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Calcium-induced calcium release mechanism in vascular smooth muscles – assessments based on contractions evoked in intact and saponin-treated skinned muscles

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Introduction

In vascular smooth muscle tissues, the contraction–relaxation cycle is dependent on the free Ca in the myoplasm, under physiological conditions, i.e. the minimum concentration of Ca required to produce contraction is 1×10^{-7} M and an almost maximum amplitude of contraction is evoked by 1×10^{-6} M Ca due to activation of either calmodulin or leiotonin C[1,7,8,10,46,49,63,64,66].

Increases in free Ca in the myoplasm are induced from two main sources, i.e. influx of Ca through the sarcolemma and release of Ca from store sites. The former may be through passive influx of Ca, voltage dependent Ca influx and receptor activated Ca influx[5,6,35,41], and the latter may be due to voltage dependent Ca release and Ca-induced Ca release mechanism[33]. These Ca influx and release mechanisms can be subclassified further (details described later).

It is uncertain whether the increased influx of Ca during activations of the sarcolemma directly increases the free Ca in the myoplasm in vascular smooth muscle cells. In 1970, Endo et al.[13], and independently Ford and Podolsky[16], presented the hypothesis that the Ca release from sarcoplasmic reticulum (SR) of striated muscles is a Ca-induced Ca release mechanism. Endo[11] reviewed this mechanism for skeletal and cardiac muscles and concluded that in skeletal muscle it may not play an important role under physiological conditions, but Fabiato[14] reported that in cardiac muscles this Ca-induced Ca release mechanism may play a physiological role in triggering contraction.

We proposed that the Ca-induced Ca release mechanism in vascular smooth muscles triggers the contraction under physiological conditions, i.e. Ca entering through the plasma membrane may not directly increase the free Ca in the myoplasm but may be accumulated into a store site and after a certain amount of Ca has been stored, activate the release of Ca. The present article will focus on this hypothesis which is based on results of experiments using intact and chemically skinned muscles[28,31,33,52].

Chemical skinning of smooth muscle tissues

Skinning of smooth muscle tissues can be done using various agents and different procedures, which are not only used for vascular muscles but have been commonly used for skeletal and cardiac muscles as substitute for mechanical skinning (so-called 'Natori's muscle'), except for the polyene antibiotics.

Glycerin. Glycerin was employed by Filo et al.[15]. A disadvantage was that glycerination requires the smooth muscle to be kept 1–3 months in 50% glycerol and, therefore, the contents of the myoplasm may be altered. Furthermore, the amplitude of contraction evoked by Ca may not be comparable with that evoked in intact conditions.

Triton X-100. Gordon[18] prepared skinned muscles using Triton X and the following procedures: a $5 \times 2 \times 0.2$ mm smooth muscle segment was excised, and the tissue was pre-soaked in EGTA, morpholinopropanesulphonic acid, KCl, sucrose and $10^{-7.5}$ M Ca containing solution for 30 min, after which the tissue was immersed in the skinning solution containing Triton X-100 with dithioerythritol or dithiothreitol for 16 h. Modifications of the procedure were tried, for example the exposure time to the detergent was shortened to 2 h, and EDTA was substituted for EGTA and Brij-58 was substituted for Triton X-100.

Combined use of Triton X and glycerol. Ruegg et al.[51] further modified the skinning procedure introduced by Gordon[18], i.e. after treatment with Triton X-100, the tissue was stored in ATP-salt solution containing 50% glycerol at −20 °C. Using this procedure[55], application of calmodulin to skinned muscles enhanced the amplitude of the Ca-induced contraction, presumably because the intracellular components (calmodulin) had to some extent been lost.

Polyene antibiotics. Filipin[3] or Nystatin[3], have also been used.

Homogenization. Kerrick et al.[39] and Kerrick and Hoar[38] used a light homogenization of the tissue. Here small dissected tissues were homogenized in relaxing solution placed in a glass tissue-homogenizing tube. The teflon pestle was rotated at less than 100 rpm for 5 sec while 5–10 lb of pressure was applied with the palm of the hand. This process was repeated two or three times. The homogenized muscle cells were then immersed in Triton X-100 containing solution for 30 min. In skinned muscles of the chicken gizzard, the Ca contraction reached a peak amplitude after several minutes. This result indicates that the Ca receptor (calmodulin), contractile proteins and other substances are probably left intact.

