# Topical Review

# Hypoxia and smooth muscle function: key regulatory events during metabolic stress

Michael J. Taggart and Susan Wray

*Physiology Department, University of Liverpool, Crown Street, Liverpool L69 3BX, UK*

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Hypoxia rapidly reduces force in many smooth muscles and we review recent data that shed light on the mechanisms involved. As many regulated cellular processes are integrated to co-ordinate smooth muscle contractility, the processes responsible for decreased force output with altered metabolism are also likely to be many, acting in concert, rather than the actions of one altered parameter. Nevertheless the aim of this study is to elucidate the hierarchical series of events that contribute to reduced smooth muscle force production during altered metabolism. We conclude that in many phasic smooth muscles the decrease in force can be attributed to impaired electro-mechanical coupling whereby the $Ca^{2+}$ transient is reduced. A direct effect of hypoxia on the $Ca^{2+}$ channel may be of key importance. In tonic vascular smooth muscles $K_{ATP}$ channels may also play a role in the integrated functional responses to hypoxia. There are also many examples of force being reduced, in tonically activated preparations, without a fall in steady-state $Ca^{2+}$; indeed it usually increases. We examine the roles of altered [ATP], pH, myosin phosphorylation, inorganic phosphate and proteolytic activity on the $[Ca^{2+}]$–force relationship during hypoxia. We find no defining force-inhibitory role for any one factor acting alone, and suggest that force most probably falls as a result of the combination of myriad factors.

Smooth muscle contraction is essential for many homeostatic functions, e.g. the regulation of blood pressure, gastro-intestinal motility and the behaviour of the urinogential tract. It is now apparent that many smooth muscles are markedly affected by hypoxia on a rapid time scale. The resulting effect on contraction may be beneficial, e.g. the dilatation of a blood vessel, thereby increasing blood flow to a hypoxic region or preservation of ATP during times of metabolic stress. However, hypoxia can also cause dysfunction, which has been associated with many disease states including atherosclerosis, vasospasm, cerebral haemorrhage and uterine dystocia. The purpose of this review is to draw together the recent data pertaining to hypoxia (or metabolic inhibition with cyanide, which will not generally be distinguished from true hypoxia, apart from when direct effects of low $O_2$ on ion channels are discussed), and develop mechanistic explanations for its effects. We do not consider hypoxic contraction, the most notable example of which is that which occurs in the lungs, or hypoxic pulmonary vasoconstriction, but confine ourselves to considering how hypoxia reduces force. We begin therefore with a brief overview of smooth muscle force production.

## Contraction in smooth muscle

For many of the details and references to this section we recommend the review by Horowitz, Menice, LaPorte & Morgan (1996). The pattern of contractile activity in smooth muscles can be divided into two sorts: phasic, where cycles of contraction and relaxation occur, and tonic, where a maintained level of force exists. Phasic contractions are due to electrical excitation at the surface membrane, leading to action potential generation, and spreading from cell to cell via gap junctions. This depolarization results in $Ca^{2+}$ entry, via L-type $Ca^{2+}$ channels and in the presence of agonists, and $IP_3$-induced $Ca^{2+}$ release from the sarcoplasmic reticulum (SR) (Somlyo & Somlyo, 1994). Tonic smooth muscles lack spontaneous action potential generation, and force follows a rise in $Ca^{2+}$ following stimulation by neurohormonal agonists. Sustained depolarization can often cause tonic contraction in phasic muscles. The relationship between electrical activity, $[Ca^{2+}]$ and force has not been clearly established in intact smooth muscle, as simultaneous measurement of these three parameters has only recently been made (Burdyga & Wray, 1997). When $[Ca^{2+}]_i$ rises it combines with calmodulin and activates myosin light chain kinase, which phosphorylates myosin light chains (MLC).

There is, then, significant actin-activated myosin ATPase activity for cross-bridge cycling and force production and MLC phosphorylation is thus an important determinant of contractile initiation. During periods of sustained tone MLC phosphorylation levels often decline and force is maintained by slowly cycling cross-bridges (Murphy, 1994). Upon stimulus cessation, $[Ca^{2+}]_i$ declines, primarily by plasmalemmal $Ca^{2+}$ extrusion via the $Ca^{2+}$-ATPase (Kosterin, Burdyga, Fomin & Grover, 1994), with possible contributions from the $Na^+$–$Ca^{2+}$ exchanger (Suzuki, 1991; McCarron, Walsh & Fay, 1994; Taggart & Wray, 1997*b*) and sequestration by intracellular organelles such as the SR and mitochondria (see later). As $[Ca^{2+}]_i$ declines, $MLC_{20}$ dephosphorylation is accomplished by a MLC phosphatase and precedes relaxation (Khromov, Somlyo & Somlyo, 1996).

### The effect of hypoxia on smooth muscle force

Figure 1 shows typical examples of the effects of hypoxia (simulated with cyanide) in a phasically contracting (Fig. 1*A*) and tonically activated preparation (Fig. 1*B*). The effects of hypoxia on force are both rapid and marked, and in this example from the ureter, such effects would reduce the flow of urine from the kidneys to the bladder (Bullock & Wray, 1997). A similar rapid attenuation of force with hypoxia or cyanide occurs in many phasic smooth muscles including uterus (Wray, Duggins, Iles, Nyman & Osman, 1992; Earley & Wray, 1993), stomach (Huang, Chowdhury, Kobayashi & Tomita, 1993) and portal vein (Sward, Josefsson, Lydrup & Hellstrand, 1993) and decline in force such as seen in Fig. 1*B* can be seen in many tonically activated preparations including uterus (Taggart, Menice, Morgan & Wray, 1997), taenia caeci (Ishida & Paul, 1990), portal vein (Sward *et al.* 1993) and cerebral arterioles (Taguchi, Heistad, Kitazono & Faraci, 1997). Given the dependence of force on $Ca^{2+}$ it is natural to ask if the contractile inhibition seen in Fig. 1 is due to a reduction in $[Ca^{2+}]$.

