The actions of extracellular H^+ on the electrophysiological properties of isolated human detrusor smooth muscle cells

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1. The influence of extracellular pH changes on intracellular pH and [Ca^{2+}], as well as on L-type Ca^{2+} currents, has been investigated in isolated human detrusor smooth muscle cells.
2. Alteration of extracellular pH by changing superfusate P_{CO_2} also changed intracellular pH. A change of superfusate pH made by altering the [$NaHCO_3$] at constant P_{CO_2} was not reflected in a change in intracellular pH.
3. Extracellular acidosis attenuated the magnitude and rate of change of intracellular [Ca^{2+}] evoked by raising the extracellular [KCl].
4. Extracellular acidosis attenuated the rate of rise and amplitude of the action potential, as well as the magnitude of the L-type Ca^{2+} current. In the pH range 6·78–7·62 no alteration to the voltage dependence of Ca^{2+} current activation or inactivation was recorded.
5. A close proportional relationship between tension generated by multicellular strips and the magnitude of peak inward Ca^{2+} current in isolated cells was noted over a wide range of the two variables using a number of interventions, including alteration to extracellular pH, [Ca^{2+}] and [Mg^{2+}].
6. Extracellular acidosis attenuated the magnitude of caffeine-dependent intracellular Ca^{2+} transients and the resting [Ca^{2+}]$_i$ between transients. Acidosis was without effect on the rise of [Ca^{2+}]$_i$ induced by carbachol.
7. The results suggest that the negative inotropic effect of extracellular acidosis can be accounted for by attenuation of the L-type Ca^{2+} current. The results also imply that intracellular stores are influenced by transmembrane Ca^{2+} fluxes at rest and that such fluxes are also attenuated by extracellular H^+.

The pH of the extracellular and intracellular environments of detrusor smooth muscle can vary under a number of conditions: (i) CO_2 is freely diffusible across the urothelium and urinary P_{CO_2} can vary greatly, rising to 20 kPa (Pitts, Ayers & Schiess, 1948), (ii) the urothelium may be damaged under certain conditions and become permeable to other urine constituents such as H^+ (Eldrup, Therup, Nielsen, Hald & Hainau, 1983), and (iii) H^+ ions are accumulated in the bladder wall during ischaemia, generated either by filling the lumen with physiological quantities of fluid or by systemic hypotension (Dunn, 1974; Bellringer, Ward & Fry, 1993).

Changes in pH in the vicinity of detrusor smooth muscle exert significant effects on contractile function. *In vivo*, instillation of an alkaline solution into the bladder increases fluid capacity, which may be interpreted as a reduction of detrusor tone (Sethia & Smith, 1987). *In vitro* pH changes exert complex effects and differ from those seen in some other muscle types such as myocardium. Field-stimulated contractions are attenuated by extracellular acidosis; however, when the acidosis is confined to the intracellular compartment contractility is increased. Moreover, an equivalent intracellular pH change has a larger effect than one confined to the extracellular space (Liston, Palfrey, Raimbach & Fry, 1991). This can lead to complex contractile changes when both intracellular and extracellular pH are expected to alter. For example, during hypoxic superfusion *in vitro* detrusor strips undergo a transient increase of contractility, followed by a decline of contractile function (Thomas & Fry, 1992). These changes

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have been explained by the development of an initial intracellular acidosis followed by a more substantial extracellular change. The objective of this paper is to investigate the electro-mechanical actions of extracellular H^+ on detrusor smooth muscle function over a range of pH values (7·6–6·6) that have significant effects on contractility both *in vivo* and *in vitro*. The results suggest that depression of the inward L-type Ca^{2+} current may be an important factor.

METHODS

Preparation

Single, isolated detrusor smooth muscle cells were prepared from human bladder biopsy samples obtained from a total of thirty-five patients, either at open operation or by cold-cup biopsy during endoscopic procedures. Approval for sample collection was obtained from St Thomas's Hospital ethical committee; informed patient consent was also obtained. Samples were taken at least 1 cm from the trigone and placed in a Ca^{2+}-free solution (pCa > 7·0) of the following composition (mM): NaCl, 105·4; $NaHCO_3$, 20·0; KCl, 3·6; $MgCl_2.6H_2O$, 0·9; $NaH_2PO_4.2H_2O$, 0·4; glucose, 5·5; sodium pyruvate, 4·5; *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulphonic acid] (Hepes), 4·9; pH 7·0. Isolated detrusor myocytes were produced using a collagenase-based enzyme mixture, as previously described (Montgomery & Fry, 1991).

Solutions

Isolated cells were superfused at 37 °C with a Tyrode solution gassed with 5% CO_2, 95% O_2 of the following composition (mM): NaCl, 118·0; $NaHCO_3$, 24·0; KCl, 4·0; $MgCl_2.6H_2O$, 1·0; $NaH_2PO_4.2H_2O$, 0·4; $CaCl_2.6H_2O$, 1·8; glucose, 6·1; sodium pyruvate, 5·0; pH 7·33 ± 0·02. Superfusate pH was altered by addition or partial omission of $NaHCO_3$ (equimolar replacement with NaCl) or by increasing the CO_2 content of the gassing mixture. $NaHCO_3$ and Ca^{2+} form ion pairs in solution so that the added $CaCl_2$ must be varied when the [$NaHCO_3$] is altered to maintain a constant [Ca^{2+}] (Fry & Poole-Wilson, 1981). Solutions containing 6 and 48 mM $NaHCO_3$ were titrated with $CaCl_2$, using a Ca^{2+}-selective electrode, to a constant free [Ca^{2+}] equal to that in normal Tyrode solution (24 mM $NaHCO_3$). $CaCl_2$ concentrations of 1·50 and 2·34 mM were required in 6 and 48 mM $NaHCO_3$ solutions respectively. Additions of $CaCl_2$, KCl and $NiCl_2$ were made from 1 M stock solutions. Measurement of the effects of pH_o changes on experimental variables were all measured at steady state unless otherwise stated, i.e. 10–15 min after introduction of the test solution.

