ONLINE SUPPLEMENT

Length-Dependent Modulation of Cytoskeletal Remodeling and Mechanical Energetics in Airway Smooth Muscle

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MATERIALS AND METHODS

Isometric Contractions

The procedure has been described previously (E1). Briefly, one end of each muscle strip was clamped to a stainless steel clip connected to a force transducer (Grass; Quincy, MA), and the other end was clamped to a stainless steel clip connected to a length manipulator (Narishige; Japan). Each muscle strip was stretched quickly to a peak force of ~12.5 g, and then allowed to equilibrate for 2 hr in PSS bubbled with air and maintained at 37°C. Isolated airway smooth muscle shortens dramatically during dissection. The stretching of unstimulated airway smooth muscle is designed to readjust the muscle length to near in vivo muscle length, and is not expected to cause damage to the muscle, because airway smooth muscle has a unique ability to adapt to a relatively large range of muscle length for maximal force development (E2). After equilibration, each muscle strip was adjusted to reference length (Lo) for maximal active force development by releasing the muscle strip quickly to a passive force of ~2.5 g. Each muscle strip was then stimulated for 10 min by K^+-depolarization with a solution similar to PSS in composition, except that 105 mM NaCl was substituted by KCl. The force (Fo) developed in this contraction was used to normalize force developed in subsequent contractions.

Reagents

Antibodies against α-SM actin (Clone 1A4), α-actinin (Clone BM-75.2), vinculin (hVIN-1), talin (Clone 8D4) and, peroxidase-conjugate secondary antibodies were purchased from Sigma Chemical Co (St. Louis, MO); Erk 1/2 and phospho-Erk 1/2 (Thr202/Tyr204) from Cell Signaling Technology (Beverly, MA); and anti-Arp3 (Human Arp3) from Upstate Biotechnology (Lake Placid, NY). All other chemicals were of reagent grade.

Statistics

Data are presented in means ± SE; n represents the number of animals. Student's *t*-test was used for the comparison of two means ($P < 0.05$ considered significant). We performed two-way ANOVA ($p < 0.05$ considered significant) on each data set to differentiate the effects of the two variables - 1 μM carbachol vs. muscle length and U0126 vs. muscle length - on pellet/supernatant ratios of cytoskeletal proteins.

REFERENCES

E1. Kim HR, Hoque M, Hai CM. Cholinergic receptor-mediated differential cytoskeletal recruitment of actin- and integrin-binding proteins in intact airway smooth muscle. *Am J Physiol Cell Physiol* 2004; 287: C1375-C1383.

E2. Bai TR, Bates JHT, Brusasco B, Camoretti-Mercado B, Chitano P, Deng LH, Dowell M, Fabry B, Ford LE, Fredberg JJ, Gerthoffer WT, Gilbert SH, Gunst SJ, Hai CM, Halayko AJ, Hirst SJ, James AL, Janssen LJ, Jones KA, King GG, Lakser OJ, Lambert RK, Lauzon AM, Lutchen KL, Maksym GN, Meiss RA, Mijailovich SM, Mitchell HW, Mitchell RW, Mitzner W, Murphy TM, Pare PD, Schellenberg PR, Seow CY, Sieck GC, Smith PG, Smolensky AV, Solway J, Stephens NL, Stewart AG, Tang DD, Wang L. On the terminology for describing the length-force relationship and its changes in airway smooth muscle. *J Appl Physiol* 2004; 97: 2029-2034.