The importance of maturational studies in airway smooth muscle

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ASTHMA AFFECTS 17.7 million Americans, 5 million of them children (41). Its incidence has more than doubled in the general population since 1980, with a disproportionate increase in children under the age of 4 (41). Measures of the yearly burden of this disease, 5,500 deaths (31), nearly $6 billion in direct treatment costs, and a total of >$25 billion in all costs, including lost productivity, costs of concomitant conditions, as well as treatment costs, place asthma among the most burdensome diseases in the United States (6). But even these numbers fail to capture the costs of impaired development and the emotional toll taken by this chronic illness in children. These facts are marshaled to call attention to the importance of the study of the basic mechanisms of asthma, particularly in two papers bearing upon these mechanisms (Refs. 43 and 44, see pp. L902 and L909, respectively, in this issue).

Although asthma and reactive airway disease are commonly believed to result from immune responses in the lungs, manifest by eosinophils and mast cells in the bronchi, there is ample evidence that airway smooth muscle hyperresponsiveness is equally important. Perhaps the most direct evidence is that hyperreactive airways are defined by their augmented response to methacholine, which stimulates the muscle directly and not through immune or inflammatory pathways. Other evidence for nonimmune causes of wheezing include the net infrequent absence of immune-reactive cells in the bronchi (45) and the observation that nonspecific airway inflammation, such as a new viral infection, can initiate prolonged periods of wheezing (26). Evidence reviewed at the end of this editorial suggests that there is at least one “asthma phenotype” resident within the muscle that is expressed in response to immune stimuli and that remains dormant until the muscle is activated by nonimmune stimuli.

Asthma frequently begins at a young age and lasts for life. But reactive airway disease of children may have three distinct clinical courses (25): one begins in infancy and disappears during early childhood; a second begins in infancy and continues for the life of the affected person; and a third begins in later childhood and continues into adulthood. These observations raise intriguing questions regarding maturational changes in the airway that occur during childhood. Understanding the nature of these changes could identify targets for therapies both to curtail the continuation of infantile wheezing into adulthood and to prevent the development of asthma in childhood and later life. Wang et al. (43, 44) describe maturational changes in the contractile mechanics of airway smooth muscle, which is likely to become a fertile area of investigation.

Most muscle scientists would likely agree that striated muscle is better understood than smooth muscle. This better understanding comes largely from the correlations between muscle function and the structures that produce the striations. Because these structures are common to all striated muscle, information gained in one muscle is often transferable to another, so that for many purposes, there is a single large community of striated muscle investigators. The situation is different in smooth muscle, which is richer in the diversity of its characteristics and mechanisms. Examples include: 1) the type of contractions produced [tonic in vascular muscle, phasic in visceral muscle (39)], 2) the different responses to agonists and antagonists, and 3) the functional length range [long in muscles of the urinary bladder (42) and airway muscle (30), short in arterial muscle (29)]. These large tissue differences have understandably fragmented the study of smooth muscle into distinct tissue groups with much less cross fertilization of ideas. There is a robust community of scientists studying airway muscle, but given their relatively small number, only scant attention has been paid to maturational changes in this muscle. This deficiency is now being rectified by the Pediatric Research Unit at Duke University, which contributed the two articles in focus here.

In discussing these two articles, we call on our prior experience in striated muscle to draw parallels with the newer work in smooth muscle. Finally, we suggest how a theory of skeletal muscle contraction can explain one model of an asthma phenotype.

Maturational changes in airway smooth muscle velocity. The first of the two papers (43) extends a prior study by the same group (2) showing that isolated airway smooth muscle from juvenile (3-wk-old) guinea pigs shortened faster than muscles from both newborn (1-wk-old) and adult (3-mo-old) animals. Maximum velocities were assessed from complete force-velocity curves, which is a more reliable way of assessing shortening capability than single measurements of unloaded shortening (7). Analysis of the curves suggested that the higher velocity in juvenile muscle was associated with an increase in stiffness and viscosity in the unstimulated muscle. This leads to the logical hypothesis that the reduced passive stiffness and viscosity in the juvenile group impose less load on contractile elements of the muscles in this age group.

But a different interpretation is suggested by similar maturational changes in cardiac muscle discovered decades ago (reviewed in Ref. 47). There are two cardiac myosin heavy chain isoforms, α (fast) and β (slow), that are expressed in different proportions to alter the speed of muscle contraction as animals mature. Could it be that there are similar maturational differences in myosin isoforms in airway smooth muscle? There are known to be different myosin isoforms in airway smooth muscle (38), and the isoforms produce different shortening velocities in motility assays (32). These considerations raise the question of the relative preponderance of different isoforms in muscles from different age groups. If substantial differences are found, intact muscles from animals of different ages could
be used to explore the physiology of the different isoforms in whole muscle, as they were in cardiac muscle.

