Relaxation of activated airway smooth muscle: relative potency of isoproterenol vs. tidal stretch

ALISON GUMP, LAURA HAUGHNEY, AND JEFFREY FREDBERG

Physiology Program, Harvard School of Public Health, Boston, Massachusetts 02130

Received 21 July 2000; accepted in final form 16 January 2001

Gump, Alison, Laura Haughney, and Jeffrey Fredberg. Relaxation of activated airway smooth muscle: relative potency of isoproterenol vs. tidal stretch. J Appl Physiol 90: 2306–2310, 2001.—Both isoproterenol and tidal fluctuations of muscle length inhibit active force development in activated airway smooth muscle. In this study, we show that length fluctuations in the range of amplitudes expected during quiet tidal breathing produce force inhibition that is equipotent with high concentrations of isoproterenol. Active force fell to 50% of its isometric value when the amplitude of the tidal stretch was 4% of muscle length. The relaxing effects of length fluctuations were insensitive to the specific contractile agonist, suggesting that the mechanism of action is largely independent of the particular signal transduction pathway and lies instead at the level of bridge dynamics. This idea is reinforced by the results of combining the relaxation effects of tidal fluctuations with those produced by isoproterenol at all but the highest concentrations studied (10−5 M). Such a combination produces multiplicative effects, indicating largely separate modes of action. These observations suggest that the tidal muscle stretches that are attendant to spontaneous breathing comprise the first line of defense against bronchospasm and that tidal muscle stretches may be the most important of all known bronchodilating agencies.

ISOPROTERENOL (Iso) is a highly selective β-adrenoceptor agonist that is well known for its ability to relax bronchial smooth muscle. It is efficacious, short acting, and reasonably potent. Its putative action lies in its ability to produce coupling between the β2-adrenergic receptor and the stimulatory G-protein, which activates adenyl cyclase, increasing cAMP levels and ultimately resulting in relaxation of airway smooth muscle through changes including Ca2+ sequestration, Ca2+ sensitivity of myosin, and IP3 formation (10). Tidal stretch of airway smooth muscle has also been shown to reduce muscle tone far below the static force level (3, 4, 6). The mechanism may lie in a direct effect of stretch on bridge dynamics, causing a shift away from the mechanical equilibrium conditions that prevail during isometric steady-state contraction (1, 3).

Although it is known that both of these agencies, β-agonists and muscle stretch, can cause nearly complete relaxation of airway smooth muscle if given in sufficient amounts, determining the relative importance of one with respect to the other is conceptually difficult. For example, the relative potency of two agonists might be assessed by comparing the molar concentrations necessary for each to reduce active force to 50% of its maximum value (the EC50), but no such direct comparison is possible between chemical vs. mechanical stimuli. Therefore, we pose the question in the following way: Compared with the effects of a potent bronchodilating agonist, such as Iso, present in therapeutic concentrations, are the bronchodilating effects of muscle stretch (in the physiological range of stretch) negligible, moderate, or dominant? Our results show that tidal stretch amplitudes in the physiological range produce force inhibition that is equipotent with Iso in concentrations of 10−7 to 10−5 M.

METHODS

Tissue preparation. Strips of bovine tracheal smooth muscle were isolated and suspended from a servo-controlled lever arm that allowed for force and length measurements to be taken and length changes to be applied. A muscle bath was perfused with a Krebs-Henseleit solution (in mM: 118 NaCl, 4.59 KCl, 1.0 KH2PO4, 0.050 MgSO4, 0.18 CaCl2, 11.1 glucose, 23.8 NaHCO3; pH 7.4), aerated with 95% O2-5% CO2, and maintained at 37°C by a surrounding water jacket. After dissection and equilibration, the muscle strips were set to the optimal length by using electric field stimulation.

Isometric dose-response curve to Iso. Under isometric conditions, the muscle strip was stimulated with 10−6 M ACh at time (t) = 100 s. At t = 400 s, 10−6 M Iso was added to the bath. Iso continued to be added to the bath in increasing log increments every 500 s until a final concentration of 10−5 M was reached.

