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^{-5} 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, PKcs, MYPT1, CPI-17, and MLC20 before and after Y 27632 and Gö 6850.

Results

Figure 2. A, B. RT-PCR analysis shows higher expression of RhoA, ROCK II, CPI-17, MLC20 and lower expression of MYPT1 and PP1cδ in the IAS than RSM.

Figure 3. A, B, C. Immunofluorescence analysis shows higher levels of PKcs, RhoA and ROCK II in the Phasic vs. the tonic of the IAS vs. RSM. D. Data show higher relax. of the IAS SMcs with Y 27632 as compared with calphosin C and Gö 6850.

Figure 4. WB analysis shows significant decrease in p-CPI-17 following both Gö 6850 and Y 27632.

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

Figure 6. WB analysis shows conc. dependent decrease in p-MLC20 in IAS vs RSM.

Figure 7. WB analysis shows conc. dependent decrease in p-CPI-17 following Y 27632. 27632.

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

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

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

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.