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# THE EFFECTS OF pH CHANGES ON HUMAN AND FERRET DETRUSOR MUSCLE FUNCTION

BY T. G. LISTON*, E. L. H. PALFREY*, S. J. RAIMBACH† AND C. H. FRY

*From the Departments of Physiology and *Urology, St Thomas's Hospital and Medical School (UMDS), Lambeth Palace Road, London SE1 7EH and the †Department of Physiology, University College, Gower Street, London WC1E 6BT*

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SUMMARY

1. The effects of altering extracellular pH on the electrically evoked contractions of ferret and human bladder (detrusor) smooth muscle have been investigated. pH was varied by changing superfusate $P_{CO_2}$ or $NaHCO_3$ concentration. Acidosis increased force when superfusate $P_{CO_2}$ was raised but decreased force when the $NaHCO_3$ concentration was reduced.

2. Intracellular pH ($pH_i$) in isolated ferret detrusor cells was measured separately by epifluorescence microscopy. Extracellular pH changes caused by altering superfusate $P_{CO_2}$ were accompanied by similar changes of $pH_i$, whereas variation of the $NaHCO_3$ concentration had smaller effects on $pH_i$.

3. It was proposed that intracellular acidosis increased contraction but extracellular acidosis depressed contraction.

4. Other interventions, such as addition and removal of $NH_4Cl$, $Cl^-$ replacement, and $NaHCO_3$ replacement with HEPES, changed $pH_i$ and had predictable effects on force. It was possible to describe unique relationships between tension and either intracellular or extracellular pH regardless of the means whereby pH changes were brought about.

5. Resting tension was reduced whether brought about by either intracellular or extracellular acidosis. $K^+$ contractures were similarly affected by acidosis. Ferret preparations showed low levels of spontaneous activity, which was reduced by acidosis and enhanced by alkalosis.

## INTRODUCTION

The smooth muscle comprising the bulk of the bladder wall (detrusor muscle) may experience changes of extracellular pH for a variety of reasons. The pH of urine can vary from 4 up to 8 or 9 and although the urothelium, which lines the urinary tract, is considered to be impermeable to the constituents of urine there are several situations where urinary pH might influence detrusor function. For example, the $P_{CO_2}$ of urine is generally greater than in blood, can rise to 150 mmHg (Pitts, Ayer & Schiess, 1949) and can readily diffuse across the urothelium. Under certain conditions the urothelium can also be damaged and rendered leaky to urine constituents (Eldrup, Thorup, Nielsen, Hald & Hainau, 1983; Monson & Wein, 1989). In addition,

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bladder filling reduces blood flow to the organ (Dunn, 1974) and leads to transient, local acidification – a phenomenon which may be exacerbated when there is detrusor hypertrophy as a result, say, of outflow tract obstruction. Some clinical observations also suggest that changes in luminal pH influence bladder function. Infusion of alkaline solutions into the lumen of patients with bladder instability increased compliance, which could be interpreted as a decrease of detrusor tone (Sethia & Smith, 1987).

Alteration of pH is known to affect function in many muscle types. Acidosis depresses function in cardiac muscle and it is likely that intracellular pH changes are predominantly important (Fry & Poole-Wilson, 1981), and virtually every process involved in contractile activation has been shown to be attenuated (Orchard & Kentish, 1990). With smooth muscle the actions of pH changes are less well described; acidosis has been shown to depress contraction in some preparations (Twort & Cameron, 1986) whilst in others increased function has been reported (Spurway & Wray, 1987). These different responses may result from the variety of smooth muscle preparations that have been used and the different ways in which tension has been evoked, i.e. by agonist-induced contractures or by direct electrical stimulation.

In the bladder, phasic contraction is under the control of motor nerves, the terminals of which are embedded in the muscle. In man these are entirely cholinergic and in smaller animals predominantly so (Palfrey, Fry & Shuttleworth, 1984; Sibley, 1984). *In vitro*, muscle contractions can be elicited by tetanic field stimulation via activation of the nerve network and this may represent the most physiological method of stimulation. Tonic contraction can also be elicited in detrusor muscle, for example, by raising the extracellular $[K^+]$ (Fry & Palfrey, 1986; Sibley, 1987) and a local increase of the extracellular $[K^+]$ can be demonstrated *in vitro* when the tissue is rendered hypoxic (T. G. Liston and C. H. Fry, unpublished data). We show here that intracellular acidosis enhances electrically induced, phasic muscle contraction, whilst extracellular acidosis is depressant and alkalosis produces the opposite responses. Tonic tension, however, is reduced by acidosis in either compartment.

Some of these results have been reported to the Physiological Society (Baro, Eisner, Fry, Liston, Montgomery & Raimbach, 1989; Fry & Liston, 1989).

## METHODS

*Preparations.* Strips of detrusor muscle, 3–4 mm length and 0·5 mm diameter, were dissected from either human bladder biopsies or whole ferret bladders. Human biopsies were obtained from adult patients undergoing endoscopic or open bladder surgery and were taken at least 1 in from the trigone. Approval from the Ethical Committee of St Thomas's Hospital was granted. Ferret bladders were immediately removed from animals that had been exsanguinated after anaesthetization with sodium pentobarbitone (May & Baker, 5 ml/kg). A smaller number of experiments were also performed on guinea-pig and rat detrusor strips. The results were qualitatively similar to those described for human and ferret preparations. The rats and guinea-pigs were killed by cervical dislocation.

*Experimental recording.* The strips were placed in a horizontal superfusion trough, tied at one end to a fixed hook in the base of the trough and at the other to an isometric force transducer (Statham UC2, Oxnard, CA, USA). The force transducer was mounted on a micro-manipulator to permit adjustment of muscle length. The output of the force transducer was conditioned via an instrumentation amplifier (CMRR > 110 dB), low-pass filtered at 5 Hz and displayed on a moving-paper chart recorder.

Preparations were initially superfused with a Tyrode solution of the following composition (in mM): NaCl, 118; KCl, 4; $NaHCO_3$, 24; $NaH_2PO_4$, 0·4; $MgCl_2$, 1; $CaCl_2$, 1·8; sodium pyruvate, 5; glucose, 6. The solutions were warmed in reservoirs to 37 °C and gassed with a mixture containing 5% $CO_2$ and 95% $O_2$ to give a pH of 7·33±0·02. The superfusion chamber was gravity fed from these reservoirs via water-jacketed tubes at a flow rate of 4 ml/min and a chamber temperature of 37±0·5 °C.

TABLE 1. Composition and pH of various superfusates

| $CO_2$% | $O_2$% | [$NaHCO_3$] | [NaCl] | [$CaCl_2$] | [HEPES] | pH | *n* |
|---|---|---|---|---|---|---|---|
| 5 | 95 | 24 | 118 | 1·80 | 0 | 7·33±0·02 | 107* |
| 2 | 98 | 24 | 118 | 1·80 | 0 | 7·65±0·07 | 46 |
| 10 | 90 | 24 | 118 | 1·80 | 0 | 7·15±0·04 | 26 |
| 20 | 80 | 24 | 118 | 1·80 | 0 | 6·86±0·03 | 93 |
| 5 | 95 | 48 | 94 | 2·45 | 0 | 7·62±0·04 | 65 |
| 5 | 95 | 12 | 130 | 1·58 | 0 | 7·14±0·04 | 8 |
| 5 | 95 | 6 | 136 | 1·50 | 0 | 6·78±0·03 | 23 |
| 10 | 90 | 48 | 94 | 2.45 | 0 | 7·34±0·03 | 26 |
| 0 | 100 | 0 | 118 | 1·80 | 24 | 7·36±0·04 | 13 |

* Tyrode solution.
Concentrations are in units of mmol/l.
pH values are expressed as means±S.D. of observations.

Stimulation of the preparations was via platinum-plate electrodes embedded in the walls of the trough. For human preparations a train of pulses, 3 s duration, was delivered every 90 s. Each pulse train was delivered at 20 Hz with an individual pulse width of 0·2 ms. Stimulation parameters for ferret preparations were similar except that the pulse train was delivered at 4 Hz. The potential difference (PD) across the plates was generally 20 V which was supramaximal for the given stimulation parameters. Contractile activation of the preparation using such a protocol ensured constant responses for 5–8 h. Under these conditions tension was about 30% of the maximal value achieved using high-frequency stimulus trains (> 40 Hz) composed of long-duration pulses (> 20 ms). The contractions could be completely abolished with tetrodotoxin ($10^{-7}$ g/ml) indicating that activation was via the nerve network embedded in the smooth muscle (Palfrey *et al.* 1984). Stimulation using individual pulses in each train greater than 1 ms directly stimulated the muscle, as assessed by a residual response to tetrodotoxin. Some experiments described in the Results were obtained with direct muscle stimulation and were quantitatively similar to those activated with short duration pulses. The majority of experiments reported were obtained using short pulses.

