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Aberrant Ca^{2+} oscillations in smooth muscle cells from overactive human bladders

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ABSTRACT

Overactive bladder (OAB) syndrome is highly prevalent and costly, but its pathogenesis remains unclear; in particular, the origin of involuntary detrusor muscle activity. To identify the functional substrate for detrusor muscle overactivity, we examined intracellular Ca^{2+} oscillations in smooth muscle cells from pathologically overactive human bladders. Basal cytoplasmic Ca^{2+} concentration was elevated in smooth muscle cells from overactive bladders. Unprovoked, spontaneous rises of Ca^{2+} were also identified. These spontaneous Ca^{2+} oscillations were Ca^{2+}-dependent, sensitive to L-type Ca^{2+} channel antagonist verapamil and also attenuated by blocking SR Ca^{2+} reuptake. The fraction of spontaneously active cells was higher in cells from overactive bladders and the magnitude of spontaneous Ca^{2+} oscillations also greater. Spontaneous action potentials or depolarising oscillations were also observed, associated with Ca^{2+} rise; with a higher percentage of cells from idiopathic OAB, but not in neurogenic OAB. Low concentrations of $NiCl_2$ attenuated both spontaneous electrical and Ca^{2+} activation. This study provides the first evidence that spontaneous, autonomous cellular activity—Ca^{2+} and membrane potential oscillations, originates from detrusor smooth muscle in human bladders, mediated by extracellular Ca^{2+} influx and intracellular release. Such cellular activity underlies spontaneous muscle contraction and defective Ca^{2+} activation contributes to up-regulated contractile activity in overactive bladders.

1. Introduction

Overactive bladder (OAB) is a symptom complex characterised by an increase of the frequency of micturition and feelings of urgency, accompanied sometimes by incontinence [1]. It is a highly prevalent disorder, especially in the elderly and women [2,3], has a profound impact on the quality of life [4,5], and is economically very burdensome to health providers [6]. The symptoms of overactive bladder can be attributed to up-regulated activity of detrusor smooth muscle, and detrusor overactivity is associated with spontaneous, involuntary contractions. The exact pathophysiology remains unclear, although neurological disorders may be causative in a proportion of patients [7,8]. Moreover, functional changes are heterogeneous in isolated smooth muscle from OAB patients [9]. However, a myogenic mechanism, whereby there are fundamental changes to the electromechanical properties of detrusor muscle has been proposed as a cause of detrusor overactivity [10]. Unprovoked, spontaneous muscle activity could be a significant pathophysiological process as it would explain enhanced bladder activity and is more prevalent in tissue from patients with overactive bladders [11], as well as in animal models of detrusor overactivity [12].

However, the cellular basis for enhanced spontaneous activity in detrusor smooth muscle from patients with overactive bladders has not been explored and in particular whether spontaneous activity originates from smooth muscle itself or other cell types [13]. The role of intracellular Ca^{2+} is of particular importance, as it is the main determinant of detrusor contractile activity. The aims of this study were: to test the hypothesis that spontaneous intracellular Ca^{2+} oscillations are increased in human detrusor smooth muscle from overactive bladders; and to elucidate the cellular mechanisms underlying such activity.

2. Materials and methods

2.1. Tissue samples, cell isolation and solutions

Detrusor samples were obtained after approval of the Joint UCL/UCLH Committees on the Ethics of Human Research and with informed patient consent in accordance with the Declaration of

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Helsinki. Samples were either from control patients with no symptoms of detrusor overactivity—stable bladders, but undergoing open operations for bladder cancer or other disorders; or from patients undergoing clam cystoplasty who had detrusor overactivity, as assessed by urodynamics. For the stable group 48 samples were used (31 males, 17 females, age 58.3 ± 16.4 years, ±S.D.), and for the overactive group 66 samples were obtained (21 males, 45 females, age 42.2 ± 16.7 years). Of the overactive group, 38 were idiopathic—bladder overactivity with no clinically detectable neurological disorders (8 males, 30 females, age 49.3 ± 15.1 years), and 28 neuropathic—bladder overactivity with a neurological disorder (13 males, 15 females, age 32.6 ± 13.8 years). Data from the idiopathic and neurogenic OAB groups are compared to control either separately or collectively. Neither gender- nor age-dependent differences were observed for any experimental variables within or between groups.

All samples were treated similarly: they were placed immediately in a Ca-free HEPES-buffered solution after excision and transported to the laboratory within 60 min. The mucosa was removed and the underlying muscle tissue was disrupted into isolated cells with a collagenase-containing medium [14] and stored in a Ca-containing HEPES-buffered medium. The yield of cells from samples of both experimental groups was similar, so that the procedure was equally effective in all samples. Viability of the cells was confirmed by trypan blue extrusion and their Ca^{2+} response to stimuli such as carbachol and high-K solutions. The HEPES-buffered solution contained (mM): NaCl, 105.4; $NaHCO_3$, 22.3; KCl, 3.6; $MgCl_2{\cdot}6H_2O$, 0.9; $NaH_2PO_4{\cdot}2H_2O$, 0.4; HEPES, 19.5; Na pyruvate, 4.5; adjusted to pH 7.1 with NaOH, and with 1.8 $CaCl_2$ as required. For experiments cells were perfused with Tyrode's solution (mM): NaCl, 118; $NaHCO_3$, 24; KCl, 4.0; $MgCl_2{\cdot}6H_2O$, 1.0; $NaH_2PO_4{\cdot}2H_2O$, 0.4; $CaCl_2$, 1.8; Na pyruvate, 5.0; glucose, 6.1, gassed with 95% O_2:5% CO_2 to pH 7.35. Ca-free Tyrode's solution (0-Ca solution) had no added $CaCl_2$ and 0.1 mM EGTA to buffer Ca^{2+} activity at pCa >8. All chemicals were from Sigma, UK.

2.2. Measurement of the intracellular free Ca^{2+} concentration, $[Ca^{2+}]_i$

$[Ca^{2+}]_i$ was measured by epifluorescence microscopy as described previously [15]. Cells were loaded with the Ca^{2+}-sensitive fluorochrome Fura-2 AM (Calbiochem, Germany) at 25 °C for 30 min and, following Fura-2 AM washout, for 20 min, to de-esterify the intracellular fluorochrome to Fura 2 acid, used for experiments. Myocytes were then continuously superfused with normal Tyrode's solution at 37 °C, at 2 ml min^{-1}. The cell of interest was illuminated alternately (50 Hz) at 340 or 380 nm, fluorescent light collected between 410 and 510 nm. The ratio, *R*, of fluorescent light when illuminated at 340 and 380 nm was used as an index of the intracellular Ca^{2+} concentration and calibrated as described previously [15].

