Simultaneous Recording of Mechanical and Intracellular Electrical Activity in Urinary Bladder Smooth Muscle

Gelijktijdige registratie van mechanische en intracellulaire elektrische activiteit in glad spierweefsel van de blaas

Proefschrift

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voor mijn ouders

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I

Introduction

In the 1940's, an elegant and exciting new technique was introduced: the penetration of a delicate cell membrane, enabling the monitoring of the electrical activity of living cells. A very fine tip of a thin glass tube filled with a conducting solution, called a microelectrode, was inserted into a cell, connecting the interior of the cell to the outside world. The idea itself originated around 1902, but the technique was not applied successfully until halfway through the 20th century. Following the pioneering work, refinements were made and the method was used in an increasing number of laboratories.

In the beginning, attention was mainly concentrated on larger cells without abundant connective tissue: striated muscle and neurones, but gradually, the technique was also applied to smooth muscle. With every action potential recorded, knowledge about the underlying electrophysiological processes increased, although the progress was slow. Even in 1968, the ionic basis of smooth muscle action potentials was not known, although by then it was suspected to be calcium instead of sodium, as in neurones [46].

The urinary bladder consists of a mucosal layer on the lumen side, a connective tissue layer on the outside and the detrusor, a smooth muscle layer, in-between. It is clinically relevant to unravel the mechanism behind detrusor contraction. Normally, urine produced in the kidneys is stored in the urinary bladder under low pressure conditions. At a convenient moment, the urine is then expelled rapidly at a high pressure, under cholinergic control. In some patients however, the detrusor spontaneously and involuntarily contracts during the storage phase. This may lead to urine loss, and additionally, the rise in pressure may lead to reflux of urine to the kidneys with a possibility of kidney damage. Electrophysiology is an essential tool to uncover the physiological basis of this disorder.

The detrusor muscle has all characteristics of jeopardising successful intracellular recordings: it consists of an integrated network of muscle bundles, orientated randomly. The muscle bundles, in turn, are formed by long, thin cells, varying from 150 to 300 μm length, and 6 to 8 μm in diameter. In addition, the cells are amply embedded in connective tissue. Apart from these features, which are a hindrance for impalement, the cells display constant mechanical activity, increasing the chance of dislodging the electrode from the cell, once it is impaled.

Still, attempts were made and the first, and for a long time also the only article to be published on combined intracellular electrical and mechanical activity of urinary bladder smooth muscle originates from 1961, describing experiments made in rabbit detrusor [72].

Presently, there are still very few publications of microelectrode recordings in intact urinary bladder smooth muscle from any large mammal [10], let alone man. To avoid the inherent difficulties, animal models with less connective tissue, especially guinea-pig, were used. To reduce the chance of dislodging the electrode from a cell during a contraction, the medium in which the recordings were performed, was made hypertonic by adding sucrose to the solution. This inhibits movement of the tissue. Alternatively, another method, the sucrose-gap technique, was used to simultaneously study mechanical and electrical activity of muscle bundles. This method works by the

grace of electrical connectivity of a tissue type. Guinea-pig detrusor smooth muscle is very well electrically coupled, in contrast to human detrusor muscle.

Since functional and behavioural differences between species have been reported [67], the results obtained in animal models cannot unconditionally be extrapolated to the human situation. Consequently, to gain more insight into the physiology of human detrusor contraction, experiments with human tissue are essential. To study human detrusor cells while circumventing the previously mentioned problems, the cells were isolated from their surrounding tissue. In this way, the fundamental electrophysiological properties of detrusor myocytes were investigated [56].

These experiments greatly increased the knowledge of human detrusor cells, but their contribution to a general physiological view of detrusor contraction is limited. From experiments in other tissues it is known that isolated cells may behave intrinsically different, or their properties may have been altered due to the isolation procedure. For this reason, research into the mechanisms of detrusor contraction must be done in strips of bladder muscle, which is the scope of this thesis.

In **Chapter II**, we describe the spontaneous electrical activity recorded in human urinary bladder strips and we compare it to the results obtained in isolated cells by Montgomery & Fry [56]. Furthermore, in an appendix, we describe a method for automatic recognition and feature detection of spike-shaped potentials.

After thus establishing the microelectrode technique, we combined the recordings with simultaneous registrations of the mechanical activity of the strips, in an attempt to investigate the exact sequence of events upon cholinergic activation (**Chapter III**) and the role of the different sources of calcium in the force development (**Chapter IV**).

The results we obtained with intracellular recordings of human urinary bladder smooth muscle cells differed from those published in animal studies. One of the explanations could be that the mechanical activity in our experiments influenced the quality of the recordings. This was observed earlier in rabbit detrusor, where the parameters of action potentials recorded in high sucrose medium [11] differed considerably from those in normal Krebs' solution [72]. Hence, we performed experiments in Krebs' solution made hypertonic with sucrose (**Chapter V**).

Still, even in hypertonic solution, the intracellular electrical recordings we made in human urinary detrusor muscle resembled the ones in normal Krebs' solution more closely than those of other investigators in guinea-pig detrusor under high sucrose conditions. Since the guinea-pig is the most used model to study the mechanism of human bladder contraction, it is important to establish possible functional differences between both species. Accordingly, we simultaneously recorded intracellular electrical and mechanical activity in guinea-pig detrusor smooth muscle strips. Additionally, we studied the effect of sucrose in the medium on the intracellular activity of these guinea-pig detrusor smooth muscle cells (**Chapter VI**).

II

Intracellular recording of spontaneous electrical activity in human urinary bladder smooth muscle strips

Adapted from: Visser and van Mastrigt
Archives of Physiology and Biochemistry, volume 107, no 3: 257-270, 1999

Summary

We studied the spontaneous electrical activity of human urinary bladder smooth muscle strips, with the eventual aim of uncovering the mechanism underlying the clinical syndrome of the unstable bladder. Intracellular microelectrodes were used to record the membrane potentials of detrusor cells in muscle bundles originating from biopsy samples. Spontaneous spike-shaped potential fluctuations were analysed automatically. The membrane potential at the start of the potential change (V_{start}), amplitude, duration at 10% of the amplitude ($d_{10\%}$), and upstroke velocity ($\Delta V/\Delta t$) were estimated and statistically analysed. The mean resting membrane potential of the detrusor strip cells was -42 mV (s.d.: 9 mV). Different types of spontaneous activity could be recorded. On successive impalements within one preparation, the type of activity varied from one single spike-shaped potential to extensive bursts and was not correlated to the resting membrane potential. There was a large variation in size and shape of the spike-shaped potentials between the biopsy samples and even within one cell. The results provide the first description of spontaneous electrical activity in human detrusor smooth muscle strips. This activity is asynchronous, which could aid to maintain a low pressure in the bladder. Most likely, this spontaneous detrusor activity is myogenic in origin.

Introduction

In the micturition cycle, urine produced in the kidneys is temporarily stored in the urinary bladder under low pressure conditions. The spherical shape of the bladder is maintained by constant activity of the muscle cells. This activity has to be asynchronous to prevent high pressures during the storage phase. The necessary asynchrony could arise from a lack of coupling between the cells [5]. The urine is expelled at a convenient moment by synchronous activation of the detrusor smooth muscle cells via the muscarinic pathway. In some patients however, detrusor contractions occur during the storage of urine [review: 60], which may lead to involuntary urine loss. Research into the initiation of detrusor contraction is necessary to uncover the physiological basis of this disorder. Skeletal muscle contraction is preceded by an action potential [55]. This is not necessarily the case in smooth muscle. Human detrusor smooth muscle contraction is predominantly controlled by the muscarinic receptor [40]. Both the M_2 and M_3 muscarinic receptor subtypes are expressed in the urinary bladder [19]. These receptor subtypes are connected to second messenger systems, and can initiate contraction through Ca^{2+} release from intracellular stores without membrane potential changes [19]. It is thus far unknown if human detrusor cells display spontaneous activity and if action potentials are involved in human urinary bladder smooth muscle contraction. It is therefore of interest to demonstrate the presence of action potentials under physiological conditions to gain a better understanding of detrusor contraction.

Most knowledge of detrusor smooth muscle electrophysiology has been gathered in animal preparations: guinea-pig [13; 59], rabbit [72] and pig [67]. Since important differences in functional and contractile behaviour of bladder muscle strips between species have been reported [67], the results of these experiments cannot unconditionally be extrapolated to the human situation. To gain more insight into human detrusor smooth muscle physiology, experiments with human tissue are essential.

Montgomery & Fry [56] described the fundamental electrophysiological properties of isolated single human detrusor cells, using the whole-cell patch clamp technique. Spontaneous action potentials were not normally observed, as in isolated guinea-pig bladder cells [42]. In guinea-pig detrusor strips however, spontaneous action potentials were observed by Creed [13], Mostwin [59] and Bramich & Brading [8]. Therefore, the lack of spontaneous activity in isolated human bladder cells may have been caused by the intrinsically different behaviour of isolated cells, or by altered properties due to the isolation procedure. For this reason, research into the mechanisms of spontaneous detrusor activity must be done in strips of bladder muscle, rather than in isolated cells.

In this paper we report the first electrophysiological recordings of human urinary bladder smooth muscle strips using conventional microelectrodes. Spontaneous fluctuations in the membrane potential, spike-shaped potentials, were recorded and studied. The data were compared with the results Montgomery & Fry [56] obtained in isolated single detrusor cells.

Materials and Methods

Biopsy samples

Detrusor biopsy samples were taken from 22 patients (1 woman; 21 men), ranging in age from 52 to 79 years (mean: 64 years; s.d.: 6 years), who were undergoing surgery for bladder or prostate cancer. Approval from the local ethical committee and written informed consent were acquired. The tissue was collected using either cold-cup biopsy forceps (trans-urethral biopsies) or at open surgery (radical prostatectomy). Immediately after excision, the samples were stored in 0.9% NaCl. Once outside the operating theatre, this solution was replaced by modified Krebs' solution (in mM): 1.8 $CaCl_2$; 1.2 $MgSO_4$; 118 NaCl; 4.7 KCl; 25.0 $NaHCO_3$; 1.2 KH_2PO_4; 11.0 Glucose. The pH was adjusted to 7.4 by aerating the solution with a mixture of 95% O_2 and 5% CO_2. Within ten minutes after excision, the tissue was transported to the laboratory, where the sample was pinned down on Sylgard 184 (Dow Corning, Ithaca, NY, USA) and a muscle bundle was dissected. Connective tissue was removed mechanically.

Electrophysiological recordings

The muscle bundle was pinned down in a 4 ml recording chamber on the stage of an inverted microscope (magnification 40x; Zeiss) and gradually heated to 35°C using a modified thermostat. Recordings were made using borosilicate glass microelectrodes with a flexible tip (GC120F, Clark Electromedical Instruments, Pangbourne Reading, GB). The electrodes were filled with 3 M KCl and had 40-60 MΩ resistance. They were placed in an Ag/AgCl pellet microelectrode holder connected to the input stage of a high impedance capacitance-neutralising amplifier (World Precision Instruments, Sarasota, FL, USA). An Ag/AgCl electrode with an outer diameter of 2.0 mm served as the reference electrode in the organ bath. The cells were speared in the longitudinal direction, to minimise movement artefacts. The signal was amplified 10 times and low pass filtered with a cut-off frequency of 1 kHz (Krohn-Hite Corporation, Avon, MA, USA). It was then AD converted at a sample rate of 2 kHz (DAS1800, Keithley MetraByte, Taunton, MA, USA) using a locally developed sampling program and digitally stored in a PC. In the first few measurements, the sampling rate was 5 kHz, and the signal was not filtered. However, in the course of the experiments, we found that the frequency content of the recorded signals did not warrant this high sampling rate and to reduce computer memory requirements, we subsequently reduced the sampling rate to 2 kHz and started filtering to exclude aliasing errors.

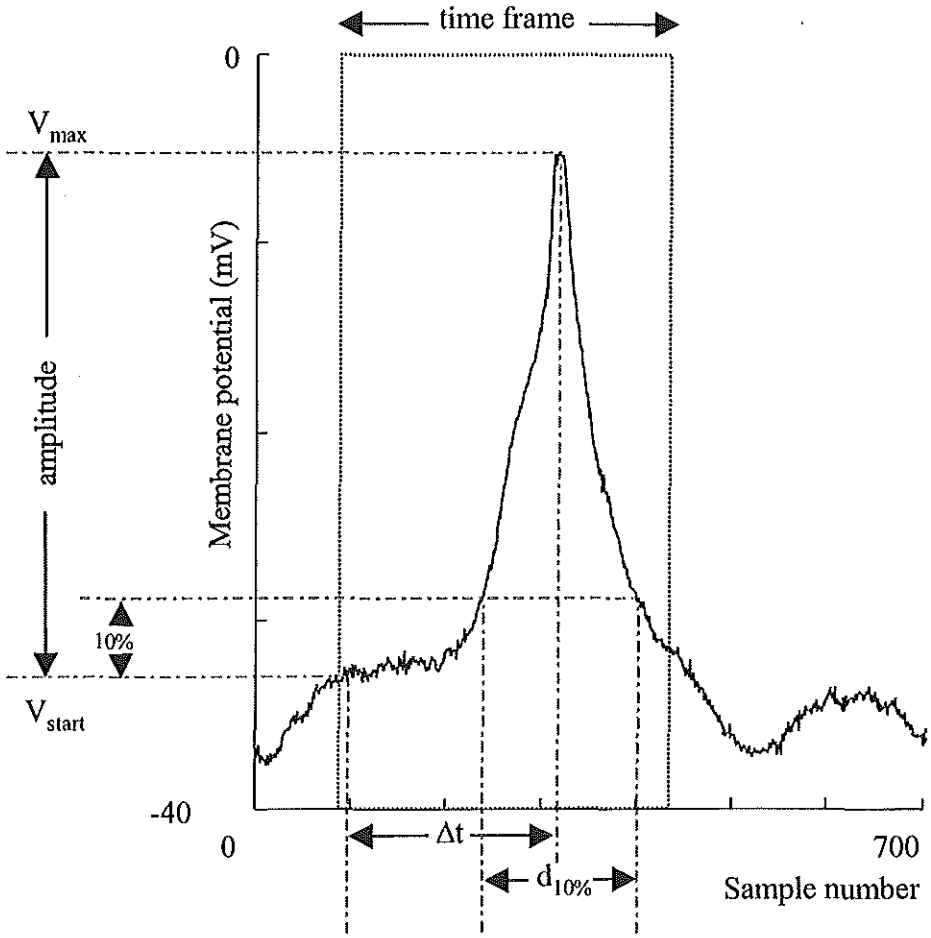

Figure 1. *Spike-shaped potential parameters measured in human urinary bladder strips. Example of a "good" spike-shaped potential. Within a predefined time frame the following parameters were determined automatically: amplitude (V_{max}-V_{start}); Δt (V_{start} to V_{max}); and $d_{10\%}$ (the duration at 10% of the amplitude). The number of samples displayed on the abscissa represent 140 ms.*

Data analysis

The resting membrane potential of the impaled cell, V_{rest}, was estimated visually from the complete recording. The data were analysed using two software programs written in Matlab® 4.2c1 (The Mathworks Inc., Natick, MA, USA), which have been described previously [54]. The first program automatically detected spike-shaped deflections of the membrane potential and displayed these one at a time,

requiring classification as *"bad"*, *"doubtful"* or *"good"*. If the deflection resembled a spike-shaped potential, showing a rapid upstroke, followed by a downstroke, it was categorised as *"good"*. The sample numbers corresponding to the maxima of the *"good"* episodes were stored and used by the second program, which determined in a predefined time frame the membrane potential at the onset of the potential change (V_{start}) and the maximum of the deflection. In the literature, the duration of spike-shaped potentials is usually measured from the first deviation from the baseline to the moment when the resting membrane potential is reached again following downstroke and afterhyperpolarisation. As such a definition is error prone even in slightly noisy data, we calculated the duration of the spike-shaped potentials at 10% ($d_{10\%}$), 25% and 50% of the maximum amplitude. Figure 1 illustrates some of the measured parameters. To determine the upstroke velocity ($\Delta V/\Delta t$) of the potential changes, the amplitude was divided by the time necessary to reach that maximum from V_{start}. The membrane potential values and sample numbers associated with the selected *"good"* signals were imported in Excel 5.0a. Statistical analysis was done using SPSS 7.0 (SPSS Inc., Chicago, IL, USA). The data was not normally distributed, therefore the median and the 95% central range (the range between the 2.5^{th} and 97.5^{th} centiles) are given. This description is preferred for summarising skewed data [1]. Differences between groups of parameters were tested for significance using the Kruskal-Wallis test and the Mann-Whitney U-test. Relationships between the parameters were studied with the Spearman's rank correlation test.

Results

General observations

35 Times we successfully impaled a cell in 13 out of 22 biopsies, sometimes for only a few seconds due to movements of the preparation. In these cases, the recorded membrane potential suddenly shifted to 0 mV. However, in some preparations recordings were possible for over 10 minutes with stable membrane potentials, indicating that movement of the preparation did not affect the quality of the recording, as long as the electrode remained in the cell. Figure 2 shows recordings in biopsy number 1 (Figure 2A) and 2 (Figure 2B) as examples. The V_{rest} of the detrusor

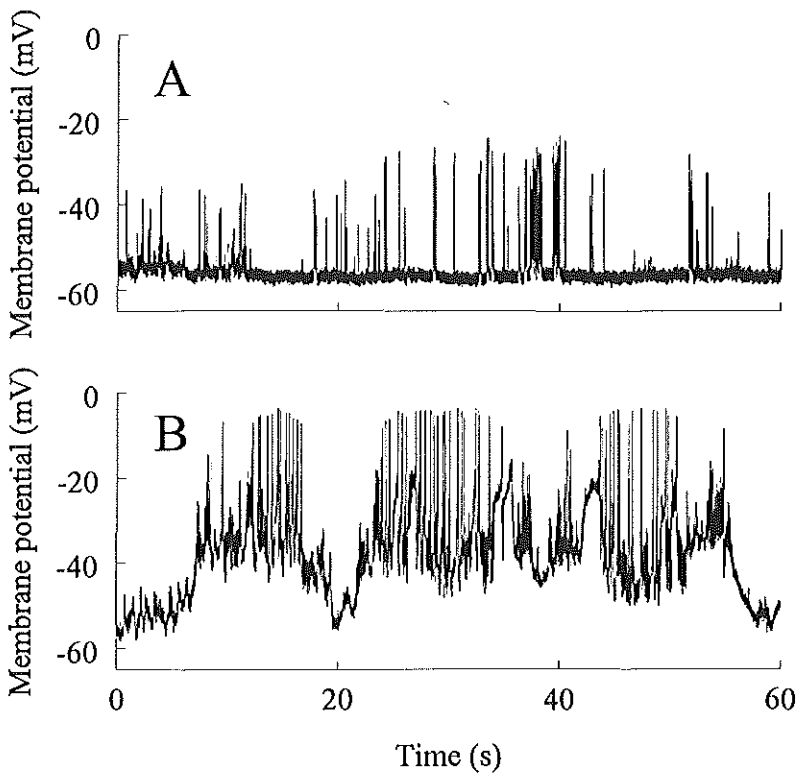

Figure 2. *Spontaneous electrical activity in human detrusor strips. A. Spike-shaped potentials and quiescent periods (biopsy 1). B. Bursts of spike-shaped potentials superimposed on slow depolarising waves (biopsy 2). Traces are at identical scale.*

strip cells ranged from -25 mV to -61 mV (mean: -42 mV; s.d.: 9 mV). V_{rest} was found to be different in different biopsies and when the same biopsy was repeatedly impaled. Spontaneous excursions from V_{rest} were found in 22 recordings in 12 strips, varying from one single spike-shaped potential to extensive bursts. The type of activity varied within and between different detrusor strips and was not correlated to V_{rest} (Kruskal-Wallis test; $p=0.171$). Table 1 gives details of the recordings of the six most active preparations. There was no significant difference between the V_{rest} and activity of muscle bundles originating from trans-urethral biopsies or radical prostatectomy operations (Mann-Whitney U-test; respectively $p=0.257$ and $p>0.999$).

Spike-shaped potential analysis

The properties of the spike-shaped potentials of the 6 most active preparations (Table 1) were statistically analysed. The other 7 preparations did not show enough spike-shaped potentials to be analysed reliably. Time frames containing a spike-shaped potential were displayed automatically one at a time, requiring a

biopsy	recording	time	spikes detected	type of activity
1	1	7:42	1	single spikes
	2	2:50	67	single spikes
	3	1:45	125	single spikes, bursts
	4	0:12	2	single spikes
	5	1:50	27	single spikes
2	1	3:54	0	quiescent
	2	11:53	465	single spikes, bursts on slow waves
3	1	12:30	1316	single spikes, bursts
	2	0:09	0	quiescent
	3	0:26	2	single spikes
4	1	12:18	343	single spikes, exceeding 0 mV
5	1	3:00	0	quiescent
	2	4:42	275	single spikes, bursts on slow waves
	3	5:03	267	single spikes
6	1	0:32	0	quiescent
	2	0:26	154	bursts
	3	4:24	506	bursts
	4	13:22	341	bursts
	5	33:19	295	bursts

Table 1. *Details of the recordings of the six most active human detrusor strips. In each biopsy sample, the type of activity and the number of detected spike-shaped potentials (spikes) are listed for each recording. The duration time is given in [minutes:seconds].*

validation. The overall percentages of potential changes qualified as *"bad"*, *"doubtful"* or *"good"* were respectively 35%, 24% and 41%. The *"good"* fluctuations were considered spike-shaped potentials and characteristic features were determined in a predefined time frame. Figure 1 gives an example of a potential change qualified as *"good"* and the determined parameters. A 10% change in duration of the time frame had only a negligible effect on the parameter values (<1%). Extreme parameter values were examined individually and were excluded from further analysis if caused by incorrect parameter estimation (e.g. a wrong amplitude was calculated). In 82% of the cases, the program determined the parameters of the *"good"* potential changes accurately. All parameters (V_{start}, amplitude, $d_{10\%}$ and upstroke velocity) were significantly different between strips (Kruskal-Wallis test; $p<0.001$) and consequently the preparations were studied separately. The parameters all had a positively skewed distribution. As an example, the distributions of the V_{start}, the amplitude and the duration of the potential changes of biopsy number 3 are shown in Figure 3.

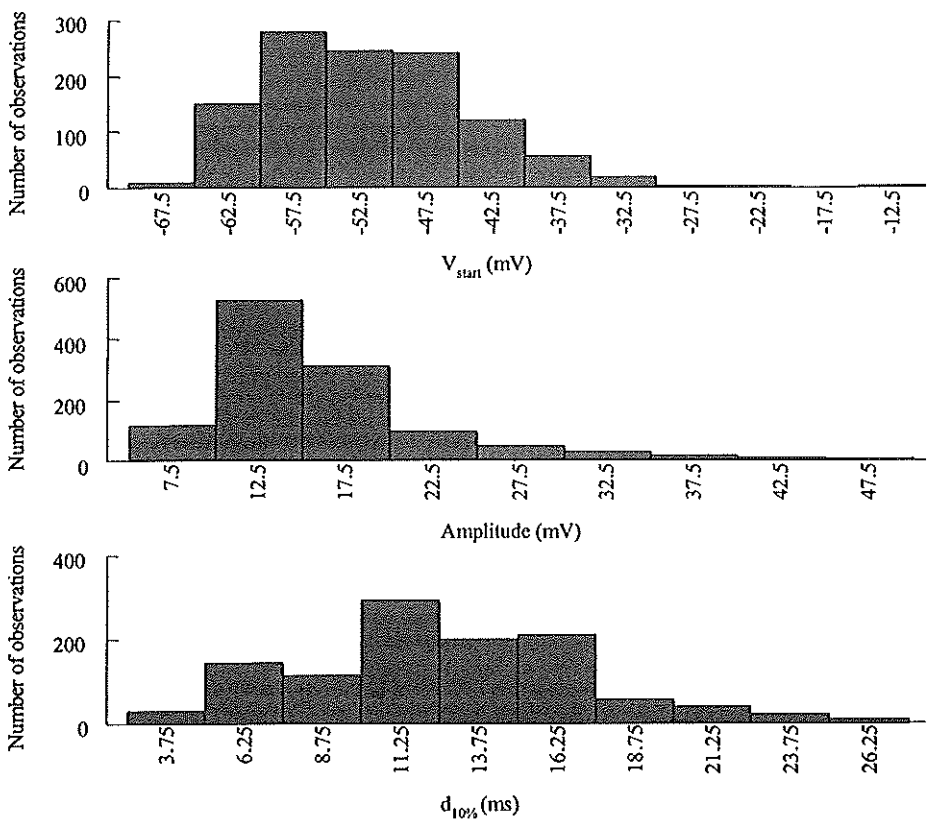

Figure 3. *The skewed distribution of the spike-shaped potential parameters. V_{start}, amplitude and duration ($d_{10\%}$) of the measured spontaneous spike-shaped potentials all had a skewed distribution. As an example, the distributions in biopsy 3 are shown.*

Parameters of spike-shaped potentials

In 4 preparations, the median V_{start} did not differ more than 2.2% from the estimated V_{rest} (Table 2). In two preparations, V_{start} was respectively 8 mV and 18 mV higher than V_{rest}, as in these biopsy samples spike-shaped potentials occurred predominantly in bursts superimposed on slow depolarising waves (see Figure 2B for a typical example). The amplitude of the spike-shaped potentials was not constant, but varied between and within all preparations (see Figure 2). The smallest amplitude detected was 5.9 mV and the largest 76.4 mV. Table 2 gives the median and the 95% central range of all parameter values. In 5 out of 6 preparations, the maximum of the spike-shaped potential approached 0 mV, but it never exceeded this value. In only one preparation did 21% of the recorded potential changes exceed the 0 mV level, featuring overshoots from +0.6 mV to +49.0 mV.

		biopsy sample					
		1	2	3	4	5	6
V_{rest} (mV)	mean	-48	-50	-55	-27	-47	-34
V_{start} (mV)	median	-51	-32	-53	-28	-39	-33
	2.5 centile	-58	-44	-65	-40	-50	-45
	97.5 centile	-25	-21	-36	-21	-23	-22
amplitude (mV)	median	13	22	14	17	13	11
	2.5 centile	7	8	8	9	6	6
	97.5 centile	26	35	32	59	36	22
$d_{10\%}$ (ms)	median	7.6	38.0	12.2	4.4	11.6	6.5
	2.5 centile	3.9	16.9	4.4	6.2	3.8	2.0
	97.5 centile	15.6	61.6	22.8	20.6	36.6	20.0
	n	183	255	1113	247	354	584
$\Delta V/\Delta t$ (V/s)	median	3.5	0.6	2.2	3.9	2.7	8.3
	2.5 centile	1.3	0.001	0.9	0.9	0.3	3.1
	97.5 centile	28.1	2.4	10.1	103.9	23.0	48.7
	n	180	245	101	236	346	583

Table 2. *Features of spike-shaped potentials of cells from human detrusor strips. The mean of the resting membrane potential (V_{rest}), and the median and 95% central range of the membrane potential at the start of the spike-shaped potential (V_{start}), amplitude, duration ($d_{10\%}$) and upstroke velocity ($\Delta V/\Delta t$) of the spike-shaped potentials of the 6 most active preparations. Note that the n for the upstroke velocity is smaller than for the other parameters, due to exclusion of spike-shaped potentials with inaccuracies in the determination of Δt caused by the shape of the fluctuation near V_{start}.*

The duration of the spike-shaped potentials was determined at different percentages of the amplitude. In some cases, this value was not reached at the end of the time frame and the duration could not be calculated at that percentage of the maximum. Only in a small number of spike-shaped potentials the duration could not be analysed at 10% of the amplitude, so that the duration of the spike-shaped potential at this percentage ($d_{10\%}$) was used in this study. The $d_{10\%}$ was variable between and within the different preparations (Kruskal-Wallis test; $p<0.001$). The fastest fluctuation lasted 1.0 ms and the slowest 67.4 ms; these values were recorded in two different preparations. In Table 2, the median and 95% central range are listed.

The upstroke velocity ($\Delta V/\Delta t$) differed significantly between the bladder strip specimens (Kruskal-Wallis test; $p<0.001$); from 8.3 V/s for the fastest to 0.6 V/s for the slowest. Table 2 displays the median and 95% central range. In a few cases, the shape of the fluctuation near V_{start} caused inaccuracies in the determination of the Δt. This was concluded after visual inspection of the extremes. Consequently, these were excluded from further analysis, explaining the smaller number of cases for this feature.

