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Review Article

Contraction and Relaxation Signaling in Gastrointestinal Smooth Muscle

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Abstract

The gastrointestinal (GI) tract is a complex multi-organ system with tissues that differ structurally and functionally. The dynamic interactions between the neuronal, muscular, immune, and glandular tissues allow the GI tract to perform its main physiological functions, which include digestion, absorption, excretion, and protection. Normal gut motility provides for the mixing and propulsion of intraluminal contents to enable efficient digestion of food, progressive absorption of nutrients, and evacuation of residues. These fine and delicate GI tract movements are generated by a highly-regulated interaction between an intricate network of neurons located within the wall of the alimentary canal (i.e. the enteric nervous system), an intrinsic pacemaker system (i.e. interstitial cells of Cajal or ICC) and the final effector cells (i.e. smooth muscle cells). GI tract smooth muscle possesses distinct regional and functional properties that distinguish it from other types of visceral and vascular smooth muscle. Gut smooth muscles contract spontaneously in the absence of neural, humoral, or hormonal stimuli and in response to stretch. They contract as a syncytium (i.e. contract in unison) and therefore classified as unitary type smooth muscle. The molecular mechanisms underlying GI smooth muscle contraction and relaxation signaling pathways are the subject of this review.

Keywords: *Smooth Muscle; Contraction; Relaxation; Myosin Light Chain (MLC20); Rho Kinase; MLC Phosphatase*

Abbreviations

AMPK: Adenosine Monophosphate Kinase; ATP: Adenosine Triphosphate; CaM: Calmodulin; CaMKII: Calmodulin-Dependent Kinase II; cAMP: Cyclic Adenosine Monophosphate; cGMP: Cyclic Guanosine Monophosphate; CPI-17: PKC Potentiated Inhibitor 17 kDa Protein; GEF: Guanine Nucleotide Exchange Factor; GI: Gastrointestinal; GSNO: S-Nitrosoglutathione; GTP: Guanosine Triphosphate; ICC: Interstitial Cells of Cajal; ILK: Integrin-Linked Kinase; IP3: Inositol Trisphosphate; IP3R: Inositol Trisphosphate Receptor; MLC: Myosin Light Chain; MLCK: Myosin Light Chain Kinase; MLCP: Myosin Light Chain Phosphatase; MRP: Multidrug Resistant Protein; MYPT1: Myosin Phosphatase Target Subunit 1; NO: Nitric Oxide; PACAP: Pituitary Adenylate Cyclase-Activating Peptide; PAK1: p21-Activated Protein Kinase 1; PDE: Phosphodiesterase; PKA: Protein Kinase A; PKC: Protein Kinase C; PKG: Protein Kinase G; PLA2: Phospholipase A2; PLC: Phospholipase C; Ppicδ: Protein Phosphatase Delta Isoform; S1P: Sphingosine-1-Phosphate; sGC: Soluble Guanylate Cyclase; SIP2: Sphingosine 1-Phosphate Receptor 2; ZIPK: Zipper Interacting Protein Kinase

Signaling for smooth muscle contraction

An essential step in smooth muscle contraction is phosphorylation of the 20 kDa regulatory myosin light chain (MLC_{20}) by a Ca^{2+}/calmodulin-dependent or -independent myosin light chain kinase (MLCK) which transfers the phosphate group from ATP to either Ser^{19} or Thr^{18} hydroxyl groups of MLC_{20}. This phosphorylation activates the actin-activated myosin ATPase and actin-myosin interaction, which thereby initiates smooth muscle contraction.

An increase in intracellular free Ca^{2+} concentration induces activation of Ca^{2+}/CaM-dependent MLCK and the phosphorylation of MLC_{20} and thus muscle contraction. The decrease in intracellular level of Ca^{2+} induces dissociation of the Ca^{2+}-CaM-MLCK complex, resulting in dephosphorylation of the MLC_{20} by myosin light chain phosphatase (MLCP) and thus smooth muscle relaxation [1,2]. Thus, the phosphorylation level of MLC_{20} is determined by the opposing activities of MLCK and MLCP and both of these enzymes' activities are well-regulated in smooth muscle.

