HHS Public Access

Author manuscript

Gastroenterology. Author manuscript; available in PMC 2017 September 14.

Published in final edited form as:

Gastroenterology. 2010 January ; 138(1): 13–8.e1-3. doi:10.1053/j.gastro.2009.11.016.

RhoA/Rho-Kinase: Pathophysiological and Therapeutic Implications in Gastrointestinal Smooth Muscle Tone and Relaxation

SATISH RATTAN[*], **BENJAMIN R. PHILLIPS**[#], and **PINCKNEY J. MAXWELL IV**[#]

[*]Department of Medicine, Division of Gastroenterology & Hepatology, Jefferson Medical College of Thomas Jefferson University, Philadelphia, PA

[#]Department of Surgery, Division of Colon and Rectal Surgery, Jefferson Medical College of Thomas Jefferson University, Philadelphia, PA

Background

Major molecular pathways affecting gastrointestinal (GI) smooth muscle function include Ca^{2+}/calmodulin (CaM)/myosin light chain kinase (MLCK), the protein kinase C (PKC)/CPI-17, and RhoA/Rho-kinase (ROCK)/myosin light chain phosphatase (MLCP).[1] Among these, RhoA/ROCK is emerging as an important mediator of smooth muscle contraction and dysfunction that cannot be explained on the basis of previously known bioenergetics.[1–3] The role of RhoA/ROCK in the pathophysiology and therapy has been investigated extensively and reviewed in organs systems other than the GI tract.[4–8] This mini-review provides current information on the role of RhoA/Rho-kinase in the pathophysiology and potential therapy for the following disorders: diffuse esophageal spasm (DES), achalasia, gastroesophageal reflux disease (GERD), gastroparesis, pylorospasm, biliary dyskinesia, adynamic ileus, colonic inertia, Hirschsprung's disease, Ogilvie's syndrome, recurrent anal fissures (RAF), hemorrhoids, rectoanal incontinence (Table 1).

The functional integration of the enteric nervous system (ENS) in conjunction with the central nervous system (CNS), smooth muscle, and perhaps interstitial cells of Cajal (ICC), is responsible for GI motility involving systematic contraction and relaxation of the smooth muscle. However, certain smooth muscle contractions or tone may occur independent of the innervation and are myogenic in nature.[9] Contraction and relaxation of the GI smooth muscle can be elicited also pharmacologically. Despite significant investigations, including the use of animal models, the exact nature of the molecular mechanisms underlying the pathophysiology of GI smooth muscle contraction and relaxation are not known. GI smooth muscles exhibiting spontaneous myogenic basal tone, such as the lower esophageal sphincter

Corresponding Author: Dr. Satish Rattan, Jefferson Medical College, Thomas Jefferson University, 1025 Walnut Street, Room # 901 College; Philadelphia, PA 19107 Tel # (215) 955-5614; Fax # (215) 503-3771; Satish.Rattan@Jefferson.edu.

Disclosures: Nothing to disclose

(LES) and internal anal sphincter (IAS), provide an opportunity to examine specific molecular pathways for contraction and relaxation in the absence of any stimulus. [9–12] Side-by-side comparison of functionally diverse yet adjoining smooth muscles (phasic vs. tonic), e.g. LES vs. the esophageal body and IAS vs. rectal smooth muscle (RSM) in the basal state, provides an excellent platform for the elucidation of these pathways (particularly RhoA/ROCK), in human health and disease. This may lead to an important target for the rational therapy for the corresponding complex and debilitating conditions, characterized by either hypo- or hypertonic states.[13–17]

Signal Transduction in GI Smooth Muscle Contractility

The mechanism of signal transduction in smooth muscle cells (SMC) involves three types of membrane-bound proteins: a membrane-spanning receptor, a GTP-binding protein that couples to the receptor, and effector enzymes capable of generating intracellular regulatory pathways.[1] Stimulatory ligands for these GI smooth muscle receptors come from several sources. Excitatory motor neurons of the ENS release acetylcholine (ACh) and tachykinins including substance P, neurokinin A, norepinephrine and neuropeptide Y.[18,19] Ligands from SMC include Angiotensin II,[12,20,21] eicosanoids,[22–24] sphingosine-1-phosphate (S1P), lysophosphatidic acid (LPA), and endocannabinoids.[25,26] Receptor ligands may also originate from adventitious cells and via circulation.[18,19] A vast range of receptors reside on the surface of GI SMC, which may activate $G\alpha_{12,13}$-proteins resulting in the activation of RhoA/ROCK (Figure 1).

RhoA Activity

The Rho family comprises twenty members distributed into eight subfamilies: Rho, Rnd, RhoD/F, RhoH, Rac, Cdc42, RhoU/V and RhoBTB.[27] The Rho subfamily includes the RhoA, RhoB, and RhoC isoforms, of which RhoA is the most studied. RhoA/ROCK is of specific interest in the regulation of smooth muscle contractility and relaxation.[1,28] At least 21 effectors of RhoA have been identified including Rho-kinase,[29] phospholipase D1 (PLD1), Rhotekin[30], and Rhophilin.[31]

Activation of RhoA depends upon its binding to the guanosine nucleotides, guanosine triphosphate (GTP) or guanosine diphosphate (GDP). Rho proteins possess a nucleotide binding pocket with an extremely high affinity for both GTP and GDP, and function as a binary signaling switch by cycling between the active GTP-bound form located in the membrane and the inactive GDP-bound cytosolic form. Three general classes of regulators of Rho protein signaling have been identified: guanine nucleotide exchange factor (GEFs), GTPase-activating proteins (GAPs), and guanine nucleotide dissociation inhibitors (GDIs) (Figure 1).[32]

GEFs control the release of GDP from the Rho protein, transiently resulting in nucleotide-free Rho. The relative intracellular abundance of GTP, relative to GDP ensures that it is predominantly GTP that fills the nucleotide binding pocket. Thus GEFs facilitate Rho activation.[33,34] Conversely, GAPs regulate Rho inactivation.[35] The balance between GEF and GAP activity determines the guanine nucleotide status of Rho, thereby regulating its activity. GDI proteins form a large complex with Rho to prevent diffusion within the plasma

membrane and into the cytosol, thus acting as an anchor and allowing for very specific spatial control of Rho activation.[32]

The lipid modification (geranyl-geranylation) of RhoA allows its interaction with the membrane suggesting that such an association is a requirement for Ca^{2+} sensitization of smooth muscle.[36] This is supported by the observation that Ca^{2+} sensitizing agonists and GTPγS cause translocation of RhoA/ROCK to the plasma membrane,[37,38] whereas inactivation of RhoA by the bacterial C3 exoenzyme inhibits this translocation, and Ca^{2+} sensitization.[38]

Rho-Kinase (ROCK)

ROCK (a cytoplasmic serine/threonine-specific kinase) translocates to the plasma membrane where it binds GTP-bound RhoA, leading to autophosphorylation and activation.[29,34,39] ROCK has two isoforms, ROCKI (ROKβ) and ROCKII (ROKα), expressed in smooth muscle.[40] ROCKII is the most common isoform involved in smooth muscle contraction.[2,41] via the prevention of naturally occurring dephosphorylation of phosphorylated-MLC_{20} (p-MLC_{20}).[1,42–44] After interaction with actin and myosin leading to an initial contraction, p-MLC_{20} undergoes rapid dephosphorylation by MLCP, thereby terminating the contraction. For sustained contraction, therefore, it is critical that this dephosphorylation is prevented, which can occur via PKC or RhoA/ROCK. RhoA/ROCK plays a major role in this regard.[45–47] ROCKII prevents dephosphorylation of p-MLC_{20} or increases its phosphorylation through three different manners (Figure 1): inhibition of the MYPT1 subunit of MLCP by its phosphorylation;[28,34,48,49] phosphorylation of CPI-17 at the threonine-38 residue (p^{Thr38}-CPI-17), which inhibits PP1c of MLCP;[28,50,51] and MLCK-like activity.[52,53]

