# The role of the sarcolemmal $Ca^{2+}$-ATPase in the pH transients associated with contraction in rat smooth muscle

E. K. Naderali, N. Buttell, M. J. Taggart, A. J. Bullock, D. A. Eisner* and Susan Wray†

*The Physiological Laboratory and *Department of Veterinary Preclinical Sciences, The University of Liverpool, Liverpool L69 3BX, UK*

1. We have investigated the origin of the intracellular acid pH transients that accompany myometrial contraction. Intra- and extracellular pH were measured with SNARF and intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) with indo-1.

2. An intracellular acidification accompanied spontaneous contractions and those elicited by KCl depolarization or the addition of the agonists carbachol or prostaglandin $F_{2\alpha}$. The size of the acidification increased with the magnitude of the contraction.

3. The intracellular acidification was accompanied by an extracellular *alkalinization*, showing that it results from proton movement across the surface membrane. Furthermore, it was decreased either by addition of $Cd^{2+}$ (20 nM, an inhibitor of the sarcolemmal $Ca^{2+}$-ATPase) or by elevating $[Ca^{2+}]_o$.

4. Extracellular alkalinization increased the magnitude of the rise of $[Ca^{2+}]_i$ and force produced by KCl.

5. An intracellular acidification was also associated with contraction in the portal vein and ureter.

6. We conclude that the sarcolemmal $Ca^{2+}$-ATPase produces a significant intracellular acidification while removing $Ca^{2+}$. Both the acidification and decrease of $[Ca^{2+}]_i$ will promote relaxation. Since $Ca^{2+}$ and protons have opposite effects on many cellular processes, this dual regulation by these two ions may be of general importance.

Alteration of intracellular pH ($pH_i$) can markedly affect smooth muscle contraction (for review see Wray, 1988). Conversely, recent work has shown that contraction causes a change of $pH_i$ in uterine smooth muscle; small acid pH transients were associated with each spontaneous contraction (Taggart & Wray, 1993). These transients did not occur when contraction was prevented by removal of $Ca^{2+}$ from the bathing solution supporting a relationship between the acidification and contraction. The precise shape of the relationship between contraction and changes of pH is, however, unknown.

The origin of the acid transient is unclear. In the myocardium an intracellular acidification has been shown to be produced by manoeuvres that increase contraction and this has been attributed to lactic acid from glycolysis (Allen, Eisner, Morris, Pirolo & Smith, 1986). However, in the myometrium, maintained contraction did not increase lactate efflux (Taggart & Wray, 1993) therefore implicating a different mechanism. In the myometrium the sarcolemmal $Ca^{2+}$-ATPase appears to be the major mechanism for lowering $[Ca^{2+}]_i$ (Enyedi, Minami, Caride & Penniston, 1988). Since this ATPase has been shown in several tissues to exchange protons for $Ca^{2+}$ (Niggli, Sigel & Carafoli, 1982; Schwiening, Kennedy & Thomas, 1993), during contraction the observed changes of $pH_i$ could be accounted for by the removal of $Ca^{2+}$ from the cell (with consequent proton entry). This hypothesis makes the following predictions, which we have tested: (i) an *extracellular* alkalinization should occur as protons enter the cell in exchange for $Ca^{2+}$ exit on the $Ca^{2+}$-ATPase; (ii) inhibitors of the sarcolemmal $Ca^{2+}$-ATPase should attenuate the intracellular acidification; and (iii) manipulating the sarcolemmal $Ca^{2+}$ gradient should affect $pH_i$ (and vice versa).

Given the ubiquitous distribution of the sarcolemmal $Ca^{2+}$-ATPase, if it were responsible for acidification associated with contraction then contraction of other smooth muscles would be expected to produce acidification.

The aims of this work were therefore (i) to establish whether the acidification in the uterus is quantitatively related to the amount of contractile activity, (ii) to determine whether

† To whom correspondence should be addressed.

other smooth muscles show an acidification with contraction, and (iii) to elucidate the origin and mechanism of the contraction-associated pH changes.

An abstract of some of this work has been presented to The Physiological Society (Naderali & Wray, 1996).

