

pH-induced changes in calcium: functional consequences and mechanisms of action in guinea pig portal vein

R. D. SMITH,¹ D. A. EISNER,² AND SUSAN WRAY¹

¹Department of Physiology, The University of Liverpool, Liverpool L69 3BX; and ²Unit of Cardiac Physiology, Department of Medicine, University of Manchester, Manchester M13 9PT, United Kingdom

Received 14 December 2001; accepted in final form 16 July 2002

Smith, R. D., D. A. Eisner, and Susan Wray. pH-induced changes in calcium: functional consequences and mechanisms of action in guinea pig portal vein. *Am J Physiol Heart Circ Physiol* 283: H2518–H2526, 2002; 10.1152/ajpheart.01102.2001.—The effects of changing extracellular (pH_o) and intracellular pH (pH_i) on force and the mechanisms involved in the guinea pig portal vein were investigated to better understand the control of tone in this vessel. When pH_o was altered, the effects on force and calcium were the same irrespective of whether force had been produced spontaneously by high-K depolarization or by norepinephrine; alkalization increased tone, and acidification reduced it. Because pH_o changes also lead to changes in pH_i , we determined whether the effects on force could be explained by these induced pH_i changes. It was found, however, that only with spontaneous activity did intracellular alkalization increase force. In depolarized preparations, force was decreased, and, with norepinephrine, force was initially decreased and then increased. Thus the effects of pH_o cannot be explained solely by changes in pH_i . The role of the sarcoplasmic reticulum (SR) and surface membrane Ca^{2+} -ATPase on the mechanism were investigated and shown not to be involved. Therefore, it is concluded that both pH_o and pH_i can have powerful modulatory effects on portal vein tone, that these effects are not identical, and that they are likely to be due to effects of pH on ion channels rather than the SR or plasma membrane Ca^{2+} -ATPase.

force; contraction; potassium

THE PORTAL VEIN is a spontaneously active vessel that transports blood from the splanchnic circulation to the liver. Alteration in blood flow through this vessel has been associated with changes in contractility and thus the development of pathological conditions such as portal hypertension (19). Further understanding of the mechanisms that determine the activity of this vessel is necessary for the development of new treatments for conditions such as portal hypertension, which at present mostly relies upon surgical intervention.

Changes in both intracellular (pH_i) and extracellular pH (pH_o) can modulate force production in many smooth muscle types, and generally acidification inhibits and alkalization increases force (1, 26). The ef-

fects of changing pH_o on force in the guinea pig portal vein are not known, despite the fact that such changes may well be important to the functioning of this portal vessel. The portal vein has been shown to be affected by pH; in rats, intracellular acidification transiently increased and then reduced electrically evoked contraction, and alkalization transiently decreased contraction and then increased it (29). However, in the human ureter (10) and guinea pig ureter (9), intracellular acidification increased force. It is not clear what mechanisms are responsible for these effects of pH_i on force.

Previous work has suggested that 1) the sarcoplasmic reticulum (SR) may contribute to the force alteration (11, 29, 32), but these data report contradictory findings on the effects of pH on SR Ca^{2+} release; 2) changes of pH may alter Ca^{2+} influx through calcium channels, and we have shown (27) in single cells from the guinea pig ureter that intracellular acidification decreased the fast transient outward K^+ current; and 3) surface membrane Ca^{2+} -ATPase transports H^+ into the cell as Ca^{2+} is removed; thus changes in either pH_i or pH_o could alter the driving force on this pump and therefore modulate the intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) and contractility (21).

Thus there are a number of mechanisms by which pH-induced effects on tone could be mediated, and an understanding of the relationship among pH, Ca^{2+} , and contraction is essential to determine the mechanism by which these effects are achieved. Few studies have simultaneously recorded force, $[\text{Ca}^{2+}]_i$, and pH in intact tissue (5, 22), although simultaneous pH and Ca^{2+} measurements have been made on cells (20, 33). Therefore, the aim of the present study was to determine how these three parameters interact in the guinea pig portal vein and to make some measurements of all three simultaneously. Specifically, we determined 1) How does pH_o influence force? 2) How does pH_o affect pH_i ? 3) What are the effects of pH on spontaneous activity and under depolarized and agonist-stimulated conditions? and 4) What is the contribution of the SR and plasmalemmal Ca^{2+} -ATPase to the effects of pH on force?

Address for reprint requests and other correspondence: R. D. Smith, The Physiological Laboratory, The Univ. of Liverpool, Liverpool L69 3BX, UK (E-mail: dsmith@liverpool.ac.uk).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Abstracts have been published on some of these data (2, 28).

METHODS

Male guinea pigs (300–400 g) were killed by cervical dislocation, and their portal veins were removed. The tissue was loaded overnight in physiological salt solution (PSS) containing the acetoxymethylester forms of carboxysemaphthorhodafluor 1 (carboxy-SNARF; 5 μ M) and indo 1 (5 μ M) to enable simultaneous measurement of pH_i and $[\text{Ca}^{2+}]_i$, respectively, to be made in some experiments. Indo 1 and carboxy-SNARF were dissolved in DMSO containing 25% pluronic acid. Loading of the indicators had no effect on the spontaneous activity of the tissue, which could be maintained for several hours. Small longitudinal strips \sim 2–3 mm in length and 1 mm in width with their endothelium removed (to rule out any effects of endothelium-derived factors) were dissected; one end was secured to the stage of an inverted epifluorescence microscope (Nikon), and the other was attached to a force transducer (Swema). The strips were continuously illuminated at 530 and 360 nm (excitation wavelengths for carboxy-SNARF and indo 1, respectively) using a series of mirrors obtained from Cairn Electronics (see Fig. 1A), and their emissions were recorded at 580 and 640 nm for carboxy-SNARF and 400 and 500 nm for indo 1, respectively. pH_i was obtained from the ratio of 640 to 580 nm and calibrated *in vitro* using the free acid form of carboxy-SNARF and a mock intracellular solution (30). Changes in $[\text{Ca}^{2+}]_i$ are represented by the ratio of 400- to 500-nm fluorescence emissions from indo 1. To ensure that our results were not influenced by contraction-associated movement artifacts, only tissues where the raw wavelength emissions (either 400/500 nm or 580/640 nm for indo 1 and carboxy-SNARF, respectively) were moving in opposite directions were analyzed. The simultaneous measurement of all three parameters proved difficult, and in some experiments data from one indicator were discarded due to poor loading or excessive movement artifacts. However, on some of these occasions, the force and data from the other indicator were utilized.

pH_i was changed by isosmotic substitution of 40 mM NH_4Cl for NaCl in the perfusate (flow rate of 2–3 ml/min in a 200- μ l chamber), and pH_o was changed by the addition of a strong acid or base, to pH 6.9 or 7.9, respectively. In some experiments (20%) where pH_i was measured, particularly strong contractions lead to a superimposition on the pH_i trace despite the individual wavelengths moving in opposite directions in response to pH alteration (see Fig. 3A). Thus, as a control, external Ca^{2+} was reduced to prevent movement artifacts and confirm that the change in the pH signal was due to changes in pH_i , which was indeed the case ($n = 3$). In experiments where the tone of the tissue was raised, 40 or 100 mM KCl was isosmotically substituted for NaCl or 2 μ M norepinephrine was applied. It is possible that the substitution of sodium for NH_4Cl may affect force production. However, when sodium was substituted for *N*-methyl-glucamine in the presence of 100 mM potassium, there was no effect on tone (results not shown); this was also demonstrated in the rat portal vein by Taggart and Wray (30). Furthermore, when the concentration of NH_4Cl was reduced to 20 mM, the same phenomenon was observed (results not shown).

