Topical Review

Rhythmicity in arterial smooth muscle

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Many arteries and arterioles exhibit rhythmical contractions which are synchronous over considerable distances. This vasomotion is likely to assist in tissue perfusion especially during periods of altered metabolism or perfusion pressure. While the mechanism underlying vascular rhythmicity has been investigated for many years, it has only been recently, with the advent of imaging techniques for visualizing intracellular calcium release, that significant advances have been made. These methods, when combined with mechanical and electrophysiological recordings, have demonstrated that the rhythm depends critically on calcium released from intracellular stores within the smooth muscle cells and on cell coupling via gap junctions to synchronize oscillations in calcium release amongst adjacent cells. While these factors are common to all vessels studied to date, the contribution of voltage-dependent channels and the endothelium varies amongst different vessels. The basic mechanism for rhythmical activity in arteries thus differs from its counterpart in non-vascular smooth muscle, where specific networks of pacemaker cells generate electrical potentials which drive activity within the otherwise quiescent muscle cells.

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Spontaneous, rhythmical contractions are generated in many different types of smooth muscle, from the gastrointestinal tract, urinary tract and lymphatic vessels through to arteries and veins (Tomita, 1981; Van Helden, 1993; Hashitani *et al.* 1996). In blood vessels, this activity, known as vasomotion, occurs in small resistance vessels of the microcirculation, as well as in larger arteries both *in vivo* and *in vitro* (see Shimamura *et al.* 1999; Nilsson & Aalkjaer, 2003 for details). While rhythmicity in non-vascular smooth muscles is often propagated, serving to actively move intraluminal contents in a peristaltic fashion, rhythmicity in vascular smooth muscle is apparently synchronous over considerable lengths of arteries. Vasomotion is thus expected to increase flow as its amplitude increases, in turn resulting in a decrease in vascular resistance (Funk *et al.* 1983; Meyer *et al.* 2002). In this case vasomotion may be seen to be beneficial and its up-regulation during pathological conditions, such as hypertension, may be considered to be protective. However the effect of vasomotion on vascular resistance is currently controversial (Gratton *et al.* 1998; Meyer *et al.* 2002) and hence its physiological significance is yet to be clearly defined.

Vasomotion occurs in arteries *in vitro* either spontaneously or in response to pressure, stretch, application of vasoconstrictor agonists or increases in extracellular potassium concentration (Duling *et al.* 1981; Hayashida *et al.* 1986; Katusic *et al.* 1988; Chemtob *et al.* 1992; Gustafsson, 1993; Lee & Earm, 1994; Stork & Cocks, 1994; Porret *et al.* 1995; Eddinger & Ratz, 1997; Hill *et al.* 1999). Since many studies have described a critical role for voltage-dependent calcium channels (VDCCs; Colantuoni *et al.* 1984; Hayashida *et al.* 1986; Hundley *et al.* 1988; Fujii *et al.* 1990; Chemtob *et al.* 1992; Omote *et al.* 1992; Gustafsson, 1993; Omote & Mizusawa, 1993, 1996; Burt, 2003; Hessellund *et al.* 2003; Takenaka *et al.* 2003) and contractions are preceded by oscillations in membrane potential (Hayashida *et al.* 1986; Segal & Beny, 1992; Gustafsson, 1993; Gokina *et al.* 1996; Hill *et al.* 1999; Bartlett *et al.* 2000; Haddock & Hill, 2002; Oishi *et al.* 2002), the traditional view of the underlying mechanism was one of a voltage-dependent membrane oscillator, analogous to that in the heart. However, more recent studies have shown that oscillations in the intracellular concentration of calcium ($[Ca^{2+}]_i$) also precede rhythmical contractions. Moreover these oscillations result from release of Ca^{2+} from intracellular IP_3 stores in all forms of rhythmicity studied to date (Mauban *et al.* 2001; Peng *et al.* 2001; Schuster *et al.* 2001; Haddock & Hill, 2002; Haddock *et al.* 2002; Sell *et al.* 2002; Lamboley *et al.* 2003; Filosa *et al.* 2004;

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Lamont & Wier, 2004; Mauban & Wier, 2004; Shaw *et al.* 2004). Thus the current view of vasomotion is that release of Ca^{2+} from IP_3 stores is essential and a regenerative mechanism of Ca^{2+}-induced Ca^{2+} release, involving either IP_3 or ryanodine receptors, establishes the oscillation in $[Ca^{2+}]_i$. Such a mechanism is sufficient in some vessels, while in others, there is the additional involvement of VDCCs, with or without a negative feedback pathway mediated by Ca^{2+}-activated potassium channels. These various mechanisms differ from those considered to explain rhythmicity in non-vascular smooth muscle where quiescent muscle cells are driven by the activity of specific pacemaker cells.

Calcium signalling in vascular smooth muscle

Recent advances in imaging technology have enabled the study of changes in $[Ca^{2+}]_i$ in individual smooth muscle cells (SMCs). This has led to the identification of localized intracellular Ca^{2+} signalling events, the most common two being Ca^{2+} sparks and Ca^{2+} waves. The former are highly localized, transient increases in Ca^{2+}, which occur in both isolated SMCs and intact arteries and are due to release of Ca^{2+} from ryanodine receptors (Nelson *et al.* 1995; Jaggar *et al.* 1998*a*; Miriel *et al.* 1999). Paradoxically, Ca^{2+} sparks lead to membrane hyperpolarization, decreased $[Ca^{2+}]_i$ and relaxation through the activation of large conductance Ca^{2+}-sensitive potassium channels (BK_{Ca}; Nelson *et al.* 1995; Jaggar *et al.* 1998*a*,*b*; Perez *et al.* 2001; Zhuge *et al.* 2002) which produce spontaneous transient outward currents (Benham & Bolton, 1986).