Measurement of intracellular pH and [Ca^{2+}]

Intracellular [H^+] and [Ca^{2+}] were measured by epifluorescence microscopy using, respectively, the intracellular indicators BCECF and fura-2 (Fluka chemicals, Glossop, Derbyshire, UK). Cells were loaded with a 1 mM solution of the cell-permeant form of the indicators for 30–60 min, then superfused at 37 °C with Tyrode solution on the stage of an inverted microscope. Cells were alternately illuminated at 430 and 510 nm (H^+) or 340 and 380 nm (Ca^{2+}) and fluorescent light collected over the ranges 530–580 nm (H^+) or 400–510 nm (Ca^{2+}). BCECF signals were calibrated using 10 μM nigericin in a high-K^+ medium to yield values of intracellular pH (Eisner, Nichols, O'Neill, Smith & Valdeolmillos, 1989). The fura-2 signals were not calibrated and results are expressed as a ratio of fluorescent light upon excitation at 340 and 380 nm (340/380 ratio).

Electrophysiological recordings

Action potentials and L-type Ca^{2+} currents were measured using patch electrodes (4–5 MΩ) in the whole-cell configuration at a sampling frequency of 4 kHz. Electrodes were filled with a solution containing (mM): CsCl, 130·0; sodium pyruvate, 5·0; sodium oxaloacetate, 5·0; sodium succinate, 5·0; Hepes, 10·0; ethylene glycol bis-(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), 1·0; pH 7·0. Action potentials were recorded under current-clamp conditions from a membrane potential of −70 mV and were elicited generally by a 40 ms, 80 pA depolarizing pulse every 3 s. Ca^{2+} current, I_{Ca}, was elicited from a holding potential of −60 mV up to +40 mV, using twelve 100 ms depolarizing steps in increments of 10 mV at 5 s intervals. Each protocol was completed every 3 min. The magnitude of I_{Ca} was taken as the difference between peak inward current and that prior to the clamp step – no leak subtraction was used on the records. The voltage dependence of activation (d_∞) was assessed by measuring the magnitude of I_{Ca} as a function of membrane potential, V; d_∞ was calculated from the chord conductance, $g = I_{Ca}/(V - E_{rev})$, where E_{rev} is the calculated value of the reversal potential of I_{Ca} from current–voltage relationships. Values were expressed as a fraction of the maximal value of g as a function of V. The voltage dependence of inactivation (f_∞) was measured using a two-pulse protocol repeated at 5 s intervals. A 2 s preconditioning pulse depolarized the membrane from −60 mV to between −50 and 0 mV in 10 mV increments, immediately followed by a 100 ms pulse to +10 mV to elicit I_{Ca}. Preliminary experiments showed that the reactivation time constant of I_{Ca} increased as the holding potential was less negative (−60 to −10 mV), but recovery was always > 95% after 2 s.

Data were fitted to the Boltzmann equation, thus:

$$d_\infty = (1 + \exp(-(V - V_{0.5})/K_d))^{-1}, \quad (1)$$

$$f_\infty = (1 + \exp((V - V_{0.5})/K_f))^{-1}, \quad (2)$$

where, $V_{0.5}$ is the voltage at which d_∞ and $f_\infty = 0.5$, and K_d and K_f are slope factors.

The decay of I_{Ca} with respect to time, $I_{Ca}(t)$, was fitted to a single exponential of the form:

$$I_{Ca}(t) = A(\exp(-t/\tau) - C), \quad (3)$$

where τ is the time constant of current decay, A is the value of $I(t)$ at $t = 0$ and C is the asymptotic value of the current trace. No significant improvement of the variance (F test) was gained by fitting the decay of the current traces with two or more exponential functions.

Statistics

All values in the text are quoted as means ± S.D., n is the number of different cells in which observations were made. Two-tailed paired or unpaired Student's t tests were used to test for significance between all normally distributed sets of data of equal variance and the null hypothesis was rejected when $P < 0.05$.

RESULTS

Changes to intracellular pH (pH_i) with alteration of extracellular pH (pH_o)

It has previously been shown, using ferret detrusor cells, that increasing superfusate P_{CO_2}, at constant [$NaHCO_3$], generates both an intracellular and extracellular acidosis, whilst alteration of the extracellular [$NaHCO_3$], at constant P_{CO_2}, generates pH alterations which are largely confined to the extracellular space (Liston *et al.* 1991). Similar results were obtained with the human detrusor cells. Initial pH_i in normal Tyrode solution was $7{\cdot}11 \pm 0{\cdot}17$ ($n = 19$). An increase of superfusate P_{CO_2} from 4·6 to 9·2 kPa (pH_o $7{\cdot}35 \pm 0{\cdot}03$ to $7{\cdot}06 \pm 0{\cdot}03$) significantly

