The involvement of hydroxyl radical and cyclooxygenase metabolites in the activation of lung vagal sensory receptors by circulatory endotoxin in rats

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Lai, Ching Jung, Ting Ruan, and Yu Ru Kou. The involvement of hydroxyl radical and cyclooxygenase metabolites in the activation of lung vagal sensory receptors by circulatory endotoxin in rats. J Appl Physiol 98: 620–628, 2005. First published October 1, 2004; doi:10.1152/japplphysiol.00539.2004.—Circulatory endotoxin can stimulate vagal pulmonary C fibers and rapidly adapting receptors (RARs) in rats, but the underlying mechanisms are not clear. We investigated the involvement of hydroxyl radicals and cyclooxygenase metabolites in the stimulation of C fibers and RARs by circulatory endotoxin (50 mg/kg) in 112 anesthetized, paralyzed, and artificially ventilated rats. In rats pretreated with the vehicle, endotoxin stimulated C fibers and RARs and caused a slight increase in total lung resistance (RL) and a decrease in dynamic lung compliance. In rats pretreated with dimethylthiourea (a hydroxyl radical scavenger) alone, indomethacin (a cyclooxygenase inhibitor) alone, or a combination of the two, C-fiber and RAR responses [C fiber: change (Δ) = −62.79, and −85%; RAR: Δ = −80, −84, and −84%, respectively] were reduced, and the RL response was prevented. The suppressive effects of a combination of dimethylthiourea and indomethacin on the C-fiber and RAR responses were not superior to indomethacin alone. In rats pretreated with isoproterenol (a bronchodilator), the C-fiber response was not significantly affected (Δ = −26%), the RAR response was reduced (Δ = −88%), and the RL response was prevented. None of these pretreatments affected the dynamic lung compliance response. These results suggest that 1) both hydroxyl radicals and cyclooxygenase metabolites are involved in the endotoxin-induced stimulation of C fibers and RARs, and 2) the involvement of these two metabolites in the C-fiber stimulation may be due to their bronchoconstrictive effects.

pulmonary C fibers; rapidly adapting receptors; reactive oxygen metabolites

ENDOTOXEMIA CAUSES TACHYPNEA, leading to hyperventilation in septic shock patients (3, 4), but the causes are not completely understood. The tachypnea induced by circulatory endotoxin in rats is prevented by bilateral cervical vagotomy, suggesting that it is a vagally mediated respiratory reflex (42). Electrophysiological studies in rats have revealed that lung C-fiber nerve endings (C fibers) and pulmonary rapidly adapting receptors (RARs), two major types of lung vagal sensory receptors, are activated by circulatory endotoxin (21). Both lung C fibers and RARs play an important role in the detection of pulmonary pathophysiological conditions and eliciting resultant respiratory reflexes (9, 25, 40). The physiological characteristics of these two types of lung receptors are different (9, 25, 40). For example, lung C fibers are very sensitive to chemical stimuli (e.g., chemical mediators) but relatively insensitive to mechanical stimuli (e.g., bronchoconstriction). In contrast, RARs can be stimulated by chemical mediators and/or changes in lung mechanics. The mechanisms underlying the activation of these lung sensory receptors by circulatory endotoxin are still unclear.

Endotoxin increases the release of a variety of chemical mediators in the lungs, including reactive oxygen metabolite (30, 33) and cyclooxygenase metabolites (5, 34). Hydroxyl radicals (·OH) are an extremely reactive oxygen metabolite formed in the lung tissues under pathological conditions (35). Cyclooxygenase metabolites, including various types of prostaglandins and thromboxane, are produced from the metabolism of arachidonic acid via an enzymatic reaction (1). Both ·OH and cyclooxygenase metabolites, either administered exogenously (2, 10, 20, 28, 38, 39) or formed endogenously (6, 7, 8, 22, 23), can chemically or mechanically stimulate lung C fibers and RARs in several species. On the other hand, endotoxin produces an increase in total lung resistance (RL) and a decrease in dynamic lung compliance (Cdyn) (21, 43, 44), both of which are potential mechanical stimuli for RARs (11, 19). The role of ·OH, cyclooxygenase metabolites, and changes in lung mechanics in the endotoxin-induced stimulation of these two types of lung sensory receptors remain to be investigated.

In this study, we recorded afferent activity arising from pulmonary C fibers and RARs in anesthetized rats to determine, first, whether ·OH and cyclooxygenase metabolites are important in the activation of lung C fibers and RARs induced by circulatory endotoxin, and, second, whether changes in lung mechanics during endotoxemia are involved in this afferent activation. To accomplish these objectives, we compared afferent and bronchomotor responses to intravenous endotoxin in animals pretreated with vehicle, dimethylthiourea (DMTU; a ·OH scavenger), indomethacin (a cyclooxygenase inhibitor), or isoproterenol (a bronchodilator).

