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Length oscillation induces force potentiation in infant guinea pig airway smooth muscle

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Abstract

Deep inspiration counteracts bronchospasm in normal subjects but triggers further bronchoconstriction in hyperresponsive airways. Although the exact mechanisms for this contrary response by normal and hyperresponsive airways are unclear, it has been suggested that the phenomenon is related to changes in force-generating ability of airway smooth muscle after mechanical oscillation. It is known that healthy immature airways of both humans and animals exhibit hyperresponsiveness. We hypothesize that the profile of active force generation after mechanical oscillation changes with maturation and that this change contributes to the expression of airway hyperresponsiveness in juveniles. We examined the effect of an acute sinusoidal length oscillation on the force-generating ability of tracheal smooth muscle from 1 wk, 3 wk, and 2- to 3-mo-old guinea pigs. We found that the length oscillation produced 15–20% initial reduction in active force equally in all age groups. This was followed by a force recovery profile that displayed striking maturation-specific features. Unique to tracheal strips from 1-wk-old animals, active force potentiated beyond the maximal force generated before oscillation. We also found that actin polymerization was required in force recovery and that prostanoids contributed to the maturation-specific force potentiation in immature airway smooth muscle. Our results suggest a potentiated mechanosensitive contractile property of hyperresponsive airway smooth muscle. This can account for further bronchoconstriction triggered by deep inspiration in hyperresponsive airways.

Keywords

actin polymerization; deep inspiration; hyperresponsive airways; ontogenesis; prostanoids

Airway hyperresponsiveness is characterized by increased sensitivity of the tracheobronchial tree to contractile stimuli as well as an increased capacity for airway narrowing. Altered contractility of airway smooth muscle (ASM) is one of the mechanisms potentially responsible for increased airway sensitivity and maximal response. The involvement of ASM in the pathogenesis of airway hyperresponsiveness has long been controversial. However, both the capacity and velocity of shortening have been shown to be elevated in ASM from hyperresponsive airways (14,17). Increased ASM contractility shown in young animals suggests a central role for ASM also in juvenile airway hyperresponsiveness (8). It becomes, therefore, particularly important to study the mechanisms by which ASM function changes with maturation and whether this has a role in juvenile hyperresponsiveness and childhood asthma.

An important feature of altered airway function in asthma is the inability of deep inspiration (DI) to improve airway function (37). DI could even induce bronchoconstriction in asthmatic subjects (2,19). It has been convincingly shown that DI reverses bronchoconstriction in normal subjects by stretching contracted ASM (22). The protective effect of DI gained considerable research interest in recent years due to reports that showed a DI before the administration of a contractile agonist attenuates the subsequent bronchoconstriction and defined this behavior as bronchoprotective effect of DI (21,29). Recent studies suggest that asthmatics are mainly lacking the bronchoprotective component of DI (26). The bronchoprotective effect of DI can be explained by the adaptive behavior that ASM displays in vitro in response to mechanical oscillation (33,34). Existing observations of normal ASM from several animal species such as canine (24), swine (34), and rabbit (35) concur that a length perturbation triggers an initial reduction of active force followed by a process called adaptation, during which active force gradually recovers to preoscillation level with periodic stimulations. The process of smooth muscle adaptation has been suggested to involve rearrangements of contractile apparatus (24) and/or noncontractile actin cytoskeleton (12).

Although ASM adaptation can explain many of the phenomena related to bronchoprotection of DI, it remains unclear if and how ASM adaptation can account for the difference in response to DI seen in normal and asthmatic subjects. A step toward the ultimate goal of understanding the response of asthmatic ASM to DI is to understand the response of hyperresponsive ASM. It is currently unknown how hyperresponsive ASM responds to a length perturbation that simulates DI. If the hyperresponsive ASM exhibits an altered expression of adaptation, it could signify an important role of ASM in airway hyperresponsiveness associated with developing or diseased airways. In fact, some reports suggested that normal and diseased ASM may respond differently to mechanical stretches. For example, it is known that stretch could induce a contraction of smooth muscle referred to as myogenic response. The stretch-induced myogenic response is a recognized phenomenon in vascular smooth muscle (9,27). Induction of myogenic response has also been shown in guinea pig ASM, and this response could be abolished by inhibiting prostanoid release with indomethacin (11). Although normal human ASM does not exhibit myogenic response, Thulesius and Mustafa (31) suggested that a myogenic response of ASM could exist in asthmatic patients. If hyperresponsive ASM responds to mechanical stretches by elevating its contractile ability, it could contribute to the further exaggerated bronchoconstriction after DI in asthmatics.

