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A. J. Visser · R. van Mastrigt

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

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Abstract 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.

Key words Detrusor · Force · Electrophysiology · Action potential

A. J. Visser (✉) · R. van Mastrigt
Department of Urology-Urodynamics,
Room EE 1630, Erasmus University Rotterdam,
PO box 1738, 3000 DR Rotterdam, The Netherlands
e-mail: visser@udn.fgg.eur.nl
Tel.: +31 10 408 7377; Fax: +31 10 408 9451

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 [3, 13, 17, 29]. Activation of the cholinergic pathway induces the release of a G-protein, which mobilises inositol (1, 4, 5)-trisphosphate (IP3) [16, 35], in turn releasing Ca^{2+} from the sarcoplasmic reticulum [10] (SR; Fig. 1). This release may occur without changes in membrane electrical activity [13]. 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 [33], 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 [26]. These spontaneous contractions observed in urodynamic studies are related to the spontaneous contractile activity seen in detrusor strips from healthy bladders [3]. 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 [18]. 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 [19]. In human bladder, a dependence of spontaneous mechanical activity on extracellular Ca^{2+} has been demonstrated [1], 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

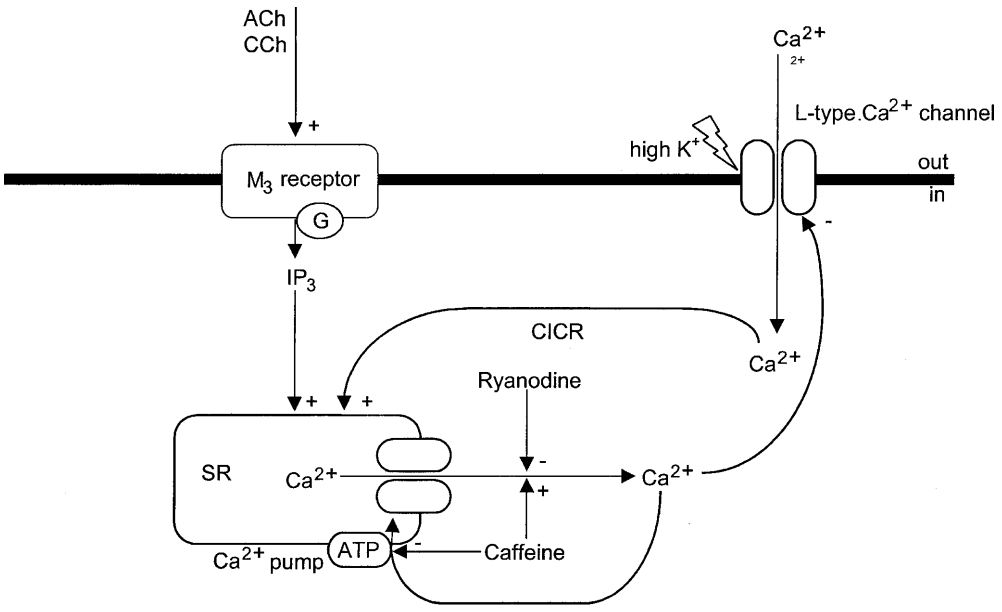

Fig. 1 A schematic representation of the pathways to control the intracellular Ca^{2+} concentration in human urinary bladder smooth muscle. The muscarinic M3 receptor, upon stimulation by carbachol (CCh), activates a second messenger system [G-protein (*G*) and inositol (1,4,5−)trisphosphate (*IP3*)], 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

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 [5], let alone man [31]. 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 [37]. Human detrusor electrical activity has been studied in single cells [24]. 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: (1) in normal Krebs' solution (100%), (2) in a solution with only 10% of the standard Ca^{2+} concentration (10%) and (3) in a solution 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 cells' ability 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.

Materials and methods

Biopsy samples

Detrusor biopsy samples were collected with approval from the local ethical committee and informed consent from 14 patients (three women and 11 men) ranging in age from 49 to 93 years (mean: 64 years SD 13 years). 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 10 min 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. Eight of the biopsy samples were stored at 40° 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 [27]. 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 40×; 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 35.0° C in standard Krebs' solution using a modified thermostat. After 30 min, 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 min 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 experi-

ment were normalised by expressing them as a percentage of the reference contraction.

