de vullingsfase nagebootst. Er bestaan verschillende medicijnen om de overactieve blaas te behandelen, maar bijwerkingen spelen hierin een grote belemmerende rol. Om nieuwe medicijnen te ontwikkelen die specifiek op de ongewenste blaasoveractiviteit ingrijpen, moet men meer weten over hoe de blaasspier eigenlijk werkt. Als men op cel- en eiwitniveau het verschil kan aantonen tussen een normale en zieke blaasspier, heeft men mogelijk een startpunt vanwaaruit specifieke medicijnen kunnen worden ontwikkeld. Men dacht altijd dat de blaas een holle spier was die werd aangestuurd door het zenuwstelsel. Pogingen om de oorzaak van de overactieve blaas te ontdekken zijn daarom met name gericht geweest op een abnormale zenuwaansturing van de blaas. Echter, de laatste tijd komen er steeds meer aanwijzingen dat er veranderingen plaatsvinden in de blaasspier zelf, die een rol zouden kunnen spelen in het ontstaan van dit ziektebeeld. Zo blijkt dat een overactieve

blaasspier vaak voorkomt bij mannen met een vergrote prostaat, hetgeen niets met een abnormale aansturing door het zenuwstelsel te maken heeft. Bij experimenten waarin de blaas werd losgekoppeld van het zenuwstelsel, blijkt de blaas spontane spieractiviteit te tonen in rust, die bovendien ritmisch van karakter is. De blaas heeft dus een interne pacemaker. Dergelijke experimenten met een geïsoleerde blaas lieten zien dat deze ritmische spieractiviteit ontregeld is bij de overactieve blaasspier. Deze bevindingen duiden op een mogelijke interne bron in de blaasspier voor het ontstaan van detrusor overactiviteit en het overactieve blaassyndroom.
Onze onderzoeksgroep startte dit project om de cellulaire eigenschappen van de blaas bij mensen met en zonder het overactieve blaas syndroom te analyseren. Ons uitgangspunt is dat de onwillekeurige samentrekkingen van de blaasspier bij het overactieve blaas syndroom hun oorsprong niet hebben in abnormale aansturing door het zenuwstelsel, maar ontstaan door structurele veranderingen in de blaasspier zelf. De blaas is dus geen ‘zwarte doos’ die simpelweg de instructies van het zenuwstelsel uitvoert.

In **hoofdstuk 1** wordt een introductie gegeven van het overactieve blaas syndroom. Tevens wordt de gedachtegang achter dit proefschrift uiteen gezet. De werking van de lagere urinewegen en de traditionele visie op de samenstelling van de blaas worden beschreven. Definities van normale en abnormale werking van het orgaan worden behandeld. We demonstreren de omvang van het probleem genaamd het overactieve blaassyndroom en bediscussiëren de beperkingen van huidige diagnostische middelen en behandelingen.

In **hoofdstuk 2** wordt de complexiteit van de terminologie, evaluatie en behandeling van klachten van de lagere urinewegen beschreven. We kunnen deze klachten indelen in klachten tijdens de opslagfase van urine, klachten tijdens het plassen en klachten na het plassen. We nemen de klachten die aanhouden na behandeling van een vergrote prostaat als voorbeeld. De plasbuis loopt door de prostaat heen. Een vergrote prostaat kan de uitstroom van urine uit de blaas belemmeren. De aanhoudende klachten na het uithollen van een vergrote prostaat, en dus het opheffen van de uitgangsbelemmering, zijn gelijk aan de klachten die voorkomen bij het overactieve blaas syndroom. Het blijkt dat andere factoren dan het zenuwstelsel dus ook een rol kunnen spelen in het ontstaan van het overactieve blaas syndroom. Bovendien rijst de vraag of de blaasspier een mogelijke intrinsieke bron heeft voor het ontstaan van detrusor overactiviteit, aangezien overactieve blaasklachten dikwijls blijven bestaan na behandeling van de uitgangsbelemmering.