Ca^{2+} waves are also transient rises in $[Ca^{2+}]_i$ which start from a specific region of the cell and are propagated along its length in a wave-like manner (Neylon *et al.* 1990; Wier & Blatter, 1991; Mayer *et al.* 1992). In contrast to Ca^{2+} sparks, Ca^{2+} waves have the potential to contribute to global cellular events since they are propagated over distance without decrement (Iino, 1999; McCarron *et al.* 2004). Thus, waves are thought to occur due to the release of Ca^{2+} from intracellular stores following activation of IP_3 receptors with the possible involvement of ryanodine receptors, since both of these channels are capable of being regulated positively and negatively by Ca^{2+} (Hagar *et al.* 1998; Wojcikiewicz & Luo, 1998; Iino, 1999; McCarron *et al.* 2004). When $[Ca^{2+}]_i$ is integrated over an entire cell with time, these Ca^{2+} waves appear as rhythmical oscillations in $[Ca^{2+}]_i$.

In 1994, Iino and colleagues observed that asynchronous Ca^{2+} waves appeared in adjacent SMCs of the rat tail artery when it was stimulated by sympathetic nerves or by the application of a vasoconstrictor agonist. Since the oscillations were abolished by caffeine and ryanodine, they were considered to be due to the release of Ca^{2+} from intracellular stores, although extracellular Ca^{2+} influx was critical for their maintenance (Iino *et al.* 1994). Ca^{2+} waves have subsequently been reported in other vascular beds, particularly following agonist application (Miriel *et al.* 1999; Jaggar & Nelson, 2000; Ruehlmann *et al.* 2000; Lee *et al.* 2001; Mauban *et al.* 2001; Peng *et al.* 2001; Sell *et al.* 2002; Lamboley *et al.* 2003; Shaw *et al.* 2004). Thus, low concentrations of agonist induce asynchronous Ca^{2+} waves which have little effect on changes in $[Ca^{2+}]_i$ across the arterial wall or on tension. However, with increasing agonist concentration, the frequency of Ca^{2+} waves increases, as does the number of responding cells (Iino *et al.* 1994; Miriel *et al.* 1999; Jaggar & Nelson, 2000; Ruehlmann *et al.* 2000; Mauban *et al.* 2001) and, in some arterial preparations, oscillations in $[Ca^{2+}]_i$ in SMCs across the vascular wall become synchronized giving rise to global oscillations in $[Ca^{2+}]_i$ and vasomotion (Mauban *et al.* 2001; Peng *et al.* 2001; Sell *et al.* 2002; Lamboley *et al.* 2003; Shaw *et al.* 2004). While tonic exogenous application of high concentrations of vasoconstrictor agonists may be considered to be unphysiological, vasomotion has also been observed spontaneously in basilar arteries and irideal arterioles *in vitro* and this activity is similarly dependent on synchronized oscillations in $[Ca^{2+}]_i$ in SMCs resulting in global oscillations in $[Ca^{2+}]_i$ across the vascular wall (Haddock & Hill, 2002; Haddock *et al.* 2002). In all of these cases, synchronization of $[Ca^{2+}]_i$ oscillations in adjacent cells occurs as a result of cell to cell coupling via gap junctions (Koenigsberger *et al.* 2004).

The voltage-independent coupled oscillator model

Inhibitors of intracellular Ca^{2+} release rapidly abolish vasomotion (Gustafsson *et al.* 1994; Hill *et al.* 1999; Bartlett *et al.* 2000; Peng *et al.* 2001; Haddock & Hill, 2002; Haddock *et al.* 2002; Burt, 2003; Takenaka *et al.* 2003) and the underlying Ca^{2+} waves in individual SMCs in all vessels examined (Peng *et al.* 2001; Haddock & Hill, 2002). On the other hand, the differential effect of inhibitors of VDCCs suggests that there is more than one mechanism in operation. The simplest mechanism is that found underlying the spontaneous vasomotion of irideal arterioles (Hill *et al.* 1999; Haddock *et al.* 2002). In these arterioles, rhythmical depolarizations precede the slow rhythmical contractions (Fig. 1*A*) as they do in other vessels; however, vasomotion is completely insensitive to inhibitors of VDCCs and the most negative membrane potential is relatively hyperpolarized at around −60 mV (Fig. 1*A*; Hill *et al.* 1999; Haddock *et al.* 2002). Moreover, voltage clamp of short segments of these vessels at a range of membrane potentials confirms that the underlying currents and vasomotion are independent of changes in membrane potential (Hill *et al.* 1999). In contrast, Ca^{2+} oscillations and vasomotion depend critically on interaction between the phospholipase C pathway and the phospholipase A2 pathway via protein kinase C (Haddock *et al.* 2002). Metabolites of arachidonic acid produced by the lipoxygenase (LOX) pathway are proposed to further stimulate the PLC pathway resulting in cyclical

oscillations in $[Ca^{2+}]_i$ due to the biphasic regulation of the IP_3 receptor by Ca^{2+} (Fig. 2; Bootman *et al.* 2001). In this case, the oscillations in membrane potential are likely to be a consequence of the oscillations in $[Ca^{2+}]_i$ rather than being caused by them. In iris arterioles, then, coupling of the individual oscillators must result from the movement of second messenger molecules through gap junctions rather than an electrical current. Interestingly, modelling of a population of coupled SMCs has demonstrated that even a weak Ca^{2+} permeability between cells is sufficient to produce synchronization of Ca^{2+} oscillations (Koenigsberger *et al.* 2004).

