Signal Transduction in Smooth Muscle

Historical perspective on airway smooth muscle: the saga of a frustrated cell

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Seow, C. Y., and J. J. Fredberg. Historical perspective on airway smooth muscle: the saga of a frustrated cell. J Appl Physiol 91: 938–952, 2001.—Despite the lack of a clearly defined physiological function, airway smooth muscle receives substantial attention because of its involvement in the pathogenesis of asthma. Recent investigations have turned to the ways in which the muscle is influenced by its dynamic microenvironment. Ordinarily, airway smooth muscle presents little problem, even when maximally activated, because unending mechanical perturbations provided by spontaneous tidal breathing put airway smooth muscle in a perpetual state of “limbo,” keeping its contractile machinery off balance and unable to achieve its force-generating potential. The dynamic microenvironment affects airway smooth muscle in at least two ways: by acute changes associated with disruption of myosin binding and by chronic changes associated with plastic restructuring of contractile and cytoskeletal filament organization. Plastic restructuring can occur when dynamic length changes occur between sequential contractile events or within a single contractile event. Impairment of these normal responses of airway smooth muscle to its dynamic environment may be implicated in airway hyperresponsiveness in asthma.

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that populate the body, we might think of airway smooth muscle as the Hell's Angel of cells, sitting on a Harley-Davidson, unshaven, a cigarette in one hand, a can of beer in the other, and a tattoo on its arm reading "Born to Lose."

For the purposes of this article, we define the basic contractile unit of airway smooth muscle as being the cross bridge, which is comprised of the motor protein myosin II and accessory proteins, and its cyclic interaction with filamentous actin. This contractile unit exerts its mechanical effects within a cytoskeletal scaffolding that is extensible (42, 70, 108, 156), in a continuous state of remodeling (48, 52, 106, 107, 133, 140), and subject to never-ending fluctuations in load that are associated with the tidal action of spontaneous breathing (31, 35, 49, 81). Considerations of the dynamic microenvironment within which the contractile unit operates have led to recent investigations of 1) acute changes in muscle mechanics that are associated with bridge-based mechanisms and their dynamic equilibration and 2) chronic changes in muscle mechanics that are associated with non-bridge mechanisms and muscle plasticity (58). These two manifestations of the dynamic microenvironment, and their mutual interactions, are the focus of this perspectives article. Because they have been covered in recent reviews, this article does not address the topics of phenotypic plasticity, cell proliferation, myosin isoform expression, accessory proteins, synthetic cellular functions, adhesion molecules, intracellular signaling, regulation of the activity of myosin II, phosphatase regulation, gene expression, or neural mechanisms in asthma (3, 12, 58, 66, 75, 76, 89, 103, 148).

THE CHANGING PICTURE OF AN ADAPTABLE CELL

What Good is Airway Smooth Muscle, Anyway?

It had been recognized quite early that lungs are irritable and that stimulation of its contractile machinery in an animal with an open chest can cause air to be expelled from the lungs and can cause a rise in intracheal pressure and an increase in airways resistance (14, 23, 104, 129). However, until the second half of the previous century, airway smooth muscle was not regarded as being a muscle of any particular significance in respiration mechanics (129). Airway smooth muscle was first described in 1804 by Reisseisen [as related by Otis (129)], and its functional properties were first considered by Einthoven (27) and Dixon and Brodie (23). More recent studies have shown that the fraction of the tissue volume that is attributable to contractile machinery is comparable for airways, alveolated ducts, and blood vessels in the lung parenchyma (124); the lung parenchyma, like the airway, is a contractile tissue (15, 24, 32, 91, 93).

Identification of the normal physiological role of airway smooth muscle remains elusive (129); in that regard, airway smooth muscle stands in contrast with other smooth muscle systems whose primary functional roles are self-evident. Mead (104) questioned the extent to which changes of smooth muscle tone might play some homeostatic role to stabilize airways and airspaces. He speculated that a moderately constricted state of airway smooth muscle may make airways behave more like the lung parenchyma in which they are embedded, thus improving the homogeneity of lung expansion; he reasoned that homogeneous lung expansion might depend on mechanical interdependence among lung structures all operating on a background of smooth muscle activity. It has been argued by others that contraction of airway smooth muscle might serve to modulate the tradeoff between dead space vs. airway resistance in a way that minimizes the work of breathing (164), serve to adjust airway caliber among parallel pathways and parenchymal compliance among peripheral lung regions in a way that optimizes the distribution of ventilation (18, 25, 124), serve to narrow the airway in a way that improves the ability of cough to expel worms or other foreign objects from the airway (P. Barnes, personal communication), serve to stiffen the airway sufficiently to prevent extreme airway collapse during forced expiration (6, 125), or serve to match the mechanical hysteresis of small airways and alveolated ducts to the rather appreciable mechanical hysteresis of the alveolar surface film in a way that allows for synchronous and uniform alveolar expansion (36, 104, 110). Schittny et al. (137) demonstrated that, throughout gestation of the fetal mouse lung, there exist peristaltic waves of airway smooth muscle contraction propagating proximal to distal in the airways; they showed that fluid displaced by this milking action maintains an appreciable positive intraluminal pressure in peripheral airways and airspaces and suggested that this fluctuating distending pressure might provide a crucial stimulus for lung growth in utero.

Each of these arguments is plausible, but evidence in each case remains less than compelling. Still another explanation for the utility of airway smooth muscle, and perhaps a better one, is that there is no explanation; that is to say, both the phylogeny and the ontogeny of the lung show that airways derive from foregut, so it cannot be ruled out that the presence of smooth muscle within this adapted piece of gut is vestigial and represents nothing more than a frozen accident of nature that finds no useful function in the lung.

Airway Hyperresponsiveness: The Story of a Good Muscle Gone Bad?

Airway hyperresponsiveness is the term used to describe airways that narrow too easily and too much in response to challenge with nonspecific contractile agonists (167). Airway hyperresponsiveness is the basic feature that underlies the excessive airway narrowing that is characteristic of asthma, but its mechanism remains unknown (82). Although asthma is usually defined as being an inflammatory disease, the link between the immunological phenotype and the resulting mechanical phenotype associated with disease presentation, including airways hyperresponsiveness, remains unclear; indeed, it is now established that airway hyperresponsiveness can be uncoupled from...
airway inflammation (10, 19, 65, 88). It remains equally unclear whether airway hyperresponsiveness is due to fundamental changes in the phenotype of the smooth muscle, structural and/or mechanical changes in the noncontractile elements of the airway wall, or alterations in the relationship of the airway wall to the surrounding lung parenchyma (82, 111).

A related paradox concerns what Brown and Mitzner (9) called the “myth” of maximal airway responsiveness. On the one hand, airway smooth muscle is now known to possess sufficient force-generating capacity to close all airways (9, 50, 102, 161). On the other hand, challenge of healthy subjects with nonspecific contractile agonists in concentrations thought to be sufficient to activate the muscle maximally results in airway narrowing that is limited in extent, and that limit falls far short of airway closure (22, 49, 166). This is reflected in the fact that normal individuals exhibit a plateau of their dose-response curve (166), indicating that some mechanism must have come into play to limit smooth muscle shortening. As anticipated by Macklem in 1987 (97), the mystery of airway hyperresponsiveness seems to be not so much why airway narrowing can become extensive in the asthmatic lung, for it would be expected to do so, but, rather, why do not all airways close during maximal bronchoprovocation of the healthy lung in vivo, since the capacity of airway smooth muscle to shorten is sufficient to close all airways.

The classical understanding of airway lumen narrowing rests on the idea that there exists a definite force-length relationship that describes the active steady-state force generated by any given muscle for any particular degree of activation. Muscle length and airway caliber are then thought to be set by a balance of static forces in which the active steady-state force generated by airway smooth muscle is in mechanical equilibrium with the passive reaction force developed by the elastic load against which that muscle shortens (100, 117). Because both the active force and the load vary with muscle length, the muscle would be predicted to accommodate itself on its force-length curve to the length at which these opposing static forces come into balance (50, 86, 100, 117).

The isometric force-generating capacity of airway smooth muscle is set principally by muscle mass, muscle contractility, and muscle position on its static force-length characteristic (100, 153, 165). Taken together, evidence available in the literature seems to suggest that there is no systematic difference in stress-generating capacity between muscle from the normal vs. the asthmatic lung, although this evidence is limited and equivocal (4, 5, 121, 146, 149). Recent studies of airway morphometry coupled with a computational model of the balance of static forces, referred to above, have led to the conclusion that changes in smooth muscle mass may play a dominant role in the emergence of airway hyperresponsiveness (86, 87, 103, 165), although the evidence is conflicting (153); moreover, this conclusion must be regarded as being tentative until it is revisited, taking into account the newly discovered dynamic factors discussed below. The passive reaction force against which the muscle shortens is set principally by the elasticity and geometry of the airway wall, tethering of the airway to the lung parenchyma, and the state of lung inflation (22, 77, 84, 105, 118, 122). Each of these factors, in turn, has its own determinants that are known to be modified with chronic airway inflammation (64, 72, 86, 111, 131).

This balance of static forces is sustained by the underlying cyclic interactions of myosin with actin. With onset of the contractile event, myosin–actin cycling begins and the number of interactions (i.e., attached bridges) increases and eventually approaches a steady state. It is widely agreed that during this process rapidly cycling cross bridges are replaced progressively by slowly cycling latch bridges if given enough time at a fixed muscle length, but the mechanisms of cycling rate regulation and the latch phenomenon remain very much an open question in the literature (11, 21, 53, 54, 66, 74, 80, 135, 163). It retrodicts, the word “latch bridge” may have been an unfortunate choice of terms, for it has sometimes been taken to connote somewhat more than the simple idea that Murphy intended, namely, bridges with very slow but nonetheless nonzero rates of bridge cycling (R. A. Murphy, personal communication). Several mechanisms are now known to come into play even farther upstream to regulate both the kinases and the phosphatases that in turn regulate phosphorylation of the 20-kDa myosin regulatory light chain (11, 80, 135, 163), and the accessory molecules caldesmon and calponin are also known to modulate bridge-cycling rates (39). Thus the simple latch scheme of Hai and Murphy (55, 57) by itself is now seen as being incomplete. Nonetheless, the latch hypothesis remains a central organizing concept because it captures the importance of phosphorylation of the myosin regulatory light chain in cycling rate regulation.

Regardless of the mechanisms of cycling rate regulation, the attainment of an isometric steady-state contraction implies that the population distribution of myosin molecules among their four possible states (attached vs. unattached to actin, phosphorylated vs. unphosphorylated regulatory light chains) and all associated processes have come to a binding equilibrium set by a balance of kinetic rate processes, many of which are ATP dependent. Once enough time has passed that this balance is attained and myosin has come to a binding equilibrium appropriate to isometric steady-state conditions (i.e., a static equilibrium of myosin binding), the muscle is then said to be in the latch state. The term “latch state” must be taken somewhat advisedly, however, because the particular distribution of attached myosin heads among phosphorylated vs. unphosphorylated species in steady-state conditions is thought to depend on the level of activation and therefore the countervailing influences of myosin kinases and phosphatases.

These central concepts, a definite active force-length curve, a corresponding balance of static forces at the mechanical level, and an underlying static equilibrium
of myosin binding at the molecular level, have formed
the three cornerstones of the classical understanding of
the role of airway smooth muscle in airway lumen
narrowing in the healthy and the asthmatic lung (100,
104, 118, 165). New evidence now shows, however,
that, for each of these three regards, the classical point
of view is probably not correct and seems to be so far
from the mark as to fail even to provide a reasonable
approximation (8, 31, 34, 35). The reason for this fail-
ure is that the classical view is limited to consider-
ations of equilibrium muscle states and static factors,
whereas regulation of airway smooth muscle length is
now known to be a nonequilibrium process that is
fundamentally dynamic. Under nonequilibrium condi-
tions, the airway need not conform to the expected
behavior of static equilibrium systems.

This is not meant to imply that equilibrium consid-
erations can be dismissed. To the contrary, Macklem
(98) has pointed out that, once the muscle has become
maximally activated (and, we would now say, come to a
static equilibrium), the forces and loads become all
important; the level of the plateau response of the
dose-response curve in normal individuals becomes
essentially uncoupled from underlying biochemistry
and cell biology. It is the absence of this plateau in the
asthmatic patients that makes asthma such a serious
disease. The static balance that Macklem spoke about
remains highly relevant because it is the static state to
which the dynamically equilibrated system would col-
lapse if destabilized, as described below, and may ac-
count for the loss of the plateau of the dose-response
curve in the asthmatic subject.

EQUILIBRATION WITHIN THE DYNAMIC
MICROENVIRONMENT

Airway smooth muscle is loaded by the oscillatory
stresses and strains that are imposed continuously by
the tidal action of spontaneous breathing. Of course,
muscular arteries and arterioles are subject to oscilla-
tory stresses caused by the pulsatile action of the
heart, and smooth muscles in the urethra, urinary
bladder, and gut are also subjected to periodic
stretches. Dynamic loading would appear to be an
intrinsic part of smooth muscle physiology.

In the case of airway smooth muscle, the effects of
time-varying loads were first addressed by Sasaki
and Hoppin (136) and later by Loring and colleagues (88a)
and Gunst and colleagues (46, 47, 49); these researchers
demonstrated that imposition of tidal changes in muscle
length depresses active force. Subsequent studies (35)
showed that imposed fluctuations of muscle length about
a fixed mean length cause depression of muscle force and
muscle stiffness (averaged over the stretch cycle); im-
posed length fluctuations also cause augmentation of the
specific rate of ATP utilization and the hysteresivity
[related to the muscle viscosity and an index of bridge
cycling rate, as described below (36)]. Imposed force fluc-
tuations about a fixed mean distending force systemati-
cally biases the airway smooth muscle toward lengthen-
ing; this phenomenon is called fluctuation-driven muscle
lengthening (35). Although tidal stretches smaller than
1% of muscle length produce only trivial mechanical
effects, tidal stretches in the range of amplitudes ex-
pected during quiet tidal breathing (about 3% of muscle
length) produce force inhibition that is equipotent with
concentrations of isoproterenol in the range $10^{-7}$ to $10^{-5}$
M (44). Molfino et al. (115) showed in humans that brief
cessation of tidal breathing causes the cross-sectional
area of central airways to decrease by about one-half
under the influence of baseline smooth muscle tone, and
when tidal breathing is resumed the airway promptly
dilates. Taken together, these findings suggest that quiet
tidal breathing is as effective in relaxing airway smooth
muscle as is a potent relaxing agonist (44).

Importantly, there is increasing evidence that the
potent bronchodilating response to periodic stretch and
deep inspirations is impaired in asthma and that this
impairment may be the proximal cause of the loss
of the plateau of the dose-response curve and the result-
ning morbidity of the disease (30, 82, 116, 144). There
exists also a bronchoprotective effect of deep inspira-
tions that is perhaps even more important than the
bronchodilating response, and it too is profoundly im-
paired in asthma (78, 101, 138, 139).

What mechanisms might account for impairment of
these salutary effects of lung inflation on airway
smooth muscle? There is a long history implicating
abnormal neural mechanisms in the pathogenesis of
asthma (3, 12, 75, 76, 115), but we know of no report
connecting neural mechanisms to impairment of the
dilating effects of a deep inspiration in asthma. Alter-
natively, we do know that there are at least two classes
of phenomena through which periodic load fluctuations
can influence airway smooth muscle shortening and
airway narrowing. These have come to be called “per-
turbed equilibria” of myosin binding and muscle “plas-
ticity,” respectively, and have different biochemical
and mechanical mechanisms as well as different con-
sequences. Nonetheless, both phenomena are condi-
tioned by the dynamic microenvironment within which
the airway smooth muscle cell operates and, therefore,
upstream factors, including the pattern of lung expan-
sion, lung recoil, transmission of forces to the airway,
and airway wall remodeling (31). Moreover, these two
classes of phenomena have the potential to interact
(82). We deal first with perturbed equilibria of myosin
binding.

PERTURBED EQUILIBRIA OF MYOSIN BINDING

Melted and Frozen Contractile States

Lung inflations strain airway smooth muscle with
each breath, and these periodic mechanical strains are
transmitted to the myosin head and cause it to detach
from the actin filament much sooner than it would
have in an isometric contraction (35, 109). Lung infla-
ton perturbs the binding of myosin to actin by the
direct effect of mechanical force acting on the actomy-
osin bond. With somewhat fewer bridges attached, the
muscle will then stretch even more with the next
breath, and so on. Eventually, a steady state is reached
in which this positive feedback process becomes dynamically equilibrated. Compared with the isometric steady state, in the dynamically equilibrated state only a small fraction of the bridges that can attach are attached. This is because premature detachments precipitated by tidal stretch reduce the duty cycle of myosin, typically by as much as 50–80% of its isometric (i.e., unperturbed) steady-state value, and depresses total numbers of bridges attached and active force to a similar extent (35, 109). As a result, the muscle can stay compliant and long even when the level of muscle stimulation is supramaximal. This perturbed state is also characterized by rapid bridge cycling and a high rate of ATP utilization per attached bridge or per unit force developed. Thus, from both the mechanical and the metabolic point of view, the perturbed state may be regarded as a hot or “melted” contractile state [S. Permutt, personal communication; (31, 35)]. Recent evidence suggests that it is this dynamically equilibrated state of affairs that is the key factor that limits the extent of narrowing during challenge of the healthy airway and thereby maintains the plateau of the dose–response curve. If so, the normal pattern of spontaneous breathing, which is punctuated by relatively frequent deep inspirations, is sufficient, but just barely (35, 81, 116, 141, 144).

In some circumstances, however, the tidal strains acting on myosin can become compromised. For example, force fluctuations impinging on airway smooth muscle are linked intimately to the peribronchial stress distending the airway and its changes in time. Any factor that lessens peribronchial stress will decrease the force fluctuations impinging on the muscle, including inflammatory thickening of the lamina reticulosa, thickening of the peribronchial adventitia, loss of lung elastic recoil, breathing at low lung volumes, and failure to take deep breaths (22, 86, 99, 100, 118); in addition, Colebatch and colleagues (13) found evidence of increased rigidity of airways in asthmatic subjects. If this is so, then the perturbed equilibrium of myosin binding can collapse toward a static binding equilibrium. For example, if for any reason the muscle should stretch a bit less, then fewer bridges would be perturbed. Because more attached bridges working in parallel are harder to break than fewer, the muscle would then become stiffer still and therefore stretch even less, and so on. Ultimately, this positive feedback process reaches the limit in which the muscle may become so stiff that the physiological forces acting on it are insufficient to stretch the muscle appreciably, leaving the muscle stuck at its static equilibrium length (31, 35). Compared with the perturbed state, this statically equilibrated contractile state is also characterized by slow bridge cycling and a small rate of ATP utilization per bridge attached or per unit force developed (35). Thus, from both the mechanical and the metabolic point of view, it is a cold or “frozen” contractile state. The muscle could be said to be frozen in the latch state.

This point of view leads to the hypothesis that airway hyperresponsiveness is associated with the failure of the underlying perturbed binding equilibrium to sustain itself and an ensuing collapse of myosin binding kinetics to the binding equilibrium that pertains in static conditions and latch (31). Clearly, this constellation of factors points toward dynamic instability, as described below.

**“Quantum Mechanics” of the Contractile Response?**

It is a well-established empirical fact that within any given lung or lung segment the response of airways to contractile agonists is always accompanied by 1) extreme heterogeneity of the response and 2) sensitivity of the response to the amplitude of the tidal volume and the magnitude of the load fluctuation (7, 33, 68, 90, 92, 94, 141, 152). It has been speculated that the observation of a smoothly graded decline of tests of lung functions (such as lung resistance) with progressively increasing doses of contractile agonist might be better explained by progressive changes in a number of open airways accommodating gas flow rather than by some smoothly changing reduction in the airway caliber of each (33). Indeed, the heterogeneity of the response is so extensive that peripheral airways have sometimes been thought of as being partitioned into only two quantum-like states, as it were, either open wide or almost closed entirely, with virtually no intermediate state (33, 130, 150). Several plausible factors have been invoked to try to account for this profound heterogeneity of the contractile response, including intrinsic inhomogeneities in airway structure, muscle amounts, muscle sensitivity to agonist, and nonuniformity of agonist delivery (9, 92, 113).

Anafi and Wilson (1) considered the narrowing of an airway containing activated airway smooth muscle subject to load fluctuations as would occur during breathing. Their mathematical analysis shows that in some circumstances such an airway must become unstable. Of course, the generic idea that airways can be unstable is not at all new (96), but the analysis of Anafi and Wilson identified a new class of airway instability that is intrinsically dynamic and, in particular, rooted in the response of airway smooth muscle to imposed load fluctuations.

To appreciate the novel aspects of this muscle instability, we imagine a lung in which there are a large number of airways that are all operating in parallel and that are in every regard identical. The airway smooth muscle is activated uniformly and subjected to identical load fluctuations caused by the tidal action of breathing. Although interregional differences are infinitesimally small at the outset, the positive feedback processes described above amplify any small differences. And, as the differences grow, the amplification grows. When the process eventually becomes dynamically equilibrated, the airways will be seen to have partitioned themselves between only two states to accommodate the total flow, one state effectively wide open and the other nearly closed. Airway smooth muscle in the closed airways would be expected to correspond to the static or “frozen” state, and muscle in the
open airways would be expected to correspond to a "melted" state with an underlying perturbed equilibrium of myosin binding. Changes in tidal volume would affect the number of airways in each state.

In the healthy lung during spontaneous breathing, tidal volume and associated force fluctuations acting on airway smooth muscle may be large enough to keep almost all units in the open/melted state and out of jeopardy of closure. However, if tidal volumes were compromised, or deep inspirations were prohibited, or transmission of force fluctuations to the airway smooth muscle were to become compromised, then a larger fraction of units would be expected to be found in the closed/frozen state. Therefore, this picture would seem to be able to explain in one stroke both a profound heterogeneity of the airway response and its sensitivity to tidal volume. In doing so, it would seem to resolve the "myth" of maximal airway responsiveness and its associated paradox.

This insight on instability of smooth muscle dynamics does not preclude important contributions from intrinsic heterogeneities that exist among airways (26, 113). The Áanfi-Wilson instability is attractive, though, because it shows that it is not necessary to postulate intrinsic airway heterogeneity of any kind to account for both a profound heterogeneity of the airway response and its sensitivity to tidal volume.

In this connection, Jensen and colleagues (73) have shown in normal individuals challenged with nonspecific contractile agonists that a single deep inspiration causes a prompt and dramatic decrease in airway resistance followed by a slow return of airway resistance to the level observed before the deep inspiration. By contrast, in individuals with severe asthma, a single deep inspiration causes a prompt but only modest decrease in airway resistance followed by a rapid return of airway resistance to the level observed before the deep inspiration. The findings in challenged normal individuals are consistent with the following interpretation. In at least a large fraction of the narrowed airways, the stiffness of airway smooth muscle was small enough that the muscle stretched in response to the deep inspiration, bridges were disrupted, and the muscle slowly recontracted thereafter. In asthmatic individuals, by contrast, the muscle in narrowed airways stretched substantially less in response to the deep inspiration (presumably because the muscle was stiffer, the physiological forces impinging on the muscle were smaller, or both). Bridges were stretched but not enough to disrupt an appreciable fraction of actomyosin bonds. Immediately after the deep inspiration, the rapid recovery of airway resistance to pre-deep-inspiration levels could reflect either a rapid elastic recoil of the stretched muscle, an altered cytoskeletal remodeling response to the deep inspiration (described below), or recontraction of bridges that were disrupted at a rate that is faster than that observed in healthy individuals. This latter idea brings us to shortening velocity.

**Speed Kills?**

A consistent association has been noted between airway hyperresponsiveness and shortening velocity even when the force-generating capacity of the muscle is the same (2, 26, 28, 157). This association suggests the possibility that the problem with airway smooth muscle in asthma may be that it is too fast rather than too strong. How shortening velocity, a dynamic property of the muscle, might cause excessive airway narrowing (a parameter that was thought to be determined by a balance of static forces) was not clear. One potential explanation looks to changes of the muscle load rather than to changes of the contractile machinery itself. Faster shortening velocities could reflect reduced intracellular or extracellular loads against which the contractile machinery is shortening (26). A reduced load would explain both the higher shortening velocity and the greater degree of shortening.

An alternative explanation suggests that higher shortening velocity implies intrinsically faster myosin cycling (31), probably due to increased intracellular concentrations of myosin light-chain kinase (28). The faster the myosin cycling, the more difficult it would be for imposed load fluctuations to perturb the actomyosin reaction; the faster the intrinsic rate of cycling, the faster a bridge, once detached, reattach and contribute again to active force and stiffness. Load fluctuations are as potent a bronchodilator as the most potent pharmacological agencies that we know of (44), but their effects would be expected to be compromised in faster muscle (31, 146).

**Myosin Bond-Length Distributions**

The behavior of airway smooth muscle operating within a dynamic microenvironment, as described above, seems to be explained in large part by the length distribution of the myosin bond and changes in that distribution that are induced by tidal stretch. The myosin molecule is composed of a globular head (the S1 subfragment), which can bind to the actin filament, and a neck region (the S2 subfragment), which acts as a simple spring connecting the S1 subfragment to the myosin backbone (tail region). According to Huxley's sliding filament model of muscle contraction (69), if a myosin head happens to be attached to actin at a position where the extension of S2 spring is zero, then, logically, that bridge contributes to muscle stiffness, but the force generated by that bridge must be zero. Bridges that find themselves attached with greater extension of the S2 spring (whether due to the myosin power stroke or an imposed muscle stretch) would contribute to force in simple proportion to that extension. The distribution of S2-subfragment extensions across the myosin population is called the bond-length distribution. Because the S2 subfragment links the globular head to the myosin backbone, it bears the active force created by the actomyosin interaction. Accordingly, if this bond-length distribution is known, then the macroscopic mechanical properties, metabolic...
properties, and heat release are readily determined (69, 109).

Computational analysis of the effects of load fluctuations on the myosin bond-length distribution suggests that imposed load fluctuations decrease the mean number of attached bridges (both phosphorylated and nonphosphorylated), depress muscle force and stiffness, and increase force-length hysteresis (35, 109). At frequencies in the breathing range (<0.1 Hz), the bond-length distribution of slowly cycling latch bridges changes little over the stretch cycle and contributes almost elastically to muscle force, but the rapidly cycling cross-bridge distribution changes substantially over the stretch cycle and dominates the hysteresis. By contrast, at lower frequencies (<0.033 Hz), this dynamic behavior is reversed; the rapid cycling cross-bridge distribution changes little over the stretch cycle and functions as a constant force generator, whereas the latch bridge bond distribution changes substantially and dominates the stiffness and hysteresis (109).

Interestingly, conflicting evidence has arisen as to whether the muscle hysteresivity is (36) or is not (141, 142) an index of bridge cycling rate and its changes. This conflicting evidence seems to be reconciled by examination of the bond-length distributions, which shows that a dissociation of muscle hysteresivity from cross-bridge cycling rates occurs when the strain amplitude is bigger than a few percent of muscle length. In the case of stretches that are large enough to perturb myosin binding (141, 142), there is only a weak coupling between net external mechanical work and the ATP consumption required for cycling cross-bridges during the oscillatory steady state. By contrast, in the case of stretches that are so small that myosin binding is not appreciably perturbed (i.e., stretches of less than 1% of muscle length), mechanical work, hysteresis, bridge cycling rate, and ATP utilization are closely associated with one another (36).

Filament Extensibility

It is known that smooth muscle conforms to Hill’s equation relating force and shortening velocity, and it is generally presumed that smooth muscle conforms to Huxley’s sliding filament model of muscle contraction, even though the structural basis for this presumption is invalid (56, 119, 120).

It was reported only recently that actin and myosin filaments are rather extensible (70, 156). Goldman and Huxley (42) were quick to point out that this finding invalidates a cornerstone on which the sliding filament model of muscle contraction rests. This left the venerable theory of muscle contraction teetering at the precipice, and therefore represented a rather disconcerting state of affairs. Of course, over the years that followed Huxley’s 1957 report, there followed a series of increasingly sophisticated models of myosin binding dynamics that replaced Huxley’s original description, but these were also in jeopardy because they all rest on the same implicit assumption of sliding filaments that are inextensible. Fortunately, though, the sliding filament theory was quickly pulled from the precipice by a reformulation that took into account filament extensibility (108) but not simply and not without ramifications. This reformulation turned out to explain phenomena that were previously unexplained or, worse, misunderstood and led to unexpected complications that bear directly on airway smooth muscle.

The reformulated theory of muscle contraction shows that, during force development, growing cross-bridge tractions transfer loads locally from thick to thin filaments, causing them to extend and therefore to slide locally relative to one another, even during a contraction in which the muscle is not allowed to shorten. On a local scale, then, the very notion of “isometric” force development becomes a logical impossibility, even when spot-follower servo technology is brought into play to regulate sarcomere length. Such behavior implies that even with a slight degree of filament extensibility 1) relative displacement between the two filaments must be nonuniform along the region of filament overlap, 2) cross-bridge strain must vary systematically along the overlap region, and, importantly, 3) the local shortening velocities, even at constant overall sarcomere length, reduce force well below the level that would have developed if the filaments had been inextensible.

EMERGENCE OF PLASTIC PHENOMENON AND ITS DIFFERENT FACES

Hill (63) noted that, more than being of interest merely for its own sake, muscle contraction may be an instructive model of a wider class of cellular processes because, in the special case of muscle, mechanical stress and unloaded shortening velocity happen to allow direct assessment of the number of molecular interactions and their rate of turnover. As discussed below, Hill’s idea is now being extended to questions not anticipated at the time of his statement, such as alterability of the cytoskeletal scaffolding, in which airway smooth muscle has proved to be an interesting model of cell plasticity that is of broader interest in cell biology.

The plastic behavior of smooth muscle becomes obvious when one uses the relatively “nonplastic” striated muscle model as a point of reference (132). For instance, smooth muscle in urinary bladder can tolerate a manifold length change without losing its contractile function (155); in striated muscle, by contrast, a 60% stretch from optimal length will reduce the filament overlap, and hence active force, to zero (43). In single isolated smooth muscle cells from toad stomach, Fay and co-workers (29) observed a large length range over which significant force can be maintained. In the same preparation, Harris and Warshaw (59) found a broad plateau in the length-force relationship when muscle length is set in the relaxed state. Plasticity of smooth muscle at the subcellular level is now thought to be an important factor accounting for both the extremely wide range of operating lengths and the ability of the
cell to reorganize itself over time to create near-optimal force at any fixed length.

Such plasticity of smooth muscle is consistent with the large volume changes that are accommodated with the function of the hollow organs that contain the smooth muscle cells. It may be reasonable to assume that different smooth muscles possess different degrees of plasticity, with the most plastic muscle cells lining the organs that undergo the largest volume change.

Although airway smooth muscle, in particular, is not known for a large range of working lengths, it has been found that the muscle is able to maintain maximal isometric force production over a threefold length range (133). Why would the ability to generate force over a large length range occur in a cell that is not required to undergo such length change in its normal function? A possible explanation is that, although different smooth muscle systems may have evolved to possess different properties to fulfill their specific functions, they all retain some basic properties, including plasticity. From the functional point of view, it is not important or even desirable for airway smooth muscle to generate maximal force over a large length range, but it is a simple fact that the muscle is capable of undergoing plastic restructuring of its contractile and cytoskeletal organization in response to external mechanical stimuli. Ordinarily, airway smooth muscle presents little problem because unending perturbations provided by tidal breathing and deep inspiration put airway smooth muscle in a perpetual state of “limbo,” keeping its contractile machinery off balance and unable to achieve its maximum potential. It is important to point out that mechanical perturbation affects airway smooth muscle contractility in two ways: it disrupts the cross-bridge attachment in activated muscle, as discussed above, and it also causes longer lasting effects through induction of plastic restructuring. Ironically, it is the “limbo” state of airway smooth muscle that seems to represent a healthy state for the airways. Plasticity in airway smooth muscle therefore is not only characterized by its ability to generate maximal force over a large length range (when given a chance to do so) but also by the ability of the tissue to disassemble its contractile apparatus when an appropriate stimulus is given and by the ability of it to reassemble when accommodated at a fixed length. It is important to point out that the structural evidence for the assembly and disassembly of contractile filaments in smooth muscle is weak; many of the structural changes discussed below are inferred from functional observations.

**Adaptations Within a Single Contractile Event**

Perturbed equilibria are necessary but not sufficient. When activated muscle in the muscle bath is subjected to progressively increasing load fluctuations approaching the magnitude and frequency expected during normal breathing, the muscle lengthens appreciably in response (35). But when load fluctuations are progressively reduced, the muscle reshortens somewhat but fails to return to its original length. Incomplete reshortening after exposure to tidal loading is not accounted for by muscle injury; the original operating length can be recovered simply by removing the contractile agonist and allowing the muscle a short interval before recontracting. Neither can incomplete reshortening be accounted for by myosin dynamics; myosin dynamics by themselves predict complete reshortening when the load fluctuations are removed (35). Thus the failure of activated muscle to reshorten completely is evidence of a plasticity of the contractile response. During a sustained contraction, the operational length of the muscle for a given loading, or the force at a given length, can be reset by loading and the history of that loading (35, 48, 51, 52, 133, 140, 158). In healthy individuals, this plasticity seems to work in a favorable direction, allowing activated muscle to be reset to a longer length. The asthmatic patient, it has been argued, never manages to melt the contractile domain in the airway smooth muscle, and, as such, the benefits of this plastic response are not attained.

Several hypotheses have been advanced to explain smooth muscle plasticity. Ford and colleagues (133) suggested that the architecture of the myosin fibers themselves may change, whereas Gunst and colleagues (45, 106, 107) argued that it is the connection of the actin filament to the focal adhesion plaque at the cell boundary that is influenced by loading history. Alternatively, another notion is that secondary but important molecules stabilize the cytoskeleton, and, as the contractile domain melts under the influence of imposed load fluctuations, those loads must be borne increasingly by the scaffolding itself and thus reflects plasticity of the cytoskeletal domain (31, 48, 58, 160); in this connection, a role for the RhoA pathway has been suggested (58, 107). Some evidence now suggests that the p38 mitogen-activated protein (MAP) kinase pathway may be involved (O. Lakser, unpublished observations). Airway smooth muscle incubated with an inhibitor of the p38 MAP kinase pathway demonstrates a greater degree of fluctuation-driven muscle lengthening than does control muscle; on removal of the force fluctuations it remains at a greater length. Moreover, force fluctuations themselves activate the p38 MAP kinase pathway. It is noteworthy in that connection that heat shock protein 27 has been implicated as an essential element in the motility of airway smooth muscle cells and is downstream target of Rho and p38 (39, 60–62, 169). These findings are consistent with the hypothesis that stress response pathways may somehow stabilize the airway smooth muscle cytoskeleton and limit the bronchodilating effects of deep inspirations.