2.3. Electrophysiological recordings

Electrophysiological recordings used patch-type pipettes (3–5 MΩ) made from borosilicate glass and filled with a high-K based intracellular medium (mM): KCl 20; aspartic acid, 110; $MgCl_2$, 5.45; Na_2ATP, 5.0; Na_4GTP, 0.2; EGTA, 0.1; HEPES, 5.0; pH adjusted to 7.1 with KOH. Membrane potentials were recorded in current-clamp mode; resting potentials recorded with no current passed into the cells ($I_h = 0$). An Axopatch-1D system (Axon Instruments) was used and data recorded via an A/D converter (Digidata 1200, Axon Instruments) at 4 kHz, and filtered with a low-pass filter, corner frequency 2 kHz.

2.4. Simultaneous recording of $[Ca^{2+}]_i$ and membrane potential

To record simultaneously electrical activity and intracellular Ca^{2+} Fura-2 was used in its non-ester form as a pentapotassium salt (Fura-2 K_5, Calbiochem). 100 μM Fura-2 K_5 was included in the intracellular medium as above, but with the EGTA concentration reduced to 0.05 mM, and dialysed into the cell via the patch pipette [15]. These concentrations of Ca^{2+} buffers contribute about 10% to the total Ca^{2+}-buffering power of detrusor smooth muscle cells [16].

2.5. Data analysis and statistical methods

Results are expressed as means ± S.E.M., except where otherwise stated; *n* is the number of preparations. Student's *t*-test and ANOVA (Bonferroni post hoc test) were used to test the difference between two and multiple mean values, respectively, a chi-square test used for two incidences. A Kolmogorov–Smirnov test was used to test the normal distribution of a data set, where $|d|$ is the statistic denoting the maximum difference between two cumulative fraction curves, one for a normal distribution and the other for the experimental data curve with the same mean. The null hypothesis was rejected for $p < 0.05$. A Clampfit program (Axon Instruments) was used for data processing.

3. Results

3.1. Resting intracellular $[Ca^{2+}]_i$ in human detrusor cells

With cells from stable bladders, the resting $[Ca^{2+}]_i$ was 66 ± 36 nM (mean ± S.D., $n = 196$ cells from 48 patients). The value was not significantly different from a separate study using guinea-pig cells [15]. The variation of data values could be described by a normal distribution ($|d| = 0.086$, $p > 0.05$). However, the resting $[Ca^{2+}]_i$ in myocytes from overactive bladders was significantly higher (75 ± 47 nM, $p < 0.05$, $n = 256$ cells from 66 patients). Fig. 1 presents data on per patient basis and shows no significant difference in cells from idiopathic and neurogenic overactivity; both data sets were significantly greater than control set.

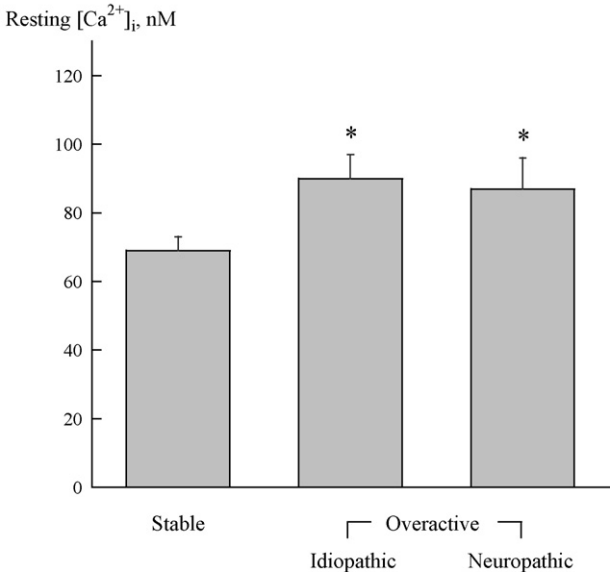

Fig. 1. Resting intracellular $[Ca^{2+}]$ in human detrusor smooth muscle cells. Mean ± S.E.M., $n = 48$, 38, 28 patients, for stable, idiopathic and neurogenic groups; $^*p < 0.05$, vs. stable, ANOVA (Bonferroni post hoc test).