MLCK regulation

As mentioned previously, smooth muscle tone is regulated by signaling cascades initiated by enteric neurotransmitters acting directly on smooth muscle receptors. In circular smooth muscle cells, contractile agonists activate PLC-β1 via Gαq coupled receptors (e.g. muscarinic m3 receptors) by Gαq binding to PLC-β1's COOH-terminal tail [3]. PLC-β1 hydrolyzes PIP2 into DAG and IP3. An increase in IP3 leads to the binding of IP3 to the high affinity IP3 receptor/Ca^{2+} channel on the sarcoplasmic reticulum, resulting in the release of Ca^{2+} into the cytosol. Of the two IP3 receptor isoforms (IP3R-I and IP3R-III) expressed in smooth muscle cells, only IP3R-I mediates Ca^{2+} release [4].

In longitudinal smooth muscle cells, Ca^{2+} mobilization is dependent on a mandatory step involving Ca^{2+} influx via voltage-gated Ca^{2+} channels[4]. Upon contractile agonist stimulation, both Gq- and Gi-coupled receptors activate cytoplasmic PLA2 resulting in phosphatidylcholine hydrolysis into arachidonic acid which induces membrane depolarization and opening of voltage-gated Ca^{2+} channels. The entry of Ca^{2+} stimulates cyclic ADP ribose formation and induces synergistic Ca^{2+}- and cyclic ADP ribose-induced Ca^{2+} release via ryanodine receptors/Ca^{2+} channels [5].

MLCK activity is strictly dependent on $[Ca^{2+}]i$ which, upon agonist stimulation, increases as a result of Ca^{2+} influx into the cytosol through voltage-gated channels and/or the release of Ca^{2+} from intracellular stores. Resting levels of $[Ca^{2+}]i$ (70-100nM) increases up to 8-fold during maximum contraction.

Four Ca^{2+} ions bind to calmodulin (CaM) cofactor which then binds to and activates MLCK [6]. The transient high levels of $[Ca^{2+}]i$ are then extruded from the cell and/or up taken into the sarcoplasmic Ca^{2+} stores. This decrease in the intracellular level of Ca^{2+} induces dissociation of the Ca^{2+}-CaM-MLCK complex, which thereupon decreases MLCK activity. MLC_{20} phosphorylation and contraction, however, are maintained by Ca^{2+}-independent MLCKs and regulated inhibition of MLCP activity in a process called Ca^{2+} sensitization [1].

Moreover, MLCK activity was shown to be regulated by protein kinases that act to provide negative feedback mechanisms. Stull and coworkers have found that MLCK is phosphorylated by CaMKII, and this phosphorylation decreases the activity of MLCK by decreasing the affinity of the enzyme for calmodulin. It was found that $[Ca^{2+}]i$ required for the half-maximum activation of CaMKII equals 500 nM, whereas that for MLCK activation is only 250 nM, suggesting a role for CaMKII in inactivation of MLCK when smooth muscle is hyperactivated and $[Ca^{2+}]i$ rises above some critical level [7]. A similar mechanism has been proposed recently with AMP kinase (AMPK) in which AMPK phosphorylates MLCK at Ser^{815} leading to decreased activity of MLCK. It was found that ablation of AMPK augmented contraction, suggesting rapid MLCK attenuation and suppression of contraction by AMPK [8]. In addition, p21-activated protein kinase 1 (PAK1) was shown to attenuate smooth muscle contraction by phosphorylating and inactivating MLCK [9] (Figure 1).

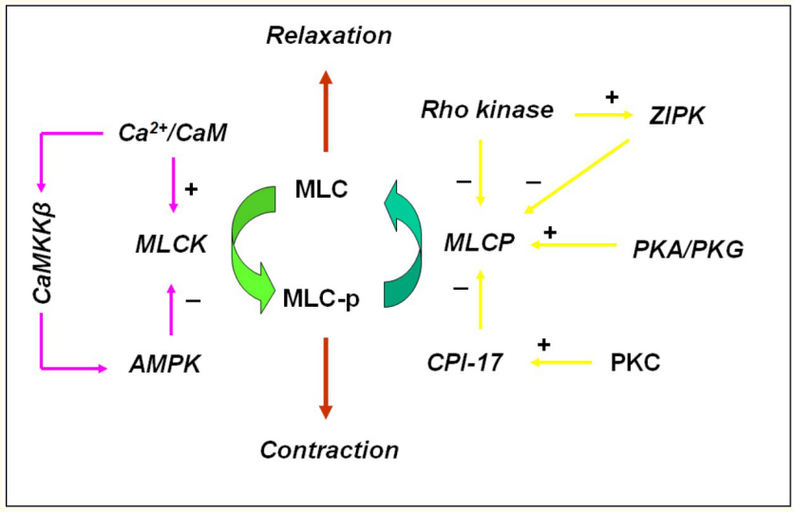

Figure 1: *Regulation of MLCK and MLCP by various signaling pathways in the gastrointestinal smooth muscle.*

MLCP regulation

While MLCK-mediated contraction is strictly dependent on $[Ca^{2+}]i$, agonist-induced MLC_{20} phosphorylation and contraction can be maintained even after $[Ca^{2+}]i$ returns to basal levels via two ways; MLC_{20} phosphorylation by Ca^{2+}-independent MLCKs and regulated inhibition of MLCP (Figure 1).