During the experiments, 1 ml 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 0.1 mM 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, UK). Filled with 3 M KCl, the electrodes had a 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, Fla., 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, Mass. 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, Mass., 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), caffeine (ICN Biomedicals, USA), KCl and ryanodine (ICN Biomedicals, USA). The 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 min. 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 was no significant correlation 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 [33]. An overview of the stimuli applied is given in Table 1.

Data analysis

The average resting membrane potential of the impaled cell was calculated for 30 s before and for 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 4.2 c1 [32] 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 [32] was used to determine the parameters that describe the spike-shaped potentials and the long lasting depolarisations. The amplitude of spike-shaped potential or the 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 [32]. This occurred in 29% of the total number of recorded spike-shaped potentials ($n = 22{,}851$) and in 20% of the total number of recorded long lasting depolarisations ($n = 2419$).

Force values were read as the average force level for 30 s 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, Ill., 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+}

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

Stimulus	Preparation (n)	Extracellular Ca^{2+} concentration		
		100%	10%	0%
ACh/CCh	13	10	15	5
KCI	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 2 Median and interquartile range (i.q.r) of parameter values recorded in human detrusor strips in the three different Ca^{2+} concentrations

Ca^{2+} concentration	Resting membrane potential (mV)			Difference between resting force and reference resting force in standard Krebs' solution (mN)			Spike-shaped potential frequency (/s)			Long lasting depolarisation frequency (/s)		
	Median	I.q.r.	n	Median	I.q.r.	n	Median	I.q.r.	n	Median	I.q.r.	n
100%	−54	34	117	10	103	118	0.40	1.4	118	0.00	0.1	118
10%	−41	14	23	10	190	23	0.03	0.8	23	0.00	0.0	23
0%	−43	31	67	−10	185	69	0.63	1.2	69	0.03	0.2	69

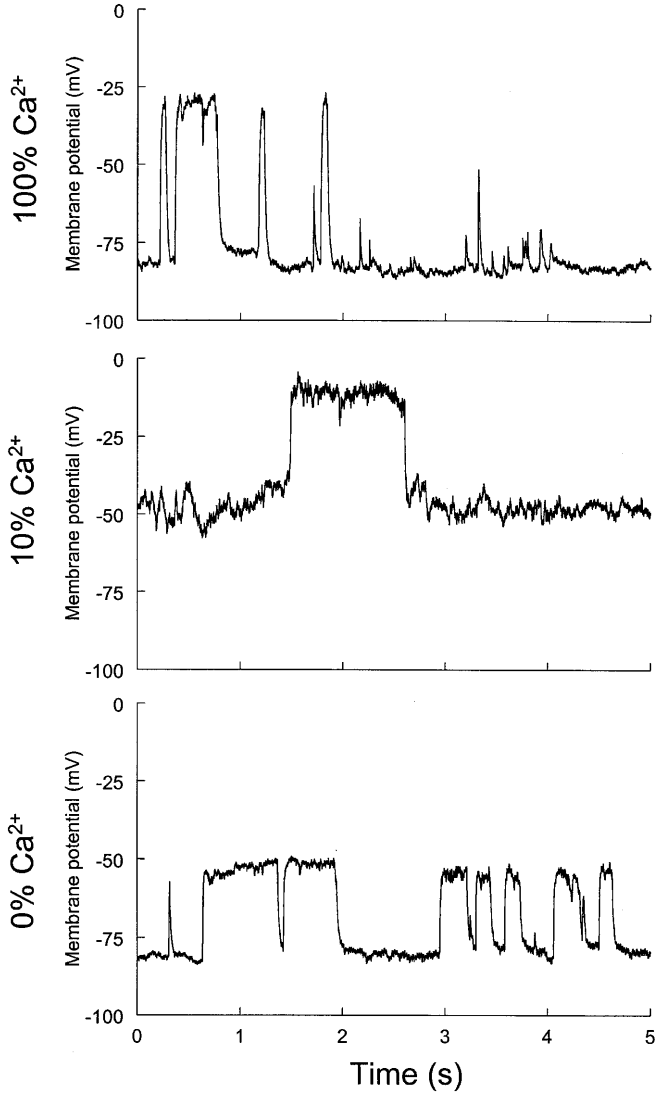

Fig. 2 Typical examples of long lasting depolarisations in human detrusor cells recorded in three different extracellular Ca^{2+} concentrations (i.e. 100%, 10% and 0%). Recordings were made in three different biopsies

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 Fig. 2. The size of both the spike-shaped potentials and the long lasting depolarisations were 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 for 30 s 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 when long lasting depolarisations ($P = 0.001$ occurred; 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 also between different cells within one strip ($P \leq 0.016$). The impalement of a cell never 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 Fig. 3a, there may 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 Fig. 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 (Fig. 3b), and there was a dramatic decrease in $d_{10\%}$ of the long lasting depolarisations when the extracellular Ca^{2+} concentration was increased (Fig. 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, two strips in 0% Ca^{2+}, one in 10% Ca^{2+} and four in 100% Ca^{2+} displayed spontaneous contractile activity: Fig. 4 shows a typical example of a spontaneous contraction in 100% Ca^{2+}

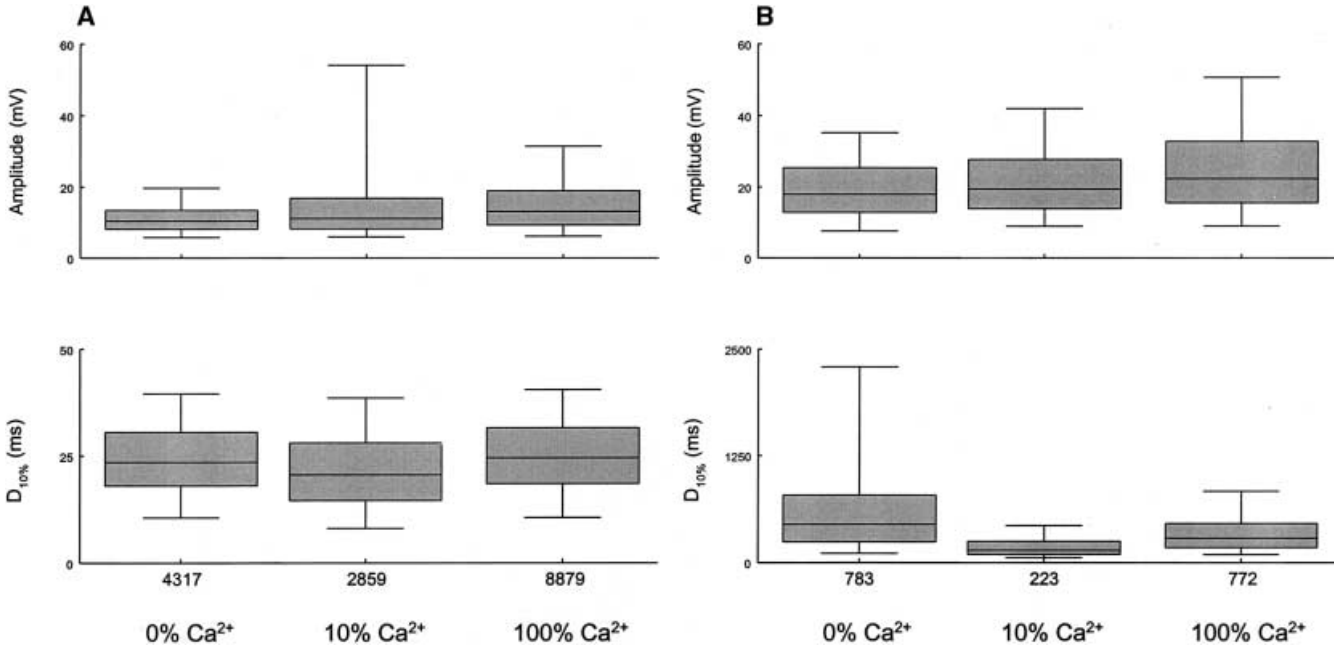

Fig. 3A, B Features of spike-shaped potential parameters (**A**) and long lasting depolarisations (**B**) measured in cells of human detrusor strips in three different extracellular Ca^{2+} concentrations. The values at 2½, 25, 50 (median), 75 and 97½ cumulative relative frequencies (centiles) are given. The numbers of events are displayed at the bottom of the graph

