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RhoA/ROCK Pathway is the Major Molecular Determinant of Basal Tone in Intact Human Internal Anal Sphincter

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Backgrounds & Aims

Knowledge of molecular control mechanisms underlying the basal tone in the intact human IAS is critical for the pathophysiology and rational therapy for debilitating rectoanal motility disorders.

Methods

We determined the effects of ROCK and PKC-selective inhibitors Y 27632 and Gö 6850 (10^{-8} to 10^{-4} M), respectively on the basal tone in the IAS vs. the RSM. We performed Western blot analysis, confocal microscopy and enzymatic activity assay to determine the levels, membrane distribution and enzyme activity of RhoA/ROCKII, PKCα, MYPT1, CPI-17, and MLC_{20} before and after Y 27632 and Gö 6850.

Results

Fig. 1

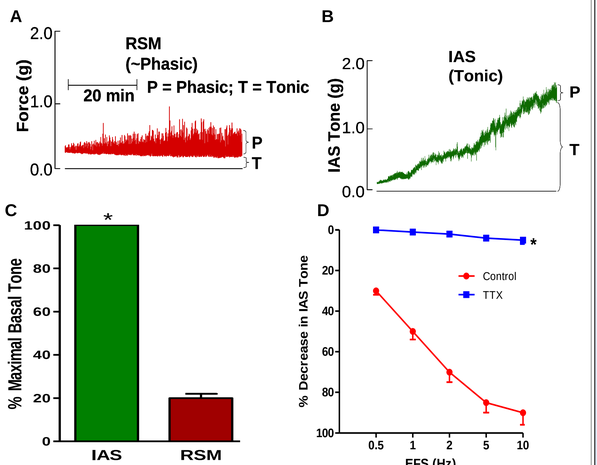


Figure 1. A,B. Phasic and tonic activities of RSM and IAS respectively. C. % maximal basal tone in IAS and RSM. D. EFS response in IAS and RSM.

Fig. 2

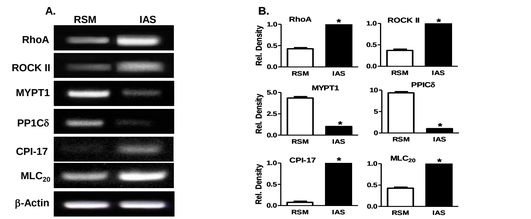


Figure 2. A, B. RT PCR analysis shows higher expression of RhoA, ROCK II, CPI-17, MLC_{20} and lower expression of MYPT1 and PP1c δ in the IAS than RSM.

Fig. 3

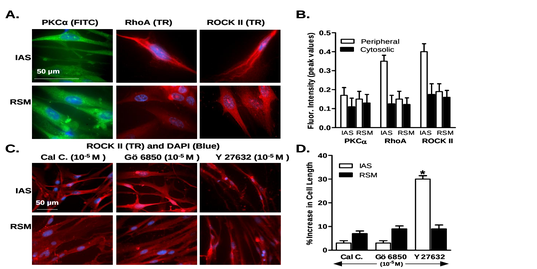


Figure 3. A, B, C. Immunofluorescence analysis shows higher levels of PKCα, RhoA and ROCK II in the periphery vs. the cytosol of the IAS vs. RSM SMCs. **D.** Data show higher relax. of the IAS SMCs with Y 27632 as compared with calphostin C and Gö 6850.

Fig. 4

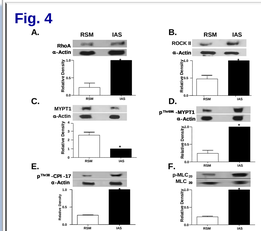


Figure 4. WB analysis shows higher levels of RhoA, ROCKII, p-CPI17, p-MYPT1 and p-MLC20 in IAS vs RSM.

Fig. 5

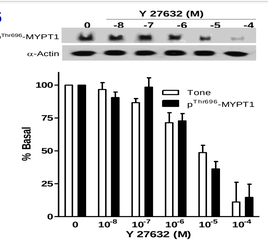


Figure 5 WB analysis shows conc.-dependent decrease in p-MYPT1 by ROCK inhibitor Y 27632.

Fig. 6

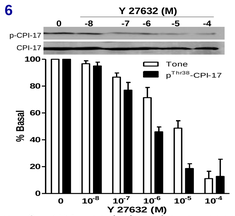


Figure 6. WB analysis shows conc.-dependent decrease in p-CPI-17 following Y 27632.

Fig. 7

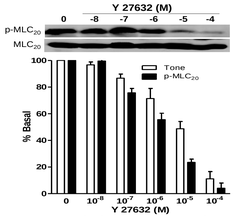


Figure 7. WB analysis shows conc.-dependent decrease in p-MLC_{20} following Y27632.

Fig. 8

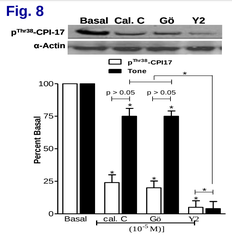


Figure 8. WB analysis shows significant decrease in p-CPI-17 following both Gö 6850 and Y 27632.

Fig. 9

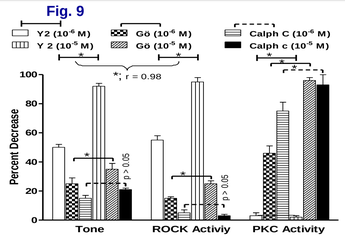


Figure 9. Data show decrease in ROCK activity with Y 27632 closely correlate with the decreases in the IAS tone.

Fig. 10

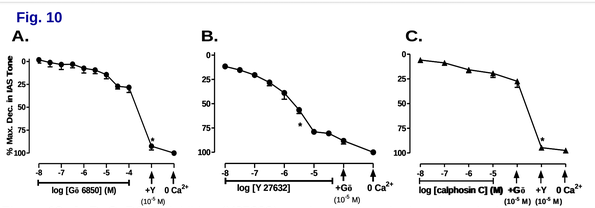
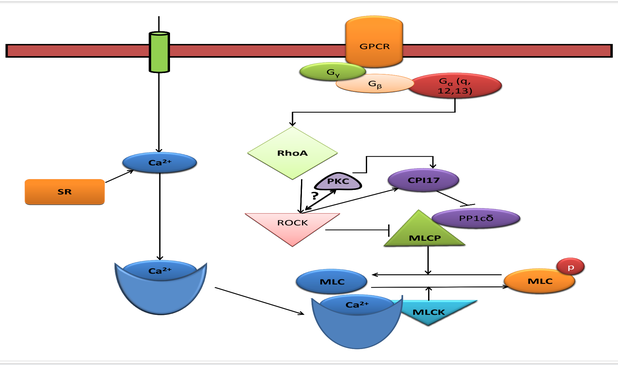


Figure 10. A, B, C. ROCK inhibitor (Y27632) nearly abolishes the basal tone in the IAS while PKC inhibitors Gö 6850 and calphostin C have limited effects.

MOLECULAR PATHWAY FOR BASAL IAS TONE



Conclusions

- RhoA/ROCK are constitutively active in the IAS, and this pathway (in contrast with PKC) is the critical determinant of the basal tone in the human IAS.
- Therefore, RhoA/ROCK are novel therapeutic targets for a number of rectoanal motility disorders in humans.