*Solutions.* The superfusate pH was altered by changing the $P_{CO_2}$, [$NaHCO_3$] or replacement of $NaHCO_3$–$CO_2$ with HEPES–$O_2$. The pH of these solutions was measured by collecting samples in gas-tight syringes at the entrance to the superfusion trough and introducing them to an acid–base analyser (ABL 30, Radiometer). The $P_{CO_2}$ was varied by gassing Tyrode solution with $CO_2$–$O_2$ mixtures containing 2, 10 and 20% $CO_2$. The [$NaHCO_3$] was either increased to 48 mM or reduced to 12 and 6 mM, at constant $P_{CO_2}$. Changes to the [$NaHCO_3$] were compensated by equimolar removal or addition of NaCl. Alteration of the [$NaHCO_3$] also alters the free [$Ca^{2+}$] (Fry & Poole-Wilson, 1981) so that the quantity of $CaCl_2$ added to the solutions was varied. The pH values of these solutions as well as the total [$CaCl_2$] are shown in Table 1. In some experiments $P_{CO_2}$ and [$NaHCO_3$] were both doubled to maintain constant superfusate pH. In other experiments 24 mM-HEPES replaced $NaHCO_3$ and the solution was gassed with 100% $O_2$. The pH of this solution was achieved by titration with a 2 M-NaOH stock solution. Superfusate [$K^+$] was increased to 40 mM by addition of solid KCl to the solution. Control experiments, adding 80 mM-sucrose to the superfusate, elicited a small, slow contracture, less than 5% of the peak response with raised [$K^+$].

All chemicals were Analar grade from BDH except the following which were obtained from Sigma; tetrodotoxin (TTX), 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulphonic acid (SITS), sodium isethionate, HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid).

*Measurement of intracellular pH.* Intracellular pH was measured in isolated ferret detrusor cells by epifluorescence microscopy. Cells were prepared by incubating, for 20–60 min at 37 °C, small

cubes (1 $mm^3$) of tissue in a medium of the following composition (in mM): NaCl, 104·8; KCl, 4·2; $NaHCO_3$, 22·2; HEPES, 22·2; $MgSO_4$, 1·1; $KH_2PO_4$, 1·1; $CaCl_2$, 1·6; glucose, 9·8; collagenase (Worthington), 3·0 mg/ml; bovine serum albumin, 10·0 mg/ml and the pH adjusted to 7·2 using 1 M-NaOH. Samples were intermittently examined under a microscope and incubation terminated when isolated, viable cells were observed. Such cells had a clear cytoplasm and a halo around the edge under phase-contrast illumination.

Cells were loaded with a 3 $\mu$M-solution of the acetoxymethyl ester of BCECF (2′,7′-bis(carboxyethyl)5-6-carboxyfluorescein, Molecular Probes, OR, USA) in the above medium, with collagenase omitted, by incubation at room temperature for 10–15 min. The loaded cells were placed on the stage of an inverted microscope over the objective ( × 40, numerical aperture 1·3) and superfused, at room temperature, with Tyrode solution at 1 ml/min for at least 5 min before recordings were started. Excitation of the fluorochrome was switched at 1 Hz between 430 and 500 nm and the intensity of emitted light at 530 nm recorded. Details of the recording system are given in Eisner, Nichols, O'Neill, Smith & Valdeolmillos (1989).

Intracellular pH, $pH_i$, was calculated from the ratio ($R$) of emitted light elicited by excitation at 500 and 430 nm using the formula (Eisner *et al.* 1989):

$$pH_i = pK + \log\frac{R-R_{min}}{R_{max}-R} + \log(430_{min}/430_{max}), \quad (1)$$

where $R_{min}$ is the light ratio of 500/430 nm at extreme acid values (pH 4·5) and $R_{max}$ the corresponding ratio at pH 9·5. The ratio $430_{min}/430_{max}$ is the ratio of light at 430 nm measured at pH 9·5 and 4·5. The p$K$ value was calculated by superfusing the cell suspension with a solution containing 10 $\mu$M-nigericin (Sigma) and buffered to pH 4·5, 7·0 and 9·5 and utilizing eqn (1). The value from nine determinations was 6·89 ± 0·30 (S.D. of an observation).

## RESULTS

### *Effects of superfusate pH on detrusor contractility*

Figure 1 shows the effect of reducing superfusate pH from 7·33 to 6·80 on electrically evoked contractions in human detrusor muscle. In part A superfusate pH was reduced by increasing, from 5 to 20%, the $CO_2$ content of the gas equilibrating with the Tyrode solution (see Table 1). Contraction in the new solution was greater than control, overshooting before a stable, but augmented, level was achieved. Upon return to control solution, tension declined as rapidly to the original level, showing a small undershoot before attaining a level the same as the previous control.

The increase in $CO_2$ content of the gas mixture was accompanied by a reduction of $O_2$ content. To discount the possibility that the fall of superfusate $P_{O_2}$ is responsible for the increase of force, control experiments were performed where the superfusate was equilibrated with 5% $CO_2$, 80% $O_2$ and 15% $N_2$. The pH of this solution was the same as when equilibrated with 5% $CO_2$–95% $O_2$ (7·35 ± 0·03 and 7·33 ± 0·02 respectively) and no change in contraction strength was observed when changing between them. Thus, the increase of force can be attributed to the reduced superfusate pH, consequent upon raising the $P_{CO_2}$.

Alteration of superfusate pH by changing the [$NaHCO_3$], at constant $P_{CO_2}$, also altered contraction strength and the results are illustrated in Fig. 1*B*. In contrast to the above, an acidosis from 7·33 to 6·80 reduced contractile force and the attainment of a steady state as well as recovery was as rapid as when $CO_2$ was used to alter superfusate pH.

Alkalinization of the superfusate produced opposite results from acidosis. Reduction of the $CO_2$ content of the equilibrating gas from 5 to 2% depressed tension, whilst an increase of the [$NaHCO_3$] from 24 to 48 mM augmented force.

Similar results were also observed in all animal preparations that were used and in Fig. 2 a summary of the data obtained with human (part *A*) and ferret (part *B*) detrusor muscle is shown. In both, progressive acidosis from 7·6 to 6·6, induced by increasing superfusate $CO_2$ content, raised the force of contraction. On the other

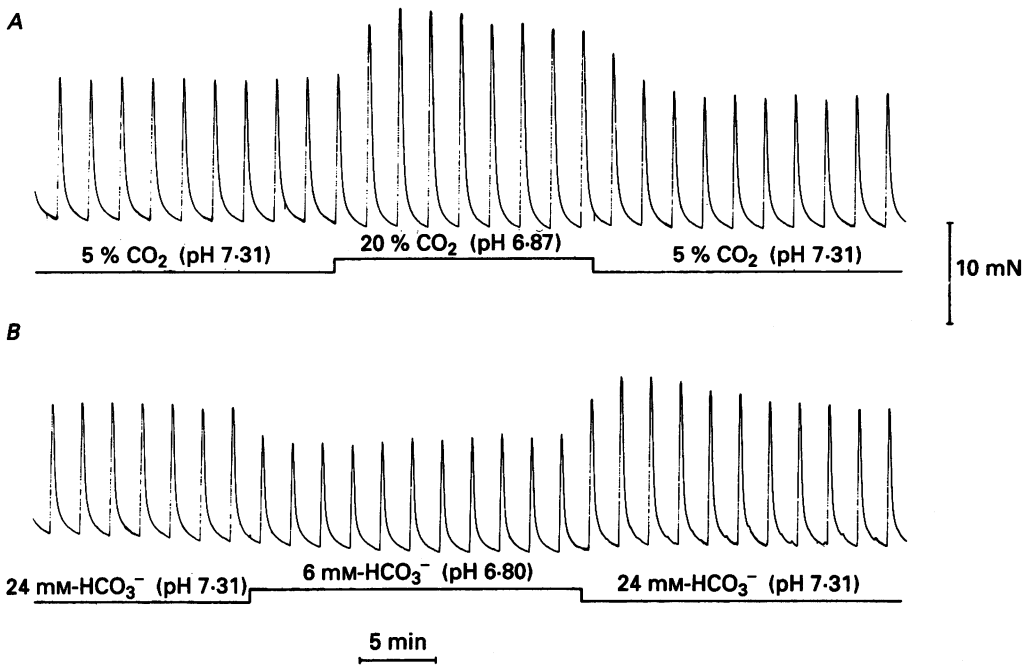


Fig. 1. Contractions recorded from a strip of human detrusor muscle stimulated every 90 s. *A*, extracellular pH lowered by increasing the superfusate $P_{CO_2}$ at constant [$NaHCO_3$]. *B*, extracellular pH lowered by decreasing superfusate [$NaHCO_3$] at constant $P_{CO_2}$. The pH values are stated beneath the experimental recordings. See Methods for composition of solutions. Temperature 37 °C.

hand, similar acid changes produced by reducing the $NaHCO_3$ concentration, at constant $P_{CO_2}$, gave the opposite result, namely a reduction of force. The straight lines through the data points were obtained by least-squares regression of the data points, but have no special significance.