When cyclopiazonic acid (CPA) was used to inhibit the actions of SR Ca^{2+} -ATPase, the tissue was preincubated with 20 μ M CPA for 20 min and then exposed to 100 mM KCl, and 5 min later 40 mM NH_4Cl was added.

To determine whether Ca^{2+} -ATPase on the plasmalemma was involved in the pH-induced effects on tone, we used

carboxy-eosin diacetate (Molecular Probes) to inhibit its action (7). The tissue was loaded for 15 min at room temperature with 20 μ M carboxy-eosin and then perfused with control PSS for 20 min to allow deesterification. In some experiments, the tissue was loaded with carboxy-eosin overnight at 5°C.

The composition of PSS was (in mM/l) 154 NaCl, 5.4 KCl, 1.2 MgSO_4 , 11.7 glucose, 11 HEPES, and 3.0 CaCl_2 . All chemicals were from Sigma except for carboxy-eosin, which was custom synthesized by Molecular Probes.

All experiments were performed at 35°C. Values represent means \pm SE; n is the number of animals. Significance was tested by means of paired or unpaired Student's *t*-tests. *P* values < 0.05 were considered significant.

RESULTS

Dual indicator methodology. Simultaneous measurement of $[\text{Ca}^{2+}]_i$ and pH as used in some experiments involves loading the tissue with indo 1 and carboxy-SNARF, respectively. Because there are few previous studies, we initially investigated possible limitations to this methodology; namely, effects on force and discrimination of the emission signals. Loading of these dyes had no effect on spontaneous activity or force production, as judged by recordings before and after loading (results not shown). The portal veins had a resting pH of 6.96 ± 0.04 ($n = 20$), which is normal for vascular smooth muscles (31), and spontaneously contracted at a frequency of 1–10 contractions/min for many hours, indicating that they were healthy and undamaged. There was also no cross-talk between the two indicators. Figure 1B clearly shows that, in nominal 0 extracellular Ca^{2+} , a large change in pH_i had no effect on either the 400-to-500-nm ratio or force. This also suggests that the pH range that we are working in does not affect the K_d of indo 1, in agreement with previous studies (6, 18). Similarly, changes in indo 1 emissions did not affect the SNARF wavelengths (results not shown). Thus, having established that our methodology was appropriate, we proceeded to investigate the effects of pH_o on force. While successful recordings of force, $[\text{Ca}^{2+}]_i$, and pH_i were obtained in the majority of data presented here, it was the case, however, that acceptable simultaneous recordings of all three parameters could not be obtained in all tissues (see METHODS). When this was the case, data from either force and $[\text{Ca}^{2+}]_i$ or force and pH_i only were included.

Effects of pH_o on spontaneous activity. External pH alkalization (from 7.4 to 7.9) produced a gradual and significant increase in the amplitude of the spontaneous contractions (control: 1.39 ± 0.32 mN; alkalization: 1.81 ± 0.37 mN, $P < 0.05$, $n = 5$) and increased their frequency (control: 1.23 ± 0.10 contractions/min; pH 7.9: 1.42 ± 0.15 contractions/min, $n = 6$; Fig. 2A). Normal activity quickly followed upon return to pH 7.4. Acidification to pH 6.9 produced a significant decrease in the amplitude of the spontaneous contractions (control: 1.39 ± 0.32 mN; acidification: 0.9 ± 0.32 mN, $P < 0.05$, $n = 5$) and a small but significant increase in frequency (control: 1.23 ± 0.10 contractions/min; pH 6.9: 1.69 ± 0.20 contractions/min, $P < 0.05$, $n = 6$). These effects reversed upon recovery to resting pH.

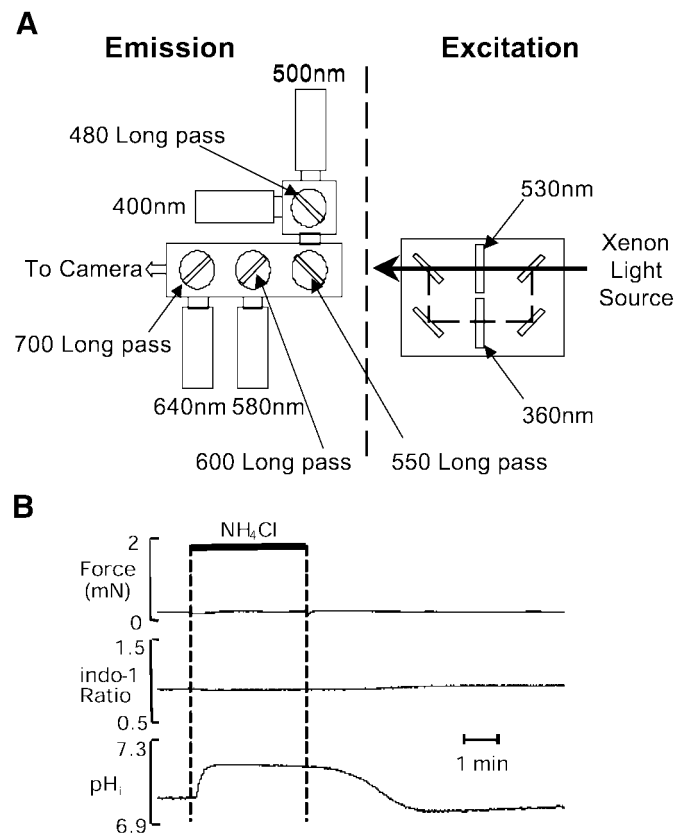


Fig. 1. *A*: apparatus for simultaneous measurement of intracellular pH (pH_i) and intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$). The tissue was continuously excited at 360 and 530 nm. The emissions were collected at 400 and 500 nm for indo 1 and 580 and 640 nm for carboxysemaphthorhodafluor 1, respectively. *B*: effects of 40 mM NH_4Cl on the indo 1 ratio: simultaneous measurement of force, the indo 1 ratio, and pH_i . The tissue was bathed in nominal Ca^{2+} -free solution to eliminate any changes in Ca^{2+} due to pH perturbation.

The changes in pH were accompanied by changes in the amplitude of the intracellular Ca^{2+} signals (Fig. 2A). Alkalinization produced an increase in the basal level of Ca^{2+} and the peak amplitude ($194 \pm 18\%$ of the control, $P < 0.05$), whereas acidification reduced both the basal level of Ca^{2+} and the amplitude of the Ca^{2+} transient ($39.4 \pm 7.9\%$ of the control, $P < 0.05$; see Fig. 2A).

