What does the following have in common?

Expulsion of newborn from the uterus
Wheeze of asthma
Spasm of coronary arteries

Basics of muscle contraction

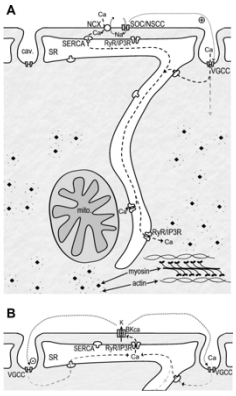
- Control of intracellular Ca^{2+} - principal mechanism that initiates contraction and relaxation in smooth and striated muscle
- Regulatory pathways:

  striated muscle-Ca^{2+} activates contraction by binding to thin filament associated protein, troponin

  smooth muscle-Ca^{2+} binds to calmodulin, which then associates with the catalytic subunit of myosin light chain kinase-phosphorylates serine 19 on the regulatory light chain of myosin (rMLC). Phosphorylation of Ser19 allows the myosin ATPase to be activated by actin and the muscle to contract.

Basics of muscle contraction

- Calcium regulation is vital
- In smooth muscle, the cytosolic free Ca^{2+} concentration is ~ 0.1 μM in basal state; ~ 10,000 times lower than that present in the extracellular space (mM)
- Activation of cells induces an increase in cytosolic concentration up to ~1-10 μM.
- Ca^{2+} diffuses in cell much more slowly than predicted from its small volume; Ca^{2+} atom migrate 0.1-0.5 μm, lasting only ~ 50 μs before being bound.
- Ca^{2+} used by different vasoactive agents comes from extracellular and/or intracellular space.
- Intracellular Ca^{2+} is localized in the mitochondria and SR
- Location is most important



Cytoplasmic microdomains permit specific regulation of components

For instance, extracellular Ca^{2+} entry typically appears as a uniform increase in Ca^{2+} signal (non-wavelike)

In contrast, when the ER/SR is the immediate source of Ca^{2+} , Ca^{2+} typically rises in a specific cellular locus, which then propagates in a wavelike fashion throughout the length of the cell.

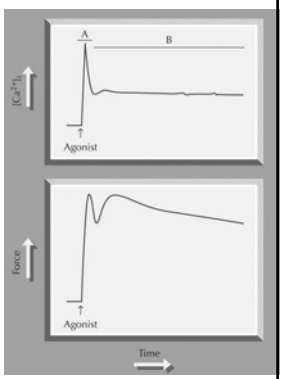
Lee et al, Am J Physiol Heart Circ Physiol (2002) 282:H1571

α1-adrenergic agonists, angiotensin II, vasopressin, endothelin elicit a rapid transient increase in $[Ca^{2+}]_i$ which subsequently declines to a steady state level that is higher than unstimulated.

Resultant force is biphasic; rapid phasic component and slow sustained tonic component.

Phasic contraction is activated by release of Ca^{2+} from intracellular stores.

Tonic contraction requires the influx of Ca^{2+} from extracellular space, which serves to maintain MLCK in a partially activated state.



Sward et al, Curr Hypertens Rep 2003 Feb;5(1):66-72

- The degree of interaction is determined by the net level of phosphorylation of the 20 kDa regulatory light chains of myosin II (rMLC).
- MLC is regulated by MLC kinase (MLCK) and MLC phosphatase (MLCP or PP1M).
- The extent of the rMLC phosphorylation and the amplitude of force production depends on the balance of the activities of MLCK and MLCP.
- Under certain conditions, force is also regulated independent of the changes in rMLC phosphorylation levels perhaps by thin filament associated proteins (caldesmon and calponin), which can be phosphorylated by MAP kinase and/or other kinases.
- Thin filament associated proteins might modulate the effect of rMLC phosphorylation, which is alone sufficient to initiate and maintain contraction.
- MLCP is a trimer comprising a 130 kD regulatory myosin binding subunit (MBS), a 37 kD catalytic subunit (PP1c), and a 20 kD protein of uncertain function (M20).

Precise coupling between force and rMLC phosphorylation is quite variable and non-linear

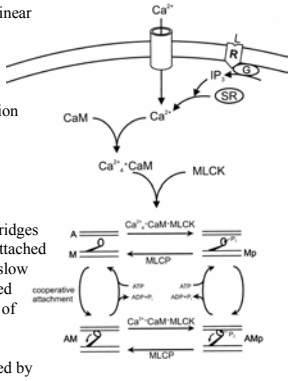
Maximal force can be attained at ~ 0.2-0.3 mol P_i/mol rMLC.

Phosphorylation often declines during tension maintenance.

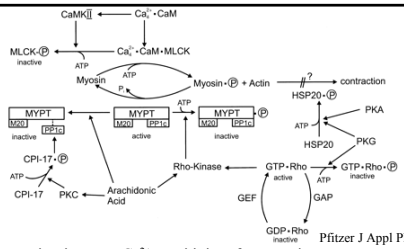
Nonphosphorylated myosin cross bridges contribute to force generation

Force generating dephosphorylated cross bridges could be generated by dephosphorylating attached cross bridges, which are thought to have a slow detachment rate compared to phosphorylated cross bridges or by cooperative attachment of dephosphorylated cross bridges.

