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Chronic exposure to sidestream tobacco smoke augments lung C-fiber responsiveness in young guinea pigs

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Chronic exposure to sidestream tobacco smoke augments lung C-fiber responsiveness in young guinea pigs. J. Appl. Physiol. 87(2): 757–768, 1999.—Children chronically exposed to environmental tobacco smoke (ETS) may have more coughs, wheezes, and airway obstruction, which may result in part from stimulation of lung C fibers. We examined the effect of chronic exposure to sidestream tobacco smoke (SS, a surrogate for ETS) on lung C-fiber responsiveness in guinea pigs, in which dynamic compliance (Cdyn), lung resistance, tracheal pressure, arterial blood pressure, and heart rate were also monitored. Guinea pigs were exposed to SS (1 mg/mm³ total suspended particulates) or filtered air 5 days/wk from 1 to 6 wk of age. They were then anesthetized, and lung C fibers (n = 55), identified by a conduction velocity of >2.0 m/s, were tested for responsiveness to chemical and mechanical stimuli. SS exposure doubled C-fiber responsiveness to left atrial capsaicin (P < 0.03) but had no effect on responsiveness to inhaled capsaicin or bradykinin on baseline activity. The data indicate that chronic exposure to sidestream smoke enhances C-fiber sensitivity to certain stimuli and may help explain respiratory symptoms in children exposed to ETS.

bronchopulmonary C fibers; airways; environmental tobacco smoke

EXPOSURE TO ENVIRONMENTAL tobacco smoke (ETS), the product of the smoldering end of cigarettes mixed with exhaled mainstream smoke, adversely affects the respiratory health of children. Children living in homes where they are exposed to ETS have more coughing (12, 13), wheezing (12), and airway obstruction (35), increased airway reactivity (13, 15) and sputum production (12), and increased risk of lower respiratory illnesses (32). These children also exhibit an increased rate and earlier onset of asthma (36).

Some of the respiratory symptoms may be caused, at least in part, by repeated acute stimulation of vagal sensory C fibers in the lungs during ETS exposure. First, some of the same respiratory symptoms associated with ETS exposure (e.g., cough, bronchoconstriction, increased mucus secretion, and increased microvascular leak) are elicited by acute stimulation of the bronchopulmonary C fibers via a local axon or a central reflex (8, 29). Second, bronchopulmonary C fibers are vigorously stimulated by acute exposures to components of ETS, including nicotine (29), acrolein (22), and oxidants (9), as well as by acