Part IV

# Smooth Muscle

## Differentiation Markers of the SMC Lineage

The unique physiological properties of SMCs are ultimately attributable to the expression of a discrete subset of SMC lineage-restricted contractile proteins, signaling molecules and cytoskeletal elements including smooth muscle (SM)-α-actin (*Acta2*), SM-myosin heavy chain (*Myh11*), SM22α (*Tagln*), SM-calponin (*CnnI*), telokin (*Mylk*), and smoothelin (*Smtn*) (for review see 2). Multiple studies have shown that the muscle-restricted transcriptional co-activator myocardin plays a critical role in regulating the "contractile SMC gene program" acting via its capacity to physically associate with the transcription factor, SRF, which in turn binds to CArG box-containing regulatory elements controlling the transcription of these SMC lineage-restricted genes (for review see 5). Recent studies have shown that alternatively spliced isoforms of myocardin regulate transcription in smooth and cardiac muscle cells, respectively (6). Myocardin sits at a nodal point regulating SMC phenotype which is influenced by multiple signaling pathways that converge upon the myocardin-SRF complex in the nucleus of SMCs influencing its activity (for review see 5).

There is ongoing debate over whether a true SMC lineage-specific marker exists. Most SMC-restricted proteins are expressed at least transiently in other muscle cell

lineages. Smooth muscle myosin heavy chain (SM-MyHC) is considered the most definitive marker of the SMC lineage (7). In situ hybridization studies performed in the developing mouse have revealed SM-MyHC is restricted to vascular and visceral SMCs (7). Cell fate-mapping studies utilizing the SM-MyHC promoter/enhancer demonstrated restricted expression of the *LacZ* reporter gene in vascular and visceral SMCs with the exception of a small population of cells within the right atrium of the heart (8). However, the failure to detect SM-MyHC gene or protein expression does not preclude a cell from belonging to the SMC lineage. In the mouse embryo, there is at least a 48-hour temporal delay between the expression of early SMC markers, such as SMA and SM22$\alpha$, and the expression of SM-MyHC in the embryonic aorta (7). Similarly, SM-MyHC is frequently not observed in synthetic SMCs following vascular injury or in other pathological circumstances (9).

Smooth muscle $\alpha$-actin (SMA) is one of the earliest markers and it is the most abundant protein expressed in SMCs (for review see 3). However, SMA is transiently expressed in embryonic cardiomyocytes and skeletal muscle and in transforming growth factor-beta (TGF-$\beta$)-stimulated endothelial cells and myofibroblasts (10,11).

and myo

## SMC PROGENITORS AND STEM CELLS

### Embryonic Stem Cells

An understanding of the processes whereby SMCs are specified and differentiate from embryonic stem (ES) cells and/or progenitor cells is required in order to elucidate and distinguish lineage relationships underlying the function(s) of SMCs during embryonic and postnatal development. ES cells are pluripotent cells derived from the inner cell mass of the blastocyst possessing the capacity for self-renewal and the ability to differentiate into all cell types. Multiple laboratories have shown that under specific cell culture conditions, embryonic stem cells may be induced to differentiate into a population of cells enriched for definitive SMCs. Exposure to TGF-β or collagen promotes ES cells to differentiate toward the SMC fate suggesting that differentiation of SMCs is dependent, at least in part, upon signals transduced from the extracellular matrix (26,27). At a transcriptional level, the forced expression of the SMC lineage-restricted transcriptional co-activator myocardin activates most, but not all, SMC lineage-restricted genes in undifferentiated embryonic stem cells (28,29). However, myocardin is not required for differentiation of vascular SMCs from ES cells, as myocardin null (*Myocd*$^{-/-}$) ES cells differentiate into SMCs *in vitro* and contribute to the vasculature of chimeric mice (30). As such, dependent upon the specific developmental context myocardin-dependent and -independent SMC differentiation programs may be activated

well as hematopoietic cell lineages (34).

## EMBRYOLOGIC ORIGINS OF SMOOTH MUSCLE CELL LINEAGE

### Origin(s) of Vascular Smooth Muscle Cells

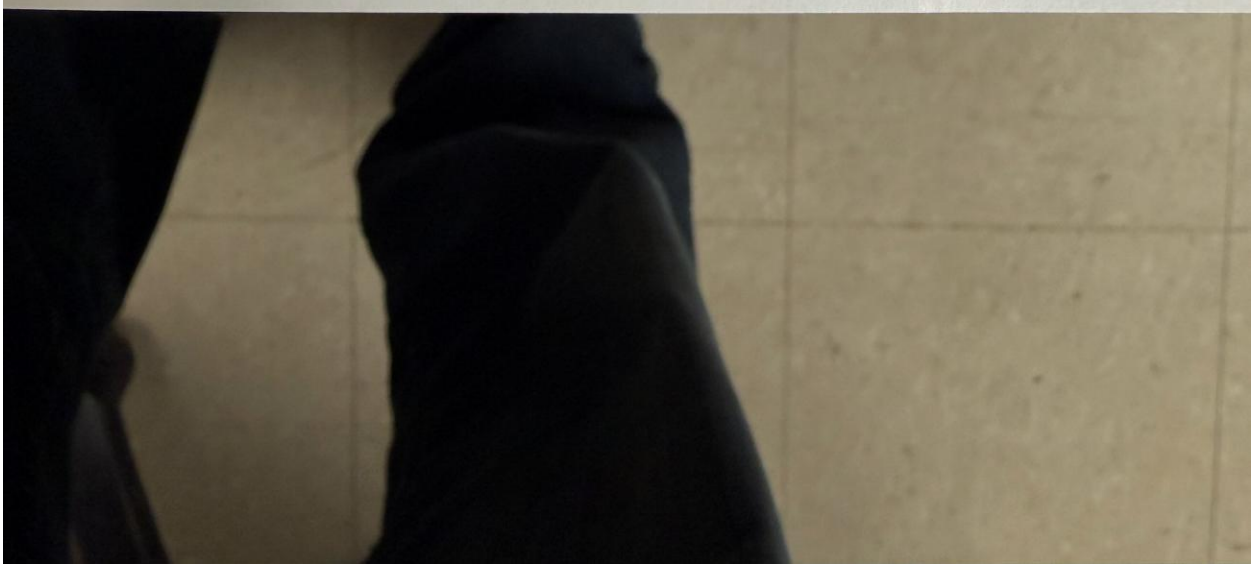
Vascular and visceral SMCs arise from multiple distinct locations and origins in the embryo in a precise spatially- and temporally-restricted manner. In the mouse, vascular SMCs are first observed on the ventral surface of the dorsal aorta at embryonic day (E) 9.0–9.5 (35). For many years it was believed that aortic SMCs were derived primarily from the splanchnic lateral plate mesoderm (36). Cell fate-mapping studies have confirmed that the first aortic SMCs in posterior regions of the embryo are derived from the lateral plate mesoderm (35). However, it is now recognized that these cells are subsequently replaced by somite-derived cells and that SMCs populating the adult aorta beyond the insertion site of the ligamentum arteriosum are derived from the paraxial mesoderm (35,37). Similarly, SMCs populating the renal and intercostal arteries are derived from the paraxial mesoderm (35). These somite-derived SMCs are believed to arise from progenitor cells in the sclerotome (37).

Cell fate-mapping studies have also revealed that SMCs populating the vasculature of the mesentery and gut are derived from a subset of Wilm's tumor protein (Wt1)-expressing serosal mesothelial cells (38). As such

precursors in other tissues and in adult tissues following stress or injury (1).

## Cardiac Neural Crest-Derived Vascular SMCs

A subpopulation of cephalic neural crest cells located between the mid-otic placode and the caudal boundary of the third somite, designated as the "cardiac neural crest", migrate ventrally through pharyngeal arches 3–6, where they invest and subsequently differentiate into vascular SMCs forming the tunica media of the great arteries and aorticopulmonary septum (for review see 40). As shown in Figure 82.1, cardiac neural crest cells delaminate from the neural tube and migrate to populate the 3rd, 4th and



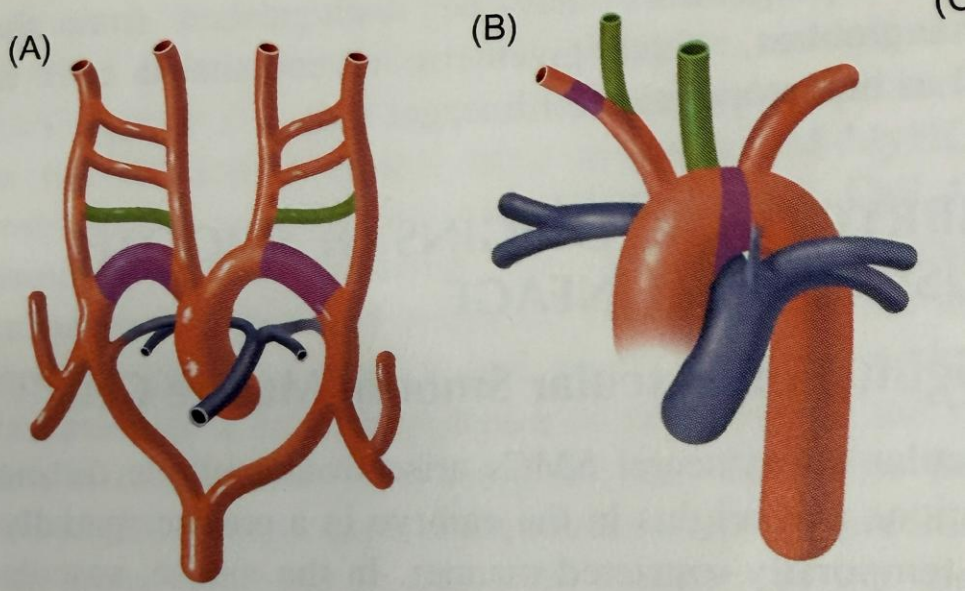



**FIGURE 82.1** Neural crest-derived SMCs populate the cardiac o
vascular system at E10.5 in the mouse. Cardiac neural crest cells mi
geal arch arteries and the cardiac outflow tract where they differen
panel B) are color coded in blue, purple, and green. (B) Patterning c
great arteries. (C) *Wnt1-Cre* transgenic mice were interbred with *R2*
gin of neural crest-derived vascular SMCs. This panel shows a post
(blue stain) in arteries populated by neural crest-derived SMCs inclu
not in the pulmonary artery (PA) or descending aorta (DAo) beyond

6th pharyngeal arch arteries. Subsequently, a subpop
tion of cells invades the cardiac outflow tract where
condense and contribute to the aortico-pulmonary sep
(41). Neural crest cells populating the pharyngeal
arteries differentiate into vascular SMCs expressing a
dant levels of SMC-restricted contractile pro
(Figure 82.1A). Subsequently, in response to the
release of growth factors

heart, proliferate, and migrate in a precise temporal and spatial pattern to form the epicardium. The proepicardial organ disappears by the end of the 5th week of human gestation.

In response to Friend of GATA (FOG)-2-dependent signals from the myocardium (59,60), a sub-population of epicardially-derived cells lose their epithelial character, undergo epithelial-to-mesenchymal transformation (EMT) and invaginate into the myocardium (61). It is not known whether there are separate populations of epicardially-derived mesothelial cells that differentiate into endothelial, smooth muscle or fibroblast sub-populations. In the embryonic heart, the coronary vessels develop from the blood islands which are aggregates of endothelial cells and erythrocytes that are not connected to the systemic circulation (58). Blood islands coalesce to form capillaries which grow within the subepicardium. Subsequently, coronary SMCs arise from the epicardial mesothelium via EMT and undergo SMC differentiation (62). These cells approach the vascular wall before the connection with the aorta is established (58). The coronary arterial circulation is established by directional capillary growth toward the sinuses of Valsalva ultimately establishing luminal patency via apoptosis of the aortic wall. Connection between the coronary arteries and aorta is established at day 44–49 in the human embryo (63). Further differentiation of mesenchymal cells into vascular SMCs occurs after blood flows within the patent coronary vasculature. Formation of the tunica media of larger coronary vessels starts at the proximal end of the coronary artery by building up a layer of mesenchymal cells.

SM with striated muscle, emphasizing the similarities but emphasizing the differences that account for the unique SM phenotype. Many of these features are also relevant to migrating non-muscle cells.

## THE CONTRACTILE APPARATUS

The rise of intracellular $Ca^{2+}$ serves as the initial switch to turn on the contractile apparatus in smooth, cardiac, and skeletal muscle. The pathways subsequently diverge with $Ca^{2+}$ binding to the actin filament-associated protein, troponin in striated muscles, whereas in SM $Ca^{2+}$ binds to calmodulin (CaM) forming an active complex with myosin light chain kinase (MLCK). MLCK/CaM/$Ca_4$ phosphorylates Ser19 of the 20 kDa myosin regulatory light chain ($RLC_{20}$) permitting actin activation of the actomyosin ATPase, cross-bridge cycling and contraction (1). A myosin light chain phosphatase (MLCP) dephosphorylates $RLC_{20}$. The magnitude of force output depends on the balance of MLCK and MLCP activities. Stimulus specific secondary levels of $Ca^{2+}$-independent regulation occur through multiple signaling pathways that modify MLCK and MLCP activities (2). Ultimately, all of these processes converge on the contractile cytoskeleton responsible for the mechanical contractile event. Thus, an appreciation of the players involved and the organization of the contractile apparatus is necessary to understand the unique mechanical properties of SM cells needed to carry out their specialized functions in the body.

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## General Organization and Relationship to Mechanical Properties

The thin actin filaments, thick myosin filaments and dense bodies constitute a contractile unit in SM (3,4). In cross-sections of portal vein SM cells myosin filaments form a 60–80 nm lattice (Figure 83.1), with each myosin surrounded by an orbit of actin filaments. The measured actin to myosin filament ratio is 15:1 (5) in agreement with ratios determined biochemically (6). At higher magnification, cross-bridges project from the myosin filaments (Figure 83.1, lower left panel). Stereoscopic imaging of longitudinal sections revealed a longitudinal order of 3–5 neighboring myosin filaments 2.2 μm in length (3) that are considered to be the A-band of a mini-sarcomere (Figure 83.2). As the maximal force developed by muscle is proportional to the sarcomere length or to the number of cross-bridges acting in parallel, the greater length of SM compared with skeletal myosin filaments (2.2 vs. 1.5 μm) contributes to the ability of SM to develop nearly the same amount of force as striated muscle, in spite of the lower concentration of myosin (3). Actin filaments emanating from plasma membrane and cytosolic dense bodies can be followed to the myosin filaments and make up the equivalent of the I-bands and Z-bands of striated muscles (4) (Figure 83.2). Importantly, the polarity of the actin filaments determined by decoration with subfragment 1 of myosin, point away from the dense bodies just as observed at Z-bands and are thus correctly positioned for a sliding filament mechanism of contraction (4). In cultured SM cells, membrane-dense bodies become focal adhesion sites consisting of large dynamic protein complexes where the cytoskeleton connects to the extracellular matrix. The actin cross-linking protein, α-actinin, also found at striated muscle Z-bands, and vimentin localize to dense bodies

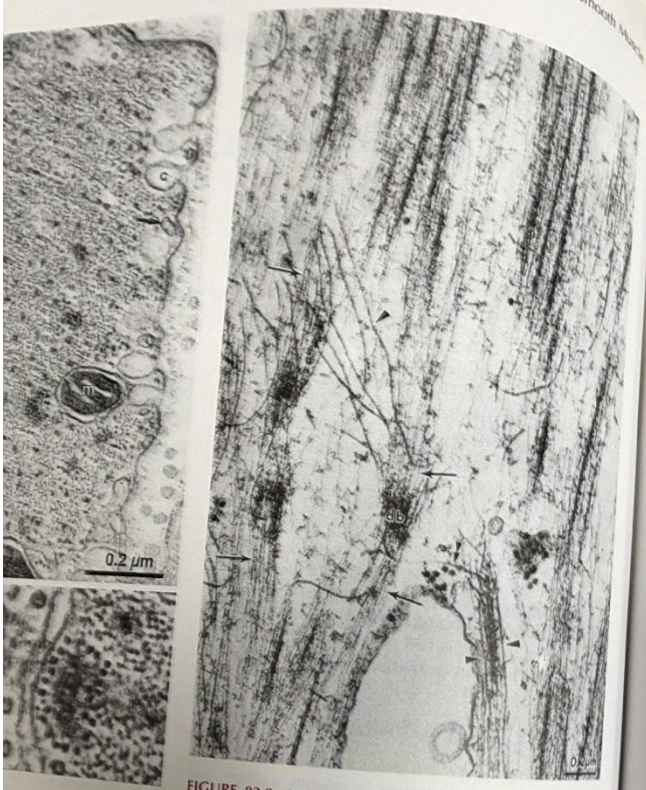


FIGURE 83.2 Mini-sarcomeres in a longitudinal section of a saponin-skinned portal vein SM cell. Filaments are spread out, revealing relationships of dense bodies with associated actin to neighboring myosin filaments. Thin filaments (*arrows*) that emerge from cytoplasmic dense bodies (db) can be traced to where they are adjacent to (overlap) myosin filaments. The 10 nm filaments (arrowheads) do not run parallel to the mini-sarcomere-like units, but appear to interconnect dense bodies (double arrowhead). *(From Bond and Somlyo, 1982 (4), with permission.)*

rabbit vas deferens SM cell
sin) filaments surrounded by
bodies (db) with associated
plings, where the sarcoplas-
membrane, are also present
ondria (m) are indicated.
(320,000×) of a transverse
filament surrounded by 16
at different levels can be
ell as a suggestion of other
ment. (Lower right panel)
nsversely sectioned dense
material is surrounded by
actin filaments are visible
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ing myofibrillogen-
mechanosensing,
ee Chapter 87).
up a third filament
Figure 83.1, lower

right panel). They do not run parallel to the mini-sarcomeres but are obliquely oriented linking up to dense bodies of different sarcomeres forming a non-contractile cytoskeleton. SM 10 nm filaments consist of two proteins, desmin and vimentin, which can be expressed individually or together in the same cell. They can massively increase in hypertrophied vascular SM (9).

## THE MYOSIN MOTOR

Smooth, cardiac, skeletal, and non-muscle myosins constitute class II of the myosin superfamily that form



filaments through self-association o
coiled-coil tails forming the filar
motor domain hydrolyzes ATP and
neck project from the backbone.
stroke of the cross-bridge, the moto
actin and the lever arm swings fron
of fulcrum point at the base of the m
ing the actin filament by 10 nm (10).
two light chains, the essential light c
regulatory light chain ($RLC_{20}$), both
modulin superfamily. $ELC_{17}$ wrap
α-helical segment of the lever arm
verter region at the fulcrum of the
with $RLC_{20}$ stabilize the lever arm.
forms, acidic ($ELC_{17a}$) and basic (E
five of the nine COOH-terminal ami
are products of a single gene (11).
with fast phasic and $ELC_{17b}$ with sl
The role of the two isoforms on contr
clear, although $ELC_{17a}$ isoform exc
$ELC_{17b}$ in SM lead to an increase in
ing and rate of force development by
cross-bridges (12). Regulation of S
$RLC_{20}$ is discussed below.

There is a single myosin heavy
Alternative splicing of two sites give
forms: SMB and SMA with or witho
insert near the ATP-binding pocket
rate of ATP hydrolysis and ADP relea
with 9 or 34 amino acids respectively
C-terminal tail thought to contribute
and possibly filament formation (1
were found in the ability of SM1 an
propel actin filaments, reviewed in (1
heterodimer pairing of all four MHC
individual SM cells may express one
forms. The 7 amino acid insert confer
ences being absent in the tonic aorta bu
SM such as intestine reviewed in (13).

## MYOSIN FILAMENTS

Myosin is organized into filaments in
tracted SM and does not depend on p
$RLC_{20}$ for assembly (5,14,15). Further
myosin in mature SM is filamentous in
agreement between SDS gels and qua
microscopy of relaxed SM (3). Therefo
posed pools of non-assembled myosin
when trachealis muscle is activated
lengths and that contributes to force deve
very small (16). The difficulty in these
from the technically challenging coun
profiles where cell length cannot be ea

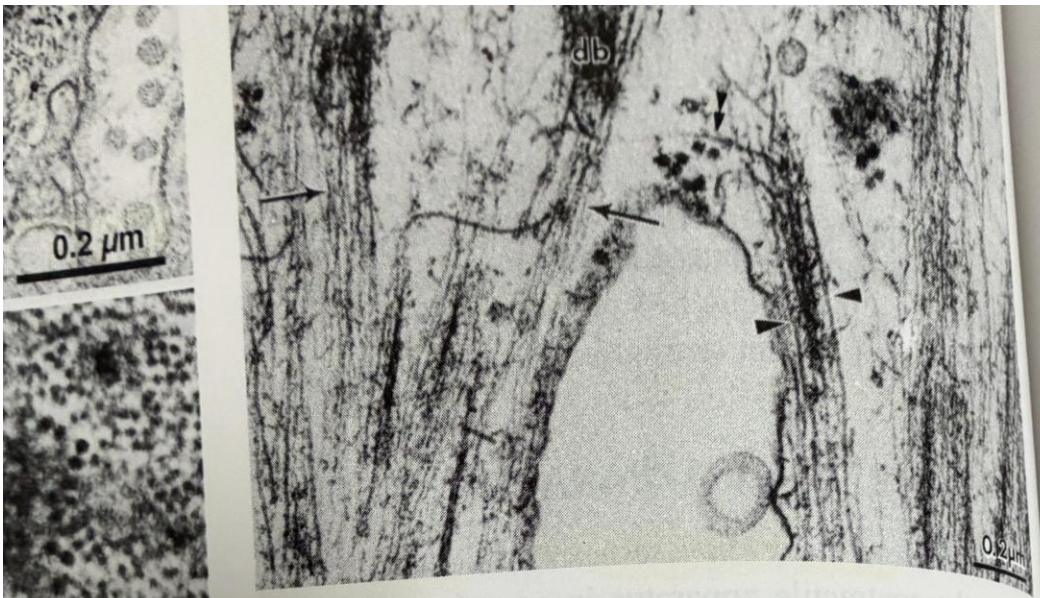


**FIGURE 83.2** Mini-sarcomeres in a longitudinal section of a saponin-skinned portal vein SM cell. Filaments are spread out, revealing relationships of dense bodies with associated actin to neighboring myosin filaments. Thin filaments *(arrows)* that emerge from cytoplasmic dense bodies (db) can be traced to where they are adjacent to (overlap) myosin filaments. The 10 nm filaments (arrowheads) do not run parallel to the mini-sarcomere-like units, but appear to interconnect dense bodies (double arrowhead). *(From Bond and Somlyo, 1982 (4), with permission.)*

right panel). They do not run parallel to the mini-sarcomeres but are obliquely oriented linking up to dense bodies of different sarcomeres forming a non-contractile cytoskeleton. SM 10 nm filaments consist of two proteins, desmin and vimentin, which can be expressed individually or together in the same cell. They can massively increase in hypertrophied vascular SM (9).

## THE MYOSIN MOTOR

Smooth, cardiac, skeletal, and non-muscle myosins constitute class II of the myosin superfamily that form

The ... clear, although $ELC_{17a}$ isoform exchange for the slow ... kinetics is not $ELC_{17b}$ in SM lead to an increase in velocity of shortening and rate of force development by non-phosphorylated cross-bridges (12). Regulation of SM myosin through $RLC_{20}$ is discussed below.

There is a single myosin heavy chain (MHC) gene. Alternative splicing of two sites gives rise to four isoforms: SMB and SMA with or without a 7 amino acid insert near the ATP-binding pocket which modifies the rate of ATP hydrolysis and ADP release; SM1 and SM2 with 9 or 34 amino acids respectively in the non-helical C-terminal tail thought to contribute to filament stability and possibly filament formation (13). No differences were found in the ability of SM1 and SM2 myosins to propel actin filaments, reviewed in (13). Homodimer and heterodimer pairing of all four MHCs may occur and individual SM cells may express one or more of the isoforms. The 7 amino acid insert confers functional differences being absent in the tonic aorta but present in phasic SM such as intestine reviewed in (13).

## MYOSIN FILAMENTS

Myosin is organized into filaments in relaxed and contracted SM and does not depend on phosphorylation of $RLC_{20}$ for assembly (5,14,15). Furthermore, most of the myosin in mature SM is filamentous in view of the close agreement between SDS gels and quantitative electron microscopy of relaxed SM (3). Therefore, recently pro- ... polymerizes ... muscle ... must be

filaments on either side of myosin filaments. Both bipolar and side polar filaments are compatible with the ability of SM to undergo extreme shortening to 25% of initial length (21). Actin filaments of opposite polarity from opposing dense bodies could be pulled along the side polar filament whereas in the case of bipolar filaments, the high actin to myosin ratio of 13:1 (21) could also bring into play additional correctly oriented actin filaments as the muscle shortens.

## CONTRACTILE REGULATION

The contractile regulation in SM is distinctly different than in striated muscles where $Ca^{2+}$ binding to troponin on the actin filament acts as a derepressor removing the inhibitory effect of troponin. In SM, $Ca^{2+}$ is a true activator increasing the low ATPase activity of the dormant myosin motor (1). This activation is through phosphorylation of the $RLC_{20}$ by the $Ca_4CaM$-dependent MLCK upon an increase in cytosolic $Ca^{2+}$. Double-headed myosin is needed for regulation (22). Phosphorylation of both

Regulatory light chains

through its actin-binding interface abutting the converter ... of one head is "blocked" domain of its partner head preventing hydrolysis. Upon phosphorylation the heads straighten. This conformation resembles the much-studied folded 10S myosin molecules, distinctive of smooth and non-muscle myosins, that upon phosphorylation assume an extended 6S active conformation (reviewed in 26). It remains to be determined whether this asymmetric head–head interaction occurs in myosin filaments either isolated or *in vivo*. Interestingly, cooperatively cycling non-phosphorylated cross-bridges can develop up to 40% of maximal force indicating that under some circumstance unphosphorylated heads are not "blocked" (27). Using cryoelectron microscopy and fitting of the SM HMM atomic structure, this asymmetric interaction of the heads has also been visualized on isolated non-phosphorylated cardiac and tarantula myosin filaments, which are stable in contrast to SM. This is surprising as the major "on" switch in cardiac muscle is regulated through $Ca^{2+}$ binding to troponin with light chain phosphorylation only playing a modulatory role under some conditions. Thus, this asymmetric head state may be a general relaxed state of the heads on the filament applicable to myosins in general and being more stable in SM (26). The detailed interactions of the off state are not yet established and await an atomic structure. A recent model, based on single ATP turnover data and on the functional dependence of the two SM myosin heads with one strongly and one weakly bound at any point in time, proposes a phosphorylation-dependent equilibrium between the compact inhibited state and the active state

ylated inhibitory protein CPI-17 (30). On the other hand, phosphorylation of telokin (31) or binding to the C-termini leucine zipper of MYPT1, reviewed in (2). Ultimately interaction cyclic nucleotides can activate MLCP through phosphorydomains and high-resolution structures are needed for insight into the molecular mechanisms underlying these processes.

## ACTIN

Thin filaments are two-stranded helical polymers of actin with the two strands crossing at ~36 nm encompassing seven actin monomers. Asymmetric actin monomers (G-actin) polymerize to form actin filaments (F-actin) 5–8 nm in diameter. Based on weight, the ratio of actin to myosin is strikingly different in SM compared with rabbit skeletal muscle, 3:1 vs. 1:3 respectively. The ratio of actin to myosin filaments is ~13:1 in SM (Figure 83.1) in contrast to 2:1 in striated muscle. Arterial has a somewhat higher ratio than venous SM. The total myosin content in SM is ~5 times less than in skeletal muscle yet it can develop equivalent maximal force/myocyte cross section (6). In motility assays the rate of movement of smooth and skeletal actin over phosphorylated or non-phosphorylated myosins, with and without a load was not different (32) in keeping with their similar biochemical properties. Thus, SM actin is not making a major contribution to the different contractile properties of smooth and striated muscle.

### Actin Isoforms

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38 nm periodicity along the actin filament. $Ca^{2+}$ binding to troponin results in a shift in the position of TM allowing interaction of actin and myosin, known as the "steric blocking model". Troponin is absent in SM yet the shift in the actin layer lines of the X-ray pattern considered to reflect the movement of TM occurs when SM is activated (40). If this interpretation is correct other Ca-binding proteins such as caldesmon (CD) may play a role to control TM (41). Nevertheless, while there is evidence for $Ca^{2+}$ regulation of the thin filament, albeit largely *in vitro*, Ca/CaM/MLCK phosphorylation of myosin $RLC_{20}$ is the dominant regulator for activation of force in SM while striated muscles are $Ca^{2+}$-regulated through the troponin switch on the actin filament. On the other hand CD regulation of thin filaments has been proposed to explain relaxation that under some conditions can occur at high levels of $RLC_{20}$ phosphorylation (reviewed in 42) and in the presence of significant basal $RLC_{20}$ phosphorylation (reviewed in 42). Conditional knock down of CD isoforms targeted to SM has yet to be achieved. Caldesmon first identified as a CaM binding protein, is a widely expressed 75 nm long molecule bound to the thin filament in a ratio of 1 CD:2 TM:14 actin in SM (reviewed in 41). the heavy isoform h-CD is restricted unphosphorylated,

force and shorten. This is complicated further in disease, since smooth muscles readily adapt to changes in functional demand by remodeling, with structural changes in contractile and passive elastic elements that modify the length-dependences of active and passive force production, resting compliance and the ability to shorten (62–64).

## F–V Relationship

The hyperbolic relationship between shortening velocity and force first described by Hill (65) for skeletal muscle also applies to smooth muscle tissues and single cells (56,66). This similarity in shape of the relationship in the two muscle types was taken as strong evidence for the operation of qualitatively similar cross-bridge mechanisms. Although maximum force production is the same or greater in smooth muscle, the maximum velocities of

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be similar

scales for the two muscles ... times faster, and its rate of chemical usage is 50 times greater. □, data for frog sartorius; ○, data for taenia coli. *(From Butler and Davies 1980 (77), with permission.)*

## Latch

Certain invertebrate smooth muscles, such as the anterior byssus retractor muscle (ABRM) of *Mytilus edulis*, show the catch state, which is similar in many ways to vertebrate smooth muscles. Catch is a condition that ensues following force development and the cessation of stimulation, characterized by a prolonged period (minutes to hours) of high force maintenance, high resistance to stretch, no force recovery on quick-release and extremely low energy cost when intracellular calcium concentrations have returned to just supra-basal concentrations. This is in contrast to the initial period of stimulation, when there is a high intracellular calcium concentration and force development, high rate of energy usage and the muscle shows force redevelopment following quick release. Unlike vertebrate smooth muscles, where the economy is based on cross-bridge kinetics, we now know that the high economy in catch derives from the fact that the mini-titin twitchin acts as a force-maintaining tether between actin and myosin filaments (see review in 79). Nevertheless, catch-like mechanical behavior, expressed as the absence of force redevelopment following quick release during force maintenance, was noted during tonic contractions in vertebrate vascular smooth muscle by the Somlyos (80). It is not yet known whether there is a counterpart to twitchin in vertebrate smooth muscles that show catch-like behavior. It is interesting that there are many structural and functional similarities between twitchin and MyC-protein of cardiac muscle (79).

Dillon and colleagues (81) measured shortening velocity at a low fixed afterload in arterial smooth muscle and found a time-dependent slowing of velocity during the

course of a contraction. The similarity in the time course of changes in shortening velocity to the time course phosphorylation of the 20 kD light chains of myosin together with energetic evidence (72) led to the development of a model for what is known as the "latch" state of mammalian smooth muscle. The mechanical and energetic similarities to the catch state inspired the term "latch." The latch-bridge hypothesis has evolved into one in which the unique properties of smooth muscle result from the dephosphorylation of an attached cross-bridge. The idea that high force output with low levels of myosin light chain phosphorylation could be explained on the basis of phosphatase activity was independently proposed by Driska (82) and Hai and Murphy (83). They suggested that myosin light chain phosphorylation is required for the transition of the cross-bridge into the force-producing state, and that phosphorylated myosin goes through the normal cross-bridge cycle in which there is attachment and detachment of the cross-bridge with concomitant splitting of ATP. A cross-bridge that is dephosphorylated while attached to actin and generating force, a latch-bridge, has a detachment rate that is very slow and would alter the kinetics of the completion of the cross-bridge cycle. The dephosphorylated cross-bridges would act as an internal load on the remaining phosphorylated cross-bridges, producing a decrease in velocity.

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these singly phosphorylated myosin molecules are still capable of generating significant force (97) and thus potential contributors to the latch state.

The observation that velocity of actin filament movement in the *in vitro* motility assay depended on the ratio of phosphorylated to unphosphorylated myosin suggests that the two cross-bridge populations can interact mechanically. The experiments were also interesting in that they showed that unphosphorylated smooth muscle myosin could impede skeletal muscle myosin to a greater extent than it does phosphorylated smooth muscle myosin, strongly suggesting that smooth muscle cross-bridges spend a greater fraction of their cycle time in the strongly bound, high-force producing state than skeletal muscle cross-bridges, which was directly confirmed at the molecular level using single molecule force assays (98,99). This, together with a longer myosin filament (3), would explain the ability of smooth muscles to generate as much force as striated muscles.

Chapter 84

# Potassium, Sodium, and Chloride Channels in Smooth Muscle Cells

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## INTRODUCTION

The plasma membrane of a vascular smooth muscle cell (SMC) expresses unique populations of $K^+$ channels, and the $K^+$ currents generated by these channels regulate electromechanical coupling and contraction of the blood vessel wall. The opening of $K^+$ channels in the SMCs results in $K^+$ efflux, and this hyperpolarizing current contributes to the resting membrane potential ($E_m$) of the SMC and promotes vasodilation. Multiple types of $K^+$ channels often work in concert to regulate the level of resting $E_m$ and to establish excitation patterns in a single SMC. Additionally, the vascular SMCs can form electrical syncytia with adjacent endothelial cells to establish an optimal level of tone in the blood vessel wall that, under normal circumstances, will permit tissues to be perfused commensurate with their needs.

This chapter primarily focuses on the structure and physiological role of $K^+$ channels in vascular SMCs. Two other types of ion channels, the voltage-gated $Na^+$ channel and the $Cl^-$ channels, also are considered since there is growing evidence for their role in regulating vascular tone. Finally, a short mention of these ion channels in the respiratory and gastrointestinal (GI) tracts is included to extend the discussion to non-vascular SMCs. Notably, the ion channel families discussed here (Figure 84.1) interact extensively with the $Ca^{2+}$-permeable channels discussed elsewhere in this book. In particular, the intracellular $Ca^{2+}$ contributed by $Ca^{2+}$-permeable channels can bind to $K^+$ and $Cl^-$ channels to dramatically alter their activity. Ultimately, a complex interaction between hundreds of ion channel subunits and signaling molecules will help to finely tune the level of excitability and contraction in SMC-containing organs.

## POTASSIUM CHANNELS

Under resting conditions, $K^+$ efflux across the plasma membrane is the primary driving force that confers a negative level of membrane potential ($E_m$) to the SMCs of the blood vessel wall. There are several unique characteristics that distinguish the electrical properties of vascular SMCs from those of striated muscle cells. First, the range of resting $E_m$ in vascular SMCs is generally between −60 mV and −35 mV, which is considerably more depolarized than the $E_m$ range between −90 mV and −70 mV generally observed in cardiac and skeletal muscle cells. Importantly, the more positive resting $E_m$ of the SMCs is near or even resides within the lower $E_m$ range for opening of voltage-gated $Ca^{2+}$ channels. Thus, the level of $E_m$ in SMCs is regarded as the primary determinant of contraction because even small reductions in $E_m$ corresponding to SMC depolarization will result in the opening of voltage-gated $Ca^{2+}$ channels, $Ca^{2+}$ influx, and SMC activation. Second, vascular SMCs rely more predominantly than striated muscle on the inhibition of resting $K^+$ efflux for depolarization, because the plasma membrane of SMCs often lacks the dense expression of fast $Na^+$ channels that mediate the initial excitatory inward current in cardiac and skeletal myocytes. Finally, SMCs appear to be electrically coupled to adjacent endothelial cells that line the blood vessel lumen by gap junction proteins, permitting ionic crosstalk between these two cell types to coordinate electrical events and the level of excitability within the blood vessel wall. In healthy individuals, the endothelial cells exert a hyperpolarizing effect on the vascular SMCs that promotes vasodilation and blood flow to distal tissues.

### Voltage-Gated $K^+$ Channels

The voltage-gated $K^+$ ($K_v$) channels are multi-protein complexes that share the common properties of $K^+$ selectivity and voltage-dependent activation. More than 40 mammalian genes encode the pore-forming α subunits of $K_v$ channels which are the transmembrane proteins that mediat conduction of $K^+$ across the plasma membrane (1 Structurally, these α-subunits contain six transmembra

Muscle. DOI: http://dx 78-0-12-381510-1.00084-3

## INTRODUCTION

The plasma membrane of a vascular smooth muscle cell (SMC) expresses unique populations of $K^+$ channels, and the $K^+$ currents generated by these channels regulate electromechanical coupling and contraction of the blood vessel wall. The opening of $K^+$ channels in the SMCs results in $K^+$ efflux, and this hyperpolarizing current contributes to the resting membrane potential ($E_m$) of the SMC and promotes vasodilation. Multiple types of $K^+$ channels often work in concert to regulate the level of resting $E_m$ and to establish excitation patterns in a single SMC. Additionally, the vascular SMCs can form electrical syncytia with adjacent endothelial cells to establish an optimal level of tone in the blood vessel wall that, under normal circumstances, will permit tissues to be perfused commensurate with their needs.

This chapter primarily focuses on the structure and physiological role of $K^+$ channels in vascular SMCs. Two other types of ion channels, the voltage-gated $Na^+$ channel and the $Cl^-$ channels, also are considered since there is growing evidence for their role in regulating vascular tone. Finally, a short mention of these ion channels in the respiratory and gastrointestinal (GI) tracts is included to extend the discussion to non-vascular SMCs. Notably, the ion channel families discussed here (Figure 84.1) interact extensively with the $Ca^{2+}$-permeable channels discussed elsewhere in this book. In particular, the intracellular $Ca^{2+}$ contributed by $Ca^{2+}$-permeable channels can bind to $K^+$ and $Cl^-$ channels to dramatically alter their activity. Ultimately, a complex interaction between hundreds of ion channel subunits and signaling molecules will help to finely tune the level of excitability and [illegible] in SMC-containing organs.

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negative level of membrane potential ($E_m$) to the SMCs of the blood vessel wall. There are several unique characteristics that distinguish the electrical properties of vascular SMCs from those of striated muscle cells. First, the range of resting $E_m$ in vascular SMCs is generally between −60 mV and −35 mV, which is considerably more depolarized than the $E_m$ range between −90 mV and −70 mV generally observed in cardiac and skeletal muscle cells. Importantly, the more positive resting $E_m$ of the SMCs is near or even resides within the lower $E_m$ range for opening of voltage-gated $Ca^{2+}$ channels. Thus, the level of $E_m$ in SMCs is regarded as the primary determinant of contraction because even small reductions in $E_m$ corresponding to SMC depolarization will result in the opening of voltage-gated $Ca^{2+}$ channels, $Ca^{2+}$ influx, and SMC activation. Second, vascular SMCs rely more predominantly than striated muscle on the inhibition of resting $K^+$ efflux for depolarization, because the plasma membrane of SMCs often lacks the dense expression of fast $Na^+$ channels that mediate the initial excitatory inward current in cardiac and skeletal myocytes. Finally, SMCs appear to be electrically coupled to adjacent endothelial cells that line the blood vessel lumen by gap junction proteins, permitting ionic crosstalk between these two cell types to coordinate electrical events and the level of excitability within the blood vessel wall. In healthy individuals, the endothelial cells exert a hyperpolarizing effect on the vascular SMCs that promotes vasodilation and blood flow to distal tissues.

## Voltage-Gated $K^+$ Channels

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## Voltage-Gated $K^+$ Channels

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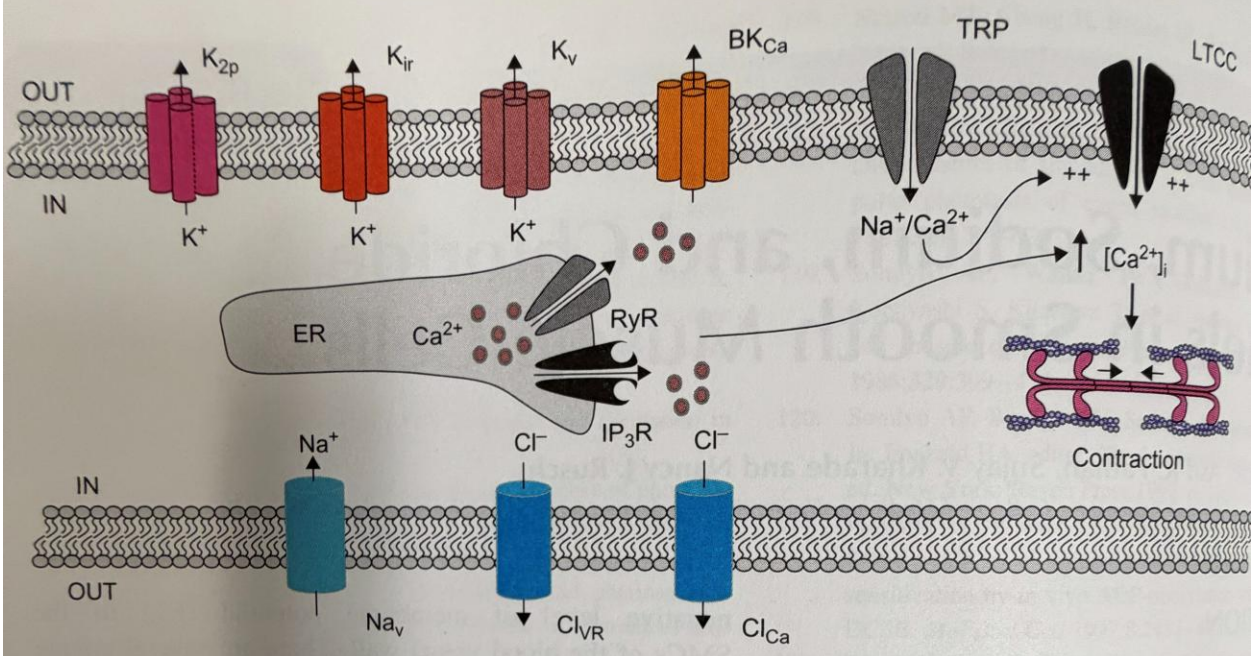
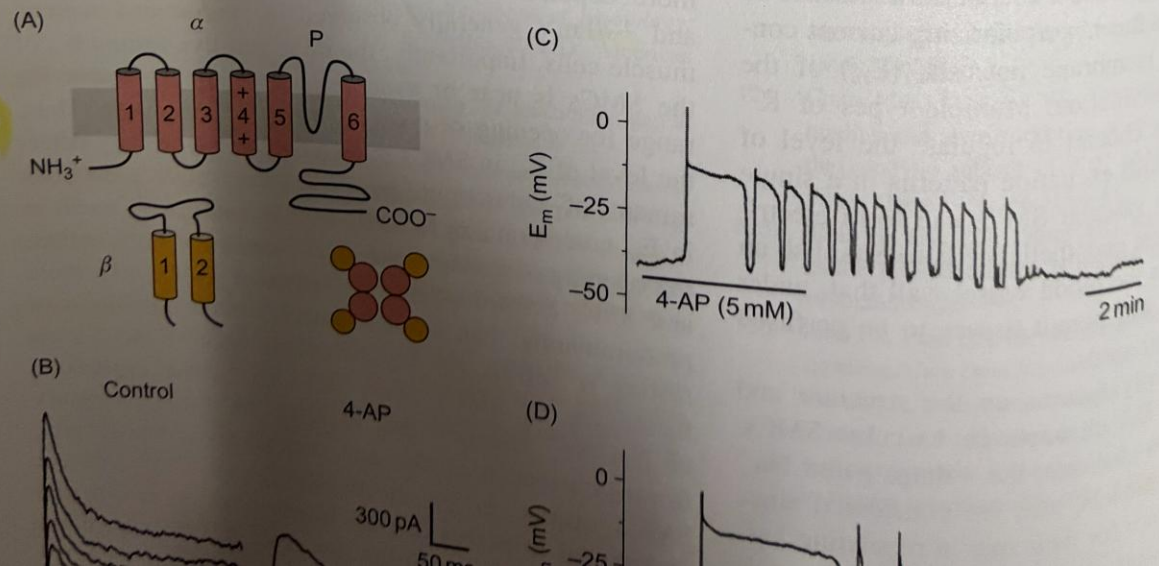



**FIGURE 84.1 Ion channels in vascular SMCs.** The $K^+$ channels include the two pore domain ($K_{2P}$), inwardly rectifying ($K_v$) and high-conductance, $Ca^{2+}$-sensitive ($BK_{Ca}$) $K^+$ channels. Voltage-gated $Na^+$ ($Na_v$) channels, volume-regulated $Cl^-$ ($Cl_{VR}$) sensitive $Cl^-$ ($Cl_{Ca}$) channels also are expressed. These $K^+$ channels interact with the ryanodine receptors (RyR), inositol tr ($IP_3R$), transient receptor potential (TRP) channels and L-type $Ca^{2+}$ channels (LTCC) to modulate SMC excitability.



of an abnormal oscillating $E_m$ pattern (Figure
A higher concentration of 4-AP (10 mM) establishes a sustained and profound depolarization of the SMCs (Figure 84.2D) (2). Similar excitatory responses occur in response to block of $K_v$ channels in the SMCs of many other vascular preparations. Not surprisingly considering their important dilator function, a loss of $K_v$ channels has been observed in pathologies of elevated vascular tone (9–11). Thus, the $K_v$ channels broadly mediate vasodilation in many vascular beds and may play a critical role in buffering abnormal vascular tone.

## High-Conductance, Calcium-Sensitive $K^+$ ($BK_{Ca}$) Channels

The high-conductance, $Ca^{2+}$-sensitive $K^+$ channels are often referred to as "Maxi-K" or "Big K" ($BK_{Ca}$) channels to recognize their high single-channel conductance

(200–300 pS), which may be 10-fold higher than the current amplitudes generated by $K_v$ channels. Thus, the opening of $BK_{Ca}$ channels unleashes a powerful hyperpolarizing force in vascular SMCs. As reviewed elsewhere in detail (12), the pore-forming α-subunit shows partial homology with the $K_v$ channels in six (S1–S6) of its seven (S0–S6) transmembrane domains that confer voltage-sensitivity and pore-formation (Figure 84.3A). However, the additional property of $Ca^{2+}$-sensitivity (Figure 84.3B) is conferred by four intracellular domains (S7–S10) and by the interaction of a unique extracellular N-terminus with a $\beta_1$-subunit. Unlike the α-subunits of $K_v$ channels that originate from multiple gene families, $BK_{Ca}$ channels appear to arise from a single *hSlo* gene, although phenotypic diversity is generated by a high level of alternative splicing. Iberiotoxin, a scorpion toxin, selectively blocks $BK_{Ca}$ channels when applied to the external membrane surface.

The $BK_{Ca}$ channels are densely expressed in vascular SMCs, and are particularly evident in the small arteries and arterioles of the cerebral, coronary, and renal circulations. These vessels show a high level of pressure-induced depolarization and constriction ("myogenic" tone). The depolarizing response to pressure in these SMCs coupled $Ca^{2+}$ act synergistically to activate

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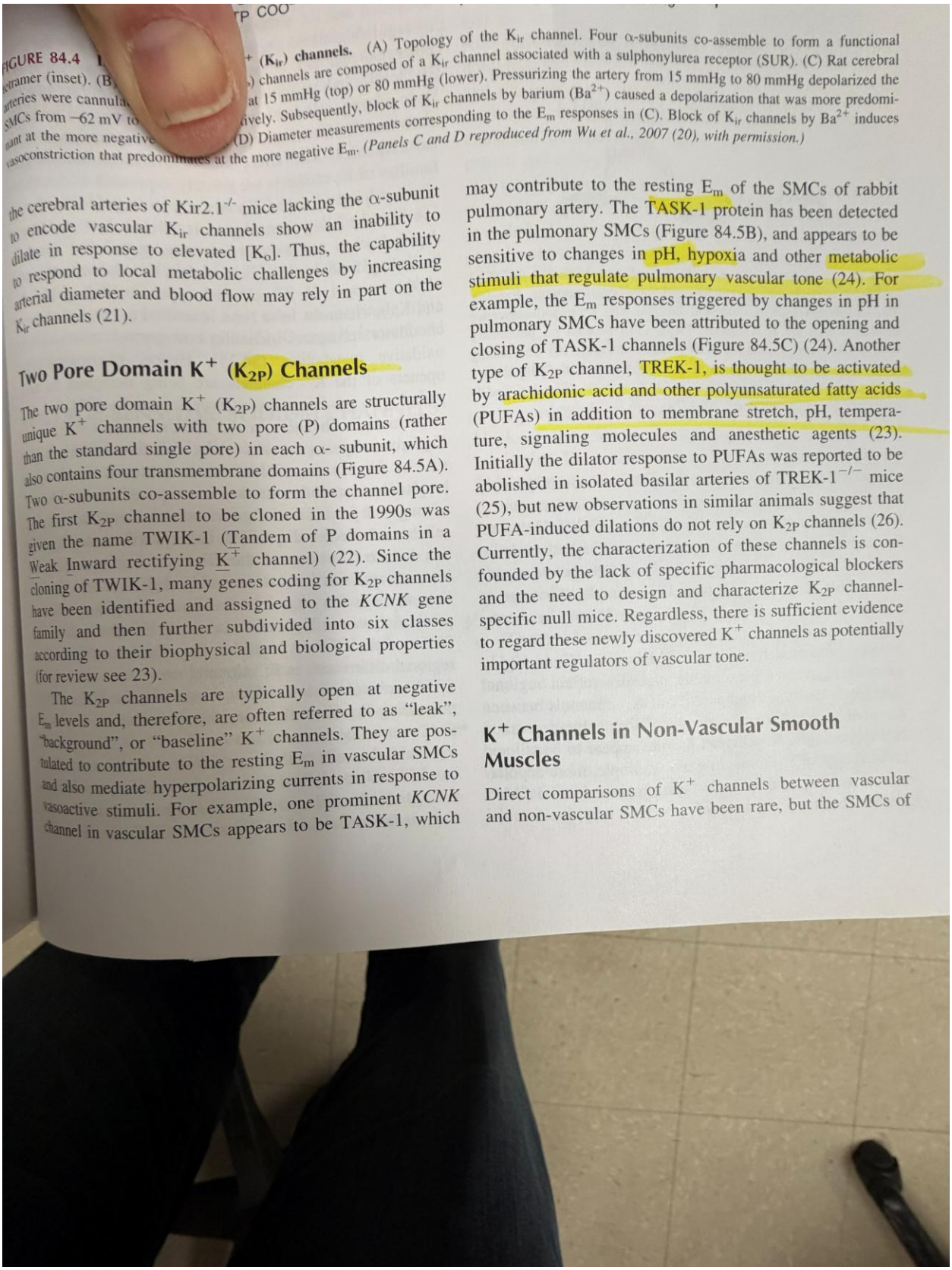


**FIGURE 84.4** I… **($K_{ir}$) channels.** (A) Topology of the $K_{ir}$ channel. Four α-subunits co-assemble to form a functional tetramer (inset). (B) …) channels are composed of a $K_{ir}$ channel associated with a sulphonylurea receptor (SUR). (C) Rat cerebral arteries were cannula… at 15 mmHg (top) or 80 mmHg (lower). Pressurizing the artery from 15 mmHg to 80 mmHg depolarized the SMCs from −62 mV to … tively. Subsequently, block of $K_{ir}$ channels by barium ($Ba^{2+}$) caused a depolarization that was more predominant at the more negative … (D) Diameter measurements corresponding to the $E_m$ responses in (C). Block of $K_{ir}$ channels by $Ba^{2+}$ induces vasoconstriction that predominates at the more negative $E_m$. *(Panels C and D reproduced from Wu et al., 2007 (20), with permission.)*

the cerebral arteries of Kir2.1$^{-/-}$ mice lacking the α-subunit to encode vascular $K_{ir}$ channels show an inability to dilate in response to elevated $[K_o]$. Thus, the capability to respond to local metabolic challenges by increasing arterial diameter and blood flow may rely in part on the $K_{ir}$ channels (21).

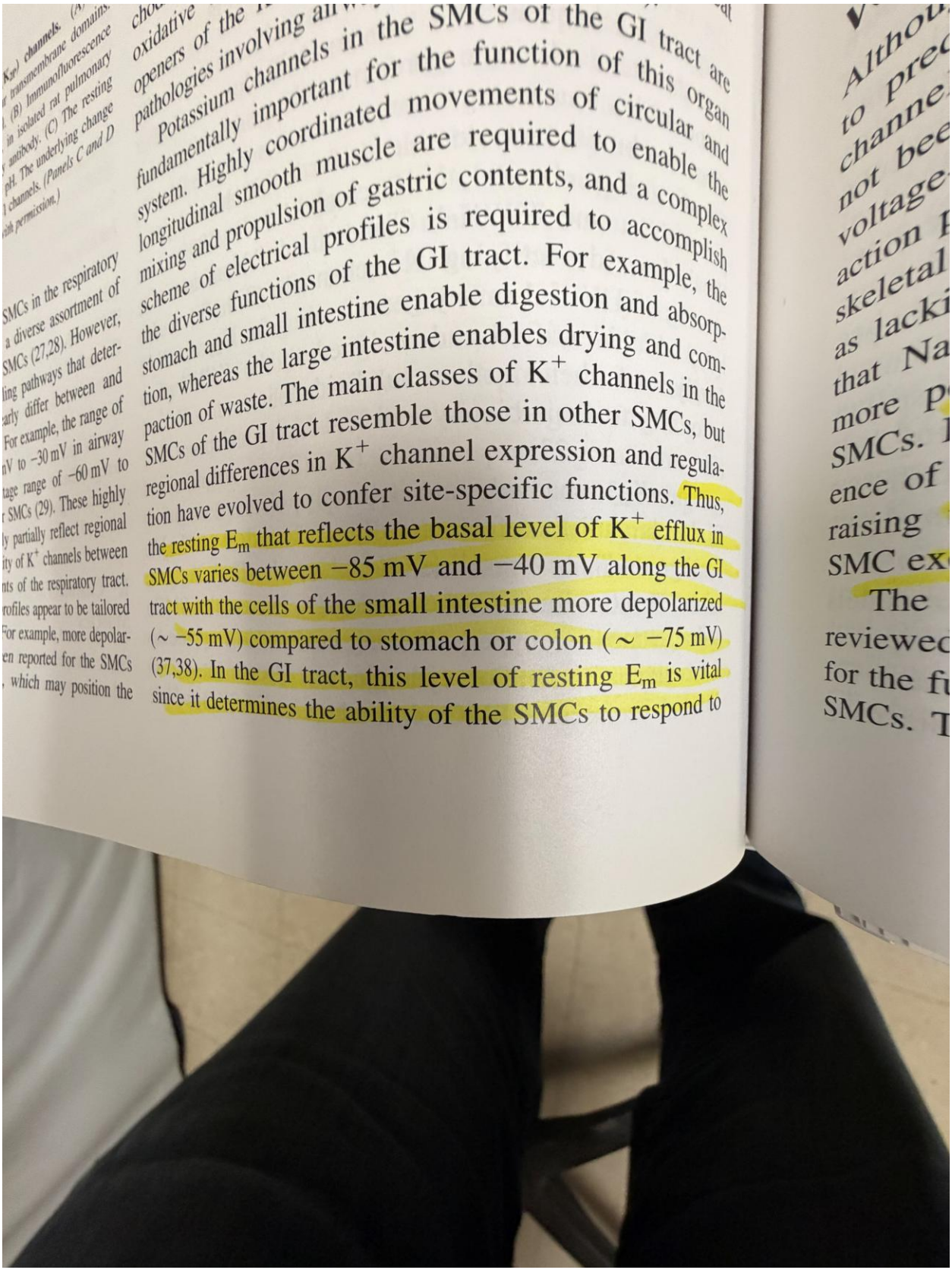
## Two Pore Domain $K^+$ ($K_{2P}$) Channels

The two pore domain $K^+$ ($K_{2P}$) channels are structurally unique $K^+$ channels with two pore (P) domains (rather than the standard single pore) in each α- subunit, which also contains four transmembrane domains (Figure 84.5A). Two α-subunits co-assemble to form the channel pore. The first $K_{2P}$ channel to be cloned in the 1990s was given the name TWIK-1 (Tandem of P domains in a Weak Inward rectifying $K^+$ channel) (22). Since the cloning of TWIK-1, many genes coding for $K_{2P}$ channels have been identified and assigned to the *KCNK* gene family and then further subdivided into six classes according to their biophysical and biological properties (for review see 23).

The $K_{2P}$ channels are typically open at negative $E_m$ levels and, therefore, are often referred to as "leak", "background", or "baseline" $K^+$ channels. They are postulated to contribute to the resting $E_m$ in vascular SMCs and also mediate hyperpolarizing currents in response to vasoactive stimuli. For example, one prominent *KCNK* channel in vascular SMCs appears to be TASK-1, which may contribute to the resting $E_m$ of the SMCs of rabbit pulmonary artery. The TASK-1 protein has been detected in the pulmonary SMCs (Figure 84.5B), and appears to be sensitive to changes in pH, hypoxia and other metabolic stimuli that regulate pulmonary vascular tone (24). For example, the $E_m$ responses triggered by changes in pH in pulmonary SMCs have been attributed to the opening and closing of TASK-1 channels (Figure 84.5C) (24). Another type of $K_{2P}$ channel, TREK-1, is thought to be activated by arachidonic acid and other polyunsaturated fatty acids (PUFAs) in addition to membrane stretch, pH, temperature, signaling molecules and anesthetic agents (23). Initially the dilator response to PUFAs was reported to be abolished in isolated basilar arteries of TREK-1$^{-/-}$ mice (25), but new observations in similar animals suggest that PUFA-induced dilations do not rely on $K_{2P}$ channels (26). Currently, the characterization of these channels is confounded by the lack of specific pharmacological blockers and the need to design and characterize $K_{2P}$ channel-specific null mice. Regardless, there is sufficient evidence to regard these newly discovered $K^+$ channels as potentially important regulators of vascular tone.

## $K^+$ Channels in Non-Vascular Smooth Muscles

Direct comparisons of $K^+$ channels between vascular and non-vascular SMCs have been rare, but the SMCs of



nitric oxide can activate both $BK_{Ca}$ and $K_v$ channels in GI SMCs (39). Other agonists may elicit more subtle hyperpolarizing responses and rely on a single type of $K^+$ channel type to buffer SMC depolarization and contraction.

## VOLTAGE-SENSITIVE SODIUM CHANNELS

Although $K^+$ and $Ca^{2+}$-permeable channels are thought to predominate in vascular SMCs, other types of ion channels also appear more sparsely, or alternatively, have not been targeted for intense study. One of these is the voltage-gated, "fast" $Na^+$ ($Na_v$) channel that mediates action potential initiation and propagation in cardiac and skeletal muscle. Historically, vascular SMCs were viewed as lacking these channels. Additionally, it was assumed that $Na_v$ channels would be mostly inactivated at the more positive levels of resting $E_m$ found in vascular SMCs. However, recent reports have confirmed the presence of $Na^+$ currents in freshly isolated vascular SMCs, raising the possibility that they contribute to vascular SMC excitability.

The molecular biology of the $Na_v$ channels is reviewed elsewhere (41), and we will focus on evidence for the functional expression of these channels in vascular SMCs. The initial reports of vascular $Na^+$ currents often

relied on findings in cultured SMCs, which are known to phenotypically drift and express ion channels not necessarily detected in freshly isolated cells. Thus, Cox et al. (42) reported that primary cultures of human aortic SMCs showed abundant tetrodotoxin-sensitive $Na^+$ currents, whereas freshly isolated SMCs from the same artery were devoid of $Na^+$ currents. Additionally, Meguro and colleagues (43) demonstrated that transcripts and currents attributed to $Na_v$ channels were absent in normal SMCs but were abundant in the SMCs of balloon-injured aortae. These reports and similar observations documenting the induction of $Na^+$ channels in vascular SMCs in short-term culture and other conditions favoring SMC proliferation generated skepticism regarding a physiological role for $Na_v$ channels in vascular SMCs *in situ*.

More recently, however, a number of investigators have directly recorded currents attributable to $Na_v$ channels in freshly isolated arterial SMCs, and have suggested that the enzymes used for cell isolation may minimize $Na^+$ currents under some conditions. For example, tetrodotoxin-sensitive $Na^+$ currents were measured in mouse and rat mesenteric arterial SMCs enzymatically dissociated with collagenase and elastase (Figure 84.6A), but could not be detected in similar SMCs dissociated with papain and collagenase (44). When steady-state activation for the $Na^+$ current were plotted,

and inactivation curves for the $Na^+$ current were plotted, a window current between −40 mV and −20 mV was revealed (Figure 84.6B), suggesting that $Na_v$ channels may be active in the range of resting $E_m$ found in vascular SMCs (43). The functional role of $Na_v$ channels in regulating vascular reactivity has been explored using the $Na_v$ channel opener veratridine, an alkaloid that slows channel inactivation. In endothelium-denuded rings of rat aorta, veratridine potentiated constrictions to low concentrations (6–8 mmol/l) of the depolarizing agent KCl, and tetrodotoxin prevented this constriction as did pharmacological block of the $Na^+/Ca^{2+}$ exchanger (45). These results imply that $Na^+$ influx through $Na_v$ channels in vascular SMCs may cause the $Na^+/Ca^{2+}$ exchanger to function in the reverse mode, i.e., extruding $Na^+$ in exchange for extracellular $Ca^{2+}$, with the resulting rise in $[Ca]_i$ contributing to SMC contraction. A similar coupling of $Na_v$ channel activation to the $Na^+/Ca^{2+}$ exchanger has been associated with vascular activation in the rat femoral artery and mouse portal vein (46,47).

There are several reports of $Na_v$ channels in the SMCs of other hollow organs, such as those found in the respiratory and GI tracts. For example, $Na_v$ channel transcript and corresponding $Na^+$ current were detected in cultured human bronchial SMCs, but this finding was not confirmed in freshly isolated SMCs from the same preparation (48,49). Additionally, currents attributed to $Na_v$ channels have been recorded from freshly isolated human jejunum circular but not longitudinal SMCs (50,51),

cle contractility act through G-protein-coupled receptors (GPCRs), the largest and most versatile receptor system in higher organisms. In this chapter, we will give an overview on GPCRs involved in the regulation of smooth muscle function, and we will describe basic signal transduction mechanisms employed by GPCRs to regulate the tone of smooth muscle cells.

## G-PROTEIN-COUPLED RECEPTORS

The mammalian genome encodes about 400 non-olfactory G-protein-coupled receptors that are activated by particular hormones, neurotransmitters or other mediators. Once a GPCR has been activated by a ligand, it couples to a heterotrimeric G-protein, which in turn then regulates one or several effectors like second messenger-producing enzymes or ion channels (1,2). This modular structure of the G-protein-mediated signaling system is the basis of the large functional versatility of the GPCR system. There are at least four basic families of G-proteins which are defined by particular G-protein $\alpha$-subunits, $G_s$, $G_i/G_o$,

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$G_q/G_{11}$, and $G_{12}/G_{13}$ (3). Each of the G-protein families regulates particular effectors after activation through a GPCR, and an activated receptor couples to a subset of individual G-protein subtypes. Also the G-protein $\beta\gamma$-complex takes part in the regulation of downstream signaling processes by regulating a variety of effector proteins (4). In smooth muscle cells, the coupling of receptors to particular G-protein subfamilies determines whether the receptor agonist induces an increase or a decrease in the smooth muscle tone. Typical smooth muscle-relaxing agonists acting through GPCRs will activate receptors coupled to the G-protein $G_s$ which in turn stimulates the activity of adenylyl cyclases and thereby mediates an increase in the intracellular cAMP concentration (5). In contrast, GPCRs mediating contraction of smooth muscle cells typically couple to G-proteins of the $G_q/G_{11}$ family which couple receptors to $\beta$-isoforms of phospholipase C resulting in the formation of diacylglycerol, an activator of protein kinase C, as well as in the formation of inositol-1,4,5-trisphosphate which releases $Ca^{2+}$ from intracellular stores (6). Many of the contraction-inducing receptors also couple to G-proteins of the $G_{12}/G_{13}$ family, which link these receptors via Rho guanine nucleotide exchange factors (RhoGEF) to the activation of the small GTPase RhoA (7). Some of the receptors mediating smooth muscle contraction also couple to $G_i/G_o$-type G-proteins which mediate an inhibition of adenylyl cyclase and thereby antagonize the activity of active receptors coupling to $G_s$.

## G-Protein-Mediated Signaling Pathways Mediating Smooth Muscle Contraction

The regulation of the phosphorylation of myosin light chain (MLC) is a central process in the control of smooth muscle cell contraction (8,9). The $Ca^{2+}$-calmodulin-activated MLC kinase (MLCK) phosphorylates MLC,

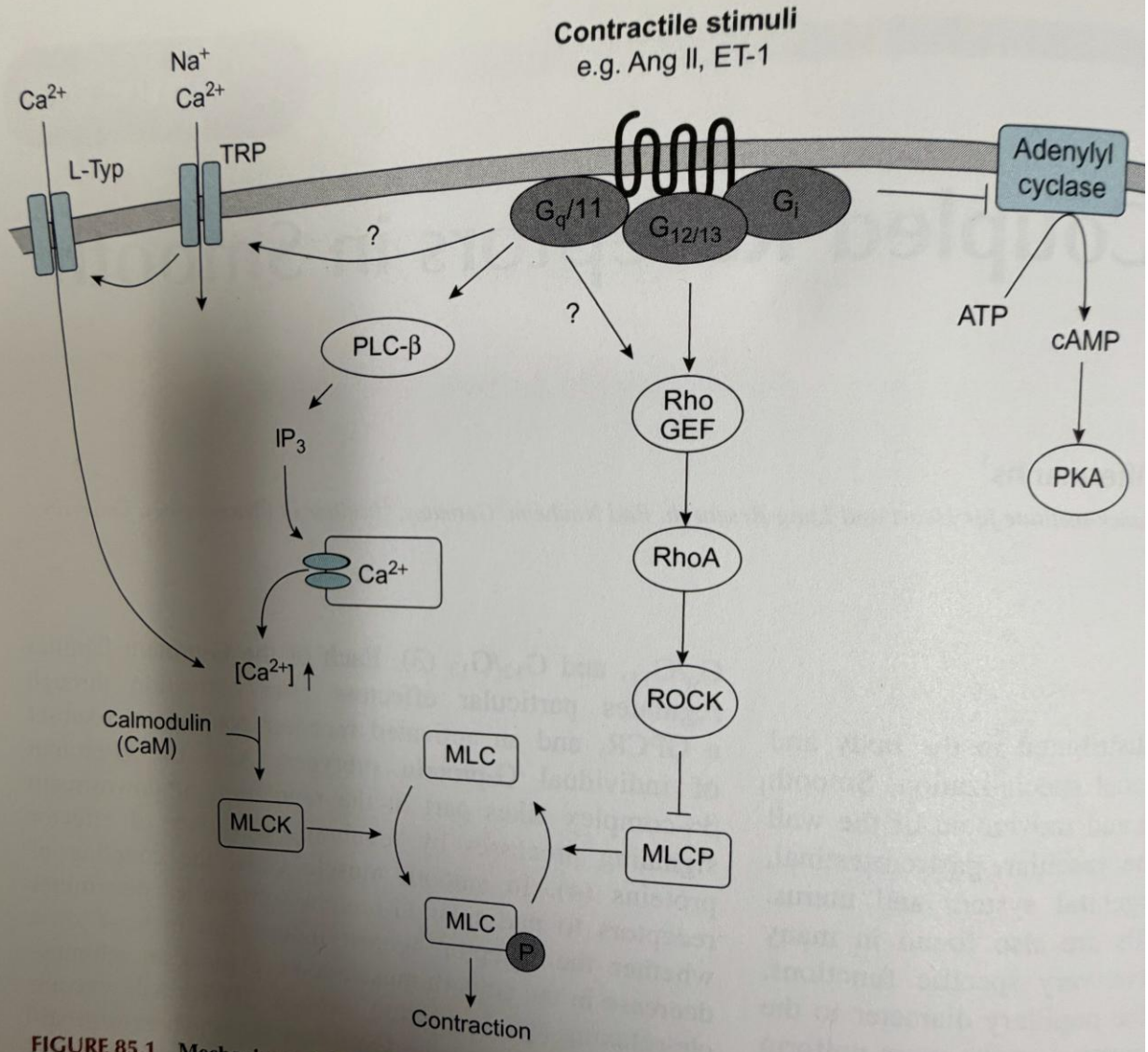



**FIGURE 85.1 Mechanisms of GPCR-mediated smooth muscle contraction.** GPCRs mediating contraction o ple to G-proteins of the $G_q/G_{11}$ family; in some cases also of the $G_{12}/G_{13}$ and $G_i$ family. Activation of β-isoforms $G_{11}$ results in inositol-1,4,5,-trisphosphate ($IP_3$) formation and release of intracellularly stored $Ca^{2+}$. $Ca^{2+}$ together sin light chain kinase (MLCK) resulting in phosphorylation of the myosin light chain (MLC). In addition, $G_q/G_{11}$ ing in depolarization and opening of voltage-dependent $Ca^{2+}$ channels. Activation of $G_{12}/G_{13}$ stimulates the nucleotide exchange factors (Rho-GEFs). The activated small GTPase RhoA via stimulation of Rho-kinase (ROC light chain phosphatase (MLCP). $G_i$ type G-proteins release relatively high amounts of βγ-subunits which may resu isoforms. In addition, α-subunits of $G_i$ type G-proteins inhibit adenylyl cyclase Figure 85.2). TRP, transient receptor potential channel. For details:

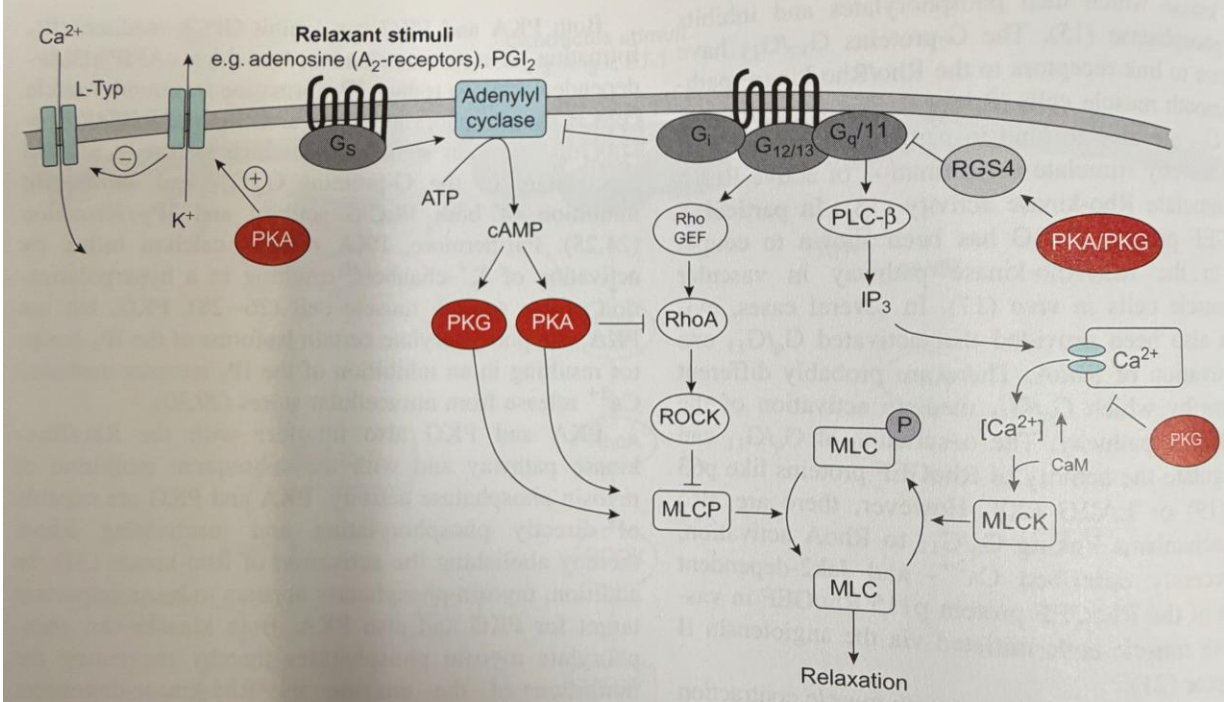
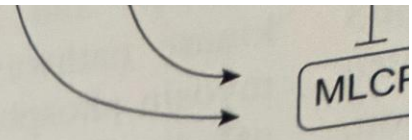



**FIGURE 85.2 Mechanisms of GPCR-mediated smooth muscle relaxation.** GPCR-mediated relaxation of smooth muscle cells involves receptors coupled to the G-protein $G_s$ which by activation of adenylyl cyclase increases cAMP levels. Cyclic AMP, primarily via activation of protein kinase A (PKA), and in part also of protein kinase G (PKG), induces relaxation. PKA inhibits the RhoA-mediated signaling pathway, activates myosin light chain phosphatase (MLCP) activity and can activate $K^+$-channels. In addition, PKA and PKG activate a regulator of G-protein signaling (RGS4) which inhibits $G_q/G_{11}$-mediated signalling. Finally, PKG has been shown to inhibit the release of intracellularly stored $Ca^{2+}$. For further text.

or during advanced atherosclerosis, this gene expressi... $G_i/G_o$ far... capable of stimulating pattern is switched to an increased formation of extra... le cell ... various intracellular lular matrix accompanied by the proliferation of sn... sses (50... muscle cells (38). The... evidence that signaling ...hus, by... ways initiated by G... critically involved ...Rs are ... trimeric G differentiation and ...iation processes of ...aling ev... muscle cells. Acti... ceptors that coup... tricate n

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or during advanced atherosclerosis, this gene expression pattern is switched to an increased formation of extracellular matrix accompanied by the proliferation of smooth muscle cells (38). There is evidence that signaling pathways initiated by GPCRs are critically involved in the differentiation and dedifferentiation processes of smooth muscle cells. Activation of receptors that couple to the G-protein families $G_q/G_{11}$ and $G_{12}/G_{13}$ tend to promote the expression of contractile genes (39–41) and to stimulate smooth muscle cell differentiation (42,43). The underlying signaling events include the activation of different MAP-kinases, the generation of reactive oxygen species (44–46) or the stimulation of the RhoA/Rho-kinase pathway (47–49).

Migration of smooth muscle cells is a fundamental process during the development of hollow organs, like, for example, blood vessels, but can also be induced in the adult organism, for example after dedifferentiation. Migration of cells, includi[illegible]ooth muscle cells, depends

**TABLE 85.1 GPCRs Mediating Contraction of Smooth Muscle Cells**

| Ligand | Receptor(s) | G-Protein Coupling | SM Expression | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Blood vessel | Airway | Uterus | GI tract | Urogenital | Ciliary |
| Acetylcholine | $M_2$ | $G_i/G_o$ | X | X | | X | X | X |
| | $M_3$ | $G_q/G_{11}$ | | X | | X | X | X |
| Adenosine | $A_1$ | $G_i/G_o$ | X | X | | X | | |
| Angiotensin | $AT_1$ | $G_q/G_{11}$, $G_i/G_o$, $G_{12}/G_{13}$ | X | | | | | |
| Apelin | APJ | $G_i/G_o$ | X | | | | | |
| ATP/UTP | $P2Y_1$ | $G_q/G_{11}$ | X | | | X | | |
| | $P2Y_2$ | $G_q/G_{11}$ | X | X | | | | |
| | $P2Y_6$ | $G_q/G_{11}$ | X | X | | X | | |
| Bradykinin | $B_1$ | $G_q/G_{11}$, $G_i/G_o$ | X | | | | | |
| | $B_2$ | $G_q/G_{11}$, $G_i/G_o$ | X | X | | | | |
| Cholecystokinin | $CCK_1$ | $G_q/G_{11}$ | | | | X | | |
| Endothelin | $ET_A$ | $G_q/G_{11}$, $G_{12}/G_{13}$ | X | X | X | X | | |
| | $ET_B$ | $G_q/G_{11}$, $G_i/G_o$ | | X | | X | | |
| Epinephrine, norepinephrine | $\alpha_{1A}$ | $G_q/G_{11}$ | X | | | | X | |
| | $\alpha_{1B}$ | $G_q/G_{11}$ | X | | | | | |
| | $\alpha_{1D}$ | $G_q/G_{11}$ | X | | | | X | |
| | $\alpha_{2A}$ and $\alpha_{2c}$ | $G_i/G_o$ | X | | X | X | | |
| | $\alpha_{2B}$ | $G_i/G_o$ | X | | X | | | |
| Histamine | $H_1$ | $G_q/G_{11}$ | X | X | | X | X | |
| Leukotriene B4 | $BLT_1$ | $G_q/G_{11}$, $G_i/G_o$ | X | | | | | |
| Leukotriene C4/D4 | $CysLT_2$ | $G_q/G_{11}$ | | X | | | | |
| LPA | $LPA_1$, $LPA_2$, $LPA_3$ | $G_q/G_{11}$, $G_{12}/G_{13}$, $G_i/G_o$ | X | X | X | X | | |
| Melatonin | $MT_1$ | $G_q/G_{11}$ | X | | | | X | |
| Motilin | MTLR | $G_q/G_{11}$, $G_{12}/G_{13}$ | | | | | X | |
| Neurokinin A | $NK_2$ | $G_q/G_{11}$, $G_i/G_o$ | X | X | | | X | |
| Neurokinin B | $NK_3$ | $G_q/G_{11}$ | | | | | X | X |
| Neuromedin U | $NMU_1$ | $G_q/G_{11}$ | X | | | | | |
| Neuropeptide S | NPS | $G_q/G_{11}$ | ? | X | | | X | X |
| Neuropeptide Y | $Y_1$ | $G_i/G_o$ | X | | | | X | |
| Neurotensin | $NTS_1$ | $G_q/G_{11}$ | | | | X | | |
| Oxytocin | OT | $G_q/G_{11}$ | | | | X | X | X |
| Prostaglandin $E_2$ | $EP_1$ | $G_q/G_{11}$ | X | X | | X | | |
| | $EP_3$ | $G_i/G_o$ | X | | | X | X | |
| Prostaglandin $F_{2\alpha}$ | FP | $G_i/G_o$ | X | X | | | | |
| S1P | $S1P_2$, $S1P_3$ | $G_q/G_{11}$, $G_{12}/G_{13}$ | | | | | | |

(Continued)

**TABLE 85.1** (Continued)

| Ligand | Receptor(s) | G-Protein Coupling | SM Expression | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Blood vessel | Airway | Uterus | GI tract | Urogenital | Ciliary |
| Serotonin | $5\text{-HT}_{1A/1B}$ | $G_i/G_o$ | X | X | | | | |
| | $5\text{-HT}_{1F}$ | $G_i/G_o$ | | | X | | | |
| | $5\text{-HT}_{2A/2B}$ | $G_q/G_{11}$ | X | X | | X | | |
| Somatostatin | $SST_2$ | $G_i/G_o$ | X | X | | X | X | X |
| Thromboxane $A_2$ | TP | $G_q/G_{11}$, $G_{12}/G_{13}$ | X | X | X | | | |
| Thrombin | PAR1,2,3 | $G_q/G_{11}$, $G_{12}/G_{13}$ | X | X | X | X | X | |
| Urotensin II | UT | $G_q/G_{11}$ | X | | | | | |
| Vasopressin | $V_{1a}$ | $G_q/G_{11}$ | X | | X | | | |

**TABLE 85.2** GPCRs Mediating Relaxation of Smooth Muscle Cells

| Ligand | Receptor(s) | G-Protein-Coupling | SM Expression | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Blood vessel | Airway | Uterus | GI tract | Urogenital | Ciliary |
| Adenosine | $A_{2A}$, $A_{2B}$ | $G_s$ | X | X | | X | | |
| Dopamine | $D_{1/5}$ | $G_s$ | X | | | | X | |
| Epinephrine, norepinephrine | $\beta_1$ | $G_s$ | | | | X | | |
| | $\beta_2$ | $G_s$ | X | X | X | X | | |
| Prostaglandin $D_2$ | $DP_1$ | $G_s$ | X | X | X | | | |
| Prostaglandin $E_2$ | $EP_2$ | $G_s$ | X | X | | | | |
| | $EP_4$ | $G_s$ | X | X | X | | | |
| Prostacyclin | IP | $G_s$ | X | | | | | |
| Relaxin | $RXFP_1$ | $G_s$ | X | X | X | | | |
| Vasopressin | $V_2$ | $G_s$ | X | | X | | | |
| VIP, PACAP | $VPAC_2$ | $G_s$ | X | X | X | X | | |

The expression of many receptors is restricted to particular smooth muscle types in certain organ systems where they mediate organ-specific regulation of smooth muscle contraction by particular hormones or transmitters (Tables 85.1 and 85.2). However, there are also GPCRs that are found widely expressed in smooth muscle tissues of different organs. As most smooth muscle organs are under the control of the autonomic nervous system, most smooth muscle cells express adrenergic, and many also muscarinic, receptors. Also other biogenic amine tors like those for serotonin and histamine many smooth muscle cells. Mo also have various re

like lysophosphatidic acid and sphingosine-1 phosph (59) which have only recently been identified, and w act through particular receptor subtypes to regulate vario cell types including smooth muscle cells.

## PHARMACOLOGICAL REGULATION OF SMO

**TABLE 85.3 Pharmacological Regulation of Smooth Muscle Cells through GPCRs**

| Receptor | Drug (Example) | Wanted Effect (Disease) |
|---|---|---|
| **Receptor agonists** | | |
| Acetylcholine ($M_3$) | Bethanechol | Contraction of *M. detrusor vesicae* → Emptying of urinary bladder |
| | Pilocarpin (local appl.) | Contraction of *M. sphincter pupillae* → Miosis |
| Vasopressin ($V_1$) | Terlipressin | Vasoconstriction |
| $\alpha_1$ adrenergic | Phenylephrine | Vasoconstriction |
| Oxytocin (OT) | Oxytocin | Induction of labor |
| Prostaglandin $E_2$ (EP) | Misoprostol | Uterus contraction |
| $\beta_2$-adrenergic | Fenoterol (e.g.) | Bronchial relaxation (bronchial asthma), tocolysis |
| Prostacyclin (IP) | Iloprost | Vasodilation |
| **Receptor antagonists** | | |
| $\alpha_1$ adrenergic | Prazosin | Vasodilation (hypertension) |
| | Tamsulosin | Relaxation of lower urinary tract smooth muscle (benign prostatic hyperplasia) |
| Angiotensin II ($AT_1$) | Losartan | Vasodilation (arterial hypertension) |
| Acetylcholine ($M_3$) | Ipratropium | Bronchial relaxation (bronchial asthma) |
| | Tropicamide | Relaxation of *M. sphincter pupillae* → Mydriasis |
| | Solifenacin | Relaxation of *M. detrusor vesicae* (urge incontinence) |
| Endothelin ($ET_A$) | Sitaxentan | Relaxation of pulmonary vessels (pulmonary hypertension) |
| Prostaglandin $D_2$ ($DP_1$) | Laropiprant | Reduced prostaglandin $D_2$-dependent cutaneous vasodilation (flushing) under nicotinic acid therapy |

obstipation are intimately linked to the regulation of peri- stinal smooth muscle. Similarly, eases, in particular bronchial ased tone of bronchial smooth ety of GPCRs expressed spe- th muscle organs makes them nterfering with organ-specific

autonomous nerves, adjacent cells or distant sites. Many of these smooth muscle regulators act through GPCRs to modulate smooth muscle function. While the basal mechanisms of GPCR-mediated smooth muscle tone regulation are very similar between different organs, the sensitivity of organ-specific smooth muscle cells to smooth muscle regulators can vary profoundly depending on the receptor subtypes expressed by a particular organ-specific More recent data also indicate that cells to organ damage,

| | |
|---|---|
| Sitaxentan | |
| Laropiprant | Relaxation of pulmon |
| | Reduced prostaglandi nicotinic acid therapy |

obstipation are intimately linked to the regulation of peristalsis by the gastrointestinal smooth muscle. Similarly, obstructive pulmonary diseases, in particular bronchial asthma, result from an increased tone of bronchial smooth muscle cells. The wide variety of GPCRs expressed specifically in particular smooth muscle organs makes them ideal targets for drugs interfering with organ-specific smooth muscle functions. In fact, many drugs have been developed as agonists or antagonists of GPCRs expressed by smooth muscle cells in order to regulate their functions (see Table 85.3).

## CONCLUSIONS

Smooth muscle cells are quite heterogeneous, depending on the organ system in which they serve their function. Their major role is to control the diameter, wall movement, and wall stiffness of hollow organs like the vascular, bronchial, gastrointestinal or urogenital system as well as the uterus. Besides a variety of myogenic regulatory mechanisms, the function of smooth muscle cells is under the control of external stimuli released from

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complex $Ca^{2+}$ signaling results from the interactions of ion transport and pump proteins located in the PM, the SR, and mitochondria. The aim of this chapter is to review the $Ca^{2+}$ entry and clearance mechanisms identified in smooth muscle cells and relate them to the hierarchy of $Ca^{2+}$ signaling.

## SOURCES OF $Ca^{2+}$ IN SMOOTH MUSCLE CELLS

In smooth muscles $Ca^{2+}$ entry into the cytosol is regulated by the influx of $Ca^{2+}$ via the plasma membrane channels and the $Na^+/Ca^{2+}$-exchanger in reverse mode (i.e., $Ca^{2+}$ in, $Na^+$ out of the cell), and/or release of $Ca^{2+}$ from intracellular $Ca^{2+}$ stores. $Ca^{2+}$ entry channels include voltage, receptor- and store-operated $Ca^{2+}$ channels.

### Voltage Operated $Ca^{2+}$ Channels

The electrical potential across the SMC membrane plays a pivotal role in control of all phasic and some tonic smooth muscle contraction, through its depolarization and the subsequent influx of $Ca^{2+}$ through voltage-gated

onic non-spiking
nce arteries and arterioles,
lly depolarized by a variety
pen DHP-sensitive $Ca^{2+}$
ustained $Ca^{2+}$ influx, thus
). The generation of the
ypes of smooth muscle is
impeded by rapid activation of a family of $K^+$ channels
sensitive to TEA (3). DHP-sensitive $Ca^{2+}$ channels are
capable of opening at moderate depolarizations (−40,
−20 mV) and generate the so called "window current",
which causes a steady increase in intracellular $Ca^{2+}$ asso-
ciated with the development of tonic contraction (3).

muscle was introduced (4,5). Therefore, at that time,
ROCs represented any plasma membrane $Ca^{2+}$-permeable
channels other than voltage-sensitive $Ca^{2+}$ channels,
which were opened as a result of the binding of an ago-
nist to its receptor. Based on new evidence, agonists can
activate voltage-independent $Ca^{2+}$ entry in smooth mus-
cle cells in three different ways that represent different
aspects of pharmaco-mechanical coupling in smooth
muscles.

The first type of ROC reported for smooth muscles is
the model of capacitative $Ca^{2+}$ entry (CCE) via store-
operated $Ca^{2+}$ channels (SOC) (6) (Figure 86.2). The

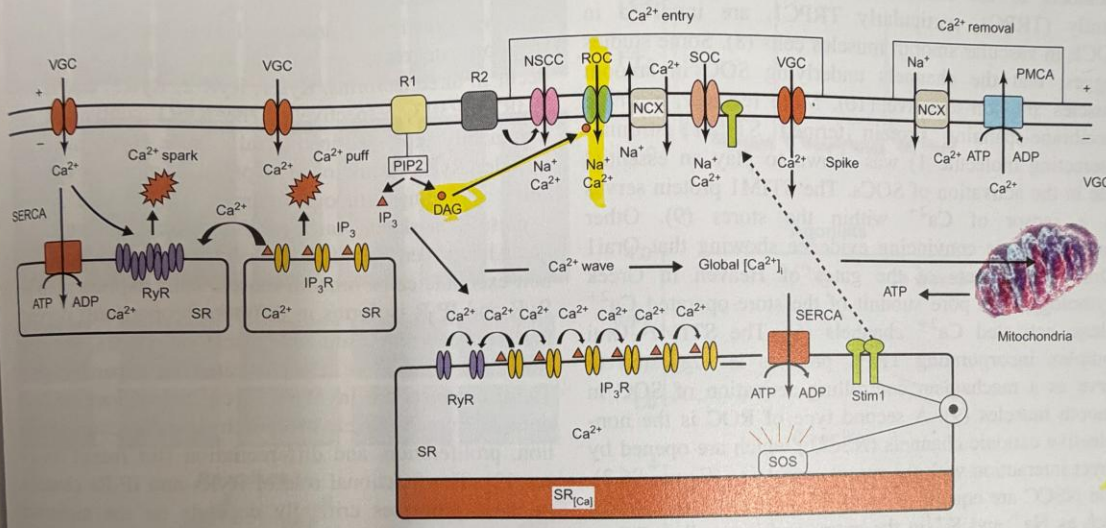



**FIGURE 86.2 $Ca^{2+}$ mobilization and clearance pathways in smooth muscle cells.** Voltage-gated, L-type Ca channels (VGC) are opened by membrane depolarization and generate $Ca^{2+}$ spikes underlying the upstroke of the propagating action potential in phasic and steady state graded $Ca^{2+}$ influx in tonic smooth muscles. $Ca^{2+}$ influx through VGC can trigger $Ca^{2+}$ sparks directly (via CICR) or indirectly (via increasing the SR $Ca^{2+}$ load). Agonist binding to G-protein-coupled receptors activates phospholipase C (PLC) generating diacylgycerol (DAG) and inositol trisphosphate (IP3). DAG activates receptor-operated channels (ROCs), eliciting $Na^+$ and $Ca^{2+}$ entry, while $IP_3$ activates $IP_3$ receptors on the SR, causing local ($Ca^{2+}$ puffs ) or global ($Ca^{2+}$ waves/oscillations) via activation of $IP_3R$. $Ca^{2+}$ release through IP3R can additionally recruit adjacent RyRs. Store depletion is sensed by stromal interacting molecule 1 (STIM1) within the SR, which translocates to and activates store-operated channels (SOCs). The elevation in subplasmalemmal $[Na^+]$ resulting from activation of non-selective cation channels (NSCC), non-selective receptor operated (ROCs) or SOCs may be sufficient to drive reverse-mode operation of $Na^+/Ca^{2+}$ exchanger (NCX), leading to $Ca^{2+}$ entry. Much of the $Ca^{2+}$ entering the cell and released from stores may be sequestered by the superficial SR through SERCAs and mitochondria. Cycling between SR $Ca^{2+}$ uptake and release mechanisms leads to $Ca^{2+}$ oscillations. $Ca^{2+}$ is removed from the cell by the plasma membrane $Ca^{2+}$ ATPase (PMCA) and forward mode operation of the NCX.

...ceptors in the genesis of localized ...contribution of $[Ca^{2+}]_i$ mobilization and waves of $[Ca^{2+}]_i$ oscillations varies in different types of SMCs, and often depends on the strength and mechanism of SMC stimulation.

## $Ca^{2+}$ CLEARANCE SYSTEMS

Separately discussing $Ca^{2+}$ sources and $Ca^{2+}$ clearance is somewhat arbitrary, as either system may play dual roles; for example, the $Na^+/Ca^{2+}$-exchanger. The advantage is seeing each from a different view point. Even though ion fluxes through channels are several orders of magnitude greater than that of clearance by ion pumps, the time course of $Ca^{2+}$ transients can be significantly underestimated without inhibition of $Ca^{2+}$ clearance pathways (37). The speed of ions through channels may dominate initial $Ca^{2+}$ transients, but the steady state brings $Ca^{2+}$ clearance mechanisms into play, and they are a major factor in determining steady state $[Ca^{2+}]_i$. A $Ca^{2+}$ influx under basal conditions estimated at 16 μmol/l per minute is more than 2 orders of magnitude greater than the resting $[Ca^{2+}]_i$. Thus, $Ca^{2+}$ clearance from the cytosol is critical to the maintenance of a quiescent baseline.

The major $Ca^{2+}$ clearance systems are sarco(endo) plasmic reticulum $Ca^{2+}$ ATPase (SERCA), $Na^+/Ca^{2+}$-exchanger (NCX) powered by the $Na^+-K^+$ ATPase (NKA), and the plasma membrane $Ca^{2+}$ ATPase (PMCA). NCX is generally considered to be a high-capacity exchanger with rapid turnover with but low affinity for $Ca^{2+}$ ($K_d \approx 1$ μM), but high turnover. SERCA and PMCA, on the other hand, have a higher affinity for $Ca^{2+}$ ($K_d \approx 0.1-0.3$ μM), but lower turnover than NCX (for review see 38). For smooth muscle, the relative

contribution of each to $Ca^{2+}$ clearance component is dependent on conditions and smooth muscle type. Estimates generally rely on inhibitors and often are linearly extrapolated from clearance rates so they must be viewed cautiously. There are also tissue, tissue preparation, and species differences.

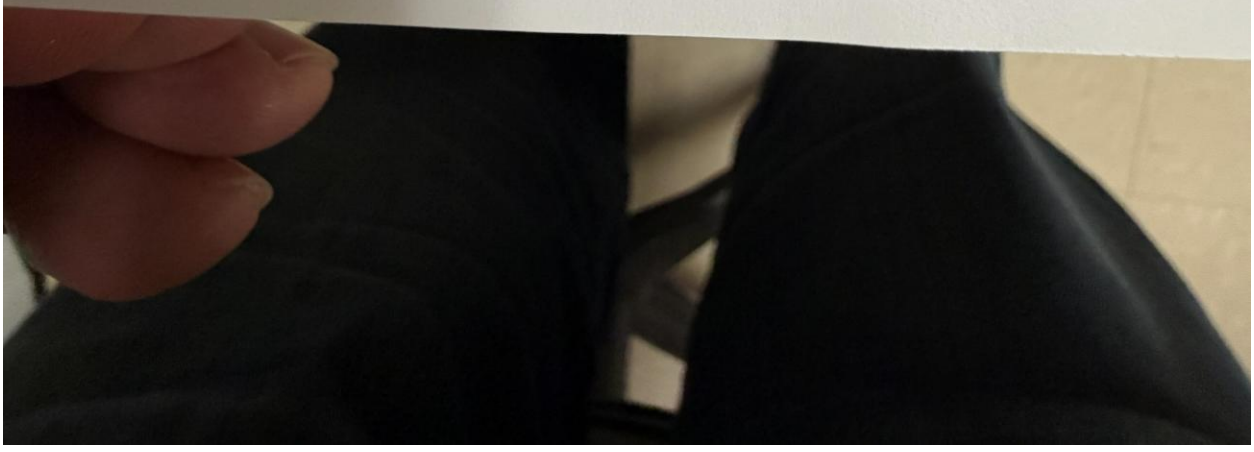
In mouse bladder, NCX accounts for about 60% of calcium clearance, while PMCA and SERCA facilitate about 20–30% each (39). In uterine smooth muscle, 35% can be attributed to NCX and the remaining attributed to PMCA (40). In cells isolated from mouse aorta, NCX accounts for 90% of the $Ca^{2+}$-extrusion following inhibition of SERCA by cyclopiazonic acid (37). Mitochondrial $Ca^{2+}$ uptake can also be a factor under certain conditions, but its apparent affinity is thought to be relatively low (~10–20 μM, (41)). Microdomains with relatively high $[Ca^{2+}]_i$ are posited to circumvent this relatively low affinity (42). Recent evidence, however, suggests mitochondria may play some role as both a buffer and/or regulator of $Ca^{2+}$ clearance (discussed below).

The distribution, localization, and interactions of these $Ca^{2+}$ clearance systems are a focus of recent interest in smooth muscle $Ca^{2+}$ handling; in particular, the role

rcolemmal space which lead to a hyperloaded SR, su
essing store-operated $Ca^{2+}$ entry and/or the activati
$Ca^{2+}$-activated $K^+$ channels via an increased $[Ca^{2+}]$
MCA4-associated sub-cellular compartments.

## $Ca^{2+}$ Clearance via $Na^+/Ca^{2+}$ Exchanger Driven by NKA

$Na^+/Ca^{2+}$-exchanger utilizing the $Na^+$ gradient powered by the $Na^+-K^+$ ATPase can be a potent $Ca^{2+}$ clearance system. The mammalian NCX family consists of at least three isoforms, NCX1–3, which, in turn, give rise to various splice variants (for reviews see 69, 70). NCX exchanges one $Ca^{2+}$ ion across the plasma membrane for



tion to the coupling of NCX and NKA discussed earlier. SERCA is also interrelated, particularly as proposed to a subsarcolemmal compartmentalization with α2-NKA and NCX. Moreover as described in the section on capacitative $Ca^{2+}$ entry (above) $SR_{[Ca]}$, modulated by SERCA is linked to $Ca^{2+}$ influx via store-operated $Ca^{2+}$ entry.

## CONCLUSION

The differences between smooth muscle types themselves are often greater than those between smooth and skeletal muscle. So it is not surprising given the wide range of functionality that the mechanisms for modulating and maintaining $Ca^{2+}$ homeostasis also differ widely. We now have a better understanding of the major players in $Ca^{2+}$ entry and clearance. We have also made gains in, if not understanding, at least recognizing the interactions between the players are central to the regulation of $[Ca^{2+}]_i$. These complex interactions, given the importance of $[Ca^{2+}]_i$ homeostasis to cell signaling and function, will continue to be a major foci of smooth muscle research.

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majority of these factors, as well as their signaling pathways, are tissue-specific, they will not be covered here in detail (for examples, see chapters in the subsection Heterogeneities).

Common chemical signals in many types of smooth muscles are neurotransmitters released by nerve activity. The most important types of neurotransmission in smooth muscle are the adrenergic and cholinergic pathways. The responses to neurotransmitters, however, can vary significantly in a tissue-specific manner. For example, norepinephrine by primarily binding to $\alpha_1$-adrenergic receptors triggers contraction in vascular smooth muscle, but by primarily binding to $\beta$-receptors causes relaxation of airway smooth muscle. Conversely, stimulation of M3 muscarinic receptors by acetylcholine leads to contraction of airway smooth muscle, but by releasing nitric oxide from the endothelium, causes relaxation of vascular smooth muscle.

## Mechanical Stimulation

In 1902 the physiologist Sir William Bayliss published his findings on an autoregulatory mechanism that controls the muscle tone in the arterial wall by a nerve-independent, stretch-dependent contraction (1). This contraction in response to enhanced pressure on the arterial wall, known as the Bayliss effect or myogenic response, is a vital mechanism that keeps blood flow constant despite

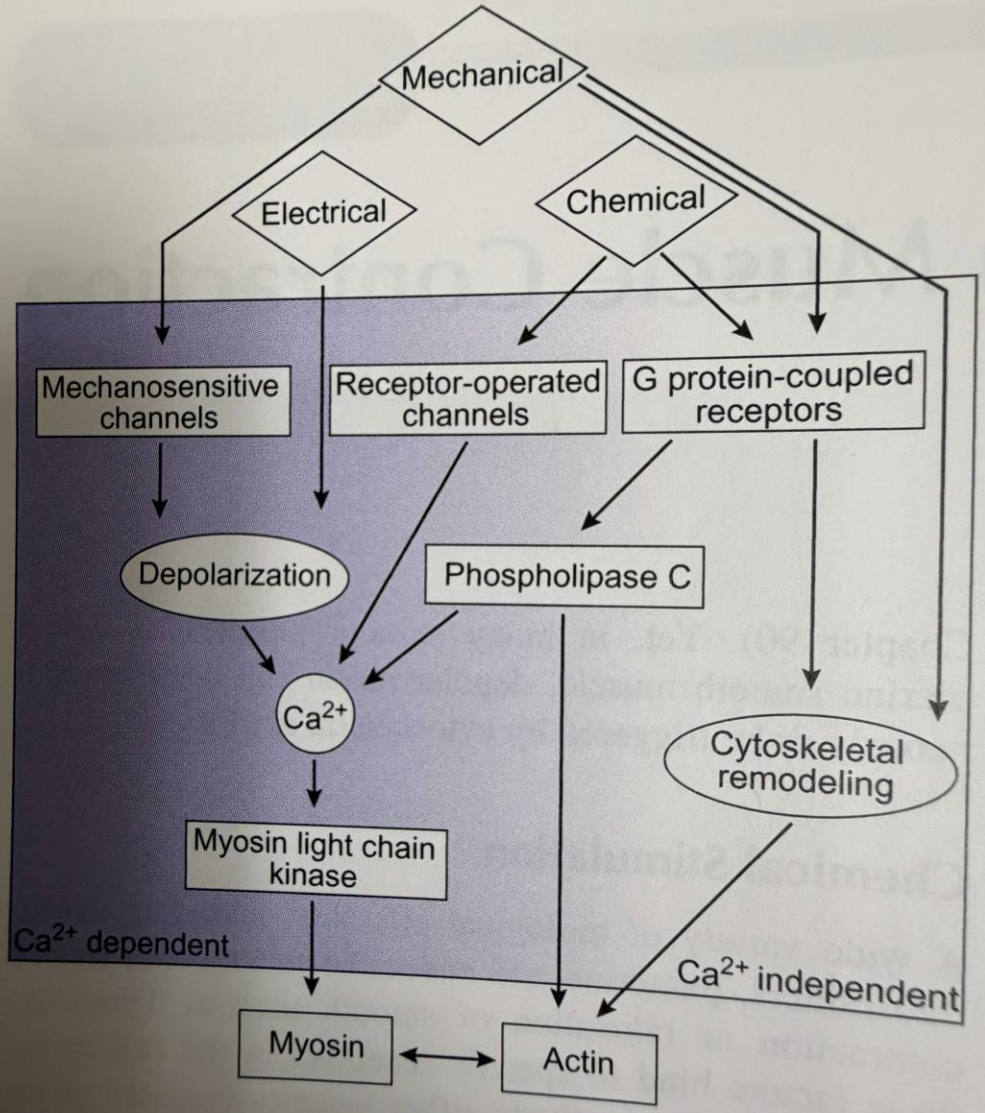


**FIGURE 87.1 Contractile signaling pathways in smooth muscle (simplified overview).** Three types of stimulation — mechanical, electrical, and chemical — target at myosin activation and/or actin–myosin interaction through pathways that show varying degrees of $Ca^{2+}$ dependency (schematically indicated by blue gradient).

