Relaxation of guinea pig trachealis during electrical field stimulation increases with age

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Chitano, Pasquale, Carrie M. Cox, and Thomas M. Murphy. Relaxation of guinea pig trachealis during electrical field stimulation increases with age. J Appl Physiol 92: 1835–1842, 2002. First published January 4, 2002; 10.1152/japplphysiol.00688.2001.—Our laboratory has previously shown that maturation of airway smooth muscle (ASM) contractility may play a role in the airway hyperresponsiveness displayed by juveniles of many species, including humans (Chitano P, Wang J, Cox CM, Stephens NL, and Murphy TM. J Appl Physiol 88: 1338–1345, 2000).ASM relaxation, which could also contribute to airway hyperresponsiveness, has neither been described nor quantified during maturation. Therefore, we studied ASM relaxation during and after electrical field stimulation (EFS) in tracheal strips from 1-wk-old, 3-wk-old, and 3-mo-old guinea pigs. Strips were stimulated (60 Hz, 18 V) at their optimal length for 15, 20, and 25 s, with and without the cyclooxygenase inhibitor indomethacin. To evaluate the role of the epithelium, deepithelialized strips from adult animals were also studied. New indexes were developed to quantify relaxation during EFS. We measured the time course of tension relaxation and its maximum rate (RTR) during the EFS, as well as the residual tension at the end of the EFS (TCTend). After EFS, we measured the maximum RTR and the time needed to half the TCTend. Relaxation during the EFS significantly increased with age. Indomethacin reduced this age difference by increasing relaxation in strips from younger animals. By contrast, removal of the epithelium in adult strips decreased relaxation. Relaxation after EFS decreased with age and was not affected by indomethacin. In adult strips, it was further reduced by epithelium removal. Our results show that during EFS 1) airway smooth muscle relaxation increases with age, 2) cyclooxygenase metabolites oppose relaxation in younger animals, and 3) epithelial removal inhibits relaxation. We suggest that a reduced ASM relaxing ability during stimulation may be involved in juvenile airway hyperresponsiveness.

Spontaneous relaxation occurs because of either an attenuation and cessation of the contractile stimulus or a release of relaxing factors during the contractile response. In the first case, relaxation consists of mechanical events driven by forces that bring the tissue to its original mechanical equilibrium (26). In the second case, relaxing mediators are actively released by nerves, epithelium, and/or the smooth muscle itself, so the contractile response is reduced and the bronchospasm limited. In certain species, both neural and nonneural components have been reported to exert a relaxing effect on airway smooth muscle. A parasympathetic nonadrenergic-noncholinergic as well as an adrenergic relaxation are present in guinea pig trachea (6, 9, 22, 27, 30), whereas, in human airways, only a nonadrenergic-noncholinergic inhibitory control has been reported (10, 11, 21). Airway epithelium has also been shown to influence ASM tone through mediator release (14, 25). It seems evident that a modulation of the several relaxing factors released in the airway tissue would influence the final outcome of the contractile response and consequently affect airway responsiveness.

Although only a few studies have addressed the issue, an altered spontaneous ASM relaxation has been reported in models of airway hyperresponsiveness. A partial relaxing phase observed during prolonged electrical stimulation in tracheal smooth muscle from control dogs has been shown to be absent in the same preparation from sensitized animals (18).

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This relaxing phase was partially inhibited by indomethacin (16) and by removal of the epithelium (14). Moreover, a longer time to attain relaxation after short electrical stimulation has been shown in ASM from sensitized dogs compared with control animals (26). These findings suggest that factors operating during electrical stimulation may affect ASM relaxation and contribute to the hyperresponsiveness of sensitized tissue.