## METHODS

Rats (~200 g) were killed by cervical dislocation under chloroform anaesthesia. Strips of longitudinal myometrium, portal vein or ureter (~100–150 $\mu$m thick) were dissected and loaded with the membrane-permeant form of the pH-sensitive indicator carboxy-SNARF (5 $\mu$M) or the $Ca^{2+}$-sensitive indicator indo-1 (20 $\mu$M), at room temperature (range, 20–22 °C) for 1–4 h (uterus and portal vein) or overnight at 4 °C (ureter). These loading protocols have been shown previously not to perturb tissue function (Taggart & Wray, 1993; Taggart, Austin & Wray, 1994; Burdyga, Taggart & Wray, 1996). After loading, the tissues were transferred to a small (200 $\mu$l) bath on the stage of an inverted microscope, with one end attached to a tension transducer. The tissues were superfused with oxygenated solution (36 ± 1 °C) of the following composition (mM): NaCl, 154; KCl, 5·4; $MgSO_4$, 1·2; glucose, 12; CaCl, 2·5. The solutions were usually buffered with 11 mM Hepes although $HCO_3^-/CO_2$ was used in some experiments; this study and previous work found there were no differences in the pH responses whether Hepes or $HCO_3^-/CO_2$ was the buffer (Taggart & Wray, 1993; Taggart *et al.* 1994). Depolarization of tissues was brought about by $K^+$ substituted isosmotically for $Na^+$, up to 80 mM. (Reduction of $Na^+$ alone has no effect on $pH_i$.) When required, $Cd^{2+}$ (20 nM), prostaglandin $F_{2\alpha}$ (1 $\mu$M) or carbachol (50 $\mu$M) were added to the solution.

### Intracellular pH and $Ca^{2+}$ measurement

The SNARF-loaded tissue was excited at 530 nm and the emission signals at 590 and 640 nm recorded. The fluorescence ratio, $F_{590}/F_{640}$, of these two signals was used to measure $pH_i$. The $pH_i$ records were calibrated using an artificial intracellular solution (Taggart & Wray, 1993), with the indicator at defined pH values, of composition (mM): NaCl, 10; KCl, 146; $MgSO_4$, 4; ATP, 4·5; Hepes, 11. For $[Ca^{2+}]_i$ measurement, indo-1-loaded tissues were excited at 340 nm and emission signals at 400 and 500 nm recorded. The ratio of these signals was used as an indicator of $[Ca^{2+}]_i$ changes. The dissociation constant of $Ca^{2+}$ binding to indo-1 was not significantly affected by pH over the small $pH_i$ range used in this study, nor were the minimum and maximum fluorescence ratios (Austin & Wray, 1995). The two emission signals from indo-1 and SNARF changed in opposite directions for all the data used; the ratio of the emission signals was used, so hence any tissue movement did not affect the $pH_i$ and $[Ca^{2+}]_i$ signals.

### Extracellular pH measurement

The membrane-impermeant form of SNARF was included in the superfusate (0·5 $\mu$M) in experiments to measure changes in external pH, and the Hepes concentration reduced to 5 mM. All other conditions and solutions were unchanged. Under these conditions the signal comes from both the extracellular space of the tissue and the bulk solution of the bath. The measured changes in pH will therefore underestimate those occurring in the extracellular space.

### Statistics

Figures given are mean values ± S.E.M. and $n$ is the number of animals. Differences were taken as significant where $P$ values were $< 0{\cdot}05$ in the appropriate paired or unpaired Student's $t$ test.

## RESULTS

### Effect of uterine contraction on the magnitude of the intracellular acidification

We found that intracellular acidification accompanies contractile activity of uterine smooth muscle, irrespective of the method used to activate contraction. In agreement with earlier findings (Taggart & Wray, 1993) it occurs with spontaneous (Fig. 1*A*) and high-$K^+$-depolarized contractions (Fig. 1*C*). In addition we found that acidification accompanied the contractions produced by physiological agonists such as carbachol (Fig. 1*B*) and prostaglandin $F_{2\alpha}$ (1 $\mu$M, 0·16 ± 0·03 pH units, $n = 3$, not shown). The magnitude of the acidification increased with that of contraction. Thus as shown in Fig. 1*D*, increasing external $[K^+]$, hence increasing the frequency of contraction and thence the time-averaged force, was accompanied by a concentration-dependent increase of acidification.

### Acidification with contraction in other smooth muscles

Figure 1*E* and *F* shows that intracellular acidification with contraction is not limited to uterine smooth muscle; ureteric and portal vein smooth muscle contraction also produced an acidification (0·30 ± 0·02 and 0·11 ± 0·02 pH units, respectively, $n = 4$).

### The mechanism of the intracellular acid transients

As mentioned in the Introduction, our previous work (Taggart & Wray, 1993) showed that the intracellular acidification was *not* accompanied by an increase of lactate efflux. We have repeated these measurements and found that high-$K^+$-activated contraction significantly *decreased* lactic acid production from 0·58 ± 0·11 to 0·37 ± 0·05 $\mu$mol $g^{-1}$ $min^{-1}$ ($n = 7$). Thus lactic acid production can be ruled out as the cause of the acidification. However, it is still possible that other intracellular sources of protons (such as those liberated by *net* ATP hydrolysis or from common buffer sites for $Ca^{2+}$ ions and protons) contribute.