Changes in pH_o were slowly transduced into pH_i changes: 0.13 ± 0.03 and 0.10 ± 0.03 pH units acidification and alkalinization, respectively ($n = 4$; see Fig. 2A). These pH_i changes are thus ~ 20 – 25% of the pH_o changes. The time to half-peak for acidification ($t_{0.5}$) was 88.7 ± 4 and 41.8 ± 7 s for alkalinization, which is in good agreement with the time course of the pH_o -induced effects on force (see Fig. 2, A and B), suggesting that transduction of pH_o changes into pH_i changes were responsible for the effects on tone.

These data show that pH_o significantly affects portal vein spontaneous contractility, pH_i , and $[\text{Ca}^{2+}]_i$. To investigate the mechanisms underlying this, we next determined whether the effects of pH_o were the same irrespective of how force was produced.

Effects of pH_o on agonist-induced tone. Application of the physiological agonist norepinephrine ($2 \mu\text{M}$) produced a marked increase in tone ($2.71 \pm 0.45 \text{ mN}$, $n = 5$) and converted the normal phasic pattern of activity to a maintained, tonic level of force (see Figs. 2B and 3B). $[\text{Ca}^{2+}]_i$ was also elevated by norepinephrine, and Ca^{2+} transients were changed to a tonic level of $[\text{Ca}^{2+}]_i$. A small acidification was also seen in some but not all preparations (3 of 7 preparations) after norepinephrine application. The effects of alteration of pH_o on force, along with $[\text{Ca}^{2+}]_i$ and pH_i , can be seen in Fig.

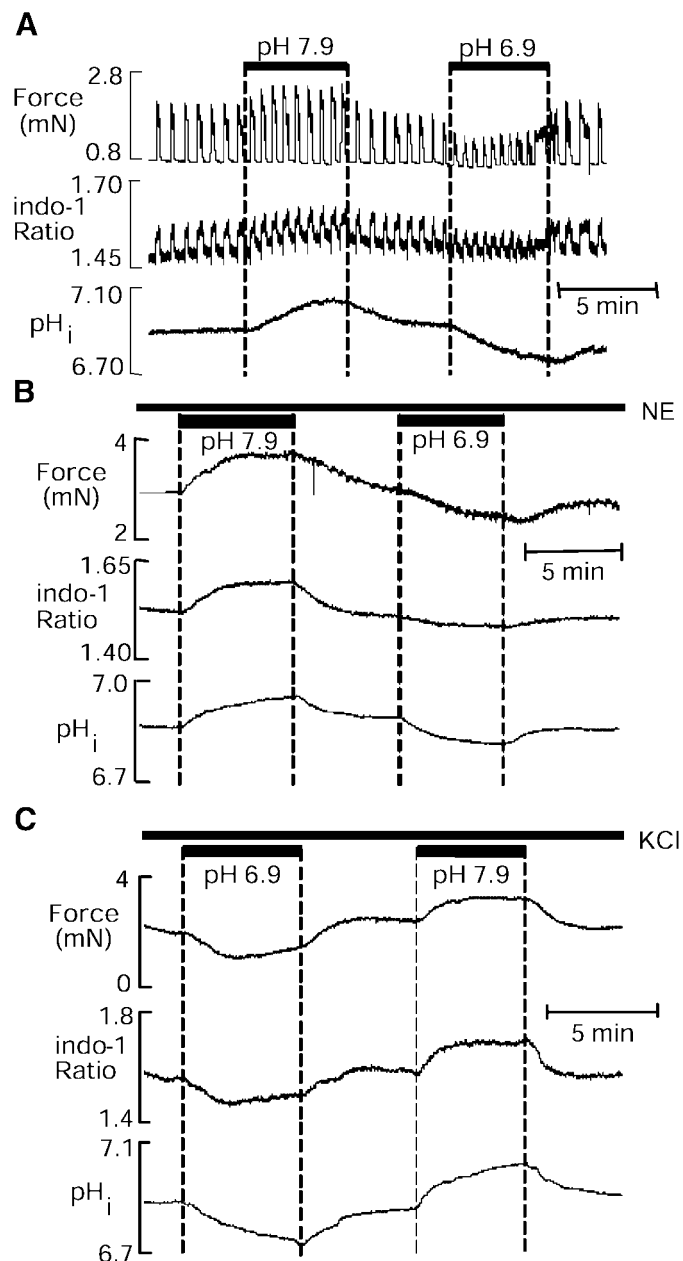


Fig. 2. Effects of changing extracellular pH (pH_o) on a spontaneously active strip from the guinea pig portal vein. *A*: effects on force, the indo 1 ratio, and pH_i . *B*: effect of pH_o on force, the indo 1 ratio, and pH_i on a $2 \mu\text{M}$ norepinephrine (NE)-precontracted strip. *C*: effects of changing pH_o on force, the indo 1 ratio, and pH_i on a 100 mM KCl-precontracted strip.