We hypothesized that, similarly to what reported for sensitized animals, a reduced relaxation during electrical field stimulation (EFS) characterizes immature tracheal smooth muscle, contributing to juvenile airway hyperresponsiveness. In the present work, we sought to study whether and by which mechanisms a relaxing phase associated with EFS varies during maturation. We used the same maturational model in which our laboratory showed ontogenetic changes in ASM contractile properties, i.e., 1-wk-old, 3-wk-old, and adult guinea pigs (8). Because this phase of the response to EFS has previously only been described qualitatively, we report here new indexes we developed to quantify it. Experiments were conducted on tracheal strips in which we measured the time course of tension relaxation and its maximum rate (RTR) during the relaxation phase of the EFS, as well as the residual tension at the end of the EFS (TCT\textsubscript{end}). We also measured, after the end of the EFS, the maximum RTR and the time needed to reduce to half the TCT\textsubscript{end}. To evaluate the role of prostanoids, we performed this study in the absence and presence of indomethacin, which abolishes intrinsic tone (2), and in the presence of indomethacin, which abolished intrinsic tone (2), and in the presence of indomethacin, which abolished intrinsic tone (2), we had to consider a form of normalization accounting for the bottom of a 80-ml double-jacketed organ bath with KH solution prepared as above (P\textsubscript{O\textsubscript{2}} 600 Torr, P\textsubscript{CO\textsubscript{2}} 40 Torr, pH 7.37). The other end was fixed to the transducer tip of an electromagnetic lever system with 4-0 braided silk surgical thread inserted through the cartilage so that the two cartilage pieces constituted the holders of the muscle via their natural structural connections. All the preparative procedures were performed in KH solution buffered to pH 7.35–7.45 by continuous aeration with 95% O\textsubscript{2}-5% CO\textsubscript{2}.

Electromagnetic Lever System

The system consists of the original apparatus by Brutsaert et al. (4) to which new electronic components have been added to improve its computerized control (Qjin Design, Winnipeg, MN, Canada), as previously described (8). Elements of the apparatus relevant to the present work consist of a force transducer, an electronic controller unit that controls the transducer performances, a power supply-stimulator, an analog-digital interface, and a computer system with dedicated software that allows data acquisition and analysis. The resolution of the transducer for force recording is 0.1 mN. The voltage signal from the transducer is converted into a digital signal by a RTDI1000 computer board with a maximum throughput of 25 kHz (Real Time Device, State College, PA). The stimulus duration is controlled by the computer program used for data acquisition and analysis (Cunningham Engineering, Lethbridge, AB, Canada). Data acquisition was carried out at 75 Hz.

Experimental Protocols

Intact tracheal strips. After equilibration for 90 min, supramaximal EFS (18 V, 60 Hz, 400 mA cm\textsuperscript{-2}) was effected by wire platinum electrodes positioned on both sides of the strip. A partial-length-tension curve was elicited by stretching the strips at increasing length and recording the isometric response to EFS so that the optimal length was identified and used in the following part of each experiment.

Three consecutive stimulations were performed with increasing stimulus duration: 15, 20, and 25 s, respectively. Then, 10-s EFS was carried out at 6-min intervals, and indomethacin (Sigma Chemical, St. Louis, MO) was added to the KH at log increments (starting at 10\textsuperscript{-8} M) immediately after each EFS until the intrinsic tone was completely abolished, which occurred at 10\textsuperscript{-6} M. Finally, three consecutive EFS were performed with 15-, 20-, and 25-s stimulus duration.

Deepithelialized tracheal strips. Strips without epithelium were obtained by gently scraping the luminal side of the trachea with the smooth edge of curved forceps immediately before the strips were dissected. Then, the same procedure followed for intact strips was employed. Only one series of 15-, 20-, and 25-s EFS without administration of indomethacin was performed for deepithelialized strips.

Data Description and Quantitative Indexes for Relaxation

Because the component of the response to EFS we wanted to analyze had not previously been described quantitatively in guinea pig ASM, which has a marked spontaneous tone, we needed to develop new appropriate indexes. For this purpose, we had to take into account that the level of tension reached by a given strip before its relaxing phase would affect the extent of its relaxation. In particular, because we wanted to compare the response in the absence and in the presence of indomethacin, which abolished intrinsic tone (2), we had to consider a form of normalization accounting for the effect of this cyclooxygenase inhibitor.

METHODS

Animals and Tissue Preparation

Hartley guinea pigs (Charles River Laboratories, Wilmington, MA) were employed for the present investigation. Three age groups were used: 1-wk-old guinea pigs [1 wk, n = 6, 138.3 ± 17.6 (SD) g, 9.3 ± 1.7 days old], 3-wk-old guinea pigs (3 wk, n = 7, 246.1 ± 33.9 g, 22.9 ± 2.9 days old), and 3-mo-old guinea pigs (adult, n = 11, 711.9 ± 92.5 g, 84.7 ± 19.9 days old). Only male animals were used for the 3 wk and adult groups.

