Review

A review of the actions and control of intracellular pH in vascular smooth muscle

G.L. Smith a,*, C. Austin b, C. Crichton a, S. Wray c

a Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK
b Department of Medicine, Manchester Royal Infirmary, Oxford Road, Manchester, M13 9WL, UK
c Physiological Laboratory, University of Liverpool, Liverpool, L69 3BX, UK

Received 8 April 1997; accepted 26 November 1997

Abstract

Objective: This review is an account of the physiological issues involved in the effects of pH on vascular smooth muscle tone. The following criteria were considered when reviewing the literature: (i) the type of smooth muscle, i.e. either tonic or phasic, (ii) the source of the smooth muscle i.e. pulmonary, systemic, large artery, resistance artery, vein or cell line, (iii) the effects of changing intracellular or extracellular pH alone, (iv) the acute or chronic effects of altered pH (v) the influence of extracellular pH on intracellular pH and (vi) the influence of altered intracellular pH on basal or agonist induced tone. Studies of the effects of pH on the individual intracellular components of vascular tone, specifically sarcoplasmic reticulum and contractile proteins function are considered. Finally, the pH sensitivity of molecular components that contribute to smooth muscle cell tone are reviewed. Conclusions: There appear to be distinct differences in the response of large arteries and resistance arteries to altered intracellular pH which may be based on the different properties of the smooth muscle within the wall of each blood vessel. Similarly, systemic and pulmonary vessels may respond differently, but no systematic study exists to allow a more definitive conclusion. Factors controlling intracellular pH such as intracellular buffering power and sarcolemmal pH regulating mechanisms may differ across the vascular bed and may contribute to some of the differences observed in response to altered extracellular pH. Finally, few studies have examined the pH sensitivity the intracellular processes involved in basal tone and pharmaco-mechanical coupling in vascular smooth muscle. More information concerning these latter aspects of smooth muscle function is required to progress the understanding of the modulator action of pH on vascular tone. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: pH; Arteries; Veins; Smooth muscle; Intracellular/extracellular ions

1. Introduction

It has been known for many years that changes in the tone of vascular smooth muscle plays an important role in the distribution of the circulation and the control of blood pressure. Gaskell [44] was probably the first to show that pH is a major determinant of tone when he demonstrated that decreases in the pH of the blood caused vasodilatation. Yet, over a century later, the effects of pH on vascular smooth muscle are poorly understood. The changes in pH can be divided into those occurring within the extracellular space (pH_e) and those occurring within the intracellular space (pH_i), although, extracellular and intracellular compartments will influence each other (see sections 5 and 6). pH_e is normally maintained within narrow limits around 7.4, but a range of disorders can lead to significant changes (e.g. pulmonary oedema, kidney damage, occlusive vascular disease). The pH_i in vascular smooth muscle is normally close to 7.1, but may vary due to: (i) changes of extracellular pH, (ii) changes in the partial pressure of CO_2 (PCO_2), (iii) hypoxic or ischaemic conditions that increase intracellular lactic acid production, (iv) increased work load (v) action of agonists. This review describes the current literature concerning (a) the influence of intracellular and extracellular pH on the tone produced by

* Corresponding author. Tel.: +44 (141) 330-5963; Fax: +44 (141) 330-4612.

0008-6363/98/$19.00 © 1998 Elsevier Science B.V. All rights reserved.
PII S0008-6363(98)00020-0
vascular smooth muscle, (b) the control of intracellular pH in vascular smooth muscle, (c) the interaction between intracellular pH and intracellular \([Ca^{2+}]\) in smooth muscle, and (d) the direct influence of pH on smooth muscle sarcoplasmic reticulum and contractile proteins. The molecular basis for the regulation of intracellular \([Ca^{2+}]\) and contraction are discussed in the context of possible targets for the influence of pH.

2. Classification of vascular smooth muscle

On the basis of the behaviour of isolated preparations, smooth muscle can be broadly classified into tonic (e.g. circular muscle from large arteries) and phasic (e.g. portal vein, and most non-vascular smooth muscle, see [26]). Both muscle types have an active basal tone which can be modulated by electrical and hormonal influences. Electrically induced contractions involve an increase in intracellular \([Ca^{2+}]\) \(([Ca^{2+}]_i)\) alone, while agonist induced contractions may involve both an increase in \([Ca^{2+}]_i\), or an increase in the responsiveness of the contractile proteins to \(Ca^{2+}\), or both. Agonist induced relaxation results from a fall in \([Ca^{2+}]_i\) and may involve a decrease in responsiveness of the contractile proteins to \(Ca^{2+}\).

Smooth muscle is present throughout the vasculature except for capillaries. We have chosen to discuss separately the effects of pH on arteries and veins, and within the arterial division, we will distinguish between conduit or large arteries (> 1 mm lumen diameter) and resistance arteries (i.e. small arteries and arterioles, 0.3–0.03 mm lumen diameter) [94]. A considerable amount of research on \(pH_1\) has used cell lines derived from smooth muscle, e.g. A10 cell line derived from rat aortic smooth muscle. These cells share phenotypic features in common with smooth muscle, but they also differ in significant ways, e.g. they are non-contractile. Caution should be taken when comparing results from these preparations to those from in vivo or freshly dissected preparations.

3. Methods used to measure pH and tone in vascular smooth muscle

Although we do not intend to review all the technical aspects to the study of pH and vascular tone, it is important that the different methodologies and preparations are understood.

3.1. Tissues

The work we are considering has all been performed on in vitro preparations. As with all in vitro work, its applicability to the in vivo system has to be examined and balanced against the mechanistic information which can probably only be obtained from studies of isolated vessels or cells. It is clear that the effects of pH may be specific to a vessel or preparation and therefore it is important to report whether the study is on arterial or venous vessels, large or resistance arteries, systemic or pulmonary vessels. Not all studies are on intact preparations; as will be discussed later, preparations with the surface membrane disrupted (‘skinned’ or permeabilised) may yield much direct information. Many studies use single vascular smooth muscle cells. Results from freshly dissociated cells should be distinguished from those of cultured cells or cell lines, as these latter cells may no longer express an in vivo phenotype [74]. Few reports on human vascular tissue are cited in this review, but increasingly biopsies of human vascular tissue are being used to study smooth muscle physiology. As will be described in more detail below, the major differences in the response of vascular smooth muscle to altered pH appears to be based on vessel type (large artery, resistance artery, vein) rather than inter-species differences.