In the present work, we studied ASM adaptation in a guinea pig maturational model and the roles independently played by prostanoids and actin polymerization. Our goal was to investigate whether and by what mechanism the mechanical adaptation is different in hyperresponsive (immature) from normal (adult) ASM.

METHODS

Animals and tissue sample preparation

Hartley guinea pigs were obtained from Charles River Laboratories (Wilmington, MA). The animals were divided into three age groups (means $\pm$ SD): 1-wk-old (1 wk, age = 7.1 ± 0.9 days, weight = 159.6 ± 31.4 g), 3-wk-old (3 wk, age = 23.0 ± 2.6 days, weight = 236.4 ± 30.2 g), and 2- to 3-mo-old (adult, age = 84.3 ± 13.1 days, weight = 687.6 ± 86.6 g). The 3 wk and adult age groups contained only male animals to avoid possible differences in ASM contractility caused by gender. Animal handling strictly followed the procedures approved by the Institutional Animal Care and Use Committee. Briefly, animals were anesthetized using pentobarbital sodium (200 mg/kg) through intraperitoneal injection. After the completion of anesthesia, confirmed by absence of reflex to toe clamping, the trachea was removed and immersed in Krebs-Henseleit (K-H) solution on ice and aerated with 95% O₂-balanced CO₂. The K-H solution contained (in mM) 115 NaCl, 1.38 NaH₂PO₄, 25 NaHCO₃, 2.5 KCl, 2.46

MgSO₄, 11.2 dextrose, and 1.9 CaCl₂. Under a dissecting microscope (SZH10 Olympus stereomicroscope), loose connective tissue was carefully removed from outside the trachea. The trachea was opened longitudinally through the center of cartilage rings. Tracheal strips were dissected with epithelium and submucosa intact. Each tracheal strip had cartilaginous attachment on both ends. One cartilage attachment was clamped in a phosphor-bronze clip that would later be inserted into the bottom of an 80-ml water-jacked organ bath filled with oxygenated K-H at 37°C. The other end of the cartilage attachment was tied to the tip of a transducer of the servo-controlled lever system using a 4.0 silk surgical thread. A minimal preload (1–3 mN) was applied to the transducer, and the transducer was clamped to keep the mounted tracheal strip isometric. Strips were then allowed to equilibrate for 1 h.

Mechanical perturbation and ASM adaptation

At the end of equilibration, strips were stimulated once with electric field stimulation (EFS; 60 Hz, 18 V, 10 s) and stretched by 0.1 mm. Six minutes later, another stimulation and stretch were applied. This stimulation-stretch cycle continued until the muscle reached a length (L_{ref}) when tension stopped increasing from that generated at the previous length. The image of the muscle strips was projected on a television monitor using a color video camera (Hitachi VK-C370 digital signal processor with Nikon c-mount adapter), and muscle length was measured. At L_{ref} , the muscle was allowed to reach maximal active force (F_{max}) with four to five EFS repeated at a 6-min interval. Active force (F), which includes both intrinsic tone and force due to external stimulation, was calculated as total force minus passive force. After F_{max} was found, an acute length perturbation (sine wave, frequency 0.5 Hz, amplitude of stretch 28% of muscle length, duration 40 s) was imposed on the relaxed tracheal strips. Upon termination of the oscillation, isometric contractions were elicited using EFS at 6-min intervals for a total of 30–40 min until active force reached a plateau. To some strips, the oscillation was also applied at lengths set at ~30% shorter or longer than L_{ref} . In these cases, F_{max} was obtained at the chosen lengths after four to five repeated EFS, and the length oscillation was initiated thereafter. In some experiments, cytochalasin D (10^{-7} M) or jasplakinolide (5×10^{-7} M) was added to the bath after finding F_{max} and 20 min before length oscillation. In separate experiments, indomethacin (10^{-5} or 10^{-4} M) was added to the bath one-half hour into the equilibration period and maintained throughout the duration of experiments.

Correction for system compliance

The muscle strips were connected to the testing system with silk thread. The compliance of the thread and the compliance of the system could dampen the actual stretch on the muscle preparations. We estimated this compliance by connecting the thread alone to the system in place of a muscle strip and recorded the length readings at each calibrated tension. To accurately document the amplitude of stretch during oscillation, we took into account the length change due to system and thread at tensions equivalent to F_{max} .

Data analysis

Force measurements were filtered with a 5-Hz digital filter to remove random noise. The contractile response after a length oscillation was expressed as 1) active force reduction caused by length oscillation and 2) active force recovery during the 30- to 40-min following the termination of the length oscillation. Active force reduction was calculated as $(F_{max} - F_i) \times 100/F_{max}$, where F_i was the force measured from the EFS immediately after an oscillation and F_{max} was the maximal active force obtained from the same strip before the oscillation. To describe the time course of active force recovery following the reduction, multiple measurements of active force were expressed in % F_{max} .

Statistics

Statistical analysis was carried out using the software Statistix (version 8; Analytical Software, Tallahassee, FL). Single parameter comparison among three age groups, such as reduction in active force caused by oscillation, active force reached 6 min after oscillation, and maximal active force reached during the recovery process, was carried out using one-way ANOVA. Single parameter comparison between any two groups was carried out using two sample *t*-tests. The entire recovery time-course curves were compared using repeated measure general ANOVA. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Reduction in active force due to length perturbation

Immediately after the sinusoidal length oscillation was terminated, the tracheal strips were stimulated isometrically using EFS. We found a reduction in active force from $F_{\max}$ in all tracheal strips, and the reduction was unaltered by the length at which the oscillation was carried out. The reduction tested at L_{ref} was found to be (means $\pm$ SE) 19.77 ± 4.22 , 18.97 ± 3.56 , and 14.18 ± 1.99 % $F_{\max}$ in 1-wk (number of animals, $n = 10$), 3-wk ($n = 9$), and adult ($n = 15$) guinea pigs, respectively (Fig. 1). In a comparison of the oscillation-induced reduction in active force among the three age groups, no statistically significant age difference was found (1-way ANOVA, $P = 0.36$).

Recovery of active force (adaptation)

Active force of 3-wk and adult strips was found to increase gradually toward the level before oscillation, whereas in 1-wk strips, the force exceeded the level before oscillation by 10% upon the second stimulation (6 min after the oscillation) and remained at that level for the entire adaptation process (Fig. 2). We termed the phenomenon of the active force exceeding that before length perturbation as force potentiation. When the entire curve of the recovery time course was compared among the three age groups, we found the curve for the 1-wk group to be significantly different from the 3-wk and adult groups (repeated measure ANOVA, $P = 0.0001$), whereas the 3-wk and adult groups were not different. One-way ANOVA also indicated that the 1-wk group differed from the other groups in active force reached at 6 min after oscillation ($P = 0.0119$) and the $F_{\max}$ reached during the entire adaptation period ($P = 0.0041$).