To obtain ASM tissue, animals were anesthetized with an intraperitoneal injection of 200 mg/kg pentobarbital sodium (Abbott Laboratories, Chicago, IL). When anesthesia was completely achieved (no reflex observed in response to a toe clamping), the trachea and the lungs were exposed, excised, and immediately put into ice-cold KH solution (PO\textsubscript{2} 600 Torr, PCO\textsubscript{2} 40 Torr, pH 7.45 by continuous aeration with 95% O\textsubscript{2}-5% CO\textsubscript{2}).

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After loose connective tissue was cleaned away, tracheal strips were dissected under a dissecting microscope (SZH10 Olympus stereomicroscope) from transverse sections of the trachea so that parallel-fibered strips were obtained. Tracheal strips were ~0.5–1 mm in width and were dissected with ~2-mm cartilaginous attachments at both ends. One cartilaginous end was clamped in a phosphor-bronze clip at the bottom of a 80-ml double-jacketed organ bath with KH solution prepared as above (P\textsubscript{O\textsubscript{2}} 600 Torr, P\textsubscript{CO\textsubscript{2}} 40 Torr, pH 7.37). The other end was fixed to the transducer tip of an electromagnetic lever system with 4-0 braided silk surgical thread inserted through the cartilage so that the two cartilage pieces constituted the holders of the muscle via their natural structural connections. All the preparative procedures were performed in KH solution buffered to pH 7.35–7.45 by continuous aeration with 95% O\textsubscript{2}-5% CO\textsubscript{2}.

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Figure 1A shows the mechanical parameters involved in the response to a 20-s EFS. In the absence of a stimulus (at rest), the level of tension that can be assessed in a strip in isotonic conditions is defined as resting tension (RT). In guinea pigs, RT consists of two components: intrinsic tone (IT) and passive resting tension (PRT) (2). The first is believed to be generated by a partial activation of contractile proteins, whereas the second would originate from the mechanical properties of the tissue. When tension is actively produced by the smooth muscle as a consequence of a stimulus, it sums up to the RT. The resulting total tension reaches a maximum (TT\text{max}) when the active tension is maximal (AT\text{max}) and then gradually declines while the stimulus is still present. After the end of the stimulus, tension rapidly decreases and transiently (for \(\sim 3\) min) reaches a lower level than the initial RT. In our experimental condition, this value of tension was equivalent to the PRT.

The analysis of the tension parameters described before reveals that the difference between TT\text{max} and PRT can be considered as the maximum potential tension drop once TT\text{max} has been reached. Therefore, we used this value to normalize the relaxation parameters measured during EFS. Considering that this value is equal to AT\text{max} + IT and that both these parameters reflect tension produced by a contractile status of the smooth muscle, we defined it as maximum total contraction tension (TCT\text{max}). Similarly, the total contraction tension measured at the end of the EFS (TCT\text{end}) could be considered as the maximum potential tension drop once the stimulus is over, and we used it to normalize the subsequent relaxation. It is immediately evident that the value of TCT\text{end} is dependent on the amount of relaxation occurring before the end of the EFS. As a consequence, TCT\text{end} decreases when the duration of the EFS is increased and vice versa, so the maximum potential tension drop after the end of the EFS varies with stimulus duration. Because this could have affected the following relaxing events, we needed to measure relaxation starting at different TCT\text{end}, and we did it by performing EFS of three different durations: 15, 20, and 25 s, as described in Experimental Protocols. In tissue from animals of all ages, both in the absence and in the presence of indomethacin, values obtained for relaxation parameters after the end of the EFS did not vary among stimuli of different duration. Therefore, we report in the result section only data obtained from 20-s EFS.

To analyze the relaxation phase during EFS, we measured the following: 1) the residual TCT\text{end}, 2) the time course of the total contraction tension from the moment TCT\text{end} started to decline, and 3) the maximum rate of tension relaxation (RTR\text{end}). RTR\text{end} was obtained by performing the derivative of the recorded force trace as a function of time and measuring its minimum value (the more negative) during the stimulus. To analyze the relaxation after the end of the EFS, we measured the maximum rate of tension relaxation after the end of stimulation (RTR\text{r}) and the time needed to reduce to half the TCT\text{end} \((T_{1/2,TCT\text{end}})\) (26). RTR\text{end} was obtained by measuring the minimum value of the force trace derivative after the end of the stimulus.