3.2. Measurements of \(pH_o\) and \(pH_i\)

For in vitro studies the measurement of \(pH_o\) is generally simple, being that of the perfusate. This assumes that there is adequate diffusion between the bulk solution and the extracellular space. In some studies it may be important to know the \(pH_o\) at the cell surface, in the past this has been obtained by using pH-sensitive microelectrodes. The measurement of \(pH_i\) proved to be problematic and did not appear in publications until the late 1980’s. This was because there was no suitable technique for measuring \(pH_i\) in small, contractile preparations (see [128]). Now two main techniques are available: magnetic resonance and fluorescent indicators. Briefly, magnetic resonance can be used on vascular tissues provided sufficient mass of tissue (~ 500 mg) can be obtained to give adequate signal. Due to the high cost of spectrometers and other factors such as low time resolution, this is not the method of choice for most investigators (see [129] for a fuller description of this method). Much more common is the use of fluorescent indicators. Of the available pH-sensitive fluorophores the choice is usually 2,7’-bis-carboxyethyl-5(6)-carboxyfluorescein (BCECF), a dual excitation single emission indicator or the seminaphthofluorescein dye carboxy SNARF (a single excitation, dual emission indicator). These are usually introduced into the cell by incubating the tissue with the membrane-permeant forms of the indicators which following intracellular enzymatic modification, to become ‘trapped’ inside the cell. Less commonly, indicators are micro-injected into the cell.

3.3. Measurements of contractility

The physiological role of smooth muscle varies in different parts of the vascular tree, altered smooth muscle contractility is responsible for changes in the distensibility
of large arteries. In resistance arteries and veins changes in smooth muscle tone influences lumen diameter and therefore vascular resistance and capacitance respectively [29]. There are several ways of measuring contractility in vitro: some methods are designed to mimic physiological conditions while others are optimised for the measurement of contractile activity. Isometric measurements of tension from strips of longitudinal or circular smooth muscle allow a measure of the activity of the contractile apparatus uninfluenced by changes in the mechanical resistance developed by the parallel elastic component (mainly due to connective tissue) within the preparation [29]. The static nature of these measurements facilitates their use in conjunction with fluorescence pH recording. Tension measurements from vessels as small as 100 μm internal diameter is possible using a wire myograph developed by Mulvaney and Halpern [95]. The advantage of this system is that lumen diameter and wall tension can be adjusted to mimic in vivo conditions. More recently, a pressure myograph system has been developed for use with small arteries, contractility is assessed by monitoring the changes in luminal diameter at a controlled intraluminal pressure [30,50].

3.4. Buffers and alteration of pH

The final methodological parameter to consider is the way pH is changed and buffered. Typically, a change of pH is simply done by addition of strong acid or base to the perfusate. Appropriate buffers of course must be employed with pK values around the desired pH. If pH is altered in CO₂/HCO₃⁻ buffered system, this can involve changes in PCO₂ or HCO₃⁻ or both. Complications with this buffer are the direct intracellular consequences of altered pH: changing [HCO₃⁻] will not immediately influence pH since movement of HCO₃⁻ across the sarcolemma is comparatively slow (due to its charge). In contrast, if PCO₂ is altered, both pH and pH will be altered due to the rapid movement of CO₂ across the surface membrane. pH can be maintained when PCO₂ is changed by altering the [HCO₃⁻] to preserve the ratio [CO₂]/[HCO₃⁻]. To selectively perturb pH (i.e. at constant pH) salts of weak acids (propionate, butyrate, lactate) or bases (ammonium, trimethylamine) are used. As explained by Thomas [121] the small amount of undissociated acid or base, in equilibrium with these salts in solution, will cross the cell membrane and then dissociate to release or absorb protons. Upon the withdrawal of these salts, these reactions and pH changes are reversed. Fig. 1A shows an example of the use of a weak base (trimethylamine) to alter pH in isolated rat mesenteric artery smooth muscle. Addition of 40 mM causes a marked intracellular alkalinisation. In Fig. 1B, addition of 60 mM butyrate (a weak acid) causes a marked acidification. Mention should be made here of studies suggesting that tone changes produced by some weak acids (e.g. lactate) may be independent of the induced pH change. McKinnon et al [87] showed that the relaxation of mesenteric resistance vessels to lactate and butyrate occurred even when the acidification was nulled. They suggested lactate may act by increasing intracellular levels of cAMP although the mechanism remains to be elucidated. All the studies mentioned above have taken the precaution of using osmotically balanced solutions which is particularly important when adding or removing high concentrations (e.g. 50 mM) of a weak acid or base. Changes of osmolarity of the bathing solution have marked effects on the contractility of smooth muscle [61,113].

3.5. Perspective

The effects of pH and its mechanism of action, may be expected to vary between vessel type. The effects of pH may alter with time or separate acute and chronic pathways may be evoked. Agonists-induced contractions may exhibit a different pH sensitivity to electrically evoked effects. The endothelium may well activate a different pathway from that of the smooth muscle and extracellular matrix pathways and there is an extensive literature on the important role played by the endothelium in mediating vascular responsiveness. It is beyond the scope of this review to consider how pH changes may influence endothelial function. In almost all the work reviewed here, the endothelium has either been removed or is absent (e.g.

![Fig. 1. Changes in vascular tone produced by A; alteration of intracellular pH (pHᵢ) and B; extracellular pH (pHᵢₒ). Rat mesenteric vascular strips were loaded with the pH-sensitive indicator carboxy-SNARF and tension and pHᵢ were measured simultaneously. Note that elevation of pHᵢ and pHᵢₒ cause an increase in vascular tone and that elevation of pHᵢₒ causes an increase in pHᵢ. When this induced increase in pHᵢ is removed e.g. by addition of the weak acid butyrate (1B) the rise in tone is abolished, suggesting that it is dependent upon pHᵢ not pHᵢₒ. From reference [11] with permission.](image-url)
isolated smooth muscle cells). At present, the role of the endothelium in mediating the response of smooth muscle to altered pH remains equivocal (e.g. [21,87]).

4. Control of pH

Resting pH in vascular smooth muscle (in CO<sub>2</sub>/HCO<sub>3</sub> buffer) ranges from 7.1–7.3, and is apparently not related to vascular site [2,9,85,98,119,122]. As we have already discussed, changes in pH are regulated by sarcolemmal processes that transport protons (or proton equivalents) across the cell membrane. In the short term (<1 s), buffering of protons at intracellular sites helps limit the magnitude of the pH changes. Both these processes will be discussed in turn.

