An investigation into the mechanism whereby pH affects tension in guinea-pig ureteric smooth muscle

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1. We have altered intracellular (pH_i) and extracellular pH (pH_o) in the smooth muscle of guinea-pig ureter and determined the effects on evoked phasic contractions. In order to investigate the mechanisms underlying the effects of pH alteration, intracellular Ca^{2+} ($[Ca^{2+}]_i$), pH_i, electrical activity and force were measured.

2. Intracellular acidification, produced by the weak acid butyrate, application of CO_2 at constant pH_o or removal of weak bases, greatly increased phasic contractions. Alkalinization with weak bases or by removal of CO_2 inhibited contractions. The results were similar whether Hepes or CO_2–HCO_3^- buffered the solutions.

3. Phasic contractions were preceded by intracellular Ca^{2+} transients in the ureter. Acidification of the cytoplasm led to an increase in the amplitude of the Ca^{2+} transient, and alkalinization decreased its magnitude.

4. In the ureter the action potential leads to Ca^{2+} influx, therefore electrophysiological recordings of its configuration were made during alteration of pH_i. Acidification led to the action potential duration and amplitude being increased, whereas alkalinization shortened the action potential and reduced its amplitude.

5. As the effects of acidification on the action potential resembled the effects of blocking of K^+ channels, we investigated whether pH_i alteration was able to alter tension when K^+ channels were blocked by tetraethylammonium. Acidification was unable to potentiate force under these conditions nor did alkalinization decrease force.

6. External pH over the range 6·8–8·0 had little or no effect on pH_i, phasic contractions and $[Ca^{2+}]_i$. Tonic contractions were enhanced, however, when pH_o was increased.

7. These data suggest that pH_i alteration in the guinea-pig ureter modulates the action potential, probably by alteration of K^+ currents. Subsequent changes in $[Ca^{2+}]_i$ and contraction then occur. A potentiating effect of acidic pH on force is not common in muscle, but may be a characteristic of the smooth muscle of the urinary tract. Changes of pH_o had little effect on phasic force or pH_i, but modulated tonic contractions. The possible physiological significance of these results is discussed.

The smooth muscle cells of the urinary tract and bladder may experience changes of pH, for example if the urinary P_{CO_2} varies (Pitts, Ayers & Schiess, 1948) or ammonium salts are present. The pH of normal urine can vary from 4·5 to 8·0, and while the urothelium will provide a largely impermeant protective lining, it may be damaged in certain disease states (Eldrup, Therup, Nielsen, Hald & Hainau, 1983) and become more permeable. In these cases, the smooth muscle cells may experience large changes in pH. As it is known that changes in intracellular pH (pH_i) can markedly influence smooth muscle contractility (Wray, 1988), any change in the pH of the urinary tract may have effects on the functioning of the ureters.

It has been shown that in human ureter an increase in P_{CO_2}, and thus presumably a decrease in intracellular pH (pH_i), leads to an increase of force while decreased P_{CO_2} has the opposite effect (Cole, Fry & Shuttleworth, 1990). In other smooth muscles of the urinogenital system the functional effects of pH change are different. In the uterus, for

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example, intracellular acidification decreased spontaneous contractions (Wray, Duggins, Iles, Nyman & Osman, 1992; Parratt, Taggart & Wray, 1994), while in detrusor (bladder) smooth muscle, intracellular acidification increased, but extracellular acidification attenuated, phasic force (Liston, Palfrey, Raimbach & Fry, 1991). In human ureteric smooth muscle, extracellular acidification was without significant effect on tension, but as pH_i was not measured, it is not known if an intracellular acidification was induced (Cole *et al.* 1990). In guinea-pig ureter it has been shown that intracellular acidification causes a small hyperpolarization, and alkalinization causes a depolarization (Aickin, 1994) and therefore it might be expected that intracellular acidification decreases, and alkalinization increases, contractility. Thus there is a need to determine the effects of known changes of pH_i on tension in ureteric smooth muscle.

The functional effects of pH_i are likely to involve changes in intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$), but there have been few studies of this in smooth muscle. In vascular cells, acidification has been associated with no change in $[Ca^{2+}]_i$ (Siskind, McCoy, Chobanian & Schwartz, 1989), an increase (Batlle, Peces, LaPointe, Ye & Daugirdas, 1993), or a transient increase then decrease (Jensen, Hughes, Boonen & Aalkjaer, 1993; Taggart, Austin & Wray, 1994*a*, *b*). It is difficult therefore to predict what the effects of pH_i alteration will be on $[Ca^{2+}]_i$ and hence what role $[Ca^{2+}]_i$ changes may play in the functional effects of pH_i alteration. Thus another aim of this work was to measure Ca^{2+} transients in the ureter simultaneously with force and to establish what effect alteration of pH_i has on $[Ca^{2+}]_i$. pH_i has been shown to be capable of influencing membrane ionic channels (Moody, 1984), which in turn may alter $[Ca^{2+}]_i$. In the smooth muscle of the ureter, the duration of the plateau phase of the action potential has been shown to play a crucial role in modulation of force (Shuba, 1977, 1981). It was therefore also important to determine if pH_i altered electrical behaviour across the ureteric cell membrane, and by what means.

In vivo, changes in external pH (pH_o) occur in the ureter. We have therefore also investigated how changes of pH_o affect pH_i, Ca^{2+} and force, to enable a direct comparison between the effects of pH_i and pH_o in the guinea-pig ureter.

We report that in guinea-pig ureteric smooth muscle, intracellular acidification increased force, $[Ca^{2+}]_i$ and the plateau component of the action potential, and alkalinization did the opposite. Experiments with the K^+ channel blocker tetraethylammonium (TEA) suggest that these effects may be due to pH_i modulating K^+ currents in the smooth muscle membrane. External pH alteration had little or no effect on force and Ca^{2+} transient. These data increase our understanding of how H^+ can act to increase force in smooth muscles, and together with previous data, suggest that increased force upon intracellular acidification may be a characteristic of the smooth muscle of the urinary system.