All contractile parameters were normalized per cross-sectional area of the strip, obtained by measuring width and thickness of each strip, while still at their optimal length in the organ bath, through a VK-C370 digital signal processor Hitachi video camera (Hitachi Home Electronics, Norcross, GA). The values of cross-sectional area were multiplied by a correction factor of 1.35, which our laboratory has previously shown to be needed when using this measure procedure (8). In strips from 1 wk, 3 wk, and adult animals, the average cross-sectional area was 0.30 ± 0.16 (SD), 0.35 ± 0.10, and 0.55 ± 0.33 mm², respectively.

Data Analysis

Data are expressed as means ± SE, except when differently indicated. Statistical analyses performed were ANOVA and Fisher’s post hoc least significant difference test to find out which groups were responsible for differences showed by ANOVA. ANOVA with repeated measures was employed when applicable. The software employed was Statistix (Analytical Software, Tallahassee, FL). \(P < 0.05\) was considered significant.

RESULTS

Contractile Response

The values obtained for parameters describing smooth muscle tone at rest and in response to EFS are shown in Table 1. For all parameters, statistically significant differences \((P < 0.05\) by ANOVA) were not observed among different age groups, even though a trend toward an increase with age of the IT was observed in the absence of indomethacin. PRT and AT\text{max} were not significantly affected by indomethacin, which, by contrast, abolished IT and significantly reduced RT and TCT\text{max} (Table 1, Fig. 1B). Table 1 clearly shows that the effect of indomethacin on RT and TCT\text{max} was determined by its action on IT.

Relaxation During EFS

TCT\text{end} is reported in Fig. 2. TCT\text{end} is expressed as percentage of TCT\text{max} so that higher values correspond
to lower relaxation and vice versa. In the absence of indomethacin, TCT\textsubscript{end} was significantly lower in strips from 3-wk and adult compared with 1-wk animals. Indomethacin almost completely abolished this age-related difference by increasing relaxation at a greater extent in younger animals. This increase was statistically significant in strips from 1-wk animals.

Because in some of the strips the relaxing phase started later during EFS, lower values of relaxation shown in Fig. 2 could be determined by a shorter time available to relax until the end of the stimulus. In other words, if relaxation was only delayed in strips from younger animals, the use of TCT\textsubscript{end} would not be an appropriate index of the absolute relaxation. For this reason, we measured TCT at 1-s intervals from the moment total contraction tension started to diminish and we expressed the values of TR as percentage drop of TCT\textsubscript{max}. This time course of TR in the absence and presence of indomethacin is shown in Fig. 3, A and B.

### Table 1. Smooth muscle tone at rest and in response to electrical field stimulation in tracheal strips from guinea pigs of different ages

<table>
<thead>
<tr>
<th></th>
<th>1 wk</th>
<th>3 wk</th>
<th>Adult</th>
<th>Adult (− Epithelium)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IT, No indomethacin</td>
<td>5.23 ± 1.50</td>
<td>6.66 ± 2.94</td>
<td>14.06 ± 5.88</td>
<td>0.27 ± 0.10</td>
</tr>
<tr>
<td>10\textsuperscript{-4} M indomethacin</td>
<td>0.21 ± 0.10</td>
<td>0.13 ± 0.03</td>
<td>0.10 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>PRT, No indomethacin</td>
<td>8.9 ± 2.7</td>
<td>7.6 ± 1.6</td>
<td>9.2 ± 3.0</td>
<td>9.6 ± 3.2</td>
</tr>
<tr>
<td>10\textsuperscript{-4} M indomethacin</td>
<td>8.1 ± 3.9</td>
<td>5.3 ± 1.2</td>
<td>7.4 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>RT, No indomethacin</td>
<td>14.1 ± 3.2</td>
<td>14.2 ± 3.8</td>
<td>23.2 ± 6.8</td>
<td>9.8 ± 3.1</td>
</tr>
<tr>
<td>10\textsuperscript{-4} M indomethacin</td>
<td>8.3 ± 4.0</td>
<td>5.4 ± 1.3</td>
<td>7.5 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>AT\textsubscript{max}, No indomethacin</td>
<td>22.7 ± 3.3</td>
<td>18.5 ± 3.4</td>
<td>18.6 ± 4.5</td>
<td>11.2 ± 1.1</td>
</tr>
<tr>
<td>10\textsuperscript{-4} M indomethacin</td>
<td>15.3 ± 2.6</td>
<td>14.3 ± 2.3</td>
<td>19.9 ± 4.5</td>
<td></td>
</tr>
<tr>
<td>TCT\textsubscript{max}, No indomethacin</td>
<td>27.9 ± 3.5</td>
<td>25.2 ± 4.1</td>
<td>32.6 ± 8.2</td>
<td>11.5 ± 1.1</td>
</tr>
<tr>
<td>10\textsuperscript{-4} M indomethacin</td>
<td>15.5 ± 2.6</td>
<td>14.4 ± 2.3</td>
<td>20.0 ± 4.5</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE given in mN/mm\textsuperscript{2} for six 1-wk-old (1 wk), seven 3-wk-old (3 wk), seven 3-mo-old (adult), and six adult without epithelium (− epithelium) animals. IT, intrinsic tone; PRT, passive resting tension; RT, resting tension; AT\textsubscript{max}, maximum active tension; TCT\textsubscript{max}, maximum total contraction tension. No significant difference was found among age groups. Both indomethacin and epithelium removal abolished IT and significantly reduced RT and TCT\textsubscript{max} (P < 0.01 by ANOVA).
respectively, and confirms that the amount of relaxation during EFS is less in younger animals and that indomethacin reduces this age-dependent difference by increasing the relaxation in younger animals.