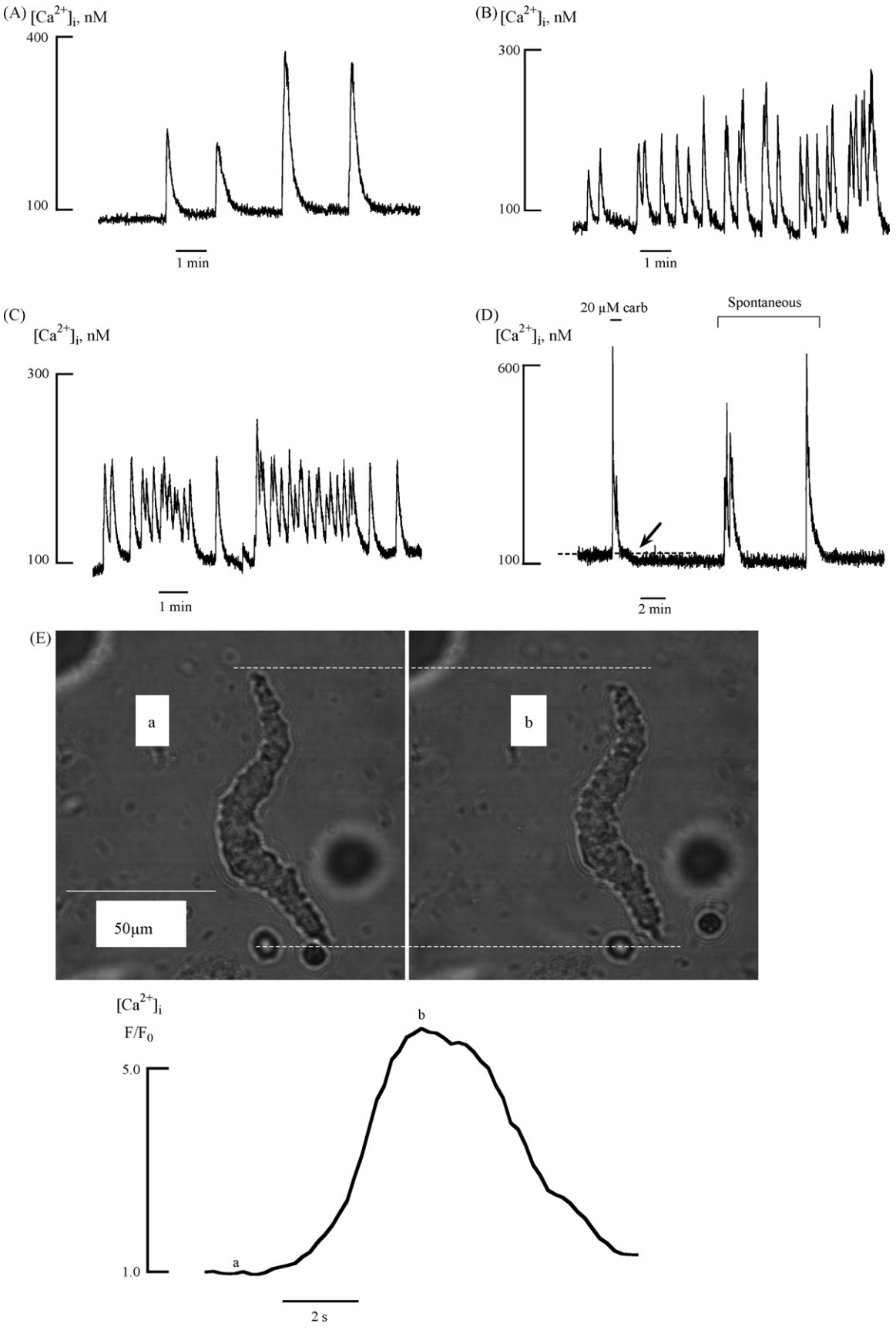

Fig. 2. Spontaneous intracellular [Ca^{2+}] transients in human detrusor myocytes. (A) Discrete transients. (B) More rapid transients. (C) Fused, tetanic responses. (D) Comparison to a maximal carbachol-evoked Ca-transient. (E) Transmitted-light image showing shortening of detrusor myocyte during a spontaneous rise of intracellular Ca; top: images taken at resting state (a) and during the peak (b) of the spontaneous Ca^{2+} rise, bottom: the time-course of such activity.

3.2. Spontaneous rises of $[Ca^{2+}]_i$

Unprovoked, spontaneous rises of $[Ca^{2+}]_i$ were observed in a proportion of cells from human detrusor samples. This spontaneous activity occurred as: discrete, isolated spike transients; frequent fast, transients; or fused tetanic rises of $[Ca^{2+}]_i$ (Fig. 2A–C). The frequency of these events varied and was on average 3.5 ± 0.3 transients per minute during phases of active firing. The frequency calculated for a longer period of observation (10–15 min), to include quiescent periods and thus the overall incidence of the activity, was 0.60 ± 0.10 per minute in cells from stable bladders ($n = 50$). The spontaneous Ca-transients were large; the majority were between one-third and two-thirds of those evoked by the muscarinic agonist carbachol and on average were not significantly different in cells from the three groups of biopsy samples. Fig. 2D shows a cell in which a maximum carbachol concentration evoked a transient initially, followed by several spontaneous transients of comparable magnitude. In general, the carbachol-transient exhibited an "undershoot" following relaxation [17], whilst the spontaneous transient did not, suggesting a different origin for the Ca^{2+}-transient. The spontaneous transients were associated with cell shortening and indicate their important functional relevance to spontaneous cell contractions (Fig. 2E).

The fraction of spontaneously active cells was greater in OAB compared to control samples (73.7 ± 3.9 and $40.2 \pm 5.2\%$; $n = 61, 44$, respectively, $p < 0.01$). This difference was maintained if the two OAB subgroups were compared separately to control (Fig. 3A). Furthermore, the frequency of spontaneous activity was greater in cells from OAB bladders; autonomous events, over the long observation period, occurred at a higher frequency of 0.93 ± 0.12 per minute ($n = 95$ cells, $p < 0.05$) compared to control cells.

The morphology of spontaneous Ca^{2+}-oscillations was variable, due to varying duration and fusion of individual events. Therefore, a standardised index was devised to quantify the overall increase