Cooperative attachment is possibly regulated by calponin and caldesmon.



Pfitzer J Appl Physiol 91:497



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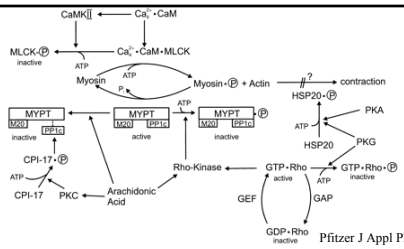
Contractile agonists increase Ca^{2+} sensitivity of contraction
Relaxation mediated by an increase in intracellular cAMP or cGMP is often associated with a decrease in Ca^{2+} sensitivity.
Two ways to modulate Ca^{2+} sensitivity:

alter balance of rMLC kinase and MLCP at constant Ca^{2+}
rMLC phosphorylation-independent regulatory mechanisms such as caldesmon or calponin or HSP20, which may disrupt phosphorylated myosin-actin cross bridges.



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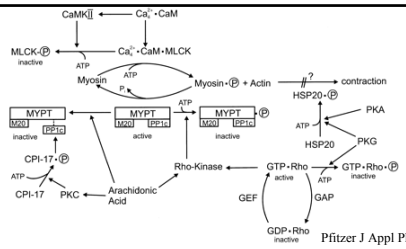
G protein-dependent inhibition of MLCP
(1) Phosphorylation of MYPT1
(2) Inhibition by endogenous smooth muscle specific phosphopeptide CPI-17
(3) Dissociation of the holoenzyme by arachidonic acid



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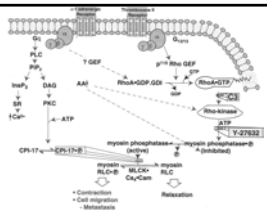
Phosphorylation of MYPT1 (MBS) by Rho kinase (ROK) leads to inhibition of MLCP. ROK inhibits MLCP by phosphorylation of MYPT1 at T695
Other substrates include CPI-17 and calponin.

CPI-17= 17 kDa PKC-Potentiated Inhibitory protein
CPI-17 becomes a potent inhibitor of MLCP when phosphorylated by PKC or ROK at T38.

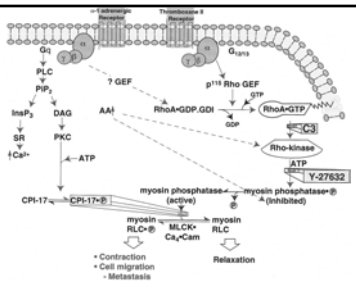
Arachidonic acid can activate ROK by interacting with the C-terminal regulatory domain of the kinase, alleviating auto-inhibition.



Tonic phase of the contractile response to thromboxane A2, endothelin, angiotensin II, vasopressin, α1-adrenergic agonists involves activation of $G_{12/13}$ family of heterotrimeric G proteins.

Activation of $G_{12/13}$ by exchange of GTP for bound GDP activates the guanine nucleotide exchange protein, p115-RhoGEF, which catalyzes the exchange of bound GDP for GTP on the small GTPase RhoA and dissociation of the guanine nucleotide dissociation inhibitor RhoGDI. Dissociation of RhoGDI enables translocation and insertion into the plasma membrane. RhoA-GTP then activates ROK, which phosphorylates MYPT1, resulting in inhibition of the phosphatase.

Shifts the balance in favor of kinase so that rMLC remains phosphorylated.

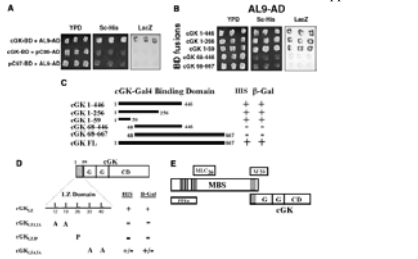


Contractile agonists acting through signaling molecules such as protein kinase C, arachidonic acid and rho kinase increase the sensitivity of vascular smooth muscle cells to contractile stimuli by inhibiting PP1M.

Signals that decrease Ca^{2+} sensitivity

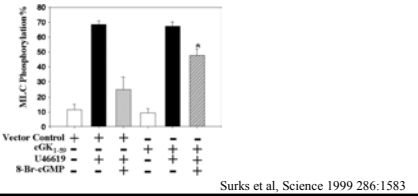
- Well-established that cAMP and cGMP decreases Ca^{2+} sensitivity of contraction in both intact and permeabilized smooth muscle.
- In vitro, PKA phosphorylates MLCK at two sites; site A decreases affinity of MLCK for Ca^{2+}/calmodulin complex.
- However, agents that elevate PKA have negligible effects on phosphorylation of site A and Ca^{2+} activation of MLCK; suggests that cAMP/PKA desensitizes smooth muscle by an alternate mechanism.
- Phosphorylation of MLCK by PKG has no effect on activity.
- Endogenous nitric oxide and related nitrovasodilators regulate blood pressure by activation of soluble guanylate cyclase, elevation of cGMP, activation of cGMP dependent kinase (cGKIα or PKG). cGMP-mediated vascular smooth muscle cell relaxation is characterized by a reduction in intracellular calcium concentration and activation of PP1M, which reduces the sensitivity of the contractile apparatus to intracellular calcium.
- The mechanism by which cGMP increases PP1M activity and myosin light chain dephosphorylation was elucidated in a series of experiments published by Surks et al.