Such results might be explained if an intracellular acidosis increased contraction strength, whilst an extracellular acidosis was depressant. $CO_2$ readily crosses cell membranes and a raised superfusate $CO_2$ would be expected also to acidify the cell interior, whilst $HCO_3^-$ and $H^+$ are less permeable, so that smaller intracellular changes would be anticipated. The depressant effect of extracellular acidosis by raising superfusate $P_{CO_2}$ is thus more than offset by the augmentation of force as a result of intracellular acidosis. An increase of both superfusate $P_{CO_2}$ and $NaHCO_3$ concentration by equal proportions, thus keeping extracellular pH constant (Table 1), should therefore increase force even more than by raising $P_{CO_2}$ alone. In a total of 13 interventions with human tissue, a Tyrode solution containing 48 M-$NaHCO_3$ and equilibrated with 10% $CO_2$ increased force to 128·8 ± 9·9 % of control. This

compares with an increase to 114·7 ± 9·4 % when gassed with 10 % $CO_2$ at normal $NaHCO_3$ and to 114·6 ± 10·3 % when superfused with 48 mM-$NaHCO_3$ and 5 % $CO_2$. Similar results were obtained with ferret detrusor preparations and these results are illustrated in Fig. 3, part *A*.

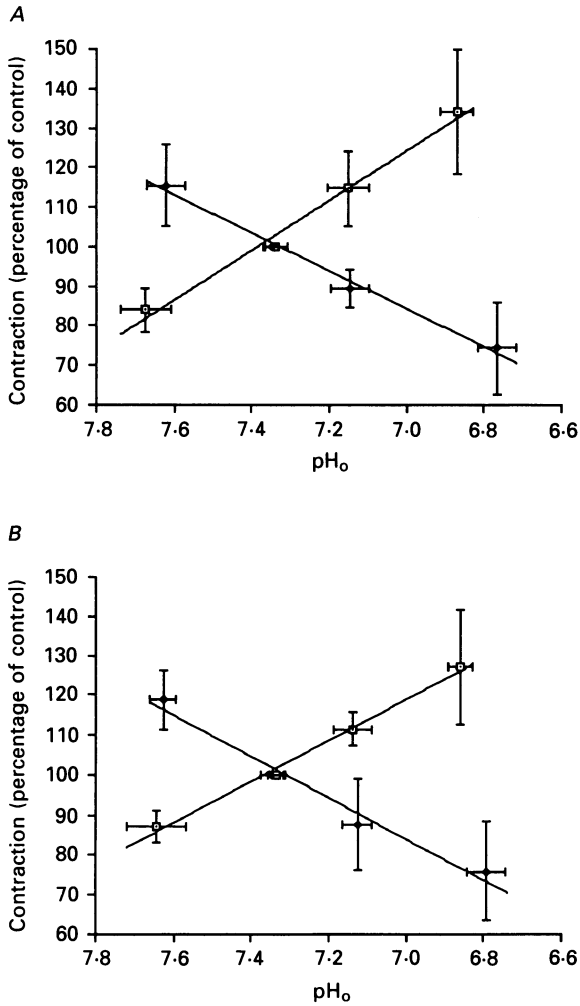


Fig. 2. *A*, a plot of phasic tension as a function of extracellular pH for human detrusor muscle. ⊡ show the relationship as superfusate $P_{CO_2}$ is varied, ■ as [$NaHCO_3$] is altered. Tension is expressed as a percentage of that in control solution (pH 7·33). Error bars represent S.D. of observations. The straight lines were obtained by least-squares regression. *B*, a similar plot obtained from ferret detrusor muscle.

*Control experiments: the effects of superfusate pH in the presence of tetrodotoxin and on the excitability of the preparation*

The results discussed above were obtained by stimulating the muscle via the attendent nerve network, that is individual pulses in the stimulating train were 0·2 ms duration (see Methods). It is possible that these effects of superfusate pH acted independently by altering the efficiency of nervous conduction or neuro-

muscular transmission rather than directly upon muscle function. In order to investigate this possibility experiments were performed using longer individual pulses in the stimulating train (20 ms) and in the presence of tetrodotoxin (10–7 g/ml). This protocol has been shown to activate smooth muscle directly rather

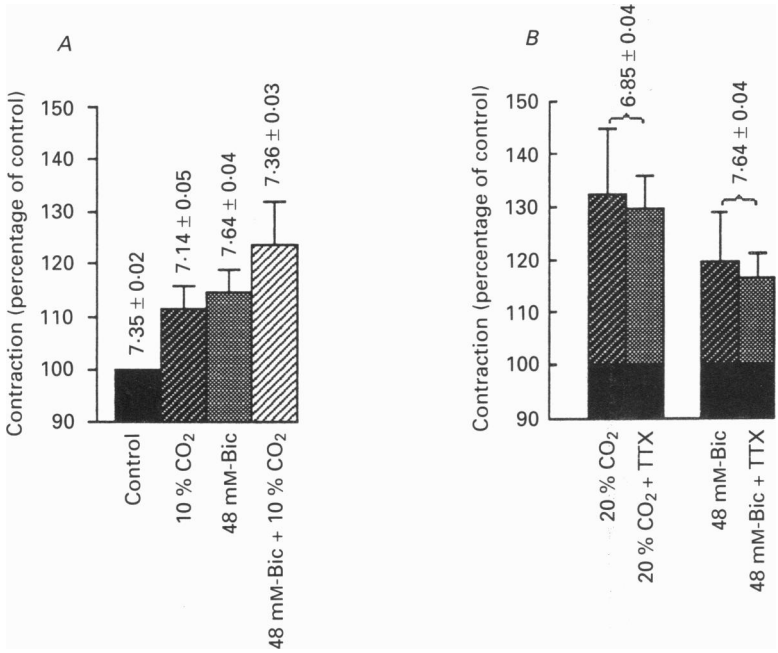


Fig. 3. *A*, bar chart summarizing the effects on electrically induced contractions of increasing superfusate $P_{CO_2}$ and [$NaHCO_3$] at constant extracellular pH (column 4). The effects of raising the two variables separately are shown in columns 2 and 3. *B*, bar chart summarizing the effect of TTX on the contractile response to raised superfusate $P_{CO_2}$ (column 2) and raised [$NaHCO_3$] (columns 4). pH values of the different solutions are shown above the appropriate column. S.D. of data is quoted. Each bar is the percentage increase of force (from 100%) compared to its own control (fully shaded section), pH 7·35 ± 0·02. These data were all obtained with ferret preparations. TTX, tetrodotoxin; Bic, bicarbonate.

than via the nervous plexus in the tissue (Palfrey *et al.* 1984). Using these stimulation parameters the contractile responses induced via nerve-mediated stimuli were of similar magnitude to those obtained by direct muscle stimulation. Results from eleven ferret preparations are illustrated in Fig. 3*B*. The increase of force as a result of increasing either superfusate $P_{CO_2}$ (columns 1 and 2) or [$NaHCO_3$] (columns 3 and 4) were identical ($P > 0{\cdot}10$) irrespective of whether stimulation was via the nervous network (columns 1 and 3) or directly on the muscle (columns 2 and 4). These experiments are consistent with the proposition that alterations to superfusate pH influence directly muscle function. Identical results were obtained with human preparations.

The effects of pH changes could be mediated by altering the relationship between force and either the frequency of pulses or the current passed in the stimulating train, i.e. there is a change in the excitability of the tissue. The force–frequency relationship

was unaltered when the $CO_2$ content of the mixture gassing the Tyrode solution was increased from 5 to 10% in two determinations. In two other experiments the relation between force and the PD across the stimulating electrodes was also unchanged during a similar rise of superfusate $P_{CO_2}$.