Voltage-dependent models

In contrast to irideal arterioles, inhibitors of VDCCs abolish vasomotion in most other arteries and also reduce or abolish the oscillations in membrane potential which precede rhythmical contractions (Gokina *et al.* 1996; Bartlett *et al.* 2000; Haddock & Hill, 2002; Oishi *et al.* 2002). These antagonists also desynchronize Ca^{2+} waves in adjacent SMCs (Miriel *et al.* 1999; Peng *et al.* 2001; Haddock & Hill, 2002). Thus VDCCs are critical for both voltage oscillations and the synchronization of Ca^{2+} waves between adjacent cells. Unfortunately most studies only measure either membrane potential changes or changes in $[Ca^{2+}]_i$ and few studies have systematically studied the ionic mechanisms underlying voltage oscillations and correlated these with the appearance and synchronization of oscillations in $[Ca^{2+}]_i$ in individual SMCs. While the largest amount of data has been amassed from studies on rat mesenteric arteries, considerable discrepancies exist in the results obtained by different groups. These differences may be attributed to different physiological conditions, for example pressurization *versus* tension. However, further confusion may have arisen due to the use of different sized segments of the mesenteric artery, as heterocellular coupling along these vessels increases as vessel size decreases (Sandow & Hill, 2000). Consequently, the reader should be aware that the conclusions reached in the present review, with the exception of the mechanism existing in the basilar artery, where both electrophysiological and imaging techniques have been used in the same study, are often based on observations made by different groups and sometimes in different vessels.

In vessels exhibiting spontaneous vasomotion, with the exception of the irideal arterioles, the membrane potential of the vascular SMCs is depolarized, the most negative potential being between −40 and −50 mV (Fig. 1*B*; Hayashida *et al.* 1986; Gokina *et al.* 1996; Bartlett *et al.* 2000; Haddock & Hill, 2002). Some vessels, like the rat mesenteric artery, are resistant to vasomotion and require induction with an agonist like phenylephrine. These arteries exhibit more hyperpolarized resting membrane potentials of around −60 mV when quiescent; however, when stimulated with a vasoconstrictor, they depolarize and initiate vasomotion (Mulvany *et al.* 1982; Yoshinaka & Uchida, 1986; Gustafsson, 1993; Gokina *et al.* 1996; Oishi *et al.* 2002). These data suggest that a depolarizing current is activated throughout the muscle and this in turn leads to the opening of VDCCs, calcium influx and synchronization of Ca^{2+} waves in individual SMCs through Ca^{2+}-induced Ca^{2+} release.

In support of this proposal, drugs which interfere with the IP_3 pathway or intracellular stores abolish spontaneous vasomotion and hyperpolarize the cell membrane (Bartlett *et al.* 2000; Haddock & Hill, 2002). In mesenteric arteries, the activation of a novel cGMP and Ca^{2+}-dependent chloride current (Matchkov *et al.* 2004*a*; Piper & Large, 2004) has been proposed, since inhibition of nitric oxide (NO) with L-NAME abolishes vasomotion in this bed and cGMP activates vasomotion in endothelium-denuded arteries (Gustafsson, 1993; Peng *et al.* 2001; Matchkov *et al.* 2004*a*). The increase in cGMP occurs following release of NO from the endothelium (Peng *et al.* 2001). In other vessels NO is unlikely to have this effect since

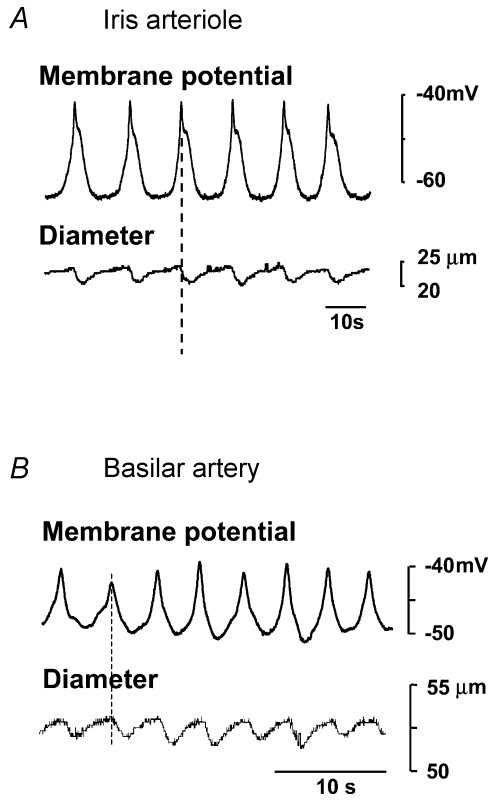

Figure 1. Membrane potential oscillations underlying vasomotion in rat irideal arterioles and basilar arteries
While depolarizations precede constrictions in both vessels, the most negative membrane potential reached in the iris is less than −60 mV while that in the basilar is considerably more positive and less stable. Note also that the oscillations in the iris are slower than those in the basilar artery. Part *B* reproduced with permission of Blackwell Publishing from Haddock & Hill (2002).

vasomotion is augmented following its inhibition. Thus, in the basilar artery traditional Ca^{2+}-dependent chloride channels (Cl_{Ca}; Large & Wang, 1996) have been suggested to underlie the depolarization, since antagonists of Cl_{Ca} abolish membrane potential oscillations and contractions and hyperpolarize SMCs (Haddock & Hill, 2002; Haddock *et al.* 2002). However these data are complicated by additional depressant effects of these Cl_{Ca} inhibitors on oscillations of $[Ca^{2+}]_i$ (Haddock & Hill, 2002; Haddock *et al.* 2002).