There are two distinct sources for the intracellular pH transients: (i) due to intracellular production of protons or (ii) from proton entry across the sarcolemma. Although both these hypotheses predict an intracellular acidification, they differ in the direction of proton movements across the surface membrane. In hypothesis (i) the protons released into the cytoplasm will be pumped out of the cell resulting in a net acid extrusion. In contrast hypothesis (ii) predicts net proton entry. By measuring the direction of proton movement it is possible to distinguish between them. In order to do this, we measured the pH of the extracellular fluid by adding the membrane-*impermeant* form of SNARF to the superfusing solution. If protons enter the cell they will come from the extracellular fluid within the myometrial strip. The geometry of the tissue means that there is not free diffusion of protons from the bulk solution and therefore proton entry into the cell will produce an external alkalinization whereas proton efflux will produce an external acidification (see also Fig. 4).

Figure 2*A* shows that spontaneous contraction was accompanied by a small extracellular *alkalinization*. The presence of the extracellular alkalinization supports the hypothesis that the intracellular acidification results from proton entry across the surface membrane. The extracellular alkalinization begins before the contraction, as does the rise in $[Ca^{2+}]_i$ as shown in Fig. 2*C*, and peaks as the contraction does. As can be seen in Fig. 2*B* and as previously found (Taggart & Wray, 1993) the intracellular acidification lags contraction. Therefore the external alkalinization precedes the intracellular acidification. The relationship of these changes in external pH, $pH_i$, $[Ca^{2+}]_i$ and force can be clearly seen in Fig. 2*D* where the three force records have been normalized to their peaks and superimposed. Although the traces are from different preparations, the similarity of the time course of the contractions makes this legitimate. The accompanying ionic changes are shown above, with the $pH_i$ record inverted for clarity. Clearly extracellular pH and $[Ca^{2+}]_i$ have a different time course to that of $pH_i$. The significance of this time course is addressed in the Discussion. In eleven preparations the extracellular alkalinization had a mean magnitude of 0·025 ± 0·008 pH units.

### Inhibition of the sarcolemmal $Ca^{2+}$-ATPase

The above results require a sarcolemmal transporter in which an increase of $[Ca^{2+}]_i$ upon excitation activates an entry of protons into the cell. As mentioned already this could result from a sarcolemmal $Ca^{2+}$-ATPase which produces $Ca^{2+}$ efflux/proton influx in response to an increase of $[Ca^{2+}]_i$. In this case inhibition of the ATPase should attenuate the intracellular acidification.