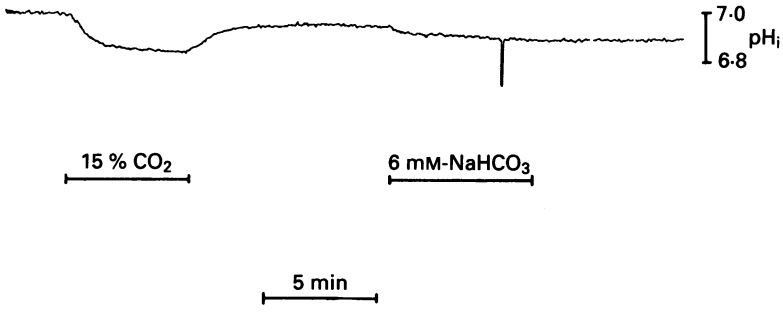


Fig. 4. Measurement of intracellular pH in an isolated ferret detrusor cell. At the first bar, the gas mixture equilibrating with the superfusate was changed from 5% $CO_2$–95% $O_2$ to 15% $CO_2$–85% $O_2$. At the second bar the solution was changed from one containing 24 mM-$NaHCO_3$ to one containing 6 mM-$NaHCO_3$, both equilibrated with 5% $CO_2$–95% $O_2$. Temperature 22 °C.

*Measurement of intracellular pH ($pH_i$)*

Intracellular pH was measured in sixteen cells with a calculated value of $pH_i$ equal to $7{\cdot}11 \pm 0{\cdot}10$ (S.D.) when superfused with normal Tyrode solution at room temperature (approximately 22 °C). At this temperature the pH of the Tyrode solution was less than that at 37 °C, namely $7{\cdot}18 \pm 0{\cdot}03$. Figure 4 illustrates the effect on $pH_i$ of first raising the $CO_2$ content and secondly reducing the [$NaHCO_3$] of the superfusate. In the former intervention the $CO_2$ content of the gas mixture was raised from 5 to 15% thus reducing superfusate pH from 7·18 to 6·70. In the latter intervention the $NaHCO_3$ concentration was lowered from 24 to 6 mM reducing pH to 6·60. Raising the $CO_2$ content caused a rapid, large fall of $pH_i$ from 7·00 to 6·83 with a nearly complete recovery, whilst a larger extracellular acidosis induced by reducing [$NaHCO_3$] had only a small effect on $pH_i$.

In a total of nine cells, reducing extracellular pH from $7{\cdot}18 \pm 0{\cdot}03$ to $6{\cdot}69 \pm 0{\cdot}02$ by equilibrating with 15% $CO_2$ reduced $pH_i$ from $7{\cdot}12 \pm 0{\cdot}07$ to $6{\cdot}88 \pm 0{\cdot}14$. A reduction of the [$NaHCO_3$] to 6 mM reduced extracellular pH from $7{\cdot}18 \pm 0{\cdot}03$ to $6{\cdot}59 \pm 0{\cdot}02$ and $pH_i$ from $7{\cdot}15 \pm 0{\cdot}04$ to only $7{\cdot}04 \pm 0{\cdot}02$.

The above results are therefore consistent with the proposal that intracellular acidosis increases detrusor contractility whilst extracellular acidosis is depressant. Alkaline changes to either compartment produce the opposite effects.

*The effects of $NH_4Cl$ on detrusor contraction and $pH_i$*

Intracellular pH was also altered by addition and removal of $NH_4Cl$ to the superfusate. In many cell types addition of $NH_4Cl$ has been shown to increase intracellular pH, and its removal induce a transient acidosis. Figure 5*A* shows that this also is true in isolated ferret detrusor cells. $NH_4Cl$ at 5, 10 and 20 mM was added

to the superfusate during the intervals shown. In each case addition was accompanied by an intracellular alkalosis, the peak value of which was related to the $NH_4Cl$ concentration. A partial recovery was observed before attainment of a steady state. Removal of $NH_4Cl$ resulted in the rapid development of a transient acidosis.

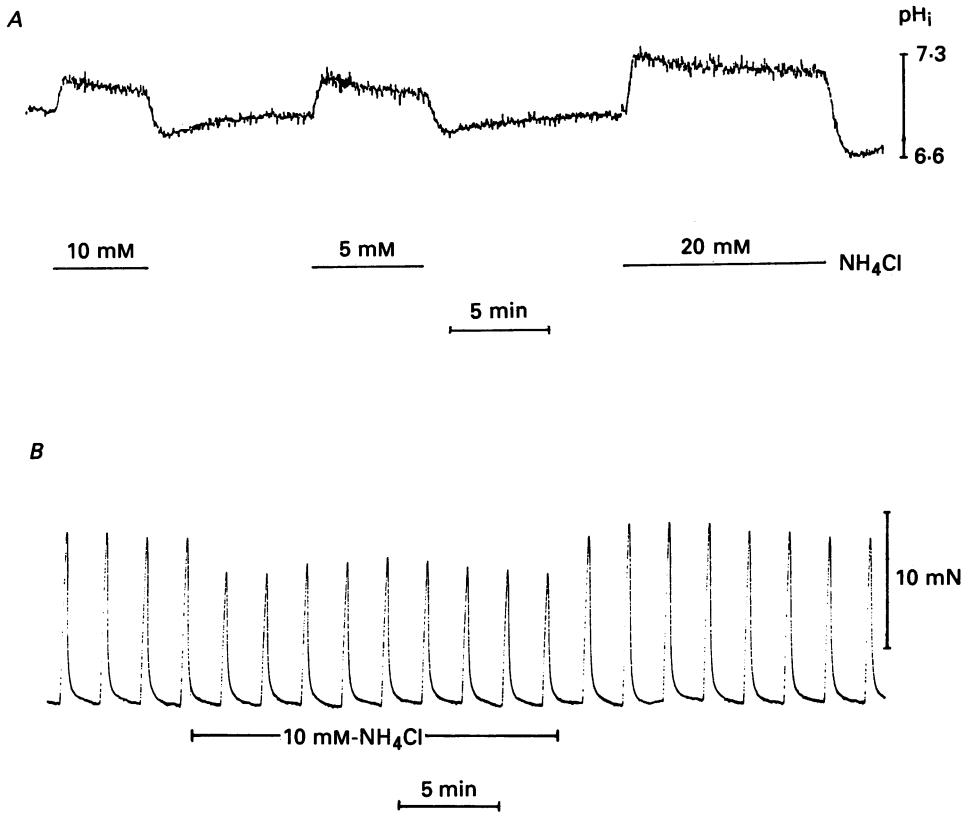


Fig. 5. The effect of $NH_4Cl$ on intracellular pH (*A*) and phasic tension (*B*). *A*, a ferret detrusor cell was exposed to 10, 5 and 20 mM-$NH_4Cl$ during the times indicated. Temperature 22 °C. *B*, 10 mM-$NH_4Cl$ was added to the superfusate as shown. Human detrusor strip, temperature 37 °C.

The contraction experiments above would predict that addition of $NH_4Cl$ would be accompanied by a reduction of developed tension and its removal would increase force. The result of an experiment on a human detrusor strip is shown in Fig. 5*B*. Addition of 10 mM-$NH_4Cl$ depressed force and its removal caused a transient overshoot, before return to control. Figure 6 compares the peak changes of tension and $pH_i$ during the addition and removal of 5, 10 and 20 mM-$NH_4Cl$ in ferret preparations.

*The action of amiloride derivatives on the $CO_2$-induced increase of force*

The existence of membrane counter-exchange mechanisms may explain the increase of force upon acidifying the intracellular space. A raised intracellular $[H^+]$ may result in $Na^+$ influx if $Na^+$–$H^+$ exchange is important in detrusor smooth

muscle. An increase of the intracellular [$Na^+$] might in turn raise the intracellular [$Ca^{2+}$] by $Na^+$–$Ca^{2+}$ exchange. There is evidence that the latter mechanism is present in this tissue as superfusion with low [$Na^+$] solutions results in a substantial contracture, rather than increased spontaneous activity (T. G. Liston & C. H. Fry, unpublished data).