An important step in maintaining contraction via MLCP inhibition involves activation of RhoA via a cascade leading to sequential agonist-mediated activation of $Gq/G\alpha_{13}$ and Rho guanine nucleotide exchange factor (Rho-GEF) [9,10]. Activated Rho A (Rho-GTP) is translocated to the plasma membrane where it activates both Rho kinase -mainly Rho kinase II, the predominant smooth muscle isoform- and PLD [11]. Hydrolysis of phosphatidylcholine by PLD yields phosphatidic acid, which is dephosphorylated to diacylglycerol, resulting in sustained activation of Ca^{2+}-dependent and -independent PKC isozymes. Rho kinase and PKC act concurrently and cooperatively to inhibit MLCP activity [2].

The same receptors that initiate Ca^{2+} mobilization and MLCK-mediated MLC_{20} phosphorylation and contraction also engage a distinct G protein-dependent pathway that mediates Ca^{2+}-independent MLC_{20} phosphorylation and contraction via negative regulation of MLCP. Some receptors (e.g. m3 receptors) are coupled to RhoA via G_{13} only, whereas others (e.g. SIP2 and motilin receptors) are coupled to RhoA via both Gq and G_{13} [9,12,13].

Structurally, MLCP holoenzyme consists of three subunits; a 37 kDa catalytic subunit of type 1 phosphatase (ppicδ), a 110 to 130 kDa regulatory subunit, known as myosin phosphatase target subunit I or MYPT1, and a 20 kDa subunit of unknown function. MYPT1 binding to the catalytic subunit enhances MLCP catalytic activity [14].

Phosphorylation of MYPT1 at Thr^{696} by Rho kinase promotes dissociation of the catalytic and regulatory subunits of MLCP and inhibits its catalytic activity [15]. Rho kinase also phophorylates Thr^{853} within the myosin-binding domain on MYPT1 upon which the enzyme dissociates from myosin and decreases the efficiency of the enzyme by decreasing availabilty of the substrate [10]. On the other hand, phosphorylation of an adjacent Ser^{695} by cAMP- or cGMP-dependent protein kinase (PKA and PKG, respectively) blocks the ability of Rho kinase to phosphorylate Thr^{696} and so restores MLCP activity [16].

PKC, mainly PKC-ε and PKC-δ, phosphorylates CPI-17, a 17 kDa endogenous inhibitor of MLCP, at Thr^{38} and greatly enhancing its ability to inhibit MLCP [17]. Thus, a dual Rho-dependent mechanism (i.e. via Rho kinase and PKC activation) causes sustained inhibition of MLCP. The relative involvement of Rho-mediated pathways-Rho kinase/MYPT1 and PKC/CPI-17- in MLCP inhibition appears to be receptor-specific. Most $Gq/_{13}$-coupled receptors (e.g. m3, S1P2, motilin) engage both pathways. ETA receptors engage only Rho kinase/MYPT1 while LPA3 receptors engage only PKC/CPI-17 [12,13,18,19].

Zipper interacting protein kinase (ZIPK) was also found to inhibit MLCP. It is a serine/threonine kinase expressed in various tissues including smooth muscle and is a member of the death–associated protein kinase (DAP) family [20]. ZIPK co-localizes with MLCP and is phosphorylated following activation of Rho kinase-dependent pathway during carbachol stimulation of rabbit bladder. The phosphorylated ZIPK, in turn, phosphorylates the myosin-binding subunit at Thr^{696}, considerably faster and even more effective than Rho kinase [21,22]. Niiro and Ikebe, however [23], have demonstrated that MYPT1 is a poor substrate for ZIPK, and instead, ZIPK acts as a Ca^{2+}-independent MLCK that directly phosphorylates MLC_{20} at both Ser^{19} and Thr^{18} in absence of Ca^{2+}. It has been suggested that the myofilament-bound ZIPK may mediate Rho kinase–dependent phosphorylation of MYPT1 and inhibition of MLCP and thus could be the link between the activated plasma membrane-bound Rho kinase and MYPT1.