Role of RhoA/ROCK/MYPT1/CPI-17 in Gastrointestinal Smooth Muscle Function

The consensus pathway for the maintenance of tone of GI smooth muscle involves receptor stimulation causing RhoA/ROCK activation to inhibit MLCP, resulting in maintained p-MLC_{20}, and sustained muscle contraction (Figure 1).[3] The IAS and LES are prototypical tonic GI smooth muscles in that they exhibit spontaneous tone in the absence of any stimulus. Higher levels of RhoA/ROCK and the corresponding signal machinery (RhoA/ROCK, p-MYPT1, CPI-17, p-CPI-17 and p-MLC_{20}) have been observed in the IAS compared to predominantly phasic RSM.[15] Also those studies[15] demonstrated the order in reverse for the levels of MYPT1 in the basal state, lower in the IAS. Additionally, the mechanism responsible for smooth muscle tone has been shown to be intrinsic to the characteristically different SMC.[16,54] Consistent with the animal data for the IAS, it has been shown recently that the RhoA/ROCK pathway is critical in the maintenance of spontaneous LES tone in humans.[13,55] Besides its role in the sustained contraction, a number of studies have shown that the active relaxation of the smooth muscle is mediated via inhibition of RhoA/ROCK signal transduction pathways.[1,42,56,57]

RhoA/ROCK Signal Transduction Machinery is Upregulated in the Spontaneously Active Basal Tone

Based on agonist responses, the smooth muscle responses have been classified arbitrarily as either tonic (displaying a protracted contraction) or phasic (contraction lasting less than one minute).[1,3,55,58–61] Sphincters of the GI tract represent truly tonic smooth muscles because they spontaneously develop tone which is largely myogenic.[11–13,15] These tonic tissues have characteristically higher levels of CPI-17 and lower levels of MYPT1. CPI-17 is an endogenous inhibitory protein for MLCP.[62,63] Collectively, the net effect is subdued MLCP leading to higher levels of p-MLC_{20} and maintained tone.[14–16] While upregulation of RhoA/ROCK is critical for the basal IAS tone, we speculate its further upregulation in the pathophysiologically hypertonic state,[17] and (Rattan et al. unpublished data). From basic research studies, we speculate renin-angiotensin system (RAS)[20,64] and cyclooxygenase especially COX-1 products (thromboxanes and prostaglandin $F_{2\alpha}$)[23,65] via the activation of specific membrane receptors activate RhoA/ROCK for a part of the basal tone in the IAS. Conceivably, therefore, RAS and COX-1 via RhoA/ROCK[14,66] may serve as important therapeutic targets for different motility disorders in humans.[17,21,66,67]

RhoA/ROCK as Novel Therapeutic Targets for Gastrointestinal Dysmotility

RhoA/ROCK and Inhibitors

ROCK inhibitors, such as Y-27632 and fasudil (HA-1077) have been evaluated across a number of organ systems,[4–8,68] while other compounds are being actively pursued.[69] Y-27632's mechanism of inhibition of ROCK appears to be by competing with ATP for its binding to the kinase.[70] Additionally, phosphorylation of CPI-17 is diminished by pretreatment with either Y-27632 or GF109203x (PKC inhibitor), suggesting a crosstalk between multiple kinases in different smooth muscle systems.[51,71] Recent studies systematically compared the selectivity vs. potency of available ROCK inhibitors (Y-27632, HA-1077, H-1152, and ROCK inhibitor II), in the IAS vs. RSM.[72] These studies using the same smooth muscle strips, determined the relationship between the basal smooth muscle tone, and the enzymatic activities of ROCK, PKC and MLCK. A comparison of IC_{50} values reveals a general agreement of the data with the existing literature in different systems (Table 2).

Specific Details of RhoA/ROCK in Different Regions of the GI Tract

Esophagus and Lower Esophageal Sphincter (LES)

ROCK inhibitors inhibit both the basal tone in the LES and the increased tone caused by Angiotensin II in humans, cats, opossum and rat.[55,73–75] In human studies, ROCK inhibitors Y-27632 and HA-1077 cause complete relaxation of the LES while the PKC inhibitors calphostin C and chelerythrine have minimal effect.[13] Additionally, ROCK inhibitors decrease nerve-evoked "on" and "off"-esophageal contractions, responsible for the peristalsis. The exact significance of RhoA/ROCK in esophageal peristalsis is not known because studies have been limited to a single esophageal site. While activated ROCK inhibits, inactivated ROCK (by the ROCK inhibitors) may unleash MLCP, thereby

facilitating relaxation. Therefore, premature unleashing of MLCP following ROCK inhibitors may not allow the development of full esophageal contraction. Targeted relaxation of the LES and EB contraction by ROCK inhibitors may provide a rational therapy in achalasia and DES.

Stomach

There is growing evidence that the RhoA/ROCK pathway has strong implications in the basal and agonist-stimulated gastric smooth muscle activity. In reconstituted smooth muscle fibers from the cultured guinea pig gastric SMCs, norepinephrine-induced contraction was found to be completely abolished by an intracellular Ca^{2+} chelator, papaverine, an MLCK inhibitor, and the ROCK inhibitor Y-27632.[76] The role of RhoA/ROCK vs. PKC pathways was examined in rabbit gastric smooth muscle tissue and the SMC. Phenylephrine and LPA-induced contraction may involve both pathways.[54,77,78] However, it was not possible to determine their relative contribution because p^{Thr695}-MYPT1 masked p^{Thr695}-MYPT1, primarily responsible for RhoA/ROCK-mediated SMC contraction. On the contrary, in mice and rats gastric smooth muscle contraction in the basal state or in response to carbachol, KCl, LPA, and field stimulation is predominantly mediated via ROCK activation.[79–81] One novel study[82] showed that under physiological conditions, the ROCK inhibitor Y-27632 causes a precipitous decrease in the basal gastric contractility and motility index in conscious rats.

Gallbladder

Although there are significant data on the role of different regulators in gallbladder motility, the molecular mechanisms that govern gallbladder motility are not known.[83] Extensive studies via molecular biology and pharmacological analyses in human gallbladder reveal that RhoA/ROCK may provide the sole molecular pathway for the spontaneous and stimulated tone.[84] Similar data were obtained in the guinea pig and sheep gallbladder.[85–87]

Small Intestine

Luminal contents of the small intestine move in an aboral direction in a highly regulated process that can result in clinical symptoms when disordered.[88] Only a few medications (but with considerable limitations) are available for small intestinal motility disorders: octreotide, erythromycin, and newer agents with activity targeted to the $5\text{-}HT_3$ and $5\text{-}HT_4$ receptors can result in decreased or increased intestinal motility.[89]

In the guinea-pig, a sustained component of intestinal smooth muscle contraction in response to carbachol, LPA, and ACh (but not K^+ depolarization) has been shown to be ROCK-dependent.[90–93] Otto et al.[93] identified another staurosporine-sensitive kinase (distinct from ROCK and PKC) which induces a large increase in the Ca^{2+} sensitivity via thiophosphorylation of MYPT1. Borman et al. in the rabbit ileum identified this kinase to be zipper-interacting protein kinase (ZIPK).[94] Follow up studies by Ihara et al.[95] in rat ileal longitudinal smooth muscle identified yet another pathway: integrin-linked kinase (ILK) for microcystin (phosphatase inhibitor)-induced contraction of smooth muscle. ILK has been shown previously to be involved in phasic esophageal smooth muscle contraction.[73] Presently, the relative contribution of these kinases in relation to RhoA/ROCK in the

physiology and pathophysiology of the small intestine is not known. Hersch et al.[96] determined the relative contribution of different pathways in SMC from the longitudinal vs. circular muscle layers of rabbit small intestine in response to endothelin. The contractile effect consisted of initial and sustained components. While the initial component was shown to be clearly MLCK-mediated, the latter sustained component was RhoA/ROCK rather than PKC-mediated. Different studies using rat ileal smooth muscle[97,98] reached a similar conclusion.