- Y2H used to identify potential cGKIα binding proteins.
  - 2.5 x 10^6 clones from human activated T cell library
  - Clone AL9 encoded the COOH terminal 181 amino acids of myosin binding subunit of myosin phosphatase. MBS is a 130 kD regulatory subunit of PP1M that confers the specificity of PP1 for MLC and is the site on PP1M that is regulated by rho kinase.
  - The COOH terminal 181 amino acids of MBS includes a leucine zipper domain.



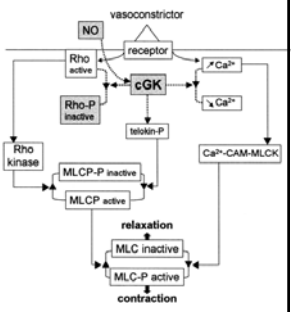
Surks et al, Science 1999 286:1583

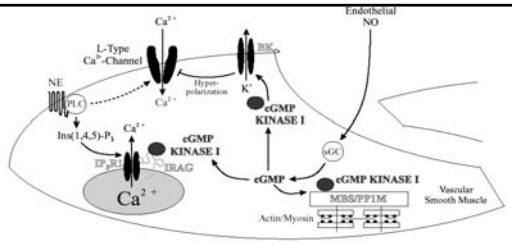
- MBS targets cGKIα to the SMC contractile apparatus and activation of cGKIα increases PP1M activity, the cGKIα increases PP1M activity.
- Thromboxane analog U46619 caused an increase in myosin light chain phosphorylation from 10 to 68% in both vector and cGK_{1-59} transfected vascular smooth muscle cells.
- In vector alone transfected SMC, 8 Br-cGMP inhibited U46619 mediated myosin light chain phosphorylation.
- Expression of cGK1-59 diminished the ability of 8 Br-cGMP to inhibit myosin light chain phosphorylation following U46619 stimulation.
- MBS assembles a multienzyme complex tethering a phosphatase and at least two kinases (Rho, cGK) with counter-regulatory effects.



Surks et al, Science 1999 286:1583

- PKG phosphorylates RhoA
- Phosphorylation may inhibit RhoA by (1) increasing association with guanine nucleotide dissociation inhibitor leading to termination of RhoA activation.
  (2) ? reduced interaction with Rho kinase
- Decreased RhoA/ROK activity would favor MLCP activity, leading to relaxation.
- Telokin-identical to the C-terminus MLCK is PKA/PKG phosphorylated. Phosphorylated telokin may increase MLCP activity, thereby mediating PKG mediated relaxation.





Hofmann et al J Cell Science 113: 1671-1676

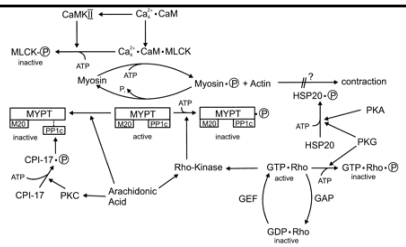
PKG phosphorylates IP3R at two sites in vitro and in vivo

Unclear whether PKG has a direct effect on Ca^{2+} release in vivo

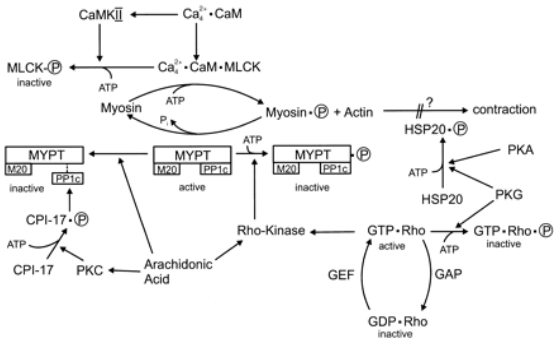
IP3R1 co-precipitates with cGKIβ and a 125-135 kDa protein termed IRAG (IP3R-associated cGKI substrate)

IRAG is located at ER membrane and is preferentially phosphorylated by CGKIβ, which inhibits IP3-induced Ca^{2+} release.

Major mechanism by which NO/cGMP reduces $[Ca^{2+}]_i$ and smooth muscle tone.



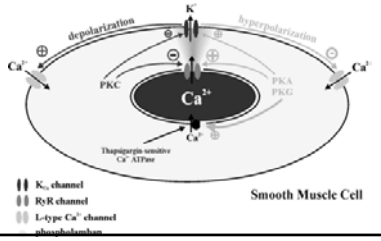
- CamKII has been reported to phosphorylate site A of MLCK; Note: although PKA phosphorylates same site in vitro, no evidence that it phosphorylates in vivo.
- Phosphorylation was associated with a decrease in Ca^{2+} sensitivity of rMLC phosphorylation.
- Suggested that this represents a negative feedback to inhibit high levels of rMLC phosphorylation.