Saponin. Ohtsuki et al.[48] prepared skinned muscles using saponin. Since saponin rather selectively makes holes in cholesterol rich membrane, the function of the SR may be preserved while the sarcolemma is made leaky. Saponin-treated muscles were prepared by Endo et al.[12] and Saida and Nonomura[53] according to the suggestion of Prof. I. Ohtsuki, Kyushu University. With 25 min application of saponin in a relaxing solution, skinned muscles were obtained[12,26,31,53]. As shown in figure 1, Itoh et al.[31] reported that after application of 50 μg/ml saponin, the Ca-induced contraction (pCa 5) was larger than the K-induced contraction (128 mM). The sensitivity of contractile proteins to Ca or Sr was much the same as obtained from

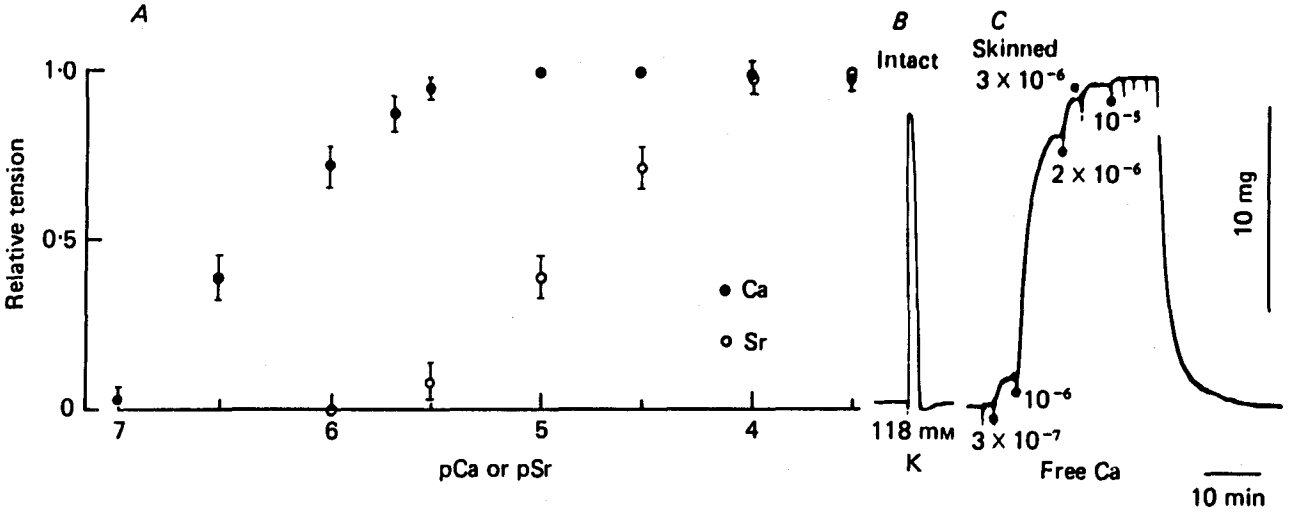

Figure 1. pCa-tension and pSr-tension relationships in skinned muscle cells. *A* pCa-tension relationship obtained from nine different skinned muscle preparations and pSr-tension relationship obtained from five preparations. The tension measured at pCa 5 was registered as 1.0. *B* Effects of 118 mM-$[K]_0$ on the intact muscle cells. *C* Effects of various concentrations of free Ca on skinned muscle cells. Cumulative applications. Free Ca concentrations inserted in C. Vertical bars indicate 2 × SD or SD (Itoh, Kuriyama and Suzuki, J. Physiol. (1981)).

superprecipitation of myosin B extracted from smooth muscles[21]. When such procedures are used, it can easily be determined whether the skinning of the muscle cell is complete, by comparing the amplitude of the Ca-induced contraction and the 128 mM K-induced contraction or the agonist-induced contraction, in intact muscles. Furthermore, since the addition of calmodulin (10^{-7} M) to the relaxing solution only slightly increased the Ca-induced contraction (to 1.2 times the control observed with 10^{-6} M Ca^{32}), the myoplasmic substrate may be adequately preserved. However, saponin is not a pure substance. Therefore, when different samples are used, the concentration and the duration of the skinning time should be carefully checked. In preparing skinned muscles by saponin treatment, the most essential factor is to use a small strip of intact muscle tissue, i.e. a piece of tissue 50 μm in width and 100–300 μm in length is adequate for skinning, but wider or thicker tissue (over 100 μm) may not be completely skinned by saponin within a limited concentration and exposure time. Tissue damage occurs with prolonged exposure.

The contraction evoked by voltage dependent Ca influxes

In vascular tissues, in vitro, spontaneously generated spikes are not usually observed except in the portal vein; however, the excitation of perivascular sympathetic nerve terminals in resistance vessels can generate spike potentials on excitatory junction potentials. In smooth muscle cells of large arteries, the spike cannot be evoked by direct muscle stimulation or by perivascular nerve stimulation. Therefore, the generation of action potentials does not play an essential role in causing contraction in most vascular smooth muscles[25,35,37,41].

Application of tetraethylammonium, 4-aminopyridine or procaine inhibits the K-conductance and depolarizes the muscle cell membrane in vascular tissues[20], and under treatment with the above agents, application of outward current evokes spikes which are blocked by application of a Ca antagonist (verapamil, diltiazem, nifedipine, nisoldipine or nicardipine), $MnCl_2$ or Ca free solution. However, the spikes persist in the absence of Na in the superfusing solution[31,36,42,61]. This means that the spike evoked in vascular smooth muscle tissues is mainly due to the influx of Ca following activation of voltage dependent Ca channels. How the spike is generated in the presence of a K-conductance inhibitor, is the subject of studies at present in progress.