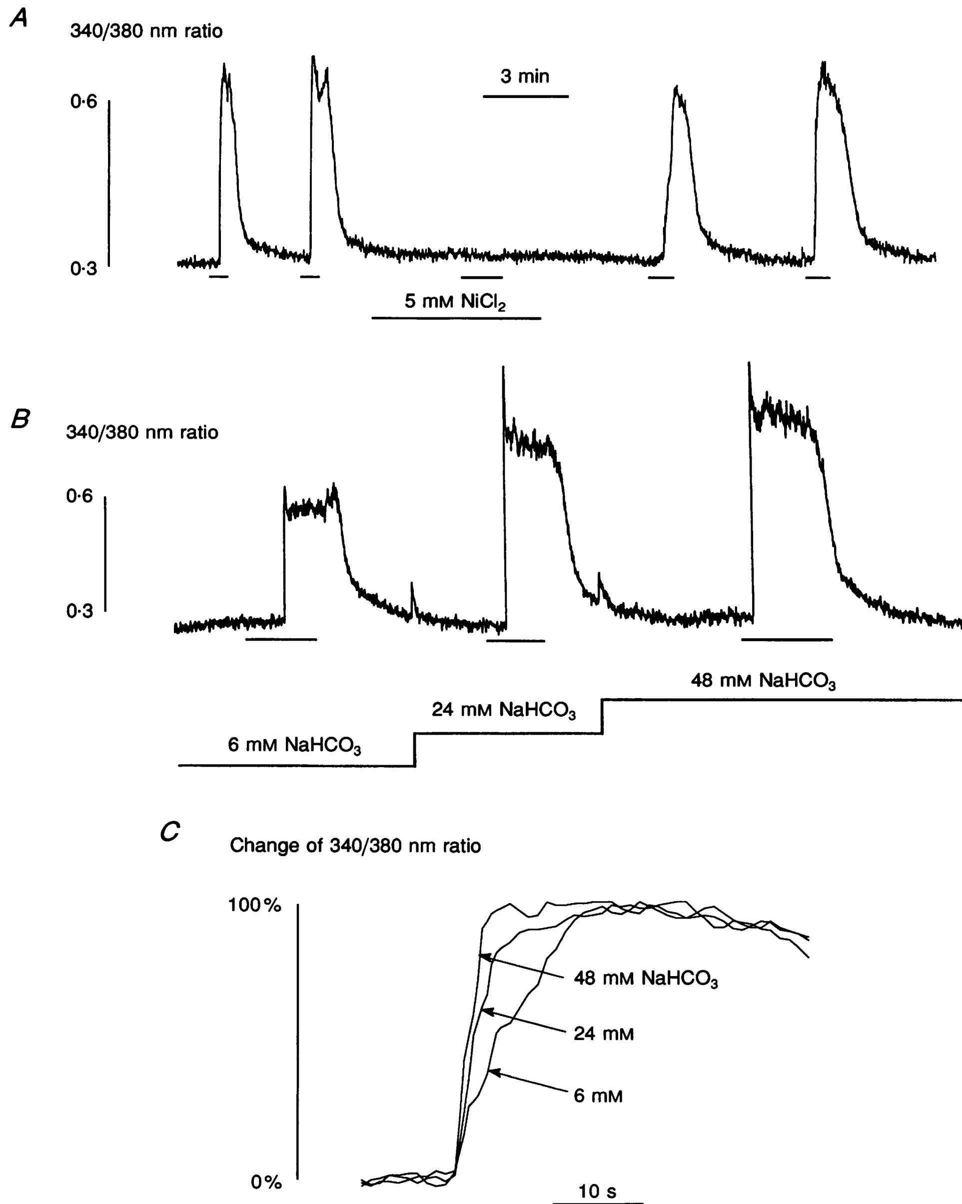

Figure 1. Intracellular Ca^{2+} transients evoked by increasing superfusate [KCl]
Intracellular Ca^{2+} transients (fura-2 fluorescence ratio at 340/380 nm) from isolated human detrusor myocytes evoked by increasing superfusate [KCl] during the periods indicated by the bars immediately below the traces. *A*, superfusate [KCl] was raised from 4 to 44 mM; 5 mM $NiCl_2$ was also added during the period indicated. *B*, high-K^+ (4–44 mM) transients evoked in the presence of 6 mM $NaHCO_3$, normal Tyrode solution (24 mM $NaHCO_3$) or 48 mM $NaHCO_3$. *C*, the initial phases of three transients evoked by increasing superfusate [KCl] to 140 mM in the presence of 6, 24 and 48 mM $NaHCO_3$. Transients have been scaled so that they are of equivalent magnitude. Experiments performed at 37 °C.

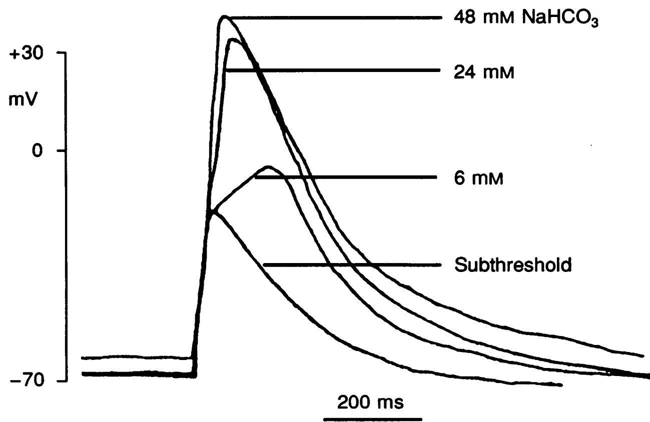

Figure 2. Superimposed action potentials from isolated human detrusor myocytes
Superimposed action potentials from isolated human detrusor myocytes recorded with CsCl-filled electrodes in the presence of 6, 24 or 48 mM $NaHCO_3$. A subthreshold pulse is also shown. Experiments performed at 37 °C.

reduced pH_i by $0{\cdot}19 \pm 0{\cdot}08$ units ($n = 13$, $P < 0{\cdot}01$). However, reduction of superfusate [$NaHCO_3$] from 24 to 6 mM (pH_o $7{\cdot}35 \pm 0{\cdot}03$ to $6{\cdot}78 \pm 0{\cdot}05$) or an increase from 24 to 48 mM (pH_o $7{\cdot}35 \pm 0{\cdot}03$ to $7{\cdot}62 \pm 0{\cdot}04$) did not significantly change pH_i ($\Delta pH_i = -0{\cdot}07 \pm 0{\cdot}11$ ($n = 6$) and $\Delta pH_i = 0{\cdot}01 \pm 0{\cdot}04$ ($n = 6$) respectively; $P > 0{\cdot}05$). The following experiments describe the result of changing superfusate [$NaHCO_3$] on detrusor myocytes.

Extracellular pH and K^+-induced intracellular Ca^{2+} transients

An increase of the superfusate [K^+] induces a contracture in human detrusor muscle strips (Liston *et al.* 1991). Figure 1 shows that in isolated cells a similar intervention is accompanied by an increase of the intracellular [Ca^{2+}], [Ca^{2+}]$_i$, as manifest by the change of fura-2 fluorescence. Figure 1*A* shows that exposure to 44 mM KCl increased the [Ca^{2+}]$_i$, which declined to control values on return to normal solution. Such observations were made in seven cells. The phenomenon could not be repeated in the presence of 5 mM $NiCl_2$ ($n = 5$), 1 μM nifedipine ($n = 3$, not shown) or zero superfusate $CaCl_2$ ($n = 4$, not shown) and suggests a transmembrane flux of Ca^{2+} in high-K^+ solution. Figure 1*B* shows three high-K^+ transients in the presence of 6 mM (pH_o 6·78), 24 mM (pH_o 7·35) and 48 mM (pH_o 7·62) $NaHCO_3$. The Ca^{2+} transient magnitude was progressively reduced as pH_o became more acid. In four cells the maximum fluorescence change in 48 mM $NaHCO_3$ was 117 ± 10% (mean ± S.D., $P < 0{\cdot}05$) of that in 24 mM $NaHCO_3$ (= 100%) and in 6 mM $NaHCO_3$ it was 64 ± 11% (mean ± S.D., $P < 0{\cdot}01$). Figure 1*C* shows that the rate of rise of such transients was also reduced in progressively acid solutions. The initial phases of three transients in 6, 24 and 48 mM $NaHCO_3$ from the same cell have been superimposed. Magnitudes of the transients were normalized to be 100% at their respective maxima. The rate of fluorescence change during the rising phase in 48 mM $NaHCO_3$ was 153 ± 18% (mean ± S.D., $n = 4$, $P < 0{\cdot}05$) of that in 24 mM $NaHCO_3$ (= 100%) and in 6 mM $NaHCO_3$ it was 37 ± 11% (mean ± S.D., $n = 4$, $P < 0{\cdot}01$). These results suggest that extracellular acidosis attenuates transmembrane movement of Ca^{2+}.