An alternative candidate for the depolarization responsible for synchronization and vasomotion is the Na^+–K^+ ATPase, an electrogenic membrane-bound enzyme, since inhibition of this pump with ouabain has been reported to block vasomotion (Gustafsson, 1993). However, other authors have suggested that these effects are non-specific, since ouabain depolarizes the cell membrane causing constriction (Oishi *et al.* 2002) and can inhibit gap junctions and cellular coupling (Harris *et al.* 2000). Interestingly, recent studies in the rat cerebral artery have demonstrated a critical role for TRPC6 and TRPM4 channels in pressure-induced SMC depolarization and vasoconstriction (Welsh *et al.* 2002; Earley *et al.* 2004). It is therefore possible that subtypes of TRP channels, such as TRPM4, which is activated by Ca^{2+} (Beech, 2005), may contribute to the depolarizing current in rhythmically active blood vessels; however, no data are currently available.

The voltage-dependent coupled oscillator model

While voltage-dependent models clearly rely on a depolarization and the involvement of VDCCs, two different models can be delineated on the basis of the hyperpolarizing phase of the membrane potential oscillation. In the voltage-dependent coupled oscillator model, which has been proposed to be responsible for agonist-induced vasomotion in mesenteric arteries (Peng *et al.* 2001), the basis for the oscillating membrane potential is proposed to be the $[Ca^{2+}]_i$ oscillation shaped by the Ca^{2+} sensitivity of the intracellular stores, as described earlier. This in turn activates an oscillating depolarization mediated via a cGMP and Ca^{2+}-dependent chloride channel (Fig. 3). The spread of current through gap junctions leads to the opening and closing of VDCCs and the synchronization of $[Ca^{2+}]_i$ oscillations in adjacent cells through Ca^{2+}-induced Ca^{2+} release without the involvement of other membrane ion channels.

The voltage-dependent membrane oscillator model

In the voltage-dependent membrane oscillator model, the membrane potential oscillation does not simply reflect

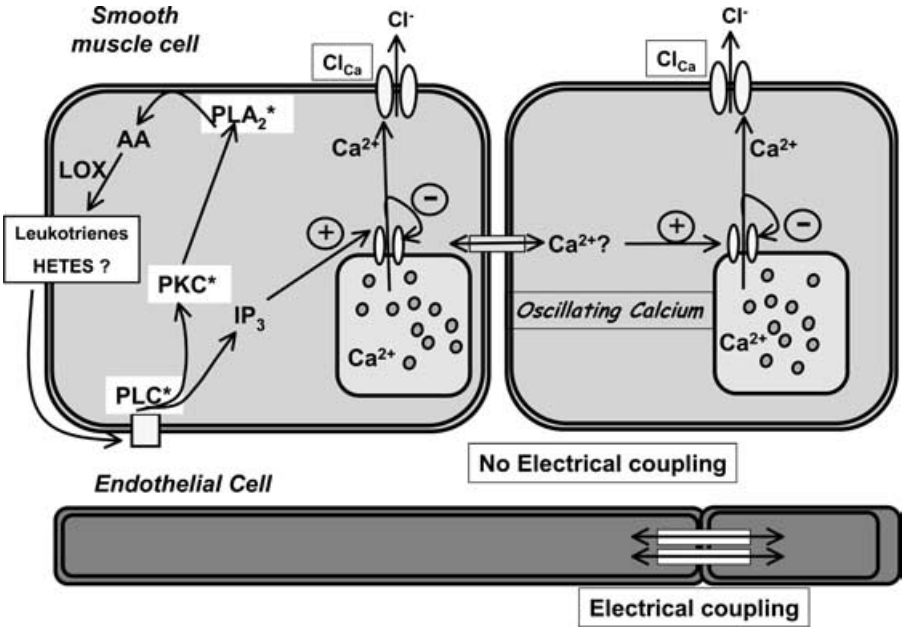

Figure 2. The voltage-independent coupled oscillator in iris arterioles
Constitutive activity of phospholipase C (PLC) leads to the production of IP_3 and basal release of Ca^{2+} from the IP_3-sensitive Ca^{2+} store. Simultaneous activation of protein kinase C (PKC) stimulates phospholipase A_2 (PLA_2) and breakdown of arachidonic acid via the lipoxygenase (LOX) pathway. The resultant metabolites further stimulate the PLC pathway resulting in cyclical oscillations in $[Ca^{2+}]_i$ due to the biphasic regulation of the IP_3 receptor by Ca^{2+}. This also produces coincident cyclical depolarizations of the cell membrane through opening of a Ca^{2+}-dependent chloride channel (Cl_{Ca}). Synchronization results from passage of Ca^{2+} through gap junctions. Voltage-dependent Ca^{2+} channels are not activated.

the oscillation in $[Ca^{2+}]_i$ but rather the alternate opening and closing of VDCCs and potassium (K^+) channels. Nevertheless, the membrane potential oscillation is still absolutely dependent on $[Ca^{2+}]_i$, which is required to initiate the depolarization necessary to activate the VDCCs.