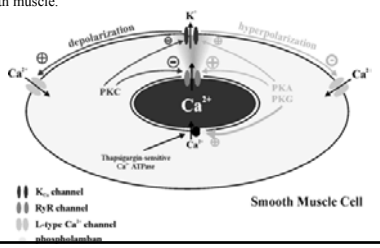
Pfitzer J Appl Physiol 91:497

Ion channels in smooth muscle

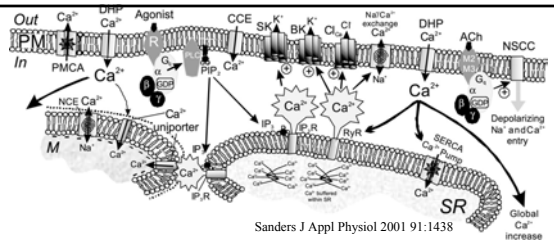
- Excitation-contraction coupling in smooth muscle is believed to occur by two mechanisms-electromechanical and pharmacomechanical coupling.
- Electromechanical coupling operates through changes in surface membrane potential; typically resting membrane potential= -40 to -70 mV.
- Primary drive for the rise in intracellular calcium is membrane depolarization, with the consequential opening of voltage operated calcium channels; neurotransmitters or hormones acting to depolarize the membrane will cause contraction while those producing membrane hyperpolarization will cause relaxation.
- Like cardiac muscle, the influx of Ca^{2+} likely causes release of Ca^{2+} from sarcoplasmic reticulum.



- Drugs that block calcium entry through VOCC will inhibit electromechanical coupling-thus the use of calcium channel blocking agents to relax vascular smooth muscle, thus producing vasodilatation and a decrease in blood pressure.
- Cell-type dependent; for instance, in asthma, Ca^{2+} blocking drugs are not effective in promoting relaxation of muscle.
- Electromechanical coupling appears to play a predominant role in phasic smooth muscle in which the membrane potential often displays marked oscillations upon which are superimposed calcium spikes
- The plasma membranes contain numerous ion channels and the distribution and properties vary among different tissues, contributing to the diversity of smooth muscle.



- Pharmacomechanical coupling- does not depend upon changes in membrane potential or calcium entry via the VOCC.
- The rise of intracellular Ca^{2+} is brought about by a combination of Ca^{2+} release from intracellular stores and Ca^{2+} entry through non-voltage gated channels, primarily receptor operated calcium channels or store operated Ca^{2+} channels
- Ca^{2+} signal often similar to that seen in many non-excitable cells, consisting of an initial rise in $[Ca^{2+}]_i$ followed by a smaller, but sustained increase dependent upon Ca^{2+} entry from the extracellular space.
- This secondary influx of Ca^{2+}, in association with the process of Ca^{2+} sensitization whereby the contractile apparatus may be activated by near-resting levels of $[Ca^{2+}]_i$, allows muscles to maintain tone over prolonged periods in the presence of an agonist; occurs in tonic smooth muscle.



Sanders J Appl Physiol 2001 91:1438

IP3R are found in central and peripheral SR, suggested that agonists can release Ca^{2+} from both sites.

Activation of the phosphatidylinositol cascade by agonists acting on trimeric G proteins or receptor tyrosine kinases and activating PLC causes the release of IP3 from PIP2

IP3 mediated activation of IP3R is the major pharmaco-mechanical coupling in SMC. Confirmed by specific inhibitors, contraction following photolytic release of caged IP3

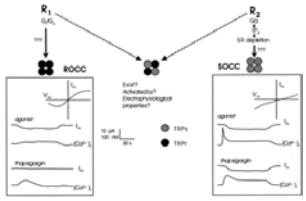
- The relative importance of electromechanical or pharmacomechanical coupling for any given smooth muscle preparation can be estimated by determining the effects of inhibitors of VOCC's on the contraction to agonists.
- For example, in guinea pig ileum, dihydropyridines such as nifedipine will virtually abolish all contractions, suggesting that electromechanical coupling predominates
- However, both mechanisms probably occur to some extent in all smooth muscle. In addition, the opening of ROCC and SOCC also produce membrane depolarization, thus activating electromechanical coupling.

- Approximately 20 years ago, it was hypothesized that receptor activation could lead to Ca^{2+} entry by a mechanism independent of membrane depolarization in smooth muscle
- Receptor operated currents have been described as non-selective cation currents rather than Ca^{2+} channel
- In the rabbit ear artery, externally applied ATP produced a rapid, transient depolarization of muscle, shown to result from activation of a non-selective cation conductance with significant Ca^{2+} permeability. Similar responses were reported to ATP in rat vas deferens, rabbit portal vein, and human saphenous veins.
- In addition to ATP, Noradrenaline, Acetylcholine, Histamine, Endothelin-1, Neurokinin A, Substance P, and Vasopressin have been shown to activate a receptor-operated cation current.

