Effects of initial length on intrinsic tone in guinea pig tracheal smooth muscle

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Bard, Martin, Sergio Salmeron, Catherine Coirault, Francois-Xavier Blanc, and Yves Lecarpentier. Effects of initial length on intrinsic tone in guinea pig tracheal smooth muscle. Am. J. Physiol. 275 (Lung Cell. Mol. Physiol. 19): L1026–L1030, 1998.—In the guinea pig, tracheal smooth muscle (TSM) exhibits intrinsic tone (IT). The active nature of IT suggests that it could be influenced by muscle length and load. In the guinea pig, IT is entirely suppressed by the cyclooxygenase inhibitor indomethacin. IT could be measured as the difference between resting tone before and after indomethacin addition. We examined, in electrically stimulated guinea pig TSM, the relationship between IT and the maximum extent of relaxation (ΔFᵢ), and the influence of indomethacin on active isometric force. When Lᵢ decreased from 100 to 75% of optimal Lᵢ, there was a significant decrease in IT (from 12.0 ± 0.2 to 5.3 ± 0.1 mN; P < 0.001). Over the range of Lᵢ studied, ΔFᵢ underestimated the amount of IT, but there was a close linear relationship between ΔFᵢ and IT (r = 0.9). Compared with the basal state, indomethacin increased active isometric force (from 9.5 ± 1.0 to 19.7 ± 2.0 mN at optimal Lᵢ; P < 0.001) and induced its length dependency. In guinea pig TSM, Lᵢ was an important determinant of IT.

IN THE BASAL STATE, human and guinea pig airway smooth muscle exhibits spontaneous tone (1, 4, 17). In vascular smooth muscle, this spontaneous tone, called “intrinsic tone” (IT), has been shown to contribute, along with the passive resting tone (PRT), to the total resting tone (RT) (15, 16).

In guinea pig tracheal smooth muscle (TSM), IT generation involves the release of prostanooids, inasmuch as indomethacin suppresses IT (1, 7, 9, 18). Moreover, spontaneous basal tone has been shown to increase after immune sensitization to ovalbumin (17), suggesting a possible role in the pathophysiology of airway hyperreactivity.

Previous reports (1, 9, 17) have shown that, in the guinea pig, electrical field stimulation (EFS) of isolated TSM induces a biphasic response: the initial contraction phase developed during EFS is followed by a relaxation phase below baseline tone levels, followed by a slow and gradual recovery of force. Both phases have been reported to be tetrodotoxin sensitive, i.e., neurally mediated (9, 17). Moreover, it has been established that the contraction phase results from the activation of cholinergic mechanisms (9, 25). Conversely, the relaxation phase results from the activation of both adrenergic and nonadrenergic noncholinergic components (1, 9).

Relaxation has been attributed, at least in part, to a transient inhibition of IT (1, 9, 17).

It has been demonstrated that initial muscle length (Lᵢ) is an important determinant of active isometric force (AF) in TSM (23). The aim of our study was to analyze, in electrically stimulated guinea pig TSM, the influence of Lᵢ on IT. We sought to determine whether the amplitude of relaxation accurately quantified the amount of IT and measured the AF-Lᵢ relationship in the presence and absence of IT.

MATERIALS AND METHODS

TSM Preparation

The experiments were performed on tracheal segments of Hartley guinea pigs weighing 300–350 g. Care of the animals conformed to the recommendations of the Helsinki Declaration, and the study was approved by our institution (Institut National de la Santé et de la Recherche Médicale, Palaiseau, France). The animals were anesthetized with intraperitoneal pentobarbital sodium (100 mg/Kg). A segment of five tracheal rings was rapidly removed and cut longitudinally. Metal clips were placed on the cartilage on either side of the posterior muscular band. The TSMs were vertically suspended at a predetermined initial tone in a physiological saline solution containing (in mM) 118 NaCl, 4.7 KCl, 1.2 MgSO₄, 7.9 H₂O, 1.1 K₂HPO₄, 24 NaHCO₃, 2.5 CaCl₂·6H₂O, and 4.5 glucose. The solution was maintained at 37°C and bubbled with a 95% O₂-5% CO₂ gas mixture at a pH of 7.40. The lower end of the tracheal strip was anchored at the bottom of the bath. The upper end of the strip was connected to a force and length transducer. The experiments were performed after a 1-h stabilization period during which the tracheal strips were electrically stimulated every 5 min by means of two platinum electrodes longitudinally arranged on either side of the muscle. Alternating square-wave pulses were delivered at a frequency of 50 Hz, a pulse width of 10 ms for 10 s, and a supramaximal voltage of 30 V/cm. During the equilibration period, preload was held constant. Preload was defined as the load stretching the muscle at rest. Afterload was defined as the load added to the preload when the muscle was electrically stimulated. Lᵢ was determined after the equilibration period with a calibrated optical system (pocket micrometer model TS-L1, Sugitoh). In the range of muscle length studied, Lᵢ had a limited effect on AF due to IT. Therefore, optimal Lᵢ (Lₒ) was defined as the Lᵢ corresponding to maximum AF after indomethacin addition.