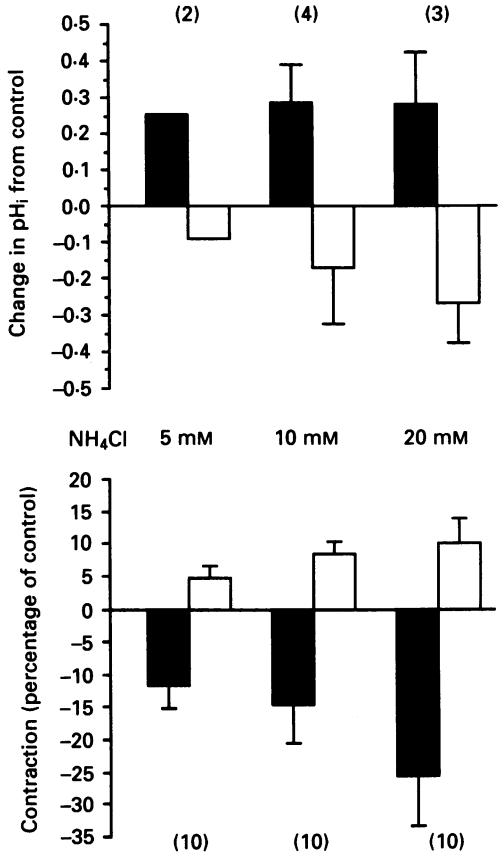


Fig. 6. Bar chart summarizing the effects of $NH_4Cl$ on intracellular pH (upper panel) and tension (lower panel) in ferret bladder. Both variables are expressed as changes from the control. The first bar at each concentration is the maximum effect in the presence of $NH_4Cl$, the second bar, the maximum effect upon removal. The number of observations are shown in parentheses by each bar.

A number of agents have been recently described which have been shown to inhibit, fairly specifically, $Na^+$–$H^+$ exchange, among these the amiloride derivative 5-[*N*-methyl-*N*-(guanidinocarbonyl methyl)] guanidine amiloride (Simchowitz & Cragoe, 1986). If the above process was significant in augmenting force when $pH_i$ was decreased, it would be expected that the amiloride derivative would inhibit the enhancement of tension as the accumulation of intracellular $Na^+$ would be prevented.

Figure 7 shows an experiment to test this hypothesis. In part *A*, a human detrusor strip was superfused with a solution of normal composition and one in which the extracellular pH was reduced, by increasing the $CO_2$ content of the gas mixture, which equilibrates with the fluid, from 5 to 20%. Force was rapidly increased in acid

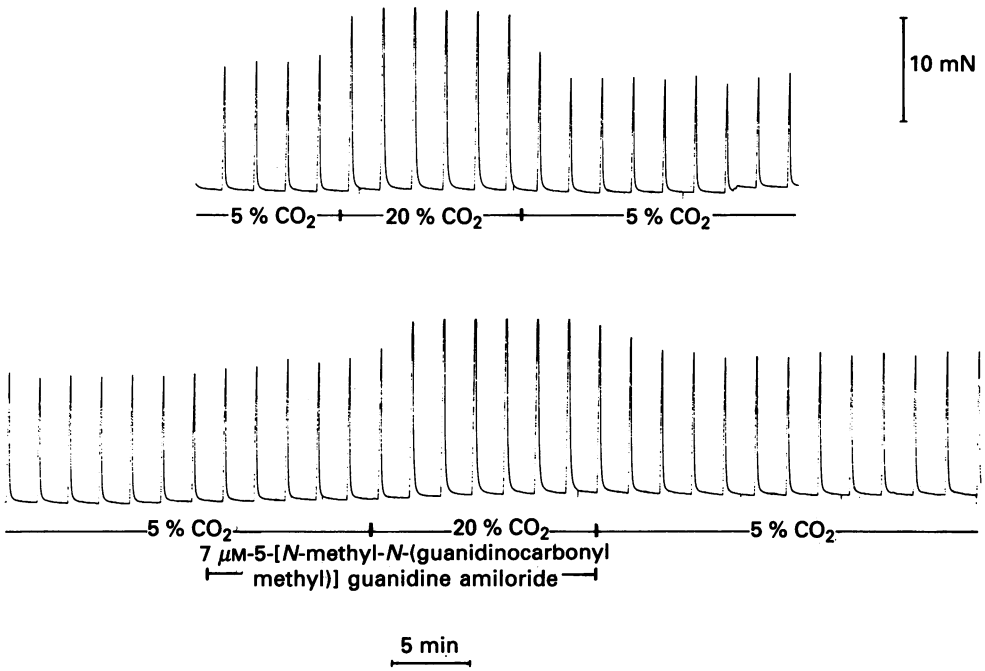


Fig. 7. The effect of 7 $\mu$M-5-[*N*-methyl-*N*-(guanidinocarbonyl methyl)] guanidine amiloride on the increase of tension induced by raising superfusate $P_{CO_2}$. The top panel shows a control response and the lower panel a second response, in the same preparation, in the presence of the amiloride derivative. Human detrusor muscle. Temperature 37 °C.

pH and recovered completely upon return to control. Addition of 7 $\mu$M-5-[*N*-methyl-*N*-(guanidinocarbonyl methyl)] guanidine amiloride to normal Tyrode solution itself (part *B*) caused a small but significant rise of tension (9·5 ± 5·3%, human; 11·9 ± 9·4, ferret). However, a reduction of extracellular pH by raising superfusate $P_{CO_2}$ produced an equivalent rise of tension compared to the situation in part *A*. In a total of thirty interventions with human tissue, raising the $CO_2$ percentage from 5 to 20% increased force by 26·6 ± 8·0% (S.D., $n = 30$). In the presence of the amiloride derivative, tension was increased by 33·0 ± 11·8% (S.D., $n = 30$). This result would suggest that if this agent is truly effective in inhibiting $Na^+$–$H^+$ exchange, the mechanism plays little role in the increase of force induced by intracellular acidosis. Similar results were found in nineteen ferret preparations: increasing $CO_2$ to 20% increased force by 27·7 ± 7·1% in the absence and by 29·2 ± 10·5% in the presence of the amiloride derivative. Similar results were obtained with another derivative of amiloride which has been shown to inhibit $Na^+$–$H^+$ exchange in various tissues, namely 5(-*N*,*N*-hexamethylene) amiloride.

*The action of* $[Cl^-]$ *and* $[HCO_3^-]$ *on tension generation and* $pH_i$

The experiments described above do not support a role for $Na^+$–$H^+$ exchange in explaining the increase in force with intracellular acidosis. The small, but significant, change of $pH_i$ when the extracellular $[HCO_3^-]$ was changed might indicate a flux of

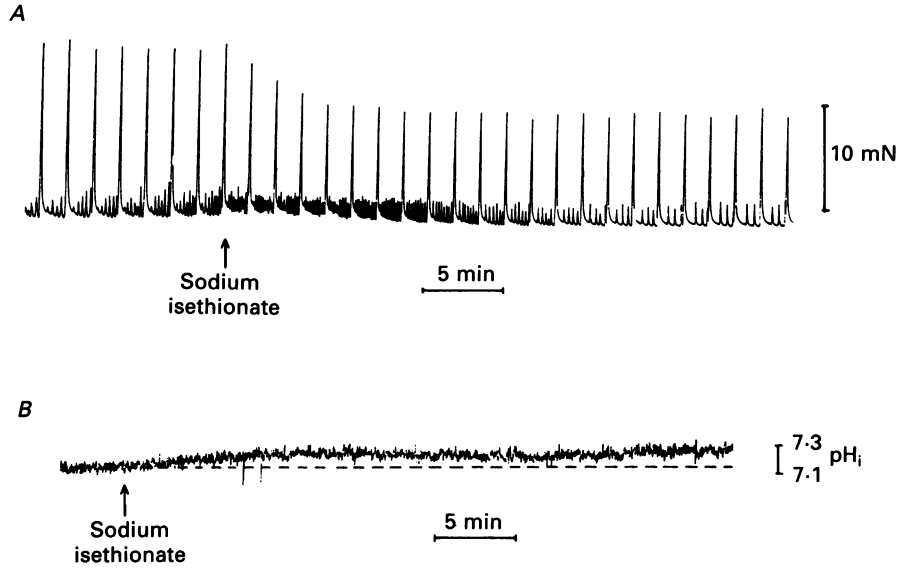


Fig. 8. The effect of low-$Cl^-$ solutions on tension (*A*) and intracellular pH (*B*). Sodium isethionate replaced NaCl in the superfusate, reducing $[Cl^-]$ from 127·6 to 9·6 mM. $[CaCl_2]$ increased from 1·80 to 2·45 mmol/l in low-$Cl^-$ solution. *A*, ferret detrusor strip, 37 °C. *B*, isolated ferret detrusor cell, 22 °C.

this ion, possibly via a $Cl^-$–$HCO_3^-$ exchanger. Further evidence for this system, and its role in determining contractile changes during alterations of pH, was gained by manipulation of the $[Cl^-]$ and $[HCO_3^-]$ cellular gradients.