Hoofdstuk 3 geeft een overzicht van de huidige theoriën die detrusoroveractiviteit proberen te verklaren. De neurogene, myogene en autonome hypotheses worden beschreven. De neurogene theorie suggereert dat detrusoroveractiviteit ontstaat ten gevolge van een verandering in de samenwerking tussen het centrale en perifere zenuwstelsel. De blaas heeft een zelfstimulerende zenuwreflexboog (perifere zenuwstelsel) die het ruggemerg kruist. Vanuit de hersenen en het ruggemerg (centrale zenuwstelsel) wordt deze zelfstimulerende reflexboog geremd. Bij bepaalde aandoeningen kan de rem vanuit het centrale zenuwstelsel wegvallen, zoals bij een dwarslaesie. Hierdoor ontstaat een

ontregeling van de blaasreflexboog, waardoor de blaas overactief wordt. De myogene theorie gaat uit van het feit dat er veranderingen plaatsvinden in de blaasspier zelf. Door veranderde eigenschappen van de spiercellen zouden de cellen makkelijker kunnen samentrekken, waardoor de blaasspier overactief wordt. De autonome theorie gaat uit van de autonome samentrekkingen van de blaas. Het blijkt dat de blaas niet alleen actief is tijdens de plasfase. Ook in de rustfase trekt de blaas samen. Deze samentrekkingen komen echter plaatselijk in de blaaswand voor, waardoor de blaasspier niet als één geheel samentrekt en er geen urine wordt verloren. De exacte betekenis van deze activiteit is onbekend, maar er wordt gespeculeerd dat de blaas op deze wijze 'aftast' in hoeverre de blaas gevuld is. Overexpressie van de autonome activiteit zou kunnen resulteren in detrusoroveractiviteit. Om deze hypotheses te toetsen, moet de blaas beschouwd worden als een complex orgaan met verscheidene regulerende componenten. Omdat er relatief weinig bekend is van de cellulaire samenstelling en werking van de blaasspier, is gedetailleerd microscopisch onderzoek van de blaasspier een vanzelfsprekende eerste stap.

In **hoofdstuk 4** onderzoeken we de verbindingen tussen de spiercellen van de menselijke blaas. In de meeste organen zijn spiercellen elektrisch en mechanisch met elkaar verbonden, waardoor een gecoördineerde samentrekking kan plaats vinden. Connexines zijn eiwitten die voorkomen in de celwand van spiercellen. Ze vormen zogenaamde gap junctions, ook wel ionkanalen genoemd. Via deze kanalen kunnen kleine geladen moleculen (ionen) zich van de ene naar de andere cel verplaatsen en ontstaat er een electrische stroom. Deze stroom is nodig voor gecoördineerde samentrekking van de spiercellen. Echter, mechanische verbinding tussen de cellen is ook nodig, omdat het orgaan anders in stukken wordt getrokken. Gap junctions belichamen slechts een passief communicatiekanaal en vormen geen mechanische verbinding. Cadherines zijn eiwitten die, net als gap junctions, ook gelokaliseerd zijn in de celwand. Er zijn verschillende subtypen van bekend. Cadherines verankeren de celwanden van aangrenzende cellen die hetzelfde cadherine subtype tot expressie brengen. We hebben daarom de expressie van verschillende cadherines in acht menselijk blazen onderzocht. De biopten zijn afkomstig van patiënten bij wie de blaas in zijn geheel is verwijderd in verband met blaaskanker. We vonden een gespikkeld patroon van cadherin-11 (een cadherine subtype) op de celwand van spiercellen in de menselijke blaas. Cadherin-11 werd ook aangetroffen in de celwand van myofibroblasten die net onder de slijmvlieslaag van de binnenzijde van de blaas gelokaliseerd zijn. Myofibroblasten zijn een recent ontdekte groep cellen die eigenschappen van spiercellen en bindweefselcellen hebben. We zagen dat cadherine-11 het interne skelet van de cellen met elkaar verbindt. Dit zogenaamde cytoskelet geeft de cel stevigheid. We concludeerden daarom dat cadherine-11 de mechanische verankering tussen spiercellen en myofibroblasten in de menselijke blaas verzorgt. Het is mogelijk dat deze myofibroblasten die onder de slijmvlieslaag liggen functioneren als een soort sensor die oprekt bij blaasvulling. Deze cellen zouden daarom sterk verankerd met elkaar moeten zijn.