Electromagnetic Apparatus

The muscle strips were anchored to an electromagnetic lever cemented to a coil and suspended in the field of an electromagnet. The load applied to the TSM segment was determined by a servo-controlled current through the coil.
The preload level, which determined the $L_0$, was electronically held constant throughout the experiment. A photoelectric transducer measured the displacement of the lever induced by muscle shortening. The equivalent moving mass of the whole system was 150 mg and its compliance was 0.2 μm/mN. The system was linear up to 5 mm of muscle shortening (12).

An adjustable electronic stop was set up to avoid muscle shortening beyond $L_0$ when afterload was applied to the muscle. Two signals, force and length, were simultaneously recorded by a computer (IPC Dynasty LE), with a base time of 50 s. The software for calculating all the mechanical parameters was developed in our laboratory. The system is not auxotonic but enables us to measure both isometric and isotonic responses.

**Mechanical Parameters**

Contraction phase. Classic mechanical parameters describing contraction in electrically stimulated TSM were obtained from fully isometric contractions. Total isometric force ($T_F$; in mN) and maximum AF ($A_F$; in mN) were measured (Fig. 1).

Relaxation phase. In electrically stimulated guinea pig TSM, the contraction phase is followed by a relaxation phase below baseline tone levels. Force then spontaneously returns to preload levels in 3–4 min. During this phase of relaxation, we measured the lowest measurable force ($\Delta F_2$; in mN) and the maximum extent of force decay below preload ($\Delta F_1$; in mN), i.e., the difference between RT and $\Delta F_2$ (Fig. 1A).

**Experimental Protocols**

Influence of $L_0$ on IT and $\Delta F_1$. To determine the effects of $L_0$ on IT and $\Delta F_1$, mechanical parameters of the isometric contraction were recorded at five different $L_0$ values ranging from 100 to 75% of $L_0$. These different $L_0$ values were obtained by reducing preload levels from 14 to 6 mN. Successive measurements of RT and $\Delta F_1$ were performed in the electrically induced isometric contractions before indomethacin addition. Thereafter, the resting length of the TSM was replaced at $L_0$, and indomethacin ($3 \times 10^{-6}$ M) was added to the Krebs solution. After an equilibrium period of 30 min, the remaining resting tone (i.e., PRT) was measured at the same corresponding $L_0$ values as before indomethacin addition. IT was calculated as the RT at baseline minus the RT after indomethacin addition (IT = RT$_{i}$ – PRT$_{i}$).

Comparison of IT and $\Delta F_1$. To determine whether the amplitude of relaxation accurately characterized IT in guinea pig TSM, baseline values of $\Delta F_1$ were compared with the corresponding values of IT at different $L_0$ values.

AF-$L_0$ relationship in presence and absence of IT. The influence of the amount of IT on AF was determined at five $L_0$ values ranging from 100 to 75% of $L_0$. For each $L_0$ value, AF was measured before indomethacin addition. Thereafter, the resting length of TSM was replaced at $L_0$, and indomethacin ($3 \times 10^{-6}$ M) was added to the Krebs solution. After an equilibrium period of 30 min, AF was recorded at the same five $L_0$ values as before indomethacin addition.

Effects of afterload level on relaxation. To determine the effects of afterload and/or muscle shortening on $\Delta F_1$, five to eight contractions with afterloads regularly increased from preload up to isometric load were applied to each muscle strip (Fig. 2). No indomethacin was added in the course of this protocol.

**Statistical Analysis**

Results are expressed as means ± SE. In all experiments, mean values were compared with analysis of variance and Student’s paired t-test with the Bonferroni correction. In all cases, significance required a P value < 0.05.