Extracellular pH and action potential configuration

Action potentials were recorded in isolated cells under current-clamp conditions using CsCl-filled patch electrodes. Under these conditions outward K^+ currents are blocked

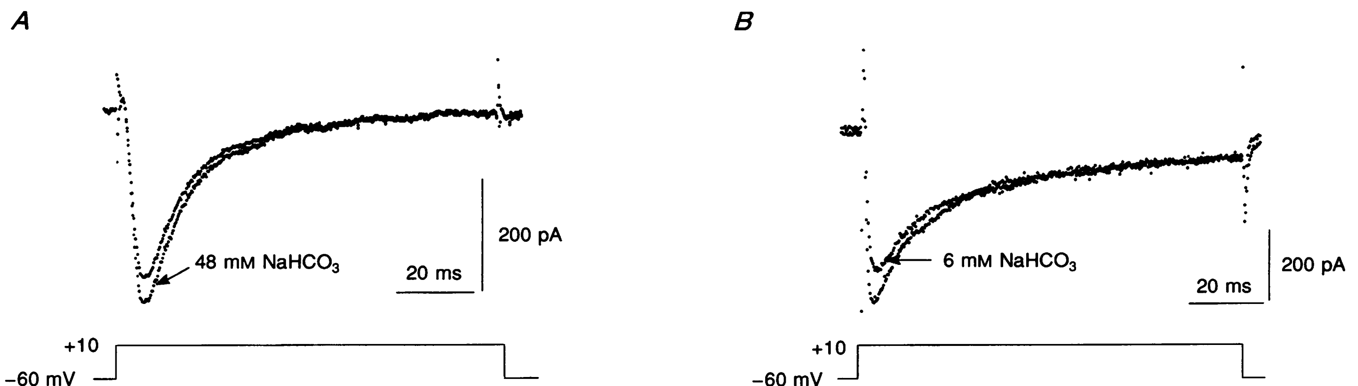

Figure 3. Ca^{2+} currents recorded from isolated human detrusor myocytes
Ca^{2+} currents recorded from isolated human detrusor myocytes with a step depolarization from −60 to +10 mV. Traces were recorded in the presence of 24 and 48 mM $NaHCO_3$ (*A*) or 24 and 6 mM $NaHCO_3$ (*B*). Experiments performed at 37 °C.

and only inward current through an L-type Ca^{2+} channel can be measured, thus generating long-duration responses (Montgomery & Fry, 1991). Figure 2 shows superimposed action potentials recorded in 6 mM (pH_o 6·78), 24 mM (pH_o 7·35) and 48 mM (pH_o 7·62) $NaHCO_3$. Decreasing pH_o resulted in a slowing of the depolarizing (upstroke) phase, reduction of the amplitude and an overall shortening of the action potential. The figure also shows a subthreshold pulse – the action potential upstroke is generated at potentials more positive than a threshold value, which is the peak of the subthreshold pulse. In this figure upstroke velocities were 3·9, 2·9 and 0·2 V s^{-1} in 48, 24 and 6 mM $NaHCO_3$. Mean results in 48, 24 and 6 mM $NaHCO_3$ were $3·8 \pm 0·7$ ($n = 10$), $2·6 \pm 0·7$ ($n = 10$) and $0·4 \pm 0·2$ V s^{-1} ($n = 5$), respectively.

Extracellular pH and the Ca^{2+} current, I_{Ca}

It has been shown previously that the predominant inward current responsible for the action potential upstroke is an L-type Ca^{2+} current. Therefore the effect of altering pH_o on this current component was investigated in isolated cells under voltage-clamp conditions, using CsCl-filled patch electrodes to eliminate outward currents. Figure 3 shows inward Ca^{2+} currents obtained by depolarizing the muscle from −60 to +10 mV and recorded in normal Tyrode solution (24 mM) and 48 mM $NaHCO_3$ (Fig. 3*A*) or in normal Tyrode solution and 6 mM $NaHCO_3$ (Fig. 3*B*). An alkalosis from $7·35 \pm 0·03$ to $7·62 \pm 0·03$ increased peak I_{Ca} by 19 ± 13% ($n = 14$, $P < 0·01$) and an acidosis to $6·78 \pm 0·05$ reduced peak I_{Ca} by 16 ± 10% ($n = 11$, $P < 0·01$).

Control experiments with extracellular Ca^{2+}

The preparation of superfusates containing variable amounts of $NaHCO_3$ required adjustment of the [$CaCl_2$] to maintain the ionized [Ca^{2+}] constant (see Methods). If the [Ca^{2+}] was not constant this might alter the relationship between magnitude of I_{Ca} and [$NaHCO_3$]. Figure 4*A* shows the relationship between I_{Ca} magnitude and superfusate [$CaCl_2$] in normal Tyrode solution; results are expressed as a proportion of the current recorded in 1·8 mM calcium Tyrode solution. I_{Ca} magnitude increased to a maximum of 152 ± 23% and could be described as having a first-order relationship with superfusate Ca^{2+} (half-maximal concentration, $0·98 \pm 0·11$ mM, $n = 10$).

Figure 4*B* shows the effect of altering the superfusate [$NaHCO_3$] on I_{Ca} when the ionized [Ca^{2+}] was maintained constant (●; see Methods). Decreasing the [$NaHCO_3$], i.e. producing acidosis, decreased the magnitude of I_{Ca}. The data points (▲) show the calculated variation of I_{Ca} if the superfusate [$CaCl_2$] was unadjusted as the [$NaHCO_3$] was altered. These latter points were derived from experimental values (●) multiplied by the proportional alteration of I_{Ca} due to the changed ionized [Ca^{2+}], as obtained from Fig. 4*A*. Thus if care is not taken to maintain constant the Ca^{2+} activity in experimental solutions the dependence of peak I_{Ca} magnitude on pH_o will be attenuated.