4.1. Buffering power in vascular smooth muscle

The buffering power of a cell (β) is defined as the concentration of protons (H<sup>+</sup> ions) required to produce a unit change in pH. The larger the value of β, the more able is the cell to resist changes in pH. Although some buffering is mediated by HCO<sub>3</sub> /CO<sub>2</sub>, much is independent of this system, and is termed intrinsic buffering power (β<sub>i</sub>). The largest contributor to β<sub>i</sub> is considered to be intracellular proteins. Few studies have examined β<sub>i</sub> of smooth muscle cells. Aalkjaer and Cragoe [2] calculated a value of 32 ± 4 mM/pH unit in mesenteric cells which compares reasonably well with values of 41 ± 4 and 33 ± 3 mM/pH unit obtained in similar vessels by other workers [14]. In cultured vascular smooth muscle cells a higher value, 58 mM/pH unit, has been found [16]. Although few studies have measured β<sub>i</sub> in other smooth muscles, values obtained in vascular smooth muscle appear to be somewhat on the larger side. In a cultured smooth muscle-like cell line, β<sub>i</sub> was found to be ~10 mM/pH unit [105], in the uterus ~12.8 ± 2.8 mM/pH unit [32], and in the vas deferens 8.6 mM/pH unit [7]. Thus vascular smooth muscle may have a greater ability to buffer changes in pH than other smooth muscles, this may be a protective mechanism against unwanted changes in tone produced when pH is altered. Of further interest is the finding in mesenteric vessels that β<sub>i</sub> is not constant but increases with acidic pH [13] in agreement with what has been seen in cardiac myocytes [125]. In mesenteric vessels, greater changes in tone are produced by acidic changes in pH than are produced by alkaline changes in pH [13]. The increasing β<sub>i</sub> with acidic values of pH may therefore be a further protective mechanism helping limit possible damage produced by acidic conditions. Clearly an understanding and knowledge of the buffering power of a vascular tissue is important when considering the effects of an acid or alkaline load on the tone.

4.2. pH-regulating mechanisms

Although intracellular buffer power will initially limit the magnitude of pH changes, in the long term (>5 min) it is changes in the activity of pH-regulating ion exchange mechanisms which restore pH to control levels. Restoration of resting pH in vascular smooth muscle is thought to involve a number of ionic exchangers. Recovery of pH following an acid load is due to activation of a Na<sup>+</</sup>-HCO<sub>3</sub> co-transporter and a separate Na<sup>+</</sup>/H<sup>+</</sup> exchange (the type 1 isof orm of the Na<sup>+</</sup>/H<sup>+</</sup> exchangers) [2,99]. Na<sup>+</</sup>HCO<sub>3</sub> co-transporters have been shown to operate in vascular smooth muscle preparations and some cultured smooth muscle cell lines, but not in others (reviewed in [1]).

The regulatory mechanisms responsible for recovery from an alkali load are uncertain. An acidifying Cl-HCO<sub>3</sub> exchanger has been found in several smooth muscle types including vascular smooth muscle and [3,45]. This consistently appears to be Na<sup>+</</sup> independent. Such an exchanger may aid recovery from alkaline loads as well as contributing to maintenance of resting pH. The exact nature and function of these ion exchangers is likely to vary throughout the vasculature and this may help to explain some of the varying effects of acidic and alkaline loads on vascular tone.

It is of interest to note that agonist activation of vascular smooth muscle may involve increasing the activity of the Na<sup>+</</sup>/H<sup>+</</sup> and the Na<sup>+</</sup>-HCO<sub>3</sub> transporters. Work, mainly on cultured cells, has shown increases of steady state pH upon stimulation with growth factors and vasoconstrictors [76,110]. In intact vessels Aalkjaer and Mulvany [4] demonstrated activation of both the Na<sup>+</</sup>/H<sup>+</</sup> and the Na<sup>+</</sup>-HCO<sub>3</sub> exchangers by vasopressin. However, no significant change of pH was observed during force production by vasopressin. Since [Ca<sup>2+</sup>], increases during agonist-induced activation, it has been speculated that Ca<sup>2+</sup> directly stimulates the exchangers [91], although protein kinase C or cGMP have also been invoked [78,80]. The physiological role of this activation remains to be established, but it could be argued that if contraction was to produce acidification inside the cell e.g. due to ATP hydrolysis and lactic acid production, then simultaneous activation of the exchanger would tend to off-set this acidification and hence negate any (unwanted) functional effect of pH change [4]. Similar activation of the Na<sup>+</</sup>-HCO<sub>3</sub> by agonists probably occurs but little is known about the pathway involved.

5. Effects of changes in pH in intact vascular preparations

5.1. Arterial smooth muscle

As described above, smooth muscle commonly experiences changes in pCO<sub>2</sub> and hence pH in vivo. Changes of
cellular metabolism, perfusion and activation may all be anticipated to have consequences for pH, while pH remains constant. Therefore, it is important to focus on the published results that describe an effect of altered pH, alone, for simplicity these have been organised into effects on basal tone and on activated tone.

5.1.1. Effects of pH on basal tone

Lloyd (77; dog and rabbit pulmonary artery) and Furtado (39; rabbit aorta) found that changes of pH had no effect on basal tone. However, Danthuluri and Deth (28) saw a sustained contraction after a significant delay on intracellular alkalisation in rat aortic smooth muscle. Studies on strips of rat mesenteric resistance artery also showed an increase in basal tone with a brief intracellular alkalisation, and a decreased tone on a brief intracellular acidification (11 and Fig. 1). Work on intact rings of rat mesenteric resistance arteries by Aalkjaer and Cragoe (2) and Matthews et al. (85) suggest that alkaline pH had no effect on basal tone, while transient acidic changes of pH appeared to cause a significant transient contraction. Similar tension responses to acidic pH were also observed in rat cerebral arteries (approximately 0.25 mm diameter) (122). Using the technique of pressure myography to study the resting diameter of isolated rat cerebral arterioles (approximately 0.1 mm diameter) Apkon and Boron (9) also observed transient vaso-constrictions accompanying transient acidic pH, and no change in vessel diameter with significant alkaline shifts of pH. In pulmonary vessels contraction has been reported to both acidification and alkalisation (72). Tension measurements on porcine coronary arteries showed that alkalisation increased basal tension levels despite a transient decrease of intracellular [Ca\(^{2+}\)] and acidification caused a decrease in basal tension (98).

In summary, across the arterial system, there appears to be no consistent pattern of effects of pH on basal tone. This may be due to the range of arterial sites studied; in particular there may be marked differences in the responsiveness of small (resistance) and large arteries. Resistance arteries and precapillary sphincters are believed to contain smooth muscle of a phasic character (88), which develop a significant degree of myogenic tone. Overall, the basal tone of these vessels appear to be insensitive to alkaline pH, yet contract to acidic shifts of pH. Smooth muscle of larger arteries are of the tonic character and yet they are not thought to develop a significant amount of intrinsic tone in vitro. The consensus view in the literature suggests that alkaline pH causes an increase tone while acidic pH causes a decreased tone. It should be noted that the majority of these studies use short (up to 15 min) changes of intracellular pH, there appears to no studies to date studying more long term effects of changes of pH.

5.1.2. Effects of pH on agonist or high-K\(^+\) induced tone

The majority of studies using agonists have shown that intracellular alkalisation depressed and acidification potentiated arterial contraction. Thus (i) Furtado (39) showed this for noradrenaline induced tone, and similar results were observed in smooth muscle from (ii) rat tail artery stimulated by phenylephrine (28); (iii) rabbit ear artery and rat tail artery stimulated by noradrenaline (119); (iv) canine coronary, mesenteric and femoral artery stimulated by prostaglandin F\(_{2\alpha}\) (38). For equal elevations of pH, Austin and Wray (13) found a greater and faster increase of force was obtained in depolarized (high-K\(^+\)) than in non-depolarized rat mesenteric arteries. In addition, they found in depolarized preparations that acidification produced a transient decrease in force followed by an increase above control levels. Other workers have also reported biphasic effects of pH in vessels precontracted either by depolarisation or agonist-induced tone, these biphasic effects were not seen during pH-induced changes in basal tone (2,39).