Krebs' solution. 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, 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, 13 and 16 spontaneous contractions were recorded, respectively (Table 1). During the spontaneous contraction in 10% Ca^{2+} Krebs' solution (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 was not significantly dependent on the Ca^{2+} 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 ($P \geq 0.438$, $P \geq 0.132$ and $P \geq 0.276$, 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, there was only a significant change in force in 10% and 100% Ca^{2+} Krebs' solution, ($P = 0.018$ and $P = 0.001$, respectively; Table 3). The spike potential frequency did not change significantly ($P \geq 0.139$). The

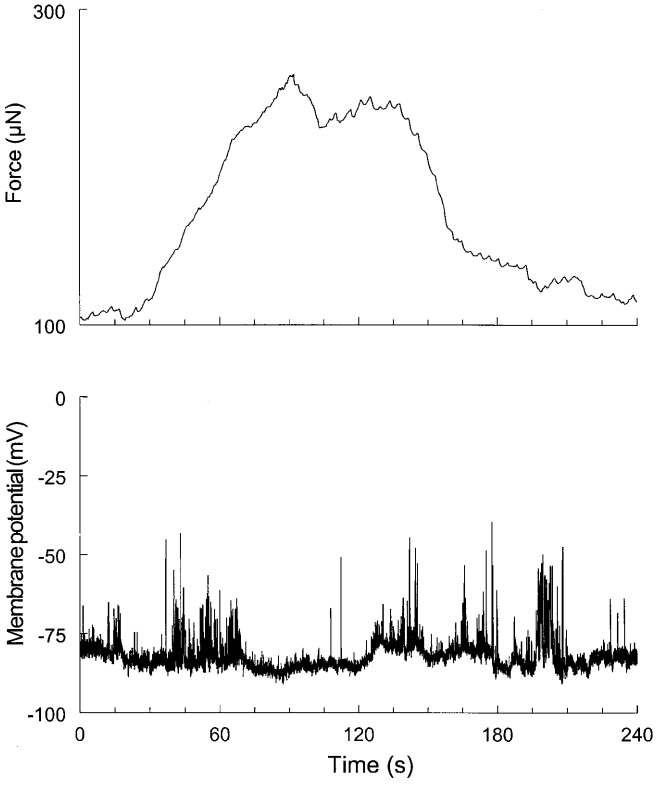

Fig. 4 A typical example of a spontaneous contraction in a human detrusor strip. The recording was made in standard Krebs' solution (100% Ca^{2+} concentration). *Upper trace*: force; *lower trace*: membrane potential

Table 3 Median and interquartile range (i.q.r.) of the changes in parameter values in response to application of KCl, muscarinic agonists acetylcholine (ACh) and carbachol (CCh), or CCh in the presence of ryanodine

Stimulus	Extracellular Ca^{2+} concentration		100%	10%	0%
KCl	Δ Normalised force (%)	Median	50	32	0
		I.q.r.	1	1	1
	Δ Vm (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 Vm (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 Vm (mV)	Median	10	–	0
		I.q.r.	9	–	18
	n		14	–	9