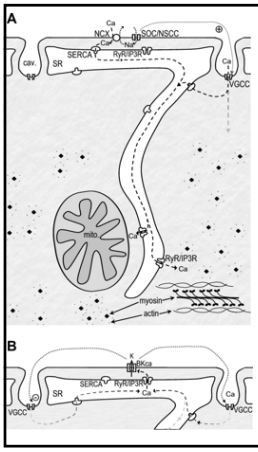
Store-operated calcium channels/currents

- In the late 1980's, Putney proposed the model for "capacitative calcium entry" in which intracellular Ca^{2+} store depletion stimulated Ca^{2+} influx across the plasma membrane to maintain a raised $[Ca^{2+}]_i$ in the face of prolonged agonist application and to aid in refilling of the stores on agonist withdrawal.
- It is not the Ca^{2+} released from the stores that activates SOCC. Thus, if the rise in $[Ca^{2+}]_i$ is prevented by inclusion of a Ca^{2+} buffer, then the store operated current would still be present. It is the fact that the stores are empty of Ca^{2+} that drives the response by an as yet unknown mechanism.
- Many of the neurotransmitters which activate ROCC simultaneously activate phospholipase C, liberating IP3. Therefore, SOCC is activated due to IP3 mediated depletion of the sarcoplasmic reticulum.

- Molecular evidence suggests that store-operated and receptor-operated channels may be formed from proteins belonging to the same family, being the mammalian homologues of the transient receptor potential (TRP) channels.
- Less clear whether they form the channels in native smooth muscle
- One putative model is that TRPC proteins may fall into two classes; one responsive to receptor activation but not store depletion and the other responsive to store depletion.



McFadzean and Gibson Br J Pharm 135: 1-13

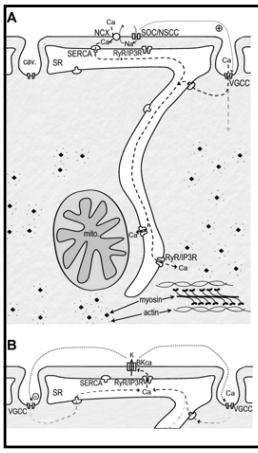


Junctional PM complex is critical for SMC contraction/relaxation

Rabbit IVC:

α-adrenergic stimulation, Ca^{2+} is transiently released from radial SR through IP3R, near the calmodulins tethered to myofilaments

Depletion of Ca^{2+} from SR/ER, which may be augmented by mitochondrial uptake causes opening of store-operated channels in the PM-SR; Na^{+} enters depolarizing membranes to activate VGCC and drives NCX in reverse direction to supply extracellular Ca^{2+} to PM-SR junctional space, which is taken up by SERCA. As SR is refilled, IP3R are activated, to start the next wave of regenerative Ca^{2+} release.

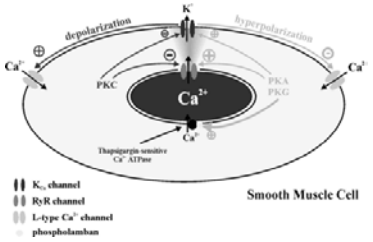


Junctional PM complex is critical for SMC contraction/relaxation

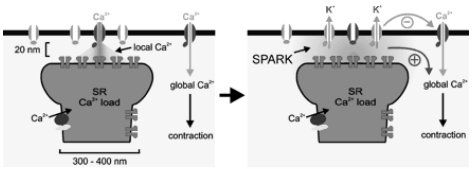
Rat cerebral resistance artery:

A different junctional complex composed of ryanodine receptor, SERCA and the large conductance Ca^{2+} activated K^{+} channel functions to relax VSMC. Recurring Ca^{2+} waves mediated by ryanodine receptor can elevate Ca^{2+} in junctional space to activate K_{Ca}, leading to hyperpolarization of membrane potential and inhibition of L-type VGCC.

Molecular organization of SMC is critical for function

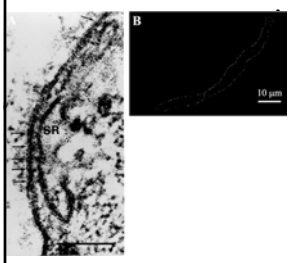


Proposed functional roles of Ca^{2+} sparks in smooth muscle cells



Unanswered questions: role of Ca^{2+} entry activating Ca^{2+} sparks

Sarcoplasmic reticulum in smooth muscle



The SR is the physiological intracellular source and sink of Ca^{2+} in smooth muscle, as in striated muscle.

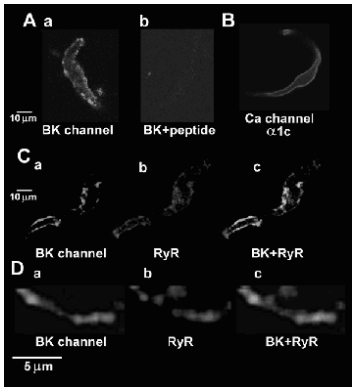
The Ca^{2+} pump of the SR is a SR/ER Ca^{2+}-ATPase of 100 kDa with isoforms 2a and 2b.