As mentioned in the previous section, Nagesetty and Paul (98) showed that intracellular alkalisation increased basal force in unstimulated tissues but in pre-contracted vessels (either with potassium or U46619), alkalisation transiently decreased tone, prior to an further increase in tone. Acid shifts of pH caused a transient promotion of force, followed by a rapid decrease. In general, these studies have studied acute effects of shifts of pH (≤ 10 mins). The chronic effects of acidic shifts of intracellular pH has yet to be studied in detail.

Recent measurements of pH, pH, and [Ca\(^{2+}\)] in a non-vascular smooth muscle indicate that a decrease of pH during contraction is accompanied by an increase of pH (97). The explanation for this result is thought to lie in the action of the sarcolemmal Ca\(^{2+}\)-ATPase. This transporter is known to carry 2 H\(^+\) ions into the cell for every Ca\(^{2+}\) ion pumped out, thus increases of [Ca\(^{2+}\)] will stimulate efflux and H\(^+\) influx via the Ca\(^{2+}\)-ATPase. Thus a fall in pH may accompany an increase of [Ca\(^{2+}\)], and provide an additional modulating influence on force production. This interesting observation has yet to be repeated in vascular tissue.

In summary, the above work suggests that intracellular acidification enhances agonist induced tone in arterial smooth muscle while alkaline pH can have variable effects. Interpretation is also complicated by the acute biphasic effects seen when pH is changed in both acidic and alkaline directions. The cellular basis for the acute and chronic effects are unknown, the complex responses may arise from a combination of effects with direct effects of pH on the contractile proteins and sarcoplasmic reticulum combining with pH-induced interference with the second messenger systems. These topics will be discussed individually in more detail later.

5.2. Venous smooth muscle

Apart from the (hepatic) portal vein, few pH studies have used smooth muscle from the venous system. The portal vein has well developed longitudinal and circular
smooth muscle layers that have electrical and mechanical properties associated with phasic smooth muscle. The spontaneous activity of this vessel is thought to be analogous to vasomotion of resistance arteries and precapillary sphincters [88]. Under ischaemic conditions, portal vein experiences intracellular and extracellular acidification. Intracellular acidification resulted in a depression in both frequency and magnitude of spontaneous contractions [67]. Recently Taggart et al. [120] studied the effects of changes of pH on contractile activity of rat portal vein. They confirmed that a decrease of pH inhibited contractile activity but noted an initial phase of increased spontaneous activity. An increase of pH also caused an increase in the frequency and strength of spontaneous contractions. However, as will be described later, some of these effects could be markedly modified by changes in pH.<ref>

6. Effects of altered pH<sub>a</sub> on pH<sub>i</sub> and vascular tone

The effects of the pH of the blood on the contractile status of vessels had been observed and documented long before those of pH<sub>i</sub>. Recently it has also become clearer how pH<sub>a</sub> and pH<sub>i</sub> may interact. As with cardiac and skeletal muscle, the permeability of smooth muscle membranes to H<sup>+</sup> (or OH<sup>-</sup>) was thought to be low, such that altered pH<sub>a</sub> would only influence pH<sub>i</sub> over 10–20 min. However, recent work suggests that the sarcolemma in certain types of vascular smooth muscle has a high H<sup>+</sup> permeability (see sub-section 6.1). An alternative route through which pH<sub>a</sub> may affect pH<sub>i</sub> is by influencing the flux of weak acids across the membrane. Metabolic monocarboxylic acids such as lactate, pyruvate and acetate can cross the sarcolemma either by passive diffusion of the protonated form or via a transporter mechanism. A specific lactic acid transporter exists in a range of cell types, and has been identified in aortic smooth muscle [60]. A reduced pH<sub>a</sub> will stimulate the influx of the protonated form of the acid. Once inside the cell, the acid will dissociate, releasing protons and acidifying the intracellular space. Anaerobic metabolism is associated with the intracellular production of lactic acid and its extrusion from the cell. Under these circumstances, pH<sub>a</sub> can markedly affect pH<sub>i</sub> simply by effecting the efflux rate of the lactic acid from the cell.

6.1. Arterial smooth muscle

The action of extracellular acidification is to cause a dilatation and alkalisation to cause a constriction of systemic vessels [69,103]. In pulmonary arterial smooth muscle external acidification has also been reported to cause relaxation [107], although an earlier report [37] found no effect. Alkalisation to 7.7 or beyond caused pulmonary vasoconstriction. Thus responses to altered pH<sub>a</sub> in arterial smooth muscle seem to be same in both systemic and pulmonary vessels. The consistency of effects of extracellular acidification contrasts with the varied effects of intracellular acidification described in a previous section (VB). In some cases similar changes in tone are observed when either pH<sub>a</sub> or pH<sub>i</sub> is reduced (e.g. rat mesenteric artery, [14]) but in general, extracellular acidification appears to cause a decrease in tone yet intracellular acidification cause an increase in tone (e.g. pulmonary artery dilates to decreased pH<sub>a</sub>, yet constricts when pH<sub>i</sub> is decreased). A more consistent story emerges for alkaline changes in pH<sub>a</sub> and pH<sub>i</sub>, in both cases increases in tone are observed, but there are exceptions, in cerebral vessels, external alkalisation induces vasoconstriction but changes in pH<sub>i</sub> alone have little or no effect on tone [9]. Therefore acidic and alkaline pH<sub>a</sub> appears to mediate effects on arterial smooth muscle that are independent of pH<sub>i</sub>.

6.2. Venous smooth muscle

Studies using the longitudinal muscle of rat portal vein has indicated that changes in pH<sub>a</sub> can have quite profound effects on the spontaneous phasic contractions. Alkaline shifts of pH<sub>a</sub> have only slight affects on contractility, despite causing marked changes in pH<sub>i</sub>. [120]. Acidic changes of pH<sub>a</sub> had a much smaller inhibitory effect on contractile activity than comparable changes of pH<sub>i</sub> alone. Therefore, as with arterial smooth muscle, changes of pH<sub>a</sub> in rat portal vein appears to attenuate the concomitant effects on pH<sub>i</sub>. In an in vivo study, responses in pial vein to altered pH<sub>a</sub> of the cerebral spinal fluid showed dilation to acidification, although larger responses were seen with pial arteries [86]. These authors suggested that the relative insensitivity of the pial veins as compared to arteries to pH makes it likely that alteration of cerebral spinal fluid pH will be of greater importance to the regulation of cerebrovascular resistance than capacitance.