Considering that the two parameters so far analyzed are dependent on our arbitrary choice of a given time point or stimulus duration, we decided to measure also \( R_T R_{st} \). This index is measured at the moment relaxation peaks and might be a more general predictor of relaxing ability, although it fails to give information about the entire occurrence of relaxation. In strips from 1 wk, 3 wk, and adult animals, it occurred 4.99 ± 0.39, 4.26 ± 0.35, and 4.60 ± 0.47 s, respectively, after maximum tension began declining. \( R_T R_{st} \) increased early with age and was increased by indomethacin (Fig. 4). Indomethacin had a stronger effect (statistically significant) on strips from 1-wk and 3-wk-old animals.

Relaxation After EFS

To evaluate the relaxing properties of ASM in strips from different age animals after the end of an EFS, we used two indexes: \( R_T R_{end} \) and half-relaxation time, i.e., \( t_{1/2,TCT_{end}} \). The results for these two indexes after 20 s EFS are reported in Table 2. In strips from 1-wk, 3-wk, and adult animals, \( R_T R_{end} \) occurred 2.26 ± 0.08, 2.61 ± 0.18, and 2.68 ± 0.13 s, respectively, after the end of the stimulation. \( R_T R_{end} \) showed a maturational change opposite to \( R_T R_{st} \), decreasing significantly with age in the absence of indomethacin.

Relaxation in Deepithelialized Strips

An epithelium contribution to the extent of relaxation in our study was likely because of its release of several mediators, including cyclooxygenase metabolites. Therefore, we performed a further series of experiments using deepithelialized strips from adult animals, to evaluate whether the epithelium could be responsible for the increased relaxation observed in that age group during EFS in the absence of indomethacin.

Also, for deepithelialized strips, the values obtained for parameters describing smooth muscle tone at rest and in response to EFS are shown in Table 1. The removal of the epithelium had a similar effect to indomethacin, abolishing IT and significantly reducing RT and \( TCT_{max} \).

Figure 5 reports \( TCT_{end} \) (A), the time course of TR (B), and \( R_T R_{st} \) (C) in intact and deepithelialized strips from adult animals. Removal of the epithelium significantly increased \( TCT_{end} \) and reduced the amount of relaxation during EFS as well as \( R_T R_{st} \).

The effect of the epithelium removal on relaxation after the end of an EFS is illustrated in Fig. 6, which shows \( R_T R_{end} \) (A) and \( t_{1/2,TCT_{end}} \) (B). This phase of tension relaxation was also significantly reduced by the removal of the epithelium.