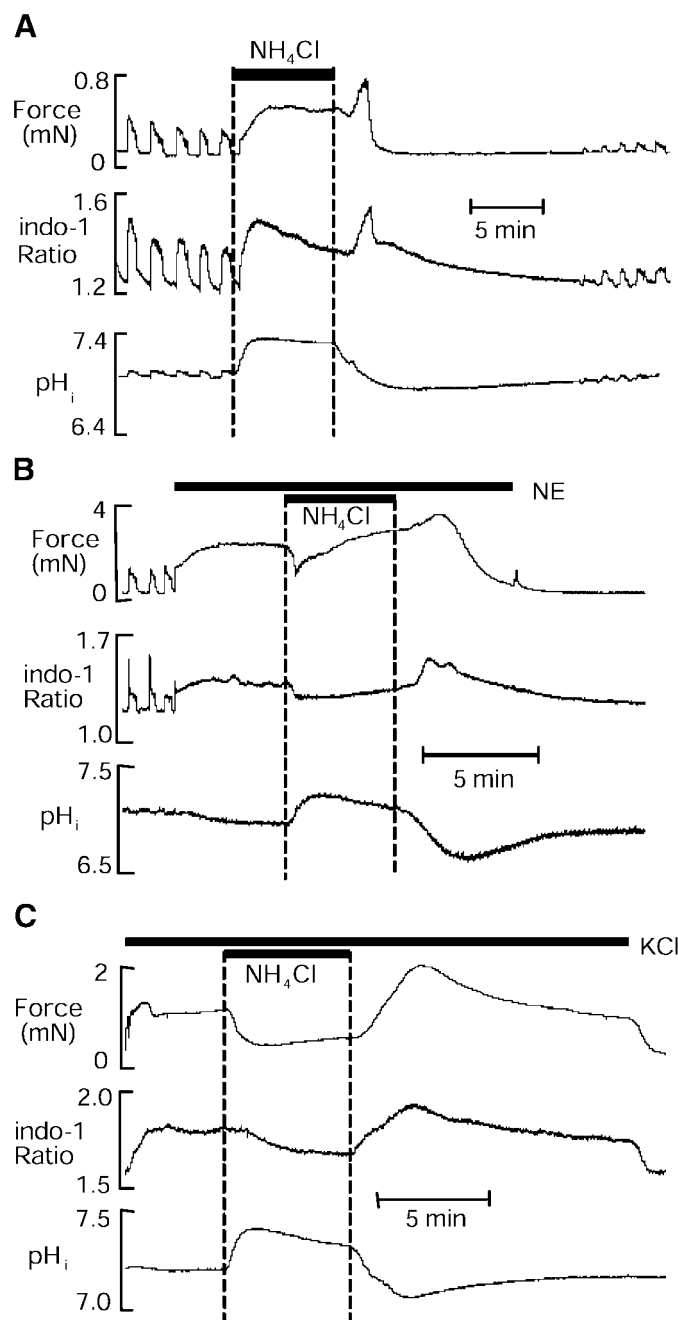


Fig. 3. Simultaneous measurement of force, the indo 1 ratio, and pH_i from a single strip of portal vein. A: effects of 40 mM NH_4Cl on spontaneous contractions. B: effects of 40 mM NH_4Cl on a $2 \mu M$ NE-precontracted strip. C: effects of 40 mM NH_4Cl on a 40 mM KCl-precontracted strip.

2B. Extracellular alkalization produced a significant increase in force, above the level of the norepinephrine-induced tone (0.43 ± 0.02 mN, $P < 0.05$, $n = 4$), and acidification produced a significant decrease (0.68 ± 0.06 mN, $P < 0.05$, $n = 4$). Restoration to control pH_o , i.e., 7.4, restored the tonic contraction to its original level (Fig. 2B). It can also be seen from Fig. 2 that the extracellular changes in pH were gradually transduced into intracellular changes ($t_{0.5}$: 82 ± 21 and 93 ± 4 s for alkalization and acidification, respectively, $n = 4$);

pH_o 6.9 produced an intracellular acidification of 0.13 ± 0.02 pH units, and pH_o 7.9 produced an intracellular alkalization of 0.16 ± 0.02 pH units ($n = 5$). These changes were not significantly different from the values obtained from the spontaneously active tissue and occurred over a similar time course.

The simultaneously recorded $[Ca^{2+}]_i$, pH_i , and force record in Fig. 2B shows that pH_o produced large changes in each parameter. The $t_{0.5}$ of pH_i , $[Ca^{2+}]_i$, and force changes were all closely associated, suggesting that the effects of pH_o on force and $[Ca^{2+}]_i$ were due to the pH_o -induced pH_i changes.

Effects of pH_o on KCl-induced tone. Depolarization with 40 or 100 mM K^+ solution produced a large increase in force in the portal vein preparations (2.05 ± 0.42 and 2.68 ± 0.55 mN, $n = 4$ and 5, respectively), similar to that seen with norepinephrine, which was maintained to varying degrees ($n = 12$; see Figs. 2C and 3C). These changes in force were accompanied by similar changes in $[Ca^{2+}]_i$ ($n = 8$). There was also a small intracellular acidification produced by 100 mM KCl (resting pH_i : 7.07 ± 1.11 ; 100 mM KCl: 7.01 ± 0.09 pH units, $n = 5$). Again, extracellular acidification was associated with a clear decrease in force (0.43 ± 0.15 mN, $P < 0.05$, $n = 4$; Fig. 2C), and extracellular alkalization produced a significant elevation of force (0.20 ± 0.03 mN, $P < 0.05$, $n = 4$). These effects were readily reversed upon return to pH 7.4 (Fig. 2C). As before, alteration of pH_o caused changes in $[Ca^{2+}]_i$ that paralleled those of force. Changes in pH_i occurred as a consequence of the alteration in pH_o (Fig. 2C). These induced changes were of similar magnitudes to those found above (0.18 ± 0.02 and 0.17 ± 0.03 pH units, $n = 4$, acid and alkaline, respectively) but were slightly quicker in onset ($t_{0.5}$: 49 ± 6 and 60 ± 10 s for alkalization and acidification, respectively, $n = 4$).

Summary of pH_o effects. These data have shown that irrespective of how force is produced, extracellular alkalization increases it, and extracellular acidification decreases it. In all cases, the pH_o change produced changes in pH_i and $[Ca^{2+}]_i$. The changes in force mirrored those in $[Ca^{2+}]_i$ and pH_i . The time course of the effects on force was much slower than when pH_i was changed (see below) but was closely associated with the pH_o -induced changes in pH_i , suggesting that the effects on force were due to transduction of the pH_o changes into pH_i changes. Because pH_i is known to be able to alter force without a change in pH_o , the question arises as to whether the alterations in force seen by altering pH_o are produced by the induced pH_i change? This was investigated next by selectively altering pH_i , using weak acids and bases, at a constant external pH of 7.4.

Effects of pH_i on spontaneous activity. The addition of 40 mM NH_4Cl produced a rapid intracellular alkalization of 0.32 ± 0.09 pH units ($n = 5$, $t_{0.5}$: 15 ± 4 s; Fig. 3A). The tissue started to regulate pH and return toward baseline, as expected in healthy tissues. These pH_i changes produced a marked effect on contractile activity transforming the phasic spontaneous activity into a large maintained increase in tone (1.88 ± 0.58

mN, $n = 6$, $P < 0.05$), an effect that was slightly preceded by a rise in $[Ca^{2+}]_i$ (see Fig. 3A). Removal of NH_4Cl produced a rebound acidification (0.22 ± 0.06 pH units, $n = 5$, $t_{0.5}$: 26 ± 5 s) and a transient increase in both $[Ca^{2+}]_i$ and force (2.64 ± 0.73 mN, $n = 6$) followed by a decrease. The initial fall in force and calcium occurred over a similar time course [10% decrease ($t_{0.1}$): 1.75 and 1.2 s, respectively]. However, after this initial phase, the decrease in force was faster ($t_{0.5}$: 21.7 s) than the fall in $[Ca^{2+}]_i$ ($t_{0.5}$: 91.9 s) but not the fall in pH_i ($t_{0.5}$: 21.3 s). Phasic activity was abolished while pH_i was acidic (Fig. 3A) and returned ~ 5 min after pH_i returned to control levels.

Effects of pH_i on agonist-induced tone. As previously described, norepinephrine increased tone. The addition of NH_4Cl produced a rapid intracellular alkalinization (0.26 ± 0.03 pH units, $n = 4$, $t_{0.5}$: 23 ± 2 s; Fig. 3B). This was accompanied by an initial, rapid decrease in tone (1.41 ± 0.39 mN, $n = 6$), followed by a transient recovery to above the norepinephrine-induced level (norepinephrine-induced level: 2.38 ± 0.47 mN; transient recovery: 2.78 ± 0.54 mN, $n = 6$, $P < 0.05$). Upon removal of NH_4Cl , there was an acid rebound (0.22 ± 0.03 pH units, $n = 4$, $t_{0.5}$: 49 ± 5 s; Fig. 3B) and a further increase in tone (0.24 ± 0.11 mN, $n = 6$, $P < 0.05$), which, after its peak, rapidly returned to the resting level of tone. pH_i also returned toward the control level (see Fig. 3B). The $[Ca^{2+}]_i$ changes closely resembled the force record. However, after the acid rebound, the fall in force was faster than that of $[Ca^{2+}]_i$ (Fig. 3B), as was the case with the spontaneously active tissue.