MATERIALS AND METHODS

General preparations. Sprague-Dawley rats (weight 330 ± 5 g; n = 112) of either sex were anesthetized with an intraperitoneal injection of chloralose (100 mg/kg; Sigma Chemical, St. Louis, MO) and urethane (500 mg/kg; Sigma Chemical). A polyethylene catheter was inserted into the jugular vein and advanced until the tip was close to the right atrium for intravenous administration of pharmacological agents. The right femoral artery was cannulated to measure arterial blood pressure. During the course of the experiments, supplemental doses of chloralose (20 mg·kg−1·h−1) and urethane (100 mg·kg−1·h−1) were administered to maintain the abolition of pain reflexes induced by pinching the animal’s tail. During the recording of

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vagal action potentials, the rats were paralyzed with pancuronium bromide (0.05 mg/kg iv; Organon Teknika, Baoxt, Holland). Periodically, the effect of pancuronium was allowed to wear off so that the depth of anesthesia could be checked. Body temperature was maintained at ~36°C throughout the experiment by means of a servo-controlled heating blanket. The experimental protocols described in this study were approved by the Institutional Animal Care and Use Committee and were in accordance with the recommendations in the “Guide for the Care and Use of Laboratory Animals” published by the National Institute of Health, Bethesda, MD.

The rats were tethered in a supine position, and the trachea was cannulated below the larynx with a short tracheal tube via a tracheotomy. A midline thoracotomy was performed, and the edges of the rib cage were retracted. The lungs were ventilated by a rodent respirator (Harvard 683; South Natick, MA) at a constant tidal volume (VT) of 2 ml. The frequency of the respirator was set at 60–65 breaths/min and was kept constant for each experiment. The expiratory outlet of the respirator was placed under 3–4 cm of water to maintain a near normal functional residual capacity. Respiratory flow was measured by using a pneumotachograph (Fleisch 4/O; Richmond, VA) coupled with a differential pressure transducer (Validyne MP45–28) via a side tap of the tracheal cannula. RL and Cdyn were determined by using the subtraction method (29). All physiological signals were recorded by a thermal array recorder (Gould TA11; Cleveland, OH) and also recorded on tape (Neurocorder DR-890; New York, NY) for later analysis.

Recording of afferent activity. Afferent activity arising from the lung vagal sensory receptors was recorded by using techniques described elsewhere (24). Briefly, the left vagus nerve was left intact, whereas the right vagus nerve was sectioned. A fine afferent filament was split from the desheathed right nerve trunk and placed on a platinum-iridium recording electrode. Action potentials were amplified (Grass PS11K; Quincy, MA), monitored by using an audio monitor (Grass AM8), and displayed on an oscilloscope (Gould 420). The fine-nerve filament was subdivided until activity from only one or two units was obtained. The strategies used to search for different types of lung vagal sensory receptors have been well-described previously (22, 23). In brief, the lungs were hyperinflated in a steplike manner to 4 VT (Fig. 1A) or by constant pressure inflation (~20 cmH2O), which was maintained for 6–15 s. Vagal pulmonary C fibers are activated by lung hyperinflation to a relatively high volume (e.g., 4 × VT; Fig. 1A), whereas RARs could be activated by a relatively low volume (e.g., 2 × VT; Fig. 1C) (9). Once the presence of a suspected single C-fiber unit was detected, capsaicin (1–2 µg/kg; Sigma Chemical), a potent chemical stimulant of C fibers, was injected as a bolus into the vein. The capsaicin solution was made from a refrigerated stock solution (5 mg/ml), which was prepared by dissolving capsaicin in 10% ethanol, 10% Tween 80, and 80% saline. Only C fibers that showed stimulation within 2 s after the injection were studied. Once the presence of a suspected single RAR was detected, the RAR response to lung deflation (Fig. 1D) was studied by exposing the expiratory outlet of the respirator to atmospheric pressure for a period of 8–10 s. This was performed because a majority of RARs are activated by lung deflation (9). Furthermore, units displaying an adaptation index to maintained lung inflation >70% were regarded as RARs (48). Finally, the conduction velocity of the majority of the afferent fibers of the lung sensory receptors was measured by a method described previously (16); the remaining fibers were not measured due to serious bleeding during the surgery. Before the end of each experiment, the general locations of the receptors studied were identified within the lung structure by gently probing the tissues with a polyethylene rod (diameter = 2 mm).