Dose-response curve to tidal strain. Force and length data were collected for 100 s while the muscle strip was oscillated with a tidal strain amplitude (ε) of 0.25% (peak-to-peak of 0.5%), expressed as a percentage of optimal length at a frequency of 0.33 Hz. This amplitude is sufficiently small that it does not perturb cross-bridge dynamics or force development (4). At t = 100 s, 10−6 M ACh was added to the bath, and strain oscillations of 0.25% were continued until t = 400 s, at which point the strain was doubled every 300 s until a final strain ε of 8% was reached.

Comparison of EC50 for tidal strain. Force and length data were collected while the muscle strip was oscillated with an
e of 0.25%. At \( t = 100 \) s, \( 10^{-4} \text{ M ACh} \) was added to the bath, and 0.25% oscillations continued until \( t = 400 \) s, at which point one of two procedures was implemented. In procedure 1, the \( e \) increased to 4%. Alternatively, in procedure 2, \( e \) was maintained at 0.25% and \( 10^{-5} \text{ M Iso} \) was added to the tissue bath. Force and length data collection continued in either case until \( t = 900 \) s, at which point the ACh and Iso were flushed from the bath.

**Differing contractile agonists.** Force and length data were acquired until \( t = 100 \) s under 0.25% strain oscillations. The muscle strip was then stimulated by use of one of three contractile agonists, \( 10^{-4} \text{ M ACh} \), \( 10^{-7} \text{ M endothelin-1 (ET-1)} \) or \( 60 \text{ mM potassium physiological salt solution (K-PSS)} \). Data acquisition continued until \( t = 400 \) s, at which point tidal strain oscillations were increased to 4%. The contractile agonists were added to the Krebs-Henseleit solution in the bath, with the exception of K-PSS, for which the bath was completely drained and refilled with the solution.

**Interactions between strain oscillations and a \( \beta \)-agonist.** A trachealis strip was contracted by using \( 10^{-6} \text{ M ACh} \) at \( t = 100 \) s under isometric conditions. A second strip from an adjacent tracheal ring was similarly contracted using \( 10^{-6} \text{ M ACh} \) while under imposed strain oscillations of 0.25%. At \( t = 400 \) s, the 0.25% strain oscillations were increased to 4%. Additions of Iso to the bath began at \( t = 900 \) s for both strips, starting at a concentration of \( 10^{-8} \text{ M} \) and increasing by a factor of 10 every 500 s until a bath concentration of \( 10^{-5} \text{ M} \) was reached.

**Tidal strain percentage.** The percent stretch was scaled from lung volume change by assuming an isotropic strain corresponding to the cube root of lung volume change above functional residual capacity. Therefore, normal tidal lung inflations are found to correspond roughly to a 4% change in length, a sigh corresponds to approximately a 12% stretch, and an inspiration from functional residual capacity to total lung capacity corresponds to a 25% stretch (4).

**Mechanical measurements.** We followed the method of Fredberg et al. (4). In brief, the total force in the muscle (\( F_T \)) is the sum of the passive force (\( F_P \)), the active force (\( F \)), the elastic force with activation (\( E\delta L \)), and the frictional force (\( R(\delta L/\delta t) \)), where \( L \) is muscle length, \( E \) is muscle elastance, and \( R \) is muscle resistance. Thus \( F_T = F + E\delta L + R(\delta L/\delta t) \). Measurements of muscle stiffness and hysteresivity (\( \eta \), defined below) require analysis of a closed force-length loop for each cyclic stretch imposed on the muscle strip, but because of ongoing force development these loops do not close. Therefore, we assumed that during force development the active force changes approximately linearly with time over the duration of a single tidal stretch; when this linear trend is removed, a closed force-length loop results for each stretch cycle, although a different trend line is used for each cycle in the sequence. From each trend line, the mean value of \( F \) over that tidal stretch is calculated. From the closed loops, the values of \( E, R, \) and \( \eta \) are computed on a loop-by-loop basis in the following manner. If \( D \) is the energy dissipated per period of imposed cyclic strain (i.e., area within the force-length loop) and \( \delta F \) is the amplitude of the phasic force variation about \( F \), then we use the following relations, which remain useful even when the loop becomes nonelliptical, which is indicative of nonlinear mechanical behavior (2, 5),