**RESULTS**

**Mechanical Characteristics of TSM**

At baseline, electrically stimulated guinea pig TSM exhibited a contraction phase followed by a phase of relaxation (Fig. 1A). The mechanical characteristics of TSM in the basal state are given in Table 1. At $L_0$, baseline values of AF and RT corresponded to 40 and 60% of TF, respectively. The effects of indomethacin are shown in Fig. 1B. As expected, indomethacin significantly reduced RT (Table 1) and abolished the phase of relaxation below the baseline tone level (Fig. 1B). Moreover, at $L_0$, indomethacin induced a 107% increase.
Comparison Between IT and 

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during relaxation were not 

in AF compared with the baseline value (Table 1). There was no significant difference in TF after indomethacin addition (23.5 ± 1.0 vs. 21.7 ± 1.9 mN; P = 0.3; Table 1).

Influence of L₁ on IT

Figure 3 depicts the relationship between L₁ and both IT and ΔF₁. At L₀, IT averaged 12.0 ± 0.2 mN and represented 86% of baseline RT. When L₁ was progressively decreased from 100 to 75% of L₀ there was a significant decrease in the amount of IT (P < 0.001; Fig. 3). Decreasing L₁ from 100 to 75% of L₀ also significantly reduced ΔF₁ (P < 0.001; Fig. 3). This indicates that in guinea pig TSM the values of both IT and ΔF₁ depend on L₁.

Comparison Between IT and ΔF₁

To determine whether ΔF₁ was a good estimate of IT, the relationship between IT and ΔF₁ was analyzed at varying L₁ values (Figs. 3 and 4). Over the range of L₁ values studied, ΔF₁ was significantly lower than IT (Fig. 3). However, there was a close linear relationship between IT and ΔF₁: the higher the values of IT, the higher the values of ΔF₁ (Fig. 4).

AF-L₁ Relationship in Presence and Absence of IT

Before indomethacin addition, the reduction in L₁ from 100 to 75% of L₀ did not significantly modify AF (Fig. 5). After indomethacin addition, decreasing L₁ from 100 to 75% of L₀ was associated with a progressive and significant reduction in AF (P < 0.001; Fig. 5). Moreover, compared with the basal state and for any L₁ value studied, AF was greater after indomethacin addition (Fig. 5).

Influence of Muscle Afterload on Relaxation

Figure 2 shows a series of afterloaded contractions obtained at baseline. When the load was increased from preload up to isometric load, the maximum amplitude of muscle shortening decreased (Fig. 2A). Conversely, relaxation was not modified by this procedure (Fig. 2B). The quantitative results of the afterloaded contractions are given in Table 2. The increase in afterload induced a decrease in the maximum amplitude of muscle shortening. However, no variations in ΔF₂ and ΔF₁ were

Table 1. Mechanical characteristics of guinea pig TSM

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Indomethacin</th>
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<tbody>
<tr>
<td>TF, mN</td>
<td>23.5 ± 1.0</td>
<td>21.7 ± 1.9</td>
</tr>
<tr>
<td>AF, mN</td>
<td>9.5 ± 1.0</td>
<td>19.7 ± 2.0</td>
</tr>
<tr>
<td>RT, mN</td>
<td>14.0 ± 0.1</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>IT, mN</td>
<td>12.0 ± 0.2</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 9 tracheal smooth muscle (TSM) strips. TF, total isometric force; AF, maximum isometric active force; RT, total resting tone; IT, intrinsic tone. Classic parameters of contraction were obtained from the fully isometric contraction before and after 3 × 10⁻⁶ M indomethacin addition. Optimal initial muscle length (L₀) is initial muscle length (1.7 ± 0.1 mm) corresponding to AF measured after indomethacin addition.

Fig. 3. Influence of initial muscle length (L₁) on both intrinsic tone (IT) and ΔF₁ in guinea pig tracheal smooth muscle (TSM; n = 9 strips). L₁ is expressed as a percentage of L₀, the L₁ corresponding to maximum AF after indomethacin addition. Results are means ± SE. Student’s paired t-test with the Bonferroni correction was used, and mean values were compared with mean values at L₀ (*P < 0.001). For range of L₁ values studied (from 100 to 75% of L₀), a significant decline in IT was observed. This indicates that, in guinea pig TSM, amount of IT depends on L₁. Moreover, over range of L₁ values studied, ΔF₁ significantly declined with L₁ and ΔF₁ was lower than IT.