Role of actin polymerization

To evaluate the role of actin polymerization, cytochalasin D (20) was added to the tissue bath for 20 min before oscillation and was maintained until the end of the adaptation period when active force reached a plateau. Cytochalasin D blocks monomer addition to the fast-growing end of actin filaments and therefore interferes with the dynamic treadmilling. One of the critical points in this study was the choice of the dose. To examine active force reduction and recovery following a length perturbation, we needed to use a concentration of cytochalasin D that would not affect active force before the perturbation. We found in a set of preliminary tests that 20-min incubation with cytochalasin D at 10^{-7} M had no effect on active force. This dose of cytochalasin D was used to evaluate the role of actin polymerization on changes in active force after oscillation. As shown in Fig. 3A, the reduction in active force in the presence of cytochalasin D was (means $\pm$ SE) 35.07 ± 4.34 , 27.78 ± 1.27 , and 24.09 ± 5.84 % $F_{\max}$ in 1-wk ($n = 5$), 3-wk ($n = 5$), and adult ($n = 5$) groups, respectively. This reduction nearly doubled that under control conditions (Fig. 1) in all three age groups (ANOVA, $P = 0.0009$), suggesting interference with actin polymerization enhanced the disruptive effect of mechanical perturbation. Figure 3B shows the time course of isometric force recovery in the presence of cytochalasin D (10^{-7} M). Cytochalasin D abolished age-related differences in ASM adaptation

after oscillation. The force potentiation seen in the 1-wk group was no longer observed. In fact, only a partial recovery was achieved in all age groups, suggesting actin polymerization is required for the recovery of active force (adaptation).

We also tested whether promoting actin polymerization of the adult strips would induce a force potentiation similar to that seen in 1-wk strips during adaptation. Adult tracheal strips from five animals were incubated with jasplakinolide (5×10^{-7} M). Jasplakinolide has been shown to increase the F/G-actin ratio in unstretched vascular smooth muscle strips (1). At this concentration, jasplakinolide promotes actin polymerization in cultured ASM cells at the site of nucleation (18). We found that treatment with jasplakinolide did not affect either active force reduction due to oscillation (*t*-test, $P = 0.1473$) or $F_{\max}$ reached during adaptation (*t*-test, $P = 0.5109$) in adult strips. The time course of active force recovery after oscillation is shown in Fig. 4. Although it appears jasplakinolide slowed down the initial progress of the recovery, no statistically significant difference was found when the two curves were compared using repeated measure ANOVA ($P = 0.2377$). Therefore, the use of a pharmacological agent known to enhance actin polymerization did not produce force potentiation in adult tracheal strips.

Role of prostanoids

We tested the effect of indomethacin at two different concentrations, 10^{-4} and 10^{-5} M, in the 1-wk group. Indomethacin lessened the active force reduction after oscillation (Fig. 5A), suggesting prostanoids play a role in the disruption produced by mechanical perturbation. At 10^{-5} M, indomethacin reduced the degree of force potentiation and delayed its onset from 6 to 18 min after an oscillation. At 10^{-4} M, the force potentiation was completely abolished (Fig. 5B). No difference was found when the time-course curve of 1 wk in 10^{-4} M indomethacin was compared with the time course of adult strips under control conditions (repeated measure ANOVA, $P = 0.27$). This suggests that inhibition of cyclooxygenase, hence blocking prostanoid release, would abolish the force potentiation in 1-wk tracheal strips so that the time course would resemble the adaptation process of normal adult tracheal strips. We further tested the effect of indomethacin at 10^{-4} M on tracheal strips from adult guinea pigs. Similar to the 1-wk group, in the presence of 10^{-4} M indomethacin, the active force reduction caused by length oscillation was $8.28 \pm 2.64 \% F_{\max}$ instead of the $14.18 \pm 1.99 \% F_{\max}$ under control conditions. In terms of adaptation, indomethacin at 10^{-4} M caused a slightly faster recovery compared with control (Fig. 6, repeated measure ANOVA, excluding the data points at *time* 0, $P = 0.0379$).

DISCUSSION

In this study, we showed that a set of DI-simulating length oscillation caused the same reduction in the subsequent active force generation in immature (1 wk) and mature (adult) ASM. However, the force reduction was followed by a distinctly different adaptation profile in immature from mature tracheal strips. Whereas the mature strips tracked a gradual recovery to reach the same level of active force as that before the perturbation, the immature strips immediately displayed force potentiation beyond the maximal active force generated before the mechanical maneuver. We also found that although actin polymerization was required for ASM force adaptation, it was probably not the cause for the force potentiation observed in immature muscle strips. On the other hand, the level of prostanoids is likely the underlying mechanism for the potentiated active force generation in immature ASM.