![Fig. 1. Comparison of dose curves for isoproterenol and strain oscillations under precontraction with \( 10^{-6} \text{ M ACh} \). F, force.](https://example.com/image1)

![Fig. 2. Comparison of the effect of the \( \beta \)-agonist isoproterenol and strain oscillations on the mechanical behavior of bovine tracheal smooth muscle. A: contractile force. B: stiffness. C: hysteresivity (\( \eta \)).](https://example.com/image2)
$E = (\delta F/\delta t) \cos \phi$, $R = (\delta \phi/\omega \delta L) \sin \phi$, and $\eta = \tan(\phi)$ where $\phi = \sin^{-1}(4D/\pi \delta F \delta L)$. With sinusoidal length changes at radian frequency $\omega$ ($= 2\pi \tau$), then $\delta F = (E + \mu R) \delta L = E(1 + j\eta) \delta L$ where $j = \sqrt{-1}$. The frictional (imaginary) part of the stress is proportional to $\omega R$ or, equivalently, $\eta E$. Alternately, $\eta$ may be regarded as the amplitude of the frictional force expressed as a fraction of the amplitude of the elastic force.

**Drugs.** The ACh, Iso, ET-1, and constituents of the Krebs-Henseleit and K-PSS solutions were all purchased from Sigma Chemical (St. Louis, MO). Because of the rapid oxidation of Iso, it was dissolved at the start of the experiment. Dilutions of ACh and Iso were done using type 1 reagent-grade water. The ET-1 was dissolved to $10^{-4}$ M in 5% glacial acetic acid, frozen in aliquots, and thawed immediately before addition to the tissue bath.

**Statistics and analysis.** Numerical data are presented as means $\pm$ SE. A Student’s $t$-test was used for comparison of data with significance at $P \leq 0.05$. The number of muscle strips used per experiment is represented by $n$.

**RESULTS**

Addition of Iso resulted in a progressive dose-effect relaxation of the contractile force produced with ACh. Increasing the amplitude of the strain oscillations produced a similar dose-effect relaxation (Fig. 1). The $\epsilon$ required to relax the muscle force by 50% was close to 4%. This degree of relaxation corresponded to an equivalent Iso concentration of slightly greater than $10^{-7}$ M.

The time course to reach the steady-state relaxation response differed substantially between the strain oscillation and $\beta$-agonist protocols. Force and stiffness decreased and $\eta$ increased immediately in response to an increase in the strain oscillation amplitude, whereas addition of Iso resulted in an equilibration time of $\approx 100$ s before relaxation to the steady state was reached. Although the force decreased to a similar extent for both interventions (Fig. 2B), the stiffness decreased substantially more in response to tidal stretch. Likewise, there was a substantial difference between the increases seen in $\eta$ (Fig. 2C).

No significant differences were found in the effectiveness of strain oscillations on steady-state relaxation values for muscle activated by ACh vs. K-PSS. Force development was slower with K-PSS (Fig. 3B), and

![Fig. 3. Changes in the strain relaxation due to precontraction with various contractile agonists. A: contractile force comparison between ACh and potassium physiological salt solution (K-PSS). B: stiffness comparison between ACh and K-PSS. C: hysteresivity comparison between ACh and K-PSS. D: contractile force comparison between ACh and endothelin-1 (ET-1). E: stiffness comparison between ACh and ET-1. F: hysteresivity comparison between ACh and ET-1.](image-url)
ACh did produce a peak in hysteresivity (Fig. 3C). Likewise, ET-1 produced similar force, stiffness, and $\eta$ values compared with ACh. Force and stiffness development were both slower with ET-1 (Fig. 3, D and E), and a similar but less prominent peak was again noted in hysteresivity with ACh (Fig. 3F). The steady-state value of $\eta$ was larger for ACh (Fig. 3F) but not significantly so.