Correlation between the spike-shaped potential parameters

The amplitude of the spike-shaped potential was significantly correlated to V_{start} in 5 biopsy samples; the Spearman's rank correlation coefficients (r_s) for each sample and corresponding p-values are listed in Table 3. In this table it can be seen that although both negative and positive correlations were found between V_{start} and spike potential amplitude, there appeared to be a strong tendency towards a negative correlation (significantly in 4 out of 6 detrusor strips). In biopsy number 4, there was even a very strong, highly significant, negative correlation between V_{start} and amplitude, r_s: -1.0 ($p<0.001$). In preparation 2, which showed predominantly potential fluctuations in a bursting pattern, the Δt was correlated to the amplitude (r_s: 0.35; $p<0.001$). In the other preparations, there was no statistical evidence that these two parameters were correlated. There was a weak correlation between $d_{10\%}$ and amplitude in 5 detrusor strips, with a trend towards a positive correlation ($p<0.05$; see Table 3).

		biopsy sample					
		1	2	3	4	5	6
V_{start} - amplitude	r_s	-0.21	-0.02	-0.09	-1.000	0.18	-0.15
	p	0.004	0.807	0.002	<0.001	0.001	<0.001
amplitude - $d_{10\%}$	r_s	0.20	0.13	0.26	-0.02	0.02	0.32
	p	0.004	0.039	<0.001	0.046	0.776	<0.001

Table 3. *Non-parametric correlation coefficients between spike-shaped potential features of the 6 most active preparations. Spearman's rank correlation coefficient (r_s) with the corresponding p-value for the correlation between the membrane potential at the start of the spike-shaped potential (V_{start}) and the amplitude, and between the amplitude and duration ($d_{10\%}$) of the spike-shaped potential.*

Discussion

Electrophysiological studies in detrusor strips may uncover the physiological basis of bladder disorders, and more specifically, the basis of detrusor instability. We have succeeded in recording membrane potentials in 13 out of 22 detrusor smooth muscle biopsies from 22 patients. As in guinea-pig urinary bladder strips [13; 59], human multicellular preparations showed different types of activity, including abundant spontaneous activity in the form of spike-shaped potentials.

Brading [5] has stated that continuous mechanical activity in the smooth muscle cells is required to maintain the necessary spherical shape. The present study demonstrates such continuous activity, possibly indicating a relationship between electrical and mechanical activity. On successive impalements of cells in one preparation, the type of activity was not constant, showing that asynchronous electrical activity may exist within one muscle bundle. This could be the result of a relatively poor cell-to-cell electrical coupling, necessary to maintain a high compliance of the detrusor muscle in the filling phase [5]. In guinea-pig, there were strong suggestions that a single smooth muscle cell is only coupled to a few very near neighbours [8]. This lack of extensive coupling enables individual cells or groups of cells to maintain a self-controlled length without compromising the overall compliance of the bladder wall. A rise in pressure of the bladder then requires synchronous activation of the smooth muscle cells upon stimulation by the innervating nerves [5].

In our study, spontaneous fluctuations in the membrane potential of the 6 most active preparations were analysed using especially developed recognition and analysis software and characterised using a set of standardised parameters. In terms of these parameters, the 6 preparations were significantly different, implying that there was biological variation in the studied biopsy samples. As in guinea-pig oviduct smooth muscle [62], the type of spontaneous electrical activity was independent of the resting membrane potential. Both parameters were also not correlated to the origin of the muscle sample. During radical prostatectomy operations, a small biopsy was removed from the bladder neck, whereas the trans-urethral biopsies originated from the bladder dome. Although both bladder areas have different functions and express different receptor types (bladder neck: mainly α-adrenergic receptors; bladder dome: mainly β-adrenergic and cholinergic receptors), no electrophysiological differences were found in this study, although we did not block nerve activity. Most probable therefore, the spontaneous activity recorded was myogenic in nature, and not resulting from any nerve activity in the smooth muscle strip preparations. In guinea-pig, other experimenters also have demonstrated that spontaneous activity is myogenic and not affected by atropine or tetrodotoxin which block respectively cholinergic activity and nerve action potential conductivity [7].

In only two preparations, spike-shaped potentials were associated with slow waves of depolarisation, unlike results obtained in rabbit detrusor preparations [72]. In this respect, human detrusor muscle resembles that of the guinea-pig, where no evidence of a true 'slow wave' mechanism underlying the spontaneous activity was found. Rather, it seemed that the cells possessed intrinsic pacemaker activity [7]. As in

the rabbit, the frequency of spike-shaped potentials within one burst of spikes was variable [72]. There was considerable variation in the shape of the spike potentials, consistent with the results obtained in rabbit [16; 72] and guinea-pig [12; 14; 62]. The size and the shape of the spike potentials varied not only between the biopsy samples but also within a single cell. Thus spike-shaped potentials in human detrusor smooth muscle cells, unlike action potentials in nerve cells, are not all-or-none responses and do not always exhibit overshoot. Similar phenomena have been observed in rabbit urinary bladder tissue [73] as well as in other smooth muscle tissue [57; 69]. A possible explanation for the variation in spike potential amplitude may be that the smaller spike potentials are the result of current leakage from firing neighbouring cells. This current leakage could also account for the fact that in general the spike potentials never exceeded the 0 mV level in our multicellular preparations, unlike the evoked action potentials in isolated detrusor cells [56]. This explanation is unlikely however, since the presence of gap junctions has thus far not been demonstrated in human detrusor muscle to substantiate the hypothesis of current leakage. Nevertheless, Garfield & colleagues [31] suggest that it might be wise to suspect that gap junctions are present until proven absent.

Spontaneous potential changes were not normally observed in isolated human detrusor cells [56], in contrast to the present results obtained in multicellular preparations. A similar finding has been described in guinea-pig, where isolated cells displayed no spontaneous electrical activity [42], in contrast to muscle strips [13; 59]. This discrepancy may be the result of intrinsically different behaviour of isolated cells, or the properties of the cells may have been altered as a result of the isolation procedure. Enzymatic treatment has been reported to have an effect on properties of smooth muscle tissue [70]. In this study, as in the guinea-pig detrusor strip experiments, the connective tissue was removed mechanically and therefore the present recordings may be considered to represent physiologically healthy cells.

The mean resting membrane potential of human detrusor smooth muscle strips was similar to the mean described in isolated human detrusor cells: -47.2 mV, (s.d.: 16.7 mV; n=10) [56]. Probably therefore, this suggests that the resting membrane potential is regulated within the cell, rather than being controlled by external factors. This suggestion is supported by the observation that V_{rest} differed between cells on successive impalements within one muscle bundle.

Fry, Montgomery & Gallegos [25] calculated a mean upstroke velocity of 5.4 V/s (s.d.: 2.4 V/s) for isolated human detrusor cells based on the average peak values of the Ca^{2+} current and membrane capacitance of the cells. They also recorded a $\Delta V/\Delta t$ in 10 cells, resulting in a mean of 2.6 V/s (s.d.: 0.7 V/s). In our study, although the $\Delta V/\Delta t$ varied considerably within and between the different strips, the median value of the upstroke velocity of 5 preparations fell in the range of the mean $\pm$ 2 s.d. measured and calculated by Fry & colleagues [25]. Only biopsy 2 fell outside this range.

In the present study, we have reported and analysed the first intracellular recordings of spontaneous spike-shaped potentials in multicellular human detrusor muscle preparations. We have demonstrated for the first time that human detrusor cells in muscle bundle strips display spontaneous activity under physiological conditions.

Although we do not want to use the term action potential for the spike-shaped potentials because of their considerable variation in shape, we assume that the spike-shaped potentials fulfil a similar role as action potentials.

The measurement technique developed in this study enables research into the mechanisms involved in the initiation of detrusor contraction, to uncover the physiological basis of bladder disorders, such as bladder instability. The difference in electrophysiological activity between multicellular and single cell preparations found, corroborates our technique. An additional advantage of this multicellular preparation is that simultaneous recordings of electrophysiological and mechanical activity can be made. The results support the view that the spontaneous detrusor activity is myogenic in origin and asynchronous, which is necessary to maintain a low pressure in the bladder during filling.

Appendix

Automatic recognition and feature detection of spontaneous spike-shaped potentials in membrane potential recordings

The membrane potential recordings presented in this study contain spontaneous fluctuations. As a result of the considerable recording time and sample rate, the measurements comprise a large number of samples. Figure 2B for instance, consists of 300902 samples. Tracing spontaneous spike-shaped potentials in such a recording is a tedious task. We developed Matlab® computer programs to automate this task.

The first of these programs recognises spontaneous spike-shaped potentials by calculating a cross covariance function. Such a function is derived by multiplying

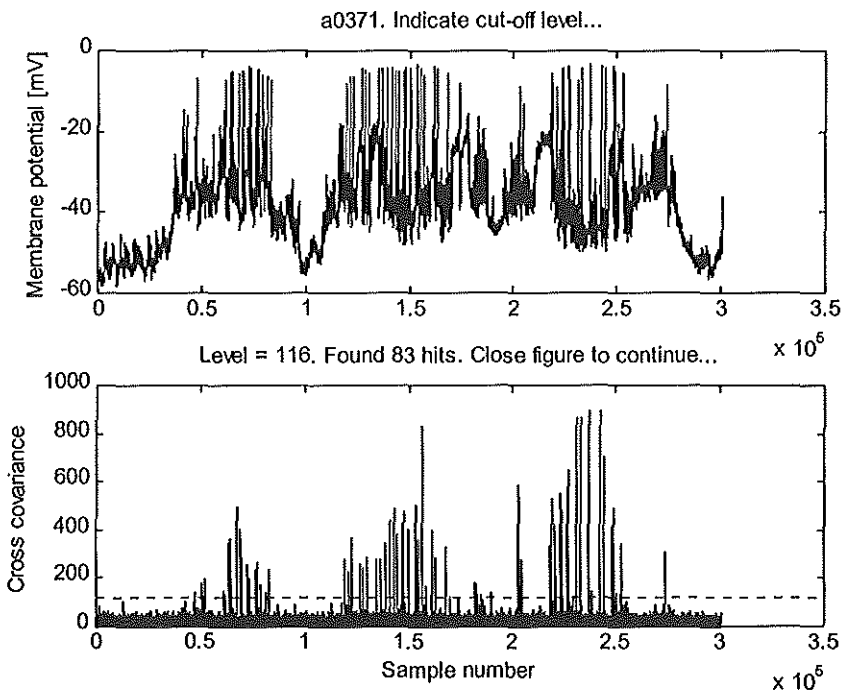

Figure 4. A cross covariance function to detect spike-shaped potentials. Output of the automatic spike-shaped potential detection program. The top panel shows the membrane potential recording from Figure 2B. The lower panel displays the cross covariance, which represents the likelihood that the recording contains a spike-shaped potential at that specific location. The dashed line in the lower panel represents a cut-off level of 116. With this level, 83 possible spike-shaped potentials are found. The number of samples on the abscissa represent 70 s.

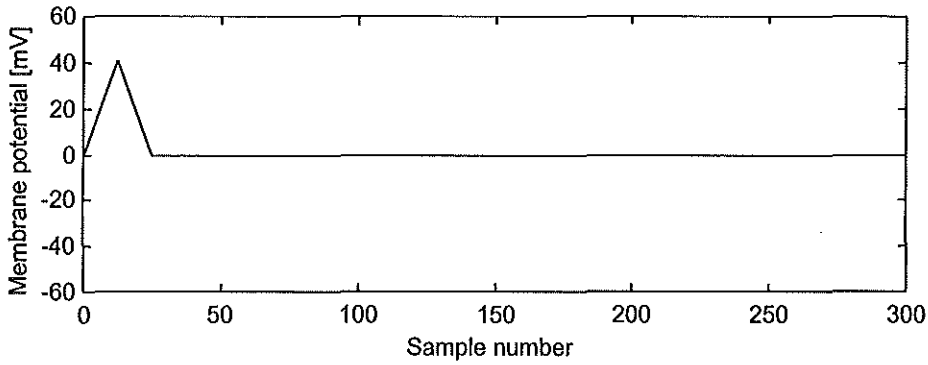

Figure 5. The triangular test signal used for the detection of spike-shaped potentials. This test signal was used to calculate the cross covariance function displayed in Figure 4. Notice that Figure 4 and Figure 5 are not at the same time scale.

the recording with a test signal, in this case an example of an idealised spike-shaped potential, and integrating the product. The resulting number represents the agreement between both signals. By shifting the test signal sample by sample along the recording and repeating the multiplication and integration process, a cross covariance function is obtained. This function represents the likelihood that the recording contains a spike-shaped potential at that specific location. Figure 4 gives an example. The top panel shows the membrane potential recording displayed in Figure 2B, the lower panel the cross covariance of this recording with a triangular test signal. The test signal, illustrated in Figure 5, represents the basic features of an spike-shaped potential, in the sense that it has an ascending and a descending flank, and has a certain duration. Notice that Figure 4 and Figure 5 are not at the same time scale. The program allows a cut-off level to be applied to the calculated cross covariance function using a mouse device. Sample numbers at which the cut-off level is superseded are stored. In the example shown in Figure 4, 83 such sample numbers were found, when the cut-off level was set at 116. Next, a small episode of recording, around each sample number found, is displayed. Figure 6 shows an example of such a presentation of the 9^{th} episode out of the 83 found. The vertical line represents the maximum of the spike-shaped potential. As this maximum is sometimes misplaced, it can be corrected using a mouse cursor. The spike-shaped potential is validated as *"bad"*, *"doubtful"* or *"good"* by clicking a button. When all episodes have been validated in turn (it is possible to revalidate previous episodes by clicking an extra button) the sample numbers corresponding to the maxima of the spike-shaped potentials are stored in a file, together with the validation.

The second computer program reads the stored file with sample numbers, retrieves the spike-shaped potentials which were validated *"good"* from the recording and calculates a number of parameters from each of these. In a predefined time frame, the minimum of the signal before the detected maximum is designated as the membrane potential at the onset of the spike-shaped potential, V_{start}. To reduce noise, the lowest-sample-but-two and not the absolute minimum is taken. The amplitude of

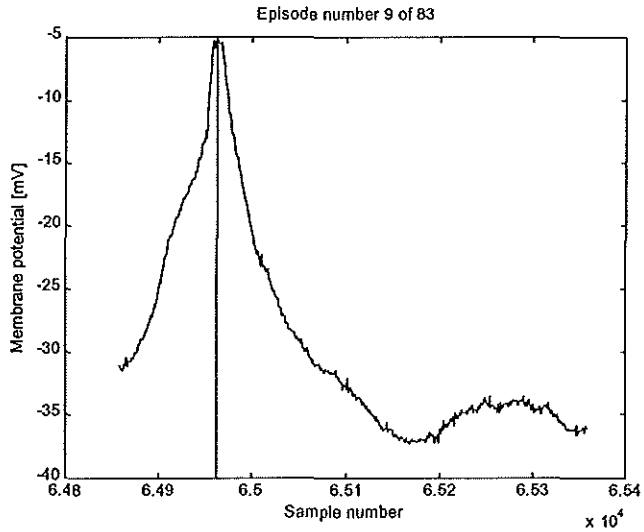

Figure 6. *Example of a "good" spike-shaped potential found with the cross covariance function. Output of the automatic spike-shaped potential detection program. The 9th episode out of the 83 found in Figure 4 by applying a cut-off level of 116 to the cross covariance function calculated with the test signal shown in Figure 5. The vertical line indicates the maximum of the spike-shaped potential. The number of samples on the abscissa represent 120 ms.*

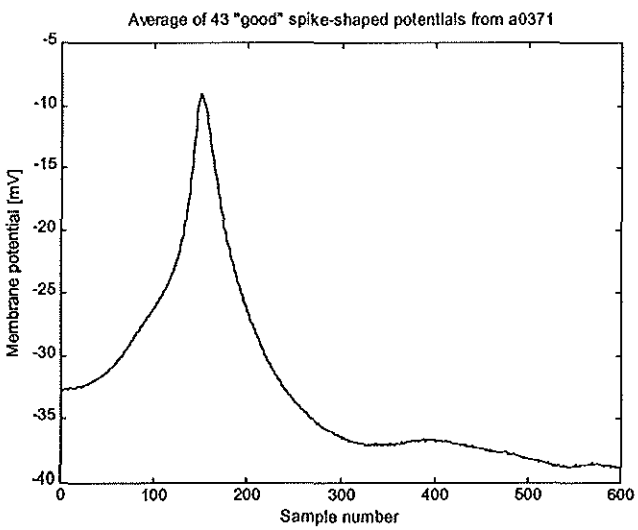

Figure 7. *Average of 43 "good" spike-shaped potentials. 43 from the 83 spike-shaped potentials found in the trace shown in Figure 4, were classified as "good". The maxima of the spike-shaped potentials were aligned and the program averaged the signals. The number of samples on the abscissa represent 120 ms.*

the spike-shaped potential is calculated by subtracting this value from the maximum. Next, a number of relative levels is applied. The two sample numbers before and after the maximum at which each level is first crossed are recorded as a measure of spike-shaped potential duration. These parameters, illustrated in Figure 1, are stored in a file. Averages and standard deviations of the signal values in the time frame around the maximum are stored in a separate file. This file therefore contains the average spike-shaped potential validated as *"good"* in the measurement. Figure 7 shows an example of the average of 43 *"good"* spike-shaped potentials from Figure 4.

A number of factors influence the automatic recognition process, most important of these are the test signal, and the cut-off level applied to the cross covariance. The test signal is independent of the software developed, and different shapes are easily generated. A recording of a *"true"* spike-shaped potential can also be used for a test signal. We experimented with using the average of a number of *"good"* spike-shaped potentials, as calculated by the averaging program, as a test signal, but found that this did not significantly increase the number of good spike-shaped potentials. Using a triangular test signal as in Figure 5, the width of the basis of the triangle and its height can be varied. In Figure 5 the width of the triangle is 20 samples, which at the used sample rate of 5 kHz (in this measurement) is equivalent to 4 ms. This compares to the width of the highest 10 mV of the spike-shaped potential shown in Figure 6. A considerably wider test signal, of 200 samples (40 ms) which compares to the basis of the spike-shaped potential yielded more artefacts and less *"good"* action potentials. Varying the height of the triangular test signal did only influence the amplitude of the cross covariance function, but not its shape, so that the same population of spike-shaped potentials and artefacts could be detected.

Figure 8 illustrates the influence of varying the cut-off level in the analysis of the measurement of Figure 4. The triangles represent the total number of episodes detected, which decreases with the cut-off level. The open squares represent the number of *"bad"* spike-shaped potentials, the closed squares the number of *"bad"* and *"doubtful"* spike-shaped potentials. In this example, above a level of 200 only *"good"* spike-shaped potentials were detected, between 100 and 200 the number of *"good"* spike-shaped potentials increased more than the number of *"bad"* and *"doubtful"* potentials, and below 100 almost exclusively *"bad"* spike-shaped potentials (i.e. artefacts) were found. In our analysis we used very low cut-off levels, to make sure that we did not miss many *"good"* spike-shaped potentials, but a possible future consideration might be to use a high level so that manual validation of the detected spike-shaped potentials is unnecessary. In the recording analysed in Figure 8 almost half of the *"good"* spike-shaped potentials will then be missed, but this might be unimportant, as long as a sufficient number of *"good"* potentials is found.

We originally used the standard Matlab® xcov function for calculation of the cross covariance function. This function however is not particularly efficient in the present application where the test signal is very short in relation to the studied measurement. Calculation of the cross covariance of a signal consisting of 10000 samples took 233 seconds on a Pentium 133 MHz PC. As it may be expected that the calculation duration increases quadratically with the number of samples, the example shown in Figure 4 would take approximately 19 hours. Using a self-written routine,

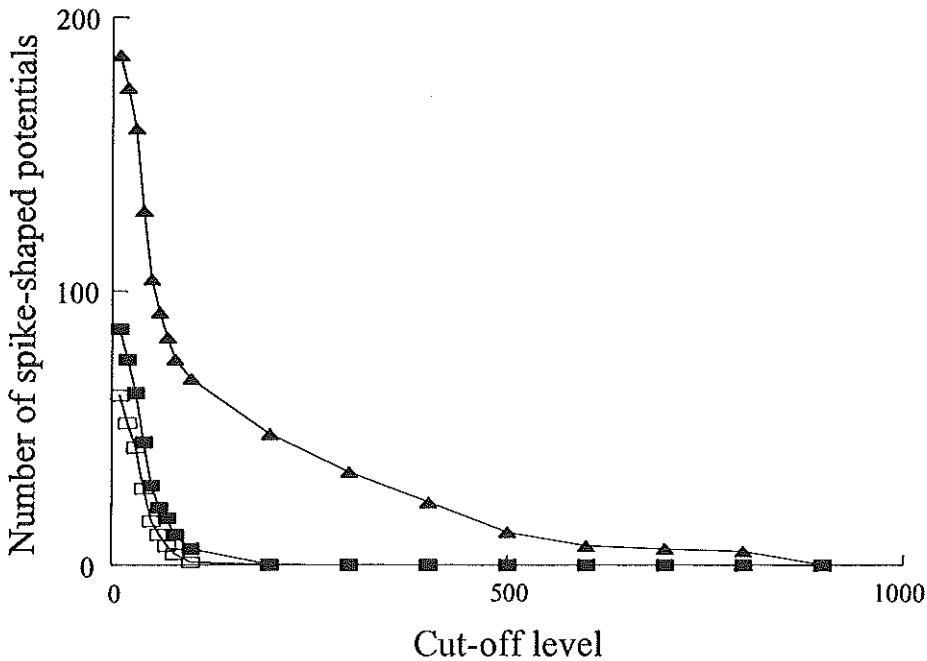

Figure 8. *Influence of the cut-off level applied to the cross covariance function on the number of spike-shaped potentials found. The open squares represent the number of "bad" spike-shaped potentials, the closed squares the number of "bad" and "doubtful" spike-shaped potentials, and the triangles the total number of spike-shaped potentials, i.e. "bad", "doubtful" and "good", found in the measurement from Figure 4, as a function of the cut-off level.*

which only multiplies and integrates the nonzero part of the test signal, this took 183 seconds. The main program described contains a graphical file preselection part to further decrease processing time by cutting large measurements in separate sections. The software runs in Matlab® 5.1 and is available from the authors.

III

Simultaneous recording of mechanical and intracellular electrical activity in human urinary bladder smooth muscle

Adapted from: Visser and van Mastrigt
the British Journal of Urology International, in press

Summary

To elucidate the role of the membrane potential in human detrusor smooth muscle contraction, we simultaneously recorded mechanical and intracellular electrical activity in muscle strips. To induce a contraction upon muscarinic receptor stimulation, the agonists acetylcholine and carbachol were applied. To block the response, atropine was added to the bath. The Ca^{2+}, necessary for activation of the contractile machinery, can be recruited via two pathways: release from intracellular stores or influx from the extracellular matrix. High potassium was applied to induce Ca^{2+} influx through voltage sensitive Ca^{2+} channels. There were significant changes in the force level upon agonist, antagonist and high potassium administration. However, significant changes in membrane potential occurred only when KCl was applied to the bath, not upon muscarinic agonist or antagonist application. Activity in the form of spike-shaped potentials never changed significantly upon application of any substance. The present results indicate that the Ca^{2+}, mobilised upon M_3 receptor stimulation, originates primarily from intracellular stores without systematic changes in membrane potential. Atropine only caused a relaxation in muscle strips previously contracted by M_3 receptor agonist stimulation. It had no effect on relaxed muscle strips.

Introduction

The urinary bladder stores urine produced in the kidneys and expels it at a convenient moment under control of the parasympathetic nervous system [23; 40]. In healthy human detrusor the neuromuscular transmission is predominantly cholinergic in nature [67], although a partially atropine-resistant response is found occasionally [68]. The transmitter release causes an increase in free cytoplasmic Ca^{2+}, activating the contractile machinery of the detrusor smooth muscle cells. There are two possible pathways for this rise in free cytoplasmic Ca^{2+} (Figure 1): influx of Ca^{2+} from the extracellular matrix through voltage sensitive L-type Ca^{2+} channels, or release from intracellular stores [26]. The human detrusor expresses the muscarinic M_3 receptor, which is coupled to a second messenger system. Stimulation of the M_3 receptor dissociates among others a G protein which mobilises inositol (1,4,5)-trisphosphate (IP_3) [37], in turn releasing Ca^{2+} from the sarcoplasmic reticulum (SR) [19]. An increased intracellular Ca^{2+} concentration in itself can also influence the release of

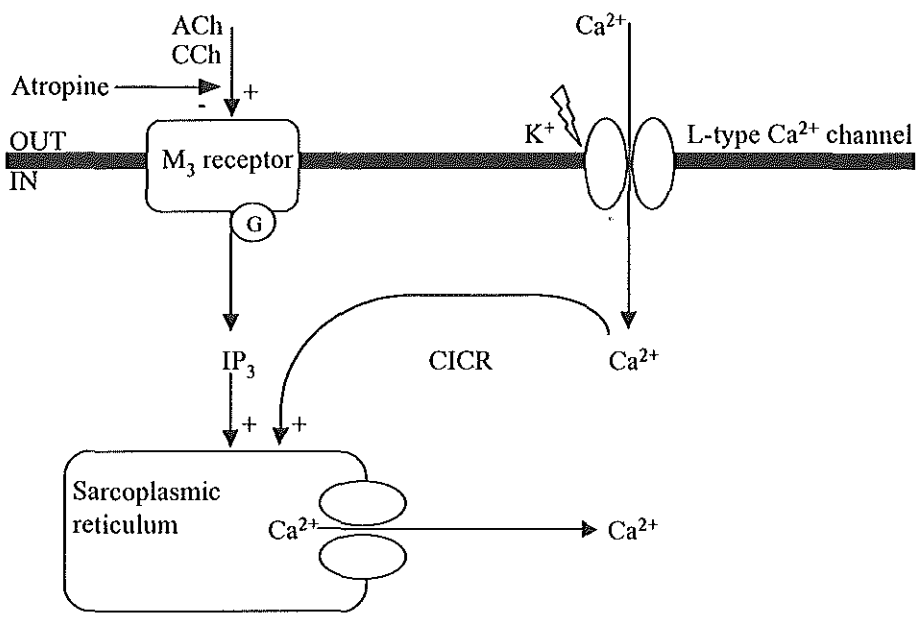

Figure 1. *A schematic representation of the two possible pathways to elevate the intracellular Ca^{2+} concentration in human urinary bladder smooth muscle. ACh and CCh stimulate the muscarinic M_3 receptor, triggering a second messenger system (G-protein (G) and inositol (1,4,5)-trisphosphate (IP_3)) that causes the release of Ca^{2+} from the sarcoplasmic reticulum (SR). Atropine blocks this response. Ca^{2+} influx through voltage sensitive Ca^{2+} channels can induce Ca^{2+} induced Ca^{2+} release (CICR) from the intracellular stores.*

Ca^{2+} (Ca^{2+} induced Ca^{2+} release, CICR) [30]. Thus far, the exact sequence of events upon cholinergic activation is unclear. Observations in isolated guinea-pig detrusor cells suggest a crucial role for the extracellular Ca^{2+} influx in determining the extent of the rise of intracellular Ca^{2+} [29]. Intracellular recordings in guinea-pig detrusor strips however, have shown that muscarinic stimulation can produce contractions with little change in membrane potential [27]. These results have been supported by experiments in isolated guinea-pig detrusor myocytes [26].

Studies in human urinary bladder have indicated that the passive force, spontaneous mechanical activity and contractions induced by muscarinic agonists and direct electrical stimulation are dependent on extracellular Ca^{2+} [3], implying Ca^{2+} channel activity. However, contractions have been induced by agonists in the absence of extracellular Ca^{2+} [49], suggesting that muscarinic agonists primarily use the second messenger route. Evidence for a combined activation of both pathways upon cholinergic innervation has also been found [23; 51].

The role of the membrane potential in human detrusor muscle contraction is thus presently unclear. The cholinergic mode of activation does not require electrical activity to generate tension but a role for electrophysiological phenomena in the contractile function of the human bladder cannot be excluded [26]. There are very few published records of electrical activity recorded from intact urinary bladder smooth muscle of any large mammal [10], including human [75]. This is probably caused by the fact that the detrusor muscle cells display continuous mechanical activity, and possess an extensive extracellular matrix containing collagen and elastin [85], which makes it exceptionally difficult to impale these cells.

Most information about human detrusor electrical activity has been obtained from single cells, using patch electrodes under current clamp conditions [56]. Spontaneous action potentials were not normally observed. This lack of spontaneous activity may have been caused by intrinsically different behaviour of isolated cells, or by altered properties due to the isolation procedure. Intracellular recordings in human detrusor strips [75] showed spontaneous activity, varying from single spike-shaped potentials to extensive bursts.

More insight in the role of the membrane potential could lead to a method to therapeutically and selectively suppress unstable detrusor contractions. In the present study, we simultaneously recorded mechanical and intracellular electrical activity in human urinary bladder strips. To induce contractions mediated by the M_3 receptor, we have applied both acetylcholine (ACh) and carbachol (CCh), as a possible difference in activation has been suggested [23]. Atropine was used to block the response. High potassium stimulation was used to induce Ca^{2+} influx through L-type Ca^{2+} channels.

Materials and Methods

Biopsy samples

Detrusor biopsy samples were collected from 11 patients (1 woman; 10 men) ranging in age from 44 to 81 years (mean: 64 years; s.d.: 11 years) with approval from the local ethical committee and informed consent. The patients were undergoing surgery for bladder or prostate cancer and the tissue was collected using either cold-cup biopsy forceps (trans-urethral biopsies) or at open surgery (radical prostatectomy). Immediately after excision, the samples were stored in 0.9% NaCl. Outside of the operating theatre, this solution was replaced by modified Krebs' solution (in mM): 1.8 $CaCl_2$; 1.2 $MgSO_4$; 118 NaCl; 4.7 KCl; 25.0 $NaHC\,O_3$; 1.2 KH_2PO_4; 11.0 Glucose. By aerating the solution with a mixture of 95% O_2 and 5% CO_2, the pH was adjusted to 7.4. The tissue was transported to the laboratory within ten minutes after excision, pinned down on Sylgard 184 (Dow Corning, Ithaca, NY, USA) and a muscle bundle of about 2 mm long and 0.2 mm wide was dissected. Three of the biopsy samples were stored at 4°C overnight before a muscle bundle was dissected. Connective tissue was removed mechanically.

