The rise of passive airway smooth muscle mechanics

Gijs Ijpma and Anne-Marie Lauzon
Meakins-Christie Laboratories, Department of Medicine, McGill University, Montréal, Québec, Canada

Submitted 28 October 2011; accepted in final form 28 October 2011

STUDIES ON AIRWAY HYPERRESPONSIVENESS and asthma have traditionally focused on changes in the airway smooth muscle (ASM) contractility and associated signaling pathways, with little attention given to the passive smooth muscle properties. As acute airway constriction in asthma is presumably the result of active contraction of the ASM, this seemed like a reasonable approach. However, as the importance of breathing dynamics in determining ASM contractility has become more evident (1), passive smooth muscle properties cannot be ignored anymore. Indeed, the lack of a bronchoprotective effect of deep inspiration (DI) in asthmatic lungs compared with healthy lungs is likely dependent on the stiffness of the airways during the DI maneuver (8). As ASM is expected to be relaxed during bronchoprotective DI, the passive viscoelastic properties of the muscle must at least partially contribute to the ASM strain experienced during a DI. Hence, the passive mechanical properties of ASM are likely to play a key role in the differential response to DI of asthmatic and normal subjects, and this may constitute an alternative target for treatment.

Passive ASM stiffness has been shown to exhibit similar length adaptation as active ASM stiffness, occurring at similar rates with repeated stimulation (2). In the present issue of the Journal of Applied Physiology, Raqeeb and colleagues (9) present their study on the regulation of the passive ASM properties and their length adaptation. The authors measured instantaneous passive stiffness using a rapid step-length increase. They reported that a significant fraction of passive stiffness is abolished in Ca$^{2+}$-depleted ASM. Furthermore, this decrease in passive stiffness is maintained at lengths shorter or longer than the reference length (in situ length). The authors also studied the redevelopment of passive stiffness after strain-induced softening. The large length oscillations used to induce this softening decreased the passive stiffness initially, but it recovered fully after equilibration. However, a decrease in passive stiffness recovery was observed in Ca$^{2+}$-depleted ASM. In addition, the passive stiffness was shown to decrease to the same extent when the measurements were performed in Ca$^{2+}$-containing Krebs solution in the presence of Ca$^{2+}$ entry blockers. Hence this regulatable passive stiffness must be intracellular in origin. Interestingly, the passive stiffness retained its ability to recover from large oscillations when myosin light chain kinase (MLCK) was inhibited by ML-7, but it was not the case in the presence of the Rho-kinase inhibitor Y-27632.

The question remains of what determines the passive stiffness of ASM. Because the regulatable passive stiffness is length independent, the authors suggest that it must come from nonstatic structures that can reorganize after the length changes. This reorganization may be attributed to nonpermanent cross-links between otherwise static cellular elements. A recent modeling study showed the potential to describe passive muscle mechanics of such a cross-linker process (3). Furthermore, the lack of change in passive stiffness with MLCK inhibition indicates that the passive stiffness, at least in ovine ASM, does not originate from phosphorylated cross bridges (9). While the binding force of unphosphorylated myosin to actin is greatly reduced compared with phosphorylated myosin (7), it is likely to contribute some of the stiffness of passive ASM. In addition, our laboratory (10) recently showed that the binding force of unphosphorylated myosin is increased in the presence of regulatory proteins, such as caldesmon and calponin. Thus it is possible that, in the absence of MLCK, Ca$^{2+}$-regulated activation of calponin and caldesmon regulates the binding of unphosphorylated myosin with actin (12). However, as pointed out by the authors, if unphosphorylated myosin were fully responsible for the regulatable stiffness, it should be greater at lengths that correspond to the maximal active force (9). Because this was not the case, other factors must come into play. Indeed, they showed that the Rho-kinase pathway is important in generating this passive stiffness. The exact role of Rho kinase will, however, require further investigations.

The authors chose to use the force change in response to a near instantaneous length change as an indicator of the muscle stiffness. This is not a true stiffness measurement, as the viscous properties of the muscle also affect this force change. The authors acknowledged this and also measured the half time of relaxation of the force after a length step. Because the viscoelasticity of ASM is highly nonlinear and not fully understood (5, 13), separating the elastic and viscous contributions to the mechanics cannot be done unambiguously. Consequently, any definition of stiffness used may include viscous components, and possibly in ASM the two cannot be distinguished as separate mechanical quantities. However, it is likely that the elastic component of the force response is primarily determined by interconnected structures, such as the cytoskeleton, while the viscous component may come from complex frictional interaction in the crowded cell environment. Furthermore, it is likely that this viscous component is not length dependent. As the regulatable stiffness was also found to be mostly length independent, it is possible that the viscous component of the measured stiffness is Ca$^{2+}$ dependent. Perhaps Ca$^{2+}$-regulated intracellular processes affect the relative jostling of the cellular particles, thus affecting the frictional interaction (4).

A previous article from the same laboratory (14) reported that one potential source for the length adaptation of passive stiffness may be the dense bodies (DB). A three-dimensional electron microscopy analysis of DB geometry showed that DB can occur as cablelike structures parallel to the cell axis. These cablelike structures adapt their lengths upon length changes. Thus, if the DB play the role of mechanical anchor points for
the contractile apparatus, as assumed in most mechanical models of smooth muscle (6, 11), then their length adaptability may be directly related to the length adaptation of the contractile apparatus. Future studies may reveal whether the DB length adaptation is Ca\(^{2+}\) dependent.

The work by Raqeeb and colleagues (9) has further cemented the importance of the passive mechanical properties of ASM and their regulation. The study raises Ca\(^{2+}\)–regulated passive stiffness as a novel target for asthma treatment. Manipulation of regulated passive stiffness could affect asthma in two ways: bronchoprotection by increasing ASM strain during DI, and decreased bronchoconstriction by affecting the structural integrity of cytoskeletal structures, such as DB cables. Although more work is needed to validate these theories directly in normal and asthmatic human ASM, this study provides a considerable advance in our understanding of the potential role of passive properties of ASM in health and disease.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: G.I. drafted manuscript; G.I. and A.-M.L. edited and revised manuscript; A.-M.L. approved final version of manuscript.

**REFERENCES**