The SR also contains phospholamban, which regulates Ca^{2+} uptake by the SR.

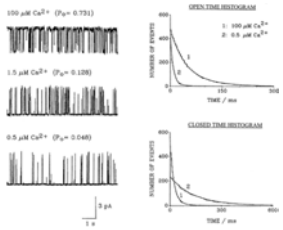
Central SR appears to form a continuous system connected with the peripheral SR.

The peripheral SR can form surface coupling with the plasma membrane; regions where the SR and plasma membranes come to within 8-10 nm of each other and are connected by elctron-dense bridging structures.

Jaggar et al, Am J Physiol Cell Physiol (2000)278:C235



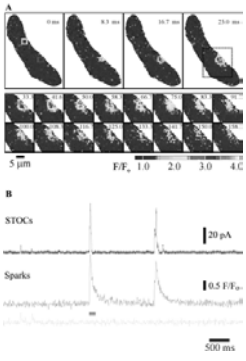
Ohi et al J Physiol 2001 534:313



Ryanodine receptors recorded in planar lipid bilayer;
Note Ca^{2+} dependence.

Jaggar et al, Am J Physiol Cell Physiol (2000) 278:C235

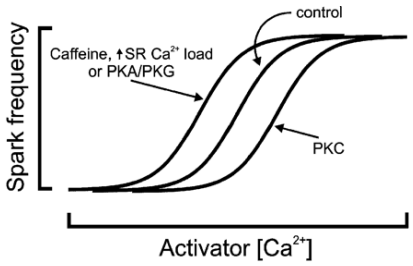
Ca^{2+} sparks activate BK_{Ca} channel currents in smooth muscle cells from cerebral arteries.



Spontaneous-transient outward current-STOC

Jaggar et al, Am J Physiol Cell Physiol (2000) 278:C235

Hypothetical modulation of Ca^{2+} spark frequency.



Jaggar et al, Am J Physiol Cell Physiol (2000) 278:C235

SR Ca^{2+} re uptake mechanisms

Few studies have addressed the role of uptake or removal of intracellular Ca^{2+}.

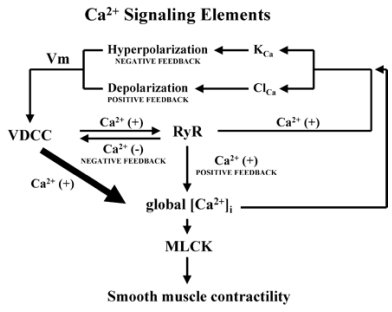
Recent studies have suggested that the $[Ca^{2+}]_{SR}$ may regulate Ca^{2+} sparks. Genetic ablation of phospholamban leads to chronic elevation in $[Ca^{2+}]_{SR}$ and Ca^{2+} spark frequency in arterial smooth muscle as compared to controls.

Elevation of $[Ca^{2+}]_{SR}$ increased Ca^{2+} sparks and transient K_{Ca} current frequency, but did not change spark amplitude, spatial spread or decay or the coupling ratio.

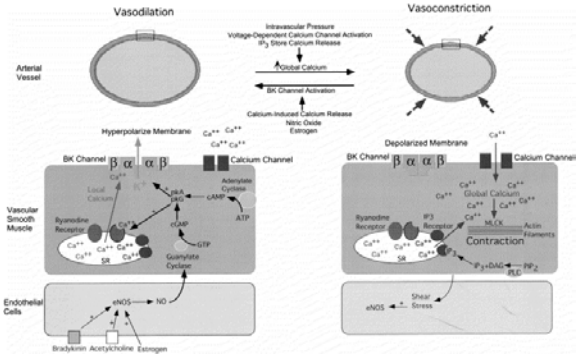
Decreasing $[Ca^{2+}]_{SR}$ reduced spark frequency, amplitude and spatial spread causing a reduction in frequency and amplitude of evoked transient K_{Ca} currents, although the coupling ratio was not affected.

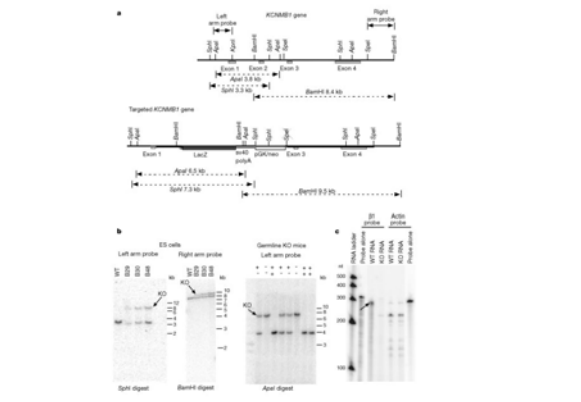
SR Ca^{2+} re uptake implications

- Elevation of $[Ca^{2+}]_{SR}$ can cause increased spark and transient K_{Ca} frequency that should lead to membrane hyperpolarization, decrease in voltage-dependent Ca^{2+} channel activity, reduction in global $[Ca^{2+}]_i$ and dilation.
- May also increase the driving force for sarcolemma extrusion mechanisms that are located in the vicinity of the release site, such as Na^+-Ca^{2+} exchanger and Ca^{2+}-ATPase. May also inactivate sarcolemmal voltage dependent Ca^{2+} channels.
- "Superficial buffer barrier hypothesis- Ca^{2+} entering SMC is buffered by the SR and is discharged vectorially towards the sarcolemma, without any effect on global $[Ca^{2+}]_i$.