Effects of pH_i on KCl-induced tone. There was a large increase in force and $[Ca^{2+}]_i$ with 40 or 100 mM KCl (see Fig. 3C). Upon the addition of NH_4Cl , there was a rapid intracellular alkalinization of 0.22 ± 0.02 pH units ($n = 4$, $t_{0.5}$: 18 ± 2 s), similar to that seen in spontaneously active and norepinephrine-stimulated tissues. Unlike the effects on spontaneous activity, however, this alkalinization produced only a decrease in both tonic force (1.52 ± 0.24 , $n = 6$, $t_{0.5}$ for the initial decrease in force: 21.4 ± 2.5 s, $n = 5$) and $[Ca^{2+}]_i$ (Fig. 3C). When NH_4Cl was removed, there was a rebound acidification of 0.17 ± 0.03 pH units ($n = 4$, $t_{0.5}$: 41 ± 12 s) compared with the resting pH. This produced an increase in both force and $[Ca^{2+}]_i$, to values above the previous level of the KCl-induced tone (1.19 ± 0.27 mN above the induced tone, $n = 6$). The increased force and $[Ca^{2+}]_i$ subsided as pH_i returned toward control levels. The time course of the force and $[Ca^{2+}]_i$ records were closely associated, with $[Ca^{2+}]_i$ slightly preceding force, suggesting that the changes in force were due to modulation of $[Ca^{2+}]_i$.

Summary of pH_i effects. In contrast to the consistent effects of pH_o on portal vein force, the effects of pH_i were found to depend on the method used to produce force. Alkalinization only increased force arising spontaneously or with norepinephrine. Acidification, in response to NH_4Cl removal, increased force produced by all these routes. Force and $[Ca^{2+}]_i$ were largely correlated, irrespective of pH_i changes. However, in some

experiments, the fall in force during acid rebound was faster than the fall in calcium (see DISCUSSION). Figure 4 shows the effects of both pH_o and pH_i changes on force presented graphically. Because different mechanisms can lead to the changes in $[Ca^{2+}]_i$ seen under different conditions, we next investigated whether the effects of pH could be due to a mechanism involving the SR or surface membrane Ca^{2+} -ATPase, which countertransports H^+ .

Effects of CPA on pH-induced changes on tone. Modulation of Ca^{2+} uptake and release from the SR is a mechanism whereby force production can be altered. We used CPA to inhibit the uptake of Ca^{2+} into the SR via Ca^{2+} -ATPase and thereby empty the SR. The addition of CPA significantly increased the frequency of contractions and produced a rise in the tone as contraction became almost tonic, presumably due to the rise in $[Ca^{2+}]_i$ occurring under these conditions (24). However, CPA had no effect on pH_i -induced ($n = 4$; Fig. 5A) or pH_o -induced ($n = 4$; Fig. 5B) changes in force or pH_i in the presence of high-K or norepinephrine ($n = 4$; results not shown).

Effects of carboxy-eosin on pH-induced changes on tone. To determine the possible role of plasmalemmal Ca^{2+} -ATPase in the pH-induced effects on tone, carboxy-eosin was used to inhibit the actions of this pump (7, 23). Carboxy-eosin produced a small rise in the basal tone of the tissue and increased the frequency of spontaneous contractions. However, carboxy-eosin had no effect on the changes in tone produced by modifying either pH_i ($n = 4$; Fig. 5C) or pH_o ($n = 5$; Fig. 5D) in the presence of high K. This was also the case when the tissue had been incubated overnight with carboxy-eosin at $5^\circ C$.

DISCUSSION

The present study used the fluorescent dyes carboxy-SNARF and indo 1 to investigate the effects of pH alteration on $[Ca^{2+}]_i$ and force in the guinea pig portal vein. The results have shown that changes in both pH_i and pH_o can modulate force in this vessel. pH_o changes produced consistent effects on force and $[Ca^{2+}]_i$ irrespective of the mechanism used to stimulate the portal vein; alkalinization increased both, and acidification decreased them. With direct alteration of pH_i , these effects were also seen with spontaneous activity and norepinephrine stimulation (acidification, after an initial increase, produced a decrease in force), whereas the opposite effects were obtained in depolarized preparations, i.e., alkalinization decreased force and $[Ca^{2+}]_i$, whereas acidification increased force and $[Ca^{2+}]_i$. The mechanism appears to be mainly via modulation of $[Ca^{2+}]_i$ rather than a direct effect on the sensitivity of the contractile machinery to Ca^{2+} . The inability of carboxy-eosin and CPA to inhibit the effects of pH on force suggests that plasmalemmal and SR Ca^{2+} -ATPases are not involved in the observed pH-induced changes in force and may be due to alterations of surface membrane ion channel activity.