METHODS

Tissue

Guinea-pigs (200–300 g) were anaesthetized with chloroform and killed by cervical dislocation. Strips of smooth muscle from the central portion of the ureter, 2–4 mm long and approximately 100 μm in thickness, were dissected after removal of any fat. For measurement of pH_i the strips were incubated with the membrane-permeant form of carboxy-SNARF, 5 μM for 2–3 h at 20 °C. For $[Ca^{2+}]_i$ measurements the strips were loaded with the permeant form of indo-1, 10 μM for 2–3 h at 20 °C. After loading, the tissue was washed and placed in a small bath (volume, ~200 μl) on the stage of an inverted epifluorescence microscope. The tissue was stimulated by silver electrodes at 3–5 V (pulse duration, 50–100 ms) and tension was measured in the strips by attaching one end to a force transducer (Grass). The tissue was constantly superfused at 37 °C with solution of the following composition (mM): Na^+, 150·3; K^+, 5·9; Ca^{2+}, 2·5; Mg^{2+}, 1·2; Cl^-, 150·2; glucose, 11·5. Solutions were buffered with either 11·1 mM Hepes and equilibrated with 100% O_2 at pH 7·4 or, as indicated in the text, with 22·4 mM HCO_3^- and equilibrated with 95% O_2–5% CO_2 at pH 7·4. The pH of such solutions was monitored continuously during experiments. In some experiments weak acid (butyrate) or bases (NH_4Cl or trimethylamine (TMA)) were used to alter pH_i at constant external pH (pH_o). They were used at concentrations up to 40 mM and isosmotically substituted for NaCl. In control experiments the effects of a 40 mM reduction in Na^+ or Cl^- by substitution with LiCl or sodium benzonate, respectively, were found to have no significant effect on tension ($n = 3$) or pH_i. In some experiments, as detailed in the text, 5 mM TEA was used to block K^+ channels. When external pH was altered, this was done by addition of strong acid or base to a Hepes-buffered solution.

Ion measurements

For pH_i measurement with SNARF, the tissue was excited at 540 nm and the fluorescent signals at 590 and 650 nm recorded. The ratio of the 590 : 650 nm signals provides a measure of pH_i. The pH_i signals were calibrated *in vitro* using a mock intracellular solution, as described previously (Taggart & Wray, 1993). For $[Ca^{2+}]_i$ signals from indo-1, the tissue was excited at 340 nm and the emission signals at 400 and 500 nm recorded. The ratio of the 400 : 500 nm signals provides a measure of $[Ca^{2+}]_i$. The Ca^{2+} records are shown as this ratio.

Electrical recordings

Simultaneous electrical and mechanical records were obtained from ureteric strips using the double sucrose gap method (Bulbring & Tomita, 1969; Burdyga & Magura, 1986). Action potentials were evoked by suprathreshold depolarizing current pulses (in the order of 10^{-7} A) of short duration (20–50 ms) (Burdyga & Magura, 1986). Anelectrotonic potentials were evoked by rectangular current pulses in the order of 10^{-7} A and 2–3 s duration.

Statistics

Mean values are quoted with standard error of the mean (S.E.M.). n is the number of animals. Statistical differences were tested using Student's paired or unpaired t tests, where appropriate; P values $\leqslant 0{\cdot}05$ were taken to be significant.

RESULTS

Changes in tension with alteration of pH_i

Initial experiments were performed in both Hepes- and CO_2–HCO_3^--buffered solutions. A mean resting value of

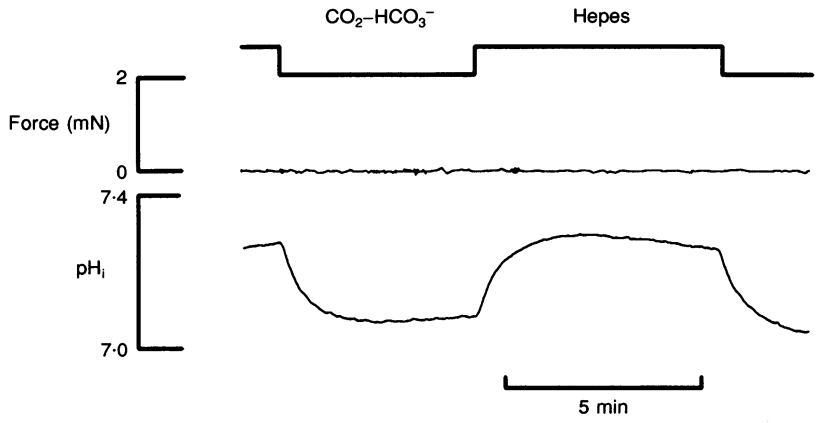

Figure 1. Simultaneous measurements of resting force (top trace) and intracellular pH (pH_i, bottom trace) in guinea-pig ureter

The buffer was changed between a CO_2–HCO_3^-- containing one and a Hepes (nominally CO_2-free) one as indicated by the bar. External pH was 7·4 throughout in this and all subsequent figures, unless stated otherwise. The tissue was loaded with carboxy-SNARF, and the fluorescence signals at 590 and 650 nm recorded to obtain the measurement of pH_i in this and subsequent figures.

pH_i in the ureteric smooth muscle of 7·22 ± 0·04 ($n = 12$) was determined in Hepes buffer, whereas CO_2–HCO_3^- buffer produced a mean steady-state pH_i of 7·10 ± 0·03 ($n = 5$). Figure 1 shows the acidification produced by changing to CO_2–HCO_3^- buffer from Hepes, and then alkalinization upon return to Hepes. It can be seen that in both cases, pH_i rapidly reached a new resting value. Figure 1 also shows that the resting tension was unaltered by these changes in pH_i. When pH_i was changed by other manoeuvres (see below), again no change in resting tension was observed. We then proceeded to examine the effect of pH_i alteration on electrically evoked contractions.

pH_i was displaced by standard procedures, such as addition and removal of weak bases (NH_4Cl or TMA) or acids (butyrate), as well as by removal and addition of CO_2–HCO_3^-. Figure 2 illustrates the effect of alkalinization produced by NH_4Cl in CO_2–HCO_3^- (Fig. 2*A*) and Hepes (Fig. 2*B*) buffers. Alkalinization produced a strong inhibition of force irrespective of the buffer used. The effect on force produced by 40 mM NH_4Cl was smaller in CO_2–HCO_3^- than Hepes buffer; thus the amplitude of force was reduced to 27 ± 5 and 8 ± 4%, respectively (control, 100%) in four paired experiments. However, the mean change in pH_i produced by NH_4Cl was also reduced in CO_2–HCO_3^- buffer, being 0·18 ± 0·2 pH units compared with 0·30 ± 0·01 pH units in Hepes. Figure 2*B* also shows that the inhibition of force was related to the absolute value of pH_i; thus more reduction in force occurs with 40 mM than 10 mM NH_4Cl and pH_i was more alkaline with 40 mM than 10 mM NH_4Cl. Figure 2 also shows that removal of NH_4Cl normally produced rebound acidification (0·10 ± 0·01 pH units in CO_2–HCO_3^- ($n = 4$) and 0·14 ± 0·01 pH units in