Regardless of the specific molecules and mechanism invoked to explain the plasticity of the contractile response, the melting of the contractile domain would appear to be a necessary (or permissive) event, but one that by itself is not sufficient to explain the effects of the history of tidal loading.
Structural and functional changes during an isometric contraction. Birefringence (41), X-ray diffraction (162), and electron microscopy (40, 168) studies have revealed an intriguing phenomenon in some smooth muscles that the myosin thick filaments grow longer during the time course of an isometric contraction. If the finding is confirmed, the implication of this structural change in terms of the muscle function will be profound. The force increase during the late phase of an isometric contraction could be attributed to the thick filament lengthening (168). More provocatively, the decrease in shortening velocity that mirrors the increase in force during the late phase of contraction could be explained by the same mechanism, that is, lengthening of the thick filaments that results in the placement of fewer thick filaments in series spanning the cell length (140). In this model, the decrease in velocity during an isometric contraction can be explained without the need to assume a change in the cycling rate of the cross bridges (55). Mitchell et al. (114), using high time-resolution freezing techniques (95), have recently found that the myosin light-chain phosphorylation in airway smooth muscle has a time course that parallels the power output of the muscle and deviates significantly from the time course of shortening velocity change. These findings suggest that myosin light-chain phosphorylation may not be the only factor regulating shortening velocity. The same conclusion is reached by Miller-Hance and Kamm (112). If indeed the myosin filaments change length during the time course of a contraction, we have to readdress the question of how smooth muscle contracts.

Adaptations Between Sequential Contractile Events

Imposing a large length change, either a stretch or a release, on a relaxed airway smooth muscle has been shown to temporarily impair the ability of the muscle to generate force in the subsequent contractions (133, 159). A similar reduction in active force is also observed in airway smooth muscle after a brief period of length oscillation before activation of the muscle (159). The amount of force reduction is linearly related to the amplitude of length change (159), suggesting that the force decrease is determined by the degree of perturbation imposed on the muscle, which could be related to the degree of induced disorganization in the contractile and cytoskeletal filaments.

The decrease in isometric force after mechanical perturbation is not permanent; the active force recovers fully over a period of ~30 min (159) when the muscle is stimulated regularly at 5-min intervals. Less frequent stimulation results in a slower recovery (158, 159). These observations could be explained by a model in which plastic restructuring (i.e., disassembly followed by assembly) of the contractile and cytoskeletal filaments is induced by mechanical strains; reorganization of the filaments occurs when mechanical perturbation is stopped. The fact that stimulation of the muscle speeds up the recovery of the ability to generate maximal force (159) suggests that phosphorylation of certain proteins may be an integral part of the muscle adaptation process.

The geometric reorganization of the contractile filaments resulting from muscle adaptation at different cell lengths can be deduced from the changes in force-velocity characteristics of airway smooth muscle at different lengths (133). The observation that isometric force is muscle length independent (after adaptation) and that the shortening velocity is proportional to muscle length has led to the proposal of a model in which the number of contractile units in series is variable and is linearly related to the adapted muscle length (133). This model requires drastic structural changes that include adding and subtracting contractile units from the serial arrangement of the contractile apparatus. The mechanism for the structural rearrangement of contractile units remains unclear, although it is not unlikely that the structural lability of the contractile and cytoskeletal filaments may facilitate the plastic reorganization. It is noteworthy that, in skeletal muscle, the strategy for long-term length adaptation is by adding or subtracting sarcomeres from the serial arrangement of the sarcomeres in myofibrils, as suggested by the study of Jakubiec-Puka and Carraro (71). Adaptation in skeletal muscle occurs at a much slower pace (many hours or even days) compared with that in smooth muscle (which occurs in seconds or minutes).

The fact that a stretch applied to a relaxed airway smooth muscle temporarily reduces the subsequent force generation provides a possible explanation for the temporary bronchoprotective effect of deep inspiration taken before bronchoconstrictor challenge (101). Another line of evidence supporting this hypothesis is that the time course of increase in airway resistance after methacholine challenge in normal subjects prohibited from taking deep breaths (81) matches well the time course of recovery of the ability of airway smooth muscle to generate force after a stretch (159).

Myosin Evanescence

A functionally adaptable cell such as airway smooth muscle must have a malleable structure to accommodate the forces that shape the geometry of the cell and at the same time retaining contractile function. Evidence for the structural lability of myosin thick filaments has been around for a long time, as discussed below, but we are just at the beginning of recognizing the importance of the thick filament evanescence and how it may facilitate the cell adaptation.

Myosin thick filament formation in solution. In vitro solution studies have revealed that polymerization of smooth muscle myosin monomers is dependent on the chemical environment as well as the state of the myosin regulatory light-chain phosphorylation (126, 151). Under conditions that mimic the intracellular environment of a relaxed muscle (e.g., low calcium concentration and dephosphorylated myosin light chains), myosin filaments depolymerize. Whereas under conditions
that mimic the intracellular environment of an activated muscle (e.g., high calcium concentration and phosphorylated myosin light chains), polymerization of the monomers occurs. A dephosphorylated myosin monomer is in a folded conformation (10S) with low ATPase activity; phosphorylation of the light chains converts the folded monomer into an extended one (6S), which can then assemble with other extended monomers to form thick filaments (17, 20, 127, 128, 154). These in vitro studies suggest that myosin thick filaments are formed on activation of a smooth muscle and dissolved when the muscle is relaxed. The observations made in intact smooth muscle cells, however, are not as conclusive.