Tension recordings

One side of the muscle bundle was pinned down in a 4 ml recording chamber with a Sylgard 184 bottom on the stage of an inverted microscope (magnification 40x; Zeiss) which was gradually heated to 35°C using a modified thermostat. The free part of the muscle bundle was connected to the forceps of a force transducer (BAM4C, Scientific Instruments, Heidelberg, Germany). The muscle bundle was stretched minimally to enable microelectrode recordings.

Electrophysiological recordings

Recordings were made using borosilicate glass microelectrodes with a flexible tip (GC120F, Clark Electromedical Instruments, Pangbourne Reading, GB). Filled with 3 M KCl, the electrodes had 40-60 MΩ resistance. They were placed in an Ag/AgCl pellet microelectrode holder connected to the input stage of a high impedance capacitance-neutralising amplifier (World Precision Instruments, Sarasota, FL, USA). An Ag/AgCl electrode (outer diameter of 2.0 mm) in an agar bridge served as the reference electrode in the organ bath. The tip potential of the microelectrode was adjusted to zero in the bath, before impaling a cell. At the end of the recording, when the tip of the electrode was no longer inside a cell, the value of the tip potential varied, if at all, less than 5 mV from the zero level. To minimise movement artefacts, the cells were speared in the longitudinal direction, at the side where the muscle

bundle was pinned to the Sylgard.

Penetration of a cell was considered successful when the reference potential rapidly shifted to a more negative value of at least -20 mV. The electrophysiological signal was amplified 10 times and low pass filtered with a cut-off frequency of 1 kHz (Krohn-Hite Corporation, Avon, MA, USA). Both this signal and the signal from the force transducer were then AD converted at a sample rate of 2 kHz (DAS1800, Keithley MetraByte, Taunton, MA, USA) using a locally developed sampling program and digitally stored in a PC. The digitised force signal was filtered with a 4^{th} order Butterworth filter, using a cut-off frequency of 2 Hz, to reduce the noise level. The shape of the signal was not affected by the filtering process.

Force induction

To induce force, three different stimuli were used: the application of ACh (Sigma), CCh (Pharmachemie B.V., the Netherlands) and KCl. Atropine (Pharmachemie B.V., the Netherlands) was used to block the muscarinic receptor. CCh was dissolved in physiological salt solution, all other pharmaca were dissolved in Krebs' solution. Stock solutions of ACh ($2.0 \cdot 10^{-2}$ M), CCh ($1.4 \cdot 10^{-3}$ M), atropine ($1.4 \cdot 10^{-3}$ M) and KCl (3.0 M) were applied in random order to the bath in units of 0.01 ml, resulting in minimal bath concentrations of $5.0 \cdot 10^{-5}$ M ACh, $3.4 \cdot 10^{-6}$ M CCh and $3.6 \cdot 10^{-6}$ M atropine. These concentrations are considered supramaximal [48; 64; 68]. To induce depolarisation of the membrane, a final KCl concentration of minimally $12.2 \cdot 10^{-3}$ M was used.

The bathing solution was refreshed constantly at $1.4 \cdot 10^{-2}$ ml/s, and the bath solution was renewed within 5 minutes. However, difficulties with making long intracellular recordings were inherent to the type of preparation, therefore substances were often applied before total washout of the previous application. In those cases, the effective concentration was calculated using a standard concentration distribution formula. In the figure legends the final concentrations of the substances in the bath are given. When Spearman's rank correlation test was performed, there were no significant correlations between the concentration of a substance and the resulting force, membrane potential (V_m) or spike potential frequency ($p \geq 0.074$), so the data were pooled. Krebs' solution without additions was used as a control for the application method.

Data analysis

The average resting membrane potential of the impaled cell, V_{rest}, was calculated from the average V_{rest} during 30 s before and during 30 s immediately after the stimulus. Resting membrane potential values more negative than -100 mV were sometimes recorded. These results were considered to be an artefact, most likely resulting from bending of the electrode and were therefore excluded from further analysis. The spike potential frequency was determined as the number of events per

second (spikes/s) in the same periods as the average resting membrane potential was determined, using especially developed software written in Matlab® 4.2c1 (The Mathworks Inc., Natick, MA, USA), as described previously [75].

Force values were read at the minimum level immediately before the stimulus and at the maximum after it. The calculated parameters were imported in Excel 5.0a and statistical analysis was done using SPSS 7.0 (SPSS Inc., Chicago, IL, USA). As values were not normally distributed, results were represented with median values and interquartile ranges. Relationships between the parameters were studied with the Spearman's rank correlation test. The goal of our study was to correlate the occurrence of force development and membrane potential activity and we therefore did not attempt to normalise force development. However, we performed the Spearman's rank correlation test on three biopsies, in which we were able to record from several cells for a longer period of time, enabling us to apply the stimuli several times (≥5 times of the same stimulus) Each biopsy was tested separately and there were no significant correlations between membrane potential, spike-shaped potential frequency and force ($p \geq 0.107$). To compare parameters before and after drug application, Wilcoxon's signed ranks test was used.

Results

Force

The median value of the passive force exerted by the human detrusor strips at the start of the experiment was 270 μN, (interquartile range: 420 μN; n=11). This passive force was to a large extent dependent on the amount of initial stretch placed on the bundle at the beginning of the experiment. Application of the muscarinic receptor agonists ACh and CCh resulted in a significant increase in force (p=0.002 and p=0.004, respectively; Figure 2), while the antagonist atropine resulted in a relaxation of the muscle strip (p=0.003; Figure 2). This relaxation only occurred if the tissue had contracted previously upon agonist stimulation and had not yet fully relaxed. If no agonist had been applied, there was no relaxation of the tissue upon antagonist application. There was no significant change in force when ACh (p=0.139) or CCh (p=0.203) were applied to the bath while atropine was present (Figure 2). KCl increased the force significantly (p<0.001; Figure 2), while the application of Krebs' solution to the bath did not have any effect (p>0.999; Figure 2).

Membrane potential and spike potential frequency

The median value of the resting membrane potential of the detrusor muscle cells upon impalement and without drugs in the recording chamber, either at the beginning of the experiment (n=10) or after a washout period of ≥ 60 minutes (n=4), was -45 mV (interquartile range: 20 mV; n_{total}=14). Any spontaneous excursion from the resting membrane potential was considered as membrane potential activity. The majority of the impaled cells displayed spontaneous activity in the form of spike-shaped potentials (Figure 3, *) varying from single spikes to extensive bursts. The type of activity varied within and between different detrusor strips. There was a large variation in size and shape of the spike-shaped potentials, between the biopsy samples and even within one cell. Figure 3 gives an example of spontaneous activity recorded. Figure 3B shows parts of the recording in Figure 3A at a faster time scale. Apart from spike-shaped potentials, fast, small depolarisations (Figure 3, +) and long lasting depolarisations (Figure 3, #) were observed.

The response to the applied substances varied greatly, and Figures 4 and 5 show examples. In Figure 4, ACh was applied to the bath, resulting in an increase in force (upper trace). The V_m (lower trace) was quiet and upon ACh application, there was no change in activity, although there was a small hyperpolarisation. Figure 5 shows an example of CCh application. There was a brief increase in membrane potential activity in the form of fast, small depolarisations after CCh application prior to an increase in force. Eventually the V_m quietened again, before the force reached its peak. Figure 6 shows an example of a change in spike-shaped potential activity after atropine application. Changes in membrane potential activity occurred in 11% of the applications of CCh and 26% of the ACh applications. The differences in V_m

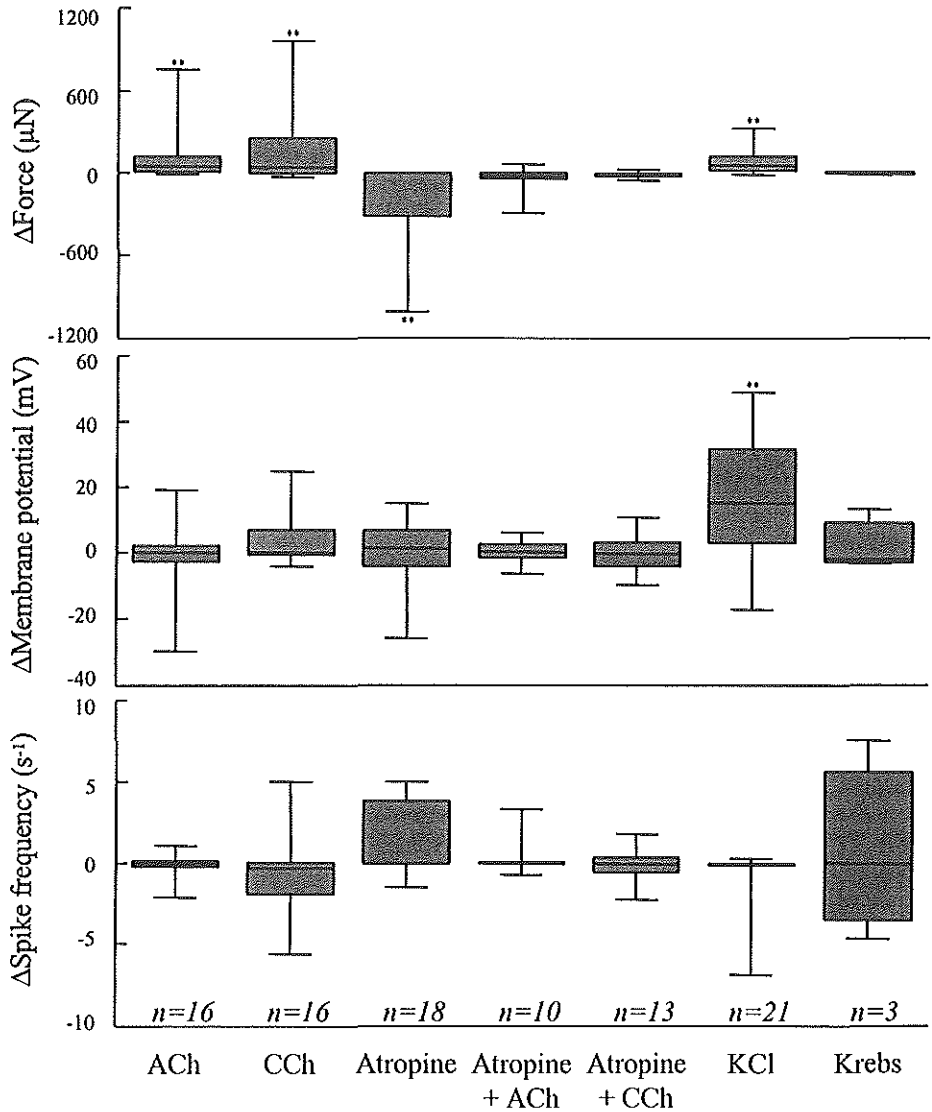

Figure 2. *The difference between parameter values before and after substance application represented in a boxplot. The values at 2½, 25, 50 (median), 75 and 97½ cumulative relative frequencies (centiles) are given. The numbers of events are displayed at the bottom of the graph. ACh, CCh and KCl resulted in a significant increase in force. Atropine caused a relaxation of muscle tissue previously contracted by muscarinic receptor stimulation. Wilcoxon's signed ranks test was used to analyse the data; ** indicates that p≤0.005; exact p-values are given in the text. The range of concentrations (in M) was: ACh: $5.0 \cdot 10^{-5}$ - $1.1 \cdot 10^{-4}$; CCh: $3.4 \cdot 10^{-6}$ - $1.7 \cdot 10^{-5}$; Atropine: $3.6 \cdot 10^{-6}$ - $2.9 \cdot 10^{-5}$; KCl: $12.2 \cdot 10^{-3}$ - $4.2 \cdot 10^{-2}$.*

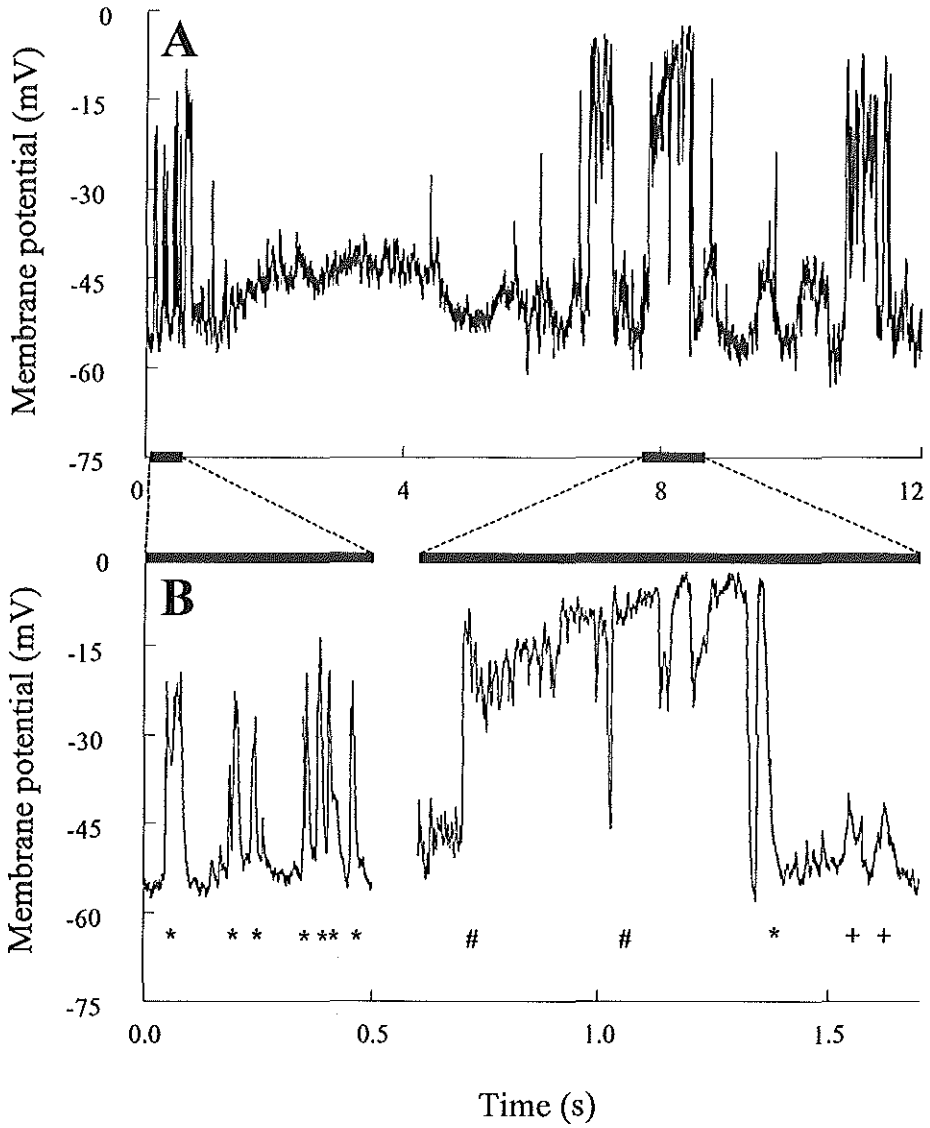

Figure 3. A typical pattern of spontaneous activity in human detrusor muscle strips consisting of spontaneous spike-shaped potentials and quiescent periods (A.). Two periods indicated by bars on the X-axis are shown at a faster time scale (B.).
** indicates spike-shaped potentials; # indicates long lasting depolarisations;*
+ indicates fast, small depolarisations.

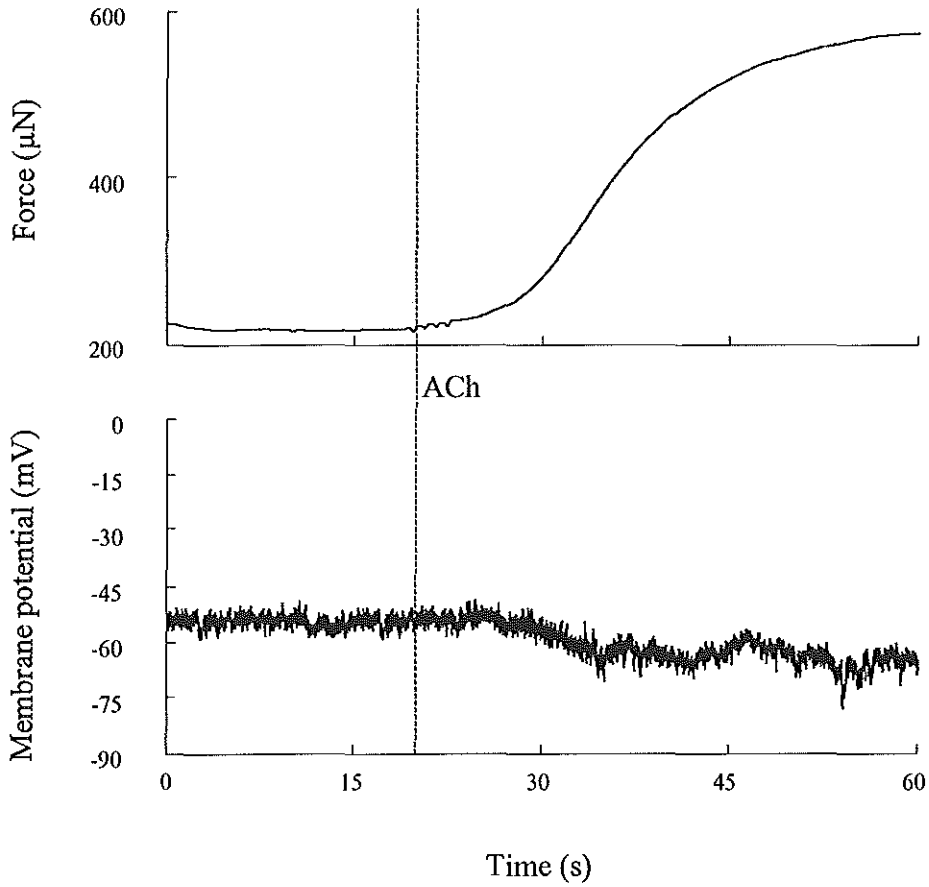

Figure 4. *A typical example of the response of human urinary bladder muscle to ACh. Upper trace: force; lower trace: membrane potential. There was a slight hyperpolarisation and no change in membrane potential activity upon stimulation with ACh ($2.6 \cdot 10^{-4}$ M).*

(p=0.959; p=0.148; p=0.320) or spike potential frequency (p=0.133; p=0.281; p=0.079) before and after application of ACh, CCh and atropine, respectively, were not significant (Figure 2). Likewise, when ACh or CCh were applied to the bath in the presence of atropine, there were no significant changes in V_m ($p \geq 0.683$; Figure 2), or spike frequency ($p \geq 0.600$; Figure 2). A significant change in V_m could only be measured when the K^+ concentration was raised (p=0.003; Figure 2). The membrane depolarised rapidly (Figure 7). In a few cases, there was a change in spike potential activity, but these changes were not significant (p=0.683; Figure 2). Adding Krebs' to the bath did not result in any difference (p>0.999; Figure 2).

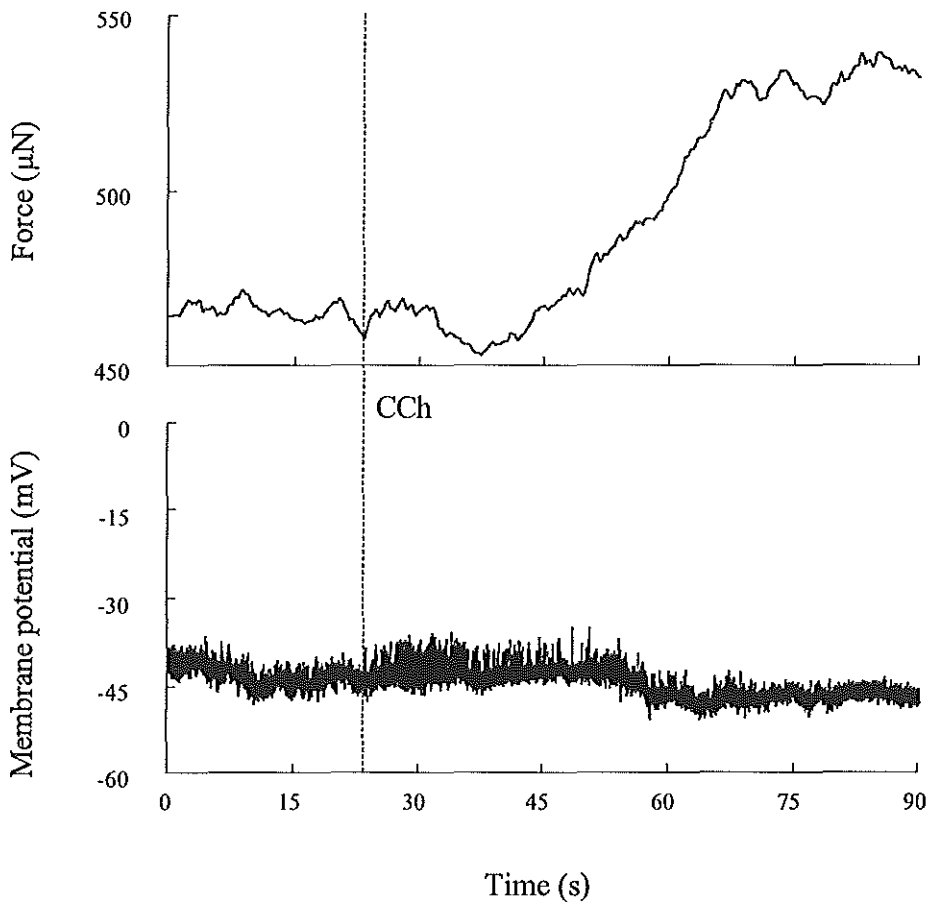

Figure 5. *An example of a response of human urinary bladder muscle to CCh. Upper trace: force; lower trace: membrane potential. A transient increase in membrane potential activity upon stimulation with CCh ($3.5 \cdot 10^{-6}$ M) is seen.*

Correlation between V_m, spike potential frequency and force

Spearman's rank correlation test showed that there was a significant correlation between the V_m of the impaled cell and the average force level in the human urinary bladder smooth muscle strip before substance application (r_s=-0.231; p=0.023; n=97), but not between the spike potential frequency and force (r_s=0.038; p=0.708; n=97). Application of muscarinic agonists, the antagonist or KCl to the bath did not result in either a significant correlation between the change in V_m and the change in force or between the latter and the change in spike potential frequency (p≥0.246 and p≥0.056, respectively).

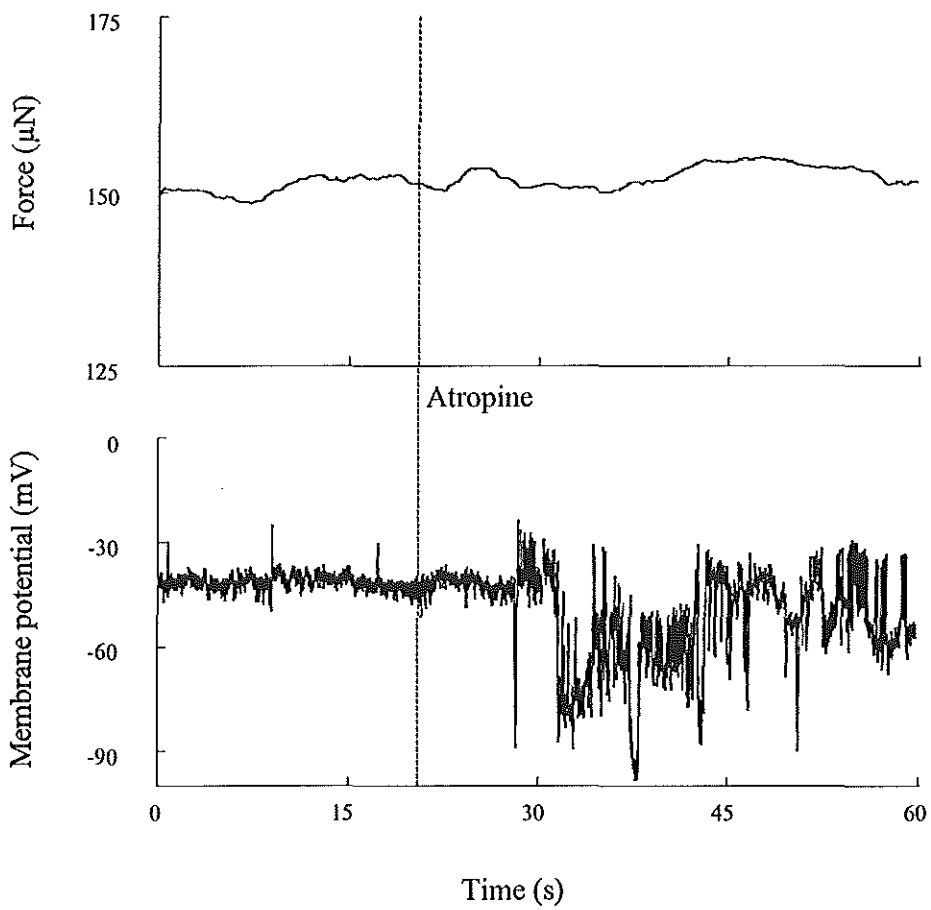

Figure 6. *An example of a response of human urinary bladder muscle to atropine. Upper trace: force; lower trace: membrane potential. A change in membrane potential activity upon stimulation with atropine ($2.1 \cdot 10^{-4}$ M) is seen.*

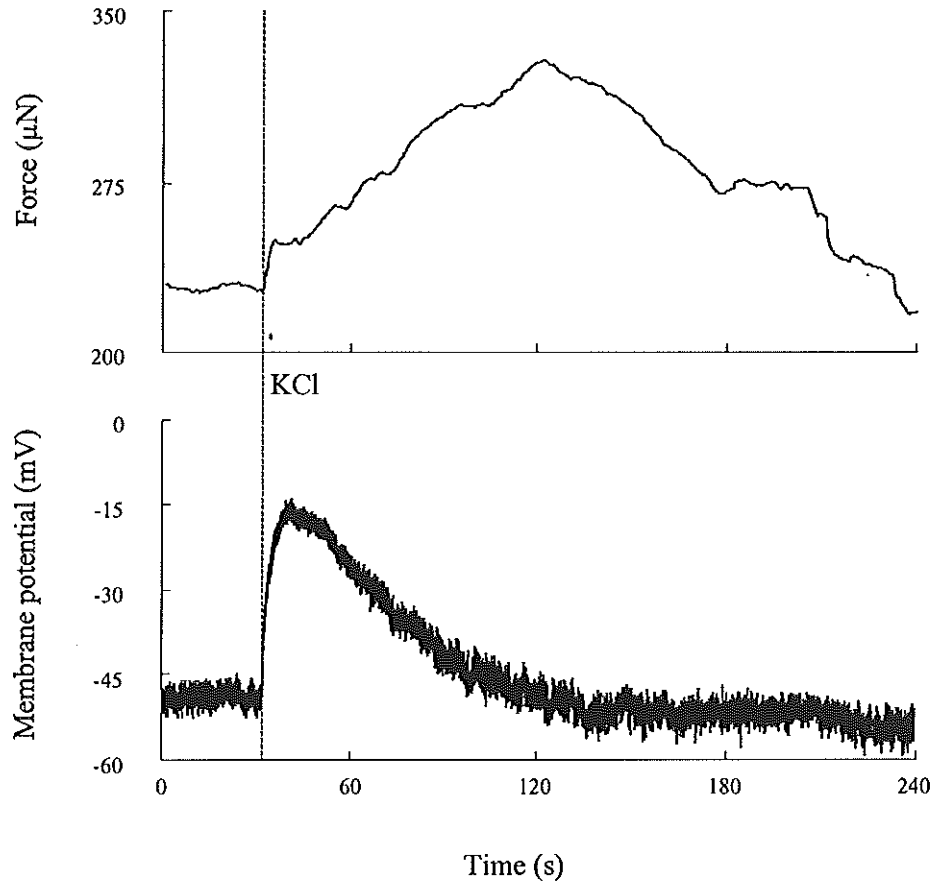

Figure 7. A typical response of human urinary bladder muscle to KCl. Upper trace: force; lower trace: membrane potential. A rapid, long lasting and large depolarisation of the membrane potential was observed upon stimulation with KCl ($3.7 \cdot 10^{-2}$ M). Figure 4 and Figure 7 were recorded in the same cell.

Discussion

Simultaneous recordings of mechanical and intracellular electrical activity are necessary to elucidate the role of the membrane potential in human detrusor contraction. In our study, the values of the resting membrane potential ranged widely. Values more negative than -100 mV were very likely caused by bending of the electrode, as such values would require a physiologically unrealistic intracellular K^+ concentration. Such bending artefacts may have been caused by the constant mechanical activity of the detrusor cells and these values were excluded from further analysis in our study.

There was a negative correlation between the V_m of the impaled cell and the average force level of a strip: the higher the resting force, the more depolarised the V_m. This is in agreement with the results obtained in rabbit detrusor [72], where stretch of the muscle strip resulted in a depolarisation of the membrane potential.