A number of mechanical studies measuring tension or contraction have demonstrated mandatory or modulatory roles for K^+ channels in vasomotion in a variety of vascular beds, including mesenteric and cerebral vessels (Jackson, 1988; Fujii *et al.* 1990; Gustafsson *et al.* 1994; Omote & Mizusawa, 1995, 1996; Huang & Cheung, 1997; Hempelmann *et al.* 1998; Dora *et al.* 2000; Okazaki *et al.* 2003; Mauban & Wier, 2004). However, few investigations have examined the role of K^+ currents during vasomotion using electrophysiology and consequently the precise role of K^+ channels in vasomotion is unknown in many arteries. However in hamster cheek pouch arterioles *in vivo*, TEA, a non-specific inhibitor of K_{Ca} channels, abolishes vasomotion, depolarizes SMCs and decreases the amplitude and increases the frequency of rhythmical depolarizations (Bartlett *et al.* 2000). In basilar arteries, a similar effect accompanied by vasoconstriction was seen after selective inhibition of intermediate conductance K_{Ca} channels, but not after inhibition of BK_{Ca}, small conductance K_{Ca} or voltage-activated K channels (Haddock & Hill, 2002). These results suggest that specific K_{Ca} channels might contribute to the hyperpolarizing and relaxing phase of the membrane potential oscillations following Ca^{2+} influx through VDCCs during the depolarizing phase. This was confirmed in basilar arteries when application of nifedipine, to block VDCCs, abolished vasomotion and all voltage oscillations and depolarized the membrane (Haddock & Hill, 2002). Thus the intracellular Ca^{2+} waves give rise to a depolarization on which is superimposed a voltage oscillation involving sequential activation of VDCCs and K_{Ca} channels (Fig. 4; Haddock & Hill, 2002). The persistence of small membrane potential oscillations and irregular contractile activity after inhibition of K_{Ca} channels (Haddock & Hill, 2002) reflects the underlying oscillations in $[Ca^{2+}]_i$, which are still synchronized in the presence of Ca^{2+} influx through VDCCs. When these VDCCs are closed, the oscillations in $[Ca^{2+}]_i$ and the resulting membrane potential oscillations are no longer synchronous and the latter disappear due to electrical coupling (Haddock & Hill, 2002).

In the voltage-dependent coupled oscillator model, oscillations in $[Ca^{2+}]_i$ produce an oscillating depolarizing current which is amplified by Ca^{2+} entry through VDCCs (Peng *et al.* 2001), with little involvement of potassium channels (Fig. 3). Indeed, Peng and colleagues have shown that depolarization by current injection can produce vasomotion in vessels primed with a low concentration of agonist, that would normally only induce asynchronous Ca^{2+} waves in individual SMCs (Peng *et al.* 2001). It is not known whether a similar depolarizing current would initiate vasomotion in quiescent vessels in the absence of agonist to provide an oscillation in the $[Ca^{2+}]_i$. In contrast, in the voltage-dependent membrane oscillator model, it would be expected that depolarization, even in the absence of intracellular Ca^{2+} waves, should initiate vasomotion (Fig. 4).

Role of ryanodine stores

Ryanodine has been reported to abolish Ca^{2+} waves in a number of arteries suggesting that amplification occurs through Ca^{2+}-induced Ca^{2+} release from ryanodine

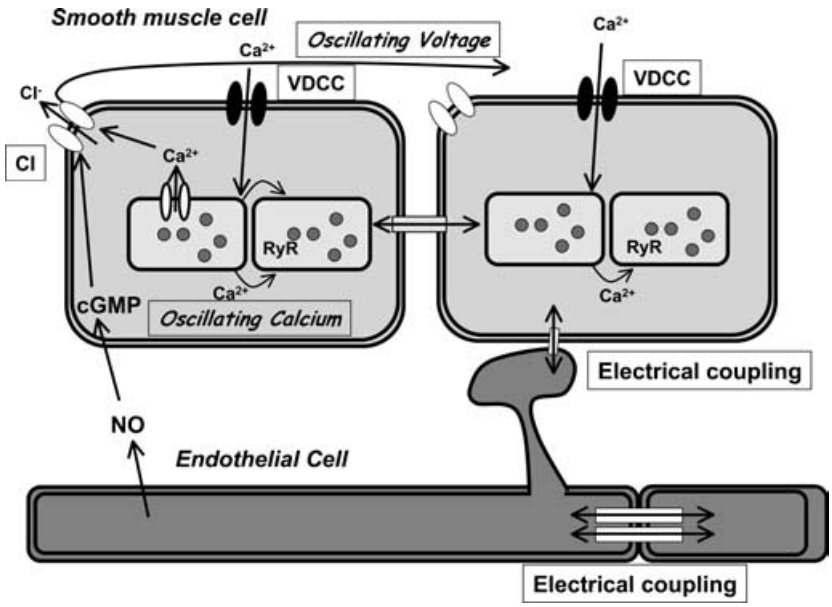

Figure 3. The voltage-dependent coupled oscillator in rat small mesenteric arteries
Application of agonist induces Ca^{2+} release from intracellular stores. This is oscillatory and, in combination with cGMP, produced by the action of endothelially derived nitric oxide (NO), activates a Ca^{2+}- and cGMP-dependent chloride channel (Cl) which intermittently depolarizes the membrane potential. If sufficient cells depolarize together the depolarization opens voltage-dependent Ca^{2+} channels (VDCC) causing extracellular Ca^{2+} influx into the cells. As the cells are all electrically coupled, this Ca^{2+} entry is simultaneous and it synchronizes the intermittent Ca^{2+} release from ryanodine receptors (RyR) in adjacent cells.

