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Smooth Muscle–Specific Myosin Phosphatase Target Subunit 1 (MYPT1): An Important Piece of the Puzzle

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Dear Sir:

We have read with a great interest a recent article by He et al in the June issue of *GASTROENTEROLOGY*.¹ The studies provide strong evidence in favor of the concept that smooth muscle–specific myosin phosphatase target subunit 1 (MYPT1) of myosin light chain phosphatase (MLCP) plays a critical role in the agonist-induced contraction/relaxation of the smooth muscle. This was shown in their studies using animals with knocked out MYPT1^{−/−}. The investigators employed the Cre-*loxP* system in which they used the promoter region and exon 1 of *Mypt1* flanked by 2 *loxP* sites to establish *Mypt1*-floxed mice. These mice were crossed with SMA-Cre transgenic mice to generate smooth muscle–specific MYPT1 knockout mice (*Mypt1*^{SMKO}).

The authors demonstrated that the phasic responses (to acetylcholine [ACh] and K⁺-depolarization) of the jejunal and ileal smooth muscles of the mutant mice in comparison with the control mice are converted into a tonic type with sustained force. The converted responses had reduced rates of shortening velocity and relaxation because of higher levels of phospho-MLC₂₀ (p-MLC₂₀). The authors observed no apparent abnormality in the intestinal motility in the mutant mice, although there was a definite trend toward the decrease in the intestinal transit (a predicted effect). The lack of significance for the abnormal intestinal motility may have been because of the huge variability among the mutant mice reflected by 49.9 ± 13.9 SEM percent transit values. The issue of intestinal motility abnormality in the *Mypt1*^{SMKO} may be resolved by studying a larger number of animals with a group beyond 16 weeks old, and by detailed examinations of the entire gut from the esophagus to the anorectum. The present studies had primarily focused on the limited regions of the gastrointestinal tract.

A critical role of MYPT1 in sustained contraction has been previously demonstrated in the phenotypically tonic versus the phasic smooth muscles of the gastrointestinal tract, in the basal state.^{2,3} Additionally, there seem to be certain similarities between *Mypt1*^{SMKO} animals and the spontaneously hypertensive rats: An increase in blood pressure and in the intestinal smooth muscle contractility with a corresponding decrease in MYPT1.^{1,4,5} An increase in the neurotransmitter (ACh)-mediated amplitude and sustained contraction of the intestinal smooth muscle in MYPT1^{SMKO} is suggestive of dysfunctional smooth muscle typified in the diffuse esophageal spasm in response to swallowing. It has been proposed that defective inhibitory neurotransmission mediated by nitric oxide and vasoactive intestinal polypeptide, unopposed excitatory neurotransmitters' (ACh; substance P) contractile actions, and increased smooth muscle sensitivity may be responsible for the

Conflicts of interest

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uncoordinated often hypertensive contractions, failure of the descending inhibition, and achalasic/hypertensive sphincteric smooth muscles.⁶

Present data with greater sensitivity of the smooth muscle in response to the excitatory agonists in the presence of similar concentrations of intracellular Ca^{2+} , suggest the role of Ca^{2+} sensitization via inhibition of MLCP via MYPT1, the primary target for RhoA/ROCK. Also, there are studies to show significantly higher levels of endogenous inhibitory protein CPI-17 (originally named so because of its targeting PKC, protein-kinase C potentiated inhibitor) in the tonic versus phasic smooth muscles. Recently, it is becoming evident that RhoA/ROCK contributes to Ca^{2+} sensitization not only by targeting MYPT1 but also by targeting CPI-17, so that CPI-17 is not exclusively targeted by PKC.^{3,7} Those data from humans and animals show significantly higher levels of CPI-17 in the spontaneously tonic smooth muscle versus the phasic, and specific decreases in the phospho-CPI-17 after selective RhoA/ROCK inhibitors. The bimodal effect of RhoA/ROCK on MYPT1 and CPI-17, however, was not appropriately discussed in the paper by He et al.

In the view of a critical role of MLCK/MYPT1-MLCP/p-MLC₂₀ in smooth muscle relaxation/contraction, it is important to determine the significance of MYPT1 in the region-specific pathophysiology in response to the corresponding reflexes, for example, swallowing in the case of esophagus and rectoanal inhibitory (defecation) reflex in the anorectum. In this regard, the potential of MYPT1 gene-deleted animal models similar to that of *Mypt1^{SMKO}* (but without compensatory genetic and adaptive physiologic responses) may go beyond the investigation of the molecular mechanisms for the agonist-induced smooth muscle contraction. Such molecular insights may further reveal the pathophysiology of certain motility disorders, with or without characteristic dysfunctional inhibitory and excitatory neurotransmissions, as discussed.⁶ These disorders may involve MYPT1-associated dearranged signal transduction cascade for the smooth muscle contraction/relaxation to explain disturbed changes in the latency gradient for the sequential contractions, a hallmark of the normal progression of the food and ingesta leading to the expulsion of waste.⁸

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Appendix

Reply. We are pleased with the keen interest in our recent work published in *GASTROENTEROLOGY* on signaling to smooth muscle myosin regulatory light chain (RLC) phosphorylation in

myosin phosphatase target subunit knockout mice.¹ Smooth muscle contractile responses converge on the regulation of the contractile machinery, involving phosphorylation of the myosin RLC subunit by the Ca^{2+} -dependent myosin light chain kinase (MLCK).^{2–3} This phosphorylation allows the myosin motor head to bind to actin filaments to initiate cell shortening and force development. The key element in smooth muscle contractile responses, including tonic and phasic gastrointestinal smooth muscles, is thus related to the extent of RLC phosphorylation, which depends on the ratio of MLCK to myosin light chain phosphatase (MLCP) activity. Both MLCK and MLCP activities are regulated in a dynamic manner with integrated signaling modules impinging on both MLCK and MLCP. We had previously shown in different smooth muscles, including intestinal smooth muscles, that knockout of MLCK, resulted in contractile failure and death in vivo and markedly attenuated contractile responses of isolated smooth muscle segments in vitro.^{5–8}

Because MLCP is composed of a protein phosphatase type 1 catalytic subunit bound stoichiometrically to the myosin phosphatase target subunit (MYPT1) and this binding to myosin is thought to be crucial for RLC dephosphorylation,^{3,4} we anticipated that the knockout of MYPT1 would lead to marked phenotypic responses, perhaps similar in principle with the MLCK knockout with contractile failure and death in vivo. Thus, we were most surprised to observe a mild phenotype in the MYPT1 knockout mice with relatively modest changes in contractile responses of isolated tissues.¹ Rattan proposes that some alterations may be revealed with additional studies involving a larger number of animals or a more extensive analysis of the entire gut from the esophagus to the anorectum. Additional signaling studies are also proposed in relation to Ca^{2+} -sensitization responses involving region specific responses, including pathophysiology of certain motility disorders with dysfunctional inhibitory and excitatory neurotransmissions. We certainly believe these types of studies are needed to understand the specific roles of Ca^{2+} sensitization under different physiologic and pathophysiologic conditions in different regions of the gut. This is why we are continuing these types of investigations beyond our initial report on MYPT1 knockout in smooth muscles of mice. We appreciate the enthusiasm Rattan exhibits for such studies.

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Conflicts of interest

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