In **hoofdstuk 5** onderzoeken we of het tellen van het aantal celverankeringen in de menselijke blaasspier gebruikt kan worden als een diagnostisch instrument om detrusor overactiviteit op te sporen. Deze celverankeringen worden ook wel adherens junctions genoemd. Tijdens eerdere onderzoeken met electronenmicroscopie werd gevonden dat de overactieve blaasspier een zogenaamd 'disjunction' patroon heeft. Dit patroon wordt gekarakteriseerd door het gebrek aan verankeringseiwitten tussen de spiercellen en een vergrote afstand tussen de cellen die is opgevuld door bindweefselstrengen (collageen). We onderzochten 23 patiënten met detrusoroveractiviteit, verdeeld in vier groepen, te weten: 5 normale, 6 met detrusor overactiviteit door een zenuwaandoening, 6 met detrusoroveractiviteit door belemmering van de blaasuitgang en 6 met detrusoroveractiviteit door onbekende oorzaak. Middels electronen microscopische technieken bevestigden we dat cadherine-11 een integraal onderdeel is van de verankeringseiwitten tussen de spiercellen. In feite belichaamt het gespikkelde patroon van cadherine-11 de adherens junctions zoals eerder aangetoond tijdens onderzoeken naar het 'disjunction' patroon. Cadherin-11 bleek minder voor te komen op spiercellen die verder uit elkaar lagen. Echter, cadherine-11 was zeer ongelijkmatig verdeeld tussen de verschillende spierbundels van de blaasspier. We vonden geen samenhang tussen de hoeveelheid cadherine-11 die voorkwam op de spiercellen en overactiviteit van de blaasspier. Daarom concludeerden we dat het tellen van het aantal adherens junctions of celverankeringen niet gebruikt kan worden als instrument om overactiveit van de blaasspier op te sporen.

In **hoofdstuk 6** onderzochten we of N-cadherine, een ander cadherine subtype, kan worden gebruikt om interstitiële cellen specifiek te markeren. Interstitiële cellen, ook wel myofibroblasten genoemd, spelen waarschijnlijk een grote rol bij het ontstaan van een overactieve blaasspier. Ze komen in het bindweefsel van de blaas voor. Men denkt dat ze een netwerk van cellen vormen die signalen van verscheidene celtypen in de blaaswand integreren. Echter, tot nu toe is er geen goede manier gevonden om deze cellen specifiek aan te kleuren en dus aantoonbaar te maken voor analyse. Cadherines fungeren als signaleringscentra tussen cellen. N-cadherine is het meest voorkomende cadherine in bindweefsel. Zestien blaasbiopten werden onderzocht welke afkomstig waren van patiënten bij wie de blaas in zijn geheel was verwijderd in verband met blaaskanker. N-cadherine werd aangetoond in sterk vertakte cellen die gelokaliseerd waren net onder het slijmlies en in de bindweefsellagen tussen de spierbundels van de blaasspier. N-cadherine bleek niet voor te komen in zenuwen of spiercellen. Middels electronenmicroscopie vonden we myofibroblasten die identiek leken aan de N-cadherine positieve cellen. Echter, er lijken verschillende soorten interstitiële cellen te bestaan in de menselijke blaas. We concludeerden daarom dat N-cadherine gebruikt kan worden om een subtype van interstitiële cellen te markeren in de menselijke blaas.

In **hoofdstuk 7** onderzochten we mogelijke veranderingen van de N-cadherine positieve celpopulatie in de overactieve blaaspier. Zoals eerder vermeld suggereert de autonome hypothese dat detrusor overactiviteit gezien kan worden als een ontsporing van autonome

activiteit van de blaas. Deze autonome activiteit ontspringt in de blaas zelf en verplaatst zich al golvend door de blaaswand. Ontsporing van deze activiteit zou niet alleen het resultaat kunnen zijn van verhoogde electrische koppeling tussen spiercellen (myogene hypothese), maar ook door veranderingen in het netwerk van interstitiële cellen. We onderzochten 23 patiënten met detrusoroveractiviteit, verdeeld in vier groepen, te weten: 5 normale, 6 met detrusoroveractiviteit door een zenuwaandoening, 6 met detrusoroveractiviteit door belemmering van de blaasuitgang en 6 met detrusor overactiviteit door onbekende oorzaak. Het onderzoek toonde aan dat het aantal N-cadherine positieve cellen veranderd leek te zijn in overactieve blaasspieren, zonder onderscheid te maken in de oorzaak van detrusoroveractiviteit. Tevens vonden we dat in spierbundels waar minder zenuwen aanwezig waren, meer N-cadherine positieve cellen leken voor te komen. Zenuwen lopen gedeeltelijk de blaaswand in om contact te maken met de blaasspier. Het verdwijnen van deze zenuwuiteinden is door meerdere onderzoekers beschreven en lijkt een eigenschap te zijn van de overactieve blaasspier, ongeacht de oorzaak. We concludeerden daarom dat de groep van N-cadherine positieve cellen in de menselijke blaas de mogelijkheid heeft om zich te uit te breiden door de blaaswand. Bovendien speculeerden we dat een hoogontwikkeld netwerk van interstitiële cellen het verlies van zenuwen in de overactieve blaasspier vervangt. Als dit nieuwe netwerk makkelijker kan worden geactiveerd, zou dit detrusoroveractiviteit tot gevolg kunnen hebben.