Reduction of either the extracellular $[HCO_3^-]$ or $[Cl^-]$ reduced tension in human and ferret detrusor strips as well as increasing $pH_i$ in isolated ferret cells. In these experiments the $[HCO_3^-]$ was reduced to nearly zero by replacing $NaHCO_3$ in the Tyrode solution by HEPES, titrating to pH 7·36 with NaOH and gassing the solution with 100% $O_2$. The superfusate $[Cl^-]$ was reduced from 127·6 to 9·6 mM by replacing the NaCl with sodium isethionate. The anion reduces the activity of free $Ca^{2+}$ in solution so that the total $[CaCl_2]$ was increased to 2·45 mM to maintain a free concentration of $Ca^{2+}$ similar to that in Tyrode solution (Band, Fry & Treasure, 1978). Figure 8 illustrates the effect on tension of replacing NaCl with sodium isethionate. In part *A*, tension measured in a strip of human tissue was reduced when the extracellular $[Cl^-]$ was lowered and in part *B* $pH_i$ slowly increased in an isolated ferret cell during application of a similar solution. Re-introduction of normal Tyrode solution allowed a gradual recovery to control conditions in both experiments. In three experiments reduction of extracellular $[Cl^-]$ to 8% increased $pH_i$ by $0{\cdot}17 \pm 0{\cdot}04$ pH units. The experiments are consistent with the presence of a

$Cl^-$–$HCO_3^-$ exchange in that reduction of the extracellular [$Cl^-$] increases the flux of $Cl^-$ from the cell and the passage of $HCO_3^-$ into the cell thus increasing sarcoplasm pH.

Several experiments were performed in the presence of 100–200 $\mu$M-SITS (4-acetamido-4′-isothiocyanatostilbene-2,2′-disulphonic acid), an agent which inhibits $Cl^-$–$HCO_3^-$ exchange turnover. Tension was reduced to 72 ± 15·6% (S.D., $n = 9$) in ferret and to 60 ± 9·5% in human strips, with a half-time of 15–20 min. A single observation in an isolated ferret detrusor cell showed that $pH_i$ rose from 7·20 to 7·31 upon addition of 100 $\mu$M-SITS. These results might imply that the exchanger is operating under normal conditions to extrude $HCO_3^-$ from the cell. However, the increase of force consequent upon raising superfusate $P_{CO_2}$ was uninfluenced by the inclusion of SITS implying that turnover of the exchange was not altered when intracellular pH was lowered.

Removal of extracellular $NaHCO_3$ and its replacement by HEPES exerted more complex changes on tension and $pH_i$. Figure 9*A* shows that, with a ferret strip, changing from a $NaHCO_3$–$CO_2$ buffered superfusate to a HEPES–$O_2$ fluid decreased force throughout the intervention. Re-introduction of normal Tyrode solution induced an overshoot of tension before return to control. In Fig. 9*B* $pH_i$ in an isolated ferret cell is increased upon addition of a HEPES medium and again overshoots when the control solution is again added.

Some differences can be seen between the tension and $pH_i$ recordings; most notable is the partial recovery of $pH_i$ in the HEPES superfusate, whilst the tension recording shows little recovery. This may reflect the damping of the transient response in the multicellular strips due to differences in diffusion delay as was seen in similar measurement with $NH_4Cl$.

*The relationship between intracellular pH, extracellular pH and tension*

The previous experiments have shown a strong relationship between an increase of force and intracellular acidosis but a decrease of force associated with extracellular acidosis. The effect of intracellular pH on tension occurs regardless of the means whereby $pH_i$ is altered. A number of the interventions, such as addition of $NH_4Cl$, HEPES substitution of $NaHCO_3$, $Cl^-$ replacement by isethionate and addition of SITS, were performed at constant extracellular pH so that a relation between $pH_i$ and tension could be established. Those interventions which altered extracellular pH, such as changing superfusate $P_{CO_2}$ and [$HCO_3^-$], also altered $pH_i$. If it is assumed that the effects of intracellular and extracellular pH changes on tension are independent and the effect of $pH_i$ changes on tension are subtracted, then the relation between extracellular pH and tension can be calculated. The results are illustrated in Fig. 10.

In part *A* of Fig. 10 the relationship between intracellular pH measured in isolated cells and isometric tension recorded from muscle strips is plotted. Each data point represents the mean value from three to nine determinations and were obtained from a variety of interventions, each of which has a different symbol (see figure legend). The common feature of these interventions was that extracellular pH remained constant. The abscissa is expressed as the mean change of $pH_i$ from the control value immediately preceding the intervention; the tension data are expressed as a

percentage of that measured under control conditions. The data show that decreasing $pH_i$ over a range of 0·6 units increases force, regardless of the means by which it was achieved, although the slope of the line should not be regarded as a quantitative description of the data as the two variables were measured in different preparations.

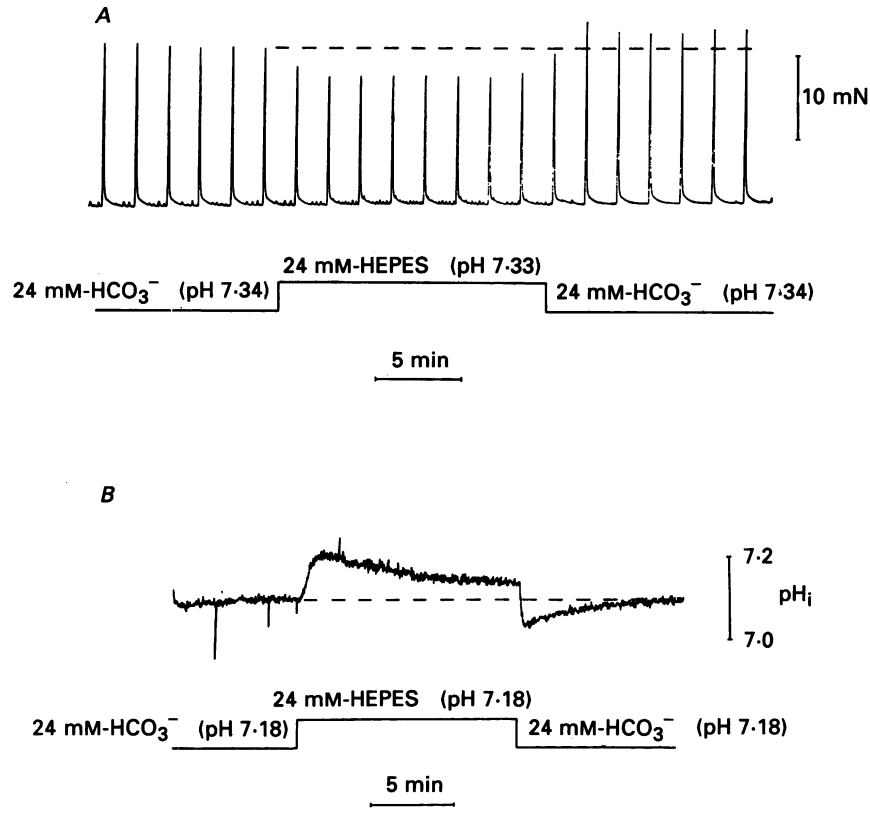


Fig. 9. The effect of replacing superfusate $NaHCO_3$ with HEPES on tension (*A*) and intracellular pH (*B*). The solutions were gassed with 5% $CO_2$–95% $O_2$ and 100% $O_2$ respectively. The HEPES solution was titrated to pH 7·34 with NaOH. *A*, ferret detrusor strip, 37 °C. *B*, isolated ferret detrusor cell, 22 °C.

In part *B* of Fig. 10 the relationship between extracellular pH and tension is shown using open symbols. The abscissa is again the change in pH from the control solution (5% $CO_2$, 24 mM-$NaHCO_3$), the magnitude of these changes being similar at 23 and 37 °C. Extracellular pH was changed by altering either superfusate $P_{CO_2}$ (○) or [$NaHCO_3$] (□). No clear relation is seen because such solutions alter $pH_i$ as well as extracellular pH. The effect that the $pH_i$ changes exert on the overall tension response can be estimated from part *A* of Fig. 10, and the effect of these $pH_i$ on the overall tension response subtracted. The result of such a correction is plotted using the closed symbols in part *B*, (●) for $P_{CO_2}$ changes and (■) for changes of the [$NaHCO_3$], and reveals a better relationship between extracellular pH and tension.