As in guinea-pig urinary bladder strips [13; 59], human multicellular preparations showed spontaneous fluctuations from the resting membrane potential in the form of spike-shaped potentials. The electrical activity was asynchronous within one muscle bundle, probably due to a relatively poor cell-to-cell coupling. As was found previously in human [75] and in rabbit [72], there was a large variation in size and shape of the spike-shaped potentials; they were not all-or-none events, unlike action potentials. Therefore, we do not use the term action potential for these events. However, we assume that the spike-shaped potentials fulfil a similar role as action potentials. Additionally, another form of spontaneous activity was recorded: fast, small depolarisations. These may correspond to the spontaneous transient depolarisations which have been recorded in guinea-pig urethra [32]. In that preparation, the spontaneous transient depolarisations are the result of spontaneous release of Ca^{2+} from intracellular stores which activates Ca^{2+} activated Cl^- channels [32]. Furthermore, we observed long lasting depolarisations that may be equivalent to the slow waves recorded in the previously mentioned study [32]. It is suggested by Hashitani & Edwards [32] that these slow waves are a summation of spontaneous transient depolarisations. From our study, we cannot draw a similar conclusion, although it is possible that the long lasting depolarisations are the result of the release of intracellular Ca^{2+}.

Stimulation of the muscarinic M_3 receptor with ACh or CCh resulted in an increase in force, but did not have a systematic effect on the membrane potential. Only in 37% of all agonist applications, was there an effect on membrane potential activity. This suggests that the primary route to mobilise Ca^{2+} upon stimulation of the M_3 receptor occurs through Ca^{2+} release from intracellular stores. Similar results were previously obtained in rabbit detrusor [72]. In contrast, Seki & colleagues [66] reported a depolarisation of the membrane with a significant increase in spike discharge in guinea-pig bladder. In the same tissue however, an increase in the spike frequency without a significant change in membrane potential upon CCh application was also reported [34]. Likewise, Wu & Fry [81] have shown that muscarinic agonists can generate a rise of intracellular Ca^{2+}, independent of changes in membrane potential

in guinea-pig detrusor myocytes. In our study, the frequency of the spontaneous spike-shaped potentials recorded in the cells did not change significantly upon any stimulus. It is therefore likely that in human detrusor this feature is neither regulated by the muscarinic receptor, nor by the intracellular Ca^{2+} concentration.

The overall mechanical activity of the muscle cells in the preparation did not show a correlation with the membrane potential or the spike potential frequency recorded in the impaled cell. There are two possible explanations for this phenomenon. It is possible that not all cells were recruited during contraction. This would be plausible for the resting tonus. However, we expect that with the supramaximal stimuli used, all cells contracted, and since we were recording from cells at the surface layer, it is unlikely that the stimuli have not reached the impaled cell. It is therefore more likely that there is a dissociation between membrane potential activity and contractile activity at the cellular level.

We found no significant differences between stimulation with CCh or ACh, when applied exogenously, as was found earlier by Anderson & colleagues [2]. However, we cannot exclude the possibility that stimulation of the M_3 receptor with exogenously applied ACh and transmitter released from nerves has different effects on the increase in free intracellular Ca^{2+}.

In the literature it has been described that muscarinic receptor stimulation may lead to both intracellular Ca^{2+} release and extracellular Ca^{2+} influx. Husted & colleagues have shown that electrically induced contractions are abolished 10 minutes after omitting Ca^{2+} from the bath solution [36], suggesting a role for extracellular Ca^{2+} upon nervous stimulation of the human detrusor. Still, the time elapsed between omitting Ca^{2+} from the bath solution and the abolishment of contractions might also indicate that the intracellular stores were depleted and could not be refilled with Ca^{2+} from the extracellular matrix. Furthermore they have shown that preventing a Ca^{2+} influx into the cell through L-type Ca^{2+} channels reduced the contraction induced by endogenously released ACh by about 40% [36]. Moreover, a role for Ca^{2+} influx via L-type Ca^{2+} channels has also been suggested in response to CCh [23]. In contrast, Maggi & colleagues demonstrated that CCh mobilises Ca^{2+} in the absence of extracellular Ca^{2+} from a caffeine and procaine-sensitive intracellular store [49]. A combination of both pathways for Ca^{2+} mobilisation upon CCh stimulation has also been reported: for small contractions almost all of the Ca^{2+} is derived from the sarcoplasmic reticulum, for intermediate contractions the Ca^{2+} is recruited from both stores evenly and for maximal contractions most Ca^{2+} is extracellular in origin [51]. Based upon this theory, one would expect Ca^{2+} from both sources to be involved in our measurements, as we stimulated supramaximally. Although we cannot exclude a combination of pathways, our data support a Ca^{2+} mobilisation primarily from the intracellular stores.

Application of the M_3 receptor antagonist atropine resulted in a significant relaxation of the smooth muscle tissue, but only if it was previously contracted by agonist stimulation and had not yet fully relaxed.

Depolarisation of the membrane potential with high potassium, presumably through stimulation of the L-type Ca^{2+} channel, resulted in a significant force development. There was no correlation between the magnitude of the depolarisation

and the developed force. Perhaps, this can be explained by the fact that the contraction of the detrusor strip was not generated only by the cell from which the recording was made. In guinea-pig detrusor, using the sucrose-gap method [59], a tight coupling between membrane potential activity and mechanical activity was reported. In human detrusor, as in rabbit detrusor, this was not found. Differences between species in functional and contractile behaviour of bladder muscle have been reported previously [67].

We conclude, that in human urinary bladder smooth muscle upon stimulation of the muscarinic M_3 receptor, electrical and mechanical activity are not correlated. This suggests that the primary route to mobilise Ca^{2+} then occurs through Ca^{2+} release from intracellular stores, without changes in membrane potential.

IV

The role of intracellular and extracellular calcium in mechanical and intracellular electrical activity of human urinary bladder smooth muscle

Adapted from: Visser and van Mastrigt
Urological Research, in press

Summary

We studied the role of extracellular and intracellular Ca^{2+} in human detrusor smooth muscle contraction. Simultaneous recordings of mechanical and intracellular electrical activity were made in three different Ca^{2+} concentrations: normal Krebs' solution (100%), 10% of the standard Ca^{2+} concentration and a solution in which Ca^{2+} was omitted from the medium (0%). Spontaneous contractions and KCl or CCh induced contractions were studied. Ryanodine and caffeine were used to manipulate the intracellular Ca^{2+} stores. The present results show that only a very small amount of Ca^{2+} in the extracellular space is sufficient to support spontaneous and induced contractions. Spike-shaped potentials and long lasting depolarisations were recorded in all three solutions, however, the prevalence of long lasting depolarisations increased when the extracellular Ca^{2+} concentration was reduced. The amplitude of the spike-shaped potentials and long lasting depolarisations appeared to be negatively affected by diminishing the extracellular Ca^{2+} concentration. Additionally, the duration of the long lasting depolarisations was reduced in 0% Ca^{2+}. The contraction upon KCl stimulation was primarily depending on the extracellular Ca^{2+}. Upon muscarinic receptor stimulation a combined activation of Ca^{2+} mobilisation from intracellular and extracellular stores may occur; the ratio of contribution of these two sources changes in accordance with the requirements of the conditions.

Introduction

The urinary bladder stores urine slowly at a low pressure and expels it rapidly at a high pressure. To accomplish this, the detrusor muscle contracts to initiate and maintain the flow of urine. In a healthy human detrusor, voiding is under cholinergic control [6; 26; 40; 67]. Activation of the cholinergic pathway induces the dissociation of a G-protein, which mobilises inositol (1,4,5)-trisphosphate (IP_3) [37; 83], in turn releasing Ca^{2+} from the sarcoplasmic reticulum [19] (SR; Figure 1). This release may occur without changes in membrane electrical activity [26]. Indeed, force development in human detrusor strips upon stimulation with acetylcholine (ACh) or carbachol (CCh) without membrane potential changes has been observed in a previous study [77], suggesting a minor role for extracellular Ca^{2+} in the initiation of contraction in human urinary bladder.

In some patients however, the detrusor spontaneously and involuntarily contracts during the storage phase [review: 60]. These spontaneous contractions observed in urodynamic studies are related to the spontaneous contractile activity seen in detrusor strips from healthy bladders [6]. It is known that muscle strips from patients with idiopathic detrusor instability or detrusor hyperreflexia exhibit larger and more frequent spontaneous contractions than strips from normal bladders [41]. It is not known if the Ca^{2+} involved in spontaneous or induced contractions originates from different sources, however, there is reason to assume this. A previous study in pig detrusor strips has shown a difference in the rate of contraction development between stimulated and spontaneous contractions, suggesting different pathways to be involved [43]. In human bladder a dependence of spontaneous mechanical activity on extracellular Ca^{2+} has been demonstrated [3], implying Ca^{2+} channel activity.

Electrophysiological recordings could aid the understanding of the role of the different Ca^{2+} sources in the mechanism of detrusor contraction and possibly lead to a clinically useful treatment of selective suppression of detrusor instability.

Presently, there are very few published records of electrical activity recorded from intact urinary bladder smooth muscle from any large mammal [10], let alone man [74]. This is probably caused by the exceptional difficulty to impale detrusor cells, with their continuous mechanical activity and extensive extracellular matrix containing collagen and elastin [85]. Human detrusor electrical activity has been studied in single cells [56], however, using this model, it is impossible to study mechanical activity.

To examine the role of extracellular Ca^{2+} in human detrusor contraction, we have recorded simultaneously mechanical and intracellular electrical activity in human urinary bladder strips under three conditions: in normal Krebs' solution (100%), in a solution with only 10% of the standard Ca^{2+} concentration (10%) and in one in which the Ca^{2+} was omitted from the medium (0%). Spontaneous contractions and contractions induced by ACh, CCh and KCl were studied. To manipulate the intracellular stores, ryanodine, which interferes with the ability of the cell to store Ca^{2+} in the SR, and caffeine, which stimulates the release and also reduces the rate of ATP-dependent Ca^{2+} uptake, were applied to the bath medium.

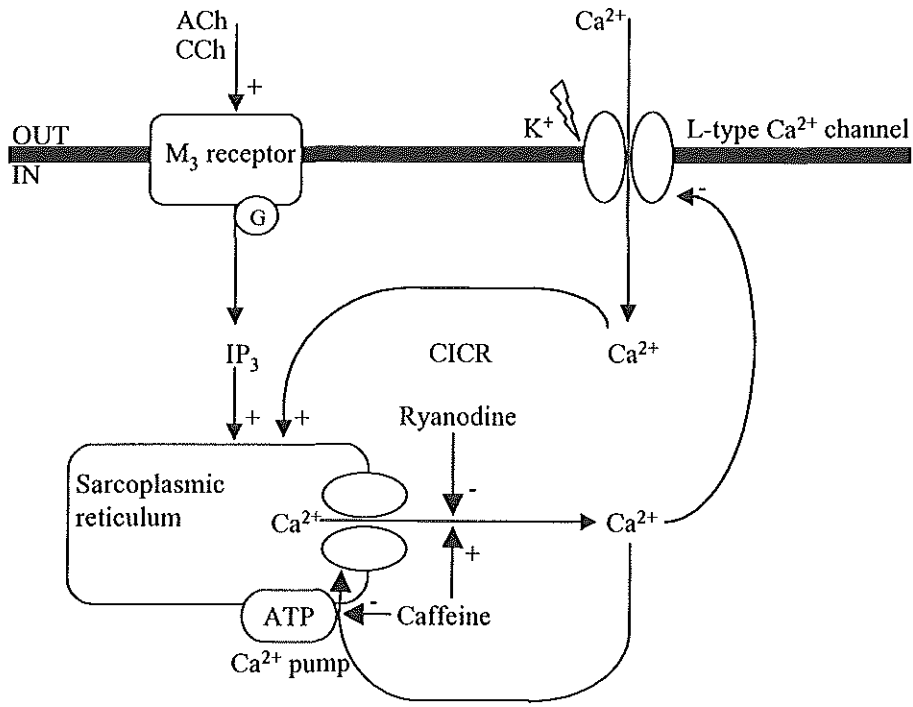

Figure 1. *A schematic representation of the pathways to control the intracellular Ca^{2+} concentration in human urinary bladder smooth muscle. The muscarinic M_3 receptor, upon stimulation by ACh or CCh, activates a second messenger system (G-protein (G) and inositol (1,4,5)-trisphosphate (IP_3)), causing the release of Ca^{2+} from the sarcoplasmic reticulum (SR). A depolarisation of the membrane, resulting from a high K^+ concentration, opens the voltage sensitive L-type Ca^{2+} channels, resulting in a Ca^{2+} influx. This may cause Ca^{2+} induced Ca^{2+} release (CICR) from the intracellular stores. Ryanodine inhibits the release of Ca^{2+} from the SR. Caffeine increases the Ca^{2+} release from the SR and at the same moment reduces the rate of ATP-dependent Ca^{2+} uptake by the Ca^{2+} pump in the membrane of the SR.*

Materials and methods

Biopsy samples

Detrusor biopsy samples were collected from 14 patients (3 women; 11 men) ranging in age from 49 to 93 years (mean: 64 years; s.d.: 13 years) with approval from the local ethical committee and informed consent. The patients were undergoing surgery for bladder or prostate cancer and the tissue was collected using either cold-cup biopsy forceps (trans-urethral biopsies) or at open surgery (radical prostatectomy). Immediately after excision, the samples were stored in 0.9% NaCl. Outside of the operating theatre, this solution was replaced by modified Krebs' solution (in mM): 1.8 $CaCl_2$; 1.2 $MgSO_4$; 118 NaCl; 4.7 KCl; 25.0 $NaHCO_3$; 1.2 KH_2PO_4; 11.0 Glucose. By aerating the solution with a mixture of 95% O_2 and 5% CO_2, the pH was adjusted to 7.4. The tissue was transported to the laboratory within ten minutes after excision, pinned down on Sylgard 184 (Dow Corning, Ithaca, NY, USA) and a muscle bundle of about 2 mm long and 0.2 mm wide was dissected. 8 Of the biopsy samples were stored at 4°C overnight before a muscle bundle was dissected. Previous work and others have shown that this does not affect the response of the biopsies to muscarinic receptor stimulation [61]. Connective tissue was removed mechanically.

Tension recordings

One side of the muscle bundle was pinned down in a 4 ml recording chamber with a Sylgard 184 bottom on the stage of an inverted microscope (magnification 40x; Zeiss). The free part of the muscle bundle was connected to the forceps of a force transducer (BAM4C, Scientific Instruments, Heidelberg, Germany) and was then stretched minimally to enable microelectrode recordings. The muscle bundle was gradually warmed to 35°C in standard Krebs' solution using a modified thermostat. After 30 minutes, CCh was applied to test the viability of the preparation. If the muscle bundle responded with a contraction, the tissue was allowed to accommodate for another 30 minutes either in standard Krebs' solution (n=5), in Krebs' solution with only 10% of the standard Ca^{2+} concentration, i.e. 0.18 mM (n=5) or one in which Ca^{2+} was omitted from the medium (n=4), before the microelectrode recordings were started. These test contractions were used as a reference point for each individual strip. To allow a comparison between the strips, the contractions measured during the experiment were normalised by expressing them as a percentage of the reference contraction.

During the experiments, 1 ml of the bathfluid was removed and analysed afterwards, using a Ca^{2+}-sensitive electrode (Orion, USA). The Krebs' solution in which Ca^{2+} was omitted, had a Ca^{2+} concentration of less than 0.1mM due to impurities of the other salts. A more accurate value of the concentration could not be determined due to limitations of the set-up.

Electrophysiological recordings

Recordings were made using borosilicate glass microelectrodes with a flexible tip (GC120F, Clark Electromedical Instruments, Pangbourne Reading, GB). Filled with 3 M KCl, the electrodes had 40-60 MΩ resistance. They were placed in an Ag/AgCl pellet microelectrode holder connected to the input stage of a high impedance capacitance-neutralising amplifier (World Precision Instruments, Sarasota, FL, USA). An Ag/AgCl electrode (outer diameter: 2.0 mm) in an agar bridge served as the reference electrode in the organ bath. To minimise movement artefacts, the cells were speared in the longitudinal direction, at the side where the muscle bundle was pinned to the Sylgard. Each cell was measured in only one Ca^{2+} concentration, because it was impossible to record from one cell for a period long enough to wash in another Ca^{2+} concentration.

The electrophysiological signal was amplified 10 times and low pass filtered with a cut-off frequency of 1 kHz (Krohn-Hite Corporation, Avon, MA, USA). Both this signal and the signal from the force transducer were then AD converted at a sample rate of 2 kHz (DAS1800, Keithley MetraByte, Taunton, MA, USA) using a locally developed sampling program and digitally stored in a PC. The digitised force signal was filtered with a 4^{th} order Butterworth filter, using a cut-off frequency of 2 Hz.

Force induction

Different stimuli were used: ACh (Sigma), CCh (Pharmachemie B.V., the Netherlands), KCl, caffeine and ryanodine (ICN Biomedicals, USA). CCh was dissolved in physiological salt solution, all other pharmaca were dissolved in Krebs' solution with a Ca^{2+} concentration corresponding to the one in the bath fluid. Stock solutions of ACh ($2.0 \cdot 10^{-2}$ M), CCh ($1.7 \cdot 10^{-3}$ M), caffeine ($7.5 \cdot 10^{-2}$ M), ryanodine ($4.0 \cdot 10^{-4}$ M) and KCl (3.0 M) were applied in random order to the bath in units of 0.01 ml, resulting in minimal bath concentrations of $5.0 \cdot 10^{-5}$ M ACh, $3.4 \cdot 10^{-6}$ M CCh, $1.9 \cdot 10^{-4}$ M caffeine and $1.0 \cdot 10^{-6}$ M ryanodine. A final concentration of $12.2 \cdot 10^{-3}$ M KCl was used to induce depolarisation of the membrane.

The bathing solution was continuously refreshed at a rate of $1.4 \cdot 10^{-2}$ ml/s, and the bath was refreshed within 5 minutes. However, difficulties with making long intracellular recordings were inherent to the type of preparation, therefore substances were often applied before total washout of the previous one. In those cases, the concentration was calculated using a standard concentration distribution formula. In the figure legends the final concentrations of the substances in the bath are given. When Spearman's rank correlation test was performed, there were no significant correlations between the concentration of a substance and the resulting force, membrane potential or spike potential frequency, so the data were pooled. There were no significant differences in the response of the tissue between ACh or CCh application, so the response to these stimuli were pooled ($p \geq 0.171$).

In a previous study, Krebs' solution without additions was used as a control for the application method; this did not result in significant changes [77]. An overview of the stimuli applied is given in Table 1.

Data analysis

		extracellular Ca^{2+} concentration		
stimulus	(n) preparations	100%	10%	0%
ACh / CCh	13	10	15	5
KCl	10	17	7	18
caffeine	7	33	0	11
ryanodine	9	32	0	11
ryanodine + CCh	8	14	0	9
spontaneous contractions	7	13	1	16

Table 1. *Number of preparations and number of applications of the used stimuli.*

The average resting membrane potential of the impaled cell was calculated during 30 s before and during 30 s immediately after the stimulus. Resting membrane potential values more negative than -100 mV were sometimes recorded. These results were not used in the analysis; they were considered artefacts, most likely bending of the electrode. An especially developed software program written in Matlab® 4.2c1 [75] was used to detect spontaneous deflections from the resting membrane potential in the form of spike-shaped potentials and long lasting depolarisations. The frequencies of both types of events were determined in the same periods as the resting membrane potential was calculated. A second program [75] was used to determine the parameters that describe the spike-shaped potentials and the long lasting depolarisations. The amplitude of a spike-shaped potential or a long lasting depolarisation was defined as the difference between the membrane potential value at the onset and the peak value of the event. The duration was calculated at 10% of the amplitude ($d_{10\%}$) to reduce noise. Negative duration values and amplitudes under 5 mV were excluded from further analysis, since previous work showed that these were caused by incorrect parameter estimation [e.g. a wrong amplitude was calculated; 75]. This occurred in 29% of the total number of recorded spike-shaped potentials (n=22851) and in 20% of the total number of recorded long lasting depolarisations (n=2419).

Force values were read as the average force level during 30 seconds before

the stimulus and at the maximum after it. The calculated parameters were imported in Excel 97 and statistical analysis was done using SPSS 8.0.2 (SPSS Inc., Chicago, IL, USA). Relationships between the parameters were studied with the Spearman's rank correlation test. To compare parameters before and after drug application, Wilcoxon's signed ranks test was used. To analyse the effect of the Ca^{2+} concentration on the parameters, the Kruskal-Wallis test and the Mann-Whitney U-test were used.

Results

The effect of extracellular Ca^{2+}

The median value of the resting membrane potential of the detrusor cells upon impalement was -54 mV. In a reduced extracellular Ca^{2+} concentration, the median value of the resting membrane potential depolarised to -42 mV in 10% Ca^{2+} and -43 mV in 0% Ca^{2+} (Table 2). There was no significant difference ($p=0.260$). The majority of the cells in any Ca^{2+} concentration displayed spontaneous activity in the form of spike-shaped potentials and long lasting depolarisations. Typical examples of long lasting depolarisations occurring in detrusor muscle cells are shown in Figure 2. The size of both the spike-shaped potentials, as well as the long lasting depolarisations was correlated with the membrane potential at the start of the event, which represents the resting membrane potential: the less negative the membrane potential, the smaller the amplitude ($p<0.001$).

The frequency (measured during 30 seconds before application of any drug) of the spike-shaped potentials was significantly affected by the Ca^{2+} concentration ($p=0.048$; Table 2). A similar effect was observed on the occurrence of long lasting depolarisations ($p=0.001$; Table 2). The amplitude and the duration at 10% of the amplitude ($d_{10\%}$) of the events were determined. The parameters of the spike-shaped potentials differed significantly, not only between biopsies ($p<0.001$), but even between different cells within one strip ($p\leq0.016$). The impalement of a cell never

		extracellular Ca^{2+} concentration		
		100%	10%	0%
resting membrane potential (mV)	median	-54	-41	-43
	i.q.r.	34	14	31
	n	117	23	67
spike-shaped potential frequency (s^{-1})	median	0.40	0.03	0.63
	i.q.r.	1.4	0.8	1.2
long lasting depolarisation frequency (s^{-1})	median	0.00	0.00	0.03
	i.q.r.	0.1	0.0	0.2
difference between resting force and reference resting force in standard Krebs' (mN)	median	10	10	-10
	i.q.r.	103	190	185
	n	118	23	69

***Table 2.** Parameter values recorded in human detrusor strips in three different Ca^{2+} concentrations. The median value and interquartile range (i.q.r.) are given for the parameters. Note that the n for resting membrane potential is smaller than the n for the other parameters in 100% Ca^{2+} and 0% Ca^{2+}, due to exclusion of potential values more negative than -100 mV.*

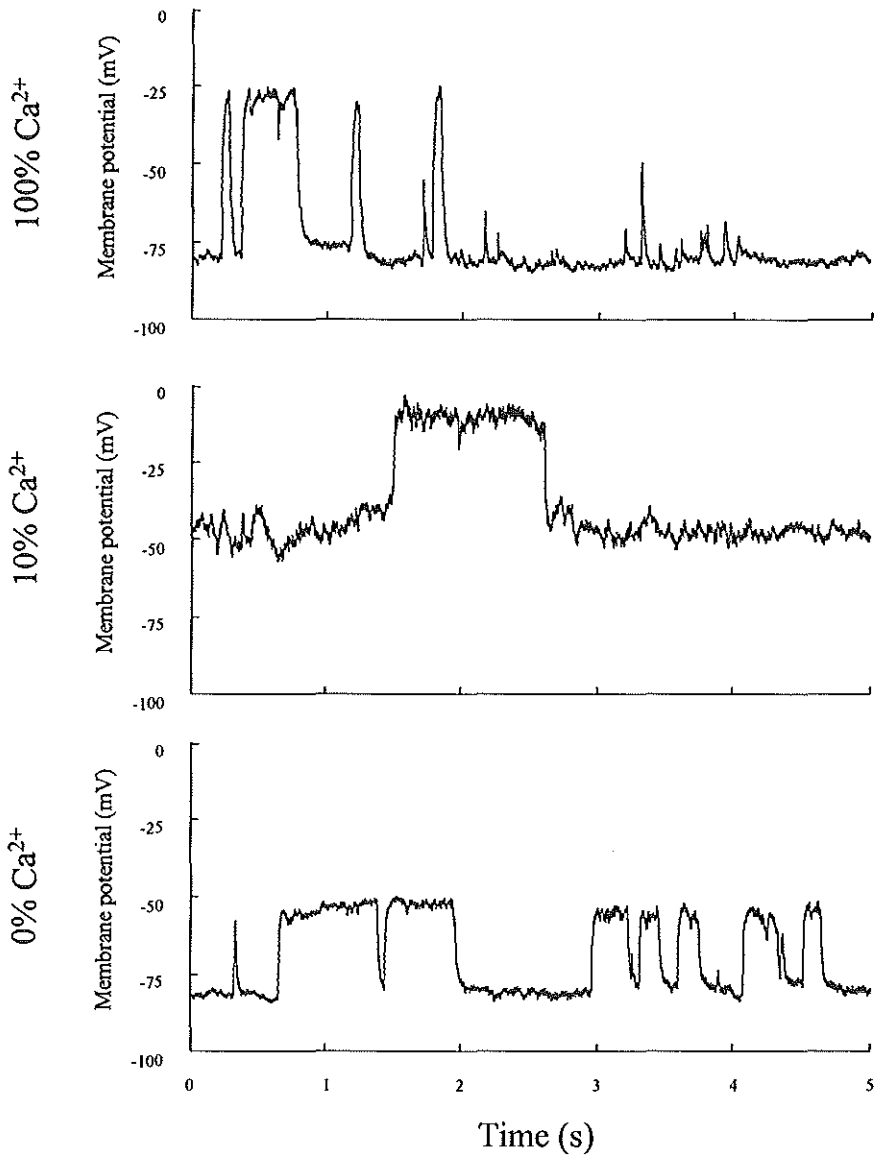

Figure 2. *Typical examples of long lasting depolarisations in human detrusor cells recorded in three different extracellular Ca^{2+} concentrations (i.e. 100%, 10% and 0%). Recordings were made in three different biopsies.*

lasted long enough to change the external Ca^{2+} concentration. Due to the large variation of the parameters between the strips and cells it was not possible to test the effect of the extracellular Ca^{2+} concentration on the parameters statistically. However, as can be seen from Figure 3A, there appears to be a slight increase in spike-shaped potential amplitude when the Ca^{2+} concentration was raised. An effect on the $d_{10\%}$ of the recorded spike-shaped potentials could not be detected. In Figure 3, the 2½, 25, 50 (median), 75 and 97½ cumulative relative frequencies (centiles) are given for the three Ca^{2+} concentrations. Similar to the parameters of the spike-shaped potentials, the parameters of the long lasting depolarisations differed between the strips ($p<0.001$) and between the cells within one muscle strip. However, on average, the amplitude increased (Figure 3B), and there was a dramatic decrease in $d_{10\%}$ of the long lasting depolarisations when the extracellular Ca^{2+} concentration was increased (Figure 3B).

The resting force varied between the strips. Therefore, the force at a given Ca^{2+} concentration was compared to the resting force of the same detrusor strip in control medium before changing the bathing fluid for the experiment. There was no significant difference between the corrected resting forces in the three extracellular Ca^{2+} concentrations ($p=0.623$; Table 2).

From the 14 preparations, 2 strips in 0% Ca^{2+}, 1 in 10% Ca^{2+} and 4 in 100% Ca^{2+} displayed spontaneous contractile activity: Figure 4 shows a typical example of a spontaneous contraction in 100% Ca^{2+} Krebs'. The normalised amplitudes of the spontaneous contractions did not differ significantly between the preparations ($p=0.086$) and were not correlated to the Ca^{2+} concentration ($p=0.135$). In our set-up,

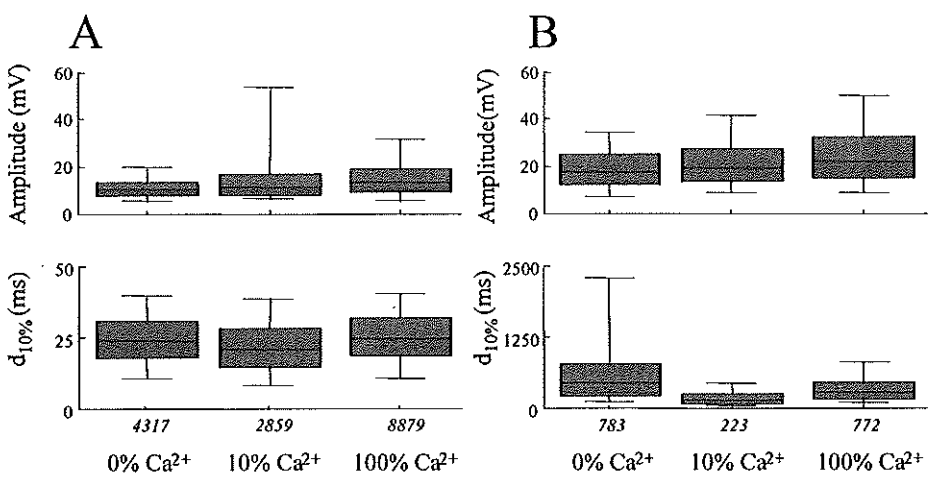

Figure 3. *Parameter values of electrical activity measured in cells of human detrusor strips in three different extracellular Ca^{2+} concentrations represented in a boxplot. A. Spike-shaped potential parameters. B. Long lasting depolarisation parameters. The values at 2½, 25, 50 (median), 75 and 97½ cumulative relative frequencies (centiles) are given. The numbers of events are displayed at the bottom of the graph.*

we used a voltage-controlled oscillator, converting the membrane potential into a tone. During spontaneous contractions, we had the impression that the noise level increased, suggesting that the spontaneous contractions were accompanied by an increase in spike potential frequency or long lasting depolarisation frequency. When the data was analysed, such an effect was found in the long lasting depolarisation frequency ($p=0.030$), but not in the spike-shaped potential frequency ($p=0.957$). In normal and 0% Ca^{2+} Krebs' solution, respectively 13 and 16 spontaneous contractions were recorded (Table 1). During the spontaneous contraction in 10% Ca^{2+} Krebs' (Table 1), the recorded cell was quiescent.