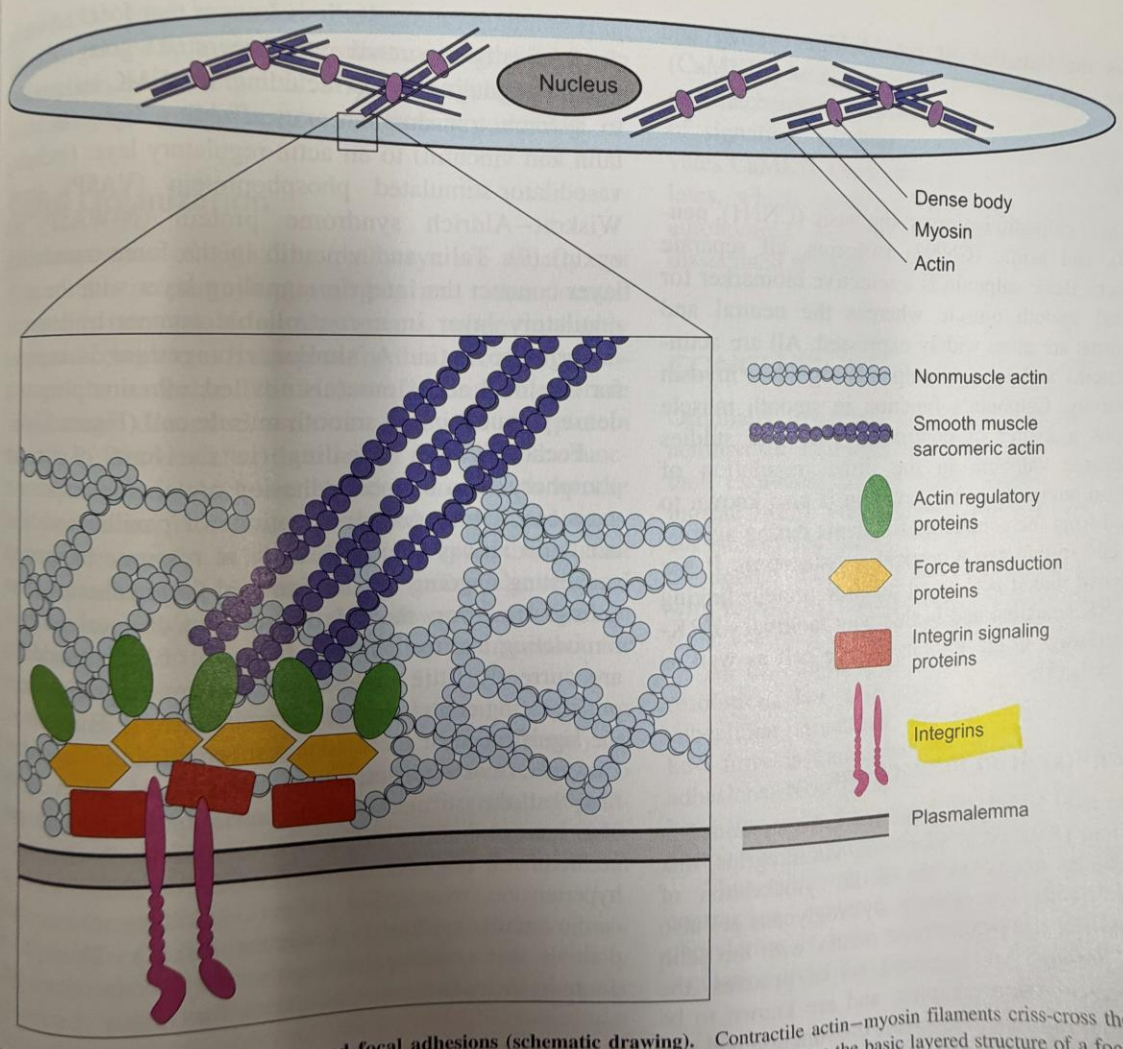


**FIGURE 87.4 Actin cytoskeleton and focal adhesions (schematic drawing).** Contractile actin–myosin filaments criss-cross the cell, anch
internally to dense bodies and externally to focal adhesions at the cell cortex. The inset illustrates the basic layered structure of a focal adhesion
integrin signaling proteins shown in red (e.g. FAK, paxillin, Src), force transduction proteins in yellow (e.g. vinculin, talin), and actin regulator
teins in green (e.g. zyxin, VASP, N-WASP) (9). Focal adhesions and bundles of smooth muscle actin (dark blue) are embedded in a cortical n
of nonmuscle actin (light blue). Please note that proteins are not drawn to scale.

isoform is a marker of differentiat

## Focal Adhesion/Actin Interactions

Over 150 proteins have been suggested to be contained in focal adhesions (8,9). Focal adhesions, via integrins, link the extracellular matrix to the actin cytoskeleton of smooth muscle cells. Interestingly, dystroglycans are also known to connect the extracellular matrix with the actin cytoskeleton through an organized set of proteins, the dystrophin–glycoprotein complex, and are known to be present in smooth muscle. However, the function and significance of the dystroglycan complex in smooth muscle are not yet fully understood.

Since smooth muscles, unlike skeletal muscles, do not possess tendons, force generated by the myosin-containing contractile filaments is funneled through the adhesion plaques to the rest of the smooth muscle tissue. In this way, the forces generated by individual "mini-sarcomeres" are added in parallel, producing a more effective force transmission than the addition of striated muscle sarcomeric forces in series at the tendon.

Focal adhesions also facilitate signaling pathways that control differentiation, apoptosis, and cell division. The "adhesome" includes actin regulatory proteins, integrin signaling proteins, and scaffolding proteins. Included in these diverse proteins are many kinases and phosphatases. Interestingly, many of the focal adhesion kinases are tyrosine kinases and in the contractile smooth muscle cell this is essentially the only major site of tyrosine phosphorylation. Other signaling pathways that directly regulate contractility (see Figure 87.1) involve primarily

dependent kinase II regulate vascular tone through vari- anisms, is involved in the temporal organization of signaling. Binding of $Ca^{2+}$/calmodulin initially activates CaMKII. Once activated, the kinase autophosphorylates, which confers a "molecular memory" in form of autonomous activity even after $Ca^{2+}$/calmodulin has dissociated from the kinase.

## CONCLUSIONS

Originally regarded as purely mechanical structures, actomyosin filaments have revealed more and more of their regulatable nature in the past decades. Since smooth muscle exerts very different functions in various organ systems of the body, it is not surprising that regulation of smooth muscle contraction is particularly complex, with regulatory mechanisms ranging from autoregulatory stretch sensing to modulation of gene expression.

As techniques evolve to allow not only for higher resolution, but also more comprehensive analysis of subcellular structures, we are beginning to understand how force is transmitted on the molecular level at focal adhesions. New perspectives on smooth muscle function have also come from the expanding collective of scaffold proteins as regulators of contraction.

Thus, although the basic control of smooth muscle contraction, centered around myosin light chain phosphorylation, is quite well understood, many aspects of its regulation are still in the process of unfolding.

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Chapter 88

# Smooth Muscle

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been refined over the past several decades in parallel with new techniques, taking into account function, morphology, organization, response to stimuli, type of innervations, and protein content. SMs have been first classified on the basis of their organization in the 1940s by Bozler into "multi-unit" and "unitary" (syncytial) (1). Multi-unit SM is found in ciliary muscle, iris muscle, piloerector muscles, but also in large airways and arteries. These types of SM are composed of separate fibers, which operate independently from one another and form few gap junctions. They are richly innervated with often one single nerve ending per fiber and are insulated by a thin basement membrane layer. Similar to skeletal muscle, multi-unit SMs are mainly controlled by nerve signals, and their stretching does not produce a contractile response. In contrast, unitary SMs are composed of bundles or sheets of fibers highly enriched with gap junctions, which allow synchronized response to stimuli. Their innervation is limited and their stimulation is controlled by factors such as hormones or mechanical factors. They respond to stretching and are found around all enteric organs and most vessels. This classification cannot, however, be regarded as rigid, since SMs in some organs, like bladder, and some blood vessels show features of both types.

With advances in electron microscopy and physiology

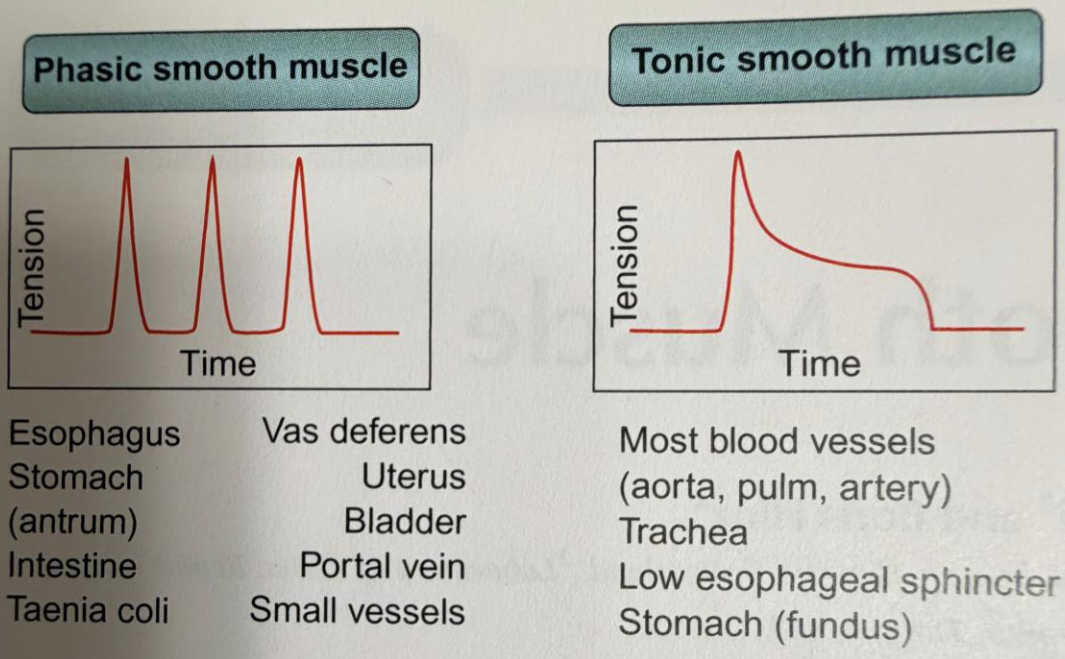



**FIGURE 88.1 Classification of SM-containing organs according to their contraction features.** SMs can be characterized by their phasic/tonic properties. Phasic SMs, which contract and relax rapidly, include most ESMs, whereas tonic SMs, which contract and relax slowly, correspond to VSMs and ASMs.

during peristalsis. The different mechanical properties of phasic and tonic SMs depend either on their signal transduction activity or on their contractile proteins composition. In tonic SMCs, initial contraction in response

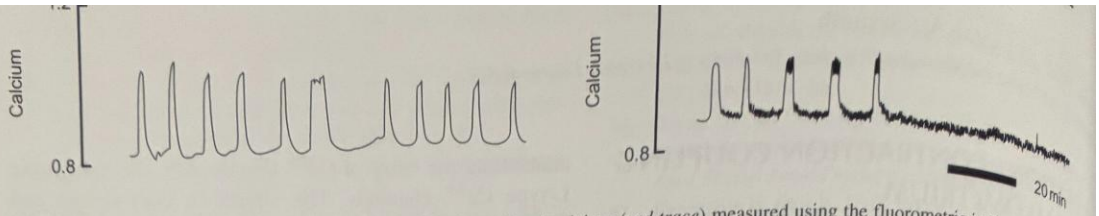



**FIGURE 90.1** Simultaneous measurement of force (*black trace*) and calcium (*red trace*) measured using the fluorometric indicator indo-1 in spon neously contracting strips of human myometrium. In (A), strips were superfused with PSS (pH7.4, 37°C) followed by high potassium (40 mM K depolarization (KCl, black bar). In (B), the effect of removing external calcium (0Ca, black bar) on inhibition of spontaneous contractions can clearly seen.

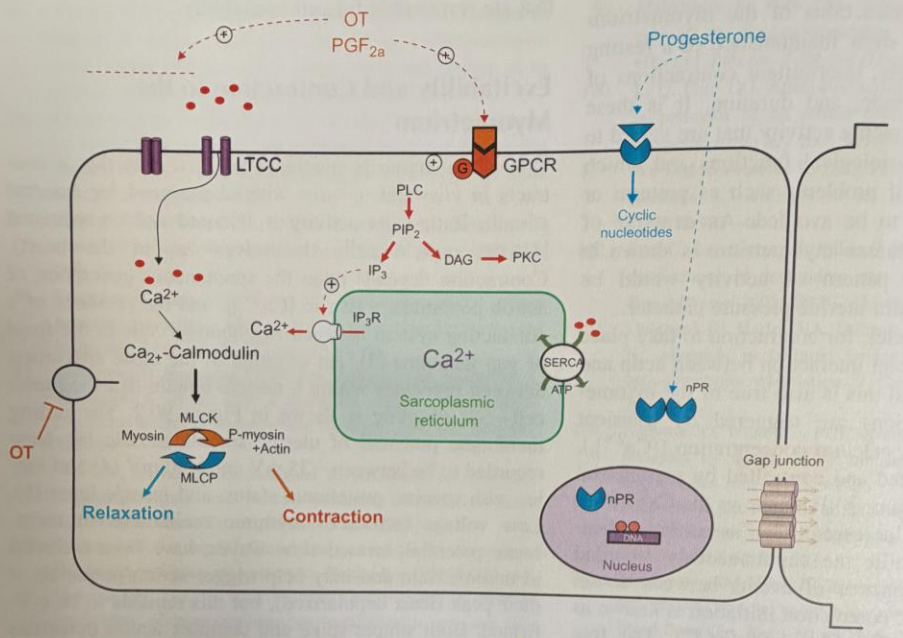



**FIGURE 90.2** Schematic to show the excitation–contraction coupling pathway in the myometrium (black arrows) and the major mechanisms of action by the hormones oxytocin, prostaglandin $F_{2\alpha}$ and progesterone. Red arrows and bars show pathways that when activated lead to stimulation of contraction whilst blue arrows and bars show those pathways leading to suppression of contraction. *(Figure adapted from Arrowsmith, Kendrick and Wray, 2010 (45), with permission.)*

predominantly mediated by $Ca^{2+}$. Individual spikes can often be grouped into bursts, with the number and frequency of spikes within each burst determining the amplitude, speed, and duration of contraction (7). The complex action potential involves an initial spike-like depolarization followed by a sustained plateau of depolarizatio

−30 and −20 mV, lasting approximately 1 minute, which may involve a weak $K^+$ but strong $Ca^{2+}$ conductance. The duration of the plateau determines the duration of contraction. Gap junctions ensure propagation of action potentials throughout the myometrium, prompting contraction syn- ity at the whole organ level, and are thus, along

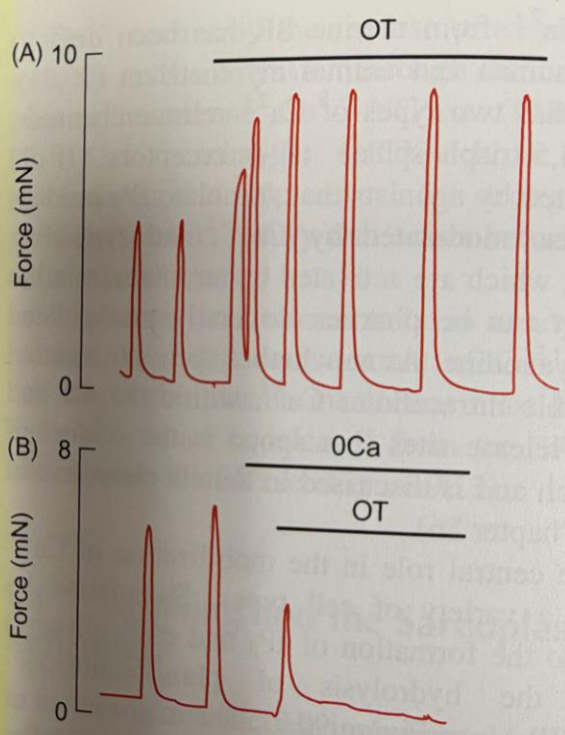
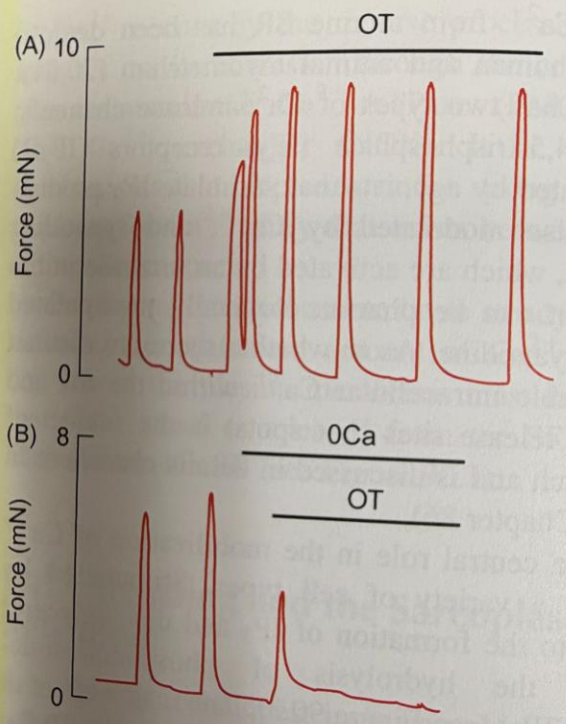



**FIGURE 90.4** The stimulatory effect of oxytocin (OT, 10 nM) in the presence (A) and absence (B) of external $Ca^{2+}$ on contractions in human myometrium. Application of OT in the presence of calcium causes significant increase in contraction amplitude, which persists for the duration of OT application. In the absence of extracellular calcium, OT is only capable of initiating one small contraction, indicating that $Ca^{2+}$ influx is a major contributor to the stimulatory effect OT.

circulating OT does not seem essential in parturition as there is no good evidence for an increase in maternal OT concentrations with labor onset. Instead it is proposed that local (uterine or decidual) increase

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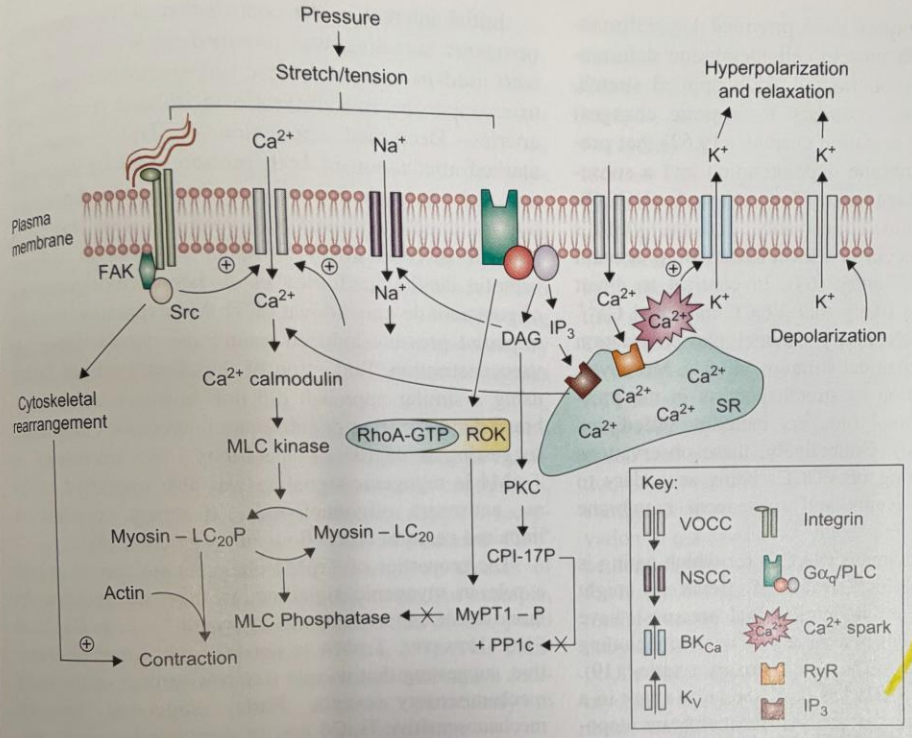



**FIGURE 93.4 Intracellular signaling pathways implicated in myogenic vasoconstriction.** Central to myogenic constriction membrane depolarization leads to opening of VGCCs, $Ca^{2+}$/calmodulin-mediated activation of myosin light chain kinase and the phosphorylation of the 20 kD myosin regulatory light chain (MLC20). Phosphorylation of $MLC_{20}$ ($MLC_{20}P$) allows acto-myosin interaction, cross-bridge cycling and contraction. Complementing this pathway, $Ca^{2+}$ sensitization via RhoA/Rho kinase-mediated myosin phosphatase inhibition potentiates $MLC_{20}P$ and enhances contraction. Further, the mechanical stimulus activates cytoskeletal rearrangement through mechanisms likely to be dependent on integrin-mediated activation of focal adhesions. $Ca^{2+}$ release from the SR may serve several distinct spatiotemporally confined roles including the regulation of $Ca^{2+}$-activated ion channels, production of $Ca^{2+}$ waves and provision of activator $Ca^{2+}$. *(From Hill et al., 2009 (57).)*

annels directly (stretch sensitive or mechanogated) but could alternately occur via secondary pathways by generation of signaling molecules that modulate ion channel gating.