Het relatief nieuwe onderzoeksveld van het overactieve blaas syndroom is uitdagend omdat de blaas veel ingewikkelder in elkaar zit dan we altijd dachten. Er wordt gespeculeerd dat overactiviteit van de blaasspier zou kunnen ontstaan in de blaaspier zelf en niet komt door een probleem in de zenuwaansturing van de blaas. Bij een grote groep patiënten die lijden aan het overactieve blaas syndroom wordt geen duidelijke oorzaak gevonden. Mogelijk dat bij hun de oorzaak in de blaas zelf ligt. Dit is echter zuiver speculatie. Ondanks dat er de laatste tijd veel vooruitgang is geboekt op wetenschappelijk gebied, blijven veel vragen over de werking van de normale en overactieve blaas nog onbeantwoord. Verscheidene hypotheses proberen het overactieve blaas syndroom te verklaren en concluderend kan worden vastgesteld dat de neurogene noch de myogene theorie het ziektebeeld volledig kunnen verklaren. De autonome hypothese is echter veelbelovend en zou het begin van een antwoord op de vele vragen kunnen zijn.

Acknowledgements / Dankwoord

List of abbreviatons

List of publications and abstracts

Curriculum vitae

Acknowledgements / Dankwoord

Dr. J.P.F.A. Heesakkers, beste John, ik weet nog goed dat ik voor het eerst met je in aanraking kwam tijdens mijn co-schappen bij de afdeling Urologie. Ik solliciteerde naar een baan als zaalarts, maar die was reeds vergeven. Ik solliciteerde daarom naar een baan als promovendus, maar….die was ook reeds vergeven. Maar John zou John niet zijn als hij niet nog een projectje zou hebben lopen. Je had contact met een Argentijn die beweerde dat hij met een pilletje de oude mannenkwaal benigne prostaat hypertrofie (oftewel een te grote prostaat) met succes kon behandelen. De geneeskundige kracht zou gebaseerd zijn op de behandeling van littekens na brandwonden. Veel meer kon je er niet over zeggen, maar je had nog wel een stapeltje verklarende artikelen, twee, in het ….Spaans. Ik spreek geen Spaans,…. en jij ook niet. Maar gelukkig, er was wel geld voor onderzoek,…. voor drie maanden. Dat zou dus wel goed komen (…). Na een dappere start bleek je gelukkig grotere plannen voor me te hebben. Je was van plan een onderzoekspijler van de Functionele Urologie binnen het urologisch laboratorium op te zetten. Ik zag het als een plek bemachtigen naast de grote Oncologie jongens. Daarvoor moest er een gedegen plan komen en natuurlijk….geld. Samen met Jack schreven we een aanvraag voor een grant. Met succes! We mochten in Parijs 250.000 dollar ophalen, begeleid door de Gipsy Kings. Kon het beter? Die Urologiewereld beviel me wel… Een goede start en het echte werk kon beginnen. Work in progress meetings, journalclubs, urogolf, je was er altijd bij. Je was laagdrempelig en je deur stond (en staat) altijd voor me open. Ik voelde me altijd door je gesteund en vond het een voorrecht om ‘voor’ je te mogen werken. Ook na mijn carrièreswitch was je er voor een goed gesprek. Mijn dank daarvoor. Ik vind je een fijne kerel. En als je geïnteresseerd bent, weet ik nog wel een mooie snoek te koop.

Prof J.A. Schalken, beste Jack. De jonge professor, het wonderkind. Met de goede ideeën en krachtige visies. De touwtjes in handen en nauwelijks te verrassen. Behalve dan door mooie dames en mijn keuze voor vervoer. Je kwam me regelmatig tegen terwijl ik stond te sleutelen aan mijn BMW E3 uit 1971; je woonde bij me om de hoek. Ik vraag me nog steeds af of de huizenprijzen in onze buurt écht zijn gestegen doordat ik mijn auto verkocht. Bedankt dat je mij de mogelijkheid gaf om promotieonderzoek te verrichten in jouw lab. Ik heb er ontzettend veel geleerd.