In all three Ca^{2+} concentrations, there was a significant increase in force upon muscarinic agonist application ($p \leq 0.007$; Table 3). The normalised force response to muscarinic agonist application was not significantly dependent on the Ca^{2+}

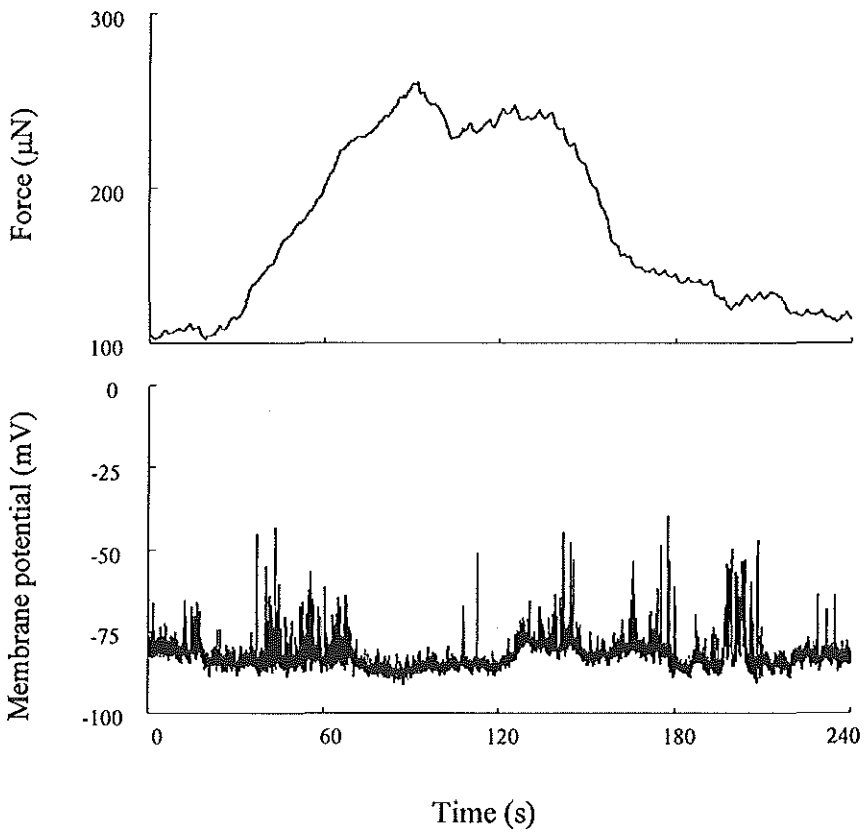

Figure 4. A typical example of a spontaneous contraction in a human detrusor strip. The recording was made in standard Krebs' solution (100% Ca^{2+} concentration). Upper trace: force; lower trace: membrane potential.

concentration (p=0.234). The membrane potential (Table 3), the spike potential frequency and the long lasting depolarisation frequency were not influenced by the administration of muscarinic agonists in any Ca^{2+} concentration (p≥0.438; p≥0.132 and p≥0.276, respectively).

In all three extracellular Ca^{2+} concentrations, there was a significant depolarisation of the membrane potential in response to KCl (p<0.018; Table 3). However, only in 10% and 100% Ca^{2+} Krebs' there was a significant change in force (p=0.018 and p=0.001, respectively; Table 3). The spike potential frequency did not change significantly (p≥0.139). The long lasting depolarisation frequency decreased

			extracellular Ca^{2+} concentration		
stimulus			100%	10%	0%
KCl	Δ normalised force (%)	median	50	32	0
		i.q.r.	1	1	1
	Δ membrane potential (mV)	median	15	7	5
		i.q.r.	9	9	23
		n	17	7	16
ACh / CCh	Δ normalised force (%)	median	33	69	43
		i.q.r.	1	1	1
	Δ membrane potential (mV)	median	2	0	-3
		i.q.r.	8	10	8
		n	10	15	5
ryanodine + CCh	Δ normalised force (%)	median	25	-	0
		i.q.r.	2	-	3
	Δ membrane potential (mV)	median	10	-	0
		i.q.r.	9	-	18
		n	14	-	9

Table 3. *Changes in parameter values in response to application of KCl, muscarinic agonists ACh and CCh, or CCh in the presence of ryanodine, in three different extracellular Ca^{2+} concentrations. The median value and interquartile range (i.q.r.) are given for the parameters.*

upon KCl stimulation only in 100% Ca^{2+} Krebs' (p=0.024) but not in 10% Ca^{2+} Krebs' (p=0.854) or 0% Ca^{2+} Krebs' (p=0.068). Figure 5 gives a typical example of the response to KCl in 10% Ca^{2+} Krebs'. Immediately after KCl application the membrane depolarised and with a delay of about one second, the force level increased. The developed force remained constant after cessation of the membrane depolarisation and eventually returned to the resting level.

The effect of intracellular Ca^{2+}

The effect of the intracellular Ca^{2+} concentration was studied using caffeine and ryanodine. Caffeine, which releases Ca^{2+} from intracellular stores, did not have a significant effect on the membrane potential ($p \geq 0.646$), the spike potential frequency ($p \geq 0.198$) or on the long lasting depolarisation frequency ($p \geq 0.240$) in 100% and 0% Ca^{2+} Krebs'. In both 0% and 100% Ca^{2+} Krebs', caffeine stimulation resulted in a statistically significant increase in force ($p \leq 0.012$), but these were not significantly different ($p=0.504$).

The extracellular Ca^{2+} concentration made a significant difference in the normalised force development ($p=0.042$), but not in the response of the membrane

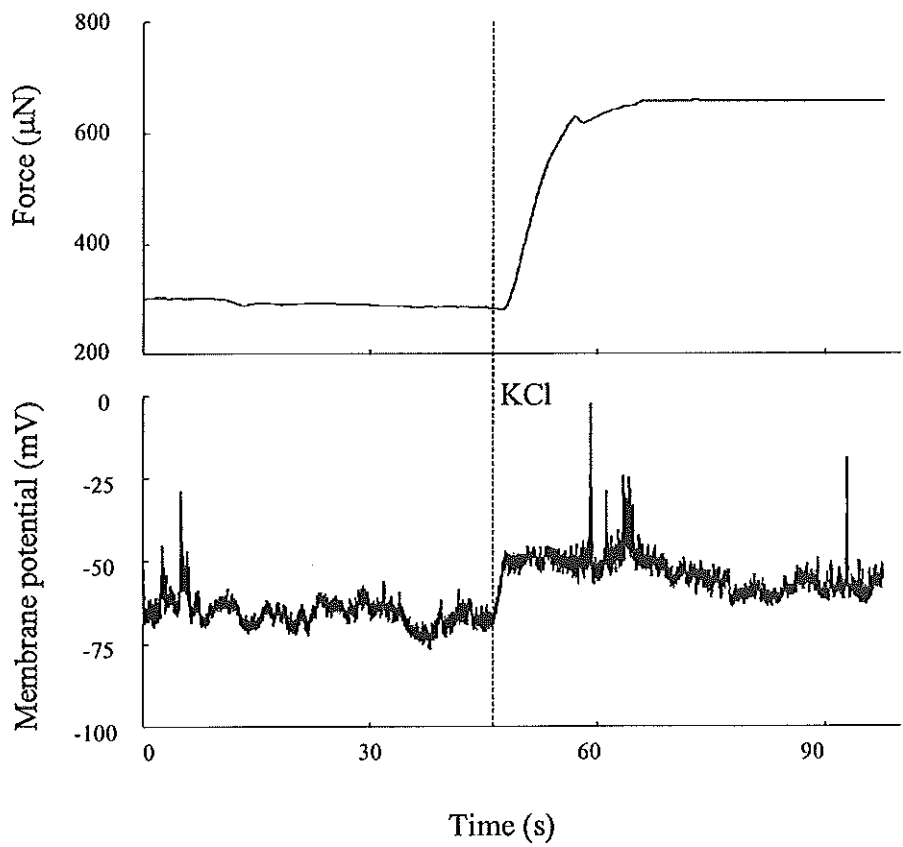

Figure 5. *A typical example of the response of a human detrusor strip to stimulation with KCl. A change in membrane potential preceding the onset of force development can be seen. The recording was made in 10% of the normal Ca^{2+} concentration. Upper trace: force; lower trace: membrane potential.*

potential (p=0.103), the spike-shaped potential frequency (p=0.519) or the long lasting depolarisation frequency (p=0.483) to application of ryanodine. An example of ryanodine application to a detrusor strip in 0% Ca^{2+} Krebs' is given in Figure 6. Immediately after application of ryanodine, there was a slight increase in force, without apparent changes in membrane potential activity.

A significant increase in force development upon CCh stimulation while ryanodine was present was found in 100% Ca^{2+} (p=0.005; Table 3), but not in 0% Ca^{2+} (p=0.068; Table 3). There was no effect on the membrane potential (p≥0.224; Table 3), the spike-shaped potential frequency (p≥0.191) or the long lasting depolarisation frequency (p≥0.341). In 100% Ca^{2+}, the normalised force development (p=0.829), the spike-shaped potential frequency (p=0.324) or the long lasting

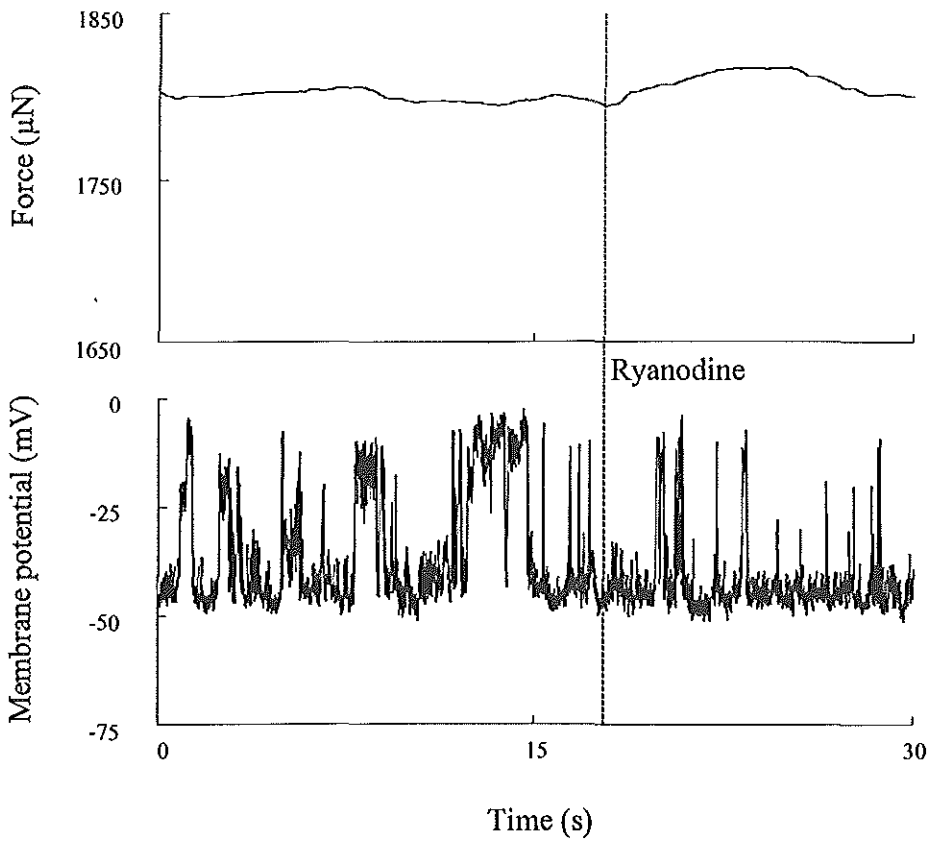

Figure 6. *A typical example of the response of a human detrusor strip to ryanodine application. A small increase in force accompanied the administration of ryanodine, without a change in membrane potential activity.* Ca^{2+} *was omitted from the bath solution (0%). Upper trace: force; lower trace: membrane potential.*

depolarisation frequency ($p=0.896$) did not differ between stimulation of the muscarinic receptor solely, or application of CCh in the presence of ryanodine. Only the response of the resting membrane potential ($p=0.019$) in 100% Ca^{2+} differed from the normal response to muscarinic agonist application when ryanodine was present in the bath. CCh or ACh application resulted in a small, non-significant depolarisation. This effect disappeared upon application in the presence of ryanodine. In 0% Ca^{2+} Krebs', none of the parameters was significantly different ($p \geq 0.083$).

Discussion

Simultaneous recordings of mechanical and intracellular electrical activity can lead to a better understanding of the roles the two different Ca^{2+} sources play in the mechanism of detrusor contraction. We studied spontaneous contractions and ACh, CCh or KCl induced contractions under three conditions: in Krebs' containing 100%, 10% and 0% of the normal extracellular Ca^{2+} concentration. The intracellular Ca^{2+} stores were manipulated with ryanodine and caffeine.

The effect of extracellular Ca^{2+}

Electrical activity

The median value of the resting membrane potential was less negative when the extracellular Ca^{2+} concentration was reduced, but this was not significant. This depolarisation upon reduction of the extracellular Ca^{2+} concentration could be caused by altered permeabilities of ions due to a different driving force for Ca^{2+}. In guinea-pig, superfusion of isolated detrusor myocytes with zero Ca^{2+} depolarised the membrane potential [82].

The prevalence of spike-shaped potentials differed significantly between the different Ca^{2+} concentrations and the frequency of long lasting depolarisations decreased when the Ca^{2+} concentration was diminished. These long lasting depolarisations may be equivalent to the slow waves recorded in the urethra of guinea-pig [32], which were suggested to result from spontaneous release of Ca^{2+} from intracellular stores activating Ca^{2+} activated Cl^- channels. From our study, we cannot unequivocally draw this conclusion, however, a study in swine tracheal smooth muscle demonstrated that removal of external Ca^{2+} decreased or eliminated Ca^{2+} induced Cl^- currents [47]. This supports the idea that the spontaneous long lasting depolarisations recorded in the present study are indeed Ca^{2+} activated Cl^- currents.

There was a large variation in size and shape of the spike-shaped potentials and long lasting depolarisations. The amplitude and duration ($d_{10\%}$) of both types of events differed significantly, not only between strips, but even within one muscle bundle, or one cell. Apart from our work in human tissue, variability in the shape and size of spontaneous action potentials has been reported in animal preparations too, by Creed [13] in guinea-pig bladder and Ursillo [72] in rabbit bladder strips.

Reducing the extracellular Ca^{2+} concentration appeared to have a negative effect on the amplitude of the spike-shaped potentials. In 0% Ca^{2+} Krebs', there was probably enough Ca^{2+} left, bound to the surface of the cells, to generate the small spike-shaped potentials. A higher extracellular Ca^{2+} concentration leads to a higher chance of free Ca^{2+} ions near the Ca^{2+} channels. When these channels are then activated, more ions can flow through the pores during the opening time, resulting in a larger inward current. There was no clear effect on the $d_{10\%}$ of the spike-shaped potentials, indicating that the opening time of the Ca^{2+} channels was not influenced by

the extracellular Ca^{2+} concentration.

Similarly, the amplitude of the long lasting depolarisations was affected by manipulation of the extracellular Ca^{2+} concentration. The $d_{10\%}$ of the long lasting depolarisations was dramatically increased upon removal of extracellular Ca^{2+}. In rabbit detrusor, it was demonstrated that when influx of extracellular Ca^{2+} was blocked, the participation of intracellular Ca^{2+} stores to a contraction increased [18]. This supports the present theory, that the long lasting depolarisations are resulting from Cl^- currents activated by intracellular Ca^{2+} release.

Mechanical activity

Reduction of the extracellular Ca^{2+} concentration had no effect on the corrected resting force of a strip. In the literature, there is an ongoing discussion on the source of Ca^{2+} responsible for resting muscle tone. In rabbit, Andersson & Forman [3] suggested that tone was dependent on extracellular Ca^{2+}. Kurihara & Sakai [45] showed similar results for guinea-pig and Maggi [50] did so for rat. However, experiments with nifedipine showed that in human urinary bladder, tone is regulated by intracellular Ca^{2+} release [21]. Our results are in agreement with this study. Important differences in functional and contractile behaviour of bladder muscle strips between species have been reported earlier [67].

In our hands, 50% of the preparations showed spontaneous contractions. The occurrence of spontaneous contractions is related to many factors: the size of the strip, the preparation and dissection methodology, oxygenation, temperature [63] and the applied tension [20]. In rabbit [3; 84] and rat [50], Ca^{2+} influx from the extracellular space is important for the generation of spontaneous contractions.

The source of Ca^{2+} involved in human urinary bladder spontaneous contractions is not known. In our study, spontaneous contractions still occurred in Krebs' solution from which Ca^{2+} was omitted. However, in this solution, Ca^{2+} bound to superficial binding sites on the cell membrane, was still present as no Ca^{2+} chelator was added to the bath. It is possible, that this was sufficient for the generation of spontaneous contractions. The intracellular Ca^{2+} threshold for contraction is 10^{-7} M and a single spike could elevate the free intracellular Ca^{2+} concentration enough to exceed this threshold in normal Krebs' solution [4]. However, Kurihara & Sakai [45] also observed spontaneous contractions in guinea-pig urinary bladder strips in Ca^{2+} free medium.

In rabbit [16; 84], rat [17] and guinea-pig [8; 58], spontaneous contractions were accompanied by spontaneous electrical activity, suggesting a major role for extracellular Ca^{2+}. As can be seen from Figure 4, there sometimes was an increase in spike frequency in the recorded cell at the onset of a spontaneous contraction, however, this effect was not significant. It should be kept in mind that the tension recording was a summation of the activity in the whole strip, while the electrode impaled only one cell. It is very likely, that not all cells were involved in the spontaneous contraction, and since it is known that human detrusor muscle is badly coupled [6], it is possible that the cell in which the recording was made was not

participating in the contraction. In rat, similarly, sometimes contractions preceding a change in electrical activity were observed [17]. If the Ca^{2+} source necessary for a spontaneous contraction is extracellular in human urinary tissue, as it is in animal detrusor, one could carefully suggest, based on the percentage in which there was a clear increase in spike frequency co-occurring with the onset of a spontaneous contraction, that about forty per cent of the cells in one muscle strip participate in a spontaneous contraction. However, from this study, we cannot exclude a role of intracellular Ca^{2+} release in spontaneous contractions.

Stimulation of the muscarinic receptor with ACh or CCh resulted in a significant increase in force in all three Ca^{2+} concentrations. Diminishing the extracellular Ca^{2+} concentration had no significant effect on the normalised force response. Increasing the extracellular K^+, resulted in a depolarisation of the membrane, which was not significantly affected by manipulation of the extracellular Ca^{2+} concentration, although it appeared that a higher extracellular Ca^{2+} concentration resulted in a larger depolarisation. Brading [6] suggested that the total tissue content of Ca^{2+} in smooth muscles may be 100 times greater than that needed for maximum contractile activity. Our results confirm this suggestion that there is enough Ca^{2+} bound to the extracellular matrix to support a depolarisation of the membrane potential in the absence of Ca^{2+} in the extracellular medium. Although there was a significant depolarisation of the membrane potential, there was no significant increase in force if Ca^{2+} was omitted from the bath (0% Ca^{2+} Krebs'). This indicates that although there was still ion influx from the bath, there was not enough Ca^{2+} entering the cell to initiate a contraction. An extracellular Ca^{2+} concentration of $\geq$10% of the normal concentration was sufficient to initiate and maintain a contraction upon KCl stimulation. The spike potential frequency was not affected by the extracellular Ca^{2+} concentration and there was an indication that the long lasting depolarisation frequency was influenced by the administration of KCl. This could be the result of a reduced spontaneous release of intracellular Ca^{2+} inducing Ca^{2+} activated Cl^- currents due to depolarisation of the membrane or an increase in free intracellular Ca^{2+} from the extracellular matrix.

The effect of intracellular Ca^{2+}

In human urinary bladder smooth muscle, the membrane potential and spike frequency are not primarily regulated by intracellular Ca^{2+} release, since ACh, CCh, caffeine and ryanodine had no significant effect on these parameters. Surprisingly, caffeine and ryanodine had no effect on the long lasting depolarisation frequency. This conflicts with the view that these events are induced by release of Ca^{2+} from intracellular stores.

There was an increase in force upon both caffeine and ryanodine application. The effect of ryanodine is caused by the mechanism of inhibition of the release of Ca^{2+} from the SR by binding to the receptor and locking it into a permanently open subconductive state, resulting into a leaky SR [30]. CCh application in the presence of ryanodine only resulted in a significant increase in force in a normal Krebs' solution.

In human urinary bladder, Masters & colleagues [53] have shown that the internal stores are the predominant source of Ca^{2+} for contraction, however, the release of intracellularly stored Ca^{2+} is highly dependent on an influx of external Ca^{2+}.

Conclusions

The present results show that only a very small amount of Ca^{2+} in the extracellular space was sufficient to support spontaneous and induced contractions. Furthermore, the results suggest that in human urinary bladder smooth muscle the ratio of participation of the two Ca^{2+} stores in the mechanism of contraction is dynamic: inhibition of the intracellular Ca^{2+} stores shifts the ratio of contribution of the two sources towards increased participation of extracellular Ca^{2+} and vice versa. Similar results have been found in rabbit detrusor [18].

V

Intracellular electrical activity in human urinary bladder smooth muscle: the effect of high sucrose medium

Adapted from: Visser and van Mastrigt submitted

Summary

The primary key to pharmacotherapy of bladder instability is in the excitation-contraction coupling of detrusor smooth muscle cells. To study this process, simultaneous recordings of mechanical and electrical activity are required. However, recordings of mechanical activity induces movement, which may affect the quality of intracellular recordings. We therefore compared the electrical activity of human detrusor smooth muscle cells in normal Krebs' and in a hypertonic solution, which immobilises the tissue, enabling us to study the effect of movement on the membrane potential. Carbachol and KCl were applied to induce contractions.

Sucrose in the medium made the tissue rigid and abolished its movement, while the electrical response was not affected. When compared with recordings in normal Krebs' solution, the average resting membrane potential was not altered. However, the membrane potential was more stable, with far less spike-shaped potentials. The spike-shaped potential amplitude was larger, while the duration was decreased. Impairing the ability of tissue movement thus resulted in changes in the electrophysiological properties of detrusor smooth muscle cells. The results suggest that stretch has an effect on L-type Ca^{2+} channels.

Introduction

Urine is stored in the bladder at low pressure, to be expelled rapidly at a convenient moment. To accomplish this, the detrusor muscle must have a high compliance in the resting phase and be capable of generating a high pressure to initiate and maintain the flow of urine. In some patients, however, detrusor muscle contractions occur during the storage phase. This may lead to involuntary urine loss, necessitating research into the mechanisms involved in the initiation of detrusor contraction. Electrophysiological techniques are tools to study the physiological basis of bladder disorders like instability and low compliance. Until now, most intracellular recordings have been made in animal preparations, mainly in guinea-pig [8; 9; 11-13; 15; 22; 24; 27; 28; 33; 34; 44; 45; 58; 59; 65; 66] and some in rabbit detrusor [11; 27; 72].

There are very few published records of electrical activity recorded from intact urinary bladder smooth muscle of any large mammal, let alone man, probably due to the fact that the detrusor muscle cells display continuous mechanical activity, and possess an extensive extracellular matrix containing collagen and elastin [85], which makes it exceptionally difficult to impale these cells. Apart from a few studies in human isolated detrusor cells [38; 56], only our group has performed intracellular electrophysiological recordings of human detrusor strips [75-77]. Reference studies are therefore lacking. The results we obtained in human studies differ from those obtained by others in animal studies. Differences between species in functional and contractile behaviour of bladder muscle have been reported previously [67], however, there could also be another explanation.

To study the initiation of bladder contraction, we made simultaneous recordings of mechanical and electrical activity. In such experiments, movement of the muscle tissue might influence the electrophysiological recording. In guinea-pig detrusor strips, most intracellular recordings have been performed in a solution made hypertonic by adding sucrose to the medium, which inhibits contractions [71].

In rabbit detrusor, there are considerable differences between the parameters of the action potentials recorded in normal Krebs' solution or high sucrose medium. In control medium, Ursillo [72] recorded spike potentials of varied size, shape and frequency without ever exceeding 0 mV. Similarly, in hypertonic solution [11], the spikes also showed considerable variation in shape. However this time, overshoot potentials of up to 18 mV were recorded. These findings suggest that movement of the tissue may cause a difference in intracellular recordings. Our previous results in human detrusor resembled the results from Ursillo [72].

In the present study, we therefore compared the electrical activity of human detrusor smooth muscle cells in normal Krebs' solution and in a hypertonic solution, which immobilised the tissue, enabling us to study the effect of movement on the membrane potential.

Materials and methods

Biopsy samples

Detrusor biopsy samples were collected from 12 patients (3 women; 9 men) ranging in age from 49 to 88 years (mean: 69 years; s.d.: 13 years) with approval from the local ethical committee and informed consent. The patients were undergoing surgery for bladder or prostate cancer and the tissue was collected using either cold-cup biopsy forceps (trans-urethral biopsies) or at open surgery (radical prostatectomy). Immediately after excision, the samples were stored in 0.9% NaCl. Outside of the operating theatre, this solution was replaced by modified Krebs' solution (in mM): 1.8 $CaCl_2$; 1.2 $MgSO_4$; 118 NaCl; 4.7 KCl; 25.0 $NaHCO_3$; 1.2 KH_2PO_4; 11.0 Glucose. By aerating the solution with a mixture of 95% O_2 and 5% CO_2, the pH was adjusted to 7.4. The tissue was transported to the laboratory within ten minutes after excision, pinned down on Sylgard 184 (Dow Corning, Ithaca, NY, USA) and a muscle bundle of about 2 mm long and 0.3 mm wide was dissected. Four of the biopsy samples were stored at 4°C overnight before a muscle bundle was dissected. Connective tissue was removed mechanically.

Electrophysiological recordings

All 12 biopsies were first tested for viability with carbachol (CCh) in normal Krebs'. Subsequently, from 6 biopsies recordings were made in normal Krebs' solution, and from 6 others in Krebs' solution made hypertonic with 15 g sucrose per 100 ml Krebs' to abolish movement [11]. One side of the muscle bundle was pinned down in a 4 ml recording chamber with a Sylgard 184 bottom on the stage of an inverted microscope (magnification 40x; Zeiss) which was gradually heated to 35°C using a modified thermostat. The free part of the muscle bundle was connected to the forceps of a force transducer (BAM4C, Scientific Instruments, Heidelberg, Germany). The muscle bundle was stretched minimally to enable microelectrode recordings.

Intracellular recordings were made using borosilicate glass microelectrodes with a flexible tip (GC120F, Clark Electromedical Instruments, Pangbourne Reading, GB). Filled with 3 M KCl, the electrodes had 40-60 MΩ resistance. They were placed in an Ag/AgCl pellet microelectrode holder connected to the input stage of a high impedance capacitance-neutralising amplifier (World Precision Instruments, Sarasota, FL, USA). An Ag/AgCl electrode (outer diameter of 2.0 mm) in an agar bridge served as the reference electrode in the organ bath. To minimise movement artefacts, the cells were speared in the longitudinal direction, at the side where the muscle bundle was pinned to the Sylgard. Penetration of a cell was considered successful when the reference potential rapidly shifted from zero to a more negative value of at least -20 mV. After the recording, when the tip of the electrode was no longer inside a cell, the value of the tip potential varied, if at all, less than 5 mV from the zero level.

The electrophysiological signal was amplified 10 times and low pass filtered

with a cut-off frequency of 1 kHz (Krohn-Hite Corporation, Avon, MA, USA). Both this signal and the signal from the force transducer were then AD converted at a sample rate of 2 kHz (DAS1800, Keithley MetraByte, Taunton, MA, USA) using a locally developed sampling program and digitally stored in a PC. The digitised force signal was filtered with a 4th order Butterworth filter, using a cut-off frequency of 2 Hz to reduce the noise level. The shape of the signal was not affected by the filtering process.

The effect of CCh (Pharmachemie B.V., the Netherlands) and KCl on the membrane potential were studied. Stock solutions of the substances were applied in random order to the bath in units of 0.01 ml, resulting in minimal bath concentrations of $3.4 \cdot 10^{-6}$ M CCh and $12.2 \cdot 10^{-3}$ M KCl.

The bathing solution was refreshed constantly at $1.4 \cdot 10^{-2}$ ml/s, renewing the bath contents within 5 minutes. Difficulties with making long intracellular recordings were inherent to the type of preparation, therefore substances were often applied before total washout of the previous application. In those cases, the effective concentration was calculated using a standard concentration distribution formula. In the figure legends the final concentrations of the substances in the bath are given.

Data analysis

The average resting membrane potential of the impaled cell, V_{rest}, was calculated during 30 s before the stimulus. There was a clear maximal depolarisation upon application of KCl, in contrast to CCh application. Therefore, in the result section, the electrical response to KCl was specified in terms of the maximal depolarisation. For CCh, the response was noted as the average V_{rest}, calculated during 30 s immediately after stimulation. Resting membrane potential values more negative than -100 mV were sometimes recorded. These results were considered to be an artefact, most likely from bending of the electrode and were therefore excluded from further analysis.