Integrin-linked kinase (ILK) is another myofilament-bound Ca^{2+}-independent MLCK. It mediates MLC_{20} phosphorylation, at both Ser^{19} and Thr^{18}, and smooth muscle contraction through Gi-coupled receptors. These receptors sequentially activate ILK through certain pathway involving PI3-kinase activation via Gβγi [24].

Signaling for relaxation

Smooth muscle relaxation is initiated by targeting MLC_{20} dephosphorylation. This involves either MLCK inactivation and/or removal of MLCP inhibition. Most agents cause relaxation by stimulating the production of cAMP (e.g. VIP and its homologue PACAP) or cGMP (e.g. nitric oxide [NO]) leading to activation of PKA, PKG or both. Cyclic AMP-activated PKA and cGMP-activated PKG are the main enzymes that induce relaxation in smooth muscle. They target different components of the contractile signaling pathways that attenuate MLCK activity and/or augment MLCP activity which eventually induce dephosphorylation of MLC_{20} and thus smooth muscle relaxation [2] (Figure 2).

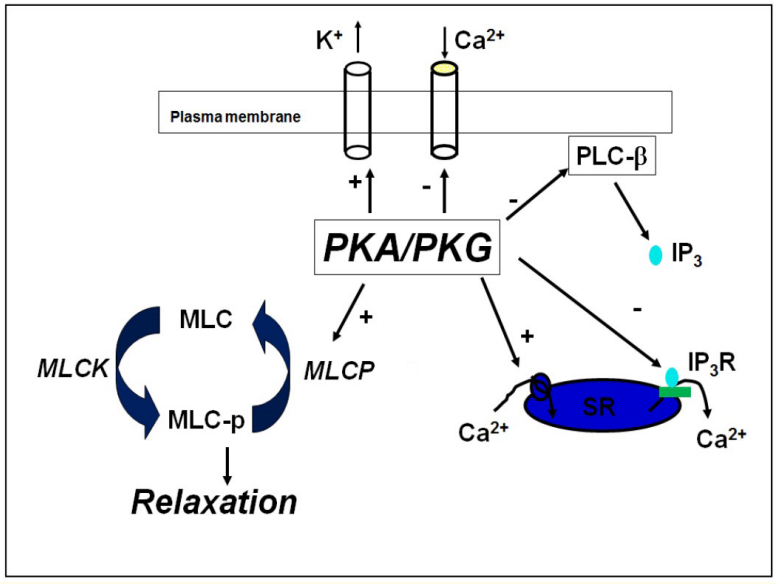

Figure 2: *Regulation of gastrointestinal smooth muscle relaxation by PKA and PKG.*

Cyclic nucleotide regulation

The levels of cAMP and cGMP in gastrointestinal smooth muscle depend on the rates of their synthesis by cyclases and degradation by phosphodiesterases (PDEs). Cyclic AMP, which is produced in ~10-fold greater amounts than cGMP, is generated from ATP via the membrane-bound adenylate cyclase (AC) -type V and VI- and is rapidly degraded by the cAMP-preferring PDE3 and cAMP-specific PDE4. On the other hand, cGMP is generated from GTP via the soluble guanylate cyclase (sGC) and is rapidly degraded by cGMP-specific PDE5. PKA inhibits AC while PKG inhibits sGC. Both PDE3 and PDE4 are activated by PKA, but only PDE3 is inhibited by cGMP. On the other hand, PDE5 is activated by PKG. When both cAMP and cGMP are present, PDE5 is also activated by PKA [25-27]. So, regulatory feedback from the protein kinases inhibits synthesis and accelerates degradation, and thereby maintains the levels of cyclic nucleotides within a narrow range.

Although cAMP preferentially activates PKA, it can, at higher concentrations, also cross-reactivate PKG [28]. An increase in both cAMP and cGMP, such as that brought about by corelease of NO, VIP, and PACAP from the same or adjacent nerve terminals, is the physiological norm during nerve-induced relaxation of the gut. Inhibition of PDE3 by cGMP enhances cAMP levels, whereas activation of PDE5 by PKA and PKG greatly increases its affinity for the more abundant cAMP. Under these conditions, PKG is activated by both cGMP and cAMP [27,29].