### What are the effects of hypoxia on $[Ca^{2+}]$ in smooth muscle?

Several studies have shown that changes in the $Ca^{2+}$ transient amplitude or duration cause similar changes in the phasic contractions (Himpens, Lydrup, Hellstrand & Casteels, 1990; Huang *et al.* 1993; Taggart, Burdyga, Heaton & Wray, 1996). Figure 1 also shows the simultaneously recorded intracellular $Ca^{2+}$ changes associated with the contractile activity during control and hypoxic conditions. Cyanide concomitantly reduces the $Ca^{2+}$ transients along with the phasic contractions; similar finding have been reported for several different smooth muscles (Sward *et al.* 1993; Taggart *et al.* 1997; Bullock & Wray, 1997). Thus we can explain the fall of force in Fig. 1*A* by a reduction in the $Ca^{2+}$ transient. In Fig. 1*B*, however, contraction is also reduced in hypoxic conditions but under conditions of unchanged steady-state $[Ca^{2+}]$. Similar findings of unchanged or even increased $[Ca^{2+}]$ (see Fig. 3) with hypoxia have been reported in many smooth muscles (see Table 1). It is therefore clear that we must discuss two main questions: (1) what are the mechanisms underlying the decreased $Ca^{2+}$ and force transients in phasic tissue? and (2) what are the mechanisms disrupting the $Ca^{2+}$–force relationship in tonically activated smooth muscle? To answer the first question we require a detailed appreciation of the control of smooth muscle membrane excitability, as this underlies the $Ca^{2+}$ transient.

### Membrane excitability

Many topics (and references) that can only be touched on here can be found in the recent book on smooth muscle excitation, edited by Bolton & Tomita (1996). Resting membrane potential in smooth muscles (approximately −75 to −45 mV) is dependent upon the balance of $K^+$, $Ca^{2+}$ and $Cl^-$ channel activity, which may change during hypoxia. Many different $K^+$ channels have been described in smooth muscle cells including those modulated by ATP, $O_2$, $Ca^{2+}$

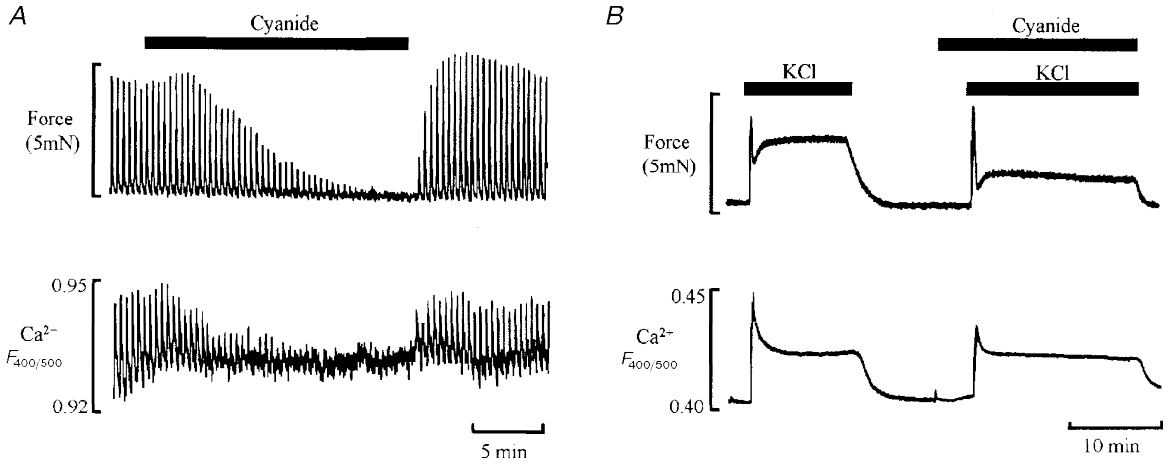


**Figure 1. Simultaneous force (top) and intracellular $[Ca^{2+}]$ (bottom) measurements before, during and after cyanide application to guinea-pig ureter**

*A*, phasic contractions produced by action potentials following electrical stimulation. *B*, tonic contraction produced by high $K^+$ depolarization. The $Ca^{2+}$ measurements were made from the ratio of the fluorescence signals of indo-1 emitted at 400 and 500 nm. Adapted from Bullock & Wray, 1998.