Colon

In circular SMC from rabbit colon, Patil et al. showed inhibition both of ACh-induced sustained contraction and of p-MLC_{20} by the ROCK and PKC inhibitor Y-27632.[99,100] The authors suggested independent roles of RhoA and PKC pathways, subsequently converging on MLCP inhibition.[100] However, recent observations that PDBu-activated contraction was attenuated by the ROCK inhibitor suggests that PKC lies upstream of ROCK (Rattan et al. unpublished data). Signal transduction for the basal and agonist-induced contraction and relaxation may be region specific. For example, in the longitudinal smooth muscle of rat proximal colon, the RhoA/ROCK pathway alone mediates Ca^{2+}-sensitization coupled to the membrane muscarinic receptors, while in the distal colon, both PKC and RhoA/ROCK mediate similar responses.[101] Later studies by the same group[102], however, showed that the differential role of ROCK vs. PKC in the rat distal colon was applicable in the longitudinal but not in the circular smooth muscle.

Internal Anal Sphincter (IAS) and Rectum

The ROCK inhibitors (Y-27632 and H-1152) were found to be more than 30-fold potent in decreasing the IAS tone than RSM phasic activity.[72] These effects correlated most closely with a decrease in ROCK activity without modifying PKC and MLCK activities. ROCK inhibitor II and HA-1077 were both found to be least potent and least selective. Therefore, selection of the ROCK inhibitor is critical in the specific therapeutic modality. Further in-depth studies in isolated SMC[16] from the IAS showed characteristically upregulated RhoA/ROCK signal transduction machinery as compared with the RSM. Additionally, direct injection of active ROCK caused further shortening of the SMC, which was selectively blocked by Y-27632. Conversely, active PKC-induced shortening of the IAS SMC was blocked not only by the PKC but also by the ROCK inhibitor. Collectively, the data suggest a role of RhoA/ROCK as molecular determinant in the basal tone. On the contrary, studies by Bitar et al. suggest a major contribution of PKC in the basal IAS tone.[103,104] These authors reached a similar conclusion using reconstructs from human IAS SMC.[105] In these studies, the effect of de-differentiation of the tonic SMC during the long-term culture is not known. Interestingly, recent data in tonic smooth muscles suggest that PKC may work partly via ROCK activation.[13,16,77,106]

RhoA/ROCK and Protein Prenyltransferase Inhibitors

RhoA undergoes post-translational prenylation to allow translocation to the membrane and functional activation.[107,108] Prenyltransferase inhibitors have been shown to selectively disrupt downstream signaling pathways of Rho GTPases.[109] Recent studies[110] reported a

decrease in the levels of basal IAS tone and membranous prenylated-RhoA in the IAS before and after the administration of prenylation inhibitor GGTI-297, which was reversible by geranylgeranyl pyrophosphate. These data suggest that RhoA prenylation is critical for RhoA translocation and for the RhoA/ROCK-mediated Ca^{2+} sensitization for smooth muscle tone.[37,59] These observations provide another avenue to explore the role of agents such as GGTI-297 and statins in the specific cases of GI hypermotility.[111,112]

Pathophysiological and Therapeutic Implications of RhoA/ROCK in the Gastrointestinal Disease

Up- or downregulation of RhoA/ROCK may contribute to different gastrointestinal motility disorders, which may, in turn, potentially be treated by ROCK inhibitors or activators, respectively. There are possible avenues for the topical application of ROCK inhibitors to minimize systemic effects,[17,113] which may have utility in relieving the IAS spasm associated with RAF to allow subsequent healing. Critical clinical data are needed to determine the exact role of RhoA/ROCK in the pathophysiology and therapeutic management of certain debilitating GI motility dysfunctions characterized by smooth muscle hypersensitivity: achalasia, DES, abnormal gastric motility, pylorospasm, irritable bowel syndrome, biliary dyskinesia, Hirschsprung's disease, RAF, hemorrhoids, megacolon, and certain forms of severe constipation; or smooth muscle hyposensitivity: GERD, gastroparesis, adynamic ileus, colonic inertia, and certain forms of rectoanal incontinence characterized by hypotensive IAS.

One of the most popular therapeutic approaches in treating GI smooth muscle hypermotility is botulinum toxin A (BTX-A).[17,114,115] This works primarily via the inhibition of release of ACh either at the end plate of the skeletal muscle or in cholinergic parasympathetic or sympathetic neurons of the smooth muscle.[116,117] A derivative of BTX, C3 exoenzyme is known to inhibit RhoA via ADP ribosylation.[118–121] Therefore, BTX may not only decrease the hyperactivity of skeletal muscle but also of GI smooth muscle.[16,34,101] In order to avoid the nonselective side effects following RhoA/ROCK inhibition, there is a need for region-specific and topically active inhibitors. Alternatively, tissue-specific silencing of the RhoA/ROCK gene using small interfering RNA is another potential approach.[41] Hypomotility disorders may be difficult as there are no specific activators of RhoA/ROCK. Therefore, one possible consideration is the molecular introduction of constitutively active RhoA/ROCK.

Acknowledgments

Grant Support and other Acknowledgements: The work was supported by National Institutes of Diabetes and Digestive and Kidney Diseases Grant DK-35385 and an institutional grant from Thomas Jefferson University. The authors thank Dr. Jagmohan Singh for his valuable comments and suggestions. We regret that space limitations did not permit us to include a number of valuable references.

Abbreviations used in this Paper

DES	diffuse esophageal spasm
IC_{50}	the concentration of the inhibitor that cause 50% decrease in ROCK activity

IAS	internal anal sphincter
LPA	lysophosphatidic acid
MLC	myosin light chain
MLCP	MLC-phosphatase
MYPT1	myosin binding (regulatory or inhibitory) subunit of MLCP
PPIc	catalytic subunit of MLCP
PKC	protein kinase C
CPI-17	protein kinase C potentiated inhibitory
RAF	recurrent anal fissures
ROCK	Rho kinase
ROCKII or ROCKα	(primary isoform of Rho kinase involved in the smooth muscle contraction)
RSM	rectal smooth muscle
SMC	smooth muscle cells
Y-27632	(R)-(+)-*trans*-N-(4-pyridyl)-4-(1-aminoethyl)-cyclohexanecarboxamide, (Rho kinase inhibitor)
Rho.GEF	GDT-GTP exchange factor
GAPs	GTPase-activating proteins
GDIs	guanine nucleotide dissociation inhibitors

References

Note: The first 5 references associated with this article are available below in print. The remaining references accompanying this article are available online only with the electronic version of the article. Visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi:[insert DOI].