6.3. Effect of pH<sub>a</sub> on pH<sub>i</sub>

Few studies have investigated the effects of changes in pH<sub>a</sub> on pH<sub>i</sub>. Previous studies on non-vascular cell types had shown that changes in pH<sub>a</sub> cause only small and slow changes in pH<sub>i</sub> and as a result the two have usually been considered separately. For example in cardiac myocytes and vas deferens the change in pH<sub>i</sub> was only around 30% 120. Therefore it appears likely that alteration of cerebral spinal fluid pH makes it likely that alteration of cerebral spinal fluid pH will be of greater importance to the regulation of cerebrovascular resistance than capacitance.
of regulating pH, or (iii) background acid loading mechanisms.

Until recently, the mechanism of action of pH on the tone described above had been poorly understood and investigated. Studies in which pH, force and Ca were simultaneously measured when pH was altered led to new insights into its mechanism of action. In rat mesenteric vessels changes in pH precede those of Ca, as nulling the effects of elevated pH on Ca prevented the effects of pH on tension [12]. However, this result may also be explained by an effect of the weak acid (e.g. butyrate) on force independent of an effect on pH (see sub-section 3.4). It has also been shown in these vessels that the changes in Ca produced by changes in pH are accompanied by changes in [Ca2+], suggesting an interaction between pH, Ca, [Ca2+], and tension. Recent work, where Ca2+ and pH were measured simultaneously, showed that when pH was altered, the changes in Ca precede those of [Ca2+], which in turn precede the changes in tension [10]. Thus in mesenteric vessels under these conditions, it appears that pH, not Ca, is the important modulator of tone. This, however, is not the case in all vascular smooth muscles, as described above, in the smooth muscle of the portal vein, changes in spontaneous activity upon alteration of pH were less marked than those found when pH alone was altered. Thus in this tissue the effects of pH can not be entirely explained by the induced changes in Ca and some other aspects of pH alteration must be influencing force [120]. Furthermore in pulmonary preparations external acidification produced relaxation, whereas reducing pH alone caused contraction [107]. Similarly in cerebral vessels, external alkalination induces vasoconstriction but changes in pH alone have little or no effect on tone [9]. It has been suggested that effects of external alkalisation on tension in cerebral vessels may be due to membrane potential depolarization as a result of a decrease in the outward K+ current [51,52] or an increase in inward Ca2+ currents [126]. In support of this view, Ahn and Hume [6] found that external acidification reduced the voltage dependent K+ current in these single pulmonary arterial, (which would presumably tend to cause constriction) while alkalination increased K+ currents (leading to relaxation).

7. pH, Ca2+ and contractile proteins

The preceding sections have reviewed the effects of pH and pH on vascular tone and the mechanisms working to buffer and regulate pH. We will now consider how pH influences vascular tone. To understand the mechanisms involved we will firstly summarise the broad category of possible mechanisms, and then outline the molecular basis for Ca2+-activated contraction in vascular smooth muscle. Evidence for the effects of changes of pH on [Ca2+] will be discussed, followed by the effects of changes of pH on force (at constant [Ca2+]).

7.1. pH, Ca2+ and tension

Both electrical (depolarization) and agonist induced changes in contractility may be modulated by pH acting on: (i) Ca2+ influx or Ca2+ extrusion (ii) the SR to change [Ca2+], and (iii) on the contractile proteins to alter tension production at a given [Ca2+]. Changes in agonist induced tone may also result from pH affecting: (i) receptor mediated generation of second messengers, (ii) the ability of second messengers to alter [Ca2+], and (iii) the ability of second messengers to induce a change in the Ca2+-activated sensitivity of the contractile proteins. These sites of action are summarised in Fig. 2.

7.2. Molecular basis for Ca2+-activated force

An increase in intracellular [Ca2+] is linked to contraction of smooth muscle via the formation of the calcium/calmodulin complex (CaCaM) and the consequent activation of myosin light chain kinase (MLCK). Once active, MLCK catalyses the phosphorylation of Ser-19 site [49] on myosin light chain (MLC). In this form, myosin can interact with actin and generate force. Dephosphorylation of MLC and the subsequent inhibition of acto-myosin interaction occurs through the activity of myosin light chain phosphatase (MLCP, [24]). Although this mechanism is common to all smooth muscle, the response of the contractile proteins to a sustained increase in [Ca2+] can vary widely. Tonic smooth muscle tend to respond with a maintained increase in MLC phosphorylation and tension, while in phasic muscle, phosphorylation and tension pro-

Fig. 2. Schematic diagram of the potential sites at which pH may affect force production. The left hand side receptor and 2nd messenger system enhance force production, while the right hand side system depress force production. Increased cytosolic [Ca2+] can occur via influx through channels or release from the sarcoplasmic reticulum.
duction is only transient [65]. Thus the differing contractile behaviour of intact smooth muscle is, in part, due to distinct properties of the contractile proteins. The major difference is believed to be in the regulation and activity of MLCP; the higher activity of this enzyme in phasic muscle may account for the phasic nature of contractility [65]. The rate of rise of force production is also more rapid in phasic muscle; again it appears that this could be explained by the higher MLCK activity [117].

Measurements from intact and permeabilised muscle suggests that in some smooth muscle types phosphorylation is transient; the amount of phosphorylated MLC returns towards prestimulation levels despite the maintenance of sustained force [40,48,73,92]. This suggests that sustained force is maintained by non or slowly cycling cross bridges which are not associated with a significant ATPase rate; this behaviour has been termed the ‘latch state’. One model suggests that the ‘latch state’ contributes to force production via dephosphorylation of attached and phosphorylated cross bridges [96].

An alternative explanation to the ‘latch state’ model is suggested by recent work by Khalil and Morgan [63] which showed that activation of protein kinase C (PKC) and the production of actin regulated force may occur after the phasic rise in phosphorylation and ATPase rate. Thin filament regulation of contraction may involve the proteins caldesmon [82] and calponin [127]. Phosphorylation of these proteins allows their disassociation from actin, and interaction with phosphorylated myosin through either Ca\(^{2+}\)/calmodulin dependent or independent pathways [5,63].

Therefore, as summarised in Fig. 3, smooth muscle has a complex series of regulatory mechanisms and an altered pH may affect any of the processes described above.