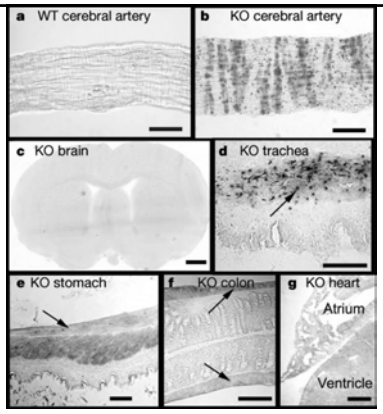


Jaggar et al, Am J Physiol Cell Physiol (2000) 278:C235

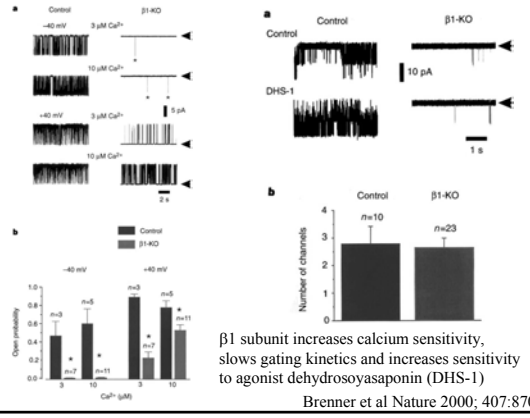




Brenner et al Nature 2000; 407:870

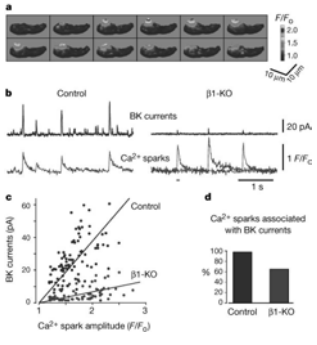


Brenner et al Nature 2000; 407:870



β1 subunit increases calcium sensitivity, slows gating kinetics and increases sensitivity to agonist dehydrosoyasaponin (DHS-1)

Brenner et al Nature 2000; 407:870



Brenner et al Nature 2000; 407:870

- Myogenic tone refers to the ability of vascular smooth muscle to alter its state of contractility in response to changes in intraluminal pressure
- The vessel constricts in opposition to an increase in intravascular pressure and dilates when the pressure decreases
- Behavior observed in a variety of vascular tissues, including veins and conduit arteries, but especially prevalent in resistance vasculature.
- Classically described as being a Ca^{2+} dependent process where pressure evoked depolarization and Ca^{2+} entry through voltage gated Ca^{2+} channels play obligatory roles
- Consistent with a role for pressure-induced depolarization, blockers of voltage gated Ca^{2+} channels have been shown to reduce myogenic responses.

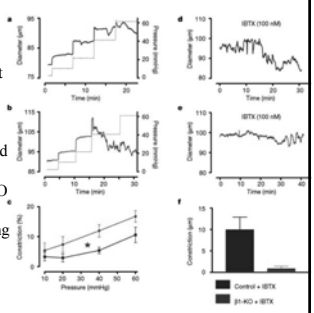
- Arteriolar SMC possess ion channels sensitive to cell membrane stretch that may be activated by vessel distension arising from an increase in intraluminal pressure.
- Have relative permeability: $K^{+}>Na^{+}>Ca^{2+}$
- Ca^{2+} influx would be relatively small- generally believed that stretch activation of these channels mainly contributes to membrane depolarization with subsequent opening of voltage gated calcium channels.
- K_{Ca} currents have been shown to attenuate the stretch-induced changes in membrane potential and myogenic constriction.
- Mechanical perturbation of cell membranes may release factors that modulate the activity of such channels.

Elevation of intravascular pressure constricts small arteries (i.e. cerebral)

Cerebral arteries that lack the β1 subunit are more constricted at a given pressure than controls

Iberiotoxin (IBTX; blocks BKCa) caused increase in constriction in control (note decrease in diameter) as compared to KO

Results indicate that BK channels lacking the β1 subunit are unable to contribute to the regulation of arterial tone.