There were spontaneous fluctuations from the resting membrane potentials in the form of spike-shaped potentials. The frequency of these events was determined in the same periods as the average resting membrane potential was determined, using especially developed software written in Matlab® 4.2c1 (The Mathworks Inc., Natick, MA, USA), as described previously [75]. The following parameters characterising these events were determined with a second program [75]: the amplitude was defined as the difference between the membrane potential value at the onset and the peak value of the event. The duration was calculated at 10% of the maximum amplitude ($d_{10\%}$) to reduce noise. Negative duration values and amplitudes under 5 mV were excluded from further analysis, since previous work showed that these were caused by incorrect parameter estimation [75]. This percentage of incorrect parameter estimation was 32% and similar to the ones in previous studies [75; 76]. Force values were read at the average level during 30 s preceding the stimulus and at the maximum after it. The calculated parameters were imported in Excel 5.0a. The Mann-Whitney U-test and Wilcoxon's signed ranks test were done using SPSS 8.0 (SPSS Inc., Chicago, USA).

Results

Force

The average force level before the experiment was 217 μN in the control group and 403 μN in the sucrose group. This difference was not significant (p=0.699; n=12). Upon testing for viability in normal Krebs' solution, both groups responded with a significant force increase upon application of CCh (p=0.028; n=6 and p=0.028; n=6), and this response did not differ significantly between the groups (p=0.310; n=12). The basal force level of both groups was higher when the experiment started and impalements of detrusor muscle cells were attempted. However, this effect did not reach the level of significance (p≥0.116). The resting force level at the beginning of the experiment did not differ significantly between the control group and the sucrose

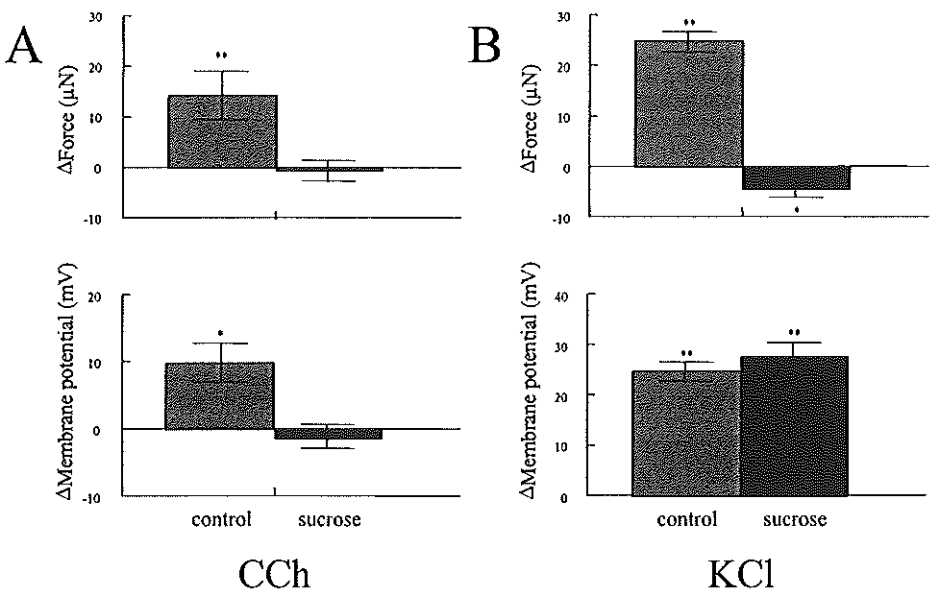

Figure 1. *The response of human urinary bladder smooth muscle strips to application of CCh (A.) and KCl (B.) in control medium (light bar) and high sucrose Krebs' (dark bar). The upper panels represent the difference in force, the lower panels show the difference in membrane potential before and after stimulation. All values are mean and s.e.m. Changes with p-values smaller than 0.05 and 0.01 are represented by * and **, respectively.*

group (p=0.093; n=12).

During the experiment, application of CCh resulted only in a significant increase in force in control medium (p<0.001; n=19), not in high sucrose medium (p=0.763; n=39; Figure 1A, upper panel). Also, application of KCl resulted in a significant increase in force level in normal Krebs' (p<0.001; n=19; Figure 1B, upper panel). However, KCl application in medium containing sucrose caused no contractile response, but resulted in a small (mean 4 μN; s.e.m.: 2 μN) but significant (p=0.031; n=39) relaxation of the tissue (Figure 1B, upper panel).

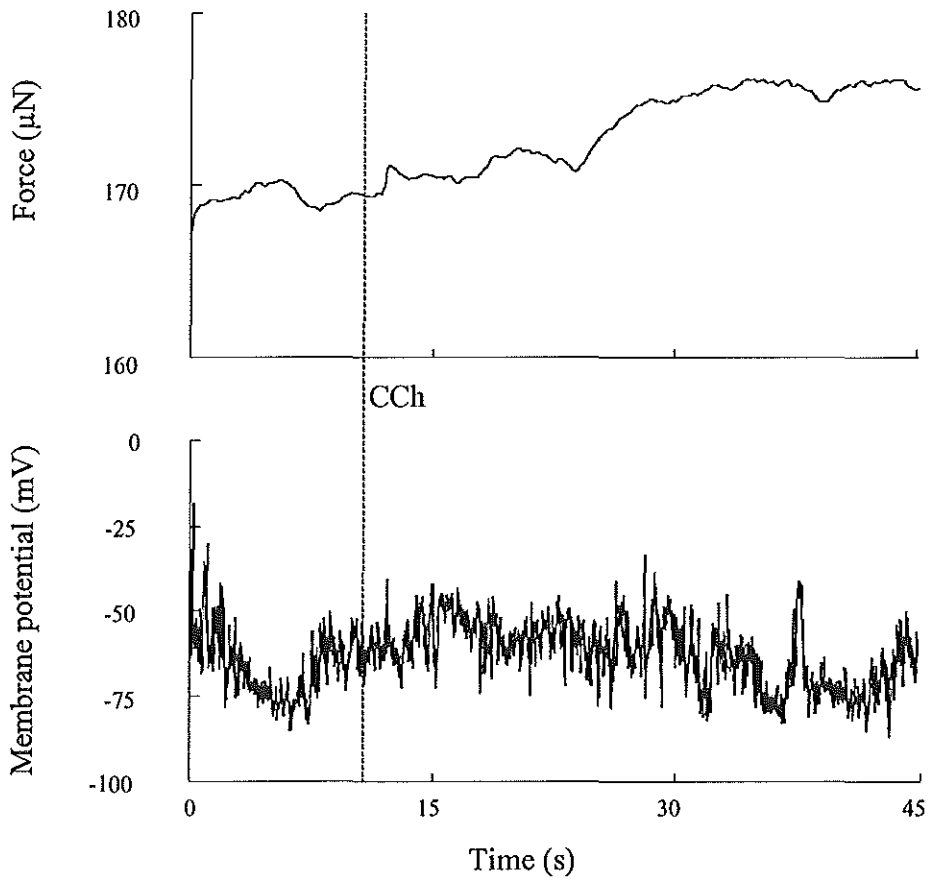

Figure 2. *Example of CCh application in control Krebs'. The concentration of CCh applied at the dotted line was $3.5 \cdot 10^{-6}$ M. Upper trace: force; lower trace: membrane potential.*

Membrane potential recordings

The average resting membrane potential, determined during 30 seconds before application of a stimulus, was -55 mV (s.e.m.: 4 mV; n=38) in control Krebs' and -52 mV (s.e.m.: 2 mV; n=78) in high sucrose medium. These values did not differ significantly (p=0.881; n=116).

Application of CCh resulted on average in a membrane potential depolarisation of 10 mV (s.e.m.: 4 mV; p=0.023, n=19) in control medium within 30 s (Figure 1A, lower panel). In high sucrose Krebs' there was no significant effect on the resting membrane potential (n=0.763; n=39; Figure 1A, lower panel). Figure 2 and Figure 3 give examples of CCh application in normal Krebs' solution and in high

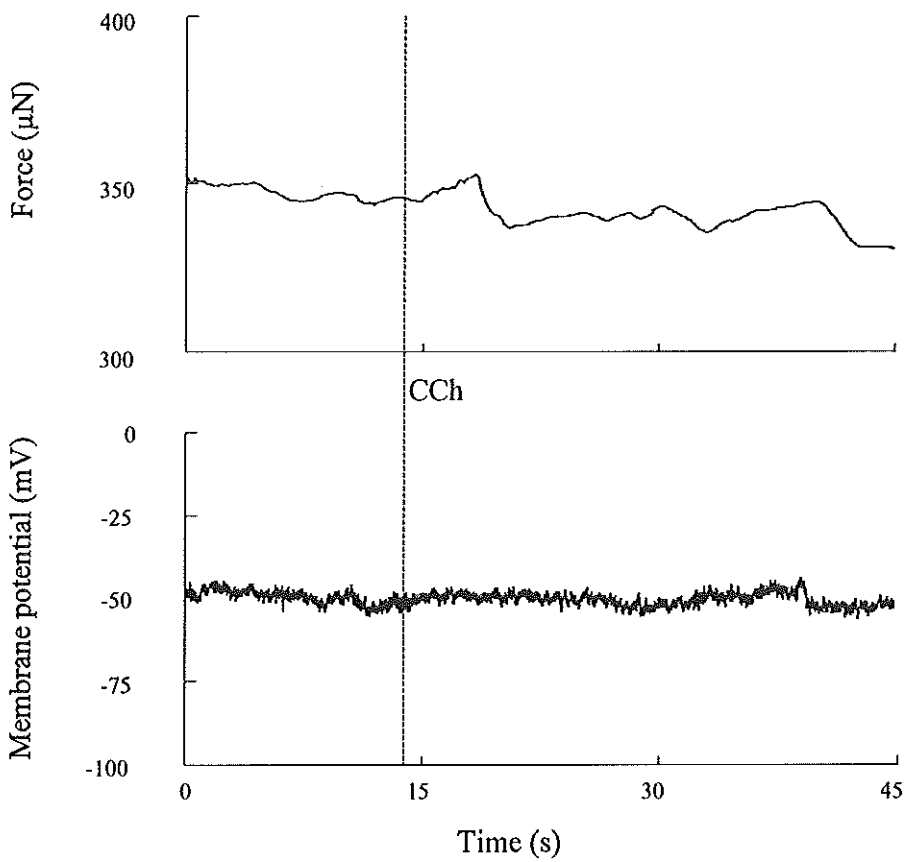

Figure 3. *Example of CCh application in high sucrose medium. The concentration of CCh applied at the dotted line was $4.3 \cdot 10^{-5}$ M. Upper trace: force; lower trace: membrane potential.*

sucrose medium, respectively.

In control Krebs', the maximal response to KCl application was on average 25 mV (s.e.m.: 3 mV) and in high sucrose the depolarisation was 28 mV (s.e.m.: 3 mV), (Figure 1B, lower panel). Adding sucrose to the medium had no significant effect on the maximal depolarisation in response to KCl application (p=0.673; n=58). Figure 4 and Figure 5 give two typical examples of the response to KCl application in normal Krebs' solution and in high sucrose medium, respectively.

In both media, the impaled cells displayed spontaneous activity in the form of spike-shaped potentials. In normal Krebs' solution, the median amplitude of the spike-shaped potentials was 10 mV and overshoot potentials were not recorded. Sucrose in the medium had a significant effect on the median amplitude and the duration of the

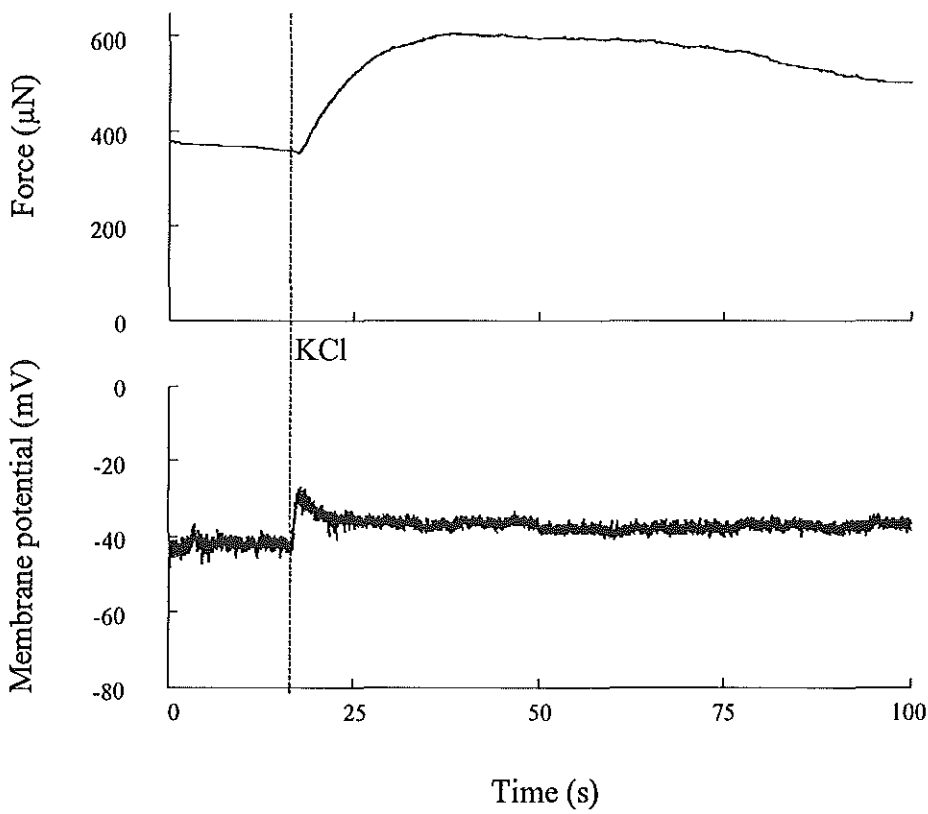

Figure 4. *Example of KCl application in control Krebs'. The concentration of KCl applied at the dotted line was $12.2 \cdot 10^{-3}$ M. Upper trace: force; lower trace: membrane potential.*

spike-shaped potentials ($d_{10\%}$; Table 1). The value of the amplitude increased by 2 mV (Table 1; $p<0.001$; n=3587) and the median value of the duration decreased by 9 ms to 23 ms (Table 1; $p<0.001$; n=3587). Also in high sucrose medium, spike-shaped potential amplitudes crossing the 0 mV were not registered. Figure 6 shows examples of spike-shaped potentials recorded in normal medium (Figure 6A) and high sucrose Krebs' (Figure 6B).

Adding KCl or CCh to the bath did not affect the frequency of the spike-shaped potentials; neither in normal Krebs' solution ($p\geq0.284$), nor in high sucrose medium ($p\geq0.091$). The presence of sucrose in the medium itself, however, decreased the spike-shaped potential frequency ($p=0.001$; n=116; Table 1).

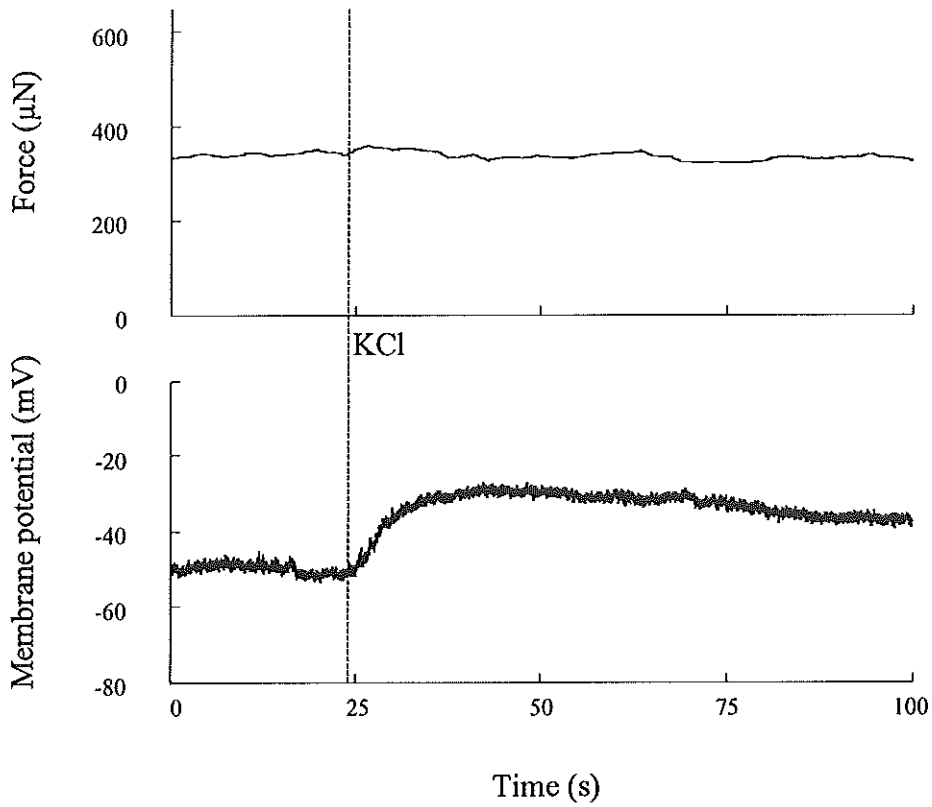

Figure 5. Example of KCl application in high sucrose medium. The concentration of KCl applied at the dotted line was $13.3 \cdot 10^{-3}$ M. Upper trace: force; lower trace: membrane potential.

spike-shaped potential parameters		control	sucrose	p-value
amplitude (mV)	median	9.7	12.0	<0.001
	i.q.r.	7.9	7.3	
$d_{10\%}$ (ms)	median	31.5	23.0	<0.001
	i.q.r.	58.0	15.5	
	n	1501	2086	
frequency (s^{-1})	median	0.40	0.02	0.001
	i.q.r.	1.55	0.17	
	n	38	78	

Table 1. *The features of spike-shaped potentials in human detrusor strips. The values of the median and interquartile range (i.q.r.) of the amplitude, duration ($d_{10\%}$) and frequency in control Krebs' and high sucrose medium are given. The p-values and number (n) are noted.*

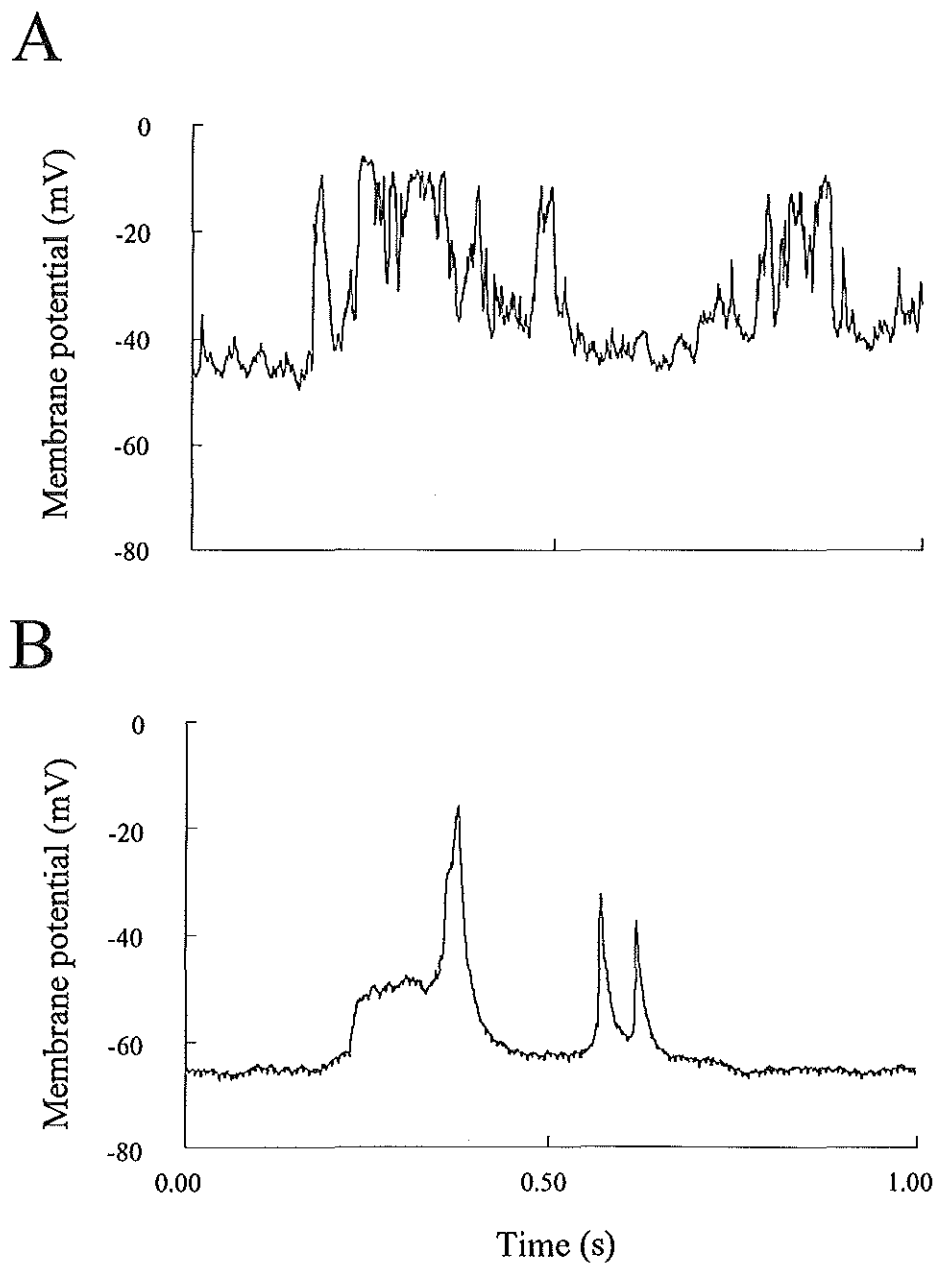

Figure 6. Examples of spike-shaped potentials recorded in human urinary detrusor strips in control medium (A.) and high sucrose Krebs' (B.).

Discussion

Electrophysiological studies provide insight into the mechanisms involved in the initiation of detrusor contraction. Previously, we made simultaneous studies of force and membrane potential of human detrusor strips. These results differed from those obtained by other groups in animal studies. Most of those studies were performed in high sucrose medium, which abolishes tissue movement. Movement may affect the recordings of intracellular electrical activity. In this study we therefore compared electrical activity recorded in human detrusor muscle cells in a hypertonic solution, with recordings made in normal Krebs' solution.

In the hypertonic solution, the muscle strips appeared stiff and rigid. The force response to application of CCh or KCl disappeared in high sucrose medium. Application of KCl even resulted in a small relaxation of the tissue, while the membrane electrical response was unaltered. This relaxation was not a side-effect of the application method, since a similar effect was not seen in response to the application of CCh.

In both media, resting membrane potentials more negative than -100 mV were sometimes recorded. These values resulted from artefacts, most likely bending of the electrode. This was not prevented by immobilising the tissue with high sucrose. The average resting membrane potential was not significantly altered by the presence of sucrose in the medium. This was observed before, in rat detrusor [17].

The response to CCh was rather variable, but in this set of experiments, in contrast to our previous study [77], on average a small depolarisation was seen in control conditions. This effect did not occur in high sucrose medium. The depolarisation of the membrane potential upon CCh application in our control group could result from dislocation of the electrode by mechanical movement of the tissue, since the effect was lacking in high sucrose medium. However, depolarisation due to CCh application has also been reported in high sucrose medium in guinea-pig intracellular recordings [34; 66]. CCh is an agonist of the muscarinic receptor, which is coupled to a second messenger system [37]. Therefore, activation of this system can produce contractions without changes in the membrane potential [27]. KCl induced a depolarisation of the membrane potential in both groups of a similar magnitude.

In hypertonic solution, the electrical recordings resembled our earlier results in human detrusor strips more closely than the results obtained in animal models by other groups, although the resting membrane potential was more stable; the frequency of the spike-shaped potentials diminished. Meanwhile, the duration of these events decreased and the amplitude increased. This effect on amplitude and frequency has been observed before in guinea-pig taenia coli [71].

These results indicate the possibility that movement of the tissue affected the electrical activity of the cells. The more stable resting membrane potential and the reduced spike-shaped potential frequency could be explained by the reduced chance of dislocation of the microelectrode by movement of the tissue, which is more likely in normal Krebs' solution. Alternatively, stretch receptors present in the cell membrane may have been activated by movement. This type of receptors has been described in human detrusor smooth muscle tissue [52]. Dislocation of the microelectrode could

easily explain a depolarisation of the membrane, but not a change in the spike-shaped potential parameters, favouring the second hypothesis. Sucrose had an effect on the spike-shaped potentials suggesting that stretch operated receptors are influencing the opening time of the L-type Ca^{2+} channels.

In conclusion, making the Krebs' solution hypertonic by adding sucrose to the medium resulted in changes in the electrophysiological properties of detrusor smooth muscle cells. In the hypertonic solution, the tissue was rigid and mechanical activity was abolished. The ability to generate spike-shaped potentials and the general shape of these events were not affected. When compared with recordings in normal Krebs' solution, the membrane potential was more stable and the spike-shaped potential amplitude was larger, while the duration was decreased. The results suggest that stretch operated receptors have an effect on L-type Ca^{2+} channels.

VI

Simultaneous recording of mechanical and intracellular electrical activity in guinea-pig urinary bladder smooth muscle; a comparison with human detrusor contraction

Adapted from: Visser and van Mastrigt
Urology, in press

Summary

The guinea-pig is the most used model to study the mechanism of human bladder contraction. We have studied the relationship between electrical and mechanical activity of guinea-pig detrusor muscle and compared this to results obtained in human tissue. We simultaneously recorded mechanical and intracellular electrical activity in muscle strips. To study the effect of tissue movement on the membrane potential, the medium was made hypertonic with sucrose. Carbachol and KCl were applied to the bath to induce contractions. Carbachol resulted in a force response without a consistent change in membrane electrical activity. KCl induced depolarisation of the membrane associated with force development. Sucrose in the medium greatly impaired the ability to contract, without affecting the electrical activity. Compared with recordings in normal Krebs' solution, the resting membrane potential was not altered. In both media, spike-shaped potentials with variable amplitudes and shapes were recorded. These events were minimally affected by sucrose. In contrast to the response to KCl, the overall mechanical response of guinea-pig detrusor strips to muscarinic receptor stimulation was not correlated to the electrical activity of a single cell. Sucrose only had a minimal effect on the electrical activity, showing that the electrical responses we measured were not affected by movement. Our intracellular recordings in guinea-pig tissue differed from the recordings obtained by other groups, but show great resemblance with those we have recorded in human urinary bladder smooth muscle strips, validating the use of guinea-pig as a model for human detrusor contraction.

Introduction

Patients with symptoms of urgency may have involuntary detrusor contractions. Combined electrophysiological and mechanical studies can lead to more insight in the initiation of detrusor contraction and might eventually lead to a cure for bladder dysfunctions. The sucrose-gap technique is the best method to study this relationship between mechanical and electrical activity. In guinea-pig it demonstrates an association between electrical activity and muscle contractions [11; 22; 45; 59].

The possibility to record electrical activity through the sucrose-gap method exists by the grace of electrical connectivity of the tissue. In contrast to guinea-pig bladder smooth muscle [24], human detrusor is badly electrically coupled [6], which vitiates the technique for this tissue. Consequently, in human detrusor an alternative method, the microelectrode technique, has to be used.

Apart from a few studies in human isolated detrusor cells [38; 56], only our group has performed intracellular electrophysiological recordings combined with force measurements of human detrusor strips [75-77]. In these studies, intracellular electrical activity and force were not correlated. Additionally, the electrical activity we recorded differed from those other groups obtained in animal studies. Differences between species in functional and contractile behaviour of bladder muscle have been reported previously [67]. Since guinea-pig is the most used model to study the mechanism of bladder contraction, it is important to know if there are differences in electrical activity between guinea-pig and human detrusor. Presently, we simultaneously recorded intracellular electrical activity and tension in guinea-pig detrusor smooth muscle strips. To induce contractions, CCh and KCl were applied. Most guinea-pig studies were performed in high sucrose medium [11; 13; 22; 34; 59; 65], which abolishes contraction [71]. Since mechanical activity is known to influence electrical activity [80], we used both normal and hypertonic media. In the discussion the present results are compared to our results obtained earlier in human detrusor strips.

Materials and methods

Guinea-pigs (343 to 610 g; n=8: 2 females, 6 males) were anaesthetised with ketamine and rompun dosed to the weight of the animal. The animals were killed by asphyxiation with CO_2. The abdominal cavity was opened, and the urinary bladder was removed and placed in cold, modified Krebs' solution (pH 7.4). The bladder was opened and pinned to the bottom of a Sylgard dish with the mucosal side up. The mucosa was carefully removed with microscissors and a single muscle bundle of about 3 mm long and 0.2 mm wide was dissected. One side was pinned down, the other side was connected to the forceps of a force transducer. The muscle bundle was tested with CCh for viability and then stretched minimally to enable microelectrode recordings. Recordings were made in a standard set-up [77]. Contractions were induced by applying KCl and CCh. During the experiment, hypertonic Krebs' solution, containing 15 g sucrose per 100 ml Krebs' [11] was washed in. Sometimes the impalements were very stable and the bathfluid could be changed during a recording. In other measurements, a new cell was impaled after washing in the hypertonic medium. The signals were stored in a PC.