7.3. Effect of pH\(_i\) on [Ca\(^{2+}\)]

A number of studies have examined the effects of pH\(_i\) on [Ca\(^{2+}\)], in cultured vascular smooth muscle cells. It was found that an increase in pH\(_i\) elicited a rise in [Ca\(^{2+}\)], [15,115] suggesting that there is indeed a relationship between the two intracellular ions. Because of the contrasting results regarding the effects of increases in pH\(_i\) on vascular tension, however, it is difficult to relate these pH\(_i\) induced changes in [Ca\(^{2+}\)], to changes in tension as force was not measured in these isolated cells. Relatively few studies have simultaneously measured [Ca\(^{2+}\)], and tension upon alteration of pH\(_i\). Jensen et al. [56] have shown that, in nor-adrenaline activated mesenteric vessels the increases in tone due to acidification and the decreases in tone due to alkalinisation were accompanied by increases and decreases in [Ca\(^{2+}\)], respectively. This suggests that pH\(_i\) alters tone by affecting [Ca\(^{2+}\)]. In contrast, in porcine coronary artery smooth muscle the alteration of [Ca\(^{2+}\)], due to a change in pH, was in the opposite direction to changes in tension i.e. an increase in tension due to alkalinisation was accompanied by a decrease in [Ca\(^{2+}\)], suggesting that it is not a change in [Ca\(^{2+}\)], which is responsible for the observed changes in tension [109]. In a recent study by Nagesetty and Paul [98], alkaline shifts of pH\(_i\) caused marked increases of tone but decreases of [Ca\(^{2+}\)]. To explain this paradoxical effect the authors suggest that pH\(_i\) may affect tension by altering the sensitivity of the contractile proteins to [Ca\(^{2+}\)], this will be discussed in more detail in a later section.

8. Direct effects of pH on smooth muscle contractile proteins

8.1. Effects of pH on Ca\(^{2+}\) activated force (permeabilised muscle studies)

The effects of altered pH on tension can be studied directly by permeabilising the sarcolemma and controlling the pH and [Ca\(^{2+}\)] around the myofibrils. Permeabilisation techniques using Triton X-100 and saponin cause extensive disruption of the sarcolemma, resulting in the possible loss of cytosolic proteins involved in contraction e.g. calmodulin and myosin light chain kinase [43,70]. The use of α-toxin from Staphylococcus aureus or the alkaloid β-escin results in much less disruption to the sarcolemma, hence retention of all cytosolic proteins [64,100]. The differential loss of regulatory proteins through the use of less selective permeabilising agents may explain the different responses reported in the literature. Precise control of the [Ca\(^{2+}\)] bathing the myofibrils allows a complete description of the relationship between [Ca\(^{2+}\)] and contractility in permeabilised vessels. This relation is commonly described by three parameters; (i) the maximal level of isometric tension production (ii) the [Ca\(^{2+}\)] required for half maximal force and (iii) the slope of the relationship between [Ca\(^{2+}\)] and force.

Early studies on caudal artery [42] and pig carotid artery [93] treated with Triton X-100 showed that acid pH enhanced the Ca\(^{2+}\) sensitivity of force production i.e. less Ca\(^{2+}\) required for a given force but decreased maximal

---

**Fig. 3.** A schematic diagram of the process that control Ca\(^{2+}\) activated force in smooth muscle. CaM = calmodulin; AA = arachidonic acid; PKA, PKC and PKG = protein kinase-A, C and G. * indicates pathways whose role in the control of activated force is speculative.
force. A study by Crichton et al. [27] compared the effect of pH in α-toxin permeabilised longitudinal smooth muscle from human umbilical artery (tonic) with rat portal vein (phasic) [27].

The results illustrated in Fig. 4 indicate the quite different responses observed in phasic and tonic muscles to altered pH. These results were obtained by exposing the preparation to short periods of altered pH (approx. 5 mins) at a set [Ca\(^{2+}\)]. To achieve more detailed information concerning the relationship between [Ca\(^{2+}\)] and force, [Ca\(^{2+}\)] can be progressively increased at a range of pH. The results, published in detail in Crichton et al. [27] are illustrated in Fig. 5. The curves associated with pH 7.7 and 6.7 were the best fit curve to the data and serve to illustrate the effects of pH on these two vascular preparations. The control curve (pH 7.2) is drawn as the best fit curve through the experimental points. Maximum Ca\(^{2+}\)-activated force was potentiated by a bathing pH of 6.7 and attenuated by a bathing pH of 7.7 in rat portal vein (phasic), whereas maximum Ca\(^{2+}\)-activated force was relatively unaffected by either pH in human umbilical artery (tonic). In rat portal vein and human umbilical artery Ca\(^{2+}\) sensitivity was not significantly effected by acidic pH, whereas alkaline pH significantly depressed the Ca\(^{2+}\) sensitivity of tension production in both and to a similar degree. A feature not addressed in detail is the effects of long term (i.e. many minutes) acidic or alkaline conditions. It is difficult to anticipate whether short or long term effects of pH are more physiologically relevant since metabolic changes will give rise to long term changes in pH [67], whereas agonist induced changes in pH have transient as well as sustained components [16].

In summary, few studies have investigated the effects of altered pH on Ca\(^{2+}\) activated force in vascular smooth muscle. Using recently developed techniques of permeabilisation which allow the soluble intracellular proteins to be retained, studies have revealed quite different response of tonic and phasic vascular smooth muscles to altered pH. Further investigation using a range of vascular preparations is required to fully characterise the pH responsiveness of contractile proteins.

8.2. Effect of pH on the components of Ca\(^{2+}\) activated force

8.2.1. Ca–Calmodulin interaction

At around neutral pH, calmodulin (CaM) is a negatively charged protein (exact charge unknown) [66]. Occupancy of all four Ca\(^{2+}\) binding sites is thought to be necessary to cause the exposure of an amphipathic domain on CaM that can interact with an enzyme to form an activated CaM–enzyme complex. Reports on the pH sensitivity of Ca\(^{2+}\) binding to CaM are not consistent. Ogawa and Tanokura [123] found that Ca\(^{2+}\) binding to CaM is not pH sensitive over the limited range of 6.8–7.2. While Tkachuk and Menshikov [123] noted a reduced affinity at more acidic pH, Crichton et al. [27] compared the effect of pH in α-toxin permeabilised longitudinal smooth muscle from human umbilical artery (tonic) with rat portal vein (phasic) [27].
pH. Measurements by Milos et al. [90] indicated that H+ is released when Ca2+ binds to CaM, suggesting that acidic pH would reduce and alkaline pH enhance the affinity of calmodulin for Ca2+. This latter observation has consequences for the interaction between CaCaM and myosin light chain kinase (see below).

8.2.2. Myosin light chain kinase

Studies suggest that CaM binds to the MLCK displacing a pseudo substrate inhibitory domain from the catalytic subunit. The interaction of CaM and MLCK is thought to be pH sensitive, in particular an increase [H+] facilitates the interaction between the two proteins. Huang and Cheung [53] suggest that H+ binding to MLCK exposes an amphipathic domain that is complementary to that on CaM and allows the two proteins to interact. They further propose that the H+ release on Ca2+ binding to CaM may play an important role in facilitating the interaction between CaM and MLCK. On the basis of this mechanism acidic pH would be expected to promote phosphorylation of myosin light chain and increase Ca2+-activated force, which corresponds to some of the experimental observations (see above).

8.2.3. Myosin light chain phosphatase

In smooth muscle this is thought to be a form of Protein Phosphatase-1 [8]. Little is known about the pH sensitivity of these enzymes, despite the fact that modulation of this enzyme by pH could markedly affect force.