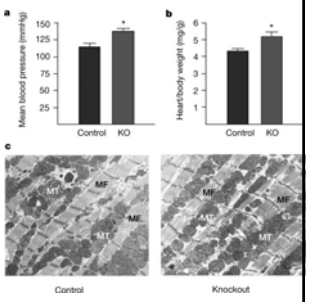


Brenner et al Nature 2000; 407:870

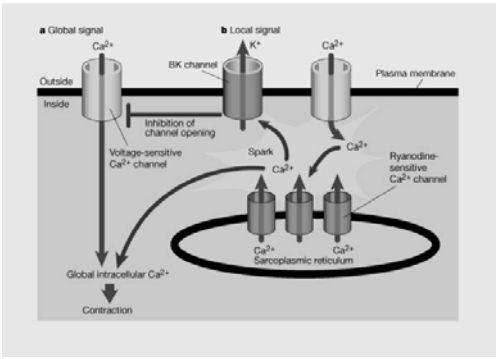
β1-KO mice demonstrated hypertension
Mean BP for control 114 mm Hg
and KO 134 mm Hg.

β1-KO mice demonstrated increased heart/body weight measurements c/w hypertension.

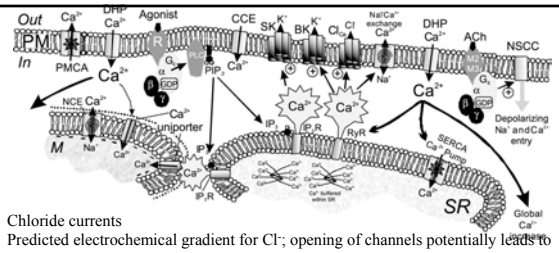
Electron microscopy demonstrated no difference between control and KO.



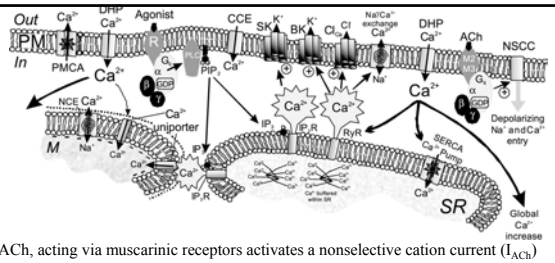
Brenner et al Nature 2000; 407:870



Standen Nature 2000; 407:845



Chloride currents
Predicted electrochemical gradient for Cl^-; opening of channels potentially leads to Cl^- efflux, membrane depolarization and vasoconstriction
Although Cl_{Ca} has been implicated in responses to agonists or neurotransmitter stimulation, controversy remains.
depolarizing effect of Cl_{Ca} could be overwhelmed by the hyperpolarizing effect resulting from activation of K_{Ca} channel
Volume-regulated Cl^- channels are expressed in VSMC; however the current generated during volume changes are not pharmacologically identified as Cl^-; therefore, the role for Cl^- channels in regulating myogenic tone requires further research.



ACh, acting via muscarinic receptors activates a nonselective cation current (I_{ACh}) in vascular and visceral smooth muscles.

At negative potentials, most of the current through this conductance is carried by Na^+; the inward Na current is responsible for a significant part of the depolarization.

I_{ACh} is voltage dependent in many cells; current reverses near 0 mV

I_{ACh} is regulated by G proteins, and activation of I_{ACh} is blocked by pertussis toxin

Unclear whether the conductance is a significant source of Ca^{2+}

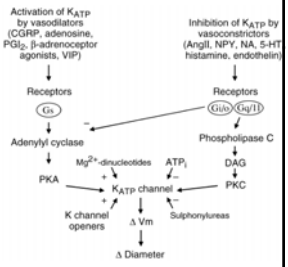
K_{ATP} channel exists as an octameric complex, containing two types of proteins subunits

Consists of 4 inwardly rectifying K^+ channel subunits , each associated with a larger regulatory sulphonylurea receptor (SUR).

Inhibited by μM $[ATP]_i$ and sulphonylurea agents

Activated by nucleotide diphosphates, in the presence of Mg^{2+}

Endogenous vasodilators, such as calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP) are mediated through PKA mediated activity of K_{ATP} channels.



Endothelium-dependent vasodilators

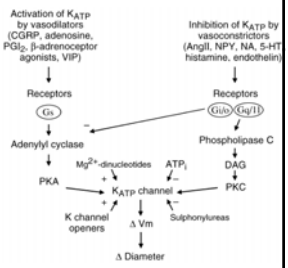
Endothelial-derived nitric oxide relaxes VSMC, in part through effects on K^+ channels.

Evidence suggests that primary mechanism is through K_{Ca}, but also via K_{ATP} (cross-activation of PKA)

Endothelium derived hyperpolarizing factor (probably distinct from endothelium derived relaxing factor/NO) may activate K_{ATP}.

Prostacyclin hyperpolarizes VSMC, probably via activation of K_{ATP}.

Adenosine activates K_{ATP} probably via activation of PKA



Vasoconstrictors may act through inhibition of K^+ channels leading to depolarization.

Endothelin, vasopressin and angiotensin II may act, in part through inhibition of K_{ATP} channels via PKC activity (both direct and indirect) through inhibition of PKA.

K_{ATP} channels may be activated in several pathologic states:
(1) Coronary, cerebral and skeletal muscle arteries dilate in response to hypoxia probably through alteration in ATP levels.
(2) Ischemia/reperfusion: Reactive hyperemia may cause increased adenosine
(3) Acidosis activates K_{ATP}
(4) Endotoxins and septic shock can activate K_{ATP}