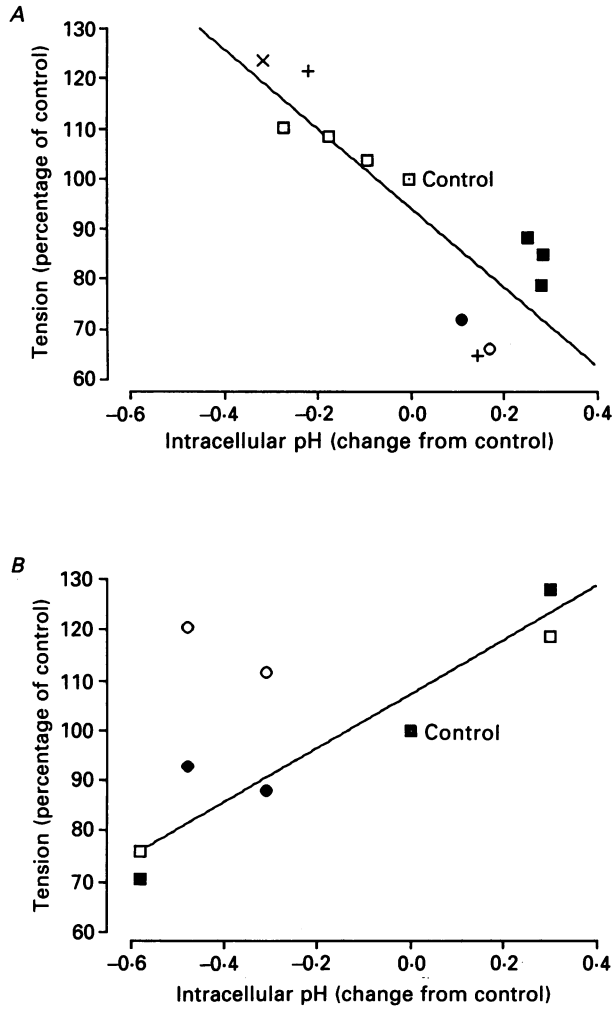


Fig. 10. The relationship, in ferret detrusor, between tension and intracellular pH (*A*) or extracellular pH (*B*). *A*, interventions at constant extracellular pH were used to construct the plot. Tension measured as a proportion of control. The intracellular pH scale is the change of $pH_i$ from the control value, negative numbers are acid shifts. Data obtained from experiments in which $NH_4Cl$ was added (■) or subtracted (□), HEPES Tyrode solution added or removed (+), in low-$Cl^-$ solution (●), in the presence of SITS (○), or solutions in which $P_{CO_2}$ and [$NaHCO_3$] were raised in equal proportions (×). *B*, open symbols refer to the experiments in which the tension scale is uncorrected for the influence of $pH_i$ changes. Closed symbols are corrected points after the contribution of $pH_i$ changes have been subtracted, using the data in *A*. The straight line is fitted through the corrected points.

In contrast to the plot in part *A*, progressive acidosis depresses function. The slope of the relation is shallower although again the precise value has no direct meaning. However, the relative value of the slopes is of importance, because it implies that changes of intracellular pH dominate extracellular effects. This is borne out by direct

observation when for example superfusate $P_{CO_2}$ is raised, which acidifies both the intracellular and extracellular spaces, but force is increased.

*The effects of pH on resting tension, high-$K^+$ contracture and carbachol contracture*

Alteration of the superfusate pH affected resting tension in both human and ferret strips and the magnitude and frequency of spontaneous activity in ferret strips. In

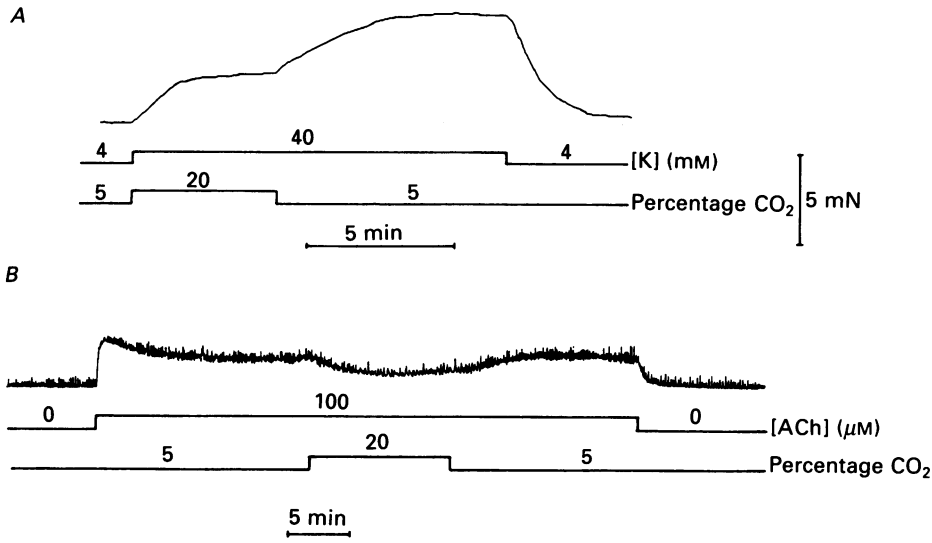


Fig. 11. The effect of raising superfusate $P_{CO_2}$ on the high-$K^+$ contracture (*A*) or the acetylcholine contracture (*B*). In the two panels acidosis was induced by changing the equilibrating gas from 5% $CO_2$–95% $O_2$ to 20% $CO_2$–80% $O_2$. *A*, human detrusor strip; the superfusate was raised from 4 to 40 mM where indicated, 37 °C. *B*, ferret detrusor strip; 100 μM-acetylcholine was added where indicated, 37 °C.

ferret preparations there was a small fall of resting tension and a reduction of spontaneous activity when the $CO_2$ gassing the superfusate was raised from 5 to 20%. Increasing superfusate pH increased resting tension in both ferret and human tissue regardless of the means by which pH was altered, i.e. changing superfusate $P_{CO_2}$ or [$NaHCO_3$]. Decreasing pH evoked the opposite response; see for example Fig. 1. These changes were small however compared to the phasic tension evoked by electrical stimulation so that they were more evident with unstimulated preparations and observed even when the muscle was in contracture during exposure to a raised superfusate [KCl]. Figure 11*A* shows a result from a strip of human detrusor muscle in which extracellular pH was reduced during exposure to 40 mM-KCl.

Raising superfusate KCl from 4 to 40 mM at control pH induced a stable contracture. During the initial period of exposure to high [KCl] superfusate pH was reduced from 7·35 to 6·80, by gassing the solution with 20% $CO_2$. A return to normal superfusate pH (5% $CO_2$) whilst maintaining the high [KCl] exposure further increased contracture tension. Force returned to normal upon introduction of normal Tyrode solution.

Acetylcholine and its analogue carbachol also evoke contracture in detrusor muscle and it has been proposed that it generates force via a mechanism partly independent of membrane depolarization (Mostwin, 1985). It was of interest therefore to see if an increase of superfusate $P_{CO_2}$ similarly altered this contracture. The result of an experiment with ferret muscle is seen in Fig. 11*B*. Application of 100 $\mu$M-acetylcholine induced contracture and an increase of spontaneous contractions. Raising the $CO_2$ from 5 to 20% in the equilibrating gas mixture reversibly reduced the contracture and slowed the frequency of spontaneous activation. The reduction of force by acidosis was not quantitatively different from that seen with the high-$K^+$ contracture.

## DISCUSSION

The results presented here indicate that intracellular acidosis increases the phasic contraction induced by electrical stimulation and extracellular acidosis attenuates such a contraction. Tonic tension, on the other hand, is reduced if either compartment is acidified, and this is observed when the preparation is superfused with a normal [$K^+$] or when contracture is elicited by increasing the [$K^+$] to 40 mM.

Data from other smooth muscle types are variable, but in view of the results presented here it is important to establish whether an intervention induces an intracellular and/or an extracellular pH change. In the ureter, an increase of extracellular $P_{CO_2}$ presumably decreasing $pH_i$, also increases force, although decreasing [$HCO_3^-$] has no effect (Cole & Fry, 1987). In rabbit ear artery intracellular acidosis increases tone whilst extracellular acidosis dilates the vessels (Ighoroje & Spurway, 1984; Spurway & Wray, 1987). In pulmonary artery an opposite situation may pertain because $CO_2$ has two actions, a constrictor effect due to carbonic acid and presumably an extracellular effect, and a dilatory action of $CO_2$ itself which may represent intracellular acidosis (Barer, 1976). In bronchial smooth muscle Stephens & Chiu (1970) found no effect of altering extracellular $P_{CO_2}$ or [$HCO_3^-$] on phasic force. Conversely, contractures induced by application of acetylcholine were enhanced by reducing extracellular $P_{CO_2}$ and attenuated by hypercapnia (Twort & Cameron, 1986). A number of other divergent responses to acid and base changes have been summarized by Wray (1988).