Prof P.F.A. Mulders, beste Peter. We hebben slechts één keer echt samen gewerkt. Maar ik wil je bedanken voor de mogelijkheid die je me gaf om onderzoek te doen bij de Urologie aan het RadboudUMC.

Dr. E. Oosterwijk, beste Egbert. De man die het lab runde als Jack er niet was. De harde werker, de turbodiesel. Overal van op de hoogte, met een zeer pienter brein. Mijn dank voor je warme ontvangsten en je wetenschappelijke blik op mijn stukken. Tot ziens in de supermarkt.

Paul Jap en Theo Hafmans. Tja, niet anders dan te noemen in één zucht. Jullie zijn geweldige kerels. Ik wilde electronenmicroscopie. Er werd mij verteld dat er een opmerkelijk koppel binnen de muren van de Radboud Universiteit opereerde die heer en meester waren op dit gebied. Deze heren zouden echter zeer druk bezet zijn en kozen hun projectpartner kritisch uit. Ik voel me daarom nog steeds zeer vereerd dat jullie met mij in zee wilden gaan. Paul, sparren gaat zoveel beter met een glas whiskey, of drie. Maar zullen we de werkoverleggen voortaan in de middag plannen in plaats van 's ochtends? Theo, geen meeting zonder "ach man! We moeten snel effe een biertje samen doen". Ik leek oneindig veel credits bij je te hebben zoals ook weer blijkt uit het feit dat je me op zondagen hielp met de layout van mijn proefschrift. Theo kerel, ge bent unne goeie. Mannen, ik heb me altijd kostelijk vermaakt met jullie. Bedankt daarvoor!

Lieve mensen van het Lab Experimentele Urologie. Maureen, Dorien, Gerald, Kees, Tilly, Mirjam, Sander en Onno. Frank, bedankt voor je begeleiding tijdens mijn landing op het lab. De vele borrels die uitliepen op stapavonden, bier, pizza en dansen. De vele lunchpauzes met slap geouwehoer en schuine moppen. Ik vond het hartstikke gezellig met jullie.

Kjeld van Houwelingen. Ik heb maar kort met je mogen werken in het lab. Maar gelukkig is daarvoor iets veel beters in de plaats gekomen. Dank voor je tips en een luisterend oor in de opstartfase van mijn onderzoek. En nog belangrijker, bedankt voor de motorrit naar Deventer, alwaar ik de moeder van mijn kinderen ontmoette.

Lieve pap en mam. Dank voor jullie steun en strategische interesse in mijn proefschrift. Het was niet makkelijk mijn proefschrift af te ronden door de veranderingen in mijn leven, maar jullie hebben mij hier zeker in gemotiveerd. Ik hou van jullie en ben dan ook blij jullie een derde kleinkind te mogen schenken.

Lieve Sophie. Dank voor je altijd luisterende oor door de jaren heen. Ik hoop dat ik jou de mijne ook altijd heb gegeven. Ik ben er trots op dat je mijn zus bent. Ik kan me geen betere paranimf bedenken.

Beste Evert-Jan. Mijn mannelijke paranimf. Gedraag je!

Max en Roos. Dank voor de warme knuffels bij de voordeur als ik thuis kom. Dank voor de slaperige glimlach bij het naar bed gaan. Dank voor de extra diepgang in mijn leven. Ik zou al mijn motoren verkopen voor jullie.

Lieve Floor. Dank voor je geduld. Niet alleen tijdens de vele avonden, weekenden en vakanties die dit proefschrift opslokte, maar ook voor je geduld met mij. Je bent de enige vrouw die me echt aan het lachen maakt. Ik hou van je.

List of abbreviations

LUTS	Lower Urinary Tract Symptoms
AOB	OverActive Bladder
DO	Detrusor Overactivity
BPE	Benign Prostatic Enlargement
CNS	Central Nervous System
PNS	Peripheral Nervous System
PAG	Peri Aqueductal Gray
PMC	Pontine Micturition Center
IC	Interstitial Cell
ICC	Interstitial Cell of Cajal
M	Muscarinic
TEM	Transmission Electron Microscopy

List of publications and abstracts

Publications

K.A.J.Kuijpers, P.F.A.Mulders
The practice guideline 'Urinary-tract infections' (second revision) from the Dutch College of General Practitioners; a response from the perspective of urology
Nederlands Tijdschrift voor Geneeskunde, 2006 April