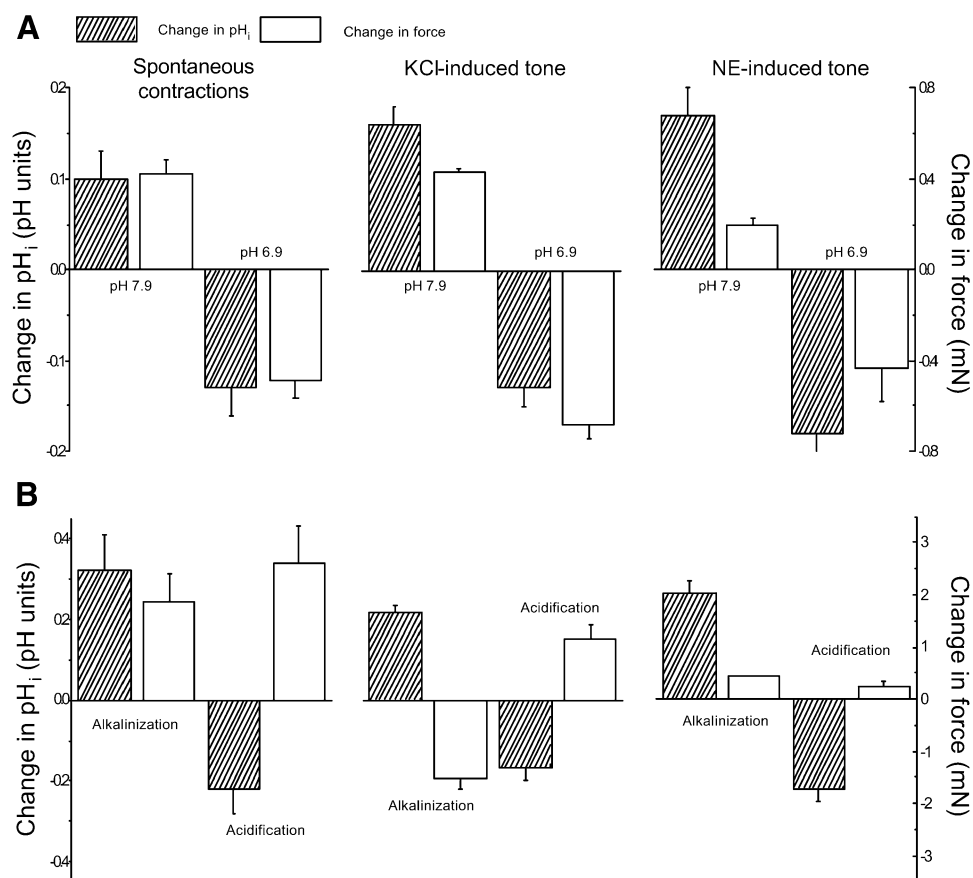


Fig. 4. Effects of pH_o changes (A) and NH_4Cl (B) on force and pH_i of spontaneously active strips and strips where the tone had been raised by NE or KCl.

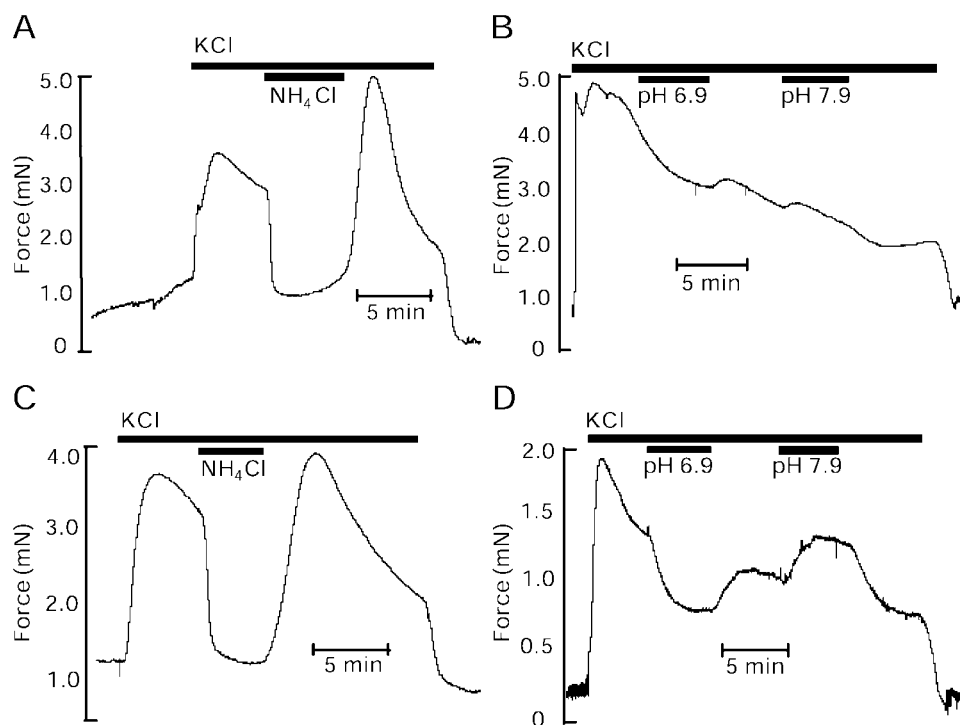


Fig. 5. Effects of cyclopiazonic acid (A and B) and carboxy-eosin (C and D) on pH_i and pH_o perturbation of 100 mM KCl-precontracted strips.

To the best of our knowledge, this is the first study to simultaneously measure force, $[Ca^{2+}]_i$, and pH_i in the portal vein. Previous studies in rat mesenteric vessels have shown the usefulness and validity of this approach (5), but few studies in any other tissue have been reported (3, 8, 20). The fluorescent spectra of the two indicators and the filters used were such that they did not interfere with each other.

pH and ion channels. A number of studies in smooth muscle have demonstrated that changes in either pH_i or pH_o can modulate Ca^{2+} influx through voltage-operated Ca^{2+} channels; acidification generally decreasing and alkalization increasing it (16, 25, 27). Iino et al. (15) showed that this was also the case in the rabbit portal vein. The effects of changing pH on the outward potassium current are less well known. Preliminary data presented by Ashcroft et al. (2) suggested that pH_i did not affect the potassium current from cells isolated from the guinea pig portal vein. However, extracellular acidification decreased, whereas extracellular alkalization increased, this current.

Effects of pH_o on spontaneous contractions. In the present study, extracellular alkalization produced a gradual increase in amplitude of the spontaneous contractions. This increase in force was accompanied by a gradual rise in both $[Ca^{2+}]_i$ and pH_i . Extracellular acidification had the opposite effect, decreasing the amplitude of the contractions and the Ca^{2+} transients. There did not appear to be any immediate effects on either $[Ca^{2+}]_i$ or force that could be attributed to the change in pH_o . Our results show that the time course of the pH_o -induced effects on force and $[Ca^{2+}]_i$ are, however, similar to the pH_o -induced changes in pH_i , suggesting that they were due to transduction into intracellular changes. Similar conclusions were drawn by Austin and Wray (4) using the mesenteric artery. The pH_o -induced effects on force are also consistent with an effect of pH_i on L-type voltage-operated Ca^{2+} channels, where acidification decreases Ca^{2+} influx and alkalization produces an increase (15).

Effects of pH_o on KCl and norepinephrine-induced tone. When pH_o was changed in the presence of high K or norepinephrine, acidification decreased force and alkalization produced an increase. As with spontaneous activity, these results could be explained by an effect on Ca^{2+} influx through voltage-operated Ca^{2+} channels. Clearly these effects must occur despite the depolarization produced by either high K or norepinephrine and are probably due to modulation of the open probability of the Ca^{2+} channels (16). pH_o changes were again transduced into intracellular changes and occurred over a similar time course to those reported by Austin and Wray (4) in the mesenteric artery. The relatively slow time course of the effects on force compared with perturbation of pH_i with NH_4Cl , and the similarity of the time course to that of the pH_i changes, suggests that transduction of the pH_o changes into pH_i changes is responsible for the observed effects on force.

Effects of pH_i on spontaneous contractions. Intracellular alkalization, produced by application of NH_4Cl , converted the spontaneous contractions into a sustained increase in $[Ca^{2+}]_i$ and force. Upon removal of NH_4Cl , the acid rebound was accompanied by a further transient increase in $[Ca^{2+}]_i$ and force, possibly due to displacement of Ca^{2+} from intracellular buffer sites (34), which was then followed by a period of quiescence. During spontaneous activity, the effects of pH on $[Ca^{2+}]_i$ and force are consistent with pH-induced effects on L-type voltage-operated Ca^{2+} channels (i.e., alkalization increases and acidification decreases Ca^{2+} influx). Interestingly, during rebound acidification, after the initial period, the fall in force consistently preceded the fall in $[Ca^{2+}]_i$. One possibility is that the acid rebound affects a phosphatase or kinase (and therefore the sensitivity of the contractile machinery to calcium) further down the excitation-contraction coupling pathway or releases a pool of Ca^{2+} that is unavailable to the contractile machinery. Further experiments are required to clarify these findings.