The suggestion that the higher shortening velocity in the juvenile muscle could result from a faster myosin isoform raises the question of how the finding of a lower stiffness and viscosity in the unstimulated juvenile muscle fits into the overall scheme. One possible explanation is that the oscillations induce more resting tone in unstimulated newborn and adult muscle. Suggestively, in the second paper in focus here (44), Wang et al. found that oscillations of newborn muscles increased force in subsequent tetani, although there is not yet data to indicate whether oscillations partially activate unstimulated muscle. Because activation is controlled by myosin light chain phosphorylation, this hypothesis could be tested by measuring phosphorylation levels in oscillated muscles from different age groups, a necessary control before stiffness measurements in unstimulated muscle can be accepted as truly passive.

**Maturational changes in airway muscle response to oscillations.** In the second article in focus (44), the same authors showed that length oscillations applied to the relaxed muscle reduced force by 15–20% in tetanic contractions immediately following the oscillations. In juvenile and adult muscles, tetanic force recovered toward its preoscillation level over several tetani to a level 7M, 20-min exposure) that did not increase force in subsequent tetani, although there is not yet data to indicate whether oscillations partially activate unstimulated muscle. Because activation is controlled by myosin light chain phosphorylation, this hypothesis could be tested by measuring phosphorylation levels in oscillated muscles from different age groups, a necessary control before stiffness measurements in unstimulated muscle can be accepted as truly passive.

Maturational changes in airway muscle response to oscillations. In the second article in focus (44), the same authors showed that length oscillations applied to the relaxed muscle reduced force by 15–20% in tetanic contractions immediately following the oscillations. In juvenile and adult muscles, tetanic force recovered toward its preoscillation level over several tetani, but in muscles from newborn animals, it increased over 2 tetani to a level ~10% above the control level and remained at that augmented level for at least 6 additional tetani. To explore possible explanations of this curious result, the authors assessed the effect of cytochalasin D, which blocks the addition of actin monomers to actin filaments. They first established conditions (10⁻⁷ M, 20-min exposure) that did not alter force during electrically stimulated tetani in nonoscillated muscle and then showed that this concentration: 1) nearly doubled the initial force deficit caused by the oscillation in all three age groups, 2) eliminated differences between muscles from different age groups, and 3) caused the level toward which force recovered in all three groups of muscles to be reduced to 80–85% of the preoscillation level. They then showed that indomethacin, known to inhibit the intracellular cyclooxygenase pathway, eliminated the oscillation-induced force potentiation in newborn muscle and reduced the initial force reduction caused by oscillation in both newborn and adult muscle.

The effects of cytochalasin D in the first set of observations suggest that the initial force reduction caused by oscillation is due, at least in part, to a disruption of the filament lattice. Some smooth muscles, including airway smooth muscle, differ from skeletal muscle in that they rapidly adapt their filament structures to maintain nearly constant force over a much wider length range than could be accommodated by the fixed array of filaments in striated muscle (30). We have proposed (9, 30) that this lattice plasticity is enabled by thick-filament evanescence. With thick filaments dissociating partially during relaxation and reforming upon activation. Our initial proposal was founded on early electron microscope studies of smooth muscle (22, 34, 35), and thick-filament evanescence has since been confirmed both by electron microscopy (11, 14, 15, 46) and by optical birefringence (11, 12, 37). Experiments similar to the present ones have been done in adult pig muscles, and the transient decline in developed force following oscillations has been correlated with a decline in thick-filament mass (24). Thus it is expected that the decline in force immediately following oscillations in the guinea pig is associated with a decline in thick-filament mass. The observation that cytochalasin D increased the force deficit following oscillations and decreased the level toward which force recovered suggests that actin filaments may also be depolymerized by the oscillations.

Our colleague at Indiana University, Dr. Susan Gunst, has proposed a theory of filament lattice plasticity based on actin filament evanescence (13, 27). It seems likely that we are all looking at different aspects of the same phenomenon, and the effects of cytochalasin D in the present experiments strengthen this likelihood. Support for Dr. Gunst’s theory of thin-filament plasticity is based on the finding that inhibitors of thin-filament formation reduce force production. The finding that cytochalasin D inhibits the recovery of force attributed to thick-filament reformation suggests that thick- and thin-filament formation are linked.

The force potentiation following oscillations in the newborn muscle and its abolition by indomethacin are likely caused by increases in activation rather than by changes in the filament lattice, and it reveals interactions between intracellular signaling pathways not present in striated muscle. The authors have previously shown that (2, 3): 1) adult muscle stimulated electrically for long periods relaxes spontaneously in spite of continued stimulation, 2) newborn muscle maintains tension during continued stimulation of the same duration, 3) several prostanoids are more abundant in newborn muscle, and 4) indomethacin causes the newborn muscle to relax spontaneously in the presence of continued electrical stimulation, in a manner similar to adult muscle. Others have shown that indomethacin inhibits stretch activation in adult guinea pig tracheal muscle (10). Together, these findings suggest that elevated intracellular prostanoids contribute to hyperreactivity of newborn guinea pig muscle and that this signaling pathway can modulate the activation produced by direct stimulation of the muscle. Although the precise mechanisms have yet to be defined and the signaling pathways may differ in humans (36), these results open new avenues of investigation and suggest possible targets for treating wheezing in infants.