Pharmacological agents. DMTU and isoproterenol were dissolved separately in isotonic saline (vehicle 1) to a concentration of 500 and 0.5 mg/ml, respectively. Indomethacin was first dissolved in polyethylene glycol and then diluted at a 1:1 ratio in isotonic saline (vehicle 2) to a concentration of 5 mg/ml. All of these pharmacological agents were purchased from Sigma Chemical.

Experimental procedures. In total, 112 rats were evenly divided into seven groups for pretreatments with vehicle 1 (the Vehicle-1 group), DMTU (the DMTU group; 500 mg/kg), vehicle 2 (the Vehicle-2 group), indomethacin (the Indo group; 5 mg/kg), a combination of vehicle 1 and 2 (the Vehicle-1+2 group), a combination of DMTU and indomethacin (the DMTU+Indo group), and finally isoproterenol (the Isop group; 0.5 mg/kg). A solution of these chemicals with a volume of 0.7 ml was slowly injected into the jugular vein over a period of 20 s. Twenty minutes later, these animals received an intravenous injection of endotoxin (E. coli lipopolysaccharide, 50 mg/kg; Sigma Chemical). Endotoxin at a volume of 0.5–0.8 ml was slowly injected into the same vein over a period of 1 min. In the Isop group, rats received a constant infusion of isoproterenol (0.05 mg·kg⁻¹·min⁻¹ iv, for 90 min) after endotoxin injection to maintain the drug’s effect. The doses of DMTU, indomethacin, isoproterenol, and endotoxin noted above have been previously used in a study of the afferent stimulation of pulmonary C fibers and RARs in rats (21–23). Each group was subdivided into two subgroups for the study of pulmonary C fibers and RARs. Only one receptor was studied in each animal. Each test period included continuous recording of the neural activity of the lung vagal sensory receptors for 10 min before and at least 90 min after the endotoxin injection. To confirm that pulmonary C fibers and RARs remained active, intravenous injection of capsaicin (1–2 µg/kg) and lung hyperinflation (4 × VT), respectively, were performed at the end of the test period. Results were discarded for

![Fig. 1. Response of a pulmonary C fiber to lung hyperinflation (A) or capsaicin (B) and afferent response of a pulmonary rapidly adapting receptor to lung hyperinflation (C) or lung deflation (D). A and C: the lungs were hyperinflated to 4 times tidal volume. B: capsaicin (1 µg/kg) was injected into the catheter at the first arrow and flushed into the vein at the second arrow. The catheter had its tip close to the right atrium. D: the lungs were deflated to atmospheric pressure. AP, action potential; Pr, tracheal pressure; ABP, arterial blood pressure.](http://jap.physiology.org/content/10.1152/jappl.00671.2004)
nine receptors that had become inactive during the test and/or were unresponsive to capsaicin or hyperinflation at the end of the test period.

Data analysis and statistics. Neural activity of the vagal sensory receptors, Rt, Cdyn, mean arterial blood pressure (MABP), and heart rate (HR) were continuously analyzed at 1-s intervals or on a breath-by-breath basis. Data of neural activity, Rt, and Cdyn were then averaged every minute to give mean values to plot responses over time. Baseline data of these physiological parameters were calculated as the average values over the 5-min period immediately preceding intravenous injection of the endotoxin. Because we found that changes in receptor discharge induced by the endotoxin challenge exhibited a bimodal pattern, responses occurring during 0–20 min and during 21–90 min after the onset of challenge were defined as the initial and delayed responses, respectively, and their peak responses were defined as the maximal values averaged over 5 consecutive min during these two phases. Peak responses of Rt, Cdyn, MABP, or HR were defined as the maximal or minimal values averaged over 5 consecutive min after vehicle or endotoxin injection. Because mean values and variabilities of their baseline activity were quite small (see RESULTS), pulmonary C fibers and RARs were judged to be activated by endotoxin when the peak response exceeded its baseline activity by at least 0.5 impulses/s. Once receptors were judged to be activated, the time of the first mean value of discharge averaged over a 1-min period that exceeded the baseline activity by at least 0.5 impulses/s was regarded as the commence time of stimulation. These physiological parameters were analyzed by using a computer equipped with an analog-to-digital converter (Gould DASA 4600) and software (Biocybernatics 1.0; Taipei, Taiwan). Data obtained from the computer analysis were routinely checked for accuracy with those calculated manually. The results were evaluated by the Student’s t-test or a two-way mixed-factorial analysis of variance followed by Duncan’s test when appropriate; the time effect was the factor for within-group comparisons, whereas the drug effect was the factor for between-group comparisons. P < 0.05 was considered significant. All data are presented as means ± SE.