receptors (Peng *et al.* 2001; Haddock & Hill, 2002). Surprisingly, though, ryanodine did not result in hyperpolarization of the cell membrane in basilar arteries, but rather produced a depolarization accompanied by an increased frequency and decreased amplitude of the voltage oscillations (Haddock & Hill, 2002). This effect is reminiscent of that found after inhibition of IK_{Ca} channels, although inhibition of these channels did not abolish Ca^{2+} waves (Haddock & Hill, 2002). Thus, Ca^{2+} from the ryanodine receptors is responsible for hyperpolarization, consistent with the demonstration of Ca^{2+} sparks, outward potassium currents and relaxation in SMCs from cerebral arteries (Nelson *et al.* 1995; Jaggar *et al.* 1998*a*,*b*, 2000). These data provide tantalizing evidence for compartmentalization of intracellular stores with particular ion channels, since the effects of Ca^{2+} release from the IP_3 receptors differ markedly from those due to ryanodine receptors (Fig. 4). Moreover, the abolition by nifedipine of voltage oscillations, without hyperpolarization, loss of Ca^{2+} waves or tone (Haddock & Hill, 2002), supports the previously reported functional grouping of K_{Ca} channels with ryanodine receptors and VDCCs (Jaggar *et al.* 2000; Fig. 4). Thus, in the basilar artery, ryanodine receptors subserve two different roles: firstly in the regenerative events involved in the formation of the intracellular Ca^{2+} waves, and secondly as an intermediary between the influx of Ca^{2+} through VDCCs and the activation of K_{Ca} channels.

In contrast to the effect in the basilar artery, inhibition of VDCCs produces relaxation in other arteries, like the mesenteric, and probably also hyperpolarization, as it does in cheek pouch arterioles (Bartlett *et al.* 2000; Oishi *et al.* 2002). These data suggest that Ca^{2+} influx through VDCCs may not be involved in activating ryanodine receptors and K_{Ca} channels in the mesenteric artery, as it does in the basilar artery. It is therefore important to determine the effect of ryanodine on membrane potential in the former vessels. If ryanodine hyperpolarizes the cell membrane, then the data suggest that Ca^{2+} released from ryanodine stores is involved in the initial depolarization and that there is little evidence for compartmentalization and classical sparks in these arteries, as there is in the cerebral vessels.

Role of cellular coupling in the synchronization of Ca^{2+} waves

It is apparent from the above discussion that vasomotion in all arteries must be critically dependent

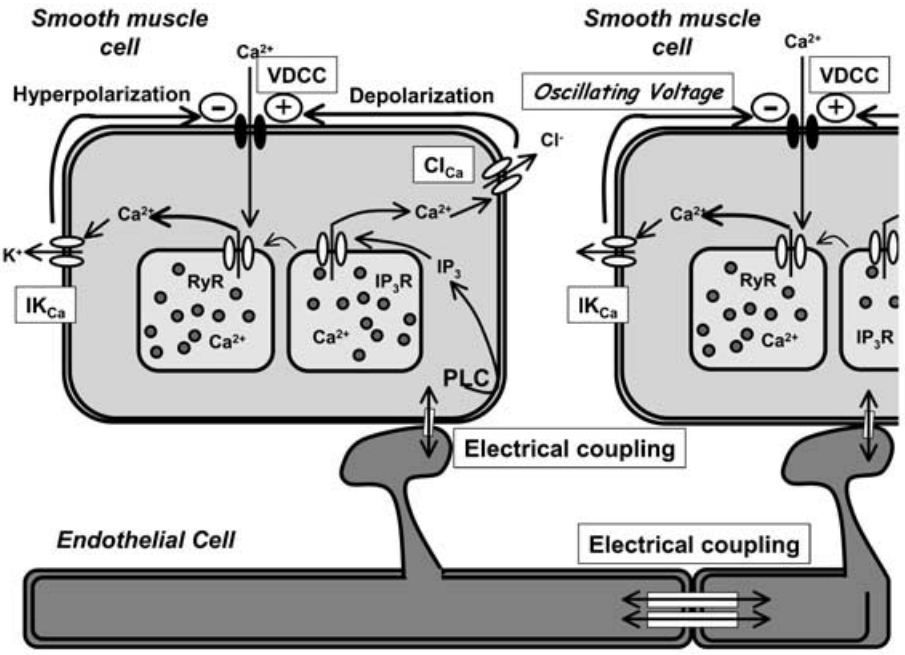

Figure 4. The voltage-dependent membrane oscillator in rat basilar arteries
Constitutive activity of phospholipase C (PLC) leads to the production of IP_3 and release of Ca^{2+} to activate a Ca^{2+}-dependent chloride channel (Cl_{Ca}). This current depolarizes the membrane potential and opens voltage-dependent Ca^{2+} channels (VDCC) causing extracellular Ca^{2+} influx. This Ca^{2+} influx activates ryanodine receptors (RyRs) causing further Ca^{2+} release which opens intermediate conductance K_{Ca} channels and the membrane hyperpolarizes, thereby closing the VDCCs. Coupling between adjacent smooth muscle cells is poor but the oscillating membrane voltage acts to synchronize adjacent cells due to electrical coupling via the endothelium. Closure of the VDCCs following SMC hyperpolarization ensures the vasodilatory part of the cycle while the tonic depolarization through the Cl_{Ca} channels ensures that the VDCCs reopen and the cycle oscillates.