**Myosin thick filaments in intact smooth muscle cells.** Early electron microscopy studies of smooth muscle ultrastructure noticed that the myosin thick filaments are labile and present only in small numbers or not at all in preparations fixed in relaxed state (79, 123, 134, 143). Some of these outcomes are results of poor fixation (79). On the whole, smooth muscle thick filaments appear to be less robust than those in striated muscle. These observations seem to be consistent with the results of the in vitro studies described above and also consistent with the hypothesis that myosin thick filaments form during contraction and dissolve (at least partially) during relaxation (123). With different preparative techniques, some later studies however have shown thick filaments present in both contracted and relaxed states (16, 37, 38, 147). A recent study also shows thick filaments in relaxed airway smooth muscle (83). There is no doubt that myosin thick filaments exist in relaxed smooth muscle. The question of whether there is an increase in the number and/or length of the thick filaments when the muscle is activated, however, is unclear and has only been addressed (in a quantitative manner) in more recent studies (40, 41, 162, 168). As mentioned earlier, these studies have found that in some smooth muscles, such as anococcygeus, the thick filaments appear to be longer in the contracted state compared with those in the relaxed state, whereas in other smooth muscles, such as taenia coli, thick filament length does not appear to be affected by the state of activation (168).

Immunofluorescent staining of chicken gizzard 10S myosins shows that there is little of the 10S form in the relaxed state and that the amount does not change significantly during contraction (67). It appears that there is a large tissue-specific variability in the degree of myosin evanescence, which could explain some of the discrepancies observed in the early studies described above. The challenge that the field faces is to sort out which smooth muscle possesses myosin evanescence and also to determine whether the difference in the degree of evanescence among various smooth muscles is of a quantitative or qualitative nature.

It is possible that myosin thick filaments only dissolve in smooth muscles that have a long quiescent period between contractions. For smooth muscles that possess a substantial active tone or contract spontaneously and frequently, the thick filaments may not disaggregate. **Myosin evanescence in airway smooth muscle.** A recent study by Kuo et al. (83) provided evidence that the thick filaments in airway smooth muscle are labile, and partial dissolution of the filaments can be induced by cyclic mechanical strains comparable to those produced by deep inspirations. As found in previous studies, the ability of the muscle to generate force is reduced substantially after a brief period of length oscillation (83, 159). The amount of force decrease coincides with a similar amount of decrease in the density of the muscle’s myosin thick filaments (83). The time course of recovery of the muscle’s ability to generate maximal force after the length oscillation is shown to parallel the recovery of the myosin thick filament density (83). This is the first study demonstrating myosin evanescence due to mechanical perturbation. It provides support for the hypothesis that the force decrease after a length change in relaxed airway smooth muscle is due to partial dissolution of the thick filaments. More importantly, it suggests a crucial role of assembly and disassembly of the thick filaments in mediating the process of mechanical adaptation. **What are the signal transduction pathways for the thick filament assembly/disassembly?** The primary signal that starts the chain reaction and eventually leads to thick filament reorganization is the mechanical force that distorts the physical dimension of the cell. Because muscle cells are usually oriented axially along the direction of force transmission, the change in physical dimension due to external force is usually the muscle length. A change in muscle cell length therefore is likely the trigger that starts the adaptation process. Unfortunately, we know very little about the regulation of the adaptation process. It is not clear what kind of receptors are involved in the signal transduction or even if there are receptors involved at all. If the purpose of the cell response is to reorganize its contractile machinery so it can function optimally at a different cell length, then the first step in the response may be the disassembly of its existing contractile apparatus. The mechanical oscillation-induced partial dissolution of the myosin thick filaments observed in airway smooth muscle supports such a hypothesis (83). The initial events in the signal transduction pathways therefore are likely those that cause the contractile machinery (which includes contractile and cytoskeletal filament networks) to be demolished. This is then followed by reconstruction of the contractile apparatus to fit within the new cell dimension. During the “demolition” and “construction” phases of the cell response, different sets of protein phosphorylation may be involved. Any theories that delineate the pathways of length change-induced cell adaptation have to be able to accommodate the temporal sequence of events that are likely present in the cell adaptation process. Undoubtedly, the signal transduction will involve numerous “upstream events” that we know almost nothing about; therefore, we will not speculate about them here. At the bottom of the cascade of signal transduction, phosphorylation of the myosin regulatory light chain may be a prerequisite for thick fila-
ment formation, if we take clues from the in vitro solution studies (17, 20, 127, 128, 154). Interestingly, phosphorylation of the light chain also activates the myosin’s ATPase activity, which enables the myosin head (cross bridge) to interact with actin to generate force (145). The same process that turns on the cross bridges therefore also promotes thick filament aggregation. The reverse process, that is, the thick filament disaggregation, is not clear. Dephosphorylation of the myosin light chain does not seem to be enough to cause total disaggregation of the thick filaments because thick filaments can be seen in relaxed and unphosphorylated smooth muscles (16, 83, 147). Dephosphorylated thick filaments, however, are susceptible to dissolution by mechanical perturbation (83). The signal transduction pathway that leads to the dissolution is not known. One possibility is that the dissolution is caused directly by the mechanical disruption. In that case, no intermediate signal transduction is required.

CONCLUDING REMARKS

Airway hyperresponsiveness is one of the cardinal features of asthma but remains largely unexplained. Currently, much attention is being focused on the upstream events that initiate and then sustain the inflammatory response, with the expectation that improved understanding of these events will help to illuminate the causes of airway hyperresponsiveness. If these initiating events can be thought of as the ultimate cause of airway hyperresponsiveness, then this review turned attention to those factors that are at the most downstream end of the inflammatory cascade: the end-effector cell, that is, airway smooth muscle. Airway smooth muscle is the proximal agent of acute airway narrowing in asthma.

It has struck us that research in airway biology and research in smooth muscle biophysics had a parting of ways many years ago. Smooth muscle biophysics took on a life of its own and pursued a deeply reductionist agenda. At the same time, airway biology focused less and less on contractile function of muscle and increasingly on cellular and molecular biology of airway inflammation. The topics addressed in this review reflect recent activities that bring airway biology and smooth muscle biophysics into the same arena once again. This is appropriate because the endpoint is fundamentally mechanical; as Julian Solway once said, if airway inflammation didn’t cause bronchospasm, asthma might be a tolerable disease (personal communication). But asthma is not a tolerable disease. As such, an integrative approach that brings together a diversity of factors will be essential.

REFERENCES


950  HISTORICAL PERSPECTIVE