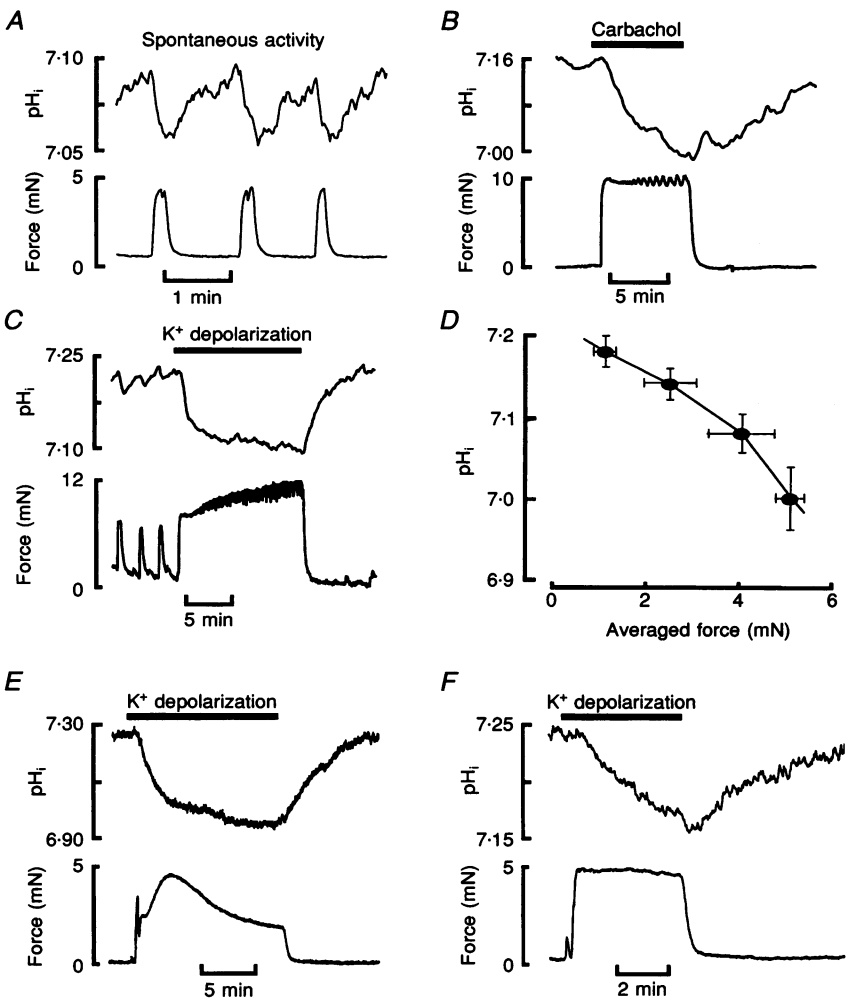


**Figure 1. Changes of $pH_i$ associated with contraction**

*A–D*, rat myometrium. In panels *A–C*, the top trace shows $pH_i$ and the lower contraction. Contraction was either spontaneous (*A*) or activated by carbachol (50 μM; *B*) or KCl (40 mM; *C*). *D*, the quantitative relationship between the average force developed (abscissa) and the intracellular acidification (ordinate). The points were obtained by elevating external KCl from 5 mM (left-hand point) to 10, 20 and 30 mM. The force records were integrated over 10 min for each of the $K^+$ concentrations and the mean value at each point from 4 preparations. *E*, rat ureter. KCl was increased from 5 to 80 mM for the period shown. *F*, portal vein. KCl was increased from 5 to 40 mM.

There are several blockers of the $Ca^{2+}$-ATPase but they may also block voltage-gated $Ca^{2+}$ channels (e.g. $La^{3+}$) or interfere with the fluorescence signals used to measure $pH_i$ (e.g. eosin; Gatto, Hale, Xu & Milanick, 1995). We used $Cd^{2+}$, which is a reasonably specific blocker of the $Ca^{2+}$-ATPase at concentrations (20 nM; Verbost, Flik, Lock & Wenderlaar-Bonga, 1988) much lower than those which affect the L-type $Ca^{2+}$ channel. Figure 3*A* shows that the application of $Cd^{2+}$ partly reversed the intracellular acidification produced by depolarization. Furthermore, if following recovery from a high-$K^+$ contraction, high $K^+$ was reapplied, but now in the presence of $Cd^{2+}$, a significantly smaller acidification (0·05 ± 0·01 pH units, not shown) occurred compared with control acidifications (0·12 ± 0·02 pH units, $n = 3$). Cadmium also significantly reduced the acidification seen in ureteric smooth muscle (not shown).

**The effects of elevating external $Ca^{2+}$ and pH**

Elevating external [$Ca^{2+}$] will increase the gradient against which $Ca^{2+}$ extrusion occurs and it would be anticipated that this would decrease the rate of $Ca^{2+}$ efflux and proton entry. Figure 3*B* shows that increasing external [$Ca^{2+}$] from 3 to 20 mM significantly reduced the acidification produced by a high-$K^+$ contraction (typical of 4 preparations).

The above data have shown that the effects of $Ca^{2+}$ on $pH_i$ are consistent with those expected from $Ca^{2+}$–$H^+$ exchange. To test further for the presence of such an exchange, we altered pH and looked for changes in $[Ca^{2+}]_i$. The tissue was first depolarized and then external pH altered. The results (Fig. 3*C*) show that elevation of external pH from 7·4 to 8·4 increased the level of both $[Ca^{2+}]_i$ and contraction (typical of 5 preparations).