1. Murthy KS. Signaling for contraction and relaxation in smooth muscle of the gut. Annu Rev Physiol. 2006; 68:345–374. [PubMed: 16460276]
2. Somlyo AP, Somlyo AV. Signal transduction by G-proteins, Rho-kinase and protein phosphatase to smooth muscle and non-muscle myosin II. J Physiol (Lond). 2000; 522:177–185. [PubMed: 10639096]
3. Gerthoffer WT. Signal-transduction pathways that regulate visceral smooth muscle function III. Coupling of muscarinic receptors to singaling kinases and effector proteins in gastrointestinal smooth muscles. Am J Physiol Gastrointest Liver Physiol. 2005; 288:G849–G853. [PubMed: 15826932]
4. Shimokawa H, Rashid M. Development of Rho-kinase inhibitors for cardiovascular medicine. Trends Pharmacol Sci. 2007; 28:296–302. [PubMed: 17482681]
5. Jin L, Burnett AL. RhoA/Rho-kinase in erectile tissue: mechanisms of disease and therapeutic insights. Clin Sci. 2006; 110:153–165. [PubMed: 16411892]

6. Oka M, Fagan KA, McMurtry IF. Therapeutic potential of RhoA/Rho-kinase inhibitors in pulmonary hypertension. Br J Pharmacol. 2009; 155:444–454.
7. Canda AE, Cinar MG, Turna B, Sahin MO. Pharmacologic targets on the female urethra. Urol Int. 2008; 80:341–354. [PubMed: 18587243]
8. Christ GJ, Andersson KE. Rho-kinase and effects of Rho-kinase inhibition on the lower urinary tract. Neurourol Urodyn. 2007; 26:948–954. [PubMed: 17696159]
9. Cook, IJ., Brookes, SJ. Colonic motor and sensory function and dysfunction. In: Feldman, M.Friedman, LS., Brandt, LJ., editors. Sleisenger and Fordtran's Gastrointestinal and Liver Disease: Pathophysiology/Diagnosis/Management. 8. Philadelphia: Saunders/Elsevier; 2006. p. 2111-2125.
10. Clouse, RE., Diamant, NE. Esophageal motor and sensory function and motor disorders of the esophagus. In: Feldman, M.Scharschmidt, BF., Sleisenger, MH., editors. Sleisenger & Fordtan's Gastrointestinal and Liver Disease: Pathophysiology/Diagnosis/Management. 8. Philadephia: W.B. Saunders Company; 2006. p. 855-904.
11. Farre R, Sifrim D. Regulation of basal tone, relaxation, and contraction of the lower esophageal sphincter. Relevance to drug discovery for esophageal disorders. Br J Pharmacol. 2008; 153:858–869. [PubMed: 17994108]
12. Rattan S. The internal anal sphincter: regulation of smooth muscle tone and relaxation. Neurogastroenterol Motil. 2005; 17:50–59. [PubMed: 15836455]
13. Sims SM, Chrones T, Preiksaitis HG. Calcium sensitization in human esophageal muscle: role for RhoA kinase in maintenance of lower esophageal sphincter tone. J Pharmacol Exp Ther. 2008; 327:178–186. [PubMed: 18628484]
14. Rattan S, De Godoy MAF, Patel CA. Rho kinase as a novel molecular therapeutic target for hypertensive internal anal sphincter. Gastroenterology. 2006; 131:108–116. [PubMed: 16831595]
15. Patel CA, Rattan S. Spontaneously tonic smooth muscle has characteristically higher levels of RhoA/ROK compared with the phasic smooth muscle. Am J Physiol Gastrointest Liver Physiol. 2006; 291:G830–G837. [PubMed: 16763289]
16. Patel CA, Rattan S. Cellular regulation of basal tone in internal anal sphincter smooth muscle by RhoA/ROCK. Am J Physiol Gastrointest Liver Physiol. 2007; 292:G1747–G1756. [PubMed: 17379756]
17. Madalinski M, Kalinowski L. Novel options for the pharmacological treatment of chronic anal fissure-Role of botulinum toxin. Curr Clin Pharmacol. 2009; 4:47–52. [PubMed: 19149501]
18. Furness JB, Young HM, Pompolo S, Bornstein JC, Kunze WAA, McConalogue K. Plurichemical transmission and chemical coding of neurons in the digestive tract. Gastroenterology. 1995; 108:554–563. [PubMed: 7835599]
19. Bornstein JC, Costa M, Grider JR. Enteric motor and interneuronal circuits controlling motility. Neurogastroenterol Motil. 2004; 16:34–38. [PubMed: 15066002]
20. De Godoy MAF, Rattan S. Autocrine regulation of internal anal sphincter tone by renin-angiotensin system: comparison with phasic smooth muscle. Am J Physiol Gastrointest Liver Physiol. 2005; 289:G1164–G1175. [PubMed: 16020656]
21. Casselbrant C, Edebo A, Wennerblom J, Lönroth H, Helander HF, Veith M, Lundell L, Fändriks L. Actions by angiotensin II on esophageal contractility in humans. Gastroenterology. 2007; 132:249–260. [PubMed: 17241875]
22. Cong P, Xiao ZL, Biancani P, Behar J. Prostaglandins mediate tonic contraction of the guinea pig and human gallbladder. Am J Physiol Gastrointest Liver Physiol. 2008; 292:G409–G418.
23. De Godoy MAF, Rattan N, Rattan S. COX-1 vs. COX-2 as a determinant of the basal tone in the internal anal sphincter. Am J Physiol Gastrointest Liver Physiol. 2009; 296:G219–G225. [PubMed: 19056763]
24. Cao W, Harnett KM, Behar J, Biancani P. $PGF_{2\alpha}$-induced contraction of cat esophageal and lower esophageal sphincter circular smooth muscle. Am J Physiol Gastrointest Liver Physiol. 2002; 283:G282–G291. [PubMed: 12121874]
25. Izzo AA, Mascolo N, Capasso F. The gastrointestinal pharmacology of cannabinoids. Curr Opin Pharmacol. 2001; 1:597–603. [PubMed: 11757815]

