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Development of Myogenic IAS Reconstructs from Human Internal Anal Sphincter (IAS) Smooth Muscle Cells (SMCs) with Functional and Molecular Properties Similar to Intact Human IAS

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Backgrounds & Aims

Rectoanal incontinence is associated with defective Internal Anal Sphincter (IAS). Current therapies are not satisfactory, raising a potential for the replacement of the dysfunctional IAS with the reconstructs. Present studies were performed to develop human IAS smooth muscle reconstructs with functional and molecular attributes similar to the intact human IAS Smooth muscle (SM).

Methods

SMCs were isolated from Human IAS tissue samples, and cultured in collagen coated tissue culture dishes with DMEM containing 10% fetal bovine serum and 50 mg/ml of sodium ascorbate, around the central Sylgard posts. Method for preparation of Sylgard posts was modified from Hecker *et al*. *Am J Physiol* 2005. Collagen I-based IAS reconstructs were made and their physiological properties were compared with intact human IAS.

Results

Fig. 1

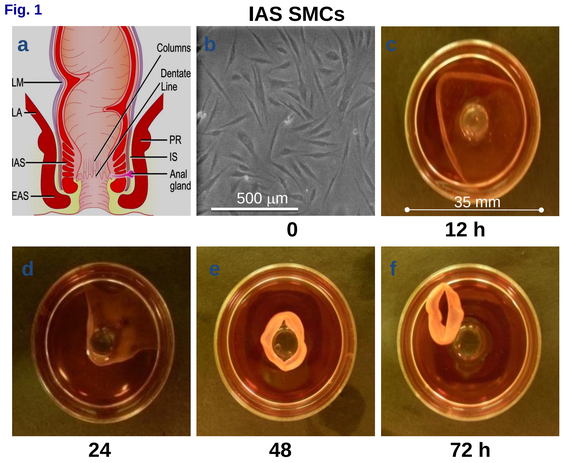

Figure 1. SMCs (1x10^7 cells/ml) were trypsinized, mixed with rat tail collagen I (5 mg/ml) and poured around the Sylgard post described above. DMEM with 15% serum was added and plates were incubated for 24 hours, and media was replaced by SMC differentiation media.

Fig. 2

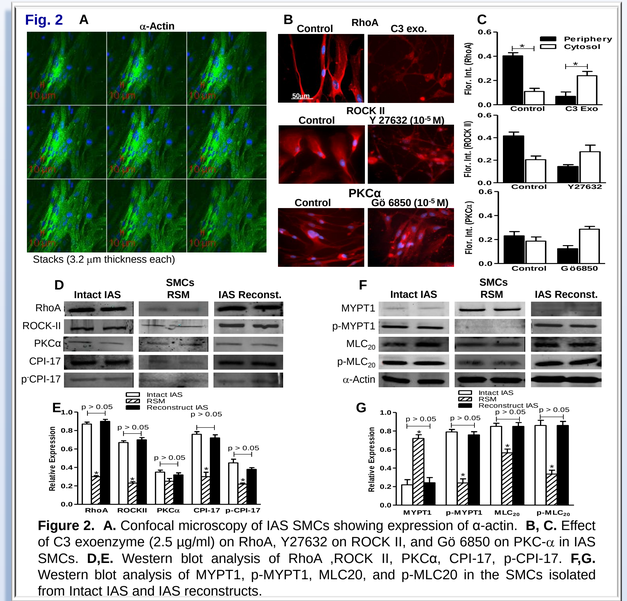

Figure 2. A. Confocal microscopy of IAS SMCs showing expression of α-actin. **B, C.** Effect of C3 exoenzyme (2.5 μg/ml) on RhoA, Y27632 on ROCK II, and Gö 6850 on PKC-α in IAS SMCs. **D,E.** Western blot analysis of RhoA ,ROCK II, PKCα, CPI-17, p-CPI-17. **F,G.** Western blot analysis of MYPT1, p-MYPT1, MLC20, and p-MLC20 in the SMCs isolated from Intact IAS and IAS reconstructs.