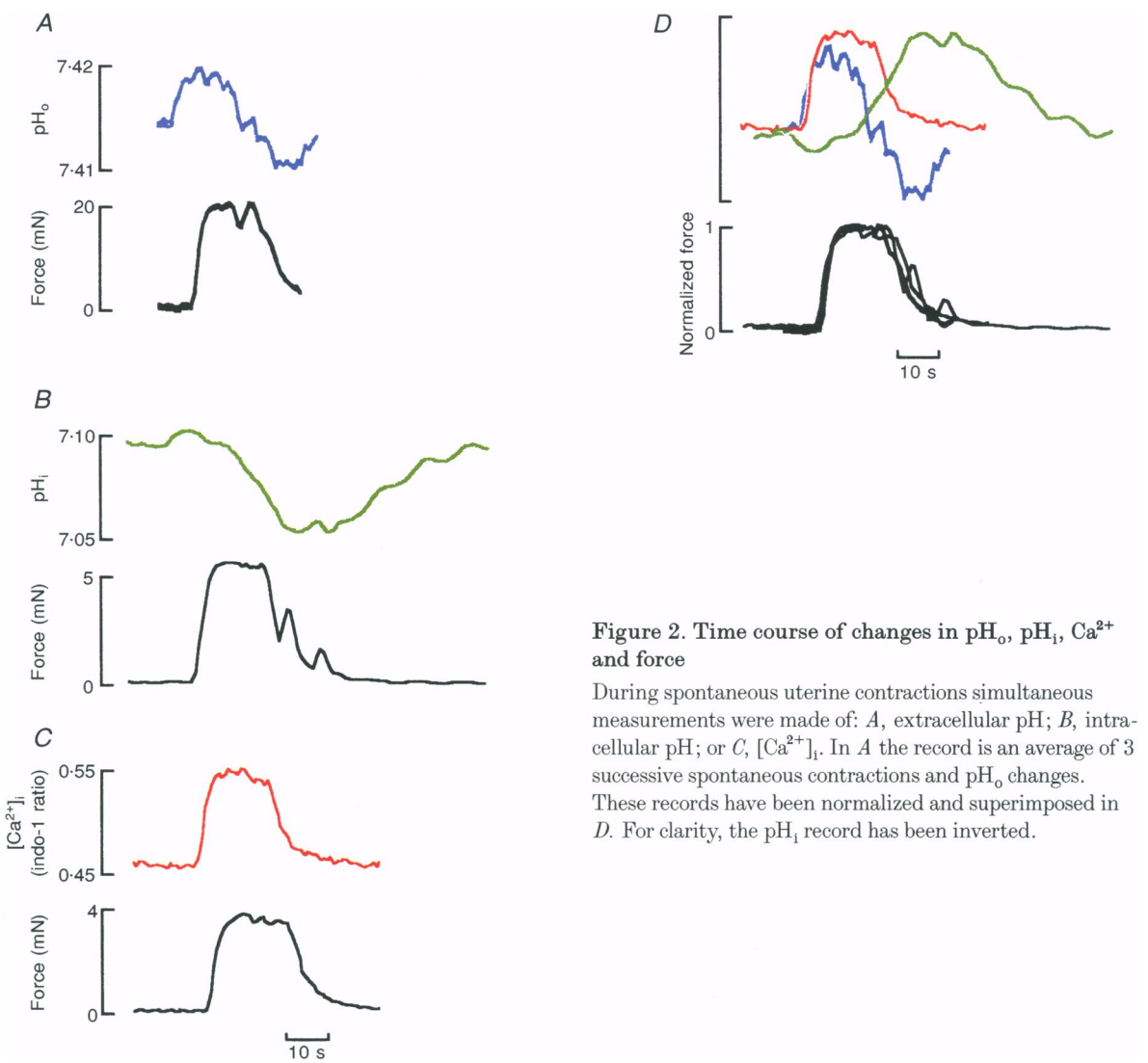


Figure 2. Time course of changes in $pH_o$, $pH_i$, $Ca^{2+}$ and force

During spontaneous uterine contractions simultaneous measurements were made of: *A*, extracellular pH; *B*, intracellular pH; or *C*, $[Ca^{2+}]_i$. In *A* the record is an average of 3 successive spontaneous contractions and $pH_o$ changes. These records have been normalized and superimposed in *D*. For clarity, the $pH_i$ record has been inverted.

## DISCUSSION

The data shown in this paper suggest that the sarcolemmal $Ca^{2+}$-ATPase produces an intracellular acidification and extracellular alkalinization during uterine contraction. Furthermore, this occurred irrespective of how force was produced in the uterus, as acidification was found during spontaneous, high-$K^+$-depolarized and agonist-stimulated contractions. The amount of acidification was also positively related to the amount of contractile activity the uterus was producing. The finding of intracellular acid transients with contraction in other smooth muscles (here portal vein and ureter) also demonstrates that this is probably a general feature of contraction in smooth muscle. The physiological implications of this and its relevance to other cellular processes will be discussed below.

The finding of acidification with contraction in the uterus is in agreement with our previous report (Taggart & Wray, 1993). In that study the role of anaerobic glycolysis was examined as it was anticipated that the ATP consumed during contraction is partly resynthesized by anaerobic glycolysis resulting in lactic acid production. The lack of any significant effect of contraction on lactate efflux (Taggart & Wray, 1993) reinforced in the present paper by a *decrease* of lactate efflux during contraction means that lactic acid production is unlikely to account for the acid transients. Other possible intracellular sources of protons are those released by net ATP hydrolysis during contraction or those displaced by an increase of $[Ca^{2+}]_i$ from sites either on soluble buffers or intracellular organelles. These explanations are difficult to reconcile with the observations that during a maintained contraction, the acidification is maintained (Fig. 1*B*, *C*, *E* and *F*). Given the presence of pH-regulating mechanisms, displacement of protons from a finite source or proton production by hydrolysis of a finite amount of ATP, would produce only a transient acidification.

Extracellular alkalinizations were detected using the membrane-impermeant form of SNARF. The pH changes