on the coordination of Ca^{2+} signals within individual SMCs leading to synchronized Ca^{2+} responses and the development of simultaneous contractions along the vessel length (Christ *et al.* 1996). Synchronization could be mediated by the transfer of small intracellular signalling molecules between cells of the vascular wall, such as Ca^{2+} or by the rapid conduction of current along the vessel length (Koenigsberger *et al.* 2004). A number of studies have demonstrated that putative gap junction uncouplers abolish synchronized contractions (Jackson *et al.* 1991; Chaytor *et al.* 1997; Hill *et al.* 1999; Bonnet *et al.* 2001), synchronized Ca^{2+} waves (Dhein, 1998; Sell *et al.* 2002; Matchkov *et al.* 2004*b*) and oscillations in membrane potential (Hill *et al.* 1999). However a growing body of evidence suggests that many of these compounds have additional non-specific effects on targets other than gap junctions (Spray & Burt, 1990; Chaytor *et al.* 1997; Hashitani & Suzuki, 1997; Yamamoto *et al.* 1998; Coleman *et al.* 2001, 2002; Tare *et al.* 2002; Matchkov *et al.* 2004*b*) and so the results of these studies must be interpreted with caution. Nevertheless, arterioles behave like electrical syncytia (Hirst & Neild, 1980) and gap junctions provide a pathway for direct or indirect chemical and electrical signals between neighbouring SMCs. In arteries in which voltage-independent mechanisms apply, synchronization is likely to occur via flux of Ca^{2+} through gap junctions, while in arteries in which voltage-dependent mechanisms apply, synchronization could result from the spread of current and coincident influx of Ca^{2+} through VDCCs.

Role of the endothelium

Considerable confusion exists in the literature concerning the role of the endothelium in vasomotion due to conflicting results from different groups even involving the same vessel. Nevertheless the endothelium could regulate vasomotion through at least two different mechanisms. Firstly, if the endothelial and smooth muscle layers are coupled through gap junctions, then the endothelium could synchronize either voltage or $[Ca^{2+}]_i$ oscillations amongst SMCs. This would be important if SMCs, unlike endothelial cells, are not well coupled and this is likely to be the case in many small arteries (Yamamoto *et al.* 2001; Rummery & Hill, 2004). Recent data suggest that Ca^{2+} does not readily pass between endothelial cells (Dora *et al.* 2003) and so the role of the endothelium may be to simply coordinate electrical responses between neighbouring SMCs.

An alternative role for the endothelium would be based on its ability to release potent vasodilatory factors which could modulate the contractile activity of the SMCs. Clearly this could occur in the absence of heterocellular coupling through myoendothelial gap junctions. In the rat mesenteric artery, removal of the endothelium has been shown to desynchronize oscillations in $[Ca^{2+}]_i$ and abolish vasomotion (Peng *et al.* 2001) and this has been proposed to be due to loss of NO and the subsequent activation of a cGMP-dependent depolarizing current in the SMCs. Addition of cell-permeant cGMP analogues was shown to restore vasomotion in endothelium-denuded tissues (Jackson *et al.* 1991; Gustafsson *et al.* 1993). However, in the same artery and in other vessels, NO has been reported to have an inhibitory effect on vasomotion and under these conditions it is unlikely to be responsible for initiating vasomotion (Watts *et al.* 1994; Tsai *et al.* 1995; Huang & Cheung, 1997; Hill *et al.* 1999, 2004; Sell *et al.* 2002; Okazaki *et al.* 2003; Mauban & Wier, 2004).

More recently, the vasodilator endothelium-derived hyperpolarizing factor (EDHF) has been suggested to initiate vasomotion (Okazaki *et al.* 2003; Mauban & Wier, 2004). Thus, vasomotion is abolished and only asynchronous oscillations in $[Ca^{2+}]_i$ can be detected in SMCs following inhibition of EDHF with the K_{Ca} channel blockers apamin and charybdotoxin (Mauban & Wier, 2004). Since Ca^{2+} and IP_3 have been reported to move through myoendothelial gap junctions from SMCs into endothelial cells (Yashiro & Duling, 2000; Budel *et al.* 2001; Oishi *et al.* 2001; Schuster *et al.* 2001; Lamboley *et al.* 2005), it has been proposed that these molecules could activate K_{Ca} channels in the endothelium. The resulting hyperpolarization would be electrotonically propagated back into the SMCs. This would constitute a modification of the voltage-dependent membrane oscillator model in which the hyperpolarizing phase occurs in the endothelium (Fig. 5).

Surprisingly, the data supporting this model were obtained from phenylephrine-stimulated mesenteric arteries in which the voltage-dependent coupled oscillator model has been previously proposed to explain vasomotion (Peng *et al.* 2001). In the basilar artery, we have also found that treatment with apamin and charybdotoxin results in asynchronous oscillations in $[Ca^{2+}]_i$ amongst SMCs, but this is accompanied by depolarization and constriction (R. E. Haddock and C. E. Hill, unpublished observations) and constriction is also observed in the mesenteric artery following inhibition of EDHF (Mauban & Wier, 2004). Removal of the endothelium would be expected to result in a loss of EDHF and a resultant depolarization; however, no change in membrane potential was found following this procedure in either mesenteric or basilar arteries (Gustafsson *et al.* 1993; Hill *et al.* 2004). It is therefore possible that the asynchronous oscillations in $[Ca^{2+}]_i$ recorded in apamin and charybdotoxin and the consequent loss of vasomotion may simply be due to inactivation of VDCCs by prolonged depolarization. The effects of apamin and charybdotoxin on voltage oscillations and membrane potential in endothelium-denuded arteries should assist in clarifying this issue.

Taken together, the role of the endothelium, in arteries like the mesenteric and basilar where myoendothelial gap junctions exist (Sandow & Hill, 2000; Hill *et al.* 2004), is to synchronize oscillations in $[Ca^{2+}]_i$ by electrotonic means between poorly coupled SMCs (Figs 3–5). In this case, factors generated by the endothelium simply modulate the frequency and amplitude of vasomotion by reducing $[Ca^{2+}]_i$ (see also Koenigsberger *et al.* 2005). On the other hand, in vessels like the iris arterioles, where heterocellular coupling does not exist, Ca^{2+} may mediate synchronization amongst SMCs and only endothelial factors which can diffuse to the SMCs will have an impact.