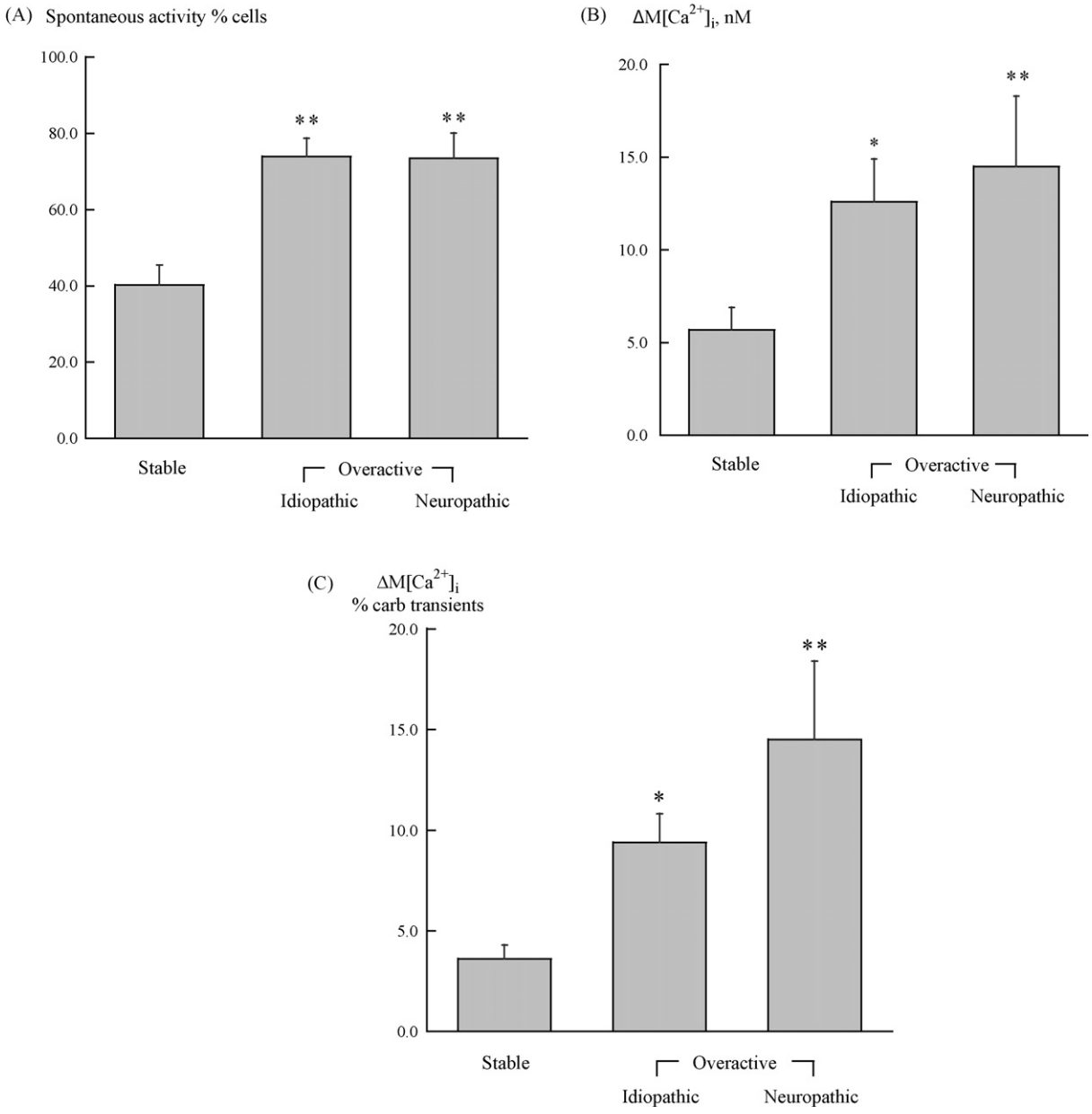

Fig. 3. Characteristics of spontaneous $[Ca^{2+}]$ transients in smooth muscle cells from stable and overactive bladders. (A) The proportion of spontaneously active cells. (B) The time-averaged increase of intracellular $[Ca^{2+}]$, $M\Delta[Ca^{2+}]_i$ (see text for details). (C) The value of $M\Delta[Ca^{2+}]_i$ normalized to the maximal carbachol-induced intracellular $[Ca^{2+}]$ rise. Data are mean ± S.E.M., ANOVA (Bonferroni post hoc test); $^*p < 0.05$, $^{**}p < 0.01$ vs. stable.

of the $[Ca^{2+}]$ over base-line—the mean magnitude of $[Ca^{2+}]_i$ rise, $M\Delta[Ca^{2+}]_i$, obtained by integrating the area under the $[Ca^{2+}]_i$ trace over 10–15 min, divided by time. This value thus reflects the magnitude, frequency and duration of Ca^{2+}-transients. The value was significantly greater in cells from OAB bladders (13.2 ± 2.0 nM vs. 5.7 ± 1.2 nM; $n = 40$, 24, respectively, $p < 0.01$). The difference was again maintained if the two OAB groups were compared separately to control (Fig. 3B). The value of $M\Delta[Ca^{2+}]_i$ to the maximum carbachol-evoked Ca-transient from the same cell was also significantly greater in the OAB group compared to control (10.2 ± 1.6 and $3.6 \pm 0.7\%$; $p < 0.01$), again the difference was preserved when each OAB subgroup was compared to control (Fig. 3C).

3.3. The cellular basis of spontaneous Ca^{2+} transients

The magnitudes of extracellular entry and intracellular release of Ca^{2+} are sufficient in detrusor muscle to cause significant increases of intracellular Ca^{2+} [15]. The sources of Ca^{2+} that generate spontaneous activity were thus investigated. Ca^{2+} influx through L-type Ca^{2+} channels is the main source of extracellular entry: it can produce large Ca^{2+} transients and is also essential to replenish intracellular Ca^{2+} stores [18]. Fig. 4A shows that spontaneous intracellular Ca^{2+} transients were rapidly and completely abolished in a 0-Ca solution, furthermore there was an equally prompt fall of the resting $[Ca^{2+}]$. Similar observations were made in 12 cells from control and OAB bladders. Similarly, the L-type Ca^{2+} channel blocker verapamil abolished intracellular Ca-transients (Fig. 4B, $n = 11$). These data suggest the importance of transmembrane Ca^{2+} fluxes through L-type Ca^{2+} channels in generating such activity. Fig. 4C implicates also the role of intracellular Ca-stores through the use of cyclopiazonic acid (CPA), which attenuates Ca^{2+} uptake into this compartment. CPA caused a maintained rise of the intracellular $[Ca^{2+}]$, which returned to control only after its removal: spontaneous activity was suppressed and subsequently returned ($n = 8$). Similar results were observed in OAB and control cells.

3.4. Electrophysiological properties of isolated cells

After membrane break-through, the resting membrane potential showed low-level fluctuations, superimposed sometimes were spontaneous depolarisations in a variety of patterns (Fig. 5). In the quiescent phases the averaged potential over several seconds was recorded as the resting membrane potential. The value was similar in cells from the control and overactive groups (-54.0 ± 2.6 mV, $n = 82$ cells vs. -51.6 ± 2.1 mV, $n = 105$ cells, $p > 0.05$); there was also no difference between subgroups of OAB (idiopath vs. neuropath: -52.3 ± 2.4 mV, $n = 81$ cells vs. -49 ± 4.5 mV, $n = 24$ cells, $p > 0.05$). Fig. 5A shows examples of spontaneous action potentials, as seen by them overshooting the 0-mV level and Fig. 5B is a fast time-base showing upstroke and repolarising phases of an action potential. In other cells smaller, more prolonged depolarisations were recorded (part C), whilst in others slow depolarisations, with occasional superimposed action potentials (part D). It is of note that the spontaneous action potentials and depolarisations were initiated from relatively negative membrane potentials, well below the threshold for the opening of the L-type Ca^{2+} channels at -40 to -35 mV [19] suggesting that other ionic currents contribute to their initiation.