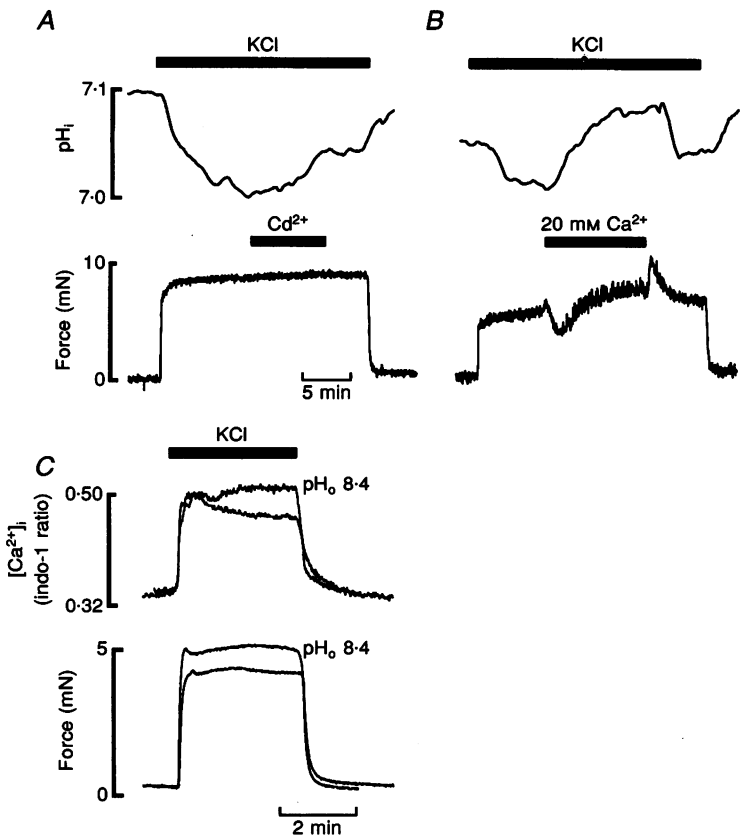


**Figure 3. Factors affecting transmembrane movements of $Ca^{2+}$ and protons in the non-pregnant rat uterus**

In both *A* and *B* the top trace shows $pH_i$ and the lower one contraction. *A*, the effects of $Cd^{2+}$. Contraction was activated by increasing KCl from 5 to 30 mM and $Cd^{2+}$ (20 nM) applied for the period indicated. *B*, the effects of elevated extracellular $Ca^{2+}$ concentration. KCl was elevated to 30 mM and $CaCl_2$ to 20 mM as shown. *C*, the effects of external pH on $[Ca^{2+}]_i$ (top) and contraction (bottom). The measurements were made on the same preparation and are shown superimposed. In both KCl was increased to 40 mM either at pH 7·4 or 8·4.

were small, hence the need to average the signals from three to five contractions in each preparation. Previous work in other tissues has used extracellular concentration changes to estimate transmembrane proton fluxes (Thomas, 1988). The extracellular pH signal measured here will be contributed to by both the extracellular space pH and a constant signal from the bulk solution surrounding the tissue. This solution signal means that our values will be an underestimate of the $pH_o$ change and we cannot therefore interpret the magnitude of these changes in a more quantitative manner. Despite this, the occurrence of the extracellular alkalinization provides direct evidence for proton movement across the sarcolemma, into the cell.

A role for the sarcolemmal $Ca^{2+}$-ATPase in the pH transients was suggested from the extracellular alkalinization. A translocation of $H^+$ by this pump has been demonstrated in a variety of preparations (Niggli *et al.* 1982; Schwiening *et al.* 1993). We demonstrated its involvement in the acidification associated with contraction by altering or blocking its activity. Cadmium, the blocker used in this study, produced significant reductions in the acidification produced by contraction. There was no additional increase in force when the acidification was reduced by $Cd^{3+}$ (Fig. 3*A*). This is consistent with work on permeabilized uterine muscle which shows that, at maximally activating $Ca^{2+}$, alkalinization does not increase force (Crichton, Taggart, Wray & Smith, 1993). Elevating external pH caused an increase in $[Ca^{2+}]_i$ and contraction (Fig. 3*C*). This potentiating effect of external pH is *not* secondary to changes of $pH_i$. If $pH_i$ alone is alkalinized then, under these depolarized conditions, there is no *increase* of either contraction or $[Ca^{2+}]_i$ (Taggart, Burdyga, Heaton & Wray, 1996). It might be argued that the effect of external alkalinization to increase $[Ca^{2+}]_i$ is due to an effect on the L-type $Ca^{2+}$ current rather than on the $Ca^{2+}$-ATPase. In this context it should be noted that previous work has shown that changes of external pH have no immediate effect on the L-type current, rather the effects develop slowly with the time course expected from the resulting changes of $pH_i$ (Shmigol, Smith, Taggart, Wray & Eisner, 1995). Elevation of external $[Ca^{2+}]$ also reduced the acidification. Force was transiently decreased, in agreement with previous findings (Kawarabayashi, Kishikawa & Sugimori, 1989). The mechanism of this effect is unclear,