26. Spiegel S, Milstien S. Exogenous and intracellularly generated sphingosine 1-phosphate can regulate cellular processes by divergent pathways. Biochem Soc Trans. 2003; 31:1216–1219. [PubMed: 14641029]
27. Boureux A, Vignal E, Faure S, Fort P. Evolution of the Rho Family of Ras-like GTPases in eukaryotes. Mol Biol Evol. 2007; 24:203–216. [PubMed: 17035353]
28. Sanders KM. Regulation of smooth muscle excitation and contraction. Neurogastroenterol Motil. 2008; 20(Suppl 1):39–53. [PubMed: 18402641]
29. Leung T, Chen XQ, Manser E, Lim L. The p160 RhoA-binding kinase ROK alpha is a member of a kinase family and is involved in the reorganization of the cytoskeleton. Mol Cell Biol. 2007; 16:5313–5327.
30. Reid T, Furuyashiki T, Ishizaki T, Watanabe G, Watanabe N, Fujisawa K, Morii N, Madaule P, Narumiya S. Rhotekin, a new putative target for Rho bearing homology to a serine/threonine kinase, PKN, and rhophilin in the Rho-binding domain. J Biol Chem. 1996; 271:13556–13560. [PubMed: 8662891]
31. Watanabe G, Saito Y, Madaule P, Ishizaki T, Fujisawa K, Morii N, Mukai H, Ono Y, Kakizuka A, Narumiya S. Protein kinase N (PKN) and PKN-related protein rhophilin as targets of small GTPase Rho. Science. 1996; 271:645–648. [PubMed: 8571126]
32. Ellenbrock SIJ, Collard JG. Rho GTPases: functions and association with cancer. Clin Exp Metastasis. 2007; 24:657–672. [PubMed: 18000759]
33. Hart MJ, Jiang X, Kozasa T, Roscoe W, Singer WD, Gilman AG, Sternweis PC, Bollag G. Direct stimulation of the guanine nucleotide exchange activity of p^{115} RhoGEF by $G\alpha_{13}$. Science. 1998; 280:2112–2114. [PubMed: 9641916]
34. Murthy KS, Zhou H, Grider JR, Brautigan DL, Eto M, Makhlouf GM. Differential signalling by muscarinic receptors in smooth muscle: m2-mediated inactivation of myosin light chain kinase via G_{i3}, Cdc42/Rac1 and p21-activated kinase 1 pathway, and m3-mediated MLC_{20} (20 kDa regulatory light chain myosin II) phosphorylation via Rho-associated kinase/myosin phosphatase targeting subunit and protein kinase C/CPI-17 pathway. Biochem J. 2003; 374:145–155. [PubMed: 12733988]
35. Vetter IR, Wittinghofer A. The guanine nucleotide-binding switch in three dimensions. Science. 2001; 294:1299–1304. [PubMed: 11701921]
36. Gong MC, Iizuka K, Nixon G, Browne JP, Hall A, Eccleston JF, Sugai M, Kobayashi S, Somlyo AV, Somlyo AP. Role of guanine nucleotide-binding proteins ras-family or trimeric proteins or both in Ca^{2+} sensitization of smooth muscle. Proc Natl Acad Sci USA. 1996; 93:1340–1345. [PubMed: 8577766]
37. Gong MC, Fujihara H, Somlyo AV, Somlyo AP. Translocation of *rhoA* associated with Ca^{2+} sensitization of rabbit smooth muscle. J Biol Chem. 1997; 272:10704–10709. [PubMed: 9099720]
38. Fujihara H, Walker LA, Gong MC, Lemichez E, Bouquet P, Somlyo AV, Somlyo AP. Inhibition of RhoA translocation and calcium sensitization by in vivo ADP-ribosylation with the chimeric toxin DC3B. Mol Biol Cell. 1997; 8:2437–2447. [PubMed: 9398666]
39. Chen XQ, Tan I, Ng CH, Hall C, Lim L, Leung T. Characterization of RhoA-binding kinase ROKα implication of the plecktrin homology domain in ROK_{α} function using region-specific antibodies. J Biol Chem. 2002; 277:12680–12688. [PubMed: 11815607]
40. Yoshii A, Iizuka K, Dobashi K, Horie T, Harada T, Nakazawa T, Mori M. Relaxation of contracted rabbit tracheal and human bronchial smooth muscle by Y-27632 through inhibition of Ca^{2+} sensitization. Am J Resp Cell Mol Biol. 1999; 20:1190–1200.
41. Wang Y, Zheng XR, Riddick N, Bryden M, Bauer W, Zhang X, Surks HK. ROCK isoform regulation of myosin phosphatase and contractility in vascular smooth muscle cells. Circ Res. 2009; 104:531–540. [PubMed: 19131646]
42. Riddick N, Ohtani K, Surks HK. Targeting by myosin phosphatase-RhoA interacting protein mediates RhoA/ROCK regulation of myosin phosphatase. J Cell Biochem. 2008; 103:1158–1170. [PubMed: 17661354]
43. Mizuno Y, Isotani E, Huang J, Ding H, Stull JT, Kamm KE. Myosin light chain kinase activation and calcium sensitization in smooth muscle in vivo. Am J Physiol Cell Physiol. 2008; 295:C358–C364. [PubMed: 18524939]

44. Berridge MJ. Smooth muscle cell calcium activation mechanisms. J Physiol. 2008; 586:5047–5061. [PubMed: 18787034]
45. Hartshorne DJ, Ito M, Erdodi F. Role of protein phosphatase type 1 in contractile functions: myosin phosphatase. J Biol Chem. 2004; 279:37211–37214. [PubMed: 15136561]
46. Kim HR, Appel S, Vetterkind S, Gangopadhyay SS, Morgan KG. Smooth muscle signalling pathways in health and disease. J Cell Mol Med. 2008; 12:2165–2180. [PubMed: 19120701]
47. Matsumura F, Hartshorne DJ. Myosin phosphatase target subunit: many roles in cell function. Biochem Biophys Res Commun. 2008; 369:149–156. [PubMed: 18155661]
48. Feng J, Ito M, Kureishi Y, Ichikawa K, Amano M, Isaka N, Okawa K, Iwamatsu A, Kaibuchi K, Hartshorne DJ. Rho-associated kinase of chicken gizzard smooth muscle. J Biol Chem. 1999; 274:3744–3752. [PubMed: 9920927]
49. Hartshorne DJ, Ito M, Erdodi F. Myosin light chain phosphatase: subunit composition, interactions and regulation. J Muscle Res Cell Motil. 1998; 19:325–341. [PubMed: 9635276]
50. Koyama M, Ito M, Feng J, Seko T, Shiraki K, Takase K, Hartshorne DJ, Nakano T. Phosphorylation of CPI-17, and inhibitory phosphoprotein of smooth muscle myosin phosphatase, by Rho-kinase. FEBS Lett. 2000; 475:197–200. [PubMed: 10869555]
51. Kitazawa T, Eto M, Woodsome TP, Brautigan DL. Agonists trigger G protein-mediated activation of the CPI-17 inhibitor phosphoprotein of myosin light chain phosphatase to enhance vascular smooth muscle contractility. J Biol Chem. 2000; 275:9897–9900. [PubMed: 10744661]
52. Amano M, Ito M, Kimura K, Fukata Y, Chihara K, Nakano T, Matsuura Y, Kaibuchi K. Phosphorylation and activation of myosin by Rho-associated kinase (Rho-kinase). J Biol Chem. 1996; 271:20246–20249. [PubMed: 8702756]
53. Kureishi Y, Kobayashi S, Amano M, Kimura K, Kanaide H, Nakano T, Kaibuchi K, Ito M. Rho-associated kinase directly induces smooth muscle contraction through myosin light chain phosphorylation. J Biol Chem. 1997; 272:12257–12260. [PubMed: 9139666]
54. Huang J, Zhou H, Mahavadi S, Sriwai W, Lyall V, Murthy KS. Signaling pathways mediating gastrointestinal smooth muscle contraction and MLC_{20} phosphorylation by motilin receptors. Am J Physiol Gastrointest Liver Physiol. 2005; 288:G23–G31. [PubMed: 15591586]
55. Harnett KM, Cao W, Biancani P. Signal-transduction pathways that regulate smooth muscle function I. Signal transduction in phasic (esophageal) and tonic (gastroesophageal sphincter) smooth muscles. Am J Physiol Gastrointest Liver Physiol. 2005; 288:G407–G416. [PubMed: 15701619]
56. Chitaley K, Webb RC. Nitric oxide induces dilation of rat aorta via inhibition of rho-kinase signaling. Hypertension. 2002; 39:438–442. [PubMed: 11882586]
57. Bolz SS, Vogel L, Sollinger D, Derwand R, de Wit C, Loirand G, Pohl U. Nitric oxide-induced decrease in calcium sensitivity of resistance arteries is attributable to activation of the myosin light chain phosphatase and antagonized by the RhoA/Rho kinase pathway. Circulation. 2003; 107:3081–3087. [PubMed: 12796138]
58. Golenhofen K, Mandrek K. Phasic and tonic contraction processes in the gastrointestinal tract. Dig Dis Sci. 1991; 9:341–346.
59. Somlyo AP, Somlyo AV. Ca^{2+} sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphatase. Physiol Rev. 2003; 83:1325–1358. [PubMed: 14506307]
60. Bitar KN, Ibitayo A, Patil SB. HSP27 modulates agonist-induced association of translocated RhoA and PKC-alpha in muscle cells of the colon. J Appl Physiol. 2002; 92:41–49. [PubMed: 11744641]
61. Bitar KN. Function of gastrointestinal smooth muscle: from signaling to contractile proteins. Am J Med. 2003; 115:15S–23S. [PubMed: 12928070]
62. Eto M, Wong L, Yazawa M, Brautigan DL. Inhibition of myosin/moesin phosphatase by expression of the phosphoinhibitor protein CPI-17 alters microfilament organization and retards cell spreading. Cell Motil Cytoskeleton. 2000; 46:222–234. [PubMed: 10913968]
63. Woodsome TP, Eto M, Everett A, Brautigan DL, Kitazawa T. Expression of CPI-17 and myosin phosphatase correlates with Ca^{2+} sensitivity of protein kinase C-induced contraction of rabbit smooth muscle. J Physiol (Lond). 2001; 535:553–564. [PubMed: 11533144]