A higher proportion of cells from overactive bladders displayed spontaneous electrical activity (54.9%, $n = 91$ cells vs. 29.5%, $n = 78$ cells from stable patients, $p < 0.01$). This excess of activity was attributable to cells from idiopathic OAB bladder samples (59.2%, $n = 71$ cells, $p < 0.01$ vs. control). With cells from the neuropathic OAB group the incidence of activity was not statistically distinguished from control (40.0%, $n = 20$ cells, $p > 0.05$ vs. control). Thus, although cells from the neuropathic and idiopathic OAB groups showed a similar increased incidence of intracellular Ca-transients, the difference of electrical spontaneous activity suggests that different mechanisms may be responsible in the two pathologies.

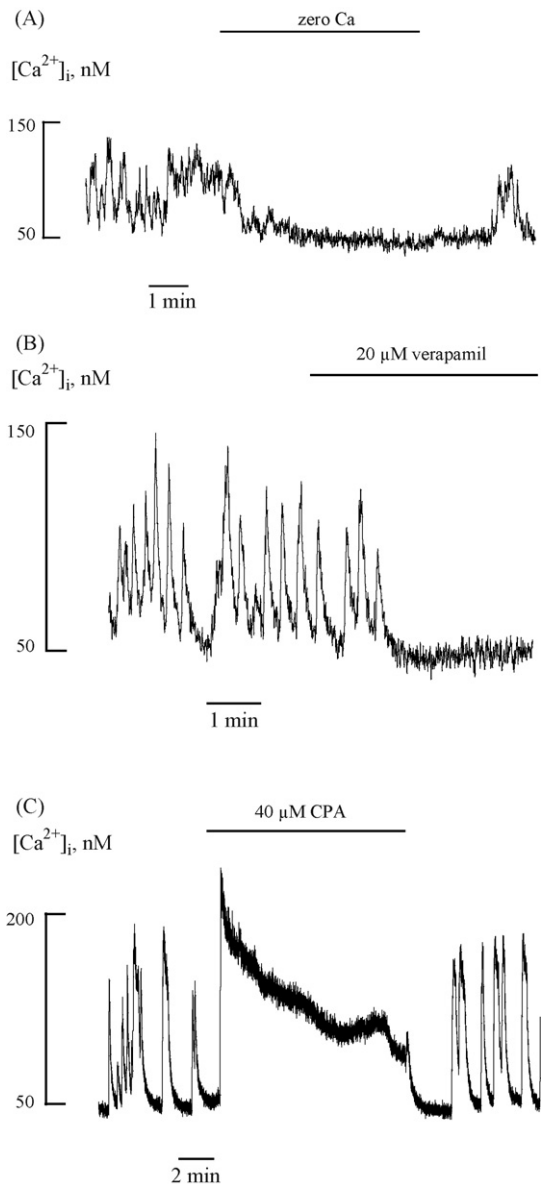

Fig. 4. Effect of interventions on spontaneous intracellular Ca^{2+} oscillations. (A) Exposure to zero-Ca solution. (B) Effect of 20 μM verapamil. (C) Effect of 40 μM cyclopiazonic acid (CPA).

3.5. The relationship between spontaneous electrical activity and intracellular Ca-transients

The previous measurements suggest some underlying interrelationship between electrical and Ca events, at least in some pathologies. A causal relationship between these events was thus investigated by simultaneous recording of both variables. Fig. 6A shows a single spontaneous action potential associated with a corresponding Ca-transient. Fig. 6B exemplifies discrete, more sustained but smaller oscillating depolarisations mirrored by similar changes to the intracellular $[Ca^{2+}]$. In part C a more random pattern of slow depolarisations was again generally accompanied by similar changes to the intracellular $[Ca^{2+}]$. The rise of Ca^{2+} was generally associated with membrane depolarisations greater than 20 mV and also related to the duration of the depolarisations.

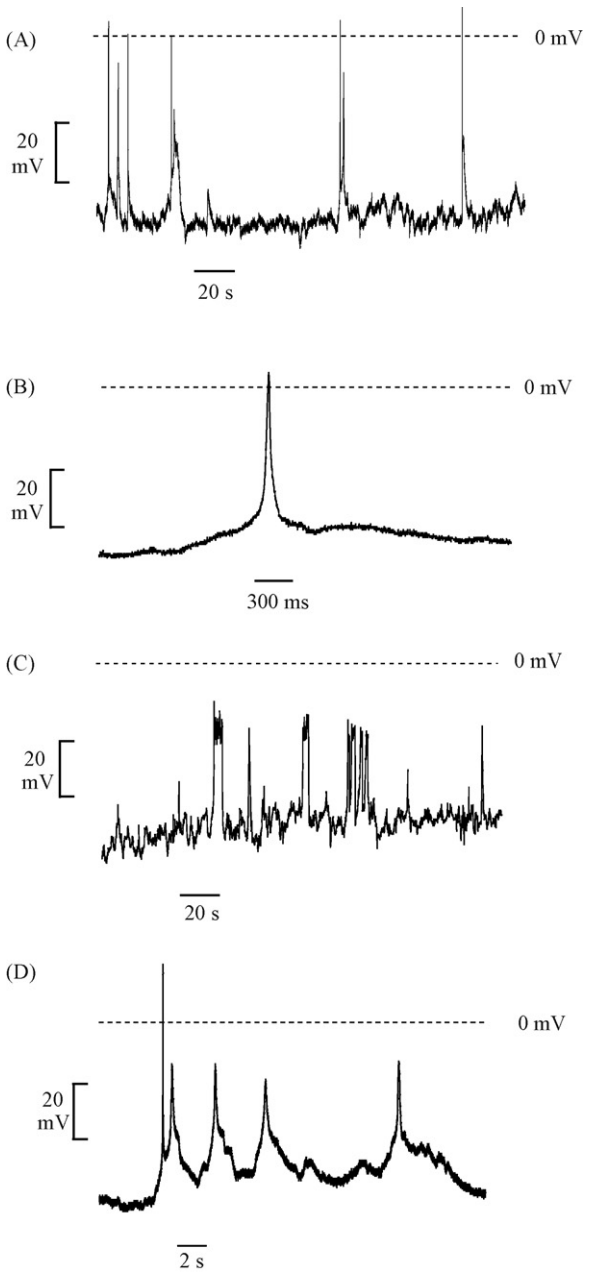

Fig. 5. Spontaneous electrical activity in human detrusor myocytes. (A) Action potentials, either in bursts, as in the start of this trace, or as individual events. (B) An action potential from part A on fast time-base. (C) Low amplitude, more sustained depolarisations. (D) Slow waves of activity.