The major finding of this study is the striking difference between immature and mature ASM in terms of force-generating ability after stretch mimicking DI. It has been consistently shown that DI is effective in reversing bronchoconstriction in healthy subjects. By contrast, DI is not effective or even further exaggerates existing bronchoconstriction in asthmatic subjects (2, 19). This contradictory response to DI became a marker of asthma severity and has been known

since the 1960s. In a recent study, Weist and colleagues (36) found that the reactivity of healthy infant airways was greater than adults in the presence of DI. Although DI helps to reduce the airway reactivity in normal adults, it is ineffective in infants, thus rendering the response of infants to resemble that of asthmatic adults. Our finding can explain at least in part this *in vivo* observation. Indeed, stretches can bring a temporary relief to the airways as indicated by a reduction in active force immediately after stretch. However, this relief could be very brief in immature ASM compared with adult, and within 5–6 min, the active force of immature ASM could rebound to 10% higher than ever before. In mature ASM, on the other hand, there may be a 30-min window before the reduced active force recovers to the same level as that before the mechanical maneuver. The specific feature of ASM adaptation demonstrated in our study suggests that smooth muscle could play a highly relevant role in determining the different effect of DI taken by mature and immature airways.

It has been shown that airway hyperresponsiveness occurs in healthy juveniles both in animal and human subjects. Immature rabbits have greater airway responsiveness and greater airway narrowing *in vivo* than adult animals (25,30). An increase in trachealis shortening velocity in the first 3 wk of life, followed by a decline toward adult life, has been recently demonstrated in guinea pigs (8). Guinea pig airways have important biological similarities to human airways such as similar orientation and distribution of ASM and similar airway innervation, including the presence of nonadrenergic, noncholinergic inhibitory fibers. This animal model showed ASM hyperresponsiveness occurs at the same stage of maturation at which airway hyperresponsiveness occurs *in vivo* in both animals and humans, thus suggesting that our results may be relevant to human airway hyperresponsiveness. Beyond interest from a maturational development point of view, our finding offers a link between the mechanical adaptability of hyperresponsive ASM and failure of bronchoprotection by DI. In this study, we obtained the first evidence that ASM from immature hyperresponsive airways carries functional differences from normal ASM in response to stretch by DI.

Another contribution of this study is toward our current understanding of ASM mechanical adaptation. ASM adaptation after length oscillation was first demonstrated in swine ASM strips (34). In that work, force reduction due to length oscillation was shown to be dependent on the amplitude and duration of the length oscillation and that the force recovery was gradual and completed within 30–40 min after the oscillation. To confirm if we are measuring the same kind of adaptive behavior here, the experimental protocol was compared with that of the previous work. Several differences are noted: 1) The choice of L_{ref} . In the previous work on swine ASM preparations, L_{ref} was chosen as the length at which passive force was 1–2% of F_{max} . However, this criterion was not used in the present study because the passive force in the guinea pig ASM preparations was not easily discernible due to the presence of intrinsic tone. Furthermore, the length-tension curve (data not shown) of guinea pig ASM was not as flat as that of swine. In other words, the plateau portion of the length-tension curve (3) covered only a small range of lengths as opposed to the wide range in the case of swine ASM. The L_{ref} chosen in this study was within the narrow plateau portion of the length-tension curve, and this served the purpose of keeping the various muscle preparations at a similar degree of stretch before a length oscillation was applied. In this respect, the principle of the choice of L_{ref} in this study agrees with that of the previous work. 2) The choice of oscillation amplitude and duration. In the previous work, multiple combinations of oscillation amplitude and duration were used. In this study, we were interested in applying length oscillations that simulated DI as closely as possible. If the airways dilate isotropically, tidal breathing would stretch the ASM by ~4% and a DI to total lung capacity would stretch the ASM by ~25% (10,13). We used 28% amplitude of stretch for 40 s to simulate several DI taken in a roll. The reduction in force was found to be slightly lower than that in the previous work. This could be partly due to the shorter duration of oscillation used and partly due to species differences. 3) The choice of stimulation interval. In the previous work, a 5-min interval was used. Unlike swine ASM, guinea pig tracheal smooth