Rhythmical contractions in non-vascular smooth muscle

Many types of non-vascular smooth muscle tissues undergo rhythmical contractions which are also preceded by rhythmical depolarizations like those described here (Tomita, 1981). The mechanism underlying these slow waves has recently been reviewed (Hirst & Ward, 2003) and while there are some similarities with the mechanisms underlying vasomotion, there are also differences. Like the rhythmical depolarizations recorded in arteries, slow waves are ultimately dependent on Ca^{2+} released from the IP_3 store and this in turn results in the activation of ion channels in the membrane and the production of small transient depolarizations (Hirst & Ward, 2003). When sufficient channels are simultaneously activated, the depolarization is large enough to activate VDCCs and the influx of Ca^{2+} produces a contraction. The spread of the depolarizing current synchronizes the potentials so that coordinated activity takes place. However, an important difference is that slow waves in the muscle cells are actually driven by specific pacemaker cells called interstitial cells of Cajal (ICCs) which, in the gastrointestinal tract, form two distinct coupled cellular networks, one inside the smooth muscle layer and one outside of it. Therefore, it is the ICCs which generate all of the depolarizing potentials and without ICCs, the muscle is quiescent. While cells with similar morphology to these ICCs have recently been observed in the rabbit portal vein and mesenteric artery, it seems likely that they are only responsible for initiating muscular activity in the portal vein, an atypical vessel with an external layer of longitudinal smooth muscle (Bolton *et al.* 2004). In contrast, in arteries the contractile rhythm depends on intrinsic activity within individual SMCs and synchronization occurs through the coupling of these individual oscillators via Ca^{2+} or voltage.

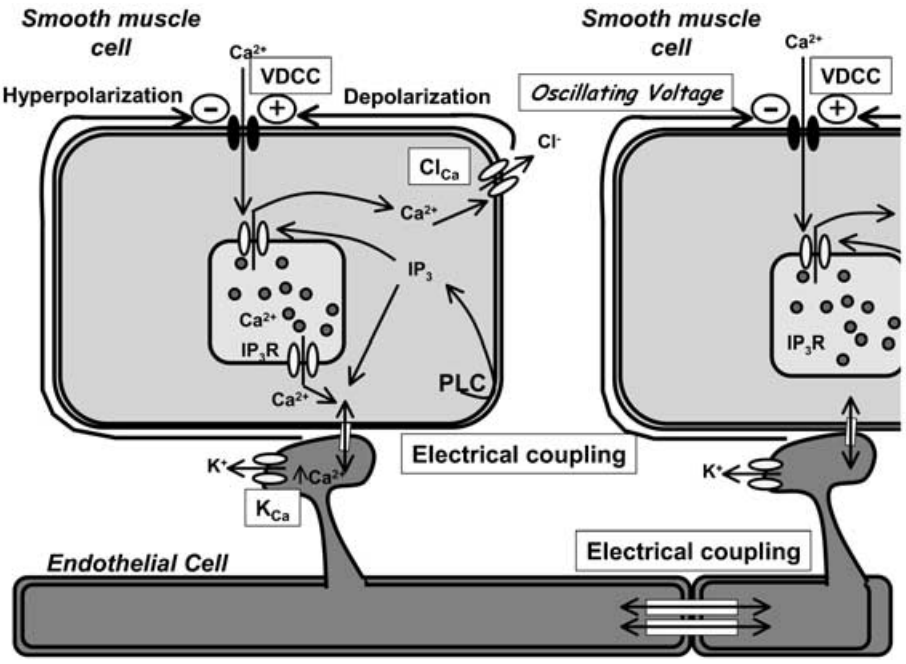

Figure 5. The voltage-dependent membrane oscillator in rat mesenteric arteries
Application of agonist induces Ca^{2+} release from intracellular stores and activation of a depolarizing current. The resulting Ca^{2+} influx through VDCCs synchronizes and augments release from intracellular stores. Either Ca^{2+} or IP_3 passes through myoendothelial gap junctions into the endothelial cells to activate K_{Ca} channels. The ensuing hyperpolarization is then transferred electrotonically back into the SMCs where it reduces Ca^{2+} entry due to the closure of VDCCs.

Conclusions

While controversies exist in the literature concerning the role of the endothelium and its vasodilatory factors, it seems clear that release of Ca^{2+} from intracellular stores and direct or indirect coupling of oscillations between SMCs form the basis of vasomotion in all vessels studied to date. In the simplest case, vasomotion within irideal arterioles results from the interaction between two intracellular pathways and there is no evidence for any involvement of voltage-dependent ion channels. In contrast, in many other arteries, depolarization and Ca^{2+} influx through VDCCs is an essential step in coordinating the individual oscillators. In mesenteric arteries, synchronous oscillations in Ca^{2+} and membrane potential may arise due to the activation of an oscillating current by the oscillating $[Ca^{2+}]_i$. Alternatively in basilar arteries, synchronous oscillations in calcium and membrane potential may arise from a tonic depolarization and the interplay between membrane ion channels. Which one of these three mechanisms exists in a particular artery may depend on the expression of specific ion channels and the degree of gap junctional coupling. The location of particular ion channels within the smooth muscle and endothelial cells, in relation to intracellular stores, other membrane ion channels and homocellular and heterocellular gap junctions may also have a significant impact.

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