RESULTS

Characteristic of the C fibers and RARs studied. The baseline activity of the pulmonary C fibers studied was irregular and sparse (0.05 ± 0.02 impulses/s; n = 56). These nerve endings were stimulated by hyperinflating the lungs up to 3 or 4 × Vr (Fig. 1A) and by bolus intravenous injection of capsaicin (Fig. 1B). The average baseline activity of RARs studied was also sparse (0.09 ± 0.02 impulses/s; n = 56). Three receptors exhibited a baseline activity in phase with ventilatory cycles, whereas the others had irregular or no baseline activity. The evoked discharge of these RARs in response to maintained lung inflation showed rapid adaptation (Fig. 1C), and the mean adaptation index of the response to the fourth Vr inflation (inflation pressure > 20 cmH2O) reached 88.7 ± 1.2% (range: 81.2–100.0%; n = 56). A majority (54 of 56) of these RARs were also activated by lung deflation (Fig. 1D). The mean conduction velocities of the afferent fibers conducting impulses from these pulmonary C fibers and RARs were 1.1 ± 0.1 m/s (range 0.5–1.7 m/s; n = 36) and 13.6 ± 0.9 m/s (range 5.2–17.8 m/s; n = 32), respectively.

Control afferent responses to endotoxin. In the Vehicle-1, Vehicle-2, and Vehicle-1 and Vehicle-2 groups (each eight receptors), intravenous injection of endotoxin stimulated seven, seven, and six pulmonary C fibers and activated eight, seven, and seven RARs, respectively. The stimulation of pulmonary C fibers (Figs. 2A and 4) and RARs (Figs. 3A and 5) started within 4.4 ± 0.8 min (range = 1–13 min; n = 20) and 3.6 ± 0.7 min
(range = 1–14 min; n = 22), respectively, after endotoxin injection, and both lasted until the end of the observation period. When stimulated, both types of lung vagal sensory receptors fired irregularly, and the evoked discharge had no obvious relation to the ventilatory cycle. The analysis of response over time revealed that receptor responses to endotoxin exhibited a bimodal pattern (Figs. 4 and 5). The initial phase had an early increase and a subsequent decrease in receptor discharge within 20 min after endotoxin injection, and the delayed phase had a second and more sustained increase in receptor discharge during the rest of the 70-min observation period (Figs. 4 and 5). As a group, the activity of pulmonary C fibers (n = 24) and RARs (n = 24) increased from their baselines of 0.09 \pm 0.02 and 0.10 \pm 0.02 impulses/s to peaks of 1.34 \pm 0.18 (time to reach the peak response = 2–15 min after injection) and 2.39 \pm 0.39 impulses/s (time to reach the peak response = 2–16 min after injection) during the initial phase, and to peaks of 0.96 \pm 0.11 (time to reach the peak response = 22–62 min after injection) and 2.96 \pm 0.38 impulses/s (time to reach the peak response = 24–57 min after injection) during the delayed phase, respectively, after endotoxin injection.

Effects of DMTU, indomethacin, or isoproterenol on afferent responses. In the DMTU, Indo, DMTU+Indo, and Isop groups (each eight receptors), endotoxin injection stimulated seven, four, three, and eight pulmonary C fibers and activated six, four, three, and five RARs, respectively. With respect to both amplitude and duration of the responses, endotoxin injection appeared to evoke a milder C-fiber stimulation in the DMTU, Indo, and DMTU+Indo groups (Figs. 2 and 4) and a milder RAR stimulation in all four groups (Figs. 3 and 5). Between-group comparisons revealed that the peak responses of pulmonary C fibers (Fig. 6A) or RARs (Fig. 6B) occurring during the initial and delayed phases were largely attenuated by pretreatment with DMTU, indomethacin, or a combination of DMTU and indomethacin. Furthermore, while pretreatment with isoproterenol largely reduced the initial and delayed peak responses of the RARs (Fig. 6B), it failed to significantly alter these two responses of the pulmonary C fibers (Fig. 6A). At the end of the test period, all pulmonary C fibers and RARs in these four groups still responded to an intravenous injection of capsaicin and lung inflation, respectively, and their mean responses had not been significantly reduced by pretreatment with these pharmacological agents (Table 1).