muscle displays intrinsic tone. After each stimulation, the intrinsic tone would first fall below the baseline tone levels and then gradually return to preload levels in 3–4 min (4). In the work on swine ASM, we also showed that a 7.5-min stimulation interval resulted in a slower recovery than stimulations at a 5-min interval. In the present work, we chose a 6-min interval to allow sufficient time for the recovery of intrinsic tone and to avoid slowing down of the recovery process.

The force potentiation after an initial reduction in immature guinea pig ASM was not only dramatic but also repeatable. This phenomenon could not be explained directly by the existing theories on ASM adaptation. Currently, there are two major hypotheses to explain the adaptive behavior of normal ASM. One is the ASM plasticity theory (24), which states that smooth muscle cells adapt to length changes by varying the number of contractile units in series leaving the number of contractile unit in parallel unchanged. According to this hypothesis, in order for smooth muscle to adapt to length perturbations, it has to first disassemble its contractile apparatus before reassembling it again to regain its optimal contractile ability. On electron microscopic examination of the cross section of ASM strips, it has been reported that the density of myosin thick filaments in smooth muscle cells reduces upon stretch or oscillation and then increases hand-in-hand with active force during repeated stimulation until it reaches the same level as that before stretch or oscillation (15). This may explain the initial decline in active force and the subsequent force recovery to the previous level as that observed in tracheal strips from adult guinea pigs. However, the labile nature of myosin thick filaments could not produce a force potentiation beyond the maximal force generated before the mechanical perturbation. In order for active force to potentiate, more contractile units need to be arranged in parallel.

The other major theory to explain smooth muscle adaptation relates to cytoskeletal, rather than contractile element rearrangement. According to this hypothesis, the arrangement of cytoskeletal β -actin and its linkages with membrane-associated dense plaques are not fixed but rather are able to reorganize when there is a passive change in muscle length to optimize the capacity for myosin and contractile α -actin to generate force. With repeated stimulation, the arrangement of the filaments would become more fixed, allowing force production and shortening to be optimized to the conformation of the cell at the time the muscle is activated (12). Mehta and Gunst (20) examined the force development in canine tracheal smooth muscle strips after inhibiting actin polymerization using latrunculin A and cytochalasin D. Their results suggested that actin polymerization plays a direct role in force development in smooth muscle independent of myosin light chain phosphorylation. However, in their experiments, only a single stimulation was applied after each length change; therefore, the role of actin polymerization in the process of ASM adaptation was not assessed. We showed that short-term, two-dimensional mechanical strain of primary cultured ASM myocytes impedes actin polymerization (32). This provides additional evidence that actin cytoskeleton of ASM responds to cyclic stretches by undergoing conformational changes. In the current study, when actin polymerization was interrupted, active force failed to recover completely, which confirms that actin polymerization is involved in the dynamics of contractile response following mechanical perturbation. On the other hand, when we incubated normal adult ASM with a pharmacological agent known to enhance actin polymerization, force potentiation was not achieved. It appears that actin is a critical component required for the process of adaptation, but actin polymerization alone may not be sufficient to instigate force potentiation.