**Table 1. Reported changes in smooth muscle steady-state or basal $[Ca^{2+}]_i$ during conditions of altered metabolism**

| Intervention | Smooth muscle | Change in steady-state calcium | Reference |
|---|---|---|---|
| Hypoxia | Coronary | ⇄ | Paul *et al.* 1991 |
| | Small pulmonary | ↑ | Salvaterra & Golfman, 1993; Post *et al.* 1995; Urena *et al.* 1996 |
| | Conduit pulmonary | ↓ | Urena *et al.* 1996 |
| | Cerebral and mesenteric | ⇄ | Aalkjaer & Lombard, 1995 |
| | Cerebral | ⇄ | Gebremedhin *et al.* 1994 |
| | Taenia coli | ⇄ | Obara *et al.* 1997 |
| Metabolic inhibition | Ileum | ↑ | Hori *et al.* 1989 |
| | Aorta | ↑ | Ishida & Honda, 1991 |
| | Stomach | ↑/⇄ | Huang *et al.* 1993; Drummond & Fay, 1996 |
| | Portal vein | ↑ | Sward *et al.* 1993; Lydrup *et al.* 1994 |
| | Pulmonary | ↑ | Yuan, 1995 |
| | Uterus | ↑ | Taggart *et al.* 1997 |
| | Ureter | ↑/⇄ | Bullock & Wray, 1997 |

and voltage. The activity and distribution of ion channels depend not just upon the particular smooth muscle preparation, but on developmental and gestational state (Khan, Smith, Morrison & Ashford, 1993; Weir & Archer, 1995; Urena, Franco-Obregon & Lopez-Barneo, 1996). Such differences may well account for much of the diversity found between smooth muscles. Any effect of hypoxia on these channels will affect resting membrane potential and is therefore likely to affect force. A general point to consider is that many ion channels require a basal level of phosphorylation for activation, and hence a fall in [ATP] may reduce current flow (Tewari & Simard, 1994; Hilgemann, 1997), although some $Ca^{2+}$ channels do not appear to be activated by phosphorylation (Klockner & Isenberg, 1985; Ohya, Kitamura & Kuriyama, 1988; Sperelakis, Xiong, Haddad & Musuda, 1994). The changes in metabolites and ions that occur with hypoxia and which may influence ion channel activity are summarized in Table 1. As will become apparent, in many smooth muscles increased outward $K^+$ current and decreased inward $Ca^{2+}$ current can be demonstrated with hypoxia or cyanide, along with increased $K^+$ efflux and decreased $Ca^{2+}$ entry.

**What are the effects of hypoxia on $K^+$ channels?** ATP-gated $K^+$ channels ($K_{ATP}$) are present in smooth muscle and have been proposed as a critical link between cell metabolism and electrical activity, and in particular as forming part of the mechanism underlying the dilatation of coronary and cerebral arterioles during hypoxia (Standen, Quayle, Davies, Brayden, Huang & Nelson, 1989; Daut, Maier-Rudolph, Von Beckerath, Mehrke, Gunther & Goedel-Meinen, 1990; Dart & Standen, 1995; Taguchi *et al.* 1997). Teleologically this makes sense; this channel is inhibited by intracellular ATP (inhibition constant, $k_i$, ~10–100 $\mu$M). In hypoxia, the fall in [ATP] and increase in [ADP], along with a decrease in intracellular pH ($pH_i$) (see Table 2), will be expected to lead to the opening of the channel and hyperpolarization of the smooth muscle cell. This will result in relaxation of the vessel and increased blood flow to the hypoxic region. Recent work has drawn attention to the gating of similar channels by other nucleotide diphosphates (Zhang & Bolton, 1996), but their role in hypoxia remains to be investigated. So, do $K_{ATP}$ channels open in hypoxia? There is good evidence that they do in the vascular studies quoted above. However, the reliance in several studies on glibenclamide to block these channels and show their involvement in the hypoxic process, has been questioned (Zhang & Bolton, 1996). It is also difficult to control for a direct effect of low oxygen on channels. It is also clear that a small membrane depolarization, rather than the predicted hyperpolarization, occurs with hypoxia or cyanide in several phasic smooth muscles (Huang *et al.* 1993; Nakayama, Chihara, Clark, Huang, Horiuchi & Tomita, 1997). In these tissues and others (Heaton, Wray & Eisner, 1993) glibenclamide is also unable to reverse the functional effects of hypoxia. Finally as can be seen in Fig. 1*A*, cyanide reduced the amplitude of the contractions but not their frequency. As $K_{ATP}$ channel opening should hyperpolarize the membrane, an effect on frequency would be expected. Thus for phasic muscles there is little compelling evidence for $K_{ATP}$ channel opening, and hyperpolarization being responsible for the fall in $Ca^{2+}$ and force. This may reflect a difference between phasic and tonic smooth muscle responsiveness to hypoxia, but it would also be useful to have more measurements of membrane potential, $Ca^{2+}$, force and $K_{ATP}$ activity in these microvessels. There is also a discrepancy between the $k_i$ for ATP and the [ATP] found in

**Table 2. A summary of the changes in some important metabolites and ions occurring as a consequence of hypoxia**

| Substance | Control | Hypoxia |
|---|---|---|
| ATP | 3 mM | 1 mM |
| ADP | 100 $\mu$M | 200 $\mu$M |
| $P_i$ | 2 mM | 7 mM |
| PCr | 4 mM | < 1 mM |
| pH | 7·2 | 6·9 |
| $Mg^{2+}$ | 100 $\mu$M | 1 mM |
| $Ca^{2+}$ (basal) | 50 nM | 200 nM |

These values are representative of those reported in many studies. $P_i$, inorganic phosphate. PCr, phosphocreatine.

smooth muscle during hypoxia, i.e. does it fall low enough to cause significant channel opening? (see Table 2).