Effects of indomethacin, DMTU, or isoproterenol on cardiopulmonary responses. In the groups pretreated with various types of vehicle, intravenous injection of endotoxin caused a slight increase in Rl (Fig. 7) and a decrease in Cdyn (Fig. 7), MABP (Figs. 2A and 3A), or HR. Rl increased to its peak in 3–9 min after endotoxin injection, subsequently declined, and was then maintained at a level higher than its baseline throughout the rest of the test period (Fig. 7). Cdyn had an initial drop within 3–6 min after endotoxin injection and progressively declined to its lowest value by the end of the test period (Fig. 7). MABP and HR initially dropped to their lowest values within 3–7 min after endotoxin injection in all animals (Figs. 2A and 3A). Subsequently, MABP and HR stayed low in 42 animals (Figs. 2A and 3A) or increased before dropping again...
in the other 6 (Fig. 3, B and C). In all animals, MABP and HR returned to a plateau level at 30–56 min after endotoxin injection and did not change further for the rest of the observation period (Figs. 2A and 3A). Pretreatment with DMTU, indomethacin, and a combination of DMTU and indomethacin prevented the increase in Rt. (Table 2 and Fig. 7), but it failed to affect the time course or reduction of the Cdyn (Table 2 and Fig. 7). MABP (Table 3), or HR (Table 3) response to endotoxin. Similarly, pretreatment with isoproterenol prevented the increase in Rt. but did not affect the decrease in Cdyn (Table 2). Particularly, pretreatment with isoproterenol produced sustained tachycardia and hypotension before the endotoxin injection, both of which persisted following the endotoxin injection because of the continuous infusion of the drug (Table 3).

Fig. 4. Mean afferent responses of pulmonary C fibers to intravenous injection of endotoxin in 7 groups of rats with various pretreatments. Pretreatments: DMTU, Indo, a combination of DMTU and Indo (DMTU+Indo), isoproterenol (Isop), Vehicle-1 (saline; vehicle for DMTU or Isop), Vehicle-2 (a mixture of polyethylene glycol and saline; vehicle for Indo), or a combination of vehicles 1 and 2 (Vehicle-1+2). Vertical dashed lines indicate onset time of endotoxin injection. FA, fiber activity. Data in each group are means ± SE of 8 animals. Note that data for Vehicle-1 in the top left panel are identical to data of Vehicle-1 in the bottom right panel.

Fig. 5. Mean afferent responses of pulmonary rapidly adapting receptors to intravenous injection of endotoxin in 7 groups of rats with various pretreatments. See legend of Fig. 4 for explanations of pretreatments. Vertical dashed lines indicate onset time of endotoxin injection. Data in each group are means ± SE of 8 animals. Note that the 2 sets of Vehicle-1 data are the same.
C-fiber, RAR, RL, and Cdyn responses to endotoxin were consistent with those reported previously (21). Furthermore, our results demonstrate that the afferent responses of these two types of lung sensory receptors to circulatory endotoxin were largely attenuated by pretreatment with DMTU alone, indomethacin alone, or DMTU and indomethacin in combination. In contrast to these effects, pretreatment with vehicle did not affect overall C-fiber and RAR stimulation. DMTU is an effective scavenger for "OH, whereas indomethacin is a cyclooxygenase inhibitor that inhibits the production of prostaglandins and thromboxane. Hence, our results suggest that both "OH and cyclooxygenase metabolites are involved in the endotoxin-stimulated activation of pulmonary C fibers and RARs.

Several investigators have demonstrated that administration of exogenous reactive oxygen metabolites or cyclooxygenase metabolites may stimulate lung C fibers and RARs (2, 10, 20, 28, 38, 39). Our previous studies have shown that endogenous "OH and/or cyclooxygenase metabolites are important activators for the stimulation of these two types of lung sensory receptors when the airway is insulted by inhaled wood smoke (22, 23) or when air emboli are lodged in the pulmonary vessels (6, 7, 8). "OH and/or cyclooxygenase metabolites also participate in the stimulation of abdominal visceral sympathetic C fibers and cardiac vagal C fibers by ischemia and/or reperfusion (27, 41, 46). Hence, it appears that these two metabolites are important activators for a variety of visceral sensory receptors under various pathological conditions. The sources of these two metabolites are not well understood. However, it is known that the lungs are a rich source of arachidonate products and the enzymes necessary for their metabolism (1). Furthermore, circulating leukocytes and lung cells have been suggested as possible sources for the production of oxygen radicals during endotoxemia (30, 49).

Fig. 6. Average afferent responses of pulmonary C fibers (A) and rapidly adapting receptors (B) to intravenous injection of endotoxin in 14 groups of rats with various pretreatments. See legend of Fig. 4 for explanations of pretreatments. The initial and delayed responses were defined as peak responses occurring within 20 min after endotoxin and during the rest of the 70-min observation period, respectively. Data in each group are means ± SE of 8 animals. Note that the 2 sets of Vehicle-1 data in both A and B are the same. * Significantly different from corresponding baseline activity; † significantly different from responses to vehicle: P < 0.05.