64. De Godoy MAF, Dunn SR, Rattan S. Evidence for the role of angiotensin II biosynthesis in the rat internal anal sphincter tone. Gastroenterology. 2004; 127:127–138. [PubMed: 15236179]

65. De Godoy MAF, Rattan N, Rattan S. Arachidonic acid metabolites follow the preferential course of cyclooxygenase pathway for the basal tone in the internal anal sphincter. Am J Physiol Gastrointest Liver Physiol. 2009; 296:G727–G734. [PubMed: 19221012]

66. De Godoy MAF, Rattan S. Angiotensin-converting enzyme and angiotensin II receptor subtype 1 inhibitors restitute hypertensive internal anal sphincter in the spontaneously hypertensive rats. J Pharmacol Exp Ther. 2006; 318:725–734. [PubMed: 16648368]

67. Khaikin M, Yebara S, Bashankaev B. Topical captopril cream: A new treatment for anal fissure? The first human study. Dis Colon Rectum. 2007; 50:747.

68. Olson MF. Applications for ROCK kinase inhibition. Curr Opin Cell Biol. 2008; 20:242–248. [PubMed: 18282695]

69. Feng Y, Yin Y, Weiser A, Griffin E, Cameron MD, Lin L, Ruiz C, Schürer SC, Inoue T, Rao PV, Schröter T, LoGrasso P. Discovery of subsitiuted 4-(pyrazol-4-yl)-phenylbenzodioxane-2-carboxamides as potent and highly selective Rho kinase (ROCK-II) inhibitors. J Med Chem. 2008; 51:6642–6645. [PubMed: 18834107]

70. Ishizaki T, Uehata M, Tamechika I, Keel J, Nonomura K, Maekawa M. Pharmacological properties of Y-27632, a specific inhibitor of rho-associated kinases. Mol Pharmacol. 2000; 57:976–983. [PubMed: 10779382]

71. Eto M, Kitazawa T, Yazawa A, Mukai H, Ono Y, Brautigan DL. Histamine-induced vasoconstriction involves phosphorylation of a specific inhibitor protein for myosin phosphatase by protein kinase C alpha and delta isoforms. J Biol Chem. 2001; 276:29072–29078. [PubMed: 11397799]

72. Rattan S, Patel CA. Selectivity of Rho Kinase inhibitors in the spontaneously tonic smooth muscle. Am J Physiol Gastrointest Liver Physiol. 2008; 294:G687–G693. [PubMed: 18187514]

73. Kim N, Cao W, Sang IS, Kim CY, Harnett KM, Cheng L, Walsh MP, Biancani P. Distinct kinases are involved in contraction of cat esophageal and lower esophageal sphincter smooth muscles. Am J Physiol Cell Physiol. 2004; 287:C384–C394. [PubMed: 15128504]

74. Park SY, Song HJ, Sohn UD. Paticipation of Rho-associated kinase in electrical stimulated and acetylcholine-induced contraction of feline esophageal smooth muscle. Eur J Pharmacol. 2009; 607:220–225. [PubMed: 19239907]

75. Rattan S, Fan Y-P, Puri RN. Comparison of angiotensin II (Ang II) effects in the internal anal sphincter (IAS) and lower esophageal sphincter smooth muscles. Life Sci. 2002; 70:2147–2164. [PubMed: 12002807]

76. Oishi K, Itoh Y, Isshiki Y, Kai C, Takeda Y, Yamaura K, Takano-Ohmuro H, Uchida MK. Agonsit-induced isometric contraction of smooth muscle cell-populated collagen gel fiber. Am J Physiol Cell Physiol. 2000; 279:C1432–C1442. [PubMed: 11029291]

77. Ratz PH, Meehl JT, Eddinger TJ. RhoA kinase and protein kinase C participate in regulation of rabbit stomach fundus smooth muscle contraction. Br J Pharmacol. 2002; 137:983–992. [PubMed: 12429570]

78. Sriwai W, Zhou H, Murthy KS. G_q-dependent signalling by the lysophosphatidic acid receptor LPA_3 in gastric smooth muscle: reciprocal regulation of MYPT1 phosphorylation by Rho kinase and cAMP-independent PKA. Biochem J. 2008; 411:543–551. [PubMed: 18237278]

79. Büyükafsar K, Levent A. Involvement of Rho/Rho-kinase signalling in the contractile activity and acetylcholine release in the mouse gastric fundus. Biochem Biophys Res Commun. 2003; 303:777–781. [PubMed: 12670478]

80. Dhaese I, Lefebvre RA. Myosin light chain phosphatase activation is involved in the hydrogen sulfide-induced relaxation in mouse gastric fundus. Eur J Pharmacol. 2009; 606:180–186. [PubMed: 19374871]

81. Mustafa S, Oriowo MA. Cooling-induced contraction of the rat gastric fundus: mediation via transient receptor potential (TRP) cation channel TRPM8 receptor and rho-kinase activation. Clin Exp Pharmacol Physiol. 2005; 32:832–838. [PubMed: 16173944]

82. Tomomassa T, Takahashi A, Kaneko H, Watanabe T, Masahiko T, Masahiko K, Morikawa A. Y-27632 inhibits gastric motility in conscious rats. Life Sci. 2000; 66:29–34.