There are $K^+$ (and $Cl^-$) channels that are activated by a rise in $Ca^{2+}$ (Carl, Lee & Sanders, 1996; Mironneau, Arnaudeau, Macrez-Lepretre & Boittin, 1996; Large & Wang, 1996). Opening of both these channels can occur spontaneously, due to brief focal releases of $Ca^{2+}$ from the SR, referred to as $Ca^{2+}$ sparks, and can be enhanced by $Ca^{2+}$ entry. The resulting currents are referred to, respectively, as spontaneous transient outward currents (STOCS) associated with hyperpolarization and relaxation (Benham & Bolton, 1986; Nelson *et al.* 1995) and spontaneous transient inward currents (STICS) associated with depolarization and contraction (Large & Wang, 1996). Clearly if there is a significant alteration in STOC activity due to hypoxia affecting SR $Ca^{2+}$ release (see later) or calcium-activated $K^+$ current ($I_{K(Ca)}$), due, e.g. to the rise in basal $Ca^{2+}$ commonly observed (see Fig. 3*A*), then this could explain the fall of force in hypoxia. However, Large & Wang (1996) suggested that there is a heterogeneous distribution of $K^+$ and $Cl^-$ channels and that when $Ca^{2+}$ rises and activates both $I_{K(Ca)}$ and calcium-activated $Cl^-$ current ($I_{Cl(Ca)}$), depolarization is the net result. Little is known about the effect of hypoxia on these currents but in pulmonary vessels the $Cl^-$ current is activated and the $K^+$ current is inhibited, and depolarization is seen (Greenwood, Helliwell & Large, 1997). The effects of hypoxia on $K^+$ channels and hence membrane potential, will influence the gating of L-type $Ca^{2+}$ channels. It is also likely that hypoxia will have direct effects on $Ca^{2+}$ channels, and these effects will be discussed next.

**Calcium channels.** Before intracellular [$Ca^{2+}$] or $Ca^{2+}$ currents could be measured during hypoxia, there was good evidence that it reduced $Ca^{2+}$ entry into smooth muscle cells (van Breemen, Wuytack & Casteels, 1975; Pearce, Ashwal, Long & Cuevas, 1989). Recent more direct approaches have shown reductions in $Ca^{2+}$ currents and relaxation by cyanide, hypoxia or oxidation (Ohya & Sperelakis, 1989; Okashiro, Tokuno, Fukumitsu, Hayashi & Tomita, 1992; Chiamvimonvat *et al.* 1995; Urena *et al.* 1996; Rekalov, Juranek, Malekova & Bauer, 1997). The first two groups of authors consider the effect of hypoxia in reducing $Ca^{2+}$ current to be a direct one on the channel, i.e. low $O_2$ directly inhibits smooth muscle $Ca^{2+}$ channels. Interestingly the evidence for such effects has followed the demonstration that low oxygen inhibits $K^+$ currents in pulmonary myocytes, causing depolarization and $Ca^{2+}$ entry, which could explain

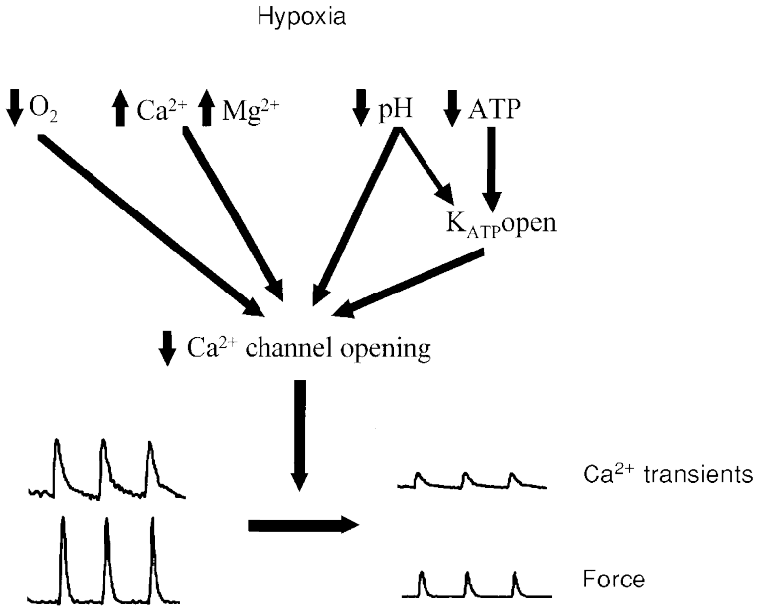


**Figure 2. A scheme to show the possible mechanism underlying the fall of phasic contractions in hypoxia**

Hypoxia reduces $O_2$, pH and ATP, and increases $Ca^{2+}$, $Mg^{2+}$ and ADP. These changes affect the opening of $Ca^{2+}$ channels, which underly the $Ca^{2+}$ transient.

hypoxic pulmonary contraction (Yuan, Goldman, Tod, Rubin & Blaustein, 1993; Franco-Obregon & Lopez-Barneo, 1996; Haddad & Jiang, 1997). An oxygen-sensitive $Ca^{2+}$ channel as a mechanism for vasodilatation is appealing, especially as it is rapidly effective, and sensitive to low oxygen in a dose-dependent manner and over the physiological range.