8.2.4. Acto-myosin ATPase

In smooth muscle the myofibrils (bundles of actin and myosin filaments) are thought to span across the cell. Their origin and insertion on the membrane is marked by a ‘dense body’ network of 10 nm filamentous proteins that contain α-actinin (the same protein is the major constituent of the Z-line in striated muscle). Blanchard and Solaro [18] have shown that reconstituted acto-myosin ATPase alone is relatively insensitive to altered pH. However, when the contractile protein filaments arranged in a regular lattice as observed in glycerol-extracted striated muscle, acidic pH depresses force production [89] by disrupting the lattice array [84]. However, it is unknown the extent to which altered intracellular pH can disrupt the arrangement of contractile filaments in smooth muscle cells.

8.2.5. Agonist induced increases in force

A variety of agonists have been shown to increase the responsiveness of the contractile proteins to Ca2+ e.g. phenylephrine and acetylcholine. Receptors for these agonists activate phospholipase C (PL-C) via a membrane bound G-protein [35]. Stimulation of PL-C generates inositol (1,4,5) trisphosphate (Ins(1,4,5)P3) and diacylglycerol (DG) [17]. DG can be produced without the accompanying Ins(1,4,5)P3 production via the activation of phospholipase D [68]. A rise in the DG content of the sarcolemma along with a rise in intracellular [Ca2+] causes the association of protein kinase C (PKC) with the membrane [101]. Although membrane bound, the activation of this kinase by intracellular [Ca2+] is thought to be able to influence a range of cytosolic processes by initiating a kinase cascade [108]. Through this route it is believed that activation of PKC causes an increase in the Ca2+-sensitivity of force production via the inhibition of MLCP [83]. If PL-D is activated, an alternative intracellular pathway has been suggested by the work of [46], who showed that arachidonic acid, a known product of PL-D activation, can directly inhibit MLCP and therefore increase force levels at a given [Ca2+]. In addition, Collins et al. [23] showed that phosphorylation of caldesmon and/or calponin and their dissociation from actin is stimulated by PKC, this effect may have a role in the maintenance of basal tone [59,63].

Clearly, in the pathways described above could be modulated by pH, yet little is known about the pH sensitivity of these individual systems. Potentially, they may possess quite distinct pH sensitivities that result in an altered predominance of signal systems at different pH.

8.2.6. Agonist induced decreases in force

A number of membrane bound receptors, including β adreno-, prostanoid and muscarinic receptors, are linked to adenylate cyclase to regulate intracellular [cAMP]. Agonists linked to guanylate cyclase e.g. atrial natriuretic factor and nitric oxide, stimulate a rise in intracellular [cGMP]. Both cAMP and cGMP have important physiological roles as intracellular mediators of smooth muscle relaxation. In intact and permeabilized smooth muscle, an increase in both intracellular [cAMP] and [cGMP] causes a relaxation [62,104]. cAMP and cGMP-dependent kinases have been shown to phosphorylate myosin light chain kinase at two separate sites [75]. Phosphorylation at Ser and Thr decreases the affinity of MLCK for the CaCaM complex and therefore reduces the tension generated at a given [Ca2+]. In one report, marked differences were seen in the pH sensitivity of the effects of cAMP and cGMP in permeabilised porcine coronary arteries [102]. cAMP mediated decrease in Ca2+ activated force was insensitive to pH, but cGMP mediated effects were absent at pH 6.5 and 7.2, but at pH 7.0, cGMP was as effective as cAMP. This suggests that G-kinase activity is markedly pH sensitive, but there is little direct evidence to support this conclusion.

In summary: Direct experimental evidence exists that acidic and alkaline pH1 modulates Ca2+-activated force production differentially in arteries and veins. In particular, the tension generated by contractile proteins of phasic smooth muscle appears markedly affected by moderate shifts of pH. These results would suggest that the direct effects of pH on Ca2+-activated force are of equal magnitude to those anticipated from changes of [Ca2+]. Little progress has been made in discovering the molecular basis of these effects which may be due to direct effects on the contractile proteins. Alternatively, the complex pathways
available to modulate Ca\(^{2+}\)-activated force in smooth muscle via a range of second messenger systems provide an other route through which altered pH may affect force.

9. Smooth muscle sarcoplasmic reticulum (SR) functioning

While the role of SR Ca\(^{2+}\) release in normal contractile behaviour of vascular smooth muscle is unclear, reports suggest that the SR can contain sufficient Ca\(^{2+}\) to initiate a maximum contraction [19,71]. Smooth muscle has the potential to release Ca\(^{2+}\) from the SR by two distinct mechanisms. One mechanism is termed Ca\(^{2+}\)-induced Ca\(^{2+}\)-release, whereby an increase in [Ca\(^{2+}\)] on the outside surface of the SR will cause Ca\(^{2+}\) release via the activation of specific (ryanodine sensitive) channels. This mechanism is well characterised in cardiac muscle, and is thought to link Ca\(^{2+}\) influx across the sarcolemma to Ca\(^{2+}\) release from the SR. Until recently, there was only indirect evidence that it existed in smooth muscle [55,112]. However, work by Iino [54] directly demonstrated Ca\(^{2+}\)-induced Ca\(^{2+}\) release in saponin-permeabilised guinea pig taenia caeci. Studies on voltage clamped single cells from guinea pig urinary bladder suggest that Ca\(^{2+}\)-induced Ca\(^{2+}\) release accounts for 70% of the total depolarisation induced increase of intracellular [Ca\(^{2+}\)] [41]. The role of this mechanism in control of intracellular [Ca\(^{2+}\)] in vascular smooth muscle is uncertain. Gregoire et al. [47] reported Ca\(^{2+}\)-induced Ca\(^{2+}\) release in single smooth muscle cells from rat portal vein, yet Kishimura and McCarron [58] failed to find evidence of Ca\(^{2+}\)-induced Ca\(^{2+}\) release from the SR during depolarisation evoked Ca\(^{2+}\) entry in the same preparation. More work is required on cells from a range of vascular sites. It is conceivable that the role of Ca\(^{2+}\)-induced Ca\(^{2+}\) release in E–C coupling may vary over the vascular tree, and in different vessels.

9.1. Effect of pH on Ca\(^{2+}\) induced Ca\(^{2+}\) release

Iino [54] demonstrated that Ca\(^{2+}\)-induced Ca\(^{2+}\) release was sensitive to pH, in that an increased pH increased the sensitivity of the channel to Ca\(^{2+}\). Similar pH sensitivities have been observed in both skeletal and cardiac muscle preparations [36]. In addition, work by Rousseau and Pinkos [111] showed that both the open probability and the single channel conductance of a cardiac muscle SR Ca\(^{2+}\) channel was reduced by acidic pH. Thus an acidic intracellular pH would depress Ca\(^{2+}\)-induced Ca\(^{2+}\) release.