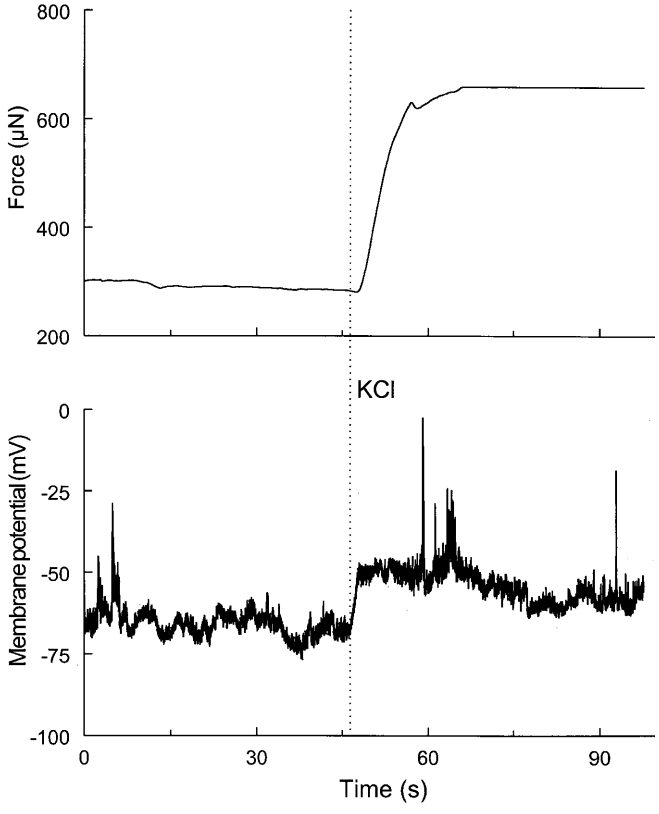

Fig. 5 A typical example of the response of a human detrusor strip to stimulation with KCl. A change in membrane potential preceding the onset of force development can be seen. The recording was made in 10% of the normal Ca^{2+} concentration. *Upper trace*: force; *lower trace*: membrane potential

long lasting depolarisation frequency reduced upon KCl stimulation only in 100% Ca^{2+} Krebs' solution ($P = 0.024$) but not in 10% Ca^{2+} Krebs' solution ($P = 0.854$) or 0% Ca^{2+} Krebs' solution ($P = 0.068$). Figure 5 gives a typical example of the response to KCl in 10% Ca^{2+} Krebs' solution. Immediately after KCl application, the membrane depolarised and with a delay of about 1 s, 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' solution. In both 0% and 100% Ca^{2+} Krebs' solution, caffeine stimulation resulted in a statistically significant increase in force ($P \leq 0.012$), but these were not statistically 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 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' solution is given in Fig. 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 ($P \geq 0.224$; Table 3), the spike-shaped potential frequency ($P \geq 0.191$) or the long lasting depolarisation frequency ($P \geq 0.341$). In 100% Ca^{2+}, the normalised force development ($P = 0.829$), the spike-shaped potential frequency ($P = 0.324$) or the long lasting 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

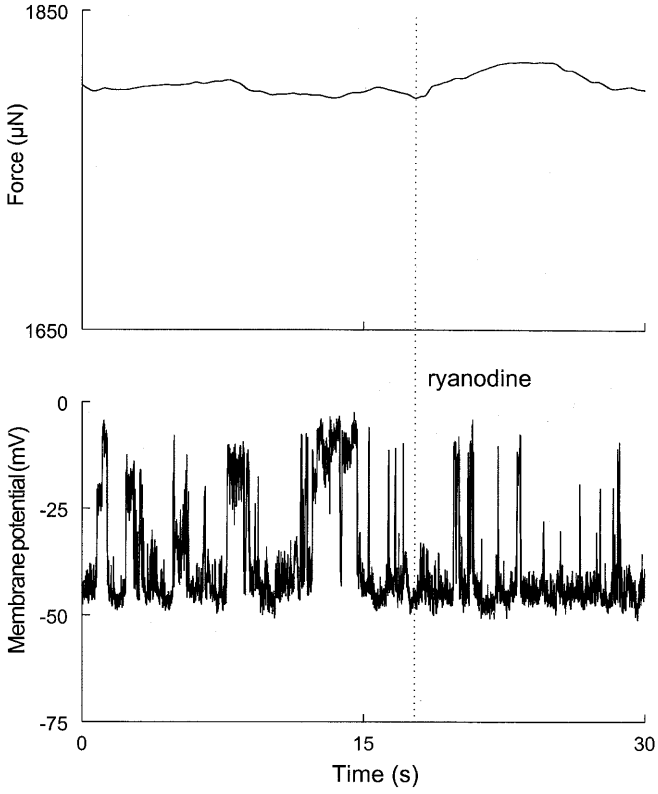

Fig. 6 A typical example of the response of a human detrusor strip to ryanodine application. A small increase in force can be observed accompanying the administration of ryanodine. There was no change in membrane potential activity. The Ca^{2+} was omitted from the bath solution (0%). *Upper trace*: force; *lower trace*: membrane potential

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' solution, 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' solution 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 0 Ca^{2+} depolarised the membrane potential [34].