In addition to degradation by phosphodiesterases, cyclic nucleotides elimination pathways comprise active export into the extracellular space via members of the multidrug resistance protein (MRP) family (the other name is ABC transporters). MRPs bind and hydrolyze ATP, providing the energy to transport their substrates across membrane barriers (Figure 3). Among the MRP family members, MRP4 and MRP5 have been shown to be competent in the transport of cAMP and cGMP respectively [30]

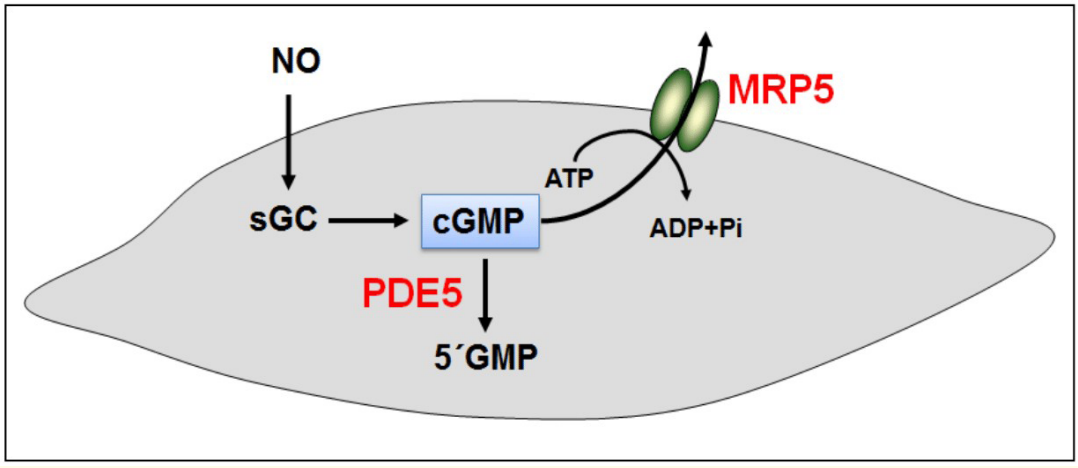

Figure 3: *Regulation of cGMP level by phosphodiesterase 5 (PDE5) and multidrug resistance protein (MRP) in the gastrointestinal smooth muscle.*

PKA and PKG targets

PKA and PKG indirectly target MLCK inactivation by primarily decreasing [Ca^{2+}]i. Both PKA and PKG can inhibit Ca^{2+} mobilization by inhibiting IP3 formation in circular muscle and arachidonic acid formation in longitudinal muscle. Inhibition of IP3 formation in circular muscle involves phosphorylation of RGS4, leading to more rapid degradation of Gαq-GTP and inhibition of PLC-β1 activity. PKG-mediated phosphorylation of SERCA2 and sarcoplasmic reticulum IP3 receptors accelerates Ca^{2+} reuptake into the stores and inhibits IP3-induced Ca^{2+} release, respectively. In addition, both kinases inhibit the activity of membrane Ca^{2+} channels and stimulate the activity of membrane K^+ channels, leading to hyperpolarization of the plasma membrane and interruption of Ca^{2+} influx into the cell, a mechanism that is important in relaxation of rhythmic contraction[2].

Moreover, PKG and PKA augment MLCP activity by different ways; first, they phosphorylate the activated form of RhoA (Rho-GTP) at Ser^{188} leading to its inactivation and translocation back to the cytosol [31]. Second, both enzymes can phosphorylate MYPT1 at Ser^{695}, preventing the inhibitory regulation of Rho kinase-mediated phosphorylation of MYPT1 at Thr^{696} [16]. Finally, both kinases are able to phosphorylate (at Ser^{13}) and enhance the activity of telokin, a smooth muscle-specific endogenous activator of MLCP [32].

Conclusion

Phosphorylation of ML_{20} is an essential step in GI smooth muscle contraction. Contractile agonists increase MLC_{20} phosphorylation by Ca^{2+}/CaM-dependent stimulation of MLCK and RhoA-dependent inhibition of MLCP activity. Relaxant agonists decrease MLC_{20} phosphorylation either by decreasing Ca^{2+} levels or increasing MLCP activity via PKA and PKG-dependent pathways. Better understanding of contraction and relaxation signaling pathways in the GI smooth muscle in different physiological and pathophysiological states will help in designing more effective drugs for treating various GI contractile diseases.

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Conflict of Interest

The author declares any conflict of interest.

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