DISCUSSION

In the present study we investigated the relaxation occurring during and after EFS in tracheal strips from 1-wk, 3-wk, and 3-mo-old guinea pigs. We found that in course of an EFS the amount and the rate of TR increases with age. Inhibition of cyclooxygenase abolished this difference by increasing relaxation in younger animals. When we removed the epithelium in strips from adult guinea pigs, this relaxation was, by contrast, diminished. We also found that, after the end of an EFS, relaxation takes longer to occur in older animals, becomes even slower when epithelium is removed, but is not affected by indomethacin. These results are the first evidence of a change in ASM relaxing ability during growth. They show that relaxation during and after stimulation depends on different mechanisms, because we found that cyclooxygenase metabolites oppose only the stimulus-dependent relaxation, whereas epithelium facilitates relaxation both during and after stimulation.
It is known that in vivo airway responsiveness to bronchoconstrictor agents decreases gradually from childhood to adulthood (5, 19). Several studies have shown that the ontogenesis of ASM contractility may be crucial in juvenile airway hyperresponsiveness (8, 13, 29) and may play a role in the differences existing between asthma in children and adults (5). Less has been accomplished with regard to ASM relaxation, a factor of ASM responsiveness commonly neglected despite its potential impact on airway responsiveness. The very few studies that have addressed the issue have shown a reduced ASM relaxation in association with increased responsiveness, thus sustaining its conceivable relevance to hyperresponsiveness.

A relaxation phase observed in canine tracheal smooth muscle during EFS was shown to be absent in strips from sensitized animals (1) and dependent on a reduced acetylcholinesterase activity in sensitized tissue (18). In normal dogs, the relaxation phase was shown in a separate study to be partially inhibited by indomethacin (16). That result, in conjunction with the observation of a stimulatory effect of indomethacin on the EFS-induced release of acetylcholine (16), suggests that prostaglandins may act on canine ASM response by inhibiting the release of acetylcholine from nerve endings. The relaxing phase during EFS was also in part inhibited by epithelium removal (14).

Our results are only partially in agreement with these previous reports, because we found that removal of the epithelium inhibits relaxation but that the inhibition of cyclooxygenase metabolites increases it. Species differences may be at the origin of these conflicting observations. Indeed, canine and guinea pig ASM differ in more than one aspect. A conspicuous IT characterizes ASM in guinea pigs, whereas none is present in dogs. Because this IT in guinea pigs is abolished by cyclooxygenase inhibitors, it is possible that a predominant contractile effect is exerted by cyclooxygenase metabolites in this species, whereas a prevalent relaxing effect is produced in canine ASM, as shown by the increased active tension produced by this tissue in the presence of indomethacin (16). Alternatively, cyclooxygenase may mainly catalyze the release of contractile metabolites in guinea pig and relaxing metabolites in dog.

Because of the presence of an elevated IT, we needed to develop new appropriate indexes to normalize relaxation in our guinea pig strips, whereas in canine strips the amount of relaxation could simply be referred to maximum active tension. From the considerations we have reported in METHODS on maximum potential tension drop, it seems evident that these two forms of normalization are in fact equivalent. Nonetheless, the relaxing events we are analyzing could theoretically be independent from the level of tension reached by the previous contraction. If that were the case, we would have overestimated the relaxation values in the presence of indomethacin, whereas, on the contrary, in canine strips the relaxation in the presence of indomethacin would have been underestimated because of
the increased active tension. This might in theory raise concern about the appropriateness of normalizing relaxation to the tension produced by the strip immediately before relaxation begins. Nonetheless, we believe that, to relate relaxation to airway responsiveness, the percentage of tension subtracted from a given level of contraction is indeed the relevant parameter that translates into reversal of bronchospasm.

With respect to maturation, the enhancing effect of indomethacin on the EFS-induced relaxing phase suggests that those cyclooxygenase metabolites that inhibit relaxation are more abundant or more effective in younger animals. Our laboratory is currently investigating the release of these metabolites in guinea pigs of different ages, and the preliminary data suggest that their spontaneous release decreases with age (7). Moreover, in guinea pig airway epithelial cells stimulated with bradykinin, our laboratory has previously observed an age-related increase in the secretion of inhibitory PGE2 accompanied by decrease in contractile thromboxane B2 and leukotriene C4 (20). Completion of the study on cyclooxygenase metabolite release and investigation of cyclooxygenase activity at different ages will be crucial for a better interpretation of the results reported in the present paper. A study on cyclooxygenase expression in ovine lung has indeed shown that its constitutive form reaches a maximum abundance in ASM at 1 mo of age and later declines (3).

