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ROLE OF RHO KINASE IN GASTROINTESTINAL MOTILITY

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The nature of distinct molecular mechanisms responsible for the phasic versus truly tonic smooth muscles is not completely understood. Similar to a number of other visceral smooth muscles, a rise in the levels of free intracellular calcium ($[Ca^{2+}]_i$) is important in the phasic contraction of the esophageal body (EB) smooth muscle (Am J Physiol Gastrointest Liver Physiol 2005;288:G407–G416; Am J Physiol Gastrointest Liver Physiol 2005;288: G849–G853). Accordingly, this involves activation of the Ca^{2+} /calmodulin (CaM)/myosin light chain kinase (MLCK) pathway. In addition, other molecular proteins such as protein kinase C (PKC) and RhoA/Rho-kinase (ROCK) play an important role in the latter component of the agonist-induced sustained contraction of the smooth muscle, and in the basal tone of the lower esophageal sphincter (LES) and other sphincteric smooth muscles (Am J Physiol Gastrointest Liver Physiol 2005;288:G407–G416; Exp Biol Med 2003;228:972–981; Annu Rev Physiol 2006;68:345–374; Physiol Rev 2003;83:1325–1358). Because of the relatively similar levels of $[Ca^{2+}]_i$ in the EB and LES smooth muscles, and the fact that the LES develops tone and the EB does not, Sims et al (J Pharmacol Exp Ther 2008;327: 178–186) used the term Ca^{2+} -sensitization being responsible for a part of the EB contraction, and for the basal tone in the LES. The data show that ROCK plays a significant role in this Ca^{2+} sensitization.

The role of ROCK in the agonist-stimulated contraction and basal tone of different gastrointestinal smooth muscles has been examined in the animal studies before. However, the role of ROCK pathway in the human gastrointestinal smooth muscle has not been examined before the present studies.

The authors simultaneously determined isometric force and the levels of $[Ca^{2+}]_i$ in intact human EB and LES smooth muscles. Levels of $[Ca^{2+}]_i$ were determined using ratiometric Ca^{2+} -sensitive dye fura-2. In the EB, spontaneous oscillations in the force were associated with transient elevations of $[Ca^{2+}]_i$. Muscarinic agonist carbachol caused a large increase in the EB force over and above the spontaneous oscillations, with smaller increases in the levels of $[Ca^{2+}]_i$. These observations suggested the role of Ca^{2+} sensitization in the EB smooth muscle contraction and in the LES tone. Two of the most commonly used ROCK inhibitors, Y-27632 and HA-1077 (Curr Opin Cell Biol 2008;20:242–248; Am J Physiol Gastrointest Liver Physiol 2008;294:G687–G693; in the maximally effective concentrations of 10 μ mol/l) caused significant decrease in the EB contraction induced by neural stimulation via electrical field stimulation (EFS) and carbachol. These decreases (~60% to 80%) in the amplitude of EB contraction were accompanied by smaller reduction in the rise

of $[Ca^{2+}]_i$. In contrast, the PKC inhibitors calphostin C and chelerythrine only had a modest effect.

In the LES, in contrast with the PKC inhibitors, the ROCK inhibitors caused significant decrease (maximal decrease of ~50%) in the basal tone in a concentration-dependent manner. In addition, ROCK inhibitors attenuated the transient increase in the LES tone during the “off response” of the EFS, and that by carbachol, but without significantly affecting the LES relaxation by EFS and sodium nitroprusside.

From these observations in the human EB and LES, the authors conclude that ROCK plays a significant role in the pathophysiology of the esophageal motility disorders.

Comment

ROCK, especially the ROCK II isoform, is the primary downstream target of the small GTP-binding protein RhoA in the sustained contraction of the smooth muscle. The role of ROCK in the gastrointestinal smooth muscle contraction in response to agonists has been known for some time (Physiol Rev 2003;83:1325–1358; Annu Rev Physiol 2006;68:345–374; J Appl Physiol 2002; 92:41–49; Gastroenterology 2006;131:108–116; Am J Physiol Gastrointest Liver Physiol 2007;292:G1747–G1756; Am J Physiol Gastrointest Liver Physiol 2005;288:G849–G853). In addition, using different animal models, the role of rho kinase in the basal tone in the gastrointestinal tract smooth muscle has been the focus of different laboratories (Am J Med 2003;115:15S–23S; Am J Physiol Gastrointest Liver Physiol 2005;288:G407–G416; Exp Biol Med 2003;228:972–981; Annu Rev Physiol 2006; 68:345–374). Major contribution of the present studies by Sims et al was the determination of the role of ROCK in the human EB and LES.