Reductions in $Ca^{2+}$ currents with secondary effects of hypoxia (e.g. changes in ATP, pH, $Ca^{2+}$ and $Mg^{2+}$) have also been reported; for example, in portal vein the $Ca^{2+}$ current was reduced when ATP was decreased (Lorenz & Paul, 1997) and acidification also reduces $Ca^{2+}$ current in many smooth muscle cells (Shmigol, Smith, Taggart, Wray & Eisner, 1995). Basal $Ca^{2+}$ is elevated during hypoxia in many smooth muscles (see Fig. 1*B*) and elevated $[Ca^{2+}]$ can also inhibit L-type $Ca^{2+}$ channels (Ohya *et al.* 1988). It is, of course, as Mg-ATP that ATP is found within the cell and a decrease in [ATP] will increase $[Mg^{2+}]$. McHugh & Beech (1996) suggested that, during blockade of oxidative metabolism, in basilar artery $Mg^{2+}$ block on the channels is responsible for the reductions in current seen. Interestingly they found no effect of inhibiting glycolysis on the inward current. Thus it differs from another report (Lorenz & Paul, 1997) which indicates a role for glycolysis in supporting $Ca^{2+}$ channel activity and directly altering $Ca^{2+}$ entry. Both studies provide a link between $Ca^{2+}$ channel function and energy production, although which mechanism predominates may well depend upon the particular smooth muscle.

Thus it appears that hypoxia, by both its direct effect on the $Ca^{2+}$ current and via secondary changes in other parameters, e.g. low pH inhibits gap junction conductance (Turin & Warner, 1980), will act to reduce the $Ca^{2+}$ current (see Fig. 2). These effects will also be reinforced by any increases in $K^+$ conductance produced by hypoxia, which will favour a decrease in L-type channel activity. The net result of these processes in phasic smooth muscles will be a decrease or abolition of the $Ca^{2+}$ transient in the cell and a decrease in force (Fig. 2). Consistent with this mechanism is the fact that if the decrease in membrane excitability is overcome, e.g. by application of high-$K^+$ depolarizing solution or agonist, then contraction, albeit of reduced magnitude, can be initiated in the continued presence of cyanide or hypoxia (Paul, 1989; Heaton *et al.* 1993). This makes it unlikely that the rapid effects of hypoxia on phasic force are due to critical changes in ATP, $pH_i$ or inorganic phosphate ($P_i$) around the myofilaments. These effects on the initiation of $Ca^{2+}$ transients, which are dependent upon electrical activity, may be distinct from those changes occurring in basal or steady-state $[Ca^{2+}]_i$ which are discussed next.

## What causes the rise in calcium?

As shown in Table 1, basal or steady-state $Ca^{2+}$ increases with hypoxia or cyanide in many smooth muscles. There is as yet no clear indication of the source of this. Several intracellular organelles are capable of storing and releasing $Ca^{2+}$ i.e. the SR, nucleus and mitochondria, and may therefore be considered likely candidates. All three organelles are often found close to one another within the cytosol (Broderick & Broderick, 1990; Nixon, Migneri & Somlyo, 1994), offering the possibility that $Ca^{2+}$ movements from each organelle may be important in co-ordinating $[Ca^{2+}]_i$ signalling, energy provision and gene expression with contractile activity.

**Sarcoplasmic reticulum**. The SR is the major intracellular storage site of releasable $Ca^{2+}$ for contractile activation (Somlyo & Somlyo, 1994). The SR is found throughout the cell and forms a continuous tubular network from regions closely apposed to the plasma membrane (peripheral SR) to deeper in the cell (central SR) and the perinuclear space (Nixon *et al.* 1994; Golivina & Blaustein, 1997; Arnaudeau, Boittin, Macrez, Lavie, Mironneau & Mironneau, 1997). $Ca^{2+}$ is accumulated in the SR by the action of a $Ca^{2+}$-ATPase. Agonist- and $IP_3$-dependent release of SR $Ca^{2+}$ can directly activate the contractile apparatus in the absence of trans-sarcolemmal $Ca^{2+}$ influx (Somlyo & Somlyo, 1990). Additionally, there is evidence for $[Ca^{2+}]_i$ amplification by ryanodine-sensitive $Ca^{2+}$-induced $Ca^{2+}$ release from some (Ganitkevich & Isenberg, 1992; Kamishima & McCarron, 1996; Arnaudeau *et al.* 1997; Kamishima & McCarron, 1997) but not all investigations (Ganitkevich & Isenberg, 1995; Kamishima & McCarron, 1996; Taggart & Wray, 1997*a*). Thus $Ca^{2+}$ released from the SR may play a role in smooth muscle contractility by directly activating the myofilaments. The direct influence of hypoxia or metabolic inhibition on SR $Ca^{2+}$ handling in smooth muscle has not been extensively studied. An early study (van Breemen *et al.* 1975) suggested that iodoacetate or dinitrophenol increased the fractional loss of $^{45}Ca^{2+}$ from intracellular stores. Ishida & Honda (1991) reported that $[Ca^{2+}]_i$ and tension responses to either noradrenaline or caffeine were inhibited by cyanide application. Indeed, conditions expected to occur with hypoxia such as decreased [ATP] and pH, and increased $Mg^{2+}$ and $[P_i]$ and altered cellular redox state, may lead to a reduction in SR-releasable $Ca^{2+}$ (Iino, 1991; Tsukioka, Iino & Endo, 1994; Kuemmerle & Makhlouf, 1995; Bootman, Missiaen, Parys, De Smedt & Casteels, 1995; Kosterin, Babich, Shlykov & Rovenets, 1996). Presently, however, there are few data to support a major role for the SR in either the rise in $Ca^{2+}$ or fall in force, during hypoxia.