The relationship between I_{Ca} magnitude and tension

Interventions which alter I_{Ca} magnitude in isolated human detrusor cells also exert effects on phasic tension development in field-stimulated strips of tissue. Figure 5 shows the relationship between these two variables during a variety of extracellular interventions: 5 mM $NiCl_2$ (■), 10 mM [$MgCl_2$] (□), acidosis to pH 6·78 (●), control (○), alkalosis to pH 7·62 (▲), and 5 mM [$CaCl_2$] (△). In control experiments addition of 5 mM $NiCl_2$ to the superfusate completely and reversibly abolished I_{Ca}. The contractile

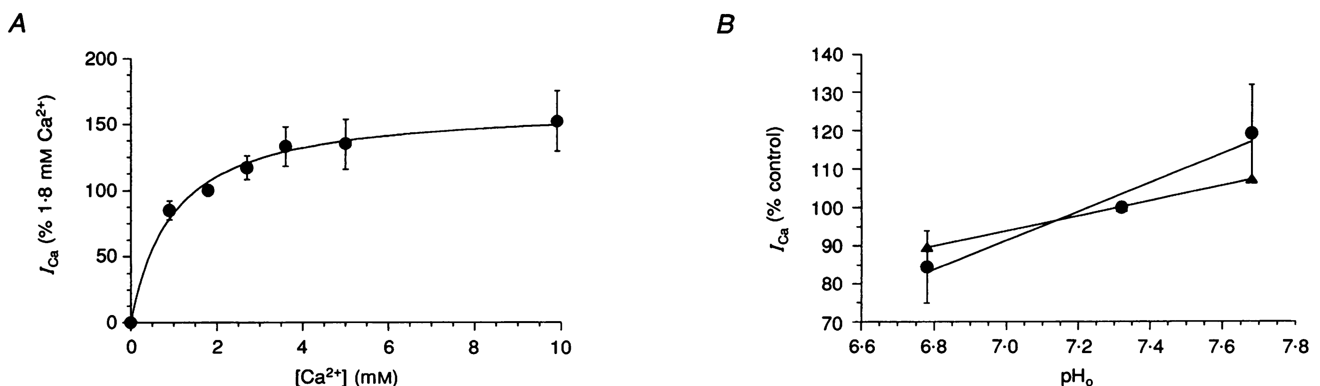

Figure 4. The influence of extracellular calcium and pH on Ca^{2+} current magnitude
A, the relationship between superfusate [Ca^{2+}] and the magnitude of peak I_{Ca} (depolarization from −60 to +10 mV). I_{Ca} is expressed as a proportion of that at pH 7·35, when [Ca^{2+}] is 1·8 mM. Experiments at 37 °C; data are given as means ± S.D. *B*, the experimental relationship between extracellular pH and the magnitude of peak I_{Ca} (depolarization from −60 to +10 mV), at constant superfusate Ca^{2+} activity (●). The figure also includes the calculated relationship if Ca^{2+} activity was uncorrected (▲). I_{Ca} is expressed as a proportion of that at pH 7·35.

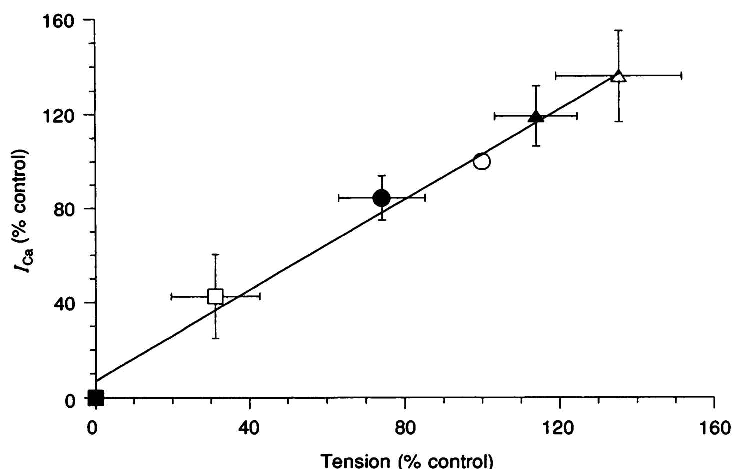

Figure 5. The relationship between peak I_{Ca} and phasic tension

The relationship between peak I_{Ca} measured in isolated myocytes (depolarization from −60 to +10 mV) and phasic tension measured in field stimulated strips for several conditions: ■, 5 mM $NiCl_2$; □, 10 mM $MgCl_2$; ●, $pH_o = 6{\cdot}78$; ○, control; ▲, $pH_o = 7{\cdot}62$; △, 5 mM $CaCl_2$. Data are expressed as percentages (means ± S.D.) of those under control conditions. Experiments performed at 37 °C.

data and the effect of 10 mM $MgCl_2$ on I_{Ca} have been reported elsewhere (Liston *et al.* 1991; Montgomery, Thomas & Fry, 1992). A close relationship was observed between the two variables, implying that the magnitude of I_{Ca} is important in determining the strength of the field-stimulated detrusor contraction.

Extracellular pH and Ca^{2+} current kinetics

Alteration of extracellular ions, especially multivalent ions, change not just the magnitude of ionic currents but also the potential dependence of channel gating. Activation (d_∞) and inactivation (f_∞) curves of I_{Ca} were therefore constructed when the extracellular [$CaCl_2$] or pH was varied. Figure 6*A* shows that raising the [$CaCl_2$] from 0·9 (◆) to 1·8 (○) and 10·0 mM (▲) shifted activation curves to more positive potentials, with no significant change to the slope factor (K_d). However, in the range of pH values tested, 7·6–6·8, no shift of either the activation or inactivation curves was observed – Fig. 6*B* illustrates activation curves of I_{Ca} at pH 7·62 (▲), 7·35 (○) and 6·78 (◆).

The time constant of I_{Ca} decay, τ_{decay}, was also unaffected by alteration of extracellular pH but was shortened by an increase of the extracellular [$CaCl_2$] to 10 mM and lengthened by a decrease to 0·9 mM. Table 1 summarizes the effects of altered extracellular pH and [$CaCl_2$] on I_{Ca} characteristics – apart from the changes mentioned above, 10 mM $CaCl_2$ caused also a small but significant increase of the slope factor, K_f.