AF-L₁ Relationship in Presence and Absence of IT

Before indomethacin addition, the reduction in L₁ from 100 to 75% of L₀ did not significantly modify AF (Fig. 5). After indomethacin addition, decreasing L₁ from 100 to 75% of L₀ was associated with a progressive and significant reduction in AF (P < 0.001; Fig. 5). Moreover, compared with the basal state and for any L₁ value studied, AF was greater after indomethacin addition (Fig. 5).

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observed. This indicates that relaxation was not influenced by load variations occurring during the initial contraction phase.

**DISCUSSION**

IT, which occurs spontaneously in guinea pig TSM, is totally abolished by the cyclooxygenase inhibitor indomethacin (1, 7, 9, 18). In this animal model, analysis of IT, which occurs spontaneously in guinea pig TSM (8), to determine whether tone level has also been reported in isolated human TSM (8). To determine whether $\Delta F_1$ was a good estimate of IT, we studied the relationship between $\Delta F_1$ and the amount of IT at various $L_i$ values. Our results showed that $\Delta F_1$ underestimated the amount of IT; i.e., $\Delta F_1$ represented ~80% of IT values. Thus IT was not totally abolished during relaxation. However, there was a close linear relationship between $\Delta F_1$ and IT (Fig. 3). These results support the hypothesis that, in electrically stimulated TSM, relaxation corresponds to a transient and incomplete inhibition of IT but that $\Delta F_1$ does not accurately quantify IT. The precise influence of IT on the relaxation process was difficult to assess because of the simultaneous changes in $L_i$, IT, and $\Delta F_1$.

In guinea pig TSM, several mechanical and pharmacological studies have analyzed initial force development and relaxation. Selective anticholinergic drugs, such as atropine, have been shown to inhibit the initial contraction phase without modifying the relaxation phase (1, 9, 11, 17). This suggests that $\Delta F_1$ is independent of the cholinergic pathway. On the other hand, it has been reported that the characteristics of EFS modulate both the contraction and relaxation phases (1, 9). The effects of loading conditions on $\Delta F_1$ (particularly afterload level and muscle shortening length) have not been previously reported. Our results show that, for a given preload, $\Delta F_1$ was not influenced by the afterload level. This suggests that intracellular mechanisms underlying relaxation remain uninfluenced by changes in muscle length and/or load during the contraction phase.

In guinea pig TSM, indomethacin induces an increase in all the mechanical parameters of contraction. Muscle shortening, velocity of contraction, and AF are all increased by indomethacin. The mechanisms underlying the effect of indomethacin may involve variations in neurotransmitter release or in contraction regula-

![Graph](Image)

**Fig. 5.** Influence of $L_i$ on AF in guinea pig TSM ($n = 9$ strips) before and after indomethacin (3 × 10$^{-5}$ M) addition. Results are means ± SE. Student's paired t-test with Bonferroni correction was used, and mean values were compared with mean values at $L_o$ ($^{*}P < 0.001$). At baseline, reduction in $L_i$ from 100 to 75% of $L_o$ did not significantly modify AF. Conversely, after indomethacin addition, decreasing $L_i$ was associated with a significant reduction in AF.

**Table 2.** Effects of afterload level on relaxation and muscle shortening in guinea pig TSM

<table>
<thead>
<tr>
<th>Load, %AF</th>
<th>$\Delta F_2$, mN</th>
<th>$\Delta F_1$, mN</th>
<th>DL, %$L_o$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–20</td>
<td>4.6 ± 0.4</td>
<td>9.4 ± 0.4</td>
<td>13.1 ± 2.0</td>
</tr>
<tr>
<td>20–40</td>
<td>4.6 ± 0.4</td>
<td>9.4 ± 0.4</td>
<td>9.1 ± 1.3</td>
</tr>
<tr>
<td>40–60</td>
<td>4.5 ± 0.4</td>
<td>9.5 ± 0.4</td>
<td>6.2 ± 0.9</td>
</tr>
<tr>
<td>60–80</td>
<td>4.5 ± 0.4</td>
<td>9.5 ± 0.4</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>80–100</td>
<td>4.5 ± 0.4</td>
<td>9.5 ± 0.4</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 9$ TSM strips. Afterload was regularly increased from preload up to the fully isometric contraction. $\Delta F_2$, lowest measurable force during phase of relaxation; $\Delta F_1$, maximum extent of force decay below preload during relaxation; DL, maximum muscle shortening during contraction process. Increase in afterload induced a decrease in DL; however, no variations in $\Delta F_2$ and $\Delta F_1$ were observed. These results show that relaxation is not influenced by afterload.