Spike generation is accompanied by contraction, and the amplitude of the contraction depends on the spike amplitude and number. However, in the presence of procaine, spikes can be evoked without contraction. According to rough calculations based on cell volume, and passive and active membrane properties, the amount of Ca influx during the action potential would increase intracellular Ca by 3×10^{-7} M[41]. We hold the view that the influx of Ca could activate the contractile proteins, provided that all this influx directly increases the amount of free Ca in the myoplasm.

In saponin-treated skinned muscles, 10^{-2} M procaine did not modify the pCa-tension relationship, as examined by cumulative applications of Ca (pCa 7–5). However, this agent (10^{-2} M) inhibited the release of Ca from store sites following application of caffeine to skinned muscles. Therefore, it seems that the contraction initiated by the spike potentials requires release of Ca from the intracellular store site[31,33,34]. We postulate that influx of Ca does not directly activate the contractile proteins but rather activate the Ca-induced Ca release mechanism.

In the porcine coronary artery[27], electrical depolarization of the membrane produced contraction without generation of action potentials and this contraction was abolished in Ca-free 2 mM EGTA containing solution, indicating that influx of Ca occurs during depolarization of the membrane. Contraction was also produced by application of excess concentrations of K and this contraction was also abolished in Ca-free EGTA containing solution but the depolarization of the membrane was unchanged.

Both depolarization-induced contractions were markedly reduced by Ca antagonists and also by procaine. The effect of Ca antagonists is due to inhibition of the voltage dependent Ca influx, while the main inhibitory action of procaine is likely to be due to the inhibition of Ca release from store sites. This idea is based on the observation that after depletion of Ca stored in the cell with repetitive applications of 10 mM caffeine in Ca free EGTA containing solution, the application of 2.5 mM Ca with 1–5 mM procaine in the presence of 5.9 mM or 128 mM K did not cause contraction. The subsequent application of 10 mM caffeine in Ca-free EGTA containing solution produced a contraction larger than that evoked if the proceeding procaine application was omitted. This means that the Ca entering the cell following the above procedure is stored, and not released in the presence of procaine.

In saponin-treated muscles from the porcine coronary artery, application of NaCl or choline Cl instead of K propionate in the relaxing solution, following storage of Ca in the cell, caused a small contraction, the amplitude being less than 0.2 times that of the contraction evoked by caffeine. If the SR membrane is polarized, reducing K would be expected to depolarize the membrane. This result suggests that if the sarcolemma is coupled to the store site, depolarization of the membrane may play a minor role in the release of Ca. Much the same small contraction can be recorded by replacement of Cl with a large anion as is also the case in skinned skeletal and cardiac muscles[11]. It has, however, been reported that the ionic composition on both sides of the SR membrane is equal, suggesting that there may be no potential difference across the SR membrane[58].

Ca entering during the excess K- or electrically induced-depolarization seems to be sequestered in the store site and activates Ca-release from the store. If this postulate is correct, the amount of Ca stored in the cell may play a key role in the activation of the Ca-induced Ca release mechanism. Actually, when the amount of Ca stores in the cell in intact tissues was reduced, the rate of rise was slowed and the time to reach the peak amplitude of the contraction evoked by 128 mM K was markedly prolonged. Figure 2A shows examples of the K-induced contractions evoked with different amounts of Ca stored in the cells. The contraction evoked by 128 mM K in 2.6 mM Ca containing solution in intact muscle tissues was taken as control (a). When 128 mM K with 2.6 mM Ca were applied following treatment with Ca-free solution containing 5.9 mM K and 2 mM EGTA (b) the contraction had a slow rate of rise and a delayed peak. Additional repetitive applications of agonist or caffeine in Ca-free solution, slowed the rate of rise further and the time to reach the peak tension was more extensively prolonged (c). Thus generation of the K-induced contraction depends on the amount of Ca stored in the cell. Therefore, it is likely that the influx of Ca caused by depolarization does not directly increase the free Ca in the myoplasm but it is accumulated in the cell and activates the Ca-induced Ca release mechanism which requires the presence of stored Ca. When Ca antagonists were applied to the intact tissue under any given condition of Ca storage, as described above, the K-induced contraction (with 2.6 mM Ca) was consistently inhibited (fig. 3). Ca antagonists (diltiazem ($< 10^{-4}$ M), nifedipine ($< 10^{-7}$ M), nimo-