### *Intracellular pH in isolated detrusor cells*

The value of $pH_i$ in isolated ferret cells ($7{\cdot}11 \pm 0{\cdot}11$) is similar to that reported in other smooth muscles (Wray, 1988). These measurements were carried out at room temperature in a 5% $CO_2$–24 mM-$HCO_3^-$ buffered system where extracellular pH was 7·18, showing that there is only a small transmembrane $H^+$ gradient. The implication therefore is that the $H^+$ is not in equilibrium across the membrane.

The change of $pH_i$ when extracellular $P_{CO_2}$ is raised allows calculation of the buffering capacity of the sarcoplasm by calculating the ratio of added acid, from $CO_2$, and the change of $pH_i$. The results obtained with ferret detrusor cells are particularly well suited for this calculation as the decrease of $pH_i$ upon raising $P_{CO_2}$ was very stable so that any processes tending to regulate $pH_i$ were relatively small and not markedly attenuating the $pH_i$ change. This is in contrast to other tissues where some recovery is observed under similar conditions. (Aickin, 1984). A value of 21·6 mequiv

$H^+$/pH unit (range 13·6–34·3, eight cells) was obtained from the data obtained in ferret detrusor cells. This value is large compared to that obtained in other smooth muscle preparations (Aickin, 1984) but comparable to that measured in isolated cardiac cells (Bountra, Powell & Vaughan-Jones, 1987). The value of the buffering capacity was independent of the initial pH (7·17–6·99) and final pH (6·98–6·58). The buffer capacity was calculated from the ratio of the change of the intracellular [$HCO_3^-$], when $P_{CO_2}$ is raised, and the change of intracellular pH. The [$HCO_3^-$] itself was obtained from the intracellular pH and superfusate $P_{CO_2}$ using the Henderson–Hasselbalch equation.

The experiments in which extracellular $Cl^-$ was reduced or SITS applied both resulted in an alkalosis. This might imply that in the normal cell $Cl^-$–$HCO_3^-$ exchange was acting to extrude $HCO_3^-$.

*The effects of extracellular acidosis on force development*

A decrease of superfusate pH reduced resting tension and ferret spontaneous activity and, if the change of $pH_i$ was small, also reduced the phasic contraction. It is of interest that in the human ureter, extracellular acidosis had no effect on phasic contraction (Cole & Fry, 1987) and in this tissue the contraction persists for some time after the nominal removal of extracellular $Ca^{2+}$. Thus, in detrusor muscle $Ca^{2+}$ movement into the cell may play a role in the development of force and can be reduced by extracellular acidosis. Experiments in guinea-pig bladder have indicated that the $K^+$ contracture is dependent on transmembrane $Ca^{2+}$ flux (Mostwin, 1985) and the above results have shown this contracture, as well as resting tension, to be diminished in acid solutions. The depolarizing phase of the detrusor action potential results from an inward $Ca^{2+}$ current (Klöckner & Isenberg, 1985*a*) and its diminution by extracellular $H^+$ would reduce the phasic contraction. Preliminary experiments show that extracellular acidosis, by reducing the superfusate [$NaHCO_3$], reduces the magnitude of the inward $Ca^{2+}$ current in isolated human detrusor cells (B. S. I. Montgomery & C. H. Fry, unpublished data).

Ferret preparations showed basal spontaneous mechanical activity at 37 °C, the frequency and/or the amplitude of which were attenuated when resting tension declined, for example when extracellular pH was reduced. Spontaneous transient outward currents have been recorded in smooth muscle, including bladder, which have been attributed to the opening of $K^+$ channels as a result of cyclical release of $Ca^{2+}$ from intracellular stores (Klöckner & Isenberg, 1985*b*; Benham & Bolton, 1986). Thus, any process which increases $Ca^{2+}$ influx would be expected to augment intracellular stores and hence the tendency for spontaneous release of $Ca^{2+}$. Several observations lend weight to this possibility; spontaneous activity was increased (1) during application of acetylcholine, which resulted also in the development of contracture, (2) upon addition of 1 μM-ouabain, (3) during intracellular and/or extracellular alkalosis and (4) metabolic blockade by addition of 5 mM-sodium azide.

*The effects of intracellular acidosis on force development*

There are in principle several reasons why an increase of the intracellular [$H^+$] could increase phasic force. One may involve an increase of the $Ca^{2+}$ sensitivity of myofibrils in acid solutions and such a phenomenon has been described in skinned rat

vascular smooth muscle between pH 6·7 and 7·1 (Gardner & Diecke, 1988), a range of $pH_i$ values reported in these experiments. Other groups, however, have reported an opposite effect of acidosis in glycerinated arterial smooth muscle (Mrwa, Achtig & Rüegg, 1974) or no effect of acidosis in taenia caeci (Iino, 1981) and it would be of interest to establish the situation in detrusor muscle itself.

An increase of the $[Ca^{2+}]_i$ when $pH_i$ is reduced might also increase force if the increase was great enough to compensate for any depressant effects of acidosis on intracellular $Ca^{2+}$ metabolism. One route that was examined was that a raised intracellular $[H^+]$ increased the intracellular $[Na^+]$ via $Na^+$–$H^+$ exchange, which in turn could raise intracellular $[Ca^{2+}]$ via a separate $Na^+$–$Ca^{2+}$ exchange; both exchangers have been demonstrated in urinary tract smooth muscle (LaBelle & Eaton, 1983; Aickin, Brading & Walmsley, 1987). In cardiac muscle such a mechanism has been found to explain an increase of force upon intracellular acidosis (Bountra & Vaughan-Jones, 1989). It would be expected therefore that attenuation of $Na^+$–$H^+$ exchange would prevent the increase of force when $pH_i$ was lowered. The failure of the amiloride derivative to achieve this result would indicate that $Na^+$–$H^+$ exchange at least plays no role in this contractile phenomenon. There are however other routes by which $[Na^+]_i$ can rise with intracellular acidosis, such as $Na^+$–$HCO_3^-$ co-transport described in several smooth muscle types including ureter (Aickin, 1988). In this tissue recovery from acidosis in the presence of $CO_2$ was unaffected by amiloride and DIDS in agreement with the experiments described above where neither amiloride derivatives or SITS (an inhibitor of anion exchange like DIDS) affected the increase of force upon increase of superfusate $P_{CO_2}$. Measurement of $[Na^+]_i$ will establish whether such a mechanism is feasible in detrusor muscle.

A second route whereby a fall of $pH_i$ could increase force is via the sarcoplasmic soluble proteins and second messengers which control contraction. It has been shown that in detrusor muscle a contracture induced by carbachol persists in a $Ca^{2+}$-free solution for a longer period than a $K^+$ contracture (Mostwin, 1985) which could be explained if carbachol activates contraction by an additional route to transmembrane $Ca^{2+}$ flux. A number of steps in these intracellular pathways are sensitive to pH changes, but the weight of evidence at present suggests that acidosis would diminish rather than enhance contraction. For example a decrease of pH from 7·5 to 6·7 reduced the ability of $IP_3$ to release $Ca^{2+}$ from platelets (Brass & Jospeh, 1985) and the binding of $Ca^{2+}$ to calmodulin, a step important in the activation of smooth muscle myosin light chain kinase, is diminished upon acidification (Busa & Nuccitelli, 1984). Further evidence against a role of such intracellular messengers in enhancing force when $pH_i$ was changed was the observation that the acetylcholine contracture, as well as the high-$K^+$ contracture, was attenuated by reducing $pH_i$. If the intracellular mechanisms activated by acetylcholine were augmented by intracellular acidification the opposite result might have been anticipated.

A final speculation centres around the observation in cardiac muscle that when intracellular $[Ca^{2+}]$ is raised, for example by application of cardiac glycosides, small spontaneous contractions occur, which is attributed to release of $Ca^{2+}$ from the sarcoplasmic reticulum (SR). The twitch however is reduced because of the depleted $Ca^{2+}$ in the SR when the normal stimulus arrives (Allen, Eisner, Pirolo & Smith,

1985). Reduction of $pH_i$ in detrusor muscle reduces resting tension and spontaneous activity and thus may conserve intracellular $Ca^{2+}$ stores for normal activation. This suggestion raises the possibility that detrusor muscle could be considered to be in some state of '$Ca^{2+}$ overload' when exhibiting spontaneous activity and near to this condition at other times. Intracellular acidosis would therefore reduce spontaneous release of $Ca^{2+}$ into the sarcoplasm so that more would be available when externally stimulated.

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