### Me... ...les of Stretch/Non-... ...nnels)

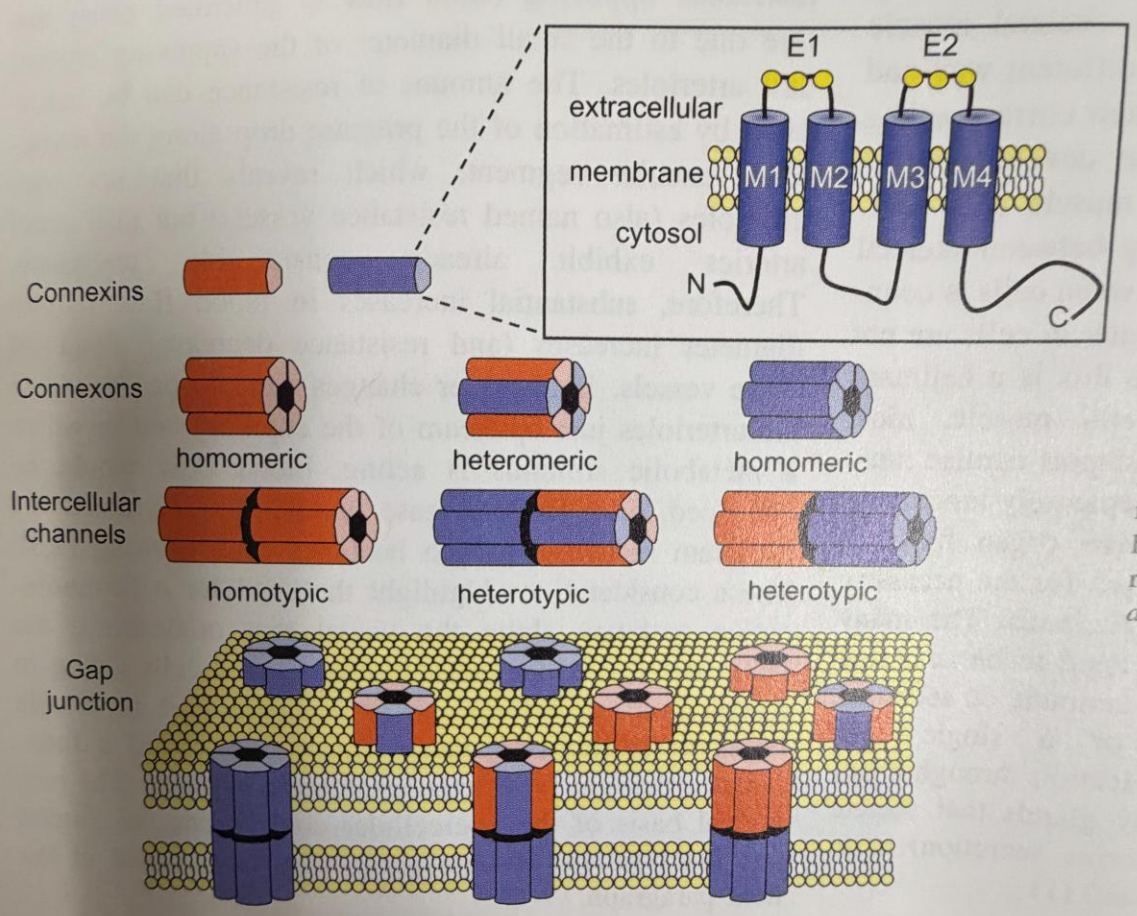
VSMC membrane potential ...ion has long been accepted. ...s in cat cerebral artery pre- ...ated that increasing intralum- ...iated with graded membrane ...lar relationships were later dem- ...ular preparations, including small ...the rat cerebral and cremaster mus- ...myogenically active arteri...

Although available data are more limited, *in vivo* measurements with glass microelectrodes support similar levels of Em (61).

Arguments have been raised to question whether a pressure-induced change in Em is an absolute requirement for myogenic constriction. These arguments are often based on myogenic responsiveness persisting in the presence of high extracellular $K^+$ (shifting the $K^+$ equilibrium potential towards 0 mV) and a plateauing of Em vs myogenic tone relationships at higher intraluminal pressures. However, it is of interest to note that permeability preparations (lacking a transmembrane potential difference) do not show myogenic reactivity... preparations can be used to de... nical coupling and... regard...

(6). How... are not actually fused but there is ... such adjacent cells, which led to the term "gap junction" instead of nexus. In these closely apposed areas of cell contact intercellular channels are clustered which bridge this gap and interconnect adjacent cells. Intercellular gap junction channels are composed of two separate hemichannels that dock face-to-face such that they interconnect

connexins that are ... These proteins ... domains named M1 ... reside in the cytopl... membrane domains ... extracellular loops ...



wall against [illegible] environment is created by the extracellular loops E1 and E2, which interact through disulfide bonds generated by the conserved cysteine residues within a single connexin molecule. However, the extracellular loops E1 and E2 also interact between different connexin molecules but this exact interaction is unclear. The two hemichannels are staggered (or rotated) against each other providing a tight docking of the two hemichannels. The aqueous pore in the center of the channel is effectively sealed against the extracellular environment by the rotation and interdigitation, which is a requirement for intercellular communication since it prevents the dilution of exchanged signaling molecules and the loss of ions (and charge) leaking into the extracellular space (7). The size of the pore allows transfer of ions as well as water and other polar molecules up to a size of 1 kilodalton (kDa). Thus, these channels create not only an electrical continuity, but also enable the diffusion of such important molecules as cAMP, inositol trisphosphat ($IP_3$), or $Ca^{2+}$-ions (14).

## Diversity of Connexin Proteins

The family of connexin genes is comprised of ~20 members (in humans 21, in mice 20) and the proteins are named according to their theoretical molecular mass; for example, connexin40 (Cx40) has a predicted molecular mass of about 40 kDa. The distinct predicted molecular mass and thus the diversity of connexin proteins results from divergencies in the cytoplasmic loop and mostly from differences in the length of the C-terminal cytoplasmic domain. The species from which the respective protein is derived is indicated by a leading small letter, e.g. mCx40 means murine connexin40 and hCX40 human

[illegible] respective connexin of the [illegible] and the human ortholog is [illegible], respectively (15). The naming of [illegible] has somewhat confusing, even more if other [illegible] are considered and a new nomenclature is being developed.

As outlined above, intercellular signalling is functionally important in diverse cell types and tissues. The diversity in the connexin gene family can be partially related to diverse cell types in that specific connexin members serve coupling in specific tissues. Thus, most members of the connexin family are expressed in specific tissues (with some exceptions). However, even in a single cell type within a certain organ multiple connexins have been identified, e.g. in smooth muscle and cardiovascular tissue Cx37, Cx40, Cx43, and Cx45 (16). This diversity generates theoretically a multiplicity of different hemichannels due to their modular design and an even larger diversity of complete intercellular channels considering the fact that a channel is composed of 12 protein subunits. These theoretical interactions can be divided in four groups with respect to the connexins forming the channel (Figure 94.1). A hemichannel composed of six identical connexins is termed homomeric whereas a hemichannel constituted by different connexins is named heteromeric. Analogously, a complete channel constructed by identical hemichannels is named homotypic and a channel made up by nonidentical hemichannels, heterotypic. This nomenclature creates four groups of channels, i.e. homomeric-homotypic (all 12 subunits are identical), homomeric-heterotypic (different hemichannels each composed of six identical subunits), heteromeric-homotypic (two identical hemichannels each composed of more than one connexin), and heteromeric-heterotypic (distinct hemichannels each composed of more than one connexin) (17). Does this diversity indeed exist? Expression of connexins in *Xenopus oocytes* or in cell lines that lack connexins physiologically along with the electrophysiological analysis of coupling between such cells expressing different connexins allows one to verify the theoretical interactions. Of the interesting connexins in the cardiovascular

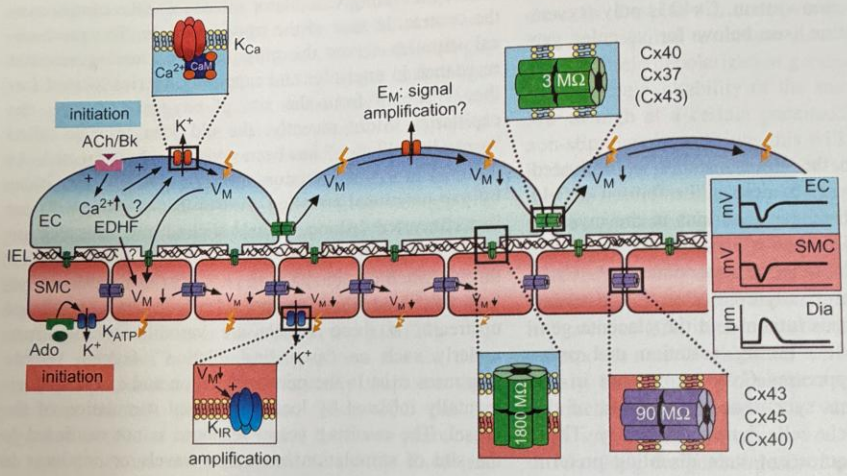



**FIGURE 94.3 Gap junctions connect separate cell layers in the vessel wall enabling conducted responses.** Local stimulation using agonists (initiation) that act on endothelial cells (EC; acetylcholine, ACh; bradykinin, Bk) or smooth muscle cells (SMC; adenosine, Ado) hyperpolarizes the cell through activation of $K^+$-channels. The hyperpolarization is transmitted along the vessel wall through gap junctions that couple either EC or SMC homocellularly by different connexins (Cx) as indicated. The conducting signal may be amplified by $K^+$-channels. In spite of amplification, the signal dissipates with distance, which is most pronounced in SMC, possibly related to higher intercellular resistances (values from reference 37) and a larger number of cell membranes to be crossed. Heterocellular coupling between EC and SMC (myoendothelial) may allow direct current transfer from EC to SMC and provide a molecular substrate for dilations accounted for by an endothelium-derived hyperpolarizing factor (EDHF). However, a distinct EDHF may be present additionally. IEL, internal elastic lamina; $V_M$, membrane potential; Dia, diameter; $K_{Ca}$, $Ca^{2+}$-activated, $K_{ATP}$, ATP-dependent, and $K_{IR}$, inwardly rectifying $K^+$ channel, respectively. *(Modified from de Wit and Griffith, 2010 (38), with permission from Springer Science + Business Media.)*

the tissue the sole destruction of the endothelial cell layer at a restrained site along the pathway abrogated the conduction of a dilation throughout this site. Thus, there are vessels in which aside from the endothelium the smooth muscle layer is also connected by low-resistance gap junctions (Figure 94.3) but this is obvic… not the case for all vessels. The reason for this di… is nc… clear but it was suggested that thi… i.e … coupling of smooth muscle cells) i… of sym… nervation, which … tight … nal communic… most … cle may res… releas… trans…

G… n the vessel … Cx40 … Cx45 with … these … In general … Cx37 … whereas C… d by … referen… m expr… was iden…

Deletion of Cx40 results in an attenuation of dilations at remote sites in case the local dilation is elicited by a substance that requires the endothelium (endothelium-dependent dilators, such as actylcholine or bradykinin) (3). Although this highlights the role of the endothelial pathway to conduct dilatory si… along the vessel wall, remote responses are not … abrogated. Thus, her a remaining endothe… (Cx37) is able to intain a less efficien… pathway or the kup smooth muscle … ns the attenuated ions at remote sites. … e second connex- x37) is also strongly … en lacking in the helium if Cx40 is … This suggests that ooth muscle lay… to transmit con- responses, alth… tly. Interestingly, lator that act… th muscle (adeno- so able to …

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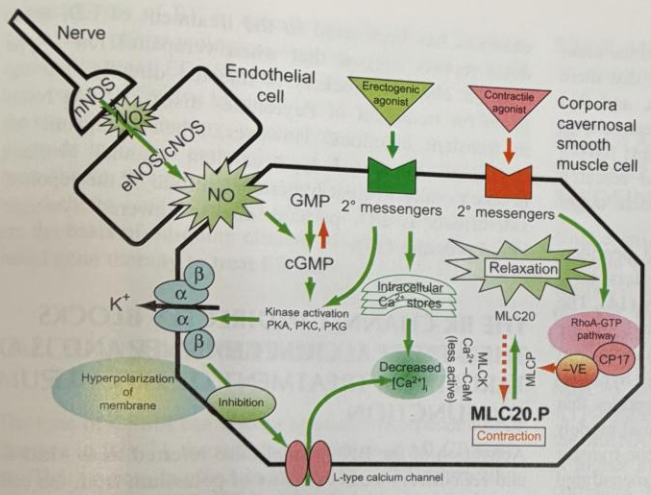


**FIGURE 102.4 The major pathways (shown with green arrows) resulting in relaxation of the corpora cavernosal smooth muscle tissue that occurs with an erection.** Smooth muscle tone is maintained through balancing pathways that either phosphorylate (and cause contraction) or dephosphorylate (and cause relaxation) myosin light chain (MLC). Nitric oxide (NO) produced by neurogenic nitric oxide synthase (nNOS) or endothelial/ constitutive NOS (eNOS/cNOS) diffuse into corporal smooth muscle cells. NO activates guanylate cyclase, raising the levels of cyclic GMP (cGMP), through its action on ion channels and intracellular $Ca^{2+}$ storage sites, lowers intracellular calcium concentrations $[Ca^{2+}]_i$. Lower $[Ca^{2+}]_i$ reduces the activity of MLC kinase (MLCK) which shifts the balance towards unphosphorylated MLC and relaxation. Calcium sensitization pathways (by contractile agonists, such as endothelin-1) result in the activation of the Rho-kinase (ROK) or CPI-17 pathway, causing heightened tone by inhibiting smooth muscle myosin light chain phosphatase (MLCP) either by directly phosphorylating MLCP or by binding to CPI-17.

## INITIATION OF AN ERECTION

The initiation of penile erection is controlled by the parasympathetic ympathetic branches of the autonomic eripherally, the balance between ervous syste factors regulates the tone of the contractile he functional state of the penis. CCSM a is considered the most impor- Neurog CCSM and penile vessels. tant l ways to direct the relaxa- W ant role is in signal trans- roduced and released from d endothelial cells where it ells and binds with intracellu- 102.4). This binding induces a uanylate cyclase, activating the zes the conversion of guanosine cyclic guanosine monophosphate cts through a cGMP-dependent pro-

suggest that cGMP also inhibits the presynaptic release and contractile effects of the adrenergic contractile neurotransmitter, noradrenaline.

The role of NO in initiating an erection has led to several groups researching treatments of ED based on increasing local levels of NO. Several approaches have been shown to be successful in animal models, such as increasing the levels of nitric oxide synthase (NOS) through gene transfer or increasing the L-arginine concentration (the substrate for NO production) either by direct supplementation or inhibition of arginase (10). A recent development which has proven effective in animal models of ED is to increase local NO concentrations by applying nanoparticles encapsulating NO which are capable of transdermal penetration directly to the dermis of the penis (11).

Breakdown of cGMP in the CCSM is achieved through the action of phosphodiesterase-5 (PDE5). The highly successful oral treatments for ED, Viagra (sildenafil), Cialis (tadalafil), and Levitra (vardenafil), are inhibitors of this enzyme, and result in increased levels of

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Breakdown of cGMP in the CCSM is achieved through the action of phosphodiesterase-5 (PDE5). The highly successful oral treatments for ED, Viagra (sildenafil), Cialis (tadalafil), and Levitra (vardenafil), are inhibitors of this enzyme, and result in increased levels of cGMP following sexual stimulation (12). All three seem to have similar efficacy profiles, being effective in 55–85% of patients. Although all these compounds have the same mechanism of action they exhibit different pharmacokinetics. Whereas sildenafil and vardenafil have a half-life of about 4 hours, that of tadalafil is much longer (approximately 17.5 hours) which is associated with a