83. Behar J, Corazziari E, Guelrud M, Hogan W, Sherman S, Toouli J. Functional gallbladder and sphincter of Oddi disorders. Gastroenterology. 2006; 130:1498–1509. [PubMed: 16678563]
84. Buyukafsar K, Akca T, Tiftik RN, Sahan-Firat S, Aydin S. Contribution of Rho-kinase in human gallbladder contractions. Eur J Pharmacol. 2006; 540:162–167. [PubMed: 16730697]
85. Quinn T, Feighery R, Baird AW. Role of Rho-kinase in guinea-pig gallbladder smooth muscle contraction. Eur J Pharmacol. 2006; 534:210–217. [PubMed: 16499905]
86. Camello-Almaraz C, Maclas B, Gomez-Pinilla PJ, Alcon S, Martin-Cano FE, Baba A, Matsuda T, Camello PJ, Pozo MJ. Developmental changes in Ca^{2+} homeostasis and contractility in gallbladder smooth muscle. Am J Physiol Cell Physiol. 2009; 296:C783–C791. [PubMed: 19211915]
87. Sahan-Firat S, Tiftik RN, Nacak M, Buyukafsar K. Rho kinase expression and its central role in ovine gallbladder contractions elicited by variety of excitatory stimuli. Eur J Pharmacol. 2005; 528:169–175. [PubMed: 16324691]
88. Jones MP, Bratten JR. Small intestinal motility. Curr Opin Gastroenterol. 2008; 24:164–172. [PubMed: 18301266]
89. Kuemmerle JF. Motility disorders of the small intestine: new insights into old problems. J Clin Gastroenterol. 2000; 31:276–281. [PubMed: 11129267]
90. Sward K, Dreja K, Susnjar M, Hellstrand P, Hartshorne DJ, Walsh MP. Inhibition of Rho-associated kinase blocks agonist-induced Ca^{2+} sensitization of myosin phosphorylation and force in guinea-pig ileum. J Physiol (Lond). 2000; 522:33–49. [PubMed: 10618150]
91. Mori M, Tsushima H. Vanadate activates Rho A translocation in association with contracting effects in ileal longitudinal smooth muscle of guinea pig. J Pharmacol Sci. 2004; 95:443–451. [PubMed: 15286430]
92. Mori M, Tsushima H. Activation of Rho signaling contributes to lysophosphatidic acid-induced contraction of intact ileal smooth muscle of guinea-pig. Can J Physiol Pharmacol. 2000; 78:729–736. [PubMed: 11007536]
93. Otto B, Steusloff A, Just I, Aktories K, Pfitzer G. Role of rho proteins in carbachol-induced contractions in intact and permeabilized guinea-pig intestinal smooth muscle. J Physiol (Lond). 1996; 496:317–329. [PubMed: 8910218]
94. Borman MA, MacDonald JA, Haystead TA. Staurosporine inhibition of zipper-interacting protein kinase contractile effects in gastrointestinal smooth muscle. Biochem Cell Biol. 2007; 85:111–120. [PubMed: 17464351]
95. Ihara E, Moffat L, Ostrander J, Walsh MP, MacDonald JA. Characterization of protein kinase pathways responsible for Ca^{2+} sensitization in rat ileal longitudinal smooth muscle 4489. American Journal of Physiology - Gastrointestinal & Liver Physiology. 2007; 293:G699–G710. [PubMed: 17656444]
96. Hersch E, Huang J, Grider JR, Murthy KS. G_q/G_{13} signaling by ET-1 in smooth muscle: MYPT1 phosphorylation via ET_A and CPI-17 dephosphorylation via ET_B. Am J Physiol Cell Physiol. 2004; 287:C1209–C1218. [PubMed: 15475516]
97. Loirand G, Cario-Taumaniantz C, Chardin P, Pacaud P. The rho-related protein Rnd1 inhiibits Ca^{2+} sensitization of rat smooth muscle. J Physiol. 1999; 516:825–834. [PubMed: 10200428]
98. González-Montelongo MC. Androgen differentially potentiate mouse intestinal smooth muscle by nongenomic activation of polyamine synthesis and rho kinase activation. Endocrinology. 2006; 147:5715–5729. [PubMed: 16946014]
99. Patil SB, Tsunoda Y, Pawar MD, Bitar KN. Translocation and association of ROCK-II with RhoA and HSP27 during contraction of rabbit colon smooth muscle cells. Biochem Biophys Res Commun. 2004; 319:95–102. [PubMed: 15158447]
100. Patil SB, Bitar KN. RhoA- and PKC-α-mediated phosphorylation of MYPT and its association with HSP27 in colonic smooth muscle cells. Am J Physiol Gastrointest Liver Physiol. 2005; 290:G83–G95. [PubMed: 16179599]
101. Takeuchi T, Kushida M, Hirayama N, Kitayama M, Fujita A, Hata F. Mechanisms involved in carbachol-induced Ca2+ sensitization of contractile elements in rat proximal and distal colon. Br J Pharmacol. 2004; 142:657–666. [PubMed: 15159278]

102. Takeuchi T, Nakajima H, Hata F, Azuma Y-T. A minor role for Ca^{2+} sensitization in sustained contraction through activation of muscarinic receptor in circular muscle of rat colon. Pflugers Arch. 2007; 454:565–574. [PubMed: 17318645]
103. Bitar KN, Hillemeier C, Biancani P, Balazovich KJ. Regulation of smooth muscle contraction in rabbit internal anal sphincter by protein kinase C and Ins(1,4,5)P_3. Am J Physiol Gastrointest Liver Physiol. 1991; 260:G537–G542.
104. Bitar KN, Hillemeier C, Biancani P. Differential regulation of smooth muscle contraction in rabbit internal anal sphincter by substance P and bombesin. Life Sci. 1990; 47:2429–2434. [PubMed: 1702175]
105. Somara S, Gilmont RR, Dennis RG, Bitar KN. Bioengineerd internal anal sphincter derived from isolated human internal anal sphincter smooth muscle cells. Gastroenterology. 2009; 137:53–61. [PubMed: 19328796]
106. Okano M, Uchikawa Y, Tanaka N, Mutoh J, Onkura M, Hisa H, Yamamoto R. Rho-kinase, but not protein kinase C, is involved in generation of the spontaneous tone in the resting phase of the isolated pig iris sphincter muscle. Curr Eye Res. 2009; 34:177–183. [PubMed: 19274524]
107. Walters CE, Pryce G, Hankey DJR, Sebti SM, Hamilton AD, Baker D, Greenwood J, Adamson P. Inhibition of Rho GTPases with protein prenyltransferase inhibitors prevents leukocyte recruitment to the central nervous system and attenuates clinical signs of disease in an anuimal model of multiple sclerosis. J Immunol. 2002; 168:4087–4094. [PubMed: 11937568]
108. Katayama M, Kawata M, Yoshida Y, Horiuchi H, Yamamoto T, Matsuura Y, Takai Y. The posttranslationally modified C-terminal structure of bovine aortic smooth muscle *rho*A p21. J Biol Chem. 1991; 266:12639–12645. [PubMed: 1905729]
109. Berzat AC, Brady DC, Fiordalisi JJ, Cox AD. Using inhibitors of prenylation to block localization and transforming activity. Methods Enzymol. 2006; 407:575–597. [PubMed: 16757354]
110. Patel CA, Rattan S. RhoA prenylation inhibitor produces relaxation of tonic smooth muscle of internal anal sphincter. J Pharmacol Exp Ther. 2007; 321:501–508. [PubMed: 17322025]
111. Li M, Li Z, Sun X. Statins suppress MMP2 secretion via inactivation of RhoA/ROCK pathway in pulmonary vascular smooth muscles cells. Eur J Pharmacol. 2008; 591:219–223. [PubMed: 18619957]
112. Chiba Y, Arima J, Sakai H, Misawa M. Lovastatin inhibits bronchial hyperresponsiveness by reducing RhoA signaling in rat allergic asthma. Am J Physiol Lung Cell Mol Physiol. 2009; 294:L705–L713.
113. Larson SD, Jackson LN, Chen LA, Rychahou PG, Evers BM. Effectiveness of siRNA uptake in target tissues by various delivery methods. Surgery. 2007; 142:262–269. [PubMed: 17689694]
114. Pasricha, PJ. Treatment of disorders of bowel motility and water flux; antiemetics; agents used in biliary and pancreatic disease. In: Brunton, LL.Lazo, JS., Parker, KL., editors. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 11. New York: McGraw-Hill; 2006. p. 983-1008.
115. Dressler D, Saberi AF. Botulinum toxin: mechanisms of action. Eur Neurol. 2005; 53:3–9.
116. Singh, BR. Molecular basis of the unique endopeptidase activity of botulinum neurotoxin. In: Brin, MF.Hallet, M., Jankovic, J., editors. Scientific and therapeutic aspects of botulinum toxin. 1. Philadelphia: Lippincott Williams & Wilkins; 2002. p. 75-88.
117. Munchau A, Bhalla KP. Uses of botulinum toxin injection in medicine today. Br Med J. 2000; 320:161–165. [PubMed: 10634738]
118. Aktories K, Just I. *In vitro* ADP-ribosylation of Rho by bacterial ADP-ribosyltransferases. Methods Enzymol. 1995; 256:184–195. [PubMed: 7476432]
119. Aktories K. Bacterial toxins that target Rho proteins. J Clin Invest. 1997; 99:827–829. [PubMed: 9062338]
120. Sekine A, Fujiwara M, Narumiya S. Asparagine residue in the *rho* gene product is the modification site for botulinum ADP-ribosyltransferase. J Biol Chem. 1989; 264:8602–8605. [PubMed: 2498316]
121. Ishida H, Zhang X, Erickson K, Ray P. Botulinum toxin type A targets RhoB to inhibit lysophosphatidic acid-stimulated actin reorganization and acetylcholine release in nerve growth factor-treated PC12 cells. J Pharmacol Exp Ther. 2004; 310:881–889. [PubMed: 15140914]