The EB smooth muscle is quiescent in the basal state and contracts transiently in response to swallowing in in vivo studies (J Clin Gastroenterol 2008;42:610–619). Such response can be mimicked in vitro in the organ bath experiments by EFS. This transient contraction in the EB may be referred to as “phasic” in nature. The responses to a contractile agonist such as muscarinic agonist may, however, be different, because it displays a combination of a phasic and tonic response. The term “tonic” with regard to an agonist refers to the latter part of the response in the form of relatively sustained contraction after the initial fading of the phasic contraction (Am J Physiol Gastrointest Liver Physiol 2005;288:G23–G31; Annu Rev Physiol 2006;68:345–374). The length of this tonic contraction may vary from tissue to tissue.

Sims et al showed that ROCK inhibitors attenuate the amplitude of esophageal contraction in a concentration-dependent manner, suggesting a role of ROCK in the EB contraction. The data may, however, not be interpreted to mean that the phasic contraction of the smooth muscle is critically dependent on ROCK activation. This is explained as follows.

In response to an appropriate agonist or neural stimulation, the release of the neurotransmitter substances such as Ach, and substance P (J Clin Gastroenterol 2008; 42:610–619) may activate Ca^{2+} /CaM/MLCK pathway. This leads to the phosphorylation of regulatory myosin light chain (phospho-MLC₂₀) initiating the EB smooth muscle

contraction (Am J Physiol Gastrointest Liver Physiol 2005;288:G407–G416). Phospho-MLC₂₀ is then dephosphorylated via myosin light-chain phosphatase (MLCP), bringing the smooth muscle back to the original state of quiescence. Although, the exact timing of the MLCP-induced dephosphorylation of MLC₂₀ is not known, ROCK is a known primary regulator of MLCP (Annu Rev Physiol 2006;68:345–374; Physiol Rev 2003; 83:1325–1358). Accordingly, activated ROCK inhibits MLCP, and inactivated ROCK may unleash it. It is conceivable therefore that premature unleashing of MLCP after ROCK inactivation (by Y-27632 and HA-1077) may decrease the time for the development of peak contraction, thereby inhibiting the ultimate EB contraction amplitude.

The data show that the ROCK inhibitors significantly attenuate the EB contraction irrespective of the stimulus used, whereas PKC inhibitors only cause a modest reduction. The present study was limited to the tissues from 1 esophageal site. Therefore, it is not possible to determine the effect of ROCK inhibitors on the speed of esophageal peristalsis, which is determined by the latency gradient in the onset and in the peak esophageal contraction after the stimulus. Future studies examining the effect of ROCK inhibitors on the multiple sites of the human esophagus should determine role of ROCK not only in the amplitude, but also the speed of esophageal peristalsis. Such information will provide a better understanding of the role of ROCK in the pathophysiology of esophageal motility disorders such as diffuse esophageal spasm.

The sphincteric tissues, because of the spontaneous tone, offer a distinct advantage to investigate the nature of molecular pathways in the basal state, alleviating the need for an exogenous agonist. Most of the currently valuable information on the signal transduction cascade, especially that involving ROCK, in the sustained contraction of the smooth muscle has been derived after an agonist response. The agonist may put different molecular pathways in a state of flux, which may even crisscross each other in different ways. This makes it difficult to track down the exact nature and the level of expression of the specific pathway during the truly tonic versus phasic contractions.

Overall, the data presented by Sims et al are in agreement with the earlier studies in the internal anal sphincter (IAS; Gastroenterology 2006;131:108–116; Am J Physiol Gastrointest Liver Physiol 2006;291:G830–G837; Am J Physiol Gastrointest Liver Physiol 2007;292:G1747–G1756). Phospho-MLC₂₀ (a primary determinant of the smooth muscle contraction) is immediately dephosphorylated by MLCP. This phenomenon may be the primary reason for the quiescent nature of most of the smooth muscles in the basal, unstimulated state. In response to a contractile agonist, the smooth muscle contracts for varying lengths of time. This time may be extremely short (measurable in seconds) or phasic in nature, as is the case in the EB in response to EFS. The sphincteric smooth muscles are truly tonic because they develop spontaneous tone in the basal state. It has been shown repeatedly that although this tone may be modulated by a number of neurohumoral agonists, it is largely myogenic in nature because of the specialized properties of the smooth muscle cells (J Pharmacol Exp Ther 2008;327:178–186; Neurogastroenterol Motil 2005;17:50–59; Br J Pharmacol 2008;153:858–869; Am J Physiol Gastrointest Liver Physiol 2006;291:G830–G837).