DISCUSSION

Our laboratory previously demonstrated that intravenous injection of endotoxin, but not its vehicle, stimulates both C fibers and RARs and causes an increase in RL and a decrease in Cdyn (21). In the present study, the characteristics of the C-fiber, RAR, RL, and Cdyn responses to endotoxin were measured immediately after the 90-min observation period. See legend of Fig. 4 for explanations of pretreatments. The initial and delayed responses were defined as peak responses occurring within 20 min after endotoxin and during the rest of the 70-min observation period, respectively. Data in each group are means ± SE of 8 animals. Note that the 2 sets of Vehicle-1 data in both A and B are the same. * Significantly different from corresponding baseline activity; † significantly different from responses to vehicle: P < 0.05.

Table 1. Average peak responses of pulmonary C fibers to intravenous capsaicin and rapidly adapting receptors to lung hyperinflation before and after various pharmacological pretreatments in the seven study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>C-Fiber Responses to Capsaicin</th>
<th>RAR Responses to Hyperinflation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before pretreatment</td>
<td>After pretreatment</td>
</tr>
<tr>
<td>Vehicle-1</td>
<td>10.5 ± 1.8</td>
<td>9.8 ± 1.3</td>
</tr>
<tr>
<td>DMTU</td>
<td>14.3 ± 2.6</td>
<td>13.8 ± 2.1</td>
</tr>
<tr>
<td>Vehicle-2</td>
<td>11.8 ± 1.6</td>
<td>12.5 ± 1.2</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>9.5 ± 1.0</td>
<td>10.3 ± 1.6</td>
</tr>
<tr>
<td>Vehicle-1+2</td>
<td>12.8 ± 2.1</td>
<td>11.0 ± 1.1</td>
</tr>
<tr>
<td>DMTU+Indomethacin</td>
<td>13.0 ± 2.3</td>
<td>11.0 ± 2.0</td>
</tr>
<tr>
<td>Isopretanol</td>
<td>12.4 ± 2.2</td>
<td>11.8 ± 1.6</td>
</tr>
</tbody>
</table>

Values are means ± SE in impulses/s; n = 8 per group. RAR, rapidly adapting receptor. Animals received pretreatment with dimethylthiourea (DMTU), indomethacin (Indo), a combination of DMTU and Indo (DMTU+Indo), isoproterenol (Isop), vehicle 1 (Vehicle-1; saline; vehicle for DMTU or Isop), vehicle 2 (Vehicle-2; a mixture of polyethylene glycol and saline; vehicle for Indo), or a combination of vehicles 1 and 2 (Vehicle-1+2). Capsaicin was injected at a dose of 1–2 μg/kg. The lung was hyperinflated to 4 × tidal volume. Responses were peak values within 5 s after capsaicin injection or after the fourth tidal volume inflation. Responses after various pretreatments were measured immediately after the 90-min observation period. *P < 0.05 vs. corresponding responses before pretreatment.
that baseline levels of lung C fibers to such chemicals. Thus a second possibility is ability to maintain or to potentiate the sensitivity of cardiac or that cascade leading to receptor stimulation. It has been suggested Therefore, one possibility is that the involvement of metabolites, has been postulated (8, 10, 20, 22, 23, 25, 40). Averaged bronchomotor responses to circulatory endotoxin in 7 groups of rats pretreated with various pharmacological pretreatments. See legend of Fig. 4 for explanations of pretreatments. Vertical dashed lines indicate onset time of endotoxin injection. RL, total lung resistance; Cdyn, dynamic lung compliance. Data in each group are means ± SE of 16 animals. Note that the 2 sets of Vehicle-1 data are the same.

The mechanisms by which -OH and cyclooxygenase metabolites are associated with the stimulation of pulmonary C fibers and RARs by circulatory endotoxin remain unclear. A direct stimulation of these two types of lung sensory receptors by metabolites affected the Cdyn response to endotoxin, this augmented pretreatment in each group. Because none of these pretreatments affected the Cdyn response to endotoxin, this augmented RAR response to lung hyperinflation was presumably due to the fact that the lung was stiffer following endotoxin challenge as evidenced by a decrease in Cdyn. An indirect stimulation of required to maintain receptor sensitivity. Consequently, administration of DMTU and indomethacin might make pulmonary C fibers and RARs less responsive to the endotoxin-related stimulus. However, this possibility is not likely because pretreatment with DMTU alone, indomethacin alone, or their combination did not reduce the C-fiber response to capsaicin and the RAR response to lung hyperinflation in this study. In fact, the response of RARs to lung hyperinflation was enhanced after pretreatment in each group. Because none of these pretreatments affected the Cdyn response to endotoxin, this augmented RAR response to lung hyperinflation was presumably due to the fact that the lung was stiffer following endotoxin challenge as evidenced by a decrease in Cdyn. An indirect stimulation of