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 [15], 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 [21]. 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 [6] in guinea-pig bladder and Ursillo [30] 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' solution, 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 [9]. 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 and Forman [1] suggested that tone was dependent on extracellular Ca^{2+}. Kurihara and Sakai [20] showed similar results for guinea pig and Maggi [22] for rat. However, experiments with nifedipine showed that in human urinary bladder, tone is regulated by intracellular Ca^{2+} release [12]. 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 [29].

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 [28] and the applied tension [11]. In rabbit [1, 36] and rat [22], 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 [2]. However, Kurihara and Sakai [20] also observed spontaneous contractions in guinea-pig urinary bladder strips in Ca^{2+} free medium.

In rabbit [7, 36], rat [8] and guinea pig [4, 25], spontaneous contractions were accompanied by spontaneous electrical activity, suggesting a major role for extracellular Ca^{2+}. As can be seen from Fig. 4, there sometimes was an increase in spike frequency in the recorded cell at the onset of a spontaneous contraction; 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 [3], 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 [8]. 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 40% 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 [3] 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' solution). 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 10% and higher 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 in a leaky SR [14]. Application CCh in the presence of ryanodine only resulted in a significant increase in force in a normal Krebs' solution. In human urinary bladder, Masters and colleagues [23] 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 toward increased participation of extracellular Ca^{2+} and vice versa. Similar results have been found in rabbit detrusor [9].