Effect of extracellular pH on intracellular Ca^{2+} release

Extracellular pH may also alter sarcolemmal Ca^{2+} fluxes in unstimulated detrusor cells and hence indirectly affect intracellular Ca^{2+} movements by varying the magnitude of Ca^{2+} stores. Figure 7 shows measurements of intracellular [Ca^{2+}] as assessed by fura-2 fluorescence. A brief exposure to 10 mM extracellular caffeine resulted in a transient increase of the intracellular [Ca^{2+}]. A linear relationship between the magnitude of fluorescence increase and log [caffeine] was recorded between 1 and

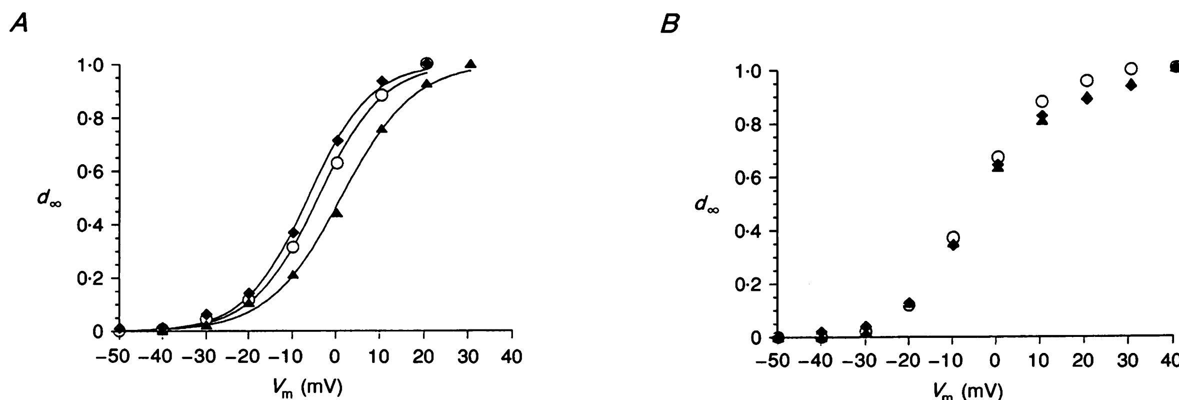

Figure 6. Activation curves of I_{Ca} from isolated human detrusor myocytes

A, data obtained in the presence of 0·9 mM (◆), 1·8 mM (○) and 10 mM (▲) superfusate $CaCl_2$. Curves were fitted according to eqn (1). *B*, data in the presence of superfusate at pH 7·62 (◆), 7·35 (○) and 6·78 (◆). Experiments performed at 37 °C; data are given as means; S.D. values omitted for clarity.

Table 1. Ca^{2+} **current** (I_{Ca}) **characteristics of human detrusor smooth muscle cells**

Variable	Control	6 mM $NaHCO_3$ ($n = 6$)	Control	48 mM $NaHCO_3$ ($n = 6$)	Control	0·9 mM $CaCl_2$ ($n = 8$)	10 mM $CaCl_2$ ($n = 8$)
Activation $V_{0.5}$ (mV)	-4.2 ± 5.7	-3.3 ± 6.0	-5.7 ± 3.9	-3.8 ± 5.3	-3.5 ± 3.9	-6.2 ± 3.6*	0.1 ± 5.5*
Activation K_d (mV)	7.2 ± 0.8	7.6 ± 1.8	7.2 ± 0.5	8.6 ± 2.2	7.7 ± 0.8	7.2 ± 0.5	8.8 ± 1.2
Inactivation $V_{0.5}$ (mV)	-31.7 ± 3.8	-29.6 ± 4.1	32.1 ± 3.8	-32.9 ± 4.1	-27.6 ± 2.8	-28.1 ± 5.6	-29.4 ± 3.9
Inactivation K_f (mV)	3.8 ± 1.4	3.9 ± 1.2	5.3 ± 1.4	5.0 ± 1.4	5.7 ± 0.7	6.4 ± 1.0	7.0 ± 0.6***
τ_{decay} (ms)	18.1 ± 2.0	18.5 ± 1.6	13.8 ± 3.4	14.4 ± 4.3	13.4 ± 4.9	18.1 ± 7.3**	10.2 ± 3.0*

Values are quoted of half-maximal ($V_{0.5}$) activation and inactivation voltages and corresponding slope factors (K_d, K_f) – see eqns (1) and (2). Time constants of I_{Ca} decay were obtained from eqn (3). Values measured in control solution (24 mM $NaHCO_3$, 1·8 mM $CaCl_2$) and in altered [$NaHCO_3$] or [$CaCl_2$]. Experimental values, given as means ± S.D., are compared to the preceding control. Experiments performed at 37 °C. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. n, number of cells.

30 mM (not shown). Fifteen second exposures to 10 mM caffeine, at 3 min intervals, were chosen as responses were reproducible for up to 1 h under these conditions. Figure 7*A* shows that removal of extracellular Ca^{2+} reduced the baseline signal and eventually abolished the caffeine-dependent transient response. The first caffeine exposure evoked a transient, but thereafter transients were greatly attenuated. Upon readdition of extracellular Ca^{2+} the baseline and transient responses returned to control levels.

A similar reversible abolition of the Ca^{2+} transient was obtained if 5 mM $NiCl_2$ was added to the superfusate.

Figure 7*B* shows the effect of altering extracellular pH on the caffeine-dependent transient response by either decreasing superfusate [$NaHCO_3$] to 6 mM or increasing it to 48 mM. Extracellular acidosis decreased and alkalosis increased the magnitude of the transient. In five cells 48 mM $NaHCO_3$ increased the transient to 162 ± 23% of control (mean ± S.E.M., $P < 0.05$) whilst 6 mM $NaHCO_3$