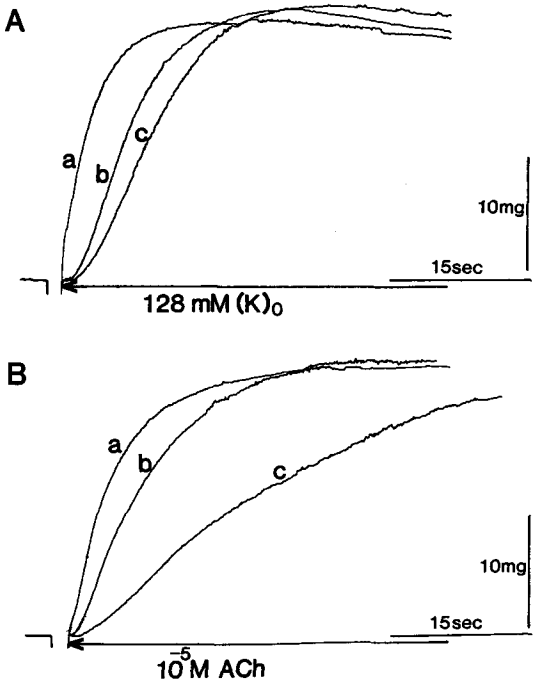

Figure 2. *A* Effects of the amount of Ca stored in cells on the 128 mM K-induced contraction in the rabbit coronary artery. a) 128 mM K was applied in Krebs solution (2.6 mM Ca). b) 2.6 mM Ca with 128 mM K applied 5 min after treatment with 5.9 mM K- and Ca-free 2 mM EGTA containing solution. c) After repetitive applications of caffeine in 128 mM K and Ca-free solution, 2.6 mM Ca was applied.
B Effects of the amount of Ca stored in cells on the 10^{-5} M acetylcholine-induced contraction in the rabbit coronary artery. a) ACh was applied in Krebs solution. b) 2.6 mM Ca with ACh were applied 5 min after application of Ca-free 2 mM EGTA containing solution. c) After depletion of Ca stored in the cell by repetitive applied caffeine in Ca-free solution, 2.6 mM Ca with ACh were applied.

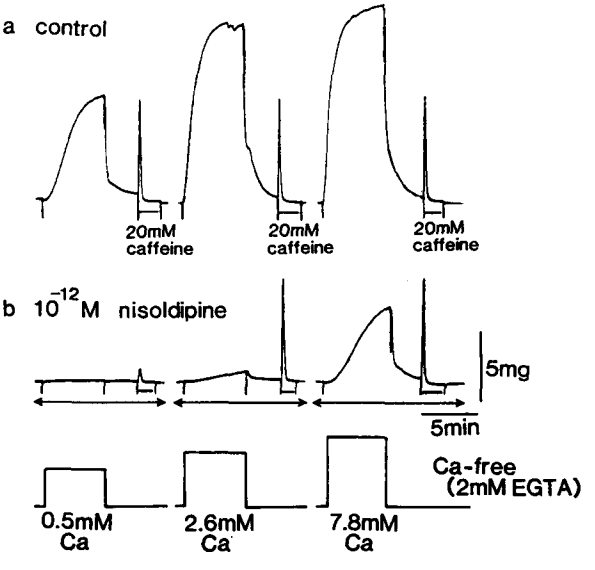

Figure 3. Effects of nisoldipine on the Ca- and caffeine-induced contraction in 128 mM K. After depletion of Ca stores in Ca-free solution containing 5.9 mM K 2 mM EGTA and by repetitive application of caffeine in 128 mM K Ca-free solution, 0.5, 2.6 and 7.8 mM Ca and 128 mM K were simultaneously applied, washed out with Ca-free 128 mM K solution for 3 min, and the 20 mM caffeine was subsequently applied (a) control (b) in the presence of nisoldipine (10^{-12} M).

dipine ($< 10^{-7}$ M) and nisoldipine ($< 10^{-7}$ M)), had no effect on calmodulin or on the release of Ca from the store site, as estimated from amplitudes of the Ca-induced and caffeine-induced contractions in skinned muscles, respectively. Therefore, the inhibition of the contraction seems to be due solely to the inhibition of Ca influx.

The possibility that influx of Ca always directly activates the contractile protein in polarized and depolarized muscles, was ruled out by the following experiments. After complete depletion of Ca stored in the cells by repetitive applications of agonist or caffeine, simultaneous application of 2.6 mM Ca, 128 mM K and nisoldipine did not evoke a contraction in Ca-free EGTA containing solution, yet the subsequently applied caffeine in Ca-free solution produced a contraction. Therefore, influxes of Ca can occur without generation of the contraction but this Ca is directly stored in cells (fig. 3).

The contraction evoked by receptor activation

a) Receptor activated mechanism

In vascular smooth muscle tissues, receptors for neurotransmitters and putative transmitters are widely distributed. For example, alpha$_1$- and alpha$_2$-adrenoceptors may occur, in both arteries and veins. Activation of the alpha$_1$-adrenoceptor does not always depolarize the membrane, the same is true for alpha$_2$-adrenoceptors. Many blood vessels also possess muscarinic receptors which may produce hyperpolarization, depolarization or no change in the membrane potential, depending on the tissues but there is a consistant production of tension. ACh-induced contraction appears with membrane hyperpolarization in guinea pig coronary artery[40], with hyperpolarization followed by depolarization in rabbit coronary artery[30], and with no changes in the membrane potential and resistance in the porcine coronary artery[27]. In this latter instance, the ACh induced contraction is larger than the 128 mM K-contraction.