The average resting membrane potential of the impaled cell was calculated during 30 s before the stimulus. There was a clear maximal depolarisation upon application of KCl. Therefore, the electrical response to KCl was specified in terms of this maximum. As there was no such obvious maximal response to CCh, in that case the average resting membrane potential was calculated during 30 s immediately after stimulation.

Resting membrane potential values more negative than -100 mV were sometimes recorded. These results were considered to be an artefact and excluded from further analysis. Spontaneous fluctuations from the resting membrane potential in the form of spike-shaped potentials were detected automatically [75]. The amplitude and duration at 10% of the maximum amplitude ($d_{10\%}$) of these events were determined as described previously [75]. The Mann-Whitney U-test and Wilcoxon's signed ranks test were done using SPSS 8.0 (SPSS Inc., Chicago, IL, USA).

Results

Force

The average force level before the experiment was 314 μN. Upon application of CCh to test for viability, the strips responded with a significant force increase ($p=0.012$; $n=8$). After washing in high sucrose medium, the basal force level increased slightly, but not significantly ($p=0.263$; $n=8$).

During the experiment, application of CCh resulted in an increase in force, both in control ($p<0.001$; $n=45$) and in high sucrose medium ($p<0.001$; $n=34$; Figure 1A, upper panel). Sucrose in the medium drastically reduced the response ($p=0.004$; $n=79$; Figure 1A, upper panel). Similarly, application of KCl resulted in an increase in force level in normal Krebs' ($p<0.001$; $n=34$; Figure 1B, upper panel), and a significantly smaller contraction in hypertonic solution ($p=0.005$; $n=63$; Figure 1B, upper panel).

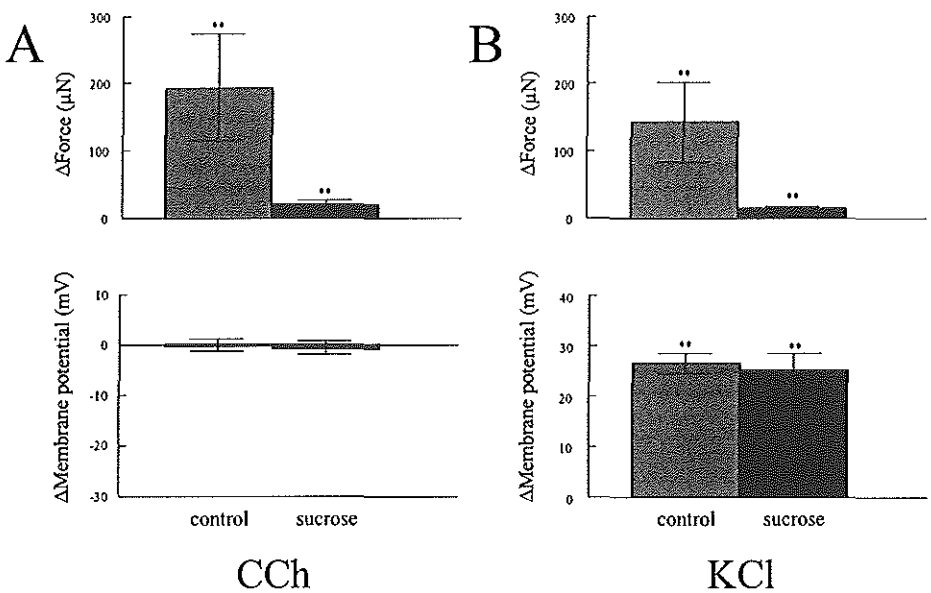

Figure 1. *The effect of CCh (A.) and KCl (B.) on force level (upper panel) and the membrane potential (lower panel) of guinea-pig detrusor smooth muscle strips in control medium and high sucrose Krebs'. The light bar represents the change upon stimulation in normal medium, the dark bar in hypertonic Krebs'. All values are mean and s.e.m. Changes with a p-value smaller than 0.01 are represented by* **.

Membrane potential recordings

The average resting membrane potential was -53 mV (s.e.m.: 2 mV; n=79) in control Krebs' and -54 mV (s.e.m.: 2 mV; n=63) in high sucrose medium. These values did not differ significantly (p=0.978; n=142). Application of CCh did not have any systematic effect on the membrane potential in control Krebs' (p=0.731; n=45); nor in hypertonic Krebs' (n=0.489; n=34); (Figure 1A, lower panel). Examples of CCh application in normal Krebs' solution and in high sucrose medium are shown in Figure 2 and Figure 3, respectively.

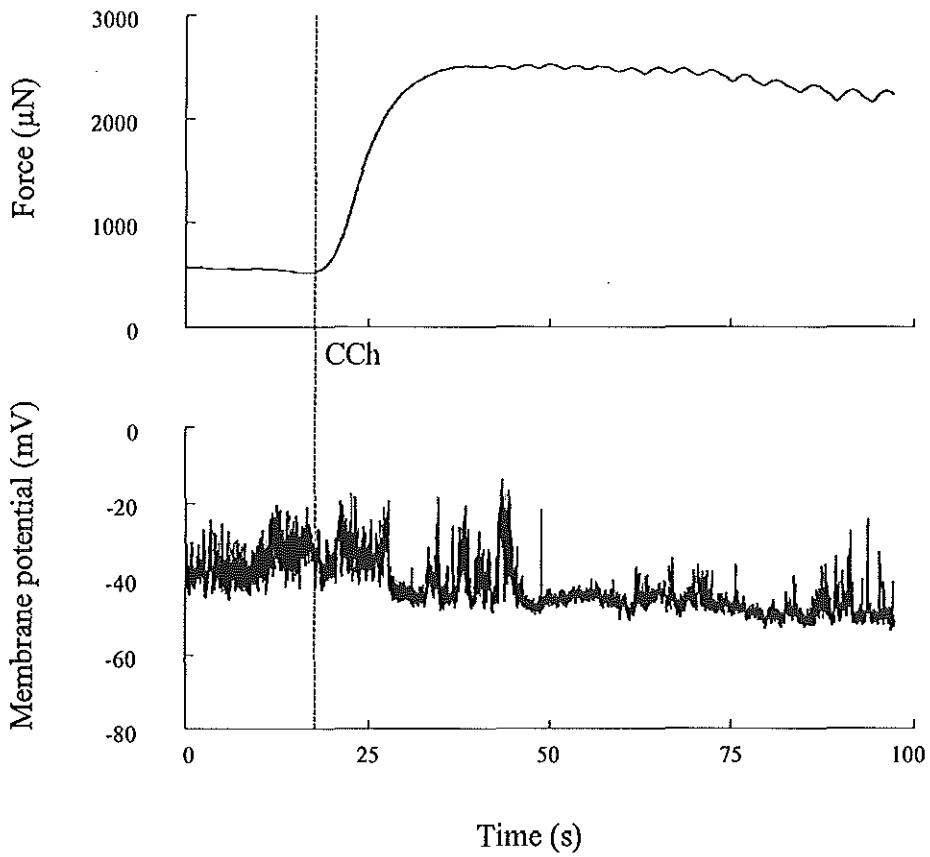

Figure 2. *Example of CCh application in control Krebs'. The concentration of CCh applied at the dotted line was $1.7 \cdot 10^{-5}$ M. Upper trace: force; lower trace: membrane potential.*

The maximal response to KCl application was on average 26 mV (s.e.m.: 2 mV) in control Krebs' and 24 mV (s.e.m.: 3 mV) in high sucrose (Figure 1B, lower panel). This difference was not significant (p=0.847; n=63). Figure 4 and Figure 5 show two typical examples of the response to KCl application recorded in the same cell, before (Figure 4) and after washing in high sucrose Krebs' (Figure 5).

In both media, the majority of the cells displayed spontaneous activity in the form of spike-shaped potentials, with considerable variation in size and duration. Figure 6 shows two examples of spike-shaped potentials recorded in the same cell before (upper trace) and after (lower trace) washing in high sucrose medium.

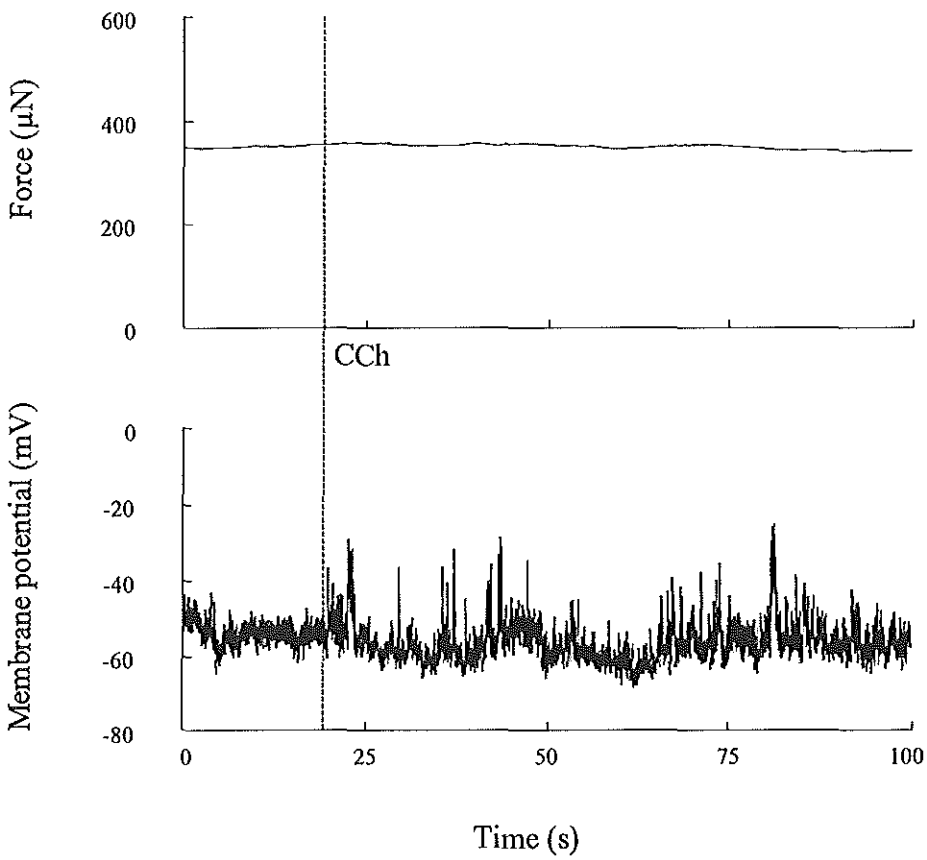

Figure 3. Example of CCh application in high sucrose medium. The concentration of CCh applied at the dotted line was $2.2 \cdot 10^{-5}$ M. Upper trace: force; lower trace: membrane potential.

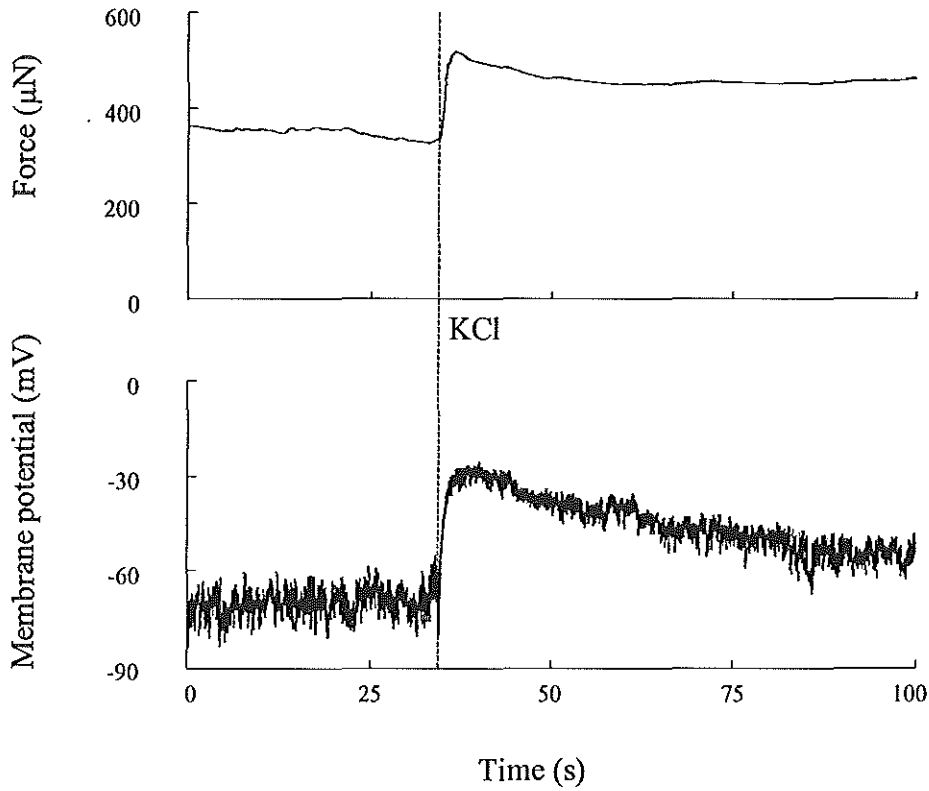

Figure 4. Example of KCl application in control Krebs'. The concentration of KCl applied at the dotted line was $4.9 \cdot 10^{-2}$ M. Upper trace: force; lower trace: membrane potential.

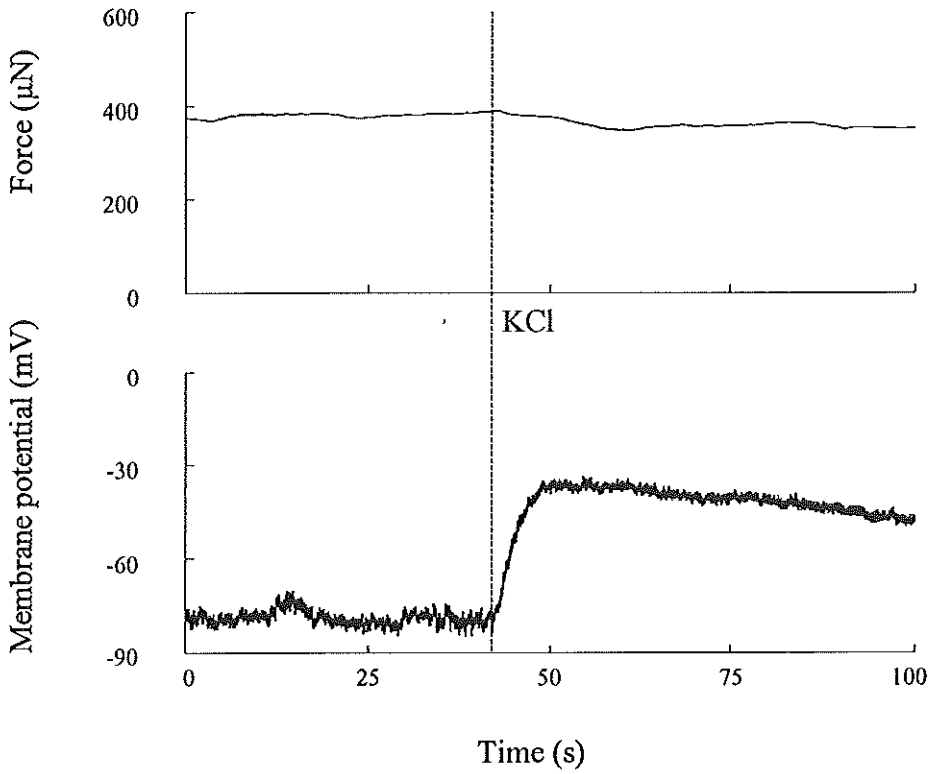

Figure 5. *Example of KCl application in high sucrose medium. The concentration of KCl applied at the dotted line was $5.8 \cdot 10^{-2}$ M. Upper trace: force; lower trace: membrane potential. Figure 4 and Figure 5 were recorded in the same cell.*

In normal Krebs' solution, the median amplitude of the spike-shaped potentials decreased ever so slightly (Table 1; $p<0.001$; $n=3337$) while the median value of the duration ($d_{10\%}$) increased (Table 1; $p<0.001$; $n=3337$). Spike-shaped potential amplitudes crossing the 0 mV were not recorded in either medium. Sucrose on itself did not affect the frequency of the spike-shaped potentials, and neither did the application of KCl or CCh in either Krebs' solution ($p\geq0.103$).

spike-shaped potential parameters		control	sucrose	p-value
amplitude (mV)	median	12	11	<0.001
	i.q.r.	7	6	
$d_{10\%}$ (ms)	median	23	24	<0.001
	i.q.r.	15	13	
	n	1797	1540	
frequency (s^{-1})	median	0.03	0.05	0.755
	i.q.r.	0.15	0.33	
	n	45	34	

Table 1. *Features of spike-shaped potentials in guinea-pig detrusor strips. The values of the median and interquartile range (i.q.r.) of the amplitude, duration ($d_{10\%}$) and frequency in control Krebs' and high sucrose medium are given. The significance levels and number (n) are noted.*

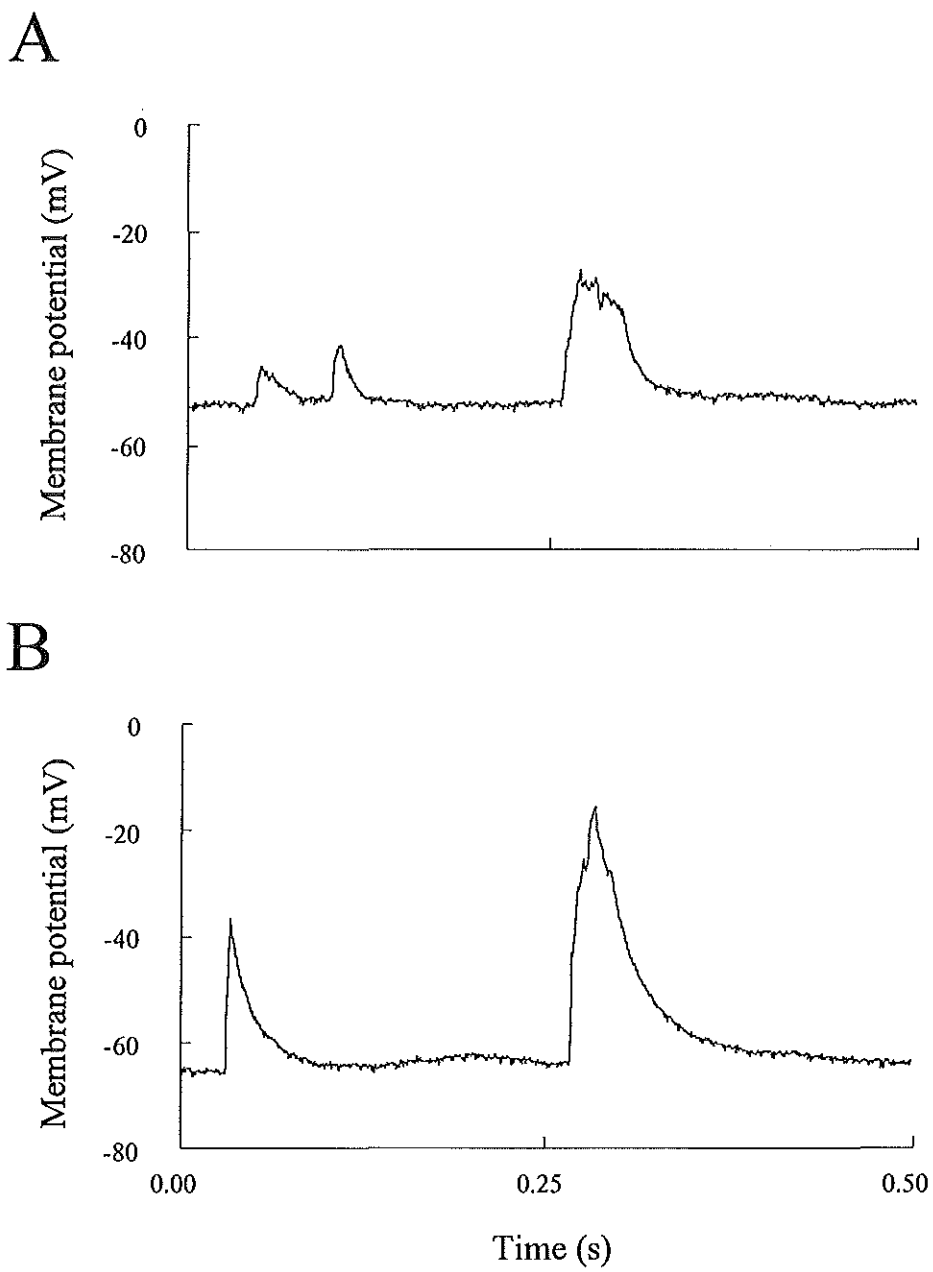

Figure 6. *Examples of intracellular electrical activity in a guinea-pig detrusor smooth muscle strip cell in control Krebs' (A.) and after washing in high sucrose medium (B.). Figure 4, Figure 5 and Figure 6 were recorded in the same cell.*

Discussion

The guinea-pig is the most used model for research into the mechanism of human detrusor contraction. It is therefore important to study possible functional differences between these species. We simultaneously recorded intracellular electrical activity and force in guinea-pig detrusor smooth muscle strips. Upon washing in hypertonic Krebs', the average resting force increased slightly but not significantly, while the ability to contract upon CCh or KCl stimulation was drastically reduced.

Sucrose in the medium did not affect the average resting membrane potential. This has been observed before, in rat detrusor [17] and in human detrusor [78]. The average resting membrane potential of -53 mV we recorded in guinea-pig detrusor was more hyperpolarised than most values described in the literature, where average resting membrane potentials between -35 mV and -45 mV are reported [11; 13; 33; 45; 58; 66]. However, a mean resting membrane potential of -61 mV has been reported too [22].

Increasing the K^+ concentration in the medium induced a contraction and a large depolarisation of the membrane potential. The electrical response was not altered by adding sucrose to the medium, while the force development was impaired. This demonstrates that the electrical responses we measured were not affected by movement.

Application of CCh induced a force response, but did not result in a consistent change in the resting membrane potential or the electrical activity in the cell, either in control Krebs' or in high sucrose medium. The muscarinic receptor is coupled to a second messenger system [37] and activation of this system may produce contractions without changes in the membrane potential. In other guinea-pig studies however, the application of the muscarinic agonists ACh [11; 12; 33] and CCh [34; 66] depolarised the membrane potential and resulted in an increase in spike frequency in high sucrose medium. In a study in which force measurements were combined with electrical activity measured using the sucrose-gap technique, CCh depolarised the membrane potential and increased the spike frequency associated with an increase in force [58]. This was not generally seen in the present intracellular study. This difference may be explained by the fact that in sucrose-gap recordings the electrical properties of a population of cells is measured, which likely reflects the overall activity more clearly than single cell intracellular recordings. The electrical activity of one single cell may not be associated with the overall changes in tension of the whole muscle bundle. In previous studies in human bladder we found similar results to the present ones in guinea-pig [76; 77].

The majority of the cells displayed spike-shaped potentials in normal and hypertonic Krebs', varying considerably in amplitude and shape. Such variation in amplitude has been published before [13; 34; 65], although considerably higher mean values of amplitudes were reported. [35; 39].

Sucrose decreased the median value of the amplitude of the spike-shaped potentials and increased their duration both ever so slightly, without affecting the frequency. As in human bladder [78], this minimal effect illustrates that the possibility

of movement exerts influence on the electrical activity of the cells. In both species, stretch activated channels have been reported [52; 79]. Longitudinal stretch activates these channels and induces a Ca^{2+} influx [52; 79]. Stretch can also modulate Ca^{2+} influx through L-type Ca^{2+} channels via changes in the membrane potential [80]. Impairing the force development with hypertonic Krebs' solution and consequently reducing the ability of tissue movement would result in a decrease in amplitude if Ca^{2+} influx through stretch operated channels is participating in the spike-shaped potential depolarisation. Indeed, the results show this effect.

In conclusion, upon muscarinic receptor stimulation, guinea-pig detrusor strips behaved similarly to human tissue [77; 78]: the electrical activity of a single cell was not correlated to the overall mechanical activity. Both in human [77; 78] and in guinea-pig tissue, movement only had a minimal effect on the electrical recordings: spike-shaped potentials were recorded in either solution and their parameters were only minimally affected by sucrose. Ursillo found small spike-shaped potentials in rabbit detrusor [72], which differed from the results other groups found in that same animal [11; 16]. Similarly, the results we obtained in guinea-pig tissue differ from those obtained by others. Still, the guinea-pig recordings in our hands showed great resemblance to our earlier measurements in human detrusor strips [75-78], validating the use of guinea-pig as a model for human detrusor contraction.

VII

General Discussion

One of the functions of the bladder is to store urine under low pressure conditions. Then, at a convenient moment, the fluid is expelled with a co-ordinated contraction under cholinergic control. In striated muscle, a contraction is preceded by an action potential, but in smooth muscle this is not necessarily the case: human detrusor contraction is initiated by the neurotransmitter acetylcholine and the matching receptor, the muscarinic receptor, is connected to a second messenger system. After the binding of the transmitter to the extracellular part of the receptor, the complex undergoes a configurational transformation, dissociating a G-protein and inositol (1,4,5)-trisphosphate. These molecules in turn trigger the release of calcium from the sarcoplasmic reticulum. The amount of intracellularly released calcium may suffice for the initiation of a contraction, without the need of additional calcium influx from the extracellular space. If that is the case, a contraction can thus be initiated without changes in the membrane potential. When we started our experiments, the exact sequence of events upon cholinergic activation and the role of the electrical activity in human detrusor contraction were unclear.

In **Chapter II**, we presented the first electrophysiological recordings of human urinary bladder smooth muscle strips. Different types of spontaneous activity were recorded, varying from one single spike-shaped potential to extensive bursts. This variation existed even between cells within one muscle bundle, indicating that the electrical activity within one muscle bundle is asynchronous. The presence of electrical activity in human detrusor smooth muscle cells thus being established, now the role of this activity in a contraction had to be determined, as this could lead to a method to therapeutically and selectively suppress unstable bladder contractions.

In **Chapter III**, we therefore combined intracellular electrical and mechanical recordings. To induce contractions we stimulated the muscarinic receptors with acetylcholine and carbachol. Consequently, we found force development, without concomitant changes in membrane potential activity. The force response could be blocked by applying the muscarinic receptor antagonist atropine to the bath, again without any effect on the electrical activity. The cells in our preparations were not unable to respond to stimuli though. Increasing the extracellular potassium concentration, and consequently changing the driving force for ions over the membrane, induced a depolarisation concurring with force development. This force resulted from activation of the contractile machinery by calcium entering the cell through voltage sensitive L-type Ca^{2+} channels. Since the electrical activity did not change upon muscarinic receptor stimulation, we concluded that the calcium necessary for a contraction induced by activation of the muscarinic receptor, originates primarily from intracellular stores.

It is known that muscle strips from patients with idiopathic detrusor instability or detrusor hyperreflexia exhibit larger and more frequent spontaneous contractions than strips from normal bladders [41]. It is unclear whether the calcium involved in spontaneous or induced contractions originates from different sources, however, there is reason to assume this. A study in pig detrusor strips has shown a difference in the rate of contraction development between spontaneous and induced contractions, suggesting different pathways to be involved [43]. If indeed the sources of calcium for both types of contractions are different, this would be a promising angle

of attack to develop a method for the treatment of unstable bladder contractions.

To investigate the role of the calcium sources in spontaneous and induced contractions in human detrusor strips, we changed the intracellular and extracellular calcium conditions. The results are presented in **Chapter IV**. Mechanical and electrical recordings were made in three different extracellular calcium conditions: in normal Krebs' solution, in a solution with only ten per cent of the standard calcium concentration and in a medium from which calcium was omitted. The intracellular stores were manipulated with ryanodine and caffeine. These agents respectively interfere with the ability of the cell to store calcium in the sarcoplasmic reticulum, and stimulate the release of calcium from the sarcoplasmic reticulum while at the same time reducing the rate of ATP-dependent calcium uptake. Under these conditions, spontaneous contractions and carbachol or potassium induced contractions were studied.

Our results were in agreement with those obtained by Forman & colleagues [21], showing that the resting tone in human detrusor is regulated by intracellular calcium release. The source of calcium involved in spontaneous human urinary bladder contractions is not known. In our study, spontaneous contractions still occurred in Krebs' solution from which calcium was omitted. However, in this solution, calcium bound to superficial binding sites on the cell membrane was still present. This indicates that only a very small amount of calcium in the extracellular space is sufficient to support spontaneous and induced contractions. Although sometimes an increase in spike frequency in the recorded cell was seen at the onset of a spontaneous contraction, this effect was not significant. It should be kept in mind though, that the electrical activity was measured in only one cell, while the mechanical activity was recorded in the whole strip. It is therefore possible that the impaled cell was not participating in the contraction. However, we could not exclude a role for intracellular calcium release in spontaneous human detrusor contractions.

Masters & colleagues [53] have shown that the internal stores are the predominant source of calcium for a voluntary contraction, although the release of intracellularly stored calcium was highly dependent on the influx of external calcium. Our results suggest that upon muscarinic receptor stimulation, a combined mobilisation of calcium from both intracellular and extracellular stores may occur. The ratio of participation of the calcium stores in the contractile mechanism is thus dynamic and changes in accordance with the requirements of the conditions: inhibition of the intracellular calcium stores shifts the ratio of contributions towards increased participation of extracellular calcium and vice versa. Non-physiological contractions, induced by an increased potassium concentration in the bath, were primarily depending on extracellular calcium.