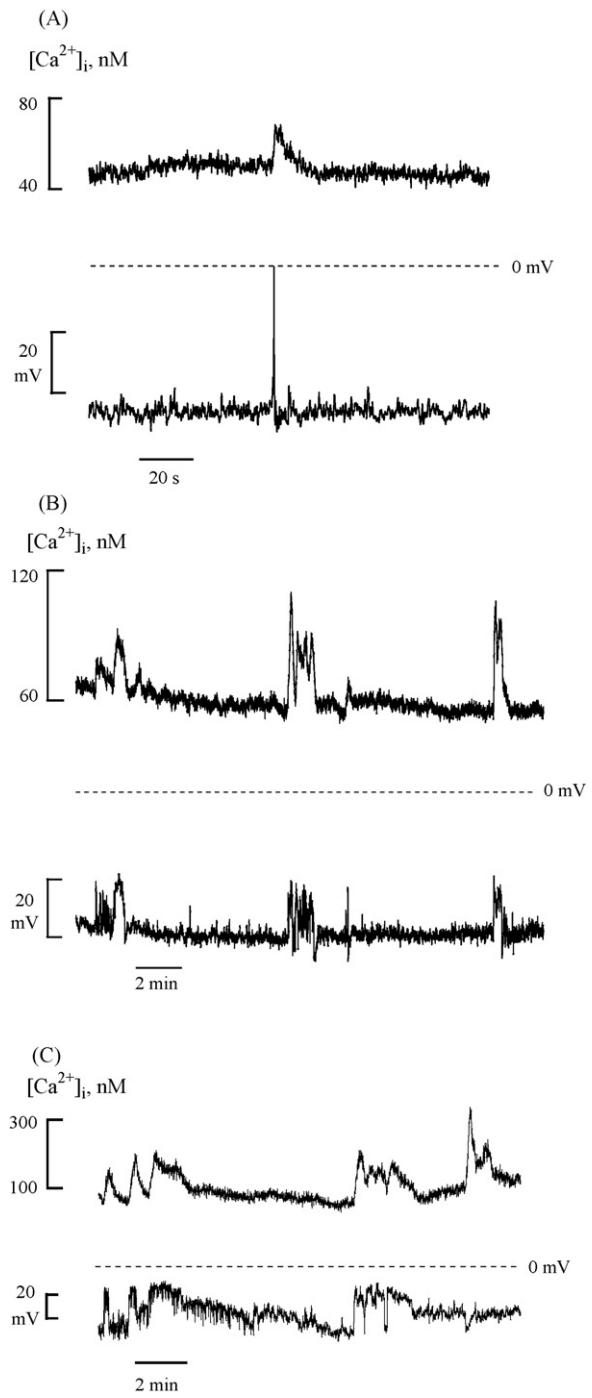

Fig. 6. The association between spontaneous membrane potential and intracellular $[Ca^{2+}]$ changes. (A) Single events. (B) Sustained changes. (C) More random changes.

To determine the causal relationship, Fig. 7A shows an example of a spontaneous depolarisation and rise of intracellular $[Ca^{2+}]$ on a faster time-base. The change of membrane potential preceded and peaked before the $[Ca^{2+}]$. Such temporal relationship was more clearly demonstrated by the phase plot in Fig. 7B in which membrane depolarisation occurred first, followed by the increase of intracellular $[Ca^{2+}]$. The plot also shows that the threshold membrane potential to trigger a detectable Ca^{2+} rise was between −30 to −40 mV, consistent with the L-type Ca^{2+} channel being pivotal in providing Ca^{2+} entry for spontaneous Ca^{2+} oscillations.

3.6. Involvement of T-type Ca^{2+} channels in spontaneous activity

The above observations suggested that spontaneous electrical activity could be initiated at relatively negative potentials by an inward current separate from the L-type Ca^{2+} channels. T-type Ca^{2+} current which operates at such negative membrane potentials is present in human detrusor muscle and sensitive to low concentrations of Ni^{+} ions [19]. Fig. 8A shows a spontaneous action potential in a cell, which fired from a relatively negative potential, and the suppression of such activity by 200 μM $NiCl_2$. Consistently, Fig. 8B demonstrates that spontaneous Ca^{2+} oscillations were also suppressed by the same intervention. Similar results were observed in six cells. Fig. 8C and D suggests that Ni^{+}-sensitive T-type Ca^{2+} current triggered Ca^{2+}-activated BK current at relatively negative membrane potentials. These observations suggest that the T-type Ca^{2+} current is an important contributor in the initiation of spon-