Table 2. Averaged bronchomotor responses to circulatory endotoxin in the seven groups of rats pretreated with various pharmacological pretreatments

<table>
<thead>
<tr>
<th>Group</th>
<th>RL, cmH2O·ml⁻¹·s⁻¹ Baseline</th>
<th>Peak</th>
<th>RL, cmH2O·ml⁻¹·s⁻¹ Baseline</th>
<th>Cdyn, ml/cmH2O Baseline</th>
<th>Maximal reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle-1</td>
<td>0.24±0.01</td>
<td>0.26±0.01*</td>
<td>0.34±0.01</td>
<td>0.26±0.02*</td>
<td></td>
</tr>
<tr>
<td>DMTU</td>
<td>0.23±0.01</td>
<td>0.24±0.01*</td>
<td>0.34±0.02</td>
<td>0.27±0.02*</td>
<td></td>
</tr>
<tr>
<td>Vehicle-2</td>
<td>0.24±0.01</td>
<td>0.26±0.01*</td>
<td>0.33±0.02</td>
<td>0.25±0.01*</td>
<td></td>
</tr>
<tr>
<td>Indo</td>
<td>0.23±0.01</td>
<td>0.24±0.01*</td>
<td>0.33±0.01</td>
<td>0.26±0.02*</td>
<td></td>
</tr>
<tr>
<td>Vehicle-1+2</td>
<td>0.24±0.01</td>
<td>0.26±0.01*</td>
<td>0.33±0.02</td>
<td>0.25±0.02*</td>
<td></td>
</tr>
<tr>
<td>DMTU+Indo</td>
<td>0.24±0.01</td>
<td>0.24±0.01*</td>
<td>0.33±0.01</td>
<td>0.26±0.01*</td>
<td></td>
</tr>
<tr>
<td>Isop</td>
<td>0.23±0.01</td>
<td>0.23±0.01*</td>
<td>0.34±0.01</td>
<td>0.26±0.02*</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SE; n = 16 per group. RL, total lung resistance; Cdyn, dynamic lung compliance. *P < 0.05 vs. corresponding baseline value.

Table 3. Averaged cardiovascular responses to circulatory endotoxin in the seven groups of rats pretreated with various pharmacological pretreatments

<table>
<thead>
<tr>
<th>Group</th>
<th>MABP, mmHg Baseline</th>
<th>Maximal reduction</th>
<th>End of test period</th>
<th>HR, beats/min Baseline</th>
<th>Maximal reduction</th>
<th>End of test period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle-1</td>
<td>91±4</td>
<td>38±6*</td>
<td>81±5</td>
<td>395±8</td>
<td>352±12*</td>
<td>387±7</td>
</tr>
<tr>
<td>DMTU</td>
<td>89±3</td>
<td>42±3*</td>
<td>77±5</td>
<td>388±10</td>
<td>344±12*</td>
<td>373±8</td>
</tr>
<tr>
<td>Vehicle-2</td>
<td>92±8</td>
<td>40±4*</td>
<td>82±5</td>
<td>400±9</td>
<td>351±15*</td>
<td>388±10</td>
</tr>
<tr>
<td>Indo</td>
<td>94±4</td>
<td>41±8*</td>
<td>81±6</td>
<td>392±7</td>
<td>323±10*</td>
<td>381±6</td>
</tr>
<tr>
<td>Vehicle-1+2</td>
<td>90±6</td>
<td>36±5*</td>
<td>81±3</td>
<td>381±9</td>
<td>343±9*</td>
<td>371±12</td>
</tr>
<tr>
<td>DMTU+Indo</td>
<td>88±3</td>
<td>37±3*</td>
<td>78±5</td>
<td>390±7</td>
<td>335±8*</td>
<td>380±8</td>
</tr>
<tr>
<td>Isop</td>
<td>45±6</td>
<td>36±4</td>
<td>43±4</td>
<td>437±9</td>
<td>413±16</td>
<td>423±9</td>
</tr>
</tbody>
</table>