Electrical activity in the form of spike-shaped potentials and long lasting depolarisations, was recorded under all conditions. Reducing the calcium concentration in the bath decreased the amplitude of the spike-shaped potentials, without affecting the duration, indicating that the opening time of the calcium channels was not influenced. The amplitude of the long lasting depolarisations was increased while the duration was dramatically decreased when the extracellular calcium concentration was raised. With the results presented in this thesis, we can only

speculate about the origin of the long lasting depolarisations, but these events may result from the spontaneous release of calcium from intracellular stores.

The amplitude of the spike-shaped potentials we recorded in human urinary bladder smooth muscle cells was more variable and smaller than those recorded by other groups in animal studies. Most of these studies were performed under conditions where the tissue was immobilised by adding sucrose to the medium. Since human detrusor tissue expresses stretch operated receptors, movement might affect the electrical activity of the cells. Additionally, it could create artefacts from dislocation of the electrode during contraction. We therefore performed experiments both in normal and in hypertonic Krebs' solution (**Chapter V**), and compared them. Medium made hypertonic by adding sucrose made the tissue rigid and abolished its movement, while the electrical response was not affected. When compared with recordings in normal Krebs' solution, the average resting membrane potential was not altered. However, the membrane potential was more stable, with far less spike-shaped potentials. The properties of these potentials were also altered: the amplitude was larger, while the duration decreased. Still, the electrical activity in the form of spike-shaped potential and long lasting depolarisation resembled closely that obtained under normal conditions; we thus found that movement only had a minimal effect on the electrical recordings of human detrusor tissue.

Another possibility to explain the differences between the results we obtained in human studies and those obtained by others in animal studies, is that they are related to the difference in species. Apart from a few studies in human isolated detrusor cells [38; 56], only our group has published intracellular electrophysiological recordings of human detrusor strips. Reference studies are therefore lacking. To validate our results, we had to bridge this gap. We therefore repeated the experiments performed in **Chapter V** in guinea-pig urinary bladder strips, the most used model for human urinary bladder contraction. The results are presented in **Chapter VI**. As in human tissue, there was no clear correlation between the electrical and mechanical activity upon muscarinic receptor stimulation in guinea-pig detrusor strips. Again, our intracellular recordings in guinea-pig tissue differed from those obtained by other groups, but greatly resembled the ones we recorded in human detrusor tissue. Although we cannot explain this difference between our data and those from other groups, it does show that, in our hands, guinea-pig detrusor intracellular electrical activity is similar to that recorded in human urinary bladder.

In conclusion, in this thesis it is demonstrated that both in human and in guinea-pig detrusor, force development upon muscarinic receptor stimulation can occur without changes in the membrane potential. This lack of correlation between mechanical and electrical activity implies that the sarcoplasmic reticulum in the cytoplasm of the cell is the source of the calcium necessary to initiate a contraction in response to cholinergic activation. It appears that the calcium involved in spontaneous contractions mainly originates from the extracellular matrix, however, a role of intracellular calcium in this type of contraction cannot be excluded totally.

S

Samenvatting

Alle organismen, zowel planten als dieren, zijn opgebouwd uit kleine bouwstenen: cellen. De cellen vormen onder andere het skelet, het zenuwstelsel, de organen en de spieren. Deze weefsels en ook de cellen waaruit ze zijn opgebouwd, verschillen sterk van elkaar. Ondanks hun diversiteit hebben cellen toch een gemeenschappelijke basisstructuur.

Een cel is te vergelijken met een zeepbel. Zo is er een heel teer omhulsel, het celmembraan, om binnen van buiten te scheiden. Het celmembraan omsluit een vloeistof, waarin zich organellen bevinden. Deze organellen vervullen dezelfde taken voor de cel als de organen voor het lichaam. In de vloeistof zijn zouten zoals natriumchloride, kaliumchloride en calciumchloride opgelost. In oplossing vallen deze zouten uiteen in natrium-, kalium-, calcium- en chloride-ionen, losse deeltjes met een elektrische lading.

Buiten de cel bevindt zich ook vocht met daarin dezelfde ionen als binnen de cel, maar in andere aantallen. Hierdoor is er een ongelijke verdeling van de elektrische lading binnen en buiten de cel: een elektrisch spanningsverschil. Deze situatie is te vergelijken met die van een stuwdam: er is een grote hoeveelheid water in het meer aan de ene kant van de dam en een kleine hoeveelheid aan de andere kant. De dam houdt het water tegen en het verschil in waterpeil in stand. Als de dam een sluis zou hebben die openstond, zou het verschil worden opgeheven en het waterpeil zou aan beide kanten op een gelijk niveau komen. In het geval van de cel is het celmembraan te vergelijken met de damwand en de ionen met het water: de verplaatsing van de ionen om het spanningsverschil op te heffen wordt verhinderd door het celmembraan. Zoals een sluis in de damwand, zijn er in het celmembraan poortjes, kanalen genoemd. Hierdoor kunnen de ionen de cel in- en uitbewegen.

Er bestaan verschillende typen kanalen voor de verschillende ionen. De meeste van deze kanalen zijn gesloten in de rusttoestand. Als het nodig is kunnen de kanalen opengaan, waarmee de ionen de mogelijkheid hebben om de cel in of uit te stromen. Daarmee verandert ook de elektrische lading, dit resulteert in een stroom: een actiepotentiaal.

Actiepotentialen zorgen voor het gecoördineerd laten werken van de cellen. Alleen als cellen samenwerken kan een organisme leven. Voor de coördinatie zorgen de hersenen; die geven opdrachten aan de rest van het lichaam. Voor het doorgeven van die commando's worden zenuwbanen gebruikt. Deze lopen als een soort elektrische bedrading door het hele lichaam. Signalen vanuit de hersenen naar cellen in het lichaam en omgekeerd worden doorgegeven als elektrische stromen, actiepotentialen, via die zenuwbanen. De actiepotentialen ontstaan doordat de kanalen die specifiek zijn voor natrium-ionen geactiveerd worden zodat deze ionen de cel binnen kunnen stromen.

Aan het eind van de zenuwbanen, bij de organen die bestuurd worden, worden de opdrachten niet doorgegeven als elektrische signalen, maar worden de actiepotentialen vertaald. Het elektrische signaal zorgt ervoor dat in de zenuwuiteinden een signaalstof wordt afgegeven. Deze signaalstof hecht zich dan aan speciale ontvangers, de receptoren, die zich in het celmembraan van de 'doel-cellen' bevinden.

Er zijn twee typen receptoren. Het ene type is gekoppeld aan een kanaal. Na

het activeren van dit type receptor wordt het kanaal opengezet en kunnen er ionen door stromen. Het andere type receptor is niet gekoppeld aan een kanaal, maar aan een boodschappersysteem. Na aankomst van een signaalstof bij het deel van de receptor aan de buitenkant van de cel, verandert de receptor van vorm, waardoor bij het gedeelte aan de binnenkant van de cel twee andere signaalstoffen vrijkomen. Deze twee stoffen geven op hun beurt het oorspronkelijke commando door.

Het was niet mogelijk om de elektrische activiteit van levende cellen te bestuderen tot 1940. In dat jaar werd een elegante nieuwe techniek geïntroduceerd in de wetenschappelijke wereld: het doorboren van een celmembraan van een levende cel met een heel dun uitgetrokken glazen buisje, een micro-elektrode.

De doorsnede van de tip van zo'n micro-elektrode is ongeveer een halve micrometer, één tweeduizendste millimeter. Aangezien de tip zo klein is, kan deze door het celmembraan geprikt worden zonder onherstelbare schade te veroorzaken. Binnen een fractie van een seconde na het aanprikken hecht het celmembraan zich om de tip van de elektrode en hiermee wordt een kanaal van de binnenkant van de cel naar de buitenwereld gecreëerd. De micro-elektrode is gevuld met een vloeistof waarin zouten zijn opgelost, zodat de vloeistof stroom geleidt, en de elektrische activiteit van de aangeprikte cel gemeten kan worden.

Het idee achter de elektrofysiologische techniek stamt uit 1902, maar het duurde tot halverwege de twintigste eeuw voordat de methode succesvol toegepast kon worden. Na het pionierswerk werd de techniek verder verbeterd en in steeds meer laboratoria gebruikt.

Aanvankelijk was de aandacht toegespitst op de hartspier, de spieren van het skelet en zenuwcellen. Deze weefseltypen bestaan uit grote cellen zonder veel bindweefsel. Bindweefsel, zoals de naam al aangeeft, vormt het cement tussen de verschillende cellen. En net als cement is het hard en taai. Deze eigenschappen zorgen ervoor dat de cellen stevig aan elkaar vastzitten, maar ze maken het doen van micro-elektrode afleidingen wel veel moeilijker: de tip van een elektrode breekt af op het bindweefsel.

Toch werd de micro-elektrode techniek gaandeweg ook toegepast op weefseltypen met meer bindweefsel, zoals gladde spieren. Dit soort spierweefsel vormt de wanden van de holle organen waaruit de ingewanden, bijvoorbeeld de maag en de darmen, bestaan.

Er werd steeds meer informatie vergaard over de elektrofysiologische processen van cellen, maar de voortgang was traag. Zelfs in 1968 was nog niet bekend dat in spiercellen de actiepotentialen veroorzaakt worden door het binnenstromen van calcium-ionen via calciumkanalen, maar ging men er nog vanuit dat natrium-ionen verantwoordelijk waren, zoals bekend was van zenuwcellen.

De blaas is ook een hol orgaan met een wand die uit glad spierweefsel bestaat. Deze spierlaag in de wand van de blaas wordt ook wel detrusor genoemd. De detrusor bevindt zich tussen twee andere lagen. Aan de binnenkant van de blaas is er een slijmlaag, om het weefsel te beschermen tegen de zure urine. Daaromheen bevindt zich de detrusor, en het geheel wordt bijeengehouden door een laag bindweefsel aan

de buitenkant.

De blaas heeft twee functies: eerst moet de urine die continu in de nieren geproduceerd wordt, worden opgeslagen. Die opslag moet onder lage druk gebeuren, omdat bij hoge tegendruk de nieren het bloed niet meer kunnen zuiveren en de urine niet meer kunnen afvoeren in de richting van de blaas. Vervolgens, als een bepaalde inhoud bereikt is en de aandrang tot plassen ontstaat, moet de urine worden geloosd. Dit gebeurt niet vanzelf, hiervoor moet de detrusor zich samentrekken. Het verkorten van de spier verhoogt de druk in de blaas en dit resulteert uiteindelijk in het uitdrijven van de urine.

Het combineren van de twee functies van de urineblaas, opslaan en uitdrijven, vereist een goede coördinatie. Bij sommige patiënten gaat deze coördinatie mis, en trekt de blaas soms ook samen tijdens de opslagfase. Dit kan leiden tot ongewenst urineverlies en eventueel zelfs tot terugstromen van urine uit de blaas in de richting van de nieren. Als dit laatste gebeurt, kunnen de nieren beschadigd raken.

Kennis van de elektrische activiteit van de spiercellen tijdens het verkorten zou misschien kunnen leiden tot een methode om de ongewenste samentrekkingen van de blaas tijdens het opslaan van urine te voorkomen. Om deze afwijking dus te kunnen begrijpen en eventueel te genezen, is elektrofysiologisch onderzoek noodzakelijk.

De menselijke detrusor is niet erg geschikt voor het maken van succesvolle micro-elektrode afleidingen: de spier bestaat uit een aaneengeschakeld netwerk van spierbundeltjes, die in willekeurige richtingen lopen. Deze bundeltjes zijn opgebouwd uit groepen van lange, dunne spiercellen: ze zijn ongeveer een derde millimeter lang en zeven duizendste millimeter in doorsnee. De spiercellen zijn rijkelijk omgeven met bindweefsel. Afgezien van deze eigenschappen, die het aanprikken op zich bemoeilijken, bewegen de cellen voortdurend, waarmee de kans vergroot wordt dat het contact tussen de micro-elektrode en het celmembraan verbroken wordt nadat de cel aangeprikt is.

Vanwege deze moeilijkheden zijn er weinig micro-elektrode studies gedaan in intacte bundels glad blaasspierweefsel van de mens of enig ander groot zoogdier. Om de problemen te omzeilen is er voornamelijk onderzoek gedaan aan blaasweefsel van kleine proefdieren, die minder bindweefsel tussen de blaasspiercellen hebben. Het meest gebruikte proefdier is de cavia. Om de kans op succesvolle micro-elektrode afleidingen te vergroten, werd in die metingen het verkorten van de spierbundels verhinderd. Dit werd bereikt door de elektrofysiologische afleidingen te maken in een meetvloeistof waaraan suiker was toegevoegd. Het suiker zorgt ervoor dat de spieren geen kracht meer kunnen ontwikkelen.

Zo werden de elektrische eigenschappen van de blaasspiercellen bestudeerd. Om de samenhang tussen die elektrische activiteit en het verkorten van de spier te bestuderen, gebruikten onderzoekers een andere techniek, de zogenaamde 'sucrose-gap' techniek. Hierbij wordt de elektrische activiteit van cellen aan de buitenkant van het weefsel gemeten. Zo is er geen kans dat een elektrode uit een cel schiet tijdens het verkorten van de spierbundel. Deze methode is dus bij uitstek geschikt om gelijktijdig beweging en elektrische activiteit van spieren te bestuderen.

De 'sucrose-gap' techniek werkt echter alleen als de elektrische activiteit van

de cellen in het weefsel gekoppeld is. De stroom die door het celmembraan van één cel loopt is namelijk heel klein. Om deze aan de buitenkant van het weefsel te kunnen meten, is het noodzakelijk dat de stroom in alle cellen precies gelijktijdig verloopt, zodat er een optelling van het signaal is. Dit is alleen mogelijk als de cellen onderling sterk elektrisch gekoppeld zijn, zoals in de caviablaas. In de menselijke detrusor is dit echter niet het geval, wat de 'sucrose-gap' techniek ongeschikt maakt voor het onderzoeken van de menselijke blaas.

Alhoewel proefdieronderzoek veel kennis oplevert, kunnen niet uit alle resultaten zonder voorbehoud conclusies over de menselijke situatie worden getrokken. Er zijn namelijk verschillen in gedrag en functie van de blaas van verschillende diersoorten beschreven. Om dus meer informatie over het mechanisme achter het samentrekken van de menselijke blaas te verkrijgen, is het noodzakelijk om experimenten te doen met menselijk weefsel.

De eerste elektrofysiologische gegevens van menselijke blaasspiercellen zijn verkregen in losse cellen. Het weefsel is dan zodanig behandeld, dat het bindweefsel is opgelost en de cellen helemaal van elkaar zijn losgeraakt. Op deze manier is het wel mogelijk om de elektrische eigenschappen van de blaasspiercellen te bestuderen, maar het levert geen inzicht in het verband tussen deze eigenschappen en het ontwikkelen van kracht. Daarbij is het ook nog mogelijk dat het oplossen van het bindweefsel geleid heeft tot beschadiging van de spiercellen, of ervoor gezorgd heeft dat hun eigenschappen veranderd zijn. Om het mechanisme van het verkorten van spieren te begrijpen is het dus noodzakelijk om onderzoek in spierbundeltjes te doen. Dat is het onderwerp van dit proefschrift.

Spiercellen hebben calcium-ionen nodig om te verkorten. Nadat het signaal om samen te trekken vanuit de hersenen is gekomen, moet het aantal calcium-ionen in de cel worden verhoogd. Deze ionen kunnen van buitenaf via calciumkanalen de cel binnenstromen. Het is echter ook mogelijk dat de calcium-ionen vrijkomen binnen de cel, vanuit een organel dat als opslagplaats dient. Nadat de hoeveelheid calcium-ionen in de cel is verhoogd, hetzij van buiten de cel, hetzij vanuit een opslagplaats, binden deze ionen zich aan de samentrekkingseiwitten binnen de cel. Deze samentrekkingseiwitten, de myosine-eiwitten, maken dan verbindingen met een ander eiwit, het actine. Actine ligt als een gevlochten touw tussen de myosine-eiwitten. De myosine-eiwitten zitten aan één kant vast aan het celmembraan en eindigen aan de andere kant in een uitsteeksel, een kopje. Dit kopje hecht zich aan het actine, knikt dan en trekt daarbij het myosine-eiwit langs het actine. Vervolgens laat het kopje weer los, knikt terug en hecht zich een stukje verder opnieuw aan het actine. Omdat veel myosine-eiwitten dit gelijktijdig doen heeft dit tot gevolg dat de spiercel zich verkort. Als het commando tot verkorten aan meer spiercellen binnen een spierbundel gegeven is, trekt de hele bundel samen. Activeren van alle bundels binnen een spier zorgt voor het ontwikkelen van kracht.

Het is bekend dat het ontwikkelen van kracht in skeletspiercellen voorafgegaan wordt door een actiepotentiaal. Nadat het elektrische signaal tot samentrekken is gegeven vanuit de hersenen, wordt dit vertaald in een signaalstof. Deze bindt zich aan een receptor die gekoppeld is aan een kanaal. Dit kanaal gaat open

en daardoor stromen calcium-ionen de cel in. Door de verplaatsing van de ionen verandert de elektrische lading binnen de cel en dit kan met een micro-elektrode gemeten worden.

In de blaasspier van de mens is de herkomst van de calcium-ionen, die betrokken zijn bij het ontwikkelen van kracht, niet bekend. Het is niet noodzakelijk dat de calcium-ionen van buiten de cel komen. De signaalstof die de commando's van de hersenen doorgeeft aan de blaas bindt zich namelijk niet aan een receptor die gekoppeld is aan een kanaal, maar aan één die verbonden is aan een boodschappersysteem. De twee boodschapperstoffen die vervolgens binnen de cel vrijkomen zorgen ervoor dat er calcium-ionen vrijkomen uit opslagplaatsen. Het is mogelijk dat op deze manier genoeg calcium-ionen vrijkomen om de spiercel te laten verkorten. Als dat zo is, dan kan dus een spiersamentrekking plaatsvinden zonder veranderingen in de elektrische activiteit van de cel. Toen wij onze experimenten begonnen, was het niet duidelijk wat er precies gebeurde met de elektrische activiteit in de blaasspiercellen wanneer er een commando tot verkorten werd gegeven vanuit de hersenen.

Tijdens urologische operaties waarbij delen van de urineblaas moesten worden verwijderd bij patiënten, bijvoorbeeld omdat ze kanker hadden, werd een klein stukje spier bewaard voor ons onderzoek. Uit dit stukje weefsel hebben wij dan één spierbundeltje gehaald. In deze spierstripjes hebben we met micro-elektrodes spiercellen aangeprikt. Er bleek allerlei spontane elektrische activiteit aanwezig te zijn in de spiercellen, variërend van één enkele actiepotentiaal tot heel veel. De gemeten elektrische activiteit verschilde per cel, en was zelfs verschillend tussen cellen in dezelfde spierbundel, wat de slechte elektrische koppeling van dit type spierweefsel bevestigt.

De elektrische activiteit was nu dus aangetoond, maar de rol van deze activiteit tijdens het samentrekken van de spier was nog altijd onduidelijk. Om die te bestuderen, moesten gelijktijdig de elektrische activiteit van een cel en het verkorten van het spierstripje gemeten worden.

De spierstripjes bevonden zich in een vloeistofbadje en waren niet meer verbonden met de hersenen. Om de commando's van de hersenen na te bootsen, voegden we zelf signaalstoffen toe aan de vloeistof in het badje. Als de boodschapperstof die normaal gesproken de receptoren op de blaas activeert werd toegediend, dan reageerde het spierbundeltje door samen te trekken. De elektrische activiteit veranderde niet. Om te testen of we de juiste receptoren bereikten, werd er ook een tegenstof toegevoegd. Deze zorgde ervoor dat de receptor niet meer geactiveerd kon worden. Als de tegenstof in het badje aanwezig was, reageerde het spierbundeltje niet op de boodschapperstof.

Nu was het niet zo dat de cellen in de spierbundel helemaal niet elektrisch konden reageren. Als op een niet-normale manier de spierbundel gedwongen werd om samen te trekken, was er een verandering in het elektrische signaal gelijktijdig met het verkorten. Voor dit niet-normale commando tot samentrekken werd de hoeveelheid kalium-ionen buiten de cel verhoogd. Om deze ongelijke verdeling op te heffen gingen er kanalen in het celmembraan open. Samen met de kalium-ionen stroomden ook

calcium-ionen de cel in. Deze calcium-ionen bonden zich vervolgens aan de samentrekkingseiwitten en er werd kracht ontwikkeld.

Omdat de elektrische activiteit van een cel niet veranderde als een normaal, ook in het lichaam voorkomend, commando tot samentrekken werd gegeven, concludeerden we dat de calcium-ionen bij deze manier van samentrekken vanuit opslagplaatsen binnen de cel komen. Deze samentrekkingen zijn te vergelijken met de gewenste samentrekkingen, die plaatsvinden als de blaas vol is. Of de calcium-ionen die betrokken zijn bij de ongewenste, spontane samentrekkingen tijdens het opslaan van urine dezelfde herkomst hebben, was niet bekend.

Uit eerder onderzoek in varkensblazen leek te volgen dat de herkomst van de calcium-ionen bij spontane samentrekkingen zou kunnen verschillen van die van gewenste spierverkortingen. Als dat inderdaad zo is, dan zou dit een veelbelovende invalshoek zijn om een methode te ontwikkelen voor de behandeling van de ongewenste samentrekkingen.

Om de herkomst van de calcium-ionen in gewenste en spontane samentrekkingen te bestuderen, hebben we de hoeveelheid calcium-ionen buiten de cel en in de opslagplaatsen binnen de cel veranderd. Zelfs als er geen calcium-ionen toegevoegd waren aan de meetvloeistof, vonden er spontane verkortingen van het spierbundeltje plaats. Het leek er dus op, dat voor deze spontane verkortingen geen calcium-ionen van buiten de cel worden gebruikt, maar hoewel er geen calcium-ionen waren toegevoegd aan de meetvloeistof, waren er wel misschien nog wel calcium-ionen gehecht aan de buitenkant van de cellen. Deze minimale hoeveelheid calcium-ionen was misschien voldoende om spontane- en gewenste samentrekkingen te laten plaatsvinden, met calcium-ionen van buiten de cel. Het kan natuurlijk ook zijn, dat er helemaal geen calcium-ionen van buiten werden gebruikt, maar dat calcium-ionen uit de opslagplaatsen werden vrijgemaakt voor de verkortingen.

De herkomst van de calcium-ionen is te meten met de elektrofysiologische techniek. Als calcium-ionen van buiten de cel nodig zijn voor het samentrekken, moeten er kanalen geopend worden. De calcium-ionen die vervolgens binnenstromen binden zich aan de samentrekkingseiwitten, wat tot verkorting leidt. Met de instroom van calcium-ionen van buiten wordt er een verandering in de elektrische activiteit van de cel gemeten en deze zal dus gelijktijdig met de verkorting plaatsvinden. Als er calcium-ionen uit opslagplaatsen gebruikt wordt, dan worden er geen kanalen in het celmembraan geopend en is er geen verandering in de elektrische activiteit van de cel. Tijdens de micro-elektrode afleidingen van sommige, maar lang niet van alle cellen was er een verband te zien tussen de elektrische activiteit en het spontaan ontwikkelen van kracht.

Nu is het zo dat de elektrische activiteit werd gemeten in één cel in een spierbundel, terwijl de kracht van de hele bundel gemeten werd. Het is dus mogelijk dat de cel die aangeprikt was met de micro-elektrode niet meedeed aan het ontwikkelen van de kracht, maar dit is een veronderstelling. We kunnen dus niet met zekerheid zeggen dat alléén calcium-ionen van buiten de cel betrokken zijn bij spontane spierverkortingen en dat calcium-ionen vanuit opslagplaatsen hierbij geen rol spelen.

De resultaten leken er verder op te wijzen dat de herkomst van de calcium-

ionen die nodig zijn voor het verkorten, nadat een in het lichaam voorkomend commando is gegeven, afhangt van de omstandigheden. Als het onmogelijk is om vanuit opslagplaatsen calcium-ionen te laten vrijkomen, dan wordt het aandeel van calcium-ionen van buiten verhoogd. Het omgekeerde is ook het geval: als er van buitenaf geen calcium-ionen de cel in kunnen stromen, worden er meer ionen vrijgemaakt vanuit de opslagruimtes.

In onze metingen zagen we altijd elektrische activiteit in de cel, onafhankelijk van de hoeveelheid calcium-ionen binnen of buiten de cel. Echter, de actiepotentialen werden wel minder hoog als de hoeveelheid calcium-ionen in de meetvloeistof minder werd. Dit kan logisch verklaard worden: als er minder calcium-ionen buiten de cel zijn, kunnen er ook minder door de kanalen de cel instromen.

De hoogte van de actiepotentialen die wij registreerden in menselijke blaasspierstripjes was erg wisselend en kleiner dan die andere onderzoekers in proefdierstudies waarnamen. Zoals gezegd werden de meeste van deze studies gedaan in een meetvloeistof waaraan suiker was toegevoegd, zodat het weefsel niet kon samentrekken.

In het celmembraan van de menselijke blaasspiercellen bevinden zich, naast de kanalen die reageren op signaalstoffen, ook kanalen die opengaan door beweging. Het is daarom mogelijk dat samentrekken er voor zorgt dat deze kanalen opengaan, er ionen gaan stromen en dat daarmee de elektrische lading van de cel verandert. Afgezien hiervan kan het ook zijn dat de micro-elektrode een beetje verschuift tijdens het samentrekken van het spierstripje, daarbij het celmembraan scheurend. Door deze scheur kunnen dan ionen de cel in- en uitstromen. Dit kan ook tot gevolg hebben dat de hoogte van de actiepotentialen verandert.

Om te onderzoeken of beweging van het weefsel het elektrische signaal van de spiercellen beïnvloedde hebben we micro-elektrode afleidingen gemaakt in de gewone meetvloeistof en in één waaraan suiker was toegevoegd. Toen de resultaten van deze metingen vergeleken werden, bleek beweging inderdaad een effect te hebben Als een spierstripje niet kon samentrekken, dan werden grotere actiepotentialen gemeten. Maar het effect was erg klein; nog altijd vertoonden de spiercellen in de meetvloeistof met suiker elektrische activiteit die meer overeenkwam met wat we onder normale omstandigheden registreerden, dan met datgene wat andere onderzoekers in proefdierstudies vonden.

Het is mogelijk dat de gemeten verschillen tussen menselijk blaasspierweefsel en dat van proefdieren te wijten zijn aan verschillen in gedrag en functie van de blaas van de verschillende diersoorten. Alleen wij hebben micro-elektrode studies gedaan in blaasspierstripjes van de mens. Dit betekent dat we ons werk niet kunnen vergelijken met het werk van anderen. Om onze resultaten te bevestigen, moest dit gat gedicht worden. Daarom hebben we ook micro-elektrode afleidingen gemaakt van blaasspierstripjes van de cavia, in een normale meetvloeistof en in één met suiker.

Net zoals in menselijk blaasweefsel, was er ook in cavia detrusor stripjes geen relatie tussen de elektrische activiteit in één spiercel en het samentrekken van de spierbundel. Opnieuw waren de actiepotentialen die wij registreerden kleiner dan die andere onderzoekers gemeten hebben, ondanks het feit dat dit keer afleidingen

gemaakt werden in blaasspierstripjes van hetzelfde proefdier, de cavia.

De activiteit die we registreerden in blaasspiercellen van de cavia leek echter heel veel op die we in menselijke blaasspiercellen zagen. Het verschil tussen onze resultaten en die van andere onderzoekers kunnen we niet verklaren. We hebben nu echter wel aangetoond dat, in ieder geval in onze experimenten, de elektrische activiteit van de menselijke blaasspiercellen lijkt op die van de cavia. Daarmee is er nu, ondanks de verschillen, de mogelijkheid gekomen om de resultaten van de metingen in caviablaas naar de menselijke situatie te vertalen.

Tot slot, uit de resultaten van de experimenten die beschreven zijn in dit proefschrift, kunnen we concluderen dat de spiercellen van zowel de menselijke- als de caviablaas kunnen verkorten zonder een verandering in elektrische activiteit, wanneer ze gestimuleerd worden met de signaalstof die ook gebruikt wordt om de commando's van de hersenen door te geven. Dit houdt in dat er vanuit opslagplaatsen binnen de cel genoeg calcium-ionen vrijgemaakt worden om een samentrekking tot stand te brengen. Ongewenste, spontane samentrekkingen lijken afhankelijk te zijn van de instroom van calcium-ionen van buitenaf, maar een rol voor calcium-ionen uit opslagplaatsen kan niet totaal worden uitgesloten bij dit type van verkorten.

R

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Curriculum vitae

De auteur van dit proefschrift werd geboren op 18 januari 1972 in Haarlem. In diezelfde plaats werd het middelbare onderwijs genoten aan het Stedelijk Gymnasium. Dit werd afgesloten met een diploma Gymnasium-β in 1990. Aansluitend volgde een studie Biologie aan de Vrije Universiteit Amsterdam tot 1996. Sinds april van dat jaar, gedurende 4 jaar, was de auteur werkzaam als Assistent in Opleiding bij de afdeling Urologie, werkgroep Urodynamica, van de Erasmus Universiteit Rotterdam, alwaar de in dit proefschrift beschreven experimenten werden uitgevoerd.

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1. Visser AJ and Mastrigt R van (1997): Action potentials recorded from human bladder smooth muscle biopsies. *Abstractbook DUA IV*: 60.
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