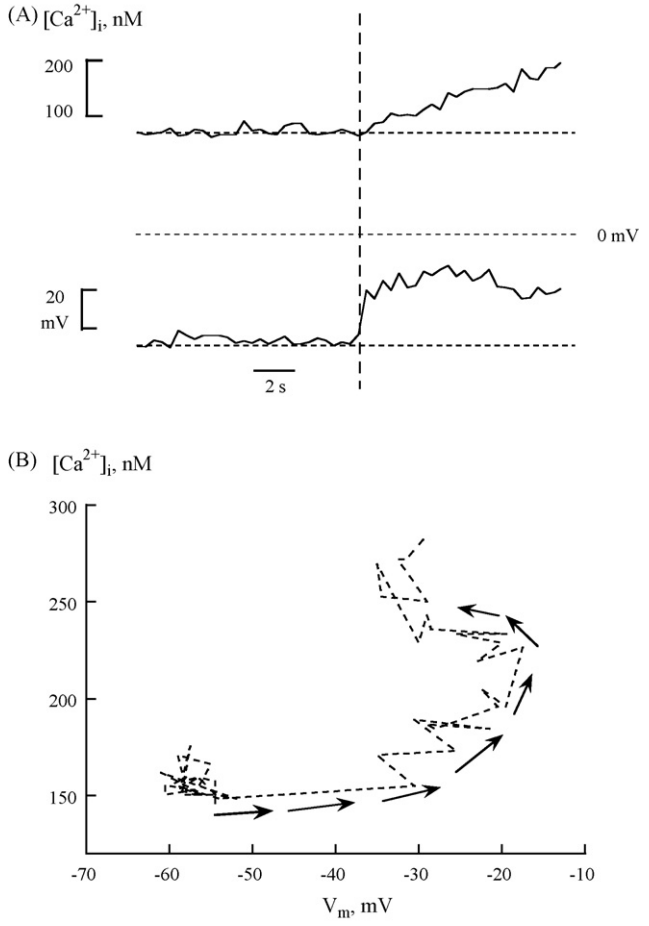

Fig. 7. The relationship between spontaneous membrane potential and intracellular Ca^{2+} changes. (A) Fast time-base of changes to membrane potential and intracellular $[Ca^{2+}]$ during the initial phase of a spontaneous activity. (B) Phase-plot of the paired event: the arrows indicate the arrow of time.

taneous electrical and hence Ca^{2+}-transients in human detrusor smooth muscle.

4. Discussion

4.1. The significance of spontaneous activity

A myogenic component to spontaneous activity has been proposed as an important contributor to the overactive bladder as evidenced by: it is increased in OAB both from human and animal models [10,20,21]; present in isolated muscle preparations and whole bladders especially from overactive bladders [22]; modulated by inotropic interventions and insensitive to neurotoxins [23]. Several histological and immunocytochemical studies have examined differences between human detrusor preparations from stable and overactive bladders to identify the underlying pathological process [24]. However, no functional investigations at the cellular level have been carried out to help understand the enhanced spontaneous activity in OAB.

This is the first study to demonstrate that spontaneous Ca^{2+}-transients exist in isolated human detrusor myocytes, and more importantly, occur at a higher frequency (unit or integrated) in cells from overactive bladder biopsies. Furthermore, the resting intracellular $[Ca^{2+}]$ was greater in overactive bladder myocytes that may facilitate the development of such transients. These differences are unlikely to be due to tissue handling or cell isolation because all tissue samples were handled similarly and the percentage of viable cells similar with all biopsy samples. Individual Ca^{2+}-transients were sufficiently large to elicit contraction. The functional relevance of this spontaneous cellular activity to in situ smooth muscle properties is indicated by the observation of spontaneous Ca^{2+} transients and associated contractile activity in multicellular preparations from both guinea-pig and human tissue [22,25,26] and the fact that such activity is enhanced in specimens from overactive human bladders [11,22]. Thus, increased spontaneous Ca^{2+}-transients may serve as a functional substrate for OAB. The deranged Ca^{2+} regulation may be a common cellular pathway for both OAB modalities and is partly attributable to hyperactive electrical activity. Whether membrane-independent mechanisms are involved in neuropathic bladder overactivity needs further investigation.

The population behaviour of individual smooth muscle cells in the bladder is likely to be more complicated than that of individual cells, as intercellular communications and the mediators in extracellular milieu would exert additional influence on their activities. However, the study has revealed fundamental mechanisms underlying such activity that would be difficult to characterize otherwise. Whilst un-coordinated contractile activity of individual cells may increase tone in the intact muscle bundle, synchronised activation of many cells will produce a bladder contraction. Synchrony may be achieved by intercellular electrical or chemical coupling, and/or stretch-activated channels. The attenuation of OAB contractions by gap junction blockers suggests intercellular co-ordination has a role in such pathological overactivity [27].

Such enhanced muscle tone and contractile activity driven by more spontaneous Ca^{2+}-transients will not just increase contractile function *per se*, but also stimulate sensory fibres in the detrusor layer by mechanical stretch, triggering an enhanced micturition reflex. Studies in cats, for example, have shown that A-δ afferents respond in a graded manner to passive distension and active contraction of the bladder [28].

The origin of the spontaneous activity in detrusor muscle bundles has been debated and non-muscle "pace making cells" have been speculated [13,29]. Recently interstitial cells within the detrusor muscle bundle have been suggested to be a potential candidate [30]. Although these interstitial cells have been extensively characterized by immunocytochemistry [31], functional evidence for pace-making activity and connectivity to smooth muscle cells is still lacking. Contrary to common belief, the present observation has confirmed that smooth muscle cells are capable of generating spontaneous activity. Furthermore, upregulation of spontaneous activity in smooth muscle accounts for the increased contraction in OABs. This not only provides an answer to pathophysiology of detrusor overactivity, but more importantly, a rationale for smooth muscle as the therapeutic target for muscle overactivity.

4.2. The origin of spontaneous Ca^{2+}-transients

Spontaneous Ca^{2+}-transients in guinea-pig detrusor muscle are importantly determined by L-type Ca^{2+} channels [25,26]. A similar involvement was suggested in human myocytes by their sensitivity to removal of extracellular Ca or addition of Ca-antagonists. In addition, Ca^{2+}-transients were associated with action potentials or smaller depolarisations. The time-course analysis indicated that membrane depolarisation preceded the rise of Ca^{2+}, opposite to the case for interstitial cells [32], and again is consistent with membrane events being ultimately responsible for spontaneous Ca^{2+}-transients. A significant observation was that small, but relatively long-lasting depolarisations, were mirrored by changes to intracellular Ca^{2+}. The prolonged depolarisations may be due to a non-inactivating or long-open state of the L-type Ca^{2+} current preparations [33], and is consistent with the efficacy of hyperpolarising agents on overactive bladders [34]. In addition, Ca^{2+} influx through Na^{+}/Ca^{2+} exchange has been shown in detrusor muscle [16]