The mechanical effects induced by changes in $L_i$ reflect changes in the intracellular Ca$^{2+}$ concentration and/or myofilament Ca$^{2+}$ sensitivity. $L_i$ may influence both Ca$^{2+}$ homeostasis and Ca$^{2+}$ sensitivity of regulatory proteins such as G proteins and inhibitor proteins (20–22). In line with this hypothesis, previous authors have demonstrated a decrease at short length in both myoplasmic intracellular Ca$^{2+}$ concentration (14) and Ca$^{2+}$ sensitivity of myosin light chain kinase (6). The length dependency of IT may be compared with the phenomenon of reduced activation at short length demonstrated in both striated and smooth muscles (10, 23, 24). The mechanisms underlying the AF decrease with muscle length may be related to a reduction in cytosolic Ca$^{2+}$ release at short length. Alternatively, an increase in prostaglandin release induced by TSM distension has been suggested (3). A decrease in myoplasmic prostaglandin content could be another putative hypothesis to explain the lower amount of IT measured at short $L_i$.

Numerous studies (1, 9, 17) have shown that, in isolated guinea pig TSM, a relaxation phase below baseline tone level follows the electrically induced contraction phase. A relaxation phase below baseline tone level has also been reported in isolated human TSM (8). To determine whether $\Delta F_1$ was a good estimate of IT, we studied the relationship between $\Delta F_1$ and the amount of IT at various $L_i$ values. Our results showed that $\Delta F_1$ underestimated the amount of IT; i.e., $\Delta F_1$ represented ~80% of IT values. Thus IT was not totally abolished during relaxation. However, there was a close linear relationship between $\Delta F_1$ and IT (Fig. 3). These results support the hypothesis that, in electrically stimulated TSM, relaxation corresponds to a transient and incomplete inhibition of IT but that $\Delta F_1$ does not accurately quantify IT. The precise influence of IT on the relaxation process was difficult to assess because of the simultaneous changes in $L_i$, IT, and $\Delta F_1$.

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tion. The close relationship between IT and AF makes it difficult to study the effect of cyclooxygenase blockade on AF generation. Linden et al. [13] have recently demonstrated the role of the level of histamine-induced tone in the response to electrical stimulation. In this study, it has not been possible to examine IT because indomethacin was systematically added in all experiments. However, this study has demonstrated that, when a high histamine-induced tone is present, the response to EFS is relaxant. Conversely, when no tone is present, a contractile response to EFS is measured. The comparison between IT and histamine-induced tone is hazardous, but this result confirms that the level of tone is an important determinant of airway response to stimulation.

In striated muscle, it is well known that L1 modulates AF (Frank-Starling relationship). Similarly, in dog TSM, in which IT is absent, AF declines with L1 [23]. Our results show that, in the presence of indomethacin, i.e., after the suppression of IT, AF significantly declines when L1 falls below L0. Conversely, before indomethacin addition, the reduction in L1 from 100 to 75% of L0 is not associated with significant changes in AF (Fig. 5). It could be hypothesized that, in the absence of indomethacin, a given proportion of cross bridges are involved in the maintenance of IT. Consequently, the remaining cross bridges that could develop AF during the initial contraction phase may be less numerous before than after IT suppression. Recently, variations in TSM plasticity have been hypothesized to explain the length dependency of mechanical parameters in canine TSM (19). Variations in muscle length may induce variations in the number of contractile units. Similar phenomena may be hypothesized to explain the length dependency of both AF and IT in guinea pig TSM. Further studies are needed to elucidate the regulation of cross bridges involved in IT generation.

In conclusion, our results show that, in electrically stimulated guinea pig TSM, L1 modulates IT and relaxation (ΔF1). Moreover, over the range of L1 values studied, relaxation reflects a transient and incomplete inhibition of IT. Finally, IT modulates the muscle length-AF relationship.

We thank D. Chena for helpful discussions and J. Kenneth Hilton for assistance in the preparation of the manuscript.


Received 4 August 1997; accepted in final form 21 August 1998.

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