A number of intracellular differences have been pointed out between the phasic and tonic smooth muscles. The tonic smooth muscles may have higher levels of $[Ca^{2+}]_i$, lower levels of CaM, MLCK and caldesmon, and higher levels of α -actin, higher levels and activity of PKC especially PKC α (Am J Physiol Cell Physiol 1998;275:C684–C692; Am J Physiol Cell Physiol 2002;282:C94–C104; Am J Physiol Gastrointest Liver Physiol 2005;288:G407–G416; J Physiol (Lond) 2001;535:553–564) and up-regulation of RhoA/ROCK (Am J Physiol Gastrointest Liver Physiol 2006;291:G830–G837).

Conceptually, higher levels of sustained phospho-MLC₂₀ by the sustained active inhibition of MLCP via constitutively active ROCK may be responsible for the major portion of the basal tone in the LES and the IAS (Gastroenterology 2006;131:108–116; Am J Physiol Gastrointest Liver Physiol 2006;291:G830–G837; Am J Physiol Gastrointest Liver Physiol 2007;292:G1747–G1756). Accordingly, the IAS smooth muscle has characteristically higher levels of RhoA/ROCK and related signal transduction machinery compared with the adjoining phasic smooth muscles. Additionally, these studies determined a close relationship between the RhoA/ROCK activity (along with the respective signal transduction cascade) and the IAS basal tone before and after ROCK inhibitors.

The other molecular protein capable of inhibiting MLCP is PKC (Am J Physiol Gastrointest Liver Physiol 2005;288:G849–G853). However, present data rule out the possibility that PKC plays a significant role in either the EB contraction or in the basal tone in the human LES because PKC inhibitors do not have a significant effect in these events.

The studies by Sims et al have important pathophysiologic implications. Down-regulation of ROCK pathway may be involved in certain forms of gastroesophageal reflux disease associated with a hypotensive LES. In addition, abnormal up-regulation of RhoA/ROCK may contribute to the pathophysiology and therapeutic potential in the esophageal motility disorders characterized by a hypertensive LES, such as achalasia.

The studies report a partial decrease in the LES tone after the maximal effective concentrations of Y-27632 and HA-1077 and no significant effect with the PKC inhibitors. In contrast, in most of the other tonic smooth muscles, these inhibitors have been shown to cause almost complete obliteration of the spontaneous tone (Am J Physiol Gastrointest Liver Physiol 2008;294:G687–G693; Gastroenterology 2006;131:108–116; Am J Physiol Gastrointest Liver Physiol 2005;288:G407–G416; Nat Med 2001;7:119–122; Neurorol Urolyn 2005;24:136–141; J Urol 2004;171:1955–1958). It is possible that these quantitative differences may be related either to the species or the limitation of the studies in the clasp fibers versus other components of the LES (Br J Pharmacol 2008;153:858–869; Br J Surg 2007;94:1427–1436).

The molecular mechanisms governing the basal tone in the LES and IAS offer an opportunity for the development of novel therapeutic modalities for the complex gastrointestinal motility disorders: achalasia, diffuse esophageal spasm, segmental Hirschsprung disease, hemorrhoids, and recurrent anal fissures. Besides surgery, a number of attempted therapeutic management approaches for such conditions have met limited success and each accompanied with certain side effects. These therapeutic modalities include

botulinum toxin A, Ca^{2+} channel blockers, muscarinic and α -adrenergic blocking agents, nitroglycerine, and phosphodiesterase type V inhibitors (Br J Pharmacol 2008;153:858–869; Ann R Coll Surg Engl 2007;89:574–579).

Besides the prominent role of ROCK in the basal tone of the LES and IAS, the role of ROCK in the pyloric sphincter, the sphincter of Oddi, and the ileocecolic sphincter has not been examined. The presence of basal tone allows these sphincters to serve as 1-way valves for the normal, forward progression of the gastrointestinal contents, in response to the appropriate reflex. Their malfunction leads to the distinct abnormalities with serious consequences (Neurogastroenterol Motil 2005; 17[Suppl 1]:1–72).

In conclusion, ROCK plays an important role in the basal LES tone and esophageal contraction. We agree with the authors' interpretation that near obliteration in the LES tone by Ca^{2+} removal or with the Ca^{2+} channel blockers is not a contradiction from the critical role of ROCK in the basal tone. It is speculated that intracellular Ca^{2+} is critical for the activation of a number of molecular events including MLCK activation (for the initial development of tone), and RhoA/ROCK activation (for the maintenance of the basal tone). Certain forms of gastroesophageal reflux disease directly related to the hypotensive LES may be associated with ROCK down-regulation. Conversely, conditions associated with the hypertensive LES such as achalasia, and DES associated with high-speed and -amplitude esophageal contractions may be associated with abnormal up-regulation of ROCK. Such concepts also warrant examining in the other parts of the gastrointestinal tract. The drug targets destined for ROCK may offer molecular therapeutic modalities of certain gastrointestinal motility disorders that are site-specific and free of systemic side effects.