It is possible that the effects of pH_i on spontaneous activity are due to modulation of pacemaker cells; alkalization increasing the rate of firing and acidification decreasing it. With changes in pH_o , which were accompanied by pH_i changes, there was also an increase in frequency of contraction (although this did not reach statistical significance).

Effects of pH_i on KCl-induced tone. We have previously shown that intracellular alkalization increases and acidification decreases potassium currents in the guinea pig ureter (27). Alkalization increases the potassium current in the portal vein (2), which would lead to hyperpolarization and thus a decrease in Ca^{2+} influx through voltage-operated Ca^{2+} channels. Therefore, we repeated these experiments using 100 mM KCl to depolarize the tissue, thereby ruling out the involvement of potassium channels.

NH_4Cl -induced alkalization produced a rapid decrease in the KCl-induced force, and this was accompanied, in a similar time frame, by a fall in $[Ca^{2+}]_i$, suggesting that the effects of pH on force were due to changes in $[Ca^{2+}]_i$ rather than the sensitivity of the contractile machinery to Ca^{2+} . Acidification had the opposite effect, increasing force. These results are opposite to those seen when pH_o was changed and with spontaneously active and norepinephrine-stimulated preparations. Clearly, these effects of pH on the KCl-precontracted portal vein cannot be explained by an effect on either voltage-operated Ca^{2+} channels (intracellular alkalization decreased KCl-induced force, but intracellular alkalization increases calcium influx through voltage-operated Ca^{2+} channels) or potassium channels. However, mitochondria or other types of ion channels that are present in this tissue (14) may have a role in these effects.

Effects of pH_i on norepinephrine-induced tone. When norepinephrine was used to raise the tone of the tissue, changes in pH_i produced similar effects to those on spontaneously active tissues apart from a transient

initial decrease in force. This difference is probably due to the mechanism by which high K and norepinephrine increase force. High K depolarizes the tissue and therefore increases force by Ca^{2+} entering the cell through voltage-operated Ca^{2+} channels, whereas norepinephrine raises tone by modulating many types of ion channels as well as release of Ca^{2+} from intracellular stores and affecting the sensitivity of the myofilaments to Ca^{2+} . It would appear from our data, and comparing the responses seen in spontaneously active tissues, that the effect on enhancing Ca^{2+} entry is the predominant one.

Acidification produced by KCl or norepinephrine. When the tone of the tissue was increased with KCl or norepinephrine, there was a small intracellular acidification (see Fig. 3B). This acidification has been seen in a number of smooth muscles and is possibly caused by hydrolysis of ATP or proton entry (4). Alternatively, a study by Naderali et al. (21) has shown that during contraction, there is an intracellular acidification that is accompanied by an extracellular alkalization. The authors proposed that this extracellular alkalization/intracellular acidification was due to plasmalemmal Ca^{2+} -ATPase removing Ca^{2+} in exchange for protons.

From the results, pH may modulate spontaneous activity of the tissue via an effect on L-type voltage-operated Ca^{2+} channels. However, when the tone of the tissue is raised by KCl or norepinephrine other, mechanisms must also exist. The possible role of plasmalemmal and SR Ca^{2+} -ATPase will now be discussed.

Role of SR Ca^{2+} -ATPase in the pH-induced changes in force. Force can be modulated by an increase or decrease in Ca^{2+} uptake or release by the SR. CPA inhibits SR Ca^{2+} -ATPase and empties the Ca^{2+} store (7). The addition of CPA produced an increase in the frequency of spontaneous contractions followed by a rise in the baseline tone. This increase in force may be due to the release of Ca^{2+} from the SR into the cytoplasm or entry of Ca^{2+} from outside the cell stimulated by depletion of the store, a phenomenon known as capacitative Ca^{2+} entry (13). When the tone of the tissue was raised using K^+ , CPA had no effect on pH_i - or pH_o -induced changes in force, suggesting that the SR Ca^{2+} store is not responsible for the pH-induced changes in force. This was also the case when pH_i was changed during norepinephrine-induced contraction. These findings suggest that even when 1,4,5-inositol trisphosphate-induced store release is activated by norepinephrine, the pH-induced effects on force were independent of release of Ca^{2+} from the store.

Role of plasmalemmal Ca^{2+} -ATPase in pH-induced changes in force. Inhibition of plasmalemmal Ca^{2+} -ATPase by carboxy-eosin (7, 12, 24) produced an increase in the basal tone of the tissue and frequency of contractions, presumably due to the inhibition of removal of Ca^{2+} from the cell by the plasmalemmal Ca^{2+} -ATPase. However, it had no effect on the pH_i - or pH_o -induced changes in force during KCl contraction. The increase in basal tone and frequency of spontaneous activity produced by carboxy-eosin suggests that plasmalemmal Ca^{2+} -ATPase may play a role in deter-

mining spontaneous activity of the tissue, but the lack of effect on the pH-induced changes in force suggests that it plays no role in these effects.

We conclude that the effects of changing pH_o on spontaneously contracting and precontracted tissues are probably mediated via modulation of L-type voltage-operated Ca^{2+} channels, with alkalization increasing influx/force and acidification decreasing both. When pH_i was modulated, the effects on spontaneous contractions could also be explained by an effect on Ca^{2+} channels; however, when the tone of the tissue was raised with either KCl or norepinephrine, the results were different. In this case, alkalization decreased force (maintained and transiently, respectively), whereas acidification produced an increase. The lack of effect of CPA and carboxy-eosin suggests that these effects were not mediated via SR or plasmalemmal Ca^{2+} -ATPase, and the experiments where the tissue was precontracted with KCl suggest that potassium channels are not involved. The similarity between the changes in force and $[\text{Ca}^{2+}]_i$ suggest that the majority of the effects are due to a direct effect on $[\text{Ca}^{2+}]_i$ and are not due to modulation of the sensitivity of the contractile machinery to Ca^{2+} . The determination of the role of mitochondria and different ion channels may lead to the further understanding of the mechanism of action by which pH_i modulates $[\text{Ca}^{2+}]_i$ during KCl or norepinephrine contracture. These new findings may be important in conditions such as portal hypertension, when the tissue is exposed to changes in its surrounding pH levels.

This work was supported by the British Heart Foundation.