Fig. 4A shows the dose-response curves for Iso when the muscle was isometric and when the muscle was simultaneously subjected to strain oscillations with an $\epsilon$ of 4%. When these data were renormalized as a percentage of the maximum force seen with 4% strain oscillations and compared with the isometric curve (Fig. 4B), the dose effect curves were almost superposable. As reasoned below in DISCUSSION, this suggests that the relaxing effects of Iso and tidal stretch are independent and multiplicative at all but the highest concentration of Iso. At $10^{-5}$ M Iso, however, tidal stretch impaired the ability of Iso to relax the muscle.

DISCUSSION

The principal findings of this report are these. When smooth muscle was activated ($10^{-4}$ ACh), the force inhibition caused by Iso ($10^{-5}$ M) and 4% strain oscillations were equipotent, with both approaching a 50% reduction in contractile force. Stiffness and $\eta$ were influenced by both agents, but more so by strain oscillations than by Iso. The changes in the muscle mechanics occurred more rapidly when strain oscillations were applied than with addition of Iso. Furthermore, the ability of tidal fluctuations to produce relaxation, inhibit stiffness, and increase $\eta$ was not affected by changing the contractile agonist. When the dose-response curve of Iso was examined with and without the influence of tidal fluctuations, the fractional degree of relaxation between modalities was equivalent at all but the highest concentration of Iso, suggesting largely independent mechanisms of action for the relaxing effects of Iso vs. those of tidal strain, i.e., deactivation vs. disruption of the myosin cross bridge.

Although the force inhibition caused by Iso is the result of a chain of signaling intermediaries, strain oscillations are believed to reduce contractile force mostly through a direct mechanical effect that perturbs the binding of myosin to actin (3). We have shown that this rapid action in the case of strain oscillations is controlled by the relatively high myosin detachment rates that prevail with appreciable displacements of the actin filament relative to the myosin backbone (8) and results in a rapid alteration of the contractile state of the muscle. Adjustment of the contractile state to the onset of tidal stretches is usually complete with one or two tidal stretches. By contrast, the slower time course seen when relaxation is brought about by $\beta$-agonist binding is controlled by kinetics of the entire signal transduction cascade and is upstream of the contractile machinery itself. The kinetics of the signaling cascade seem to be slow compared with kinetics of myosin detachment.

The ability of tidal fluctuations in muscle length to maintain fully activated muscle in a semi-relaxed state has been demonstrated in prior studies (3, 6). Here we show that this relaxation effect does not depend on the specific contractile stimulus. Insensitivity of the relaxation response to the contractile stimulus reinforces the idea that imposed length fluctuations exert their effects at the level of bridge dynamics and not further upstream. Moreover, the multiplicative rather than additive nature of the combined relaxation effects of Iso vs. length fluctuations throughout most of the range indicates two agencies acting through independent mechanisms. That is to say, the fractional relaxation that Iso was able to elicit was not affected by application of tidal stretches; for all but the highest concentration of Iso studied, the total relaxation was the fractional change produced by one intervention multiplied by the fractional change produced by the other.

The observations reported here suggest that the tidal muscle stretches that are attendant to spontaneous breathing are as potent as high concentrations of Iso and likely comprise the first line of defense against bronchospasm. In asthma, this bronchodilating mech-
anism becomes compromised (1, 7, 9, 11), but the reason for this failure remains unexplained.

We thank Dr. Gary Anderson for advice.

This work was supported by National Heart, Lung, and Blood Institute Grants P01 HL-33009 and RO1 HL-59682.

REFERENCES