Values are mean ± SE; n = 16 per group. MABP, mean arterial blood pressure; HR, heart rate. *P < 0.05 vs. corresponding baseline value.
these two types of lung sensory receptors by mechanical stimuli, such as an increase in interstitial fluid volume (15, 36) and changes in lung mechanics (8, 11, 19, 20), has been reported. Furthermore, -OH and cyclooxygenase metabolites may cause pulmonary edema (13, 26). In addition to the decreased Cdyn, circulatory endotoxin elicited an increase in Rl in this study. The increase in Rl may reflect central airway constriction, whereas a decrease in Cdyn may reflect peripheral airway constriction and/or increased interstitial fluid (12). Accordingly, a third possibility is that the involvement of -OH and cyclooxygenase metabolites is mediated through their ability to cause an increase in interstitial fluid volume or by induction of a change in lung mechanics. However, pretreatment with DMTU, indomethacin, or their combination did not affect the decrease in Cdyn, yet it did attenuate the C-fiber and RAR responses to endotoxin. In contrast, pretreatment with DMTU, indomethacin, or their combination prevented the increase in Rl induced by endotoxin. Furthermore, pretreatment with isoproterenol, a bronchodilator, also prevented this increase in Rl, as well as largely reducing the RAR response, but had no effect on the C-fiber response. The inability of isoproterenol to modify the C-fiber response was not because the drug effects had worn off, because the hypotension induced by isoproterenol persisted throughout the test period. Collectively, these observations support the notion that endotoxin-induced airway constriction plays no role in the C-fiber stimulation, whereas it is responsible for a significant portion of the RAR activation. The dissociation of the relationship between the afferent and Cdyn response to endotoxin implies that the involvements of -OH and cyclooxygenase metabolites in the stimulation of these two types of lung sensory receptors is unlikely to be mediated through increased interstitial fluid. Following the endotoxin challenge, the changes in discharge of C fibers and RARs exhibited a bimodal pattern. This may raise a question of whether C-fiber and RAR responses that occurred during the initial and delayed phases may be mediated through different mechanisms. However, pretreatment with either DMTU alone or indomethacin alone suppressed both initial and delayed responses of C fibers and RARs, indicating that either -OH or cyclooxygenase metabolites participate in the afferent stimulation during both phases. Furthermore, the bronchomotor responses to endotoxin challenge did not display a similar bimodal pattern, suggesting the possibility that measurements of lung mechanics may not completely reflect the situation of airway constriction.

In this study, the suppressive effect of pretreatment with a combination of DMTU and indomethacin was not superior to that of pretreatment with indomethacin alone. We speculate that there is an overlap in the functional contributions of -OH and cyclooxygenase system to the C-fiber and RAR responses to circulatory endotoxin. Additionally, pretreatment with DMTU alone, indomethacin alone, or a combination of DMTU and indomethacin did not completely abolish the C-fiber and RAR responses to endotoxin. Pretreatment with DMTU, indomethacin, or a combination of DMTU and indomethacin failed to attenuate the decrease in MABP and HR caused by the endotoxin. These results suggest that factors other than -OH and cyclooxygenase metabolites may also be involved in the afferent and cardiovascular responses. Similarly, pretreatment with isoproterenol did not completely abolish the RAR responses to endotoxin, implying that factors other than airway constriction may contribute to these afferent responses. Indeed, circulatory endotoxin causes the release of a wide array of chemical mediators, such as histamine (31), lipoxygenase metabolites (34), nitric oxide (18), and cytokines (17). Recent investigations have suggested that cytokine interleukins are the chemical mediators responsible for activation of abdominal vagal afferents by circulatory endotoxin, thus providing the signaling to elicit several brain-mediated illness responses, including fever (37), hyperalgesia (47), and the activation of the hypothalamic-pituitary-adrenal axis (14). Evidently, stimulation of vagal afferents by endotoxin-related mediators is not unique in the lung.

In our laboratory’s previous studies (21, 42, 43), endotoxin at doses of 30 – 50 mg/kg was used to induce experimental endotoxemia, characterized by hyperventilation, hypotension, changes in lung mechanics, and airway injury. These cardiovascular-pulmonary responses are similar to those observed during clinical endotoxemia (3, 4, 32). It is known that humans and sheep are exquisitely sensitive to endotoxin, whereas dogs and rats require 1,000- to 10,000-fold higher doses to produce cardiopulmonary responses (32). The dose of endotoxin (50 mg/kg) used in this study was at the upper limit of the range. While the high dose of endotoxin does not invalidate our results, it, nevertheless, limits the generalizability of our findings.

In conclusion, our results demonstrate that both -OH and cyclooxygenase metabolites are involved in the stimulation of pulmonary C fibers and RARs evoked by circulatory endotoxin. The stimulation of pulmonary C fibers is presumably due to the chemical action of these two metabolites, whereas the activation of RARs results from the mechanical effect of bronchoconstriction induced by these two metabolites.

GRANTS

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REFERENCES

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