In this article, we tentatively classify the receptor activated mechanisms into four devisions, including the responses to neurotransmitters or neurosecretory substances. This classification does not correspond to structural subunits but relates to different mechanisms of Ca mobilization (fig. 4).

1) Voltage dependent Ca influx resulting from receptor activation. When the agonist combines with the receptor (agonist binding site), ion channels are activated (mainly for Na or K), thus producing depolarization or hyperpolarization of the membrane. When the nerve terminal is located in close proximity to the postjunctional receptor, miniature excitatory junction potentials (mejps) can be recorded[25,62]. In vascular smooth muscles, perivascular nerve stimulation evokes excitatory junction potentials (ejps); these trigger the spike potentials, as seen for example, in the mesenteric artery. When the nerve terminal is located far from the postjunctional receptor, repetitive stimulation produces a slow depolarization[60]. The ejps are not blocked by alpha$_1$- or alpha$_2$-adrenoceptor blocking agents but are blocked by guanethidine or tetrodotoxin, however, the slow depolarization is blocked by alpha-adrenoceptor blocking agents (either alpha$_1$- or alpha$_2$-) and by guanethidine. Furthermore, exogenously applied noradrenaline (NAd), or phenylephrine also produces a contraction, with or without a slow depolarization of the membrane (due to activation of α_1- or/with α_2-adrenoceptors), and this contraction is blocked by al-

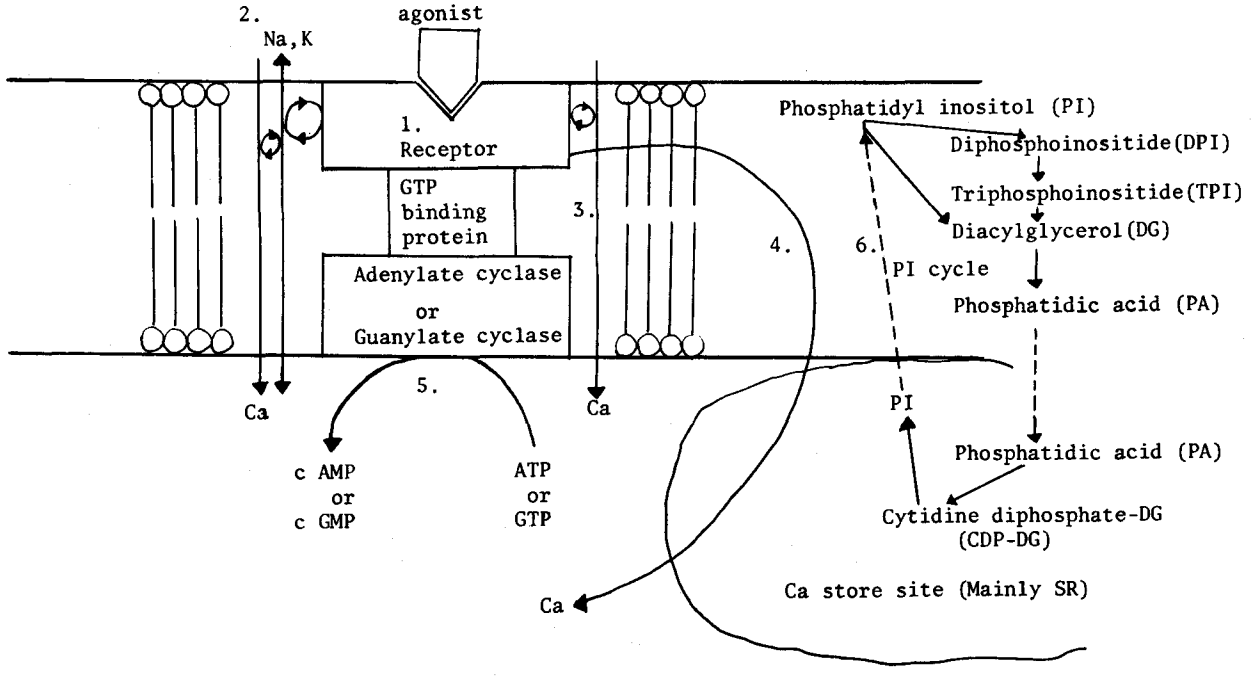

Figure 4. Receptor activated mechanisms in relation to Ca mobilization in vascular smooth muscles. *1* Agonist binding site; *2* receptor activated ion fluxes (Na, K) with resulting voltage, depending Ca influx (mechanism (1) ; *3* receptor activated Ca ionophore (mechanism (2) ; *4* receptor activated Ca release from store site (mechanism (3) ; *5* production of cyclic nucleotides (mechanism (4) ; *6* the P-I cycle.
The relationship between *4* and *6* is not yet clarified, and therefore both routes are shown separately.

pha-adrenoceptor blockers. Therefore, the nature of the receptors which generate ejps and those which cause a slow depolarization differ (22: the junctional adrenoceptor termed a 'gamma adrenoceptor'). The generation of ejps is an essential step in the propagation of excitation and generation of the Ca spikes, but the activation of extrajunctional adrenoceptors, due to the overflow of NAd released by nerve stimulation or to exogenously applied NAd, depolarizes the membrane, thus provoking the voltage dependent Ca influx (this Ca influx is inhibited by Ca antagonists).