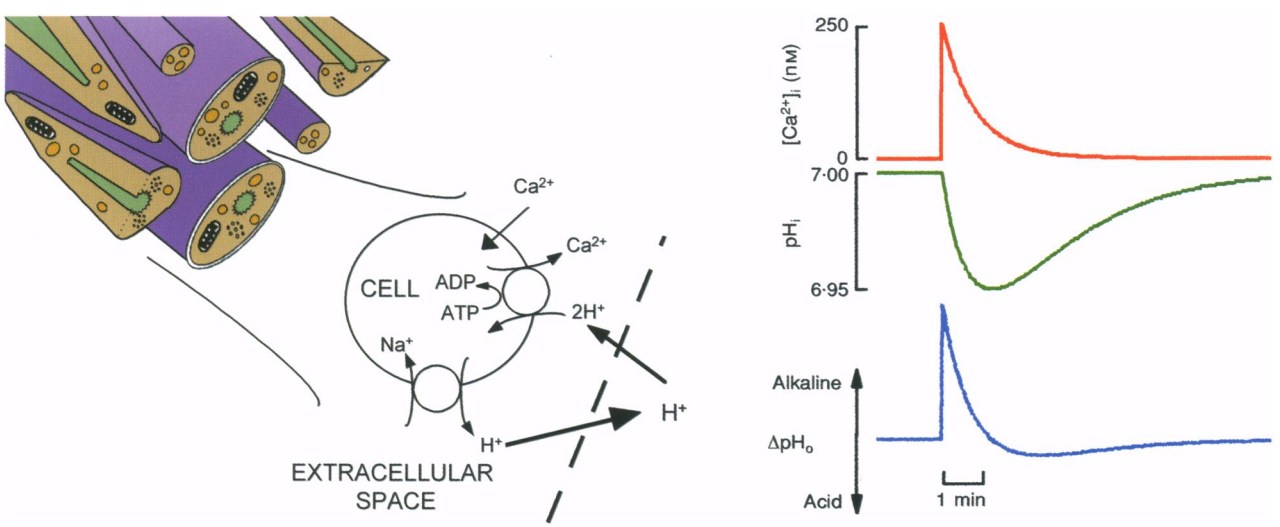


**Figure 4. A model to relate $Ca^{2+}$ and pH**

The left-hand side of the figure shows smooth muscle cells and the extracellular space schematically (based on Salmons, 1995) and the ionic movements and compartments used to generate the model shown on the right-hand side. The model shows the effects of an increase of $[Ca^{2+}]_i$ (top) on intracellular (middle) and extracellular (bottom) pH. In the model, extracellular pH reflects the balance between movements across the cell membrane and diffusion to the bulk solution. A $Ca^{2+}$-ATPase (exchanging $Ca^{2+}$ for $H^+$ ions) and a $Na^+$–$H^+$ exchange produced the proton movements across the cell membrane. $[Ca^{2+}]_i$ is controlled by the $Ca^{2+}$-ATPase and an entry of $Ca^{2+}$ that is stimulated to produce the transient. Note the fast extracellular alkalinization (associated with the rapid entry of protons on the cell) on the $Ca^{2+}$-ATPase and the smaller acid undershoot as the $Na^+$–$H^+$ exchange pumps protons out of the cell. The $Na^+$–$H^+$ exchange is assumed to produce an efflux proportional to $[H^+]$, and the $Ca^{2+}$–$H^+$ exchange flux to be proportional to $[Ca^{2+}]_i$. We have ignored effects of extracellular pH on these fluxes and of $pH_i$ on $Ca^{2+}$–$H^+$ exchange. The model assumes a proton buffering power of 10 mmol $l^{-1}$ and that 0·1 % of $Ca^{2+}$ is ionized. The $Ca^{2+}$ transient for a spontaneous contraction was simulated by allowing $Ca^{2+}$ entry into the cell to elevate $[Ca^{2+}]_i$ by 250 nM. The values of the various parameters were adjusted to give the time course shown.

but suggests that calcium influx may be transiently reduced. Force then was seen to increase. This could be due to increased influx or decreased calcium extrusion on the $Ca^{2+}$-ATPase. The latter mechanism could of course explain the decreased acidification seen. Thus data using several different experimental approaches are consistent with what would be expected from $Ca^{2+}$–$H^+$ exchange on the ATPase.

Although no previous studies have directly assessed the functional relationship between proton movements produced by the $Ca^{2+}$-ATPase and cell function, there is ample evidence in cultured vascular smooth muscle cells (Daugirdas, Arrieta, Ye, Flores & Batlle, 1995), red blood cells (Niggli *et al.* 1982) and snail neurones (Schwiening *et al.* 1993) that the $Ca^{2+}$-ATPase produces significant proton fluxes. In the snail neurone the $Ca^{2+}$-ATPase was shown to produce external alkalinization. Thus in many cells activation of cellular processes by $Ca^{2+}$ will stimulate the $Ca^{2+}$-ATPase and produce transient changes of intra- and extracellular pH which, in turn, will have both energetic and functional consequences.