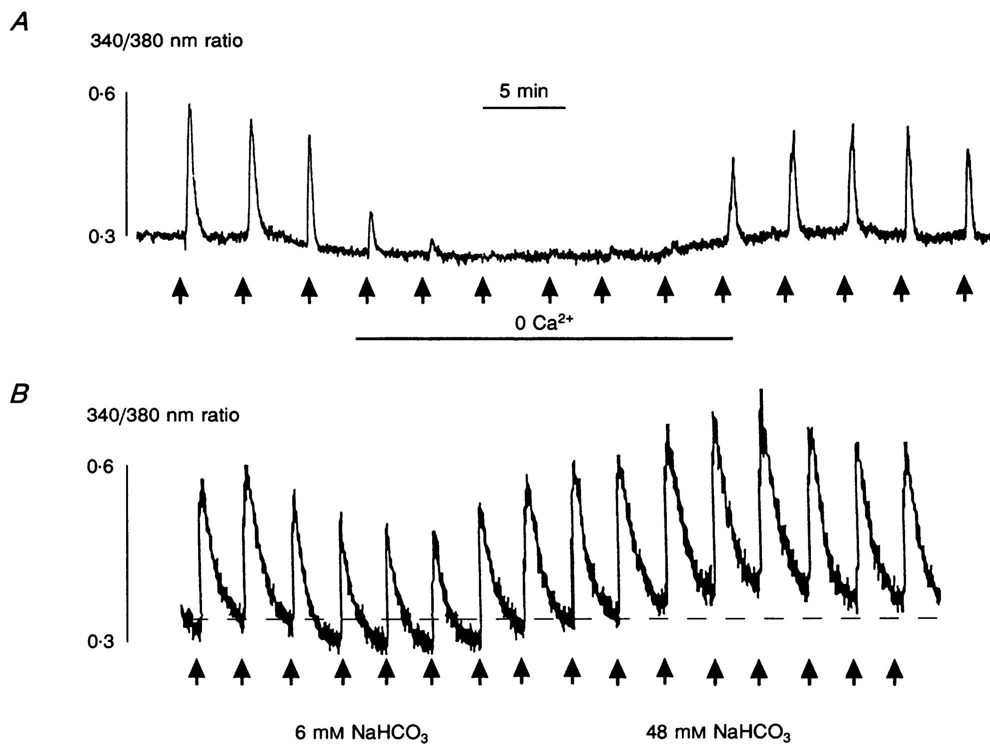

Figure 7. Intracellular Ca^{2+} transients evoked by exposure to 10 mM caffeine
Intracellular Ca^{2+} transients (fura-2 fluorescence ratio at 340/380 nm) from isolated human detrusor myocytes evoked by exposure to 10 mM caffeine for 15 s, as indicated by the arrows. *A*, the effect of extracellular Ca^{2+} removal during the horizontal bar. *B*, the effect of extracellular acidosis to pH 6·78 (6 mM $NaHCO_3$) or alkalosis to 7·62 (48 mM $NaHCO_3$) during the period indicated by the horizontal bars. Experiments were performed at 37 °C.

decreased the transient magnitude to $62 \pm 12\%$ of control (mean $\pm$ S.E.M., $P < 0.01$). These effects were fully reversible in all cases. The baseline fluorescence signal was also reduced in acid solutions and increased in alkaline solutions. The dashed line in Fig. 7*B* represents the mean baseline before application of the test solutions. In the absence of caffeine exposures there were no changes to the baseline when either extracellular Ca^{2+} was removed or extracellular pH changed.

A rise in the intracellular $[Ca^{2+}]$ can also be induced by brief (15 s) exposure of detrusor cells to 10 μM carbachol. These responses were unaffected by adding 44 mM KCl to the superfusate and thus might be presumed to be relatively independent of cell membrane potential. Addition of 5 mM $NiCl_2$ had no effect on the first carbachol transient but subsequent transients were reduced, suggesting again the importance of the extracellular space in replenishing intracellular Ca^{2+} stores. Single carbachol exposures were carried out in either acid (6 mM $NaHCO_3$) or alkaline (48 mM $NaHCO_3$) superfusates and the results compared to control. No significant effect of changing pH_o on the magnitude of this transient was recorded (105 ± 5 and $91 \pm 5\%$ of control, respectively; means $\pm$ S.E.M., $n = 5$).

DISCUSSION

Results presented here show that extracellular acidosis attenuates transmembrane Ca^{2+} flux in isolated human detrusor smooth muscle cells and that such effects can explain the reduction of phasic force recorded in multicellular preparations under similar conditions (Liston *et al.* 1991). In all cases reported above alkalosis produced opposite results. The fact that pH changes were confined to the extracellular space when superfusate $[NaHCO_3]$ was altered suggests that the permeability to HCO_3^- is small in detrusor. Evidence from vas deferens and taenia coli smooth muscles shows that HCO_3^- can cross the cell membrane by Cl^-–HCO_3^- exchange, but that the magnitude can vary between muscle types (Aickin & Brading, 1990). The small changes of pH_i reported here would suggest a minor role in detrusor muscle.

Transmembrane Ca^{2+} movement and extracellular pH

These results suggest that membrane depolarization of human detrusor, by increasing the $[K^+]_o$, causes a rise of the $[Ca^{2+}]_i$ which may be produced by a transmembrane Ca^{2+} influx, and that such a flux can be attenuated by extracellular acidosis. The observation that the rise of the intracellular $[Ca^{2+}]_i$ can be blocked by removal of extracellular Ca^{2+}, or addition of $NiCl_2$ or nifedipine, suggests it is mediated by a transmembrane flux, possibly through L-type Ca^{2+} channels. A rise of $[K^+]_o$ evokes contracture, mediated by membrane depolarization in muscle strips, and a rise of the $[Ca^{2+}]_i$ in isolated cells. Contracture can be detected at a $[K^+]_o$ as low as 12 mM, sufficient to depolarize detrusor to about -40 mV (Palfrey, Fry & Shuttleworth, 1984). In isolated cells depolarization to between -40 and -32 mV is just sufficient to elicit a Ca^{2+} current through L-type channels, whilst raising $[K^+]_o$ to 12 mM will generate a small rise of the $[Ca^{2+}]_i$. Extracellular acidosis over the range 7·6–6·8 decreased both the magnitude of the L-type Ca^{2+} current as well as the magnitude and rate of increase of $[Ca^{2+}]_i$ on raising $[K^+]_o$. These observations are consistent with the similar reduction of phasic force, induced by field stimulation, and the high-K^+ contracture, induced by the same alterations to pH_o (Liston *et al.* 1991).

A decrease of pH_o has been shown to reduce the magnitude of L-type Ca^{2+} current from a number of cell types. In several reports the weight of evidence is that reduction is largely the result of a change to the gating properties of the channel, as evidenced by a shift of the activation and inactivation curves to more positive potentials with extracellular acidosis (Iijima, Ciani & Hagiwara, 1986; Prod'hom, Pietrobon & Hess, 1987; Krafte & Kass, 1988; Gilliam, Rivas, Wendt, Starmer & Grant, 1990). Krafte & Kass (1988) showed that an acidosis of 0·3 units from pH 7·4 shifted the curves by 2 mV. The precise relationship between pH_o and the magnitude of the voltage shift is dependent, among other factors, on the association constant, K_H, of H^+ to surface groups of density σ_H. A value for pK_H of 5·8 was calculated for cardiac Ca^{2+} channels with a charge density of $-1e$ per 2·5 nm^2.