2) Receptor activated Ca ionophore (fig. 4). In the guinea pig basilar artery, it was found that 5-hydroxytryptamine (5-HT) produced a contraction which was markedly reduced in Ca-free solution. 5-HT depolarized the membrane with reduction in the ionic conductance, measured by applications of constant inward current pulses or by the current-voltage relationship. Often the 5-HT-induced depolarization led to repetitive spiking and, as a consequence, phasic contractions were superimposed on the slowly developed contraction. Application of nicaldipine, a Ca antagonist, blocked the spike potential and phasic contractions, but did not modify the amplitude of the slow contraction. This means that 5-HT can produce contraction of the guinea pig basilar artery by influx of Ca that is not related to activation of the voltage dependent Ca channels, but due to receptor activated Ca influx[17].

3) Receptor activated Ca release (fig. 4). In the procine coronary artery, ACh produced a contraction with no change in the membrane potential and resistance. The ACh-induced contraction was still preserved in Ca-free 2 mM EGTA containing solution (about 80% of the contraction evoked in the presence of Ca). In skinned muscles, ACh had no effect on the pCa-tension relationship or on the Ca release from the store site, as estimated from amplitudes of the caffeine-induced contraction in skinned muscles[29]. Therefore, in this tissue, ACh may activate a mechanism to produce an intermediate substance, which accelerates the release of Ca from the store site (details described latter).

4) Receptor activated regulation of secondary messenger (fig. 4). Activation of receptors may produce secondary messengers such as cyclic AMP (cAMP) or cyclic GMP (cGMP), which modulate the free Ca in the myoplasm and the contraction. For example, cAMP and cAMP dependent protein kinase (A kinase) inhibit activation of myosin light chain kinase, thus reducing phosphorylation of the myosin light chain, and actin-myosin interactions. Simulataneously cAMP and A kinase increase both the Ca uptake into the store site and the Ca extrusion through activation of Ca ATPase, at the sarcolemma[7,28]. cGMP with cGMP dependent protein kinase (G kinase) also inhibits the phosphorylation at pCa below 6, as estimated from the pCa-tension relationship observed in skinned muscle tissues, and accelerates the Ca extrusion by activation of the Ca ATPase at the sarcolemma[34]. Furthermore, stimulation of muscarinic or vasopressin receptors activate the phosphatidyl inositol cycle (PI cycle) and the C kinase which inhibits myosin ATPase activity in platelets (Hidaka et al., personal communications).

This tentative analysis of the mechanisms activated by the receptor may explain why Ca antagonists block the voltage dependent Ca influx resulting from activation of cation channels in (1), but not the receptor activated Ca influx (2) not the Ca release from the store site (3). Regardless of whether the agonist accelerates or inhibits cell function, the mechanisms of Ca mobilization through receptor activation can be tentatively divided into the above classes. When the receptor possesses only mechanism (1), this receptor may be located in close opposition to nerve terminals, as in the case of the endplate. However, the so-called extrajunctional receptors may possibly be equipped to activate not only mechanism (1) but also other mechanisms, as specific features of the receptor for neurosecretory substances. Therefore, multiple responses in relation to Ca mobilization may be evoked by receptor activation by a single agonist.

b) Ca-induced Ca release mechanism in relation to receptor activated Ca mobilization

How the receptor activates influx and release of Ca and how this is related to the Ca-induced Ca release mechanism in vascular smooth muscles will now be given attention.

In Ca-free solution, NAd produces a contraction of rabbit mesenteric artery. Saida and Van Breemen[54] postulated that activation of the alpha-adrenoceptor releases Ca distributed just beneath the cell membrane or the Ca bound at the membrane (sarcolemma), thus activating the Ca-induced Ca release mechanism at the intracellular Ca store site. Another hypothesis is that receptor activation may trigger a biochemical change at the sarcolemma and that this process or a product will release the Ca stored in the cell (mainly SR). The receptor activated metabolite may reduce the threshold for activation of the Ca-induced Ca release mechanism. In that case, the minute amount of Ca released from the cell membrane or SR may activate the Ca-induced Ca release mechanism. A phosphatidylinositol (P-I) breakdown at the sarcolemma has been found to produce diacylglycerol (directly or through phosphatidyl inositol di- and tri-phosphate), and this P-I breakdown stimulates the production of phosphatidic acid at the SR, and this substrate re-synthesizes to phosphatidyl inositol through cytidine diphosphate diacylglycerol at the sarcolemma (phosphatidyl inositol cycle (P-I cycle)[44]). These processes may be closely linked with the actions of a Ca ionophore. The P-I cycle is triggered by activation of muscarinic receptors α_1-adrenoceptors and others[23,24,45,47,50]. Recently, Suematsu et al.[59] reported that inositol trisphosphate releases Ca from store sites (SR) in dispersed skinned single muscles. The Ca release in Ca-free solution as induced by NAd or ACh in vascular tissues may be closely related to activation of the P-I cycle (fig. 4). Further experiments are required to clarify the interrelationship between agonist stimulation and Ca release mechanism.