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References

1. Andersson K-E, Forman A (1986) Effects of calcium channel blockers on urinary tract smooth muscle. Acta Pharmacol Toxicol 58: 193
2. Brading AF (1987) Physiology of bladder smooth muscle. In: Torrens M, Morrison JFB (eds) The physiology of the lower urinary tract. Springer, Berlin Heidelberg New York, p 161
3. Brading AF (1997) A myogenic basis for the overactive bladder. Urology 50: 57
4. Bramich NJ, Brading AF (1996) Electrical properties of smooth muscle in the guinea-pig urinary bladder. J Physiol 492: 185
5. Callahan SM, Creed KE (1985) The effects of oestrogens on spontaneous activity and responses to phenylephrine of the mammalian urethra. J Physiol 358: 35
6. Creed KE (1971) Membrane properties of the smooth muscle membrane of the guinea-pig urinary bladder. Pflugers Arch 326: 115
7. Creed KE, Ishikawa S, Ito Y (1983) Electrical and mechanical activity recorded from rabbit urinary bladder in response to nerve stimulation. J Physiol 338: 149
8. Creed KE, Malmgren A (1993) The effect of cromakalim on the electrical properties of and [86 Rb^{+}] efflux from normal and hypertrophied rat bladder. Clin Exp Pharmacol Physiol 20: 215
9. Damaser MS, Kim K, Longhurst PA, Wein AJ, Levin RM (1997) Calcium regulation of urinary bladder function. J Urol 157: 732
10. Eglen RM, Reddy H, Watson N, Challiss RAJ (1994) Muscarinic acetylcholine receptor subtypes in smooth muscle. Trends Pharmacol Sci 15: 114
11. Finkbeiner AE, Bissada NK (1980) Effect of detrusor muscle length and tension on its response to pharmacologic and electrical stimulation. part II. In vitro study. Urology 16: 650
12. Forman A, Andersson K-E, Hendriksson L, Rud T, Ulmsten U (1978) Effects of nifedipine on the smooth muscle of the human urinary tract in vitro and in vivo. Acta pharmacol et toxicol 43: 111
13. Fry CH, Wu C (1998) The cellular basis of bladder instability. Br J Urol 81: 1
14. Ganitkevich VY, Isenberg G (1992) Contribution of Ca^{2+}-induced Ca^{2+}-release to the $[Ca^{2+}]_i$ transients in myocytes from guinea-pig urinary bladder. J Physiol 458: 119
15. Hashitani H, Edwards FR (1999) Spontaneous and neurally activated depolarizations in smooth muscle cells of the guinea-pig urethra. J Physiol 514: 459
16. Iacovou JW, Hill SJ, Birmingham AT, Bates CP (1989) Calcium dependence and inositol phosphate accumulation in human detrusor smooth muscle. Neurourol Urodyn 8: 418
17. Kinder RB, Mundy AR (1985) Atropine blockade of nerve-mediated stimulation of the human detrusor. Br J Urol 57: 418
18. Kinder RB, Mundy AR (1987) Pathophysiology of idiopatic detrusor instability and detrusor hyper-reflexia. An in vitro study of human detrusor muscle. Br J Urol 60: 509
19. Koeveringe GA van, Mastrigt R van (1991) Excitatory pathways in smooth muscle investigated by phase-plot analysis of isometric force development. Am J Physiol 261: R138
20. Kurihara S, Sakai T (1976) Relationship between effects of procaine and Ca on spontaneous electrical and mechanical activities of the smooth muscle cells of the guinea pig urinary bladder. Jap J Physiol 26: 487
21. Liu X, Farley JM (1996) Acetylcholine-induced chloride current oscillations in swine tracheal smooth muscle cells. J Pharmacol Exp Ther 276: 178
22. Maggi CA, Manzini S, Parlani M, conte B, Giuliani S, Meli A (1988) The effect of nifedipine on spontaneous, drug-induced and reflexy-activated contractions of the rat urinary bladder: evidence for the participation of an intracellular calcium store to micturition contraction. Gen Pharmacol 19: 73
23. Masters JG, Neal DE, Gillespie JI (1999) The contribution of intracellular Ca^{2+} release to contraction in human bladder smooth muscle. Br J Pharmacol 127: 996
24. Montgomery BSI, Fry CH (1992) The action potential and net membrane currents in isolated human detrusor smooth muscle cells. J Urol 147: 176
25. Mostwin J (1988) Electrical membrane events underlying contraction of guinea pig bladder muscle. Neurourol Urodyn 6: 429
26. Mundy AR (1988) Detrusor instability. Br J Urol 62: 393
27. Palea S, Artibani W, Ostardo E, Trist DG, Pietra C (1993) Evidence for purinergic neurotransmission in human urinary bladder affected by interstitial cystitis. J Urol 150: 2007
28. Potjer RM, Constantinou CE (1989) Frequency of spontaneous contractions in longitudinal and transverse bladder strips. Am J Physiol 257: R781
29. Sibley GNA (1984) A comparison of spontaneous and nerve-mediated activity in bladder muscle from man, pig and rabbit. J Physiol 354: 431
30. Ursillo RC (1961) Electrical activity of the isolated nerve-urinary bladder strip preparation of the rabbit. Am J Physiol 201: 408
31. Visser AJ, Mastrigt R van (1999) The effect of ACh and CCh on the membrane potential of human detrusor smooth muscle strips. J Muscle Res Cell Motil 20: 97
32. Visser AJ, Mastrigt R van (1999) Intracellular recording of spontaneous electrical activity in human urinary bladder smooth muscle strips. Arch Physiol Biochem 107: 257
33. Visser AJ, Mastrigt R van (2000) Simultaneous recording of mechanical and intracellular electrical activity in human urinary bladder smooth muscle. Br J Urol (in press)
34. Wu C, Sui G, Fry CH (1998) The role of the membrane potential in refilling functional intracellular Ca^{2+} stores in guinea-pig detrusor smooth muscle. J Physiol 507P: 22P
35. Yoshimura Y, Yamaguchi O (1997) Calcium independent contraction of bladder smooth muscle. Int J Urol 4: 62
36. Yu HJ, Hypolite JA, Wein AJ, Levin RM (1995) Effect of magnesium ions on rabbit detrusor contractility and intracellular free calcium. Pharm 51: 186
37. Zimmern PE, Lin VK, McConnell JD (1996) Smooth muscle physiology. Urol Clin North Am 23: 211