**Mitochondria**. The mitochondrion is a high capacity, low affinity $Ca^{2+}$ storage site which several recent studies have suggested may play a role in removing $Ca^{2+}$ from the cytosol upon cell stimulation. In non-muscle cells, oxidizable substrates that increase mitochondrial membrane potential and respiration, and presumably $Ca^{2+}$ uptake, were found to alter the $IP_3$-induced $Ca^{2+}$ wave profile (Jouaville, Ichas, Holmuhamedov, Camacho & Lechlelter, 1995). In stomach smooth muscle cells, mitochondrial $Ca^{2+}$ increased upon SR $Ca^{2+}$ release by caffeine (Drummond & Fay, 1996). In addition, it has recently been reported that the decay of smooth muscle $[Ca^{2+}]_i$ transients, evoked by membrane depolarization, is impaired by a variety of agents interfering with mitochondrial function (Drummond & Fay, 1996; McGeown, Drummond, McCarron & Fay, 1996).

Furthermore, inhibition of oxidative phosphorylation slowed the time course of decay of $Ca^{2+}$-activated chloride currents elicited by membrane depolarization, again suggesting that $Ca^{2+}$ signals may be regulated by mitochondrial uptake mechanisms (Greenwood *et al.* 1997).

$Ca^{2+}$ accumulation by the mitochondria may be a means of limiting cytosolic $Ca^{2+}$ increases and thereby protecting against deleterious protease activity. It may also be a mechanism linking energy supply to demand as $Ca^{2+}$ has been implicated in the regulation of several key mitochondrial enzymes (Broderick & Broderick, 1990). Organellar swelling, and increased $Ca^{2+}$ leakiness, with mitochondrial inhibition has been reported (Somlyo & Somlyo, 1990; Piper, Null & Siegmund, 1994) and may therefore result in several important consequences for the smooth muscle cell including contributing to the rise in steady-state $Ca^{2+}$ both directly, and indirectly by activation of depolarizing currents (Greenwood *et al.* 1997).

**Nucleus.** Evidence from a large number of cell types, including smooth muscle, indicates that the nucleus can also act as a $Ca^{2+}$ store. Changes in smooth muscle nuclear $Ca^{2+}$ concentration ($[Ca^{2+}]_n$) following depolarization or agonist stimulation have been reported (Williams, Becker & Fay, 1987; Burnier, Centeno, Burki & Brunner, 1994; Bkaily *et al.* 1997). It has been suggested that $IP_3$ receptors are located on the inner surface of the nuclear envelope