The data of Fig. 2 show that contraction is associated with an extracellular alkalinization that develops somewhat before and decays over the same time scale as does contraction. In contrast the intracellular acidification peaks after contraction and decays more slowly (Fig. 2*D*). The model illustrated in Fig. 4 accounts for the different time courses of intra- and extracellular pH changes and relates them to the changes of $[Ca^{2+}]_i$. Briefly, the model contains a $Ca^{2+}$ influx that is stimulated on depolarization. $Ca^{2+}$ removal is produced by a $Ca^{2+}$-ATPase that electroneutrally exchanges $Ca^{2+}$ for protons. An active proton extruder (such as a $Na^+$–$H^+$ exchange) then removes protons. We further assume that the difference between extracellular pH and bulk superfusate pH is proportional to the flux of protons into the tissue. This then predicts that the time course of the extracellular alkalinization will be the same as that of the proton entry into the cell which (ignoring the $Na^+$–$H^+$ exchange for the moment) will be equal to the activity of the $Ca^{2+}$-ATPase. This, in turn, will have the same time course as that of $[Ca^{2+}]_i$. In contrast $pH_i$ will continue to become more acidic until the proton extrusion on $Na^+$–$H^+$ exchange is greater than the entry on the $Ca^{2+}$-ATPase.

Given that this model can qualitatively account for the different time courses of the external and internal pH changes, it is worthwhile considering whether it can *quantitatively* account for the magnitude of the acidification. Spontaneous contractions are accompanied by the same magnitude of $Ca^{2+}$ current as that which we record on depolarizing from −60 to +10 mV (Shmigol *et al.* 1995). This produces a $Ca^{2+}$ current of 100 pA that decays with a time constant of 50 ms giving an integral of 5 pC. Taking a cell volume of 3 pl and assuming a stoichiometry of $2H^+$: $1Ca^{2+}$, and an intracellular proton buffering capacity of 10 mmol $l^{-1}$ this will produce an intracellular acidification of 0·002 pH units per action potential (Eiesland, Baro, Raimbach, Eisner & Wray, 1991). Given that the train of action potentials occurs over a time scale that is much faster than that of pH regulation, the acidifications produced by successive action potentials will sum, and the observed acidification accompanying a spontaneous contraction (0·04 pH units) can be accounted for by the entry expected from twenty action potentials, a number similar to that recorded previously (Kawarabayashi, 1994). Thus it appears that this mechanism can quantitatively account for the magnitude of the acid transients occurring during spontaneous contractions.

These results have considerable physiological significance. As shown by the model in Fig. 4, the $Ca^{2+}$-ATPase produces an intracellular acidification that develops after the rise of $[Ca^{2+}]_i$. It therefore occurs at a time when relaxation has been initiated by the decrease of $[Ca^{2+}]_i$ (also produced by the $Ca^{2+}$-ATPase) and therefore further promotes relaxation. Our previous work has suggested that intracellular acidification suppresses spontaneous activity by decreasing the L-type $Ca^{2+}$ current (Shmigol *et al.* 1995). The $Ca^{2+}$-ATPase mechanism ensures that this suppressant effect occurs when $[Ca^{2+}]_i$ is decreasing. Therefore pH-induced inhibition of electrical activity is synchronized with $[Ca^{2+}]_i$-dependent mechanical relaxation. If the acidification occurred earlier it would interfere with contraction. Furthermore since the magnitude of the intracellular acidification is related to that of the contraction (Fig. 1*D*), the larger the contraction the greater the acidification, and thence its relaxing effect. In the uterus specifically, by simultaneously eliciting an acidification, a 'brake' is put on the magnitude and duration of the contraction and relaxation is promoted, preventing hypoxic damage to both uterine tissue and the fetus. It is interesting to note that $Cd^{2+}$ is associated with an increased risk of pre-term labour in both women and animals (Fagher, Laudanski, Schutz, Sipowicz & Akerlund, 1993). Although other explanations cannot be excluded, this may result from an increase in $[Ca^{2+}]_i$ and reduced intracellular acidification produced by inhibition of the $Ca^{2+}$-ATPase.

The significance of this dual regulation by $Ca^{2+}$ may well go beyond smooth muscle. It has also been noted in the past that an acidification accompanies a rise in $[Ca^{2+}]_i$ in cardiac muscle (Vaughan-Jones, Lederer & Eisner, 1983). A likely explanation of this acidification is that it is caused by $Ca^{2+}$–$H^+$ exchange on the cardiac $Ca^{2+}$-ATPase. However, other changes evoked by activation and contraction may also contribute to the net change in $pH_i$ seen in some tissues, for example agonist activation of the $Na^+$–$H^+$ exchanger (Aalkjaer & Mulvany, 1991). Interactions between intracellular protons and $Ca^{2+}$ occur in many other cellular processes in addition to contraction, e.g. metabolism, fertilization and neuronal activity (Moody, 1984). Given the ubiquitous distribution of the $Ca^{2+}$-ATPase in eukaryotic cells the dual, concerted regulation of these ions may be of general importance.

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**Author's email address**

S. Wray: s.wray@liverpool.ac.uk

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