REFERENCES

1. Aalkjaer C and Peng HL. pH and smooth muscle. *Acta Physiol Scand* 161: 557–566, 1997.
2. Ashcroft FJ, Smith RD, and Wray S. The effects of changing pH on spontaneous activity, calcium, pH and K^+ currents in guinea-pig isolated portal vein (Abstract). *J Physiol* 526: 41P, 2000.
3. Atsumi T, Sugita K, Kohno M, Takahashi T, and Ueha T. Simultaneous measurement of Ca^{2+} and pH by laser cytometry using fluo-3 and SNARF-1. *Cytometry* 24: 99–105, 1996.
4. Austin C and Wray S. Extracellular pH signals affect rat vascular tone by rapid transduction into intracellular pH changes. *J Physiol* 466: 1–8, 1993.
5. Austin C, Dilly K, Eisner D, and Wray S. Simultaneous measurement of intracellular pH, calcium, and tension in rat mesenteric vessels: effects of extracellular pH. *Biochem Biophys Res Commun* 222: 537–540, 1996.
6. Austin C and Wray S. The effects of extracellular pH and calcium change on force and intracellular calcium in rat vascular smooth muscle. *J Physiol* 488: 281–291, 1995.
7. Bassani RA, Bassani JWM, and Bers DM. Relaxation in ferret ventricular myocytes: role of the sarcolemmal Ca ATPase. *Pflügers Arch* 430: 573–578, 1995.
8. Behrendt JH, Milde H, Weber WM, Kowalik S, Zahner H, Burger HJ, and Clauss W. Intracellular calcium and pH conditions of cultured cells infected with *Eimeria bovis* or *E. separata*. *Parasitol Res* 86: 294–300, 2000.
9. Burdya TV, Taggart MJ, and Wray S. An investigation into the mechanism whereby pH affects tension in guinea-pig ureteric smooth muscle. *J Physiol* 493: 865–876, 1996.
10. Cole RS, Fry CH, and Shuttleworth KE. Effects of acid-base changes on human ureteric smooth muscle contractility. *Br J Urol* 66: 257–264, 1990.

11. **Crichton CA, Templeton AGB, and Smith GL.** Bathing pH effects Ca^{2+} -activated force in isolated a-toxin permeabilized rat portal vein and human umbilical artery to differing extents. *J Physiol* 459: 489P, 1993.
12. **Gatto C, Hale CC, Xu W, and Milanick MA.** Eosin, a potent inhibitor of the plasma membrane Ca pump, does not inhibit the cardiac Na-Ca exchanger. *Biochemistry* 34: 965–972, 1995.
13. **Gibson A, McFadzean I, Wallace P, and Wayman CP.** Capacitative Ca^{2+} entry and the regulation of smooth muscle tone. *Trends Pharmacol Sci* 19: 266–269, 1998.
14. **Hogg RC, Wang Q, and Large WA.** Effects of Cl channel blockers on Ca-activated chloride and potassium currents in smooth muscle cells from rabbit portal vein. *Br J Pharmacol* 111: 1333–1341, 1994.
15. **Iino S, Hayashi H, Saito H, Tokuno H, and Tomita T.** Effects of intracellular pH on calcium currents and intracellular calcium ions in the smooth muscle of rabbit portal vein. *Exp Physiol* 79: 669–680, 1994.
16. **Klockner U and Isenberg G.** Calcium channel current of vascular smooth muscle cells: extracellular protons modulate gating and single channel conductance. *J Gen Physiol* 103: 665–678, 1994.
17. **Klockner U and Isenberg G.** Intracellular pH modulates the availability of vascular L-type Ca^{2+} channels. *J Gen Physiol* 103: 647–663, 1994.
18. **Lattanzio FA and Bartschat DK.** The effect of pH on rate constants, ion selectivity and thermodynamic properties of fluorescent calcium and magnesium indicators. *Biochem Biophys Res Commun* 177: 184–191, 1991.
19. **MacMathuna PM.** Mechanisms and consequences of portal hypertension. *Drugs* 44: 1–13, 1992.
20. **Martinez-Zaguilan R, Martinez GM, Lattanzio F, and Gillies RJ.** Simultaneous Measurement of Intracellular pH and Ca^{2+} using the fluorescence of SNARF-1 and fura 2. *Am J Physiol Cell Physiol* 260: C297–C307, 1991.
21. **Naderali EK, Buttell N, Taggart MJ, Bullock AJ, Eisner DA, and Wray S.** The role of the sarcolemmal Ca^{2+} -ATPase in the pH transients associated with contraction in rat smooth muscle. *J Physiol* 505: 329–336, 1997.
22. **Otter D and Austin C.** Simultaneous monitoring of vascular contractility, intracellular pH and intracellular calcium in isolated rat mesenteric arteries; effects of weak bases. *Exp Physiol* 85: 349–351, 2000.
23. **Shmigol A, Eisner DA, and Wray S.** Carboxyeosin decreases the rate of decay of the $[\text{Ca}^{2+}]_i$ transient in uterine smooth muscle cells isolated from pregnant rats. *Pflügers Arch* 437: 158–160, 1998.
24. **Shmigol AV, Eisner DA, and Wray S.** Properties of voltage-activated $[\text{Ca}^{2+}]_i$ transients in single smooth muscle cells isolated from pregnant rat uterus. *J Physiol* 511: 803–811, 1998.
25. **Shmigol AV, Smith RD, Taggart MJ, Wray S, and Eisner DA.** Changes of pH affect calcium currents but not outward potassium currents in rat myometrial cells. *Pflügers Arch* 431: 135–137, 1995.
26. **Smith GL, Austin C, Crichton C, and Wray S.** A review of the actions and control of intracellular pH in vascular smooth muscle. *Cardiovasc Res* 38: 316–331, 1998.
27. **Smith RD, Eisner DA, and Wray S.** The effects of changing intracellular pH on calcium and potassium currents in smooth muscle cells from the guinea-pig ureter. *Pflügers Arch* 435: 518–522, 1998.
28. **Smith RD, Eisner DA, and Wray S.** The effects of pH on force in guinea-pig portal vein: simultaneous measurements of pH, calcium and force (Abstract). *J Physiol* 517: 63P, 1999.
29. **Taggart M, Austin C, and Wray S.** A comparison of the effects of intracellular and extracellular pH on contraction in isolated rat portal vein. *J Physiol* 475: 285–292, 1994.
30. **Taggart M and Wray S.** Simultaneous measurement of intracellular pH and contraction in uterine smooth muscle. *Pflügers Arch* 423: 527–529, 1993.
31. **Taggart MJ, Austin C, and Wray S.** Contribution of extracellular calcium to intracellular pH-induced changes in spontaneous force of rat portal vein. *Exp Physiol* 80: 69–78, 1995.
32. **Tsukioka M, Iino M, and Endo M.** pH dependence of inositol 1,4,5-trisphosphate-induced Ca^{2+} release in permeabilized smooth muscle cells of the guinea-pig. *J Physiol* 475: 369–375, 1994.
33. **Weigmann TB, Welling LW, Beatty DM, Howard DE, Vamos S, and Morris SJ.** Simultaneous imaging of intracellular $[\text{Ca}^{2+}]$ and pH in single MDCK and glomerular epithelial cells. *Am J Physiol Cell Physiol* 265: C1184–C1190, 1993.
34. **Wray S.** Smooth muscle intracellular pH: measurement, regulation, and function. *Am J Physiol Cell Physiol* 254: C213–C225, 1988.