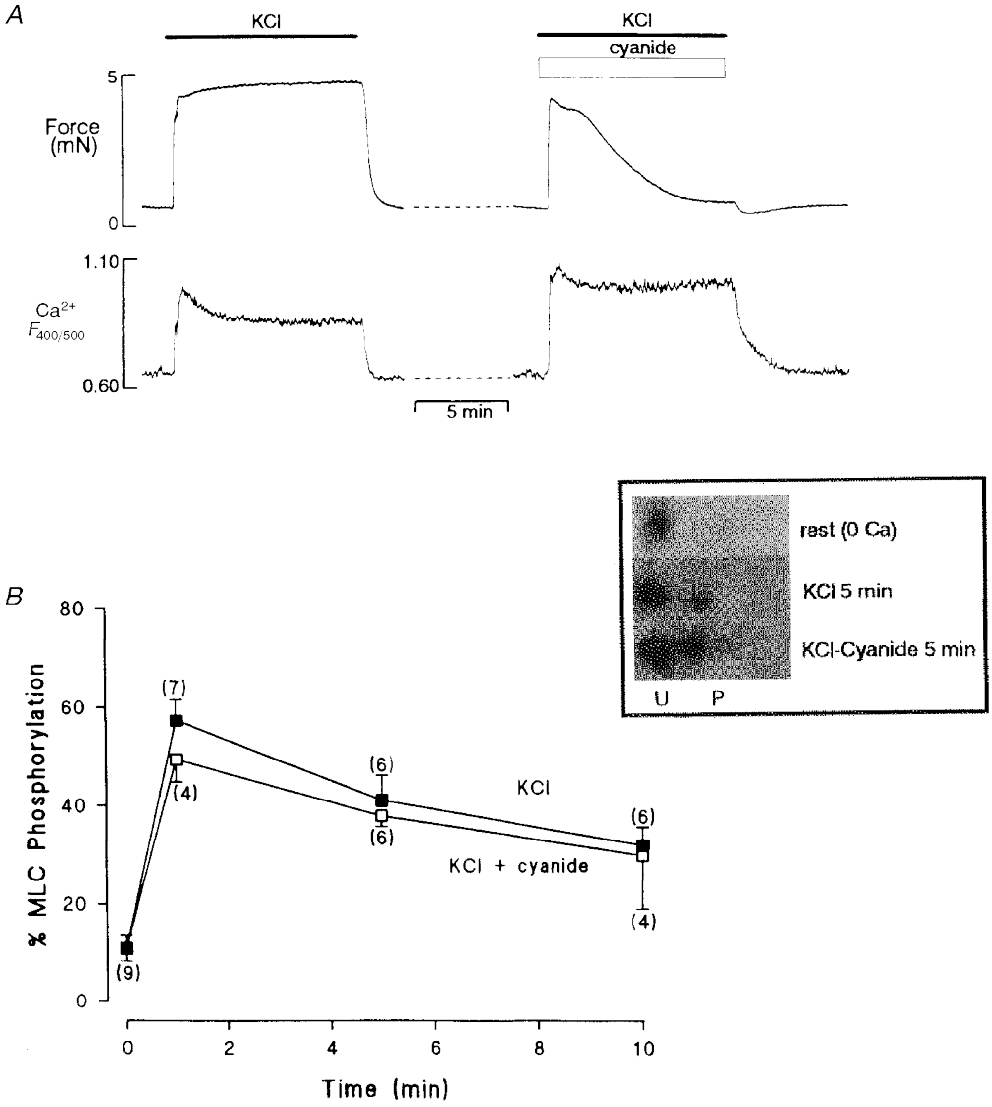


**Figure 3. Changes in uterine force and myosin phosphorylation with cyanide**

*A*, simultaneous force (top) and $Ca^{2+}$ (bottom) records showing the effect of hypoxia on a depolarized rat uterine preparation. Note the increased $Ca^{2+}$ record in hypoxia, but the fall of force. *B*, MLC phosphorylation measurements during contraction for 10 min produced by high $K^+$, in the presence and absence of hypoxia. There was no significant difference in the amount of MLC phosphorylation at any time point; mean data from densiotometric scans of silver stained 2-D gels (see inset). U, unphosphorylated; P, phosphorylated. Figure adapted from Taggart *et al.* 1997.

membrane (Gerasimenko, Gerasimenko, Tepikin & Petersen, 1995) such that any agonist-induced release of stored $Ca^{2+}$ will be directed into the nucleoplasm (Fay, Shlevin, Granger & Taylor, 1979; Himpens, De Smedt & Casteels, 1994; Gerasimenko *et al.* 1995). Although the SR forms a continuous tubular network with the perinuclear space, $Ca^{2+}$ may not be stored homogeneously throughout this region (Golivina & Blaustein, 1997). Given a dual role in $Ca^{2+}$ homeostasis and gene expression, the nucleus may perform crucial functions in the cellular responses to hypoxia, e.g. regulation of transcription of genes encoding carbohydrate metabolism (Bunn & Poyton, 1996). We can, however, find no study examining the contribution of nuclear $Ca^{2+}$ to changes in cytosolic $Ca^{2+}$ found in hypoxia. We will now discuss the possible causes of the reduction of force in hypoxia when $[Ca^{2+}]$ is not reduced.

### Altered $[Ca^{2+}]$–force relationship during hypoxia

In a variety of different smooth muscles hypoxia, or metabolic inhibition, has resulted in a significant reduction in force, but no change or increases in $[Ca^{2+}]_i$ either at rest or in activated tissues (see Table 1 for references) as illustrated in Figs 1*B* and 3*A*. These studies thus led to the conclusion that hypoxia can produce a dissociation of the $[Ca^{2+}]_i$–force relationship in smooth muscle (Taggart *et al.* 1997) and indirectly supported earlier observations that raising extracellular $Ca^{2+}$ under such conditions did not prevent hypoxic-induced reductions in force (Ishida & Paul, 1990). Therefore we will now review which other factors may be important in causing the reduction of force.

**Myosin light chain (MLC) phosphorylation**. As previously described the phosphorylation of MLCs is a critical determinant of smooth muscle force production (Somlyo & Somlyo, 1994). As hypoxia or metabolic inhibition result in a reduction of cytosolic [ATP], it is possible that the phosphorylation potential of the tissue declines, especially as $MLC_{20}$ phosphorylation has been suggested to account for up to 50% of the ATP cost associated with contraction (Wingard, Paul & Murphy, 1994). However, the findings of recent studies suggest that neither mitochondrial inhibition, nor hypoxia, result in significant changes in levels of $MLC_{20}$ phosphorylation (Taggart *et al.* 1997; Obara, Bowman, Ishida & Paul, 1997; and see Fig. 3*B*). Thus, altered smooth muscle metabolism results in a dissociation of both the $[Ca^{2+}]_i$–force and $MLC_{20}$–force relationships, as shown in Fig. 3.

**Myosin ATPase activity**. The above experiments and Fig. 3 illustrate that the fall in force with altered metabolism is unrelated to the phosphorylation of $MLC_{20}$. As cytosolic [ATP] during hypoxia or as a result of cyanide addition remains $> 1$ mM (Wray, 1990), in the absence of microdomains of extremely low ATP, it is unlikely that [ATP] surrounding the myofilaments is limiting the myosin ATPase (Arner & Hellstrand, 1985) and hence contraction. Furthermore as creatine kinase is localized to smooth muscle myofilaments it will also help maintain ATP : ADP (Clark, Khuchua, Kuznetsov, Saks & Ventura-Clapier, 1993; Clark & Dillon, 1995). Several studies are thus in agreement that energy supply to the myofilaments is not inhibiting force during hypoxia (Hardin, Wiseman & Kushmerick, 1992; Harrison, Larcombe-McDouall, Earley & Wray, 1994; Clark & Dillon, 1995; Nakayama *et al.* 1997; Taggart *et al.* 1997).