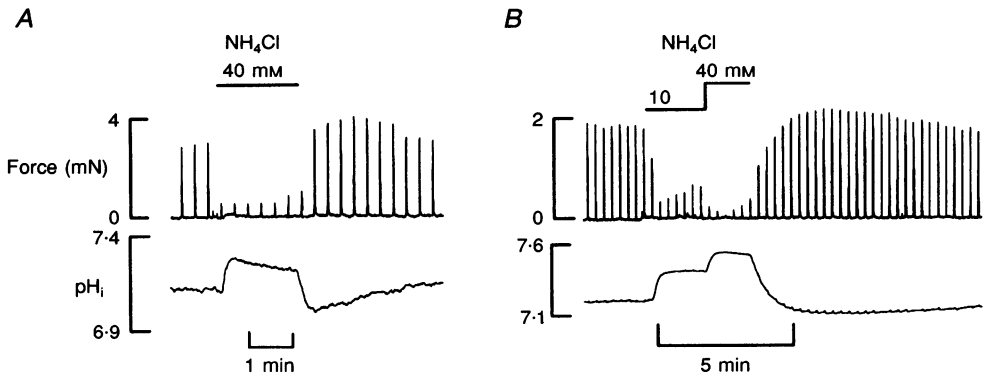

Figure 2. Simultaneous measurements of force (top traces) and pH_i (bottom traces) in guinea-pig ureter

The cytoplasm was alkalinized by the addition of NH_4Cl (40 mM) in CO_2–HCO_3^- (*A*) and Hepes (*B*) buffers. In *B*, NH_4Cl was added at 10 and then 40 mM. NH_4Cl was present for the period indicated by the bars.

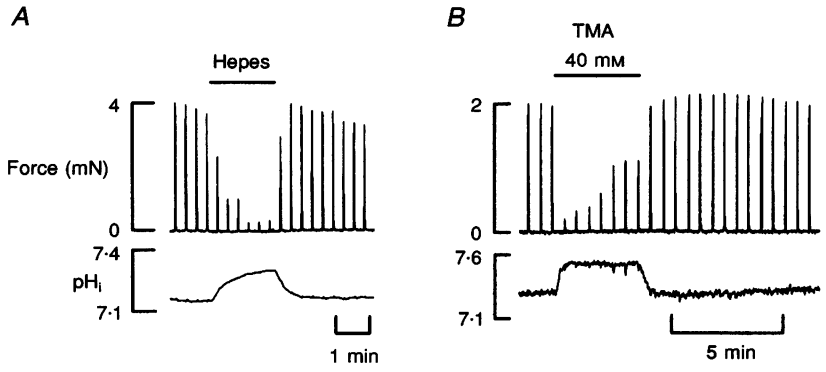

Figure 3. Intracellular alkalinization and contractile behaviour of guinea-pig ureter
The effect on force of alkalinization produced by changing from CO_2–HCO_3^- to Hepes buffer (*A*) and by 40 mM TMA (*B*).

Hepes ($n = 12$), which was accompanied by a significant potentiation of force (132 ± 4% in CO_2–HCO_3^- and 148 ± 4% in Hepes). In some preparations the inhibition of force produced by alkalinization became progressively less; this was sometimes (Fig. 2*A*) but not always (Fig. 2*B*) associated with pH_i regulation restoring pH_i towards resting levels. Figures given for force reduction or augmentation were therefore measured at the first contraction in the new solution.

Alkalinization also inhibited evoked mechanical activity when produced by changing from CO_2–HCO_3^- buffer to Hepes (Fig. 3*A*). The inhibition of force increased as the alkalinization progressed, as can be seen in Fig. 3*A*. Force was restored upon return of pH_i to resting values. As mentioned above, resting pH_i was more alkaline in Hepes buffer and consistent with this we observed that control contractions were of lower amplitude in Hepes (81 ± 4%) relative to paired CO_2–HCO_3^- control contractions (100%, $n = 5$). Figure 3*B* shows an example of alkalinization produced by TMA (40 mM), and again force was inhibited. This preparation also showed a marked decrease in the effect of alkalinization with time. The mean alkalinization produced was 0·25 ± 0·05 pH units ($n = 5$) in Hepes buffer, and this produced a mean reduction in the initial contraction to 30 ± 7% of control values. Removal of TMA produced slight rebound acidification (0·04 ± 0·02 pH units) and a small mean increase in force (107 ± 3%).

The effect of acidification on evoked contractions in the ureter was to increase force. Figure 4 shows typical results when the cytoplasm was acidified by addition of CO_2–HCO_3^- (Fig. 4*A*) or butyrate to Krebs solution buffered by either CO_2–HCO_3^- (Fig. 4*B*) or Hepes (Fig. 4*C*). The mean acidification produced by experiments such as that illustrated in Fig. 4*A* was 0·15 ± 0·01 pH units ($n = 5$) and it was accompanied by a mean potentiation of force of 160 ± 41%. Butyrate in Hepes buffer caused a mean acidification of 0·13 ± 0·02 pH units and a potentiation of force of 166 ± 5% ($n = 10$; Fig. 4*C*). There was a small rebound alkalinization upon butyrate removal which was accompanied by a small reduction in force. Qualitatively similar results were obtained with butyrate in CO_2–HCO_3^- buffer ($n = 3$, Fig. 4*B*).