Figure 2B shows the effects of Ca stored in the cell, on the ACh-induced contraction. When 2.6 mM Ca with ACh was applied after exposure to Ca-free 2 mM EGTA containing solution, a large contraction was evoked. However, the time required to reach the peak amplitude was delayed and the rate of rise of the contraction was slowed in comparison with the control. After depletion of the Ca store by repetitive applications of ACh or caffeine in Ca-free solution 2.6 mM Ca with ACh produced a con-

traction with an even lower rate of rise of the contraction and with an extended delay in reaching the peak tension. Therefore, the ACh-induced contraction also depends on the amount of Ca stored in the cell. However there is a difference between the K- and ACh-induced contractions which could be elucidated from the action of nisoldipine, i.e. after depletion of Ca from the store site, simultaneous application of 2.6 mM Ca, 10^{-5} M ACh with 10^{-8} M nisoldipine evoked a contraction with a very slow rate of rise. However, simultaneous applications of 2.6 mM Ca, 128 mM K with 10^{-8} M nisoldipine produced no contraction. Here, we want to emphasize that ACh increases the influx of Ca by a mechanism which is not completely inhibited by Ca antagonists.

Actions of caffeine and procaine on vascular smooth muscle tissues

Caffeine is known to release Ca from the SR in skeletal and cardiac muscles[4,9,11,65]. In vascular smooth muscles, caffeine also released Ca from the store site in the presence or absence of extracellular Ca. This drug also affects the sarcolemma, i.e. in intact muscle, low concentrations of caffeine hyperpolarized the membrane due to an increase in K-conductance and at high concentrations depolarized the membrane due to a decrease in the K-conductance[28,31]. In some vascular smooth muscle cells, increase in the K-conductance can be triggered by Ca distributed just beneath the membrane (Ca dependent K channel)[43]. When the internal Ca was kept at 10^{-7} M, the K channel was activated to a greater extent than at 10^{-8} M Ca, as measured by the patch clamp procedure using the rabbit portal vein. With application of procaine to the 'inside-out' membrane fragment the K channel was inhibited, which corresponds to a decrease in membrane conductance and the depolarization caused by procaine (K. Kitamura, personal communication). Caffeine, on the other hand, may increase the free Ca by release of bound Ca and thus increase the K conductance of the membrane. This means that caffeine releases Ca not only to trigger contraction but also to modulate the K-conductance of the membrane, while procaine inhibits the Ca release from the store site and also the K-conductance of the membrane.

In skinned smooth muscles, caffeine releases the Ca stored in the cell and this release is prevented by procaine (fig. 5). In skinned skeletal muscle, caffeine produces an oscillatory release of Ca from the SR in the presence of Ca and low concentrations of EGTA[13]. This oscillatory release of Ca was not apparent in vascular smooth muscles, presumably due to a lower uptake of Ca into the SR. In intact muscle cells of the rabbit mesenteric artery, NAd did produce oscillatory contractions. These contractions were attributed to activations of the Ca-induced Ca release mechanism, as the generation of oscillatory contractions depended on the amount of intracellularly stored Ca. Caffeine did not produce oscillatory contractions. Differences in responses to NAd or caffeine may be related to Ca extrusion from the cell, as this occurs more readily in the presence of caffeine than NAd and, as a