**Intracellular pH**. Significant decreases in intracellular pH occur with hypoxia and metabolic inhibition and often can be correlated with an increase in lactate production and efflux (Vogel, Lilja & Hellstrand, 1983; Wray, 1990; Ishida & Paul, 1990; Harrison *et al.* 1994; Taggart & Wray, 1995). For example, both *in vivo* and *in vitro* uterine hypoxia cause $pH_i$ to fall by around 0·3 pH units and lactate efflux to increase fivefold (Wray, 1990). Hypoxia and ATP depletion both inhibit $Na^+$–$H^+$ exchange, which would also contribute to a reduction in $pH_i$ (Burns, Homma & Harris, 1991; Noel & Pouyssegur, 1995). Decreases in $pH_i$ with metabolic inhibition were suggested to contribute to impaired mechanical output especially in spontaneously active tissues (Wray, 1990). However, altered $pH_i$ appears not to be the prime factor responsible for force inhibition for several reasons: (a) when cyanide-induced $pH_i$ changes are removed, force is still attenuated (Taggart & Wray, 1995); (b) the tonic force of many smooth muscles is often promoted by intracellular acidification (Taggart *et al.* 1996); (c) pH-induced alterations in $Ca^{2+}$-activated force of permeabilized smooth muscles cannot explain hypoxic-induced force inhibition (Crichton, Taggart, Wray & Smith, 1993; Wu & Vaughan-Jones, 1994); and (d) hypoxia can result in force inhibition without concomitant reductions in $pH_i$ (Aalkjaer & Lombard, 1995; Obara *et al.* 1997).

**Inorganic phosphate ($P_i$)**. Hypoxic-induced elevations of $[P_i]$ have been reported in both *in vivo* and *in vitro* situations (Ishida & Paul, 1990; Harisson *et al.* 1994). $P_i$ inhibits $Ca^{2+}$-activated force of permeabilized smooth muscle fibres (Itoh, Kanmura & Kuriyama, 1986; Crichton *et al.* 1993) independently of the level of $MLC_{20}$ phosphorylation (Gagelman & Guth, 1987; Osterman & Arner, 1995). $P_i$ also accelerates the relaxation from rigor upon photolysis of caged ATP, even in the presence of increased [MgADP], an effect attributable to $P_i$ driving the actomyosin equilibrium to a condition favouring weakly attached or detached cross-bridges (Somlyo & Somlyo, 1990; Nishiye, Somlyo, Torok & Somlyo, 1993). Thus, as suggested by Taggart *et al.* (1997), the hypoxia-induced increase in $[P_i]$ appears to be a suitable candidate for modulating force in the face of elevated [MgADP] (which would promote the lifespan of attached cross-bridges; Nishiye *et al.* 1993) and unaltered MLC phosphorylation. Indeed, when the increases in $[P_i]$ with altered metabolism were reduced, the resultant inhibition of force (Ishida & Paul, 1990), and rates of relaxation (Hardin *et al.* 1992; Taggart & Wray, 1997*c*), were also less. The absolute (Hardin *et al.* 1992) reduction in $Ca^{2+}$-activated force of permeabilized smooth muscle with elevated $[P_i]$, however, is less than the inhibition of force in intact arteries

during metabolic alteration (e.g. compare uterine smooth muscle studies of Crichton *et al.* 1993 and Taggart *et al.* 1997). It is unlikely, therefore, that $[P_i]$ changes alone can account for tonic force attenuation with metabolic inhibition.

**Cytoskeleton**. An additional possible force-modulatory consequence of hypoxia/metabolic inhibition of smooth muscle, especially as $[Ca^{2+}]_i$ remains elevated, is the activation of $Ca^{2+}$-dependent actin-capping proteins and/or proteases (Gailly, Lejuene, Capony & Gillis, 1990) which have been suggested to contribute to attenuated cardiac contractility following ischaemic reperfusion (Gao, Liu, Mellgren & Marban, 1995). Consistent with this notion are the findings that actin-capping agents inhibit tonic smooth muscle contraction independently of $[Ca^{2+}]_i$ and $MLC_{20}$ phosphorylation (Hori, Saito, Shin, Ozaki, Fusetani & Karaki, 1993; Obara & Yabu, 1994). Additionally, the extent of force inhibition is similar to that observed during mitochondrial inhibition (Taggart *et al.* 1997). Changes in the cytoskeleton will also affect ion channel activity (Hilgemann, 1997) and therefore also influence the response to hypoxia.

## Conclusions

There are many potential regulatory sites of the excitation–contraction coupling pathway in smooth muscle during conditions of altered metabolism. Hypoxia and cyanide can inhibit force production either with or without altering the profile of the activating $[Ca^{2+}]$. Hypoxic changes of plasma membrane channel activity contribute to reduced contractility. We propose that in phasic smooth muscles decreased electromechanical coupling and reduction of the $Ca^{2+}$ transient are responsible for the decline in spontaneous force seen in hypoxic conditions. We further speculate that this is predominantly a direct effect on the $Ca^{2+}$ channels, rather than via an increase in $K^+$ conductance. In tonically active smooth muscle, a dissociation between $Ca^{2+}$ and force is commonly encountered with hypoxia and cyanide. The decrease in force appears not to be due to limitation of ATP around the myofilaments. We suggest rather, that a combination of the factors, including increased $[P_i]$, acting directly on the myofilaments is the most likely cause. The SR, nucleus and mitochondria may play a wider role in smooth muscle $Ca^{2+}$ homeostasis than previously appreciated, and their involvement in the acute and chronic changes occurring with hypoxia pose many intriguing questions for smooth muscle homeostasis.


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**Corresponding author**

M. J. Taggart: Physiology University of Liverpool, Liverpool L69 3BX, UK.

Email: m.j.taggart@liverpool.ac.uk