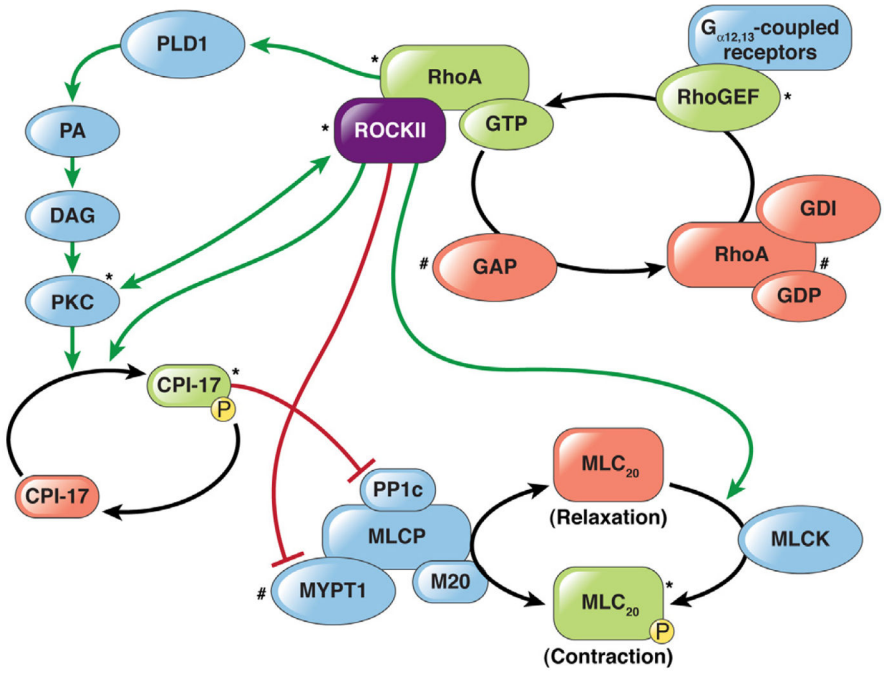

Figure 1.

Schematic representation of the major pathways involved in excitation-contraction, and relaxation coupling in GI smooth muscle. RhoA/ROCK may be activated either by G-protein coupled receptor activation or independent of it. The complex of RhoA.GDI.GDP usually reflects inactive state of RhoA. It transforms via RhoGEFs into an active RhoA.GDI.GTP complex formation. GEFs catalyze the exchange of GDP for GTP on RhoA. RhoGAP drives the reaction in the reverse direction. Activated RhoA/ROCK leads to the phosphorylation of MLC_{20}, and subsequent contraction via these different pathways. First, there is inhibition of MLCP through its regulatory subunit MYPT1. Second, there is phosphorylation of CPI-17 and subsequent inhibition of MLCP via its catalytic subunit PP1c. Finally there is MLCK-like activity. Shown on the left side, RhoA/ROCK may either activate PKC directly or via PLD1/PA/DAG. There is growing evidence in different smooth muscle systems that PKC may lie upstream of RhoA/ROCK pathway (denoted by bidirectional arrow). In contrast with the multiple pathways for the phosphorylation of MLC_{20} as in the case of RhoA/ROCK, the major target for the PKC is CPI-17. Interestingly, phosphorylation of CPI-17 is not limited to RhoA/ROCK and PKC, but other kinases, such as ZIP kinase, and ILK can mediate this event.

Green arrows and substrates indicate stimulation, red lines and substrates indicate inhibition. * = hypothetical sites of upregulation in the case to spontaneously tonic smooth muscle (e.g. sphincteric) or during the pathologically hypercontractility state of different GI smooth muscles. Conversely, these sites may be inherently downregulated in the case of phasic smooth muscle in the basal state or during the pathologically hypocontractility state; #, hypothetical sites of inherent downregulation of the tonic smooth muscle and upregulation of the phasic smooth muscle in the basal state.

Abbreviations: ROCK, Rho kinase; Rho.GDI, guanine exchange dissociation inhibitor; Rho.GEF, GDT-GTP exchange factor; GAP, GTPase-activating protein; ZIPK, zipper-interacting kinase; ILK, integrin-linked kinase

Table 1

Implications of the Role of RhoA/ROCK in the Pathophysiology and Therapeutic Target in Different Organs of the Gastrointestinal Tract

Organ	Disorders
Esophageal Body	• Diffuse esophageal spasm
Lower Esophageal Sphincter (LES)	• Achalasia • Gastroesophageal reflux disease
Stomach	• Gastroparesis • Pylorospasm
Gallbladder	• Biliary dyskinesia
Small Intestine	• Adynamic ileus
Colon	• Colonic inertia • Hirschsprung's disease • Ogilvie's syndrome
Internal Anal Sphincter (IAS)	• Recurrent anal fissures • Hemorrhoids • Rectoanal incontinence

Table 2

Alphabetical Listing of Potency Comparison of Different ROCK Inhibitors

ROCK Inhibitor	Selectivity	Potency (from different systems)	Potency in IAS vs. Rectal Smooth muscle[c]
[a] Fasudil (HA-1077)	ROCK I & ROCK II	IC_{50}[b] = 10.7 μmols/L	IAS IC_{50} = 1.82 μmols/L RSM IC_{50} = 4.57 μmols/L
GSK-269962A	ROCK I	ROCK I IC_{50} = 0.0016 μmols/L ROCK II IC_{50} = 0.006 μmols/L	
Hydroxy Fasudil (HA-1100)	ROCK I & ROCK II	IC_{50} = 1.8 μmols/L	
Rho Kinase Inhibitor (H-1152 or H-1152P)	ROCK I & ROCK II	IC_{50} = 0.0118 μmols/L	IAS IC_{50} = 0.0079 μmols/L RSM IC_{50} = 2.51 μmols/L
Rho Kinase Inhibitor II	ROCK I & ROCK II	IC_{50} = 0.2 μmols/L	IAS IC_{50} = 2.95 μmols/L RSM IC_{50} = 15.4 μmols/L
Rho Kinase Inhibitor III Rockout	ROCK I & ROCK II	IC_{50} = 25.0 μmols/L	
Rho Kinase Inhibitor IV	ROCK II	IC_{50} = 0.0118 μmols/L	
SB-772077-B	ROCK I & ROCK II	ROCK I IC_{50} = 0.0056 μmols/L	
SR-3677	ROCK II	ROCK I IC_{50} = 0.056 μmols/L ROCK II IC_{50} = 0.0072 μmols/L	
SLx-2119	ROCK II	ROCK I IC_{50} = 24.0 μmols/L ROCK II IC_{50} = 0.105 μmols/L	
Y-27632	ROCK I & ROCK II	IC_{50} = 5.0 μmols/L	IAS IC_{50} = 0.436 μmols/L RSM IC_{50} = 13.4 μmols/L

[a] There are a number of ongoing clinical trials on ROCK inhibitors especially Fasudil for different medical conditions e.g. hypertension, asthma, and cerebral vasospasm.[68] However, to our knowledge, there are no such trials for GI dysmotility.

[b] IC_{50}, the concentration of the inhibitor that causes 50% decrease in the ROCK activity.

[c] The IC_{50}s are compared in the internal anal sphincter (IAS) vs. the rectal smooth muscle (RSM). The IAS is spontaneously tonic smooth muscle while the RSM is primarily phasic with a small tonic component.