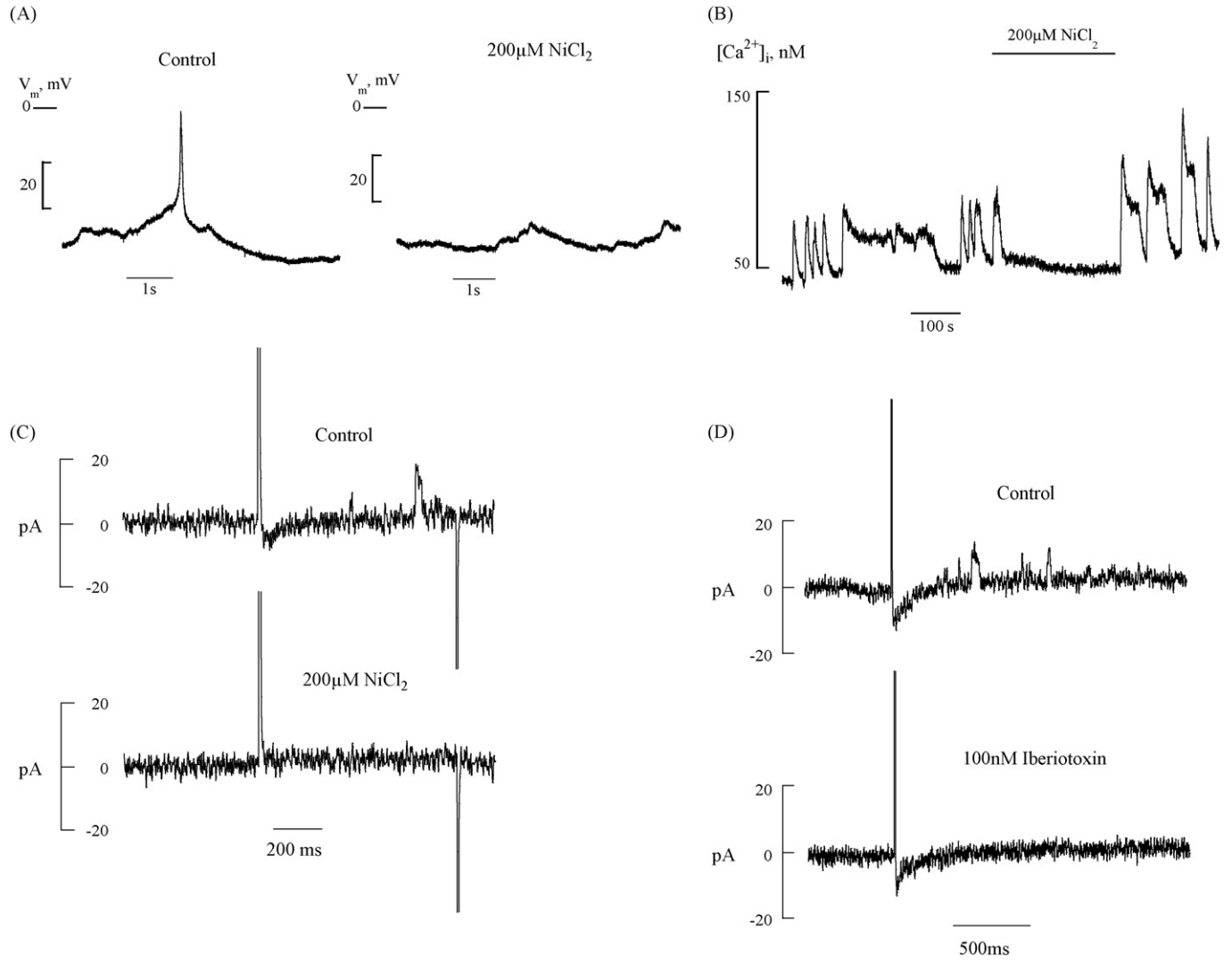

Fig. 8. Effect of 200 μM $NiCl_2$ on spontaneous activity. (A) Effect on electrical activity before and after exposure to 200 μM $NiCl_2$, 2-min interval between traces. (B) Effect on spontaneous Ca^{2+} rises, the myocyte was exposed to $NiCl_2$ during the time denoted by the horizontal bar. (C) Ni^{+}-sensitive outward current by a clamp step to selectively activate T-type current (from −90 to −50 mV). (D) Abolition of outward current by iberiotoxin.

so that a depolarisation, from whatever source, would also favour Ca^{2+} entry.

An up-regulated T-type Ca^{2+} channel activity, as demonstrated in human detrusor from idiopathic overactive bladders [35], could explain the enhanced electrical activity. T-type Ca^{2+} current is activated at more negative membrane potentials, i.e., near the resting potential recorded in these experiments. Thus, T-type channel activity would depolarize the membrane to a level where L-type channels would open and generate an action potential and enable Ca^{2+} influx. The depressant effect on spontaneous electrical activity of submillimolar $NiCl_2$ concentrations, fairly selective for T-type Ca^{2+} channels [19], would indicate their importance in initiating spontaneous activity. The ability of the T-type Ca^{2+} channel to trigger Ca^{2+}-activated current suggests its additional role in modulating BK channels of the excitation-relaxation cycle of spontaneous Ca^{2+} oscillations [36].

However, intracellular Ca-stores will also influence spontaneous activity as CPA increased the resting intracellular [Ca^{2+}] and abolished spontaneous activity. CPA prevents Ca^{2+} uptake into Ca-stores, so that membrane-evoked release is not possible. However, it was not investigated in this study if the size of intracellular Ca-stores is altered in different bladder pathologies.

4.3. Pathophysiological implications and conclusion

Ca^{2+} regulation has been widely recognised to be fundamental in a variety of biological functions [37] and thus deranged Ca^{2+} metabolism would have pathological implications. To this end, Ca^{2+} dysregulation as a pathological basis for muscular dysfunction has been recognised in skeletal muscle and cardiac muscle [38,39] and as such similar mechanisms may mediate smooth muscle disorders. Whilst data from animal models and cell culture strongly suggest a role of intracellular Ca^{2+} in vascular smooth muscle disorders [40,41], results from the present study present the first evidence that Ca^{2+} dysregulation can cause such smooth muscle dysfunction in human. Elucidation of similar mechanisms in other smooth muscle hyperactivity and identification of their modulators may reveal specific drug targets for treatment of the associated human conditions [42].

In summary, the present observations have confirmed that smooth muscle cells are capable of generating spontaneous activity and may thus serve as one origin for spontaneous activity in intact muscle, among other candidates [13,29]. Furthermore, upregulation of spontaneous activity in smooth muscle could account for the increased contraction in OABs. Distinct cellular pathways partici-

pate in such spontaneous activation. This not only provides insights to pathophysiology of detrusor overactivity, but also a rationale for smooth muscle as the therapeutic target for muscle overactivity.

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