9.2. Ins(1,4,5)P\(_3\) induced Ca\(^{2+}\) release

Agonists shown to release Ca\(^{2+}\) from intracellular stores have been functionally linked to PL-C and Ins(1,4,5)P\(_3\) production. Ins(1,4,5)P\(_3\) can release Ca\(^{2+}\) from the SR by the activation of specific Ca\(^{2+}\) channels which can be blocked by heparin [31,118]. The Ins(1,4,5)P\(_3\) gated channels have a similar secondary and tertiary structure to the Ca\(^{2+}\)-gated ryanodine-sensitive channels. Both types of channels also possess functional similarities, both have a similar biphasic dependence on [Ca\(^{2+}\)] and both are sensitive to adenine nucleotides. Work by Yamazawa et al. [130] on saponin-permeabilised smooth muscle suggests that Ca\(^{2+}\)-induced Ca\(^{2+}\)-release and Ins(1,4,5)P\(_3\)-induced Ca\(^{2+}\) release Ca\(^{2+}\) from functionally distinct parts of the SR. In addition to this, phasic smooth muscle is thought to contain a relatively larger amount of Ins(1,4,5)P\(_3\)-sensitive SR compared with tonic muscle [34].

Altered intracellular pH may influence Ca\(^{2+}\) release by (i) modulation of the coupling between the sarcolemma and the SR, i.e. affecting the Ca\(^{2+}\) influx or Ins(1,4,5)P\(_3\) generation and/or (ii) altering the ability of Ins(1,4,5)P\(_3\) to release Ca\(^{2+}\).

9.3. pH effects on Ins(1,4,5)P\(_3\) induced Ca\(^{2+}\) release

An early study by Brass and Joseph [20], in saponin permeabilised platelets, showed that release of Ca\(^{2+}\) by Ins(1,4,5)P\(_3\) was enhanced in alkaline pH. Recently, Tsukioka et al. [124] studied the pH dependence of Ins(1,4,5)P\(_3\)-induced Ca\(^{2+}\) release in saponin permeabilised guinea pig portal vein. These studies indicated that increased pH from 6.7 to 7.0 caused a marked increase in the rate of Ca\(^{2+}\) release by Ins(1,4,5)P\(_3\). Further increases in pH to 7.3 caused a further smaller enhancement of Ins(1,4,5)P\(_3\)-induced Ca\(^{2+}\) release. An increase in the binding of Ins(1,4,5)P\(_3\) and Ca\(^{2+}\) to the channel at the higher pH is thought to account for some of this affect, as Ca\(^{2+}\) is known to positively modulate the Ins(1,4,5)P\(_3\)-induced Ca\(^{2+}\) release mechanism [124]. As with Ca\(^{2+}\)-induced Ca\(^{2+}\) release, these results suggest that acidic pH would depress and alkaline pH enhance the Ins(1,4,5)P\(_3\)-induced Ca\(^{2+}\) release from smooth muscle SR.

One complication in trying to anticipate the effects of pH on Ins(1,4,5)P\(_3\) induced Ca\(^{2+}\) release is the report by Smith et al. [116] that a rapid decrease of intracellular pH causes a transient release of Ins(1,4,5)P\(_3\) and subsequent Ca\(^{2+}\) release from intracellular stores. This effect was observed in a range of cell types including rat aortic smooth muscle cells [116]. In this context, it is interesting to note that Jensen et al. [56] observed a transient increase in [Ca\(^{2+}\)], and tension after a rapid decrease of pH on rat mesenteric small arteries.

Fig. 6 illustrates the utility of the permeabilised smooth muscle technique in studying the effects of pH on agonist induced Ca\(^{2+}\) release. A strip of α-toxin permeabilised portal vein smooth muscle (approx. 100 μm wide, 30 μm thick, 2 mm long) was bathed in mock intracellular solution containing 10 μM Fura-2. Addition of nor-adrenaline caused a transient rise of [Ca\(^{2+}\)] within the permeabilised muscle and a consequent transient rise of tension. Lowering the pH from 7.2 to 6.5, caused a marked increase in the
Fig. 6. Panel A measurements of [Ca\(^{2+}\)] (using 10 mM Fura-2) and tension from an α-toxin permeabilised strip of longitudinal muscle from rat portal vein.

Under control conditions the preparation was perfused with a mck intracellular solution containing 120 mM potassium methansulphonate, 5 mM di-sodium ATP, 15 mM di-sodium creatine phosphate and 7 mM MgCl\(_2\), 0.2 mM EGTA 1 mM nor-adrenaline was applied for 40 s as indicated above the records at 5 min intervals. Panel B indicates the average of 3 fluorescence and tension records under control pH 7.2 and acidic pH 6.8 conditions.

9.4. Ca\(^{2+}\) uptake by the SR and modulation by agonists.

cAMP and cGMP may mediate relaxation of smooth muscle by stimulating Ca\(^{2+}\) uptake by the SR. These second messengers activate A-kinase and G-kinase respectively (although cross reactivity does occur [57]; which proceed to phosphorylate a range of target proteins within the cell. Modulation of the activity of the SR Ca\(^{2+}\) pump ((Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase) is primarily via the associated pentameric protein phospholamban, which is present in cardiac, slow skeletal and smooth muscle SR. Two phosphorylation sites have been identified on phospholamban that regulate the proteins association with the Ca\(^{2+}\) pump the Ser-16 site phosphorylated by A- and G-kinase [25]; the Thr-17 site is phosphorylated by CaM-kinase [22]. Thus agonist stimulated increases in cAMP and cGMP stimulates the SR Ca\(^{2+}\) pump, decreases cytosolic [Ca\(^{2+}\)] and mediates relaxation. Little is known about the pH sensitivity of the interaction between phospholamban and (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase. SERCA2b is the form of the (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase expressed in smooth muscle cells. It differs structurally and functionally [79] from that found in cardiac muscle (SERCA2a). SERCA2b has a lower turnover rate for both Ca\(^{2+}\) transport and ATP hydrolysis, but the pH dependence of the two forms is thought to be similar. Studies on isolated (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase (SERCA2a) indicate that an acidic pH would reduce the pump rate [81,114] not by affecting Ca\(^{2+}\) binding, but by a direct affect on the (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase.

In summary, on the basis of the studies cited above, acidic conditions would tend to lower both the capacity of the SR to sequester Ca\(^{2+}\) and the ability of intracellular second messengers to release Ca\(^{2+}\) from the SR. However, few studies have directly used smooth muscle preparations from the vasculature and the effects of pH particularly on SERCA2b and the ability of phospholamban to modulate the Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase is still unknown. Considering the proposed importance of this latter mechanism in mediating endothelium dependant relaxation this should be an important focus of research in the future.

Acknowledgements

We are grateful to the following for supporting the work described in this review: the British Heart Founda-
tion, the Wellcome Trust and the Medical Research Coun-

References


[88] Mellander S, Johansson B. Control of resistance, exchange, and