Fig. 3

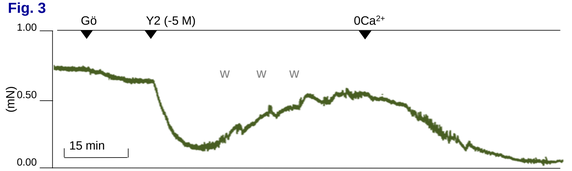

Figure 3. A typical tracing of the characteristic responses of above developed IAS reconstructs from human IAS SMCs. PKC inhibitor Gö 6850 causes minimal decrease in the basal tone, while ROCK inhibitor Y 27632 almost obliterates the tone, close to 0Ca2+.

Fig. 4

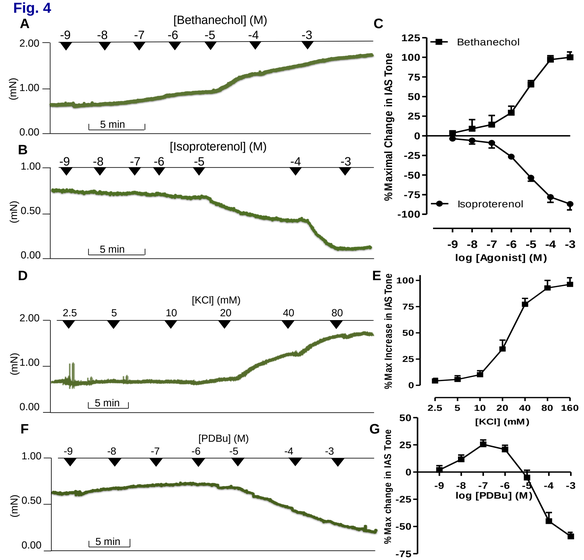

Figure 4. Typical tracings showing the effects, and data of reconstructs' responses to **A,C.** bethanechol **B, C.** isoproterenol, **D, E.** KCl and **F, G.** PDBu.

Fig. 5

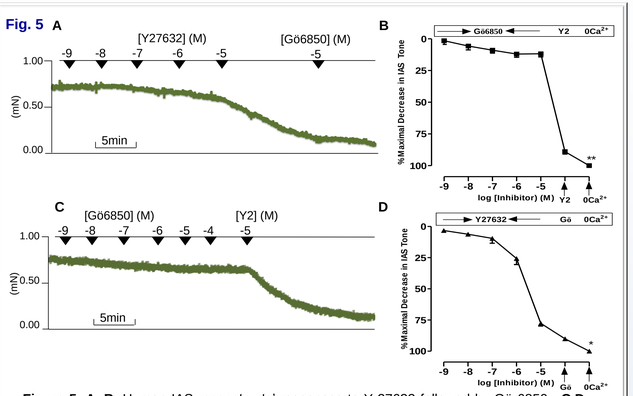

Figure 5. A, B. Human IAS reconstructs' responses to Y 27632 followed by Gö 6850. **C,D,** Above protocol in reverse.

Fig. 6

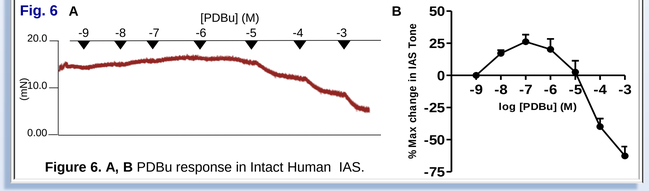

Figure 6. A, B PDBu response in Intact Human IAS.

Summary & Conclusions

- The studies for first time show that presently prepared IAS reconstructs from human IAS SMCs are functionally and molecularly similar to intact human IAS.
- Data further show that basal tone in these IAS reconstructs is primarily dependent on RhoA/ROCK pathway.