Besides a maturation of the profile of their release, a different responsiveness to prostanoids at different ages could occur, although, to the best of our knowledge, this has not been reported. Nonetheless, compared with older animals, airway tissue from immature guinea pigs showed a greater response to exogenous administration of the lipoxygenase metabolite leukotriene D4 (12). Considering that lipoxygenase and cyclooxygenase may exert a reciprocal influence on the profile of their released metabolites, both the involvement of lipoxygenase and the action of each arachidonic acid metabolites on ASM relaxation during development should be further studied.

Although cyclic nucleotides are mainly thought to be important second messengers for agonist-induced relaxation (17), guanosine 3′,5′-cyclic monophosphate has been shown to be also involved in modulating both resting tension and EFS-dependent relaxation (24), suggesting a common mechanism underlying inhibition of ASM tone in these two distinct events. Our data on the effect of indomethacin, which reduces intrinsic tone and increases relaxation during EFS, is in agreement with that suggestion. In particular, our data suggest that EFS may act either by determining a different release of arachidonic acid metabolites at different ages or by affecting the action of their different basal content on ASM tone.

Both adrenergic and nonadrenergic-noncholinergic innervation contribute to ASM relaxation (9, 10, 22). Although our data on the effect of indomethacin suggest that other mechanisms are involved, we cannot exclude, on the basis of our present results, that a maturational change of tracheal innervation is also implicated in the age-dependent increase of relaxation we have observed. Indeed, relaxation during EFS in adult guinea pigs has been reported to be inhibited by blocking β-adrenergic receptors with propranolol (22). Therefore, also the participation of this component of the response remains to be addressed to evaluate both a possible maturation of the adrenergic inhibitory effect and its interactions with the other relaxing components.

Relaxation after the removal of a stimulus has been minimally investigated in ASM. In tracheal smooth muscle from control and sensitized dogs, isotonic relaxation has been evaluated after EFS of 1- and 10-s duration and has been shown to be slower in sensitized strips only after 1-s EFS (26). As the authors pointed out, the same mechanism responsible for increased shortening velocity and capacity, i.e., a different behavior of normally cycling cross bridges, may be the cause of the early prolonged relaxation in sensitized tissue. We measured relaxation after an EFS that lasted 15–25 s, when only latch bridges are expected to be operating, and found a significant decrease of relaxation with age. We suggest that, in our maturational model, these results may be determined by changes in the viscoelastic properties of the tracheal tissue, which conceivably are involved in the process that brings back the tissue to its mechanical equilibrium. However, the removal of the epithelium reduced relaxation in adult strips, suggesting that epithelial factors are also required for an efficient relaxation. Indeed, numerous studies have documented a regulatory effect of the epithelium on ASM, with both contractile and relaxing effect (25). With respect to the main purpose of our study, our results show that epithelium and cyclooxygenase metabolites exert an opposite effect on relaxation during EFS, suggesting that a balance among different factor determine the level of this component of ASM response. Further studies will be needed to establish whether and which other factors are involved in this maturational process.

We finally need to consider that changes in tissue mechanical properties with maturation could affect the relaxing events we have studied in the present paper. Because relaxation during and after EFS increased and decreased with age, respectively, it is conceivable that mechanical factors have a different influence on the two events in conjunction or interacting with non-mechanical factors, e.g., arachidonic acid metabolites. In a previous study on ontogenesis of ASM contractility (8), our laboratory has shown data that support an age-dependent increase of the viscous components in tracheal strips, as revealed by a substantial increase of the internal resistance to shortening. Relaxation data within the present work have been obtained in isometric conditions and are therefore only marginally affected by viscosity. Nonetheless, TR translates in vivo into a reduction of ASM shortening and bronchospasm so that viscoelastic properties need to be investigated during development to evaluate the relevance of our results to an in vivo response. A recent study in rabbit has investigated steady-state stiffness of tracheal mu-
cossal membrane as a function of age (28) but has failed to show significant changes with age. A similar study would be important in our model to address the specific issue of relaxation as well as possible differences among species.

In conclusion, we have shown that the ability of ASM to relax during EFS increases with age by a mechanism at least partially mediated by cyclooxygenase metabolites and that epithelium is required for this relaxation to take place. An increased relaxing capacity associated with a reduced shortening velocity during the contractile phase of ASM response to stimulation (8) may conceivably result in a reduced level of bronchoconstriction in mature guinea pig airways. These findings support the hypothesis that ontogenetic changes in ASM relaxing ability may contribute to maturational changes in airway responsiveness.

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