**An asthma phenotype.** As discussed above, several lines of evidence suggest that airway hyperresponsiveness is partly due to changes in the contractile apparatus that lead to an asthma phenotype. Thus a scientific and clinical issue is to discover the number and nature of these phenotypes. Evidence for one possible phenotype comes from the group in Winnipeg who first developed an animal model in which one-half of a litter of mongrel dogs were injected with ragweed pollen (sensitized) while the other half served as “littermate controls” (23). When muscles from both sensitized and control animals were stimulated electrically, force rose to its plateau level in ~10 s. The rate of rise and the final level of force were similar in both types of muscle (1); however, muscles from the sensitized animals had significantly higher shortening velocities for the first 2 s of stimulation and achieved substantially shorter lengths when allowed to shorten under light load early in the tetanus (40). This potential for more extensive early shortening would facilitate greater bronchoconstriction when the smooth muscle is first stimulated and could therefore be an asthma phenotype. Chemical assays revealed that myosin light chain kinase was significantly increased in the sensitized animals (21).
We focus on this work because it is not intuitively obvious that an increase in activating enzyme would increase only the velocity and extent of shortening at light loads early in a contraction without affecting later force or shortening and without substantially altering the rise of isometric force. But these results are easily explained by the original, two-state, cross-bridge theory proposed by A. F. Huxley in 1957 (18) to demonstrate how a sliding filament mechanism produces the force, shortening, and heat rates measured in skeletal muscle (16).

In this model, force and shortening were assumed to be generated by sidepieces extending from the myosin filaments that cyclically attached to and detached from actin filaments, so that the two states were attached and detached. By endowing each bridge with an internal spring that supported force between the filaments when the bridge was attached and by judicious choice of functions to describe the attachment and detachment rates for crossbridges, Huxley (18) quantitatively reproduced the mechanical behavior of skeletal muscle. By allowing one ATP molecule to be hydrolyzed per cross-bridge attachment-detachment cycle, he reproduced the heat data.

The model has since been expanded to include additional states (19) to account for more recently discovered mechanical “transients” that occur when attached cross bridges undergo movement that alters spring tension (20) and for chemical reactions that must occur as ATP is consumed. But this recent emphasis on more complex models does not detract from the utility of the original theory in explaining steady-state behavior when attachment and subsequent force generation are lumped into a single transition, and chemical reactions are assumed not to affect these mechanical transitions.

The model is already familiar in the airway smooth muscle community, having been used by a group at the Harvard School of Public Health to model aspects of tracheal muscle behavior (28). Only one aspect of the model is needed for the current discussion. This relates to events during the rise of force in a tetanic contraction.

In his classic description of “active state,” A. V. Hill (17) proposed that muscle was activated almost instantly and that the observed slow force development was due to internal shortening of the contractile elements as they stretched the series elastic elements. Huxley (18) offered a different explanation, that the slow force development is not necessarily due to internal shortening but to slow attachment of bridges in the fully activated isometric muscle. This explanation has been confirmed by the finding that force rises relatively slowly when the sarcomeres are held rigidly isometric, so that there is no internal shortening, and that stiffness, taken as a measure of attached cross bridges, rises only slightly faster than force (8). In contrast to the slow rise of isometric force, the model predicts, and experiment confirms, that shortening velocity under a constant load achieves its steady value very rapidly, within the time taken to shorten ~0.1–0.15% of muscle length (5), equivalent to ~1 cross-bridge stroke length. Rapid attainment of steady shortening occurs because bridges detached by the shortening begin new cycles according to the new steady conditions. The disparity between the slow rise of isometric force and the rapid approach to steady shortening velocity under light loads early in a tetanus is precisely the mechanism to explain the results from Winnipeg. More rapid activation, caused by more abundant light chain kinase, will not substantially increase the rate of force development because this is determined largely by the rate of cross-bridge attachment under isometric conditions. It will also not increase the final level of force achieved because the force developed during a tetanus is near the maximum that the muscle can achieve, but it will significantly increase the ability of the muscle to shorten under light loads early in the contraction.

The importance of the Winnipeg experiments is that they explain how immune reactions can lead to expression of an altered smooth muscle phenotype that will produce bronchospasm when the muscle is first activated. Their relevance to the articles in focus is that they show how events in early development can alter the expression of the muscle phenotype to make it hyperresponsive.

The two articles in focus open a window on important changes that occur in airway smooth muscle as an animal matures. These studies emphasize both the relevance of mechanical measurements of muscle activity in the study of reactive airway disease and the importance of maturational changes in the development and maintenance of asthma. The work completed thus far already suggests likely areas to search for therapeutic targets for treating airway hyperreactivity in infants and young children and for preventing the development of hyperresponsive airways in susceptible children.

REFERENCES