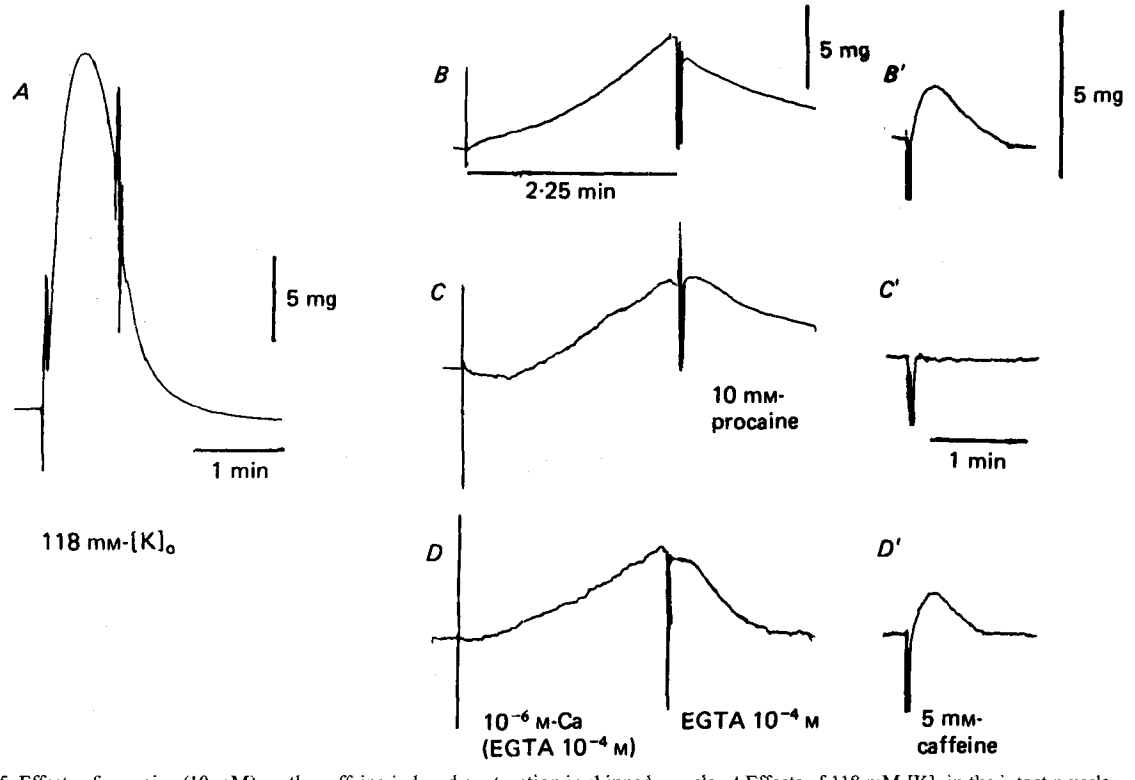

Figure 5. Effects of procaine (10 mM) on the caffeine-induced contraction in skinned muscle. *A* Effects of 118 mM-$[K]_0$ in the intact muscle. *B* and *D* Contraction and relaxation of the skinned muscle by application of 10^{-6} M-Ca with 10^{-4} M-EGTA and 10^{-4} M-EGTA containing relaxing solution, respectively. In *C*, procaine (10 mM) was added to the relaxing solution. *B'* and *D'* Caffeine-induced contraction (5 mM-caffeine). *C'* Effects of procaine (10 mM) on the caffeine-induced contraction (the relaxing solution was applied for 3 min in *B–D*) (Itoh, Kuriyama and Suzuki, J. Physiol. (1981)).

consequence, the amount of Ca stored is insufficient to activate the Ca-induced Ca release mechanism[33].

Mobilization of Ca estimated from single cell suspensions

The Ca mobilization across the sarcolemma can be measured using suspensions of single cells. When smooth muscle cells were prepared from the procine coronary artery by collagenase under Ca free conditions, trypan blue resistant isolated muscle cells (more than 90%) could be obtained. The Ca efflux estimated from the retained intracellular ^{45}Ca in Ca-free solution was temperature dependent ($Q_{10} > 3$). This Ca efflux was inhibited by oligomycin or vanadate. The findings indicate the presence of an active Ca transport mechanism at the sarcolemma (H. Ueno and H. Kuriyama, in preparation). When the Ca uptake was measured in the presence of various concentrations of ^{45}Ca after skinning the muscle cell suspension with saponin, the ^{45}Ca uptake increased in the presence of Ca over 4×10^{-8} M and reached a maximum value at 7×10^{-5} M Ca, under conditions of treatment with NaN_3 to block mitochondrial uptake. This accumulation increased 4 times in the presence of oxalate. Without treatment of NaN_3, the Ca uptake increased markedly in the presence of Ca concentrations over 10^{-6} M (8 times that observed in the presence of NaN_3 at 10^{-4} M Ca). However, at Ca concentrations below 10^{-6} M, the amount of Ca uptake was not modified by the presence of NaN_3. This means that, since Ca uptake into mitochondria occurred only in the presence of more than 10^{-6} M Ca, the uptake and release of Ca by mitochondria in this vascular smooth muscle may not play an important role in the contraction-relaxation cycle under physiological conditions.

When the saponin-treated single cell suspension was soaked in various concentrations of cold Ca, ^{45}Ca which has been previously taken up, was released in proportion to the concentration of cold Ca, and this ^{45}Ca release was inhibited by procaine. Therefore, the evidence of Ca efflux from the saponin treated single cells also supports the proposal of the existence of a Ca-induced Ca release mechanisms for mobilization of Ca in vascular smooth muscles.

Summary

This article was concerned with the role of Ca in triggering the contraction in vascular smooth muscles. Whenever Ca influx is activated, this Ca does not directly activate the contractile proteins, but rather triggers the release of Ca from the SR to activate calmodulin. This release of Ca by Ca is dependent on the amount of Ca stored within the cells.

Voltage dependent Ca influx activated by excess concentrations of K, electrical depolarization and Ca spikes is required to produce the contraction through activation of the Ca-induced Ca release mechanism. The elucidation of the contribution of the P-I response for Ca mobilization through activation of receptors under physiological conditions hopefully will lend support to our hypothesis.

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