The force potentiation in 1-wk ASM is not likely due to a myogenic response triggered by length oscillation either. If indeed a myogenic response was initiated during cyclic stretches, the force reduction immediately after the oscillation would have been further enhanced after abolition of myogenic response with indomethacin. On the contrary, in the presence of indomethacin, the oscillation-induced force reduction was significantly reduced. Interestingly, indomethacin abolished the force potentiation in 1-wk ASM preparations but induced a slight

increase in force recovery in adult. It appears that indomethacin provided ASM with a “cushion” that diminishes the effects of length oscillation. The target of indomethacin could be the key element responsible for the force potentiation observed in the immature ASM. Indomethacin inhibits prostanoid release by inhibiting cyclooxygenase. A study on cyclooxygenase expression in ovine lung has shown that its constitutive form reaches a maximal abundance in ASM at 1 mo of age and declines into adulthood (5). An elevated expression of cyclooxygenase in immature airways would produce more prostanoids. Using enzyme immunoassay, Chitano et al. (7) showed that the prostanoids thromboxane B₂, 6-keto-PGF_{1α}, and PGE₂ are more abundant in 1-wk than in adult guinea pig tracheas. It is known that prostanoids exert on ASM both relaxing and contractile actions with PGE₂ and PGI₂ mainly acting as relaxing factors (23). Chitano et al. (6) showed that whereas ASM from adult guinea pigs relaxes to the baseline level while the stimulation is ongoing, 1-wk ASM maintains the contractile state throughout the stimulation. In the presence of indomethacin, 1-wk ASM is able to spontaneously relax like adult muscle strips. The specific response of a given tissue to prostanoids depends on the abundance of each prostanoid as well as on their receptor subtype expression. Nevertheless, it is evident that the elevated level of prostanoids in immature airways may modulate ASM response and may cause the age differences reported. It is likely that the mechanical adaptation of ASM is also regulated by prostanoids, which would contribute to the force potentiation in 1-wk ASM. It remains to be identified which specific prostanoids are responsible for the phenomenon of force potentiation. Moreover, prostanoids are released as inflammatory mediators and have been shown to play a role in airway responsiveness (16,28). The results from this study suggest that the mechanisms underlying the adaptive behavior of hyperresponsive ASM include factors involved in pathological conditions such as inflammatory response.

In this study, we examined the effect of length oscillation on the force-generating ability of tracheal smooth muscle from 1-wk, 3-wk, and adult guinea pigs. We found that the length oscillation caused initial reduction of active force, which was followed by a gradual recovery in adult but a potentiated force in the 1-wk group. Although actin polymerization was required for the recovery of active force, an age-dependent prostanoid release is likely the underlying mechanism for the strikingly different response to mechanical perturbation exhibited by mature and immature ASM.

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Fig. 1. Reduction in isometric force due to length oscillation measured in 1-wk-old (1-wk), 3-wk-old (3-wk), and 2- to 3-mo-old (adult) guinea pigs. Bars indicate the mean values of reduction expressed as % F_{max} , error bars indicate SE. F_{max} , maximal active force measured before length oscillation.

Fig. 2.

Adaptation of isometric force measured in the 3 age groups. Data points show mean values and SE expressed as % $F_{\max}$. Data points at 0 min indicate force generated immediately after the termination of length oscillation. Dotted line at 100% denotes the level of $F_{\max}$ measured before oscillation.

Fig. 3. Effect of cytochalasin D (10^{-7} M) on force reduction following length oscillation (A) and adaptation of isometric force (B). Data show means and SE expressed as % $F_{\max}$. Dotted line at 100% denotes the level of $F_{\max}$.

Fig. 4. Comparison of adaptation process between control and treatment with jasplakinolide (5×10^{-7} M) measured in tracheal strips from adult guinea pigs. Data points show means and SE expressed as %F_{max}. Dotted line at 100% denotes the level of F_{max}.

Fig. 5. Effect of indomethacin (Indo; 10^{-5} and 10^{-4} M) on force reduction (A) and force adaptation (B) measured in tracheal strips from 1-wk guinea pigs. Data points show means and SE expressed as % F_{max} . Dotted line at 100% indicates the level of F_{max} .

Fig. 6. Effect of indomethacin (Indo; 10⁻⁴ M) on force adaptation in tracheal strips from adult guinea pigs. Data show means and SE expressed as %F_{max}. Dotted line at 100% indicates the level of F_{max}.