K.A.J. Kuijpers, J.P.F.A. Heesakkers
The complexities of diagnosing and treating OAB in men, new patients, new data, new treatment options
European Genito-urinary Disease, 2007; Issue I: 61-63

K.A.J. Kuijpers, J.P.F.A. Heesakkers, C.F.J. Jansen, J.A. Schalken
Cadherin-11 is expressed in detrusor smooth muscle cells and myofibroblasts of normal human bladder
European Urology 2007 Oct; 52(4): 1213-1221

K.A.J. Kuijpers, J.P.F.A. Heesakkers, T.G.M. Hafmans, J.A. Schalken
Adherens junctions and cadherin-11 in normal and overactive human detrusor smooth muscle cells
The Journal of Urology, 2011 May;185(5):1946-195

K.A.J. Kuijpers, J.P.F.A. Heesakkers, T.G.M. Hafmans, C.F.J. Jansen, J.A. Schalken
An update of the interstitial cell compartment in the normal human bladder
BioMed Research International, Volume 2014, Epub 2014 Feb 27

K.A.J. Kuijpers, J.P.F.A. Heesakkers, C.F.J. Jansen, J.A. Schalken
Alterations of the myovesical plexus of the human overactive detrusor
BioMed Research International, Volume 2014, Epub 2014 April 15

Abstracts

K.A.J. Kuijpers, J.P.F.A. Heesakkers, J.A. Schalken
Humane blaas bezit een geïntegreerd netwerk van interstitiële cellen: het substraat voor detrusor overactiviteit?
Voorjaarsvergadering Nederlandse Vereniging van Urologie, Nieuwegein, 2007

K.A.J. Kuijpers, J.P.F.A. Heesakkers, J.A. Schalken
Human bladder possesses a network of N-cadherin positive cells; the substrate for detrusor overactivity?
Annual International Continence Society Congress, Rotterdam, 2006

K.A.J. Kuijpers, J.P.F.A. Heesakkers, J.A. Schalken
Cellular characterization of cadherin within detrusor smooth muscle cells and suburothelial myofibroblasts in human bladder
Annual International Continence Society Congress, New Zealand, 2006

K.A.J. Kuijpers, J.P.F.A. Heesakkers, J.A. Schalken
Cellular characterization of detrusor smooth muscle cadherin
Annual European Association of Urology Congress, Paris, 2006

Rijkhoff N.J.M., **K.A.J. Kuijpers**, J.P.F.A. Heesakkers
Suppression of detrusor contractions using anal stimulation
Annual International Continence Society Congress, Rotterdam, 2006

K.A.J. Kuijpers, J.P.F.A. Heesakkers, J.A. Schalken
Cellulaire karakterisatie van detrusor gladde spiercellen
Najaarsvergadering Nederlands Vereniging Urologie, Utrecht, 2005

K.A.J. Kuijpers, R.J. Krom, M. Stegall
Inhibition of the Gal Alpha 1-3 Gal Xenoreactive Natural Antibodies in pig-to-baboon kidney xenotransplants using a Gal Alpha 1-3 Gal mimeotope
Mayo Clinic Rochester Meeting, Minnesota, USA, 2001

Curriculum vitae

Kamiel Kuijpers was born at 22nd June 1978 in Nijmegen, The Netherlands. In 1996 he obtained his VWO diploma at the Canisius College Mater Dei Nijmegen and was accepted for Medical School at the Radboud University Nijmegen Medical Centre. During a period of two years he participated in race rowing and comedy performances. He is an enthusiastic motorcyclist and spends much of his leisure time restoring classic cars and motorcycles. In 2003 he was a research fellow at the Department of Surgery, Mayo Clinic Rochester, Minnesota, USA and performed research in Pig-to-Baboon Kidney Xenotransplants during a 6 months period. He obtained his medical degree at the Radboud University Nijmegen in 2005 and performed research in the field of translational research concerning the overactive bladder from 2006 untill 2008. In 2006 he received the Astellas European Foundation Prize Fund Award for his research proposal. He was also awarded the prize for second best abstract (non-oncology) at the 21st annual EAU congress, Paris, France. In 2014 he wrote his thesis called ‘Disentanglement of myogenic related structures in the bladder wall of humans with and without overactive bladder syndrome’. Meanwhile, he worked as a Urologist in training for almost two years, but switched to become a Physician in Rehabilitation Medicine. In June 2010 and July 2012 he and his partner Floor became parents of their son Max and daughter Roos Kuijpers. They are now expecting their third child.