The lack of observed effect of pH_o changes on i_{Ca} activation and inactivation curves is at variance with the above reports. It is possible that with detrusor myocytes the pK_H value may be in the more acid range so the observed effects would be proportionately smaller. For example, a value of pK_H of 4·8 would predict a shift of only 2 mV for a pH_o change from 7·35 to 6·78, which is near the limits of experimental resolution. It is of interest that the proportional decline of I_{Ca} over the above pH range was also relatively modest (15·6%) compared with similar experiments with cardiac ventricular myocytes. However, it is of note that not all reports show a shift of (in)activation curves with alteration to pH_o. Using sino-atrial node cells, Satoh & Seyama (1986) found a decrease of I_{Ca} magnitude caused by acidosis without alteration of the gating properties of the current and suggested protonation of the channels. Future experiments with pH_o variations over a greater range are needed to resolve this issue in detrusor myocytes. The effect of altering extracellular Ca^{2+} on (in)activation curves was, however, similar to that measured in cardiac myocytes (e.g. Kass & Krafte, 1987).

The detrusor action potential is characterized by a rising phase dependent on an L-type Ca^{2+} current (Montgomery & Fry, 1991), so that alteration of pH_o would

be expected to slow the initial rate of depolarization. Action potentials were recorded with CsCl-filled electrodes so that K^+ currents would not interfere with this phase of the action potential. Isolated detrusor cells are uniformly polarized under such experimental conditions so that $d\dot{V}/dt_{max}$ is proportional to net membrane ionic current, i.e. $I_{Ca} = c_m d\dot{V}/dt_{max}$, where c_m is cell membrane capacitance. In seventy-two human detrusor myocytes the average values of I_{Ca} and c_m in normal Tyrode solution were 355 ± 151 pA (mean ± S.D., $n = 72$) and 66 ± 16 pF (mean ± S.D., $n = 72$) respectively, yielding a calculated value for $d\dot{V}/dt_{max}$ of $5{\cdot}4 \pm 2{\cdot}4$ V s^{-1} (mean ± S.D., $n = 72$). This compares with the experimental value of $2{\cdot}6 \pm 0{\cdot}7$ V s^{-1} (mean ± S.D., $n = 10$), which is not significantly different from the calculated value ($P > 0{\cdot}05$).

The effects of pH_o changes on action potential upstroke velocity confirmed the measurements of I_{Ca}. Extracellular alkalosis increased both peak I_{Ca} and $d\dot{V}/dt_{max}$ to 120 ± 13 and 150 ± 24% of control respectively, values not significantly different from each other. However, acidosis exerted a much greater proportional effect on $d\dot{V}/dt_{max}$ than on peak Ca^{2+} current (to 15 ± 10% of control and 84 ± 10% of control, respectively). The reason for this difference is unclear, but it may be possible that when recording action potentials at low pH a residual K^+ current persists which may attenuate upstroke velocity by hastening repolarization.

The relationship between tension and I_{Ca} magnitude

Using guinea-pig detrusor smooth muscle cells it has been shown that the rise of $[Ca^{2+}]_i$ during depolarization is closely correlated with the magnitude of I_{Ca} and that this influx of Ca^{2+} is crucial for the further release of Ca^{2+} from intracellular stores (Ganitkevich & Isenberg, 1991, 1992*b*). Figure 5 above shows that this correlation extends to a close relationship between the percentage change of I_{Ca} magnitude, measured in isolated myocytes, and phasic tension, recorded from multicellular preparations under a variety of experimental conditions including alteration of the extracellular concentration of, among other ions, H^+, Ca^{2+} and Mg^{2+}.

Caffeine- and carbachol-induced intracellular Ca^{2+} transients

Application of 10 mM caffeine resulted in a transient increase of the $[Ca^{2+}]_i$, similar to those observed in other smooth muscle preparations (Sato, Ozaki & Karaki, 1988; Pacaud & Bolton, 1991; Ganitkevich & Isenberg, 1992*a*) and presumably due to a release from intracellular stores. The responses were highly reproducible for more than 1 h with repeated caffeine applications, which differs from the rapid run-down reported by Ganitkevich & Isenberg (1992*a*) in guinea-pig bladder cells. The difference may be due to the absence of an attendant patch electrode in the above experiments, so that although it is not possible to control membrane potential during such experiments important intracellular constituents may not be perfused away, thus allowing consistent transients to be elicited.

The reduction of caffeine-dependent transients by acidosis could result from the attenuation of a transmembrane Ca^{2+} flux, which is important in maintaining the quantity of Ca^{2+} in intracellular stores. A Ca^{2+} flux via Na^+–Ca^{2+} exchange is one possibility, but needs to be established. The importance of extracellular Ca^{2+} in maintaining such responses was evident from their slow decline in Ca^{2+}-free superfusate. Similar findings have been described concerning the bradykinin-induced rise of $[Ca^{2+}]_i$ in endothelial cells. Increased pH_o enhanced the transients but had no effect on the rate of decline of the transient, implying that extrusion of Ca^{2+} from the cell was unaffected, but that the quantity of Ca^{2+} in intracellular stores was dependent on transmembrane flux of Ca^{2+} (Thuringer, Diarra & Sauvé, 1991).

Addition of 10 μM carbachol also evoked a transient rise of the $[Ca^{2+}]_i$, which has been attributed to release from those intracellular stores that are also sensitive to caffeine and which is accompanied by a transient outward current (Komori & Bolton, 1990; Pacaud & Bolton, 1991). This observation would suggest that alteration of pH_o over the range 7·6–6·8 has little effect on the binding of carbachol to the muscarinic receptor and/or the transduction mechanism for the release of intracellular Ca^{2+}. This observation is in agreement with measurements of phasic force in multicellular strips of detrusor muscle when the magnitude of force reduction by extracellular acidosis was the same whether the muscle was stimulated directly or via the embedded cholinergic nerve network (Liston *et al.* 1991). This implies that extracellular H^+ ions exert a direct action on detrusor muscle, i.e. by reducing Ca^{2+} influx through L-type channels, rather than on the cholinergic neuromuscular junction. It is also of interest that an increase of extracellular P_{CO_2}, when pH_i as well as pH_o is reduced, depresses the carbachol contracture (Liston *et al.* 1991), suggesting that the phenomenon is mediated by an intracellular rather than extracellular action of H^+.

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Acknowledgements

We are grateful to St Thomas's Hospital Special Trustees, Action MS (Northern Ireland) and the MRC for financial support, and the surgeons of St Thomas's and Guy's hospitals for supplying experimental material.

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Received 20 August 1993; accepted 4 February 1994.