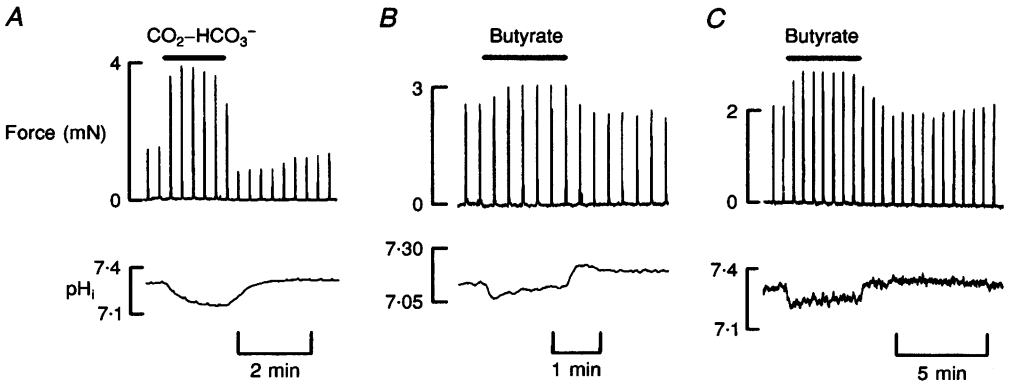

Figure 4. Intracellular acidification and contractile behaviour of guinea-pig ureter
Simultaneous recordings of force (top traces) and pH_i (bottom traces) in ureter acidified by addition of CO_2–HCO_3^- (*A*) or 40 mM butyrate in CO_2–HCO_3^- (*B*) or in Hepes (*C*) buffers.

Thus, these results show a consistent pattern of force changes irrespective of the method used to change pH_i or the buffering system: intracellular alkalinization of the guinea-pig ureteric smooth muscle was always associated with an inhibition of force, and acidification with an increase. As the effects of pH_i alteration on force were similar irrespective of the buffer used, the rest of the experiments were performed in Hepes buffer.

The changes in $[Ca^{2+}]_i$ and pH_i

As changes in $[Ca^{2+}]_i$ have been shown to underlie many of the changes in force in smooth muscle, we investigated the possibility that changes in $[Ca^{2+}]_i$ were responsible for the effects of pH_i on force.

The changes in the indo-1 emission wavelengths produced by contractions of the ureteric smooth muscle (400 and 500 nm) were in opposite directions and gave clear changes in the 400:500 nm indo-1 ratio. Ca^{2+} transients were associated with the phasic contractions, as illustrated in Fig. 5. When NH_4Cl was applied to the preparations, as seen in Fig. 5*A*, it produced a significant inhibition of the amplitude of both Ca^{2+} transients ($27 \pm 8\%$, $n = 4$) and force (see above). Removal of NH_4Cl was always associated with transient potentiation of the indo-1 ratio and force (Fig. 5*A*). Alkalinization by TMA also produced a significant reduction in the Ca^{2+} transient amplitude, to $46 \pm 9\%$ ($n = 5$) compared with control. Acidification by butyrate, as shown above, produced a large increase in force. As can be seen from Fig. 5*B* (typical of 5), the increase in force was accompanied by a significant increase in the magnitude of the Ca^{2+} transients ($150 \pm 20\%$) and a small elevation of basal Ca^{2+}. Removal of butyrate was accompanied by transient inhibition of force and Ca^{2+} transients (Fig. 5*B*).

Changes in electrical activity with pH_i

The above $[Ca^{2+}]_i$ data showed that the predominant mechanism underlying the pH_i effects on force was unlikely to be at the level of the contractile machinery, as changes in force correlated closely with changes in $[Ca^{2+}]_i$. This therefore suggested an earlier process in excitation–contraction coupling was being affected. In the ureter, the amplitude and duration of phasic contractions are closely related to the parameters of the underlying action potential (Shuba, 1977). We therefore examined the effects of acidification and alkalinization on evoked action potentials while simultaneously recording tension.

Under control conditions the action potential of the guinea-pig ureter smooth muscle, evoked by just suprathreshold depolarization, had an initial fast component consisting of repeated and gradually decaying spikes and a subsequent plateau phase, in agreement with earlier studies (Shuba, 1977, 1981; Brading, Burdyga & Scripnyuk, 1983). Butyrate (40 mM) produced a small hyperpolarization ($3{\cdot}2 \pm 1{\cdot}3$ mV, $n = 5$), but nonetheless increased the amplitude and duration of the first spike and plateau component, while additional repetitive spikes were normally abolished (Fig. 6*A* ii). The duration of the overall action potential, measured at 50% of repolarization, was increased $1{\cdot}9 \pm 0{\cdot}3$ times ($n = 5$). In addition, the rate of fall of the action potential decreased $2{\cdot}1 \pm 0{\cdot}2$ times. All these changes in the action potential produced a strong potentiation of force, as seen in previous figures. The stimulatory action of butyrate was readily reversible within 5–10 min of its withdrawal. Removal of butyrate normally produced repolarization and in most tissues a rebound transient inhibition of contraction was seen. The size of the electronic potentials was virtually unaltered (Fig. 6).

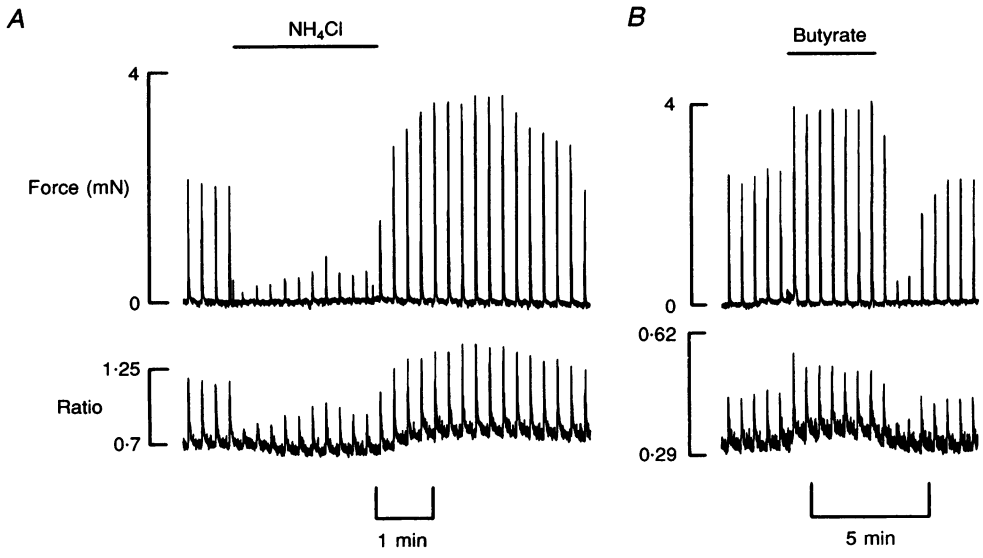

Figure 5. Calcium transients and force in the guinea-pig ureter

The effects of *A*, alkalinization produced by NH_4Cl (40 mM) and *B*, acidification produced by butyrate (40 mM) on force (top traces) and intracellular $[Ca^{2+}]$ (bottom traces) in ureter. $[Ca^{2+}]_i$ was measured from the ratio of the indo-1 fluorescence signals at 400 and 500 nm.

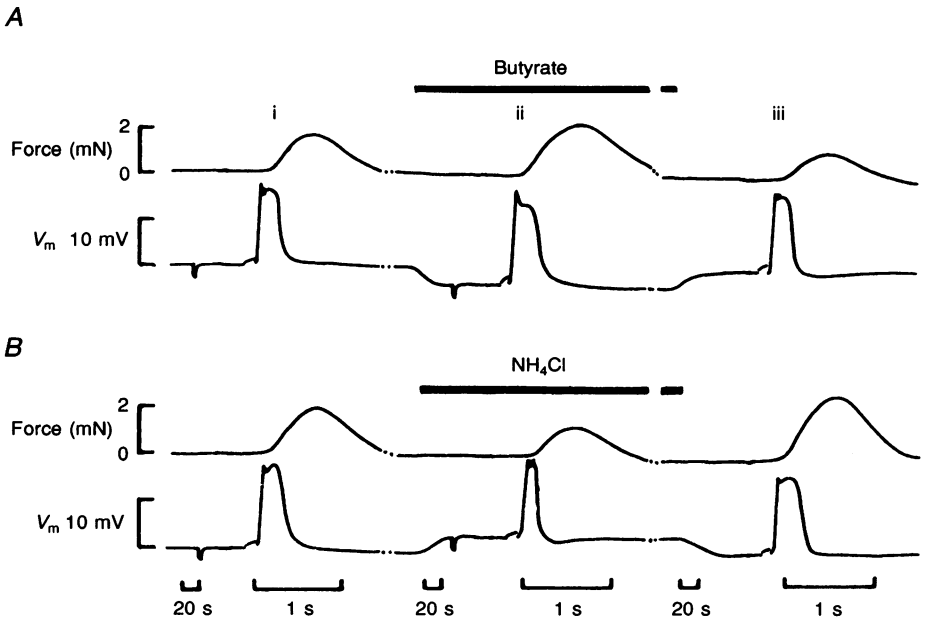

Figure 6. The effect of butyrate and NH_4Cl on resting membrane potential, membrane conductance and the evoked electrical and mechanical responses of guinea-pig ureter smooth muscle to depolarizing current pulses

In each pair of records the upper trace shows the mechanical and the bottom trace the electrical responses throughout the figure. The electrical records were obtained by the double sucrose gap method. V_m, membrane potential. In *A* and *B*, following the control record (i), specimen records in either 40 mM butyrate or 40 mM NH_4Cl were obtained after 30 s (ii) and then after return to control solution (iii).

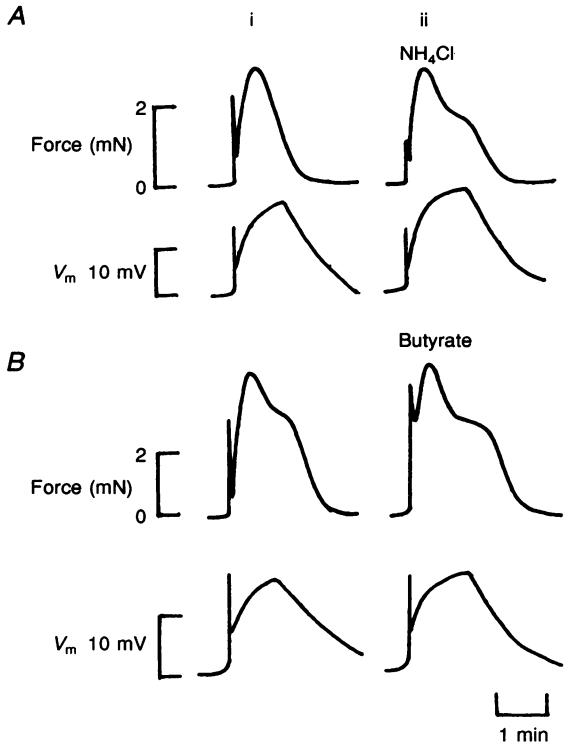

Figure 7

Effect of 40 mM NH_4Cl (*A*) and 40 mM butyrate (*B*) on the electrical (bottom trace) and mechanical (top trace) activity of guinea-pig ureter muscle evoked by 140 mM K^+. i, control record.

Both NH_4Cl (40 mM, $n = 4$; Fig. 6*B*) and TMA (40 mM, $n = 3$) produced a strong inhibitory action on the evoked electrical and mechanical activity of ureter muscle. Although both substances produced a small *depolarization* (3–5 mV), the amplitude and duration of the plateau component were decreased. These changes in the action potential were accompanied by an inhibition of force (Fig. 6*B* ii). It was noticeable that the changes in action potential pattern produced by the acidic rebound following NH_4Cl removal (Fig. 6*B* iii) were similar to those produced by direct acidification by butyrate (Fig. 6*B* ii).

These data clearly show that the mechanisms controlling the duration of the action potential are influenced by pH_i alteration. The slowing of the fall of the action potential by acidification suggested that repolarizing outward current is inhibited by acidosis. In the ureter this current is mostly Ca^{2+}-activated K^+ current ($I_{K(Ca)}$) and is sensitive to TEA (Imaizumi, Katshuhiko & Watanabe, 1989). However, modulation of the L-type Ca^{2+} current, a major contributor to inward current (Imiazumi *et al.* 1989), cannot be ruled out. In order to distinguish between these two possibilities, the effects of butyrate and TMA in high-K^+ depolarized preparations were examined. Under these conditions the initial phasic component of contraction is associated with the action potential upstroke, and the tonic component of contraction is associated with sustained depolarization and L-type Ca^{2+} channel activity (Shuba, 1981; Brading *et al.* 1983).

Sustained depolarization was produced by changing to a high-K^+ solution. NH_4Cl (40 mM) produced a selective inhibition of the phasic contractions and displayed little effect on the tonic component (Fig. 7*A* ii). Butyrate (40 mM) selectively potentiated the phasic contractions of the high-K^+ contracture but had little effect on the tonic component

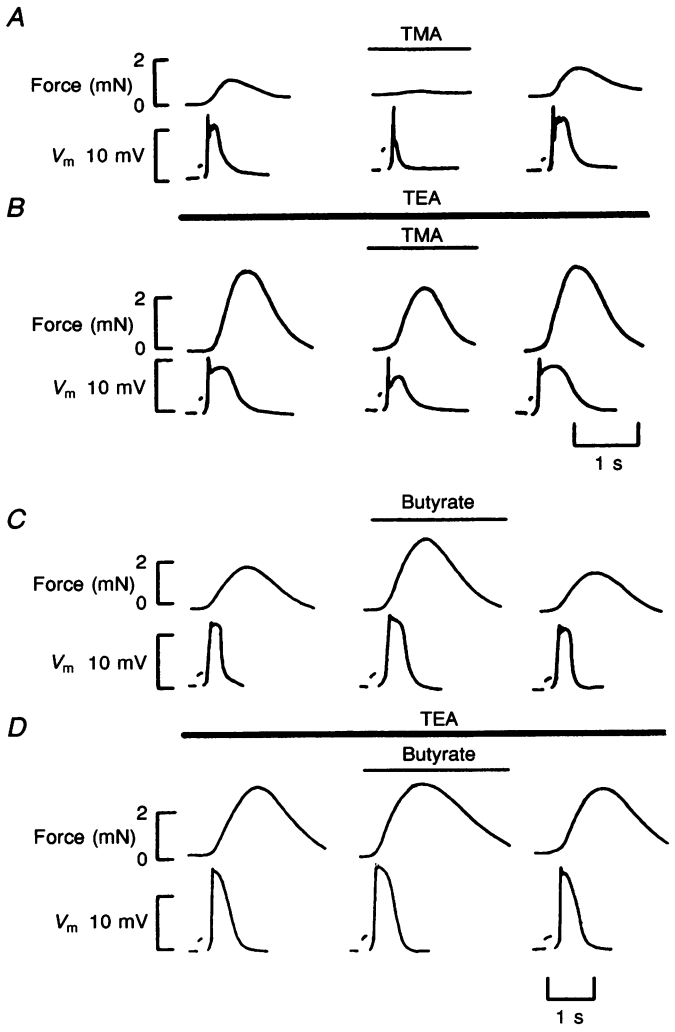

Figure 8

Changes in electrical and mechanical activity of guinea-pig ureter muscle induced by TMA (40 mM) and butyrate (40 mM) (thin bars) taken before (*A* and *C*, respectively) and after (*B* and *D*) addition of 5 mM TEA (thick bar). *A*–*D* are continuous recordings taken from the same preparation.

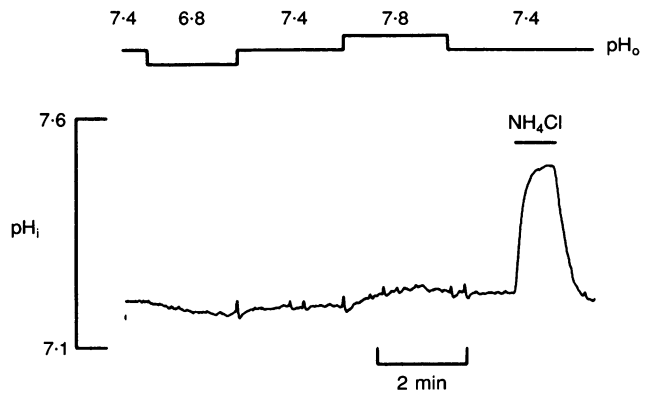

Figure 9. pH$_i$ measurements during alteration of external pH (pH$_o$) from 7·4 to 6·8 or 7·8 in guinea-pig ureter

The final part of the trace shows, for comparison, the pH$_i$ change elicited by NH_4Cl (40 mM) application.

of the high-K^+ contracture associated with sustained depolarization (Fig. 7*B* ii). These data suggest that it is K^+ channels rather than Ca^{2+} channels which are most likely to be modulated by the pH$_i$ changes. Since the repolarizing current in ureter was shown to be mostly K^+ dependent and TEA sensitive (Imaizumi *et al.* 1989) we next examined the effects of TMA and butyrate on the evoked action potentials and phasic contractions of ureter in the presence of TEA (5 mM). Application of TEA did not alter resting pH$_i$ and the pH changes produced by butyrate (40 mM) and TMA (40 mM) were similar in the presence or absence of TEA ($n = 5$). In Fig. 8, records *A–D* are from the same preparation and show the effect of TMA and butyrate first in the absence and then in the presence of TEA. These records clearly show that TEA significantly attenuated the inhibitory action of TMA and the stimulant action of butyrate. Also, it is noticeable that the stimulatory effects of TEA (e.g. in the first panels) look similar to those produced by butyrate. Similar results were obtained in four other preparations.

Thus, prior blocking of K^+ channels abolished or reduced the effects of pH$_i$ on tension in the smooth muscle of the guinea-pig ureter. This suggests the involvement of K^+ channels in the mechanism of action of H^+ in this tissue.

External pH alteration

Changes in external pH produced only small changes in the parameters measured, i.e. force, pH$_i$ and $[Ca^{2+}]_i$. There were only small and slow changes in pH$_i$ when pH$_o$ was either raised or lowered, see Fig. 9. When pH$_o$ was changed between 6·8 and 8·0 and compared with control contractions, there was a small decrease in contraction amplitude at acidic pH$_o$ (to 88 ± 2% of control, $n = 5$) and a small increase at alkaline pH$_o$ (116 ± 3%, $n = 5$), see Fig. 10. Simultaneous force and $[Ca^{2+}]_i$ measurements when pH$_o$

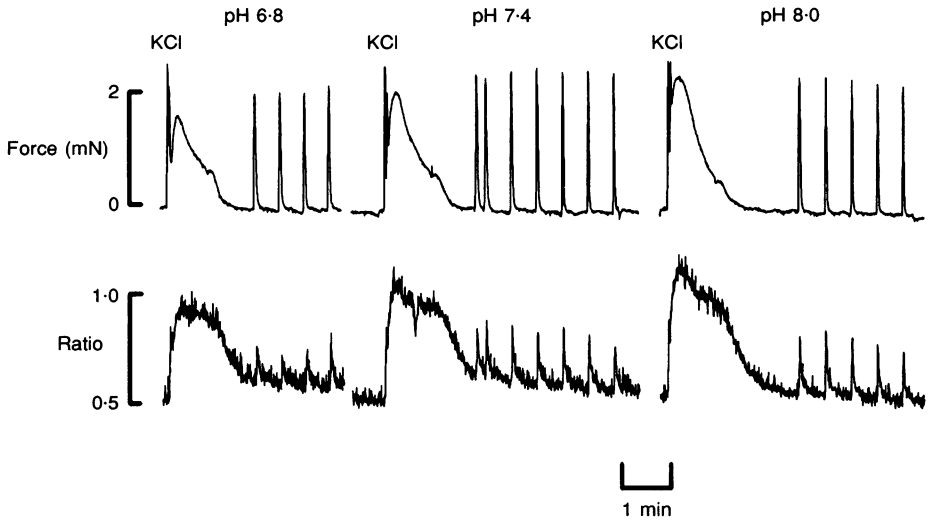

Figure 10

Changes in contractile activity and $[Ca^{2+}]_i$ evoked by electrical and high-K^+ stimulation at external pH 6·8, 7·4 and 8·0 in guinea-pig ureter. The $[Ca^{2+}]_i$ was measured as in Fig. 5.

was changed are shown in Fig. 10. Again, little effect on the phasic contractions was seen and correspondingly small changes in the Ca^{2+} transients were seen. In high-K^+ solution the phasic contractions were also found to be little altered by pH_o change. The peak amplitude of the tonic contraction, however, was raised when pH_o increased and fell when pH_o decreased (Fig. 10), and this was accompanied by corresponding changes in the measured $[Ca^{2+}]_i$ signal.

Thus it appears that when pH_o is altered it has only minor effects on phasic contraction, pH_i and Ca^{2+} in the guinea-pig ureter, but can influence the tonic component of a high-K^+ contraction.

DISCUSSION

The data in this paper show that a change in intracellular pH will produce marked alterations of contractile function, electrical activity and $[Ca^{2+}]_i$ in guinea-pig ureteric smooth muscle. The finding of increased phasic force under acidic intracellular conditions is unusual in muscle, but not unique, and consistent with an earlier study in human ureter (Cole *et al.* 1990). There have been few studies in any smooth muscle where electrical and $[Ca^{2+}]_i$ changes in response to a pH_i change have been investigated. In the guinea-pig ureter, the data suggest that the pH_i effects on phasic force can be explained in terms of the changes in electrical activity and thus $[Ca^{2+}]_i$. Extracellular pH change produced relatively small effects on phasic contractions and the other measured parameters.

To draw conclusions about the mechanisms underlying the functional changes seen in the ureter requires drawing on the data provided from three different sets of experimental measurements – pH_i, $[Ca^{2+}]_i$ and electrical activity – as it was not possible to simultaneously measure all variables. Given that: (i) all other experimental conditions were kept constant; (ii) that tension was always measured, thereby providing an indication that the tissue was responding in the same manner to pH_i alteration irrespective of what parameter was being measured; and (iii) the results were highly reproducible, it seems reasonable to interrelate the data. The preparation used consisted overwhelmingly of smooth muscle cells, and outer connective tissue was dissected and removed. There was also a good correspondence between the intracellular changes in pH or $[Ca^{2+}]$ and force, again indicating that the signals we are measuring arise from smooth muscle cells in the ureter.

pH_i and force in smooth muscle

The resting value of pH_i in the ureter was found to be 7·22 in Hepes, which is similar to that reported in many smooth muscles (Wray, 1988). In CO_2–HCO_3^- buffer, the value was 7·10. Aickin (1994), using pH-sensitive microelectrodes, reported a higher value of pH_i in CO_2–HCO_3^- of 7·22 and in Hepes a value of 6·92. It is unclear why there should be such differences, but no functional measurements were made by Aickin (1994). The changes in force elicited by pH_i alteration in our study were the same irrespective of the method used to alter pH_i. Thus, acidification potentiated force when produced by direct acidification with butyrate, by removal of a weak base and by addition of CO_2. Similarly, alkalinization, no matter how produced, was always associated with a reduction in force. Thus it seems reasonable to conclude that it is the initial excursion of pH_i that is responsible for the ensuing changes. The size of the effect on force was also shown to be correlated to the size of the pH_i change (Figs 2*B* and 3*A*). It was however found in some preparations that the initial reduction of force produced by alkalinization was not maintained. This was sometimes, but not always, associated with pH_i regulation. The mechanisms underlying these secondary effects of pH_i are unclear. For this reason we normally only altered pH_i for 2–3 min and have concentrated on the initial effects of pH_i alterations.

Changes in pH_i are recognized to be potent modulators of smooth muscle contraction (Wray, 1988). What is interesting in smooth muscle is the variety of functional responses seen compared with striated muscle. Thus intracellular acidification depresses contraction in cardiac and skeletal muscle (e.g. Fabiato & Fabiato, 1978; Fry & Poole-Wilson, 1981) but in smooth muscle it may potentiate contractions, as seen here and by others (e.g. human ureter, Cole *et al.* 1990; rabbit ear artery, Spurway & Wray, 1987; human and ferret detrusor, Liston *et al.* 1991). In other smooth muscles, tension is decreased or even abolished by acidification (e.g. uterus: Taggart & Wray, 1993; mesenteric artery: Austin & Wray, 1993) or there is a biphasic response (mesenteric artery: Jensen *et al.* 1993; portal vein: Taggart, Austin & Wray, 1994*a*, *b*). Thus unlike striated muscle, where acidification has been shown to attenuate every step in excitation coupling (Orchard & Kentish, 1990), in smooth muscle differential effects of pH_i must be occurring at some of these stages. In this paper we have identified two stages in excitation–contraction coupling that are altered in the smooth muscle of the ureter by pH_i: membrane electrical activity and $[Ca^{2+}]_i$.

Changes in evoked action potentials

The data showed that acidification produced a significant prolongation of the action potential, while alkalinization reduced the duration of the plateau of the action potential. In the smooth muscle of the ureter, the duration of the plateau component of the action potential plays a critical role in modulation of force. Thus all factors acting to prolong it (e.g. histamine, noradrenaline, TEA and local anaesthetics in charged forms) produce potentiation of force, while substances which act to shorten its duration (e.g. Ca^{2+} channel blockers, papaverine, caffeine and local anaesthetics in neutral form) act as inhibitors of force (Shuba, 1977; Brading *et al.* 1983; Burdyga & Magura, 1986). This modulation of the duration of the plateau

component produced by alteration of pH_i in ureteric smooth muscle is consistent with the observed effects on force.

The question of how acidification increases the duration of the action potential and alkalinization decreases it needs addressing. In other tissues there is evidence for acidification decreasing voltage-gated Ca^{2+} entry (Moody, 1984; Irisawa & Sato, 1986; Klockner & Isenberg, 1994). Clearly in the ureter this is not occurring (see below). The changes produced by butyrate on the evoked action potential closely resembled those produced by the K^+ channel blocker TEA on this smooth muscle (Shuba, 1977). TEA is an inhibitor of outward current in excitable cells, including smooth muscles. Its application to guinea-pig ureter showed that the amplitude and action potential duration considerably increased, the additional spikes on the action potential disappeared, and the fast inward current increased (Shuba, 1977, 1981). This description could be used to describe the data in Fig. 8 showing the effects of acidification either by TMA removal or butyrate application. This suggests that intracellular acidification is inhibiting membrane K^+ channels, which would account for the prolongation of the action potential and increase in force. There is also evidence from a wide variety of preparations that K^+ channels are blocked by low pH_i (see Moody, 1984, for a review). When we tested this idea by applying TEA directly to block K^+ channels, pH_i changes had very little or no effect on force. This is consistent with the suggestion that the predominant effect of acidification is a prolongation of the action potential by blockade of K^+ currents, in the guinea-pig ureter. From this it would follow that the effects of pH_i alteration on Ca^{2+} influx during the action potential could arise indirectly through the modulation of the K^+ outward current, although a direct effect of pH_i on Ca^{2+} channels cannot be ruled out at present. In the ureter, as with other smooth muscles, Ca^{2+} enters with the action potential, which plays a key role in excitation–contraction coupling. This Ca^{2+} entry may be sufficient to cause contraction without the release of Ca^{2+} from the sarcoplasmic reticulum (Maggi, Giuliani & Santicioli, 1994, 1995). Thus there is a strong link between electrical activity and Ca^{2+}, which in turn affects contraction.

$[Ca^{2+}]_i$ changes

There are relatively few studies in smooth muscle investigating $[Ca^{2+}]_i$ and contraction directly (Morgan & Morgan, 1984; Himpens & Somlyo, 1988). As far as we are aware these are the first simultaneous measurements in ureter. The data showed a good correlation between the intracellular Ca^{2+} transient and force in the ureter. When pH_i was altered, the Ca^{2+} transient was altered and force altered in a corresponding manner. Acidification was also associated with a small rise in the basal indo-1 signal. This has also been reported in uterine smooth muscle under similar conditions (Heaton & Wray, 1994). It may be that protons displace Ca^{2+} from common binding sites. Although pH_i in other smooth muscles has been shown to influence the influx of extracellular Ca^{2+} (Klockner & Isenberg, 1994) and release of Ca^{2+} from the sarcoplasmic reticulum (Schulz, Thevenod & Dehlinger-Kremer, 1989), it would appear that in the guinea-pig ureter, pH_i effects on the action potential may well be sufficient to explain most of its effect on Ca^{2+}.

External pH effects

Despite relatively large changes in pH_o in both acid and alkaline directions, little effect on phasic force or any of the other parameters measured was observed. This is in agreement with a previous study where force and electrical activity were monitored during changes of pH_o (Burdyga & Magura, 1986). That study showed little or no effect on phasic contractions when pH_o was varied between pH 6 and 9, although some small changes in the action potential were noted; pH_o of 9 reduced the amplitude of both the phasic and plateau components of the action potential, while at pH 6, the opposite effect was observed (Burdyga & Magura, 1986). We found that little change in the pH_i signal occurred with alteration of pH_o. It has recently been shown that extracellular acidification of human detrusor smooth muscle attenuates both the action potential and the L-type Ca^{2+} current, consistent with its ability to reduce phasic force (Fry, Gallegos & Montgomery, 1994). In high-K^+, we observed an increase of tonic force and $[Ca^{2+}]_i$ with alkalinization, also consistent with an alteration of L-type Ca^{2+} channel inward current.

Physiological significance

As mentioned earlier, different smooth muscles differ in their functional response to pH_i alteration (Wray, 1988). It may be, however, that those of the urinary system all respond to intracellular acidification with an increase in force, given the current data (guinea-pig ureter) and that from previous studies (human ureter, Cole *et al.* 1990; human and ferret bladder, Liston *et al.* 1991). This is in contrast to the genital system where acidification of the uterus decreases spontaneous force (Wray *et al.* 1992). As the pH of the urine can drop to as low as 4·5 it may be that some of this acidity would be transmitted across the urothelial lining, especially in diseased states. The pH_i may also be altered by ammonium salts present in the urine and crossing cell membranes. By responding with an increase in force, the smooth muscle may help increase the passage of urine through the tract, thereby limiting any possible damage from such acidic conditions. It would make functional sense if the different parts of the urinary system responded in a consistent way to the acidic pH change. It should, however, be noted that extracellular pH changes appear to differ in their functional effects from those associated with pH_i alteration (these data and Cole *et al.* 1990; Liston *et al.* 1991).

In summary, we have shown changes of pH_i will have marked effects on the tension of guinea-pig ureteric smooth

muscle and may therefore have consequences for its functional ability, with acidic pH increasing force production. The effects on force resembled the effects of K^+ channel blockade in this muscle. The prolonged action potential with intracellular acidification was in turn associated with an increased Ca^{2+} transient in the muscle. It is suggested that the increased levels of $[Ca^{2+}]_i$ act to promote tension in the guinea-pig ureter. Alkalinization produced functionally opposite effects.

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Acknowledgements

We are grateful to the MRC, The Wellcome Trust and The Physiological Society for supporting this work. M. J. Taggart is a Wellcome Research Career Development Fellow. T. Burdyga is a Wellcome Trust Travelling Research Fellow. We thank Ann O'Flynn for assisting with some of the experimental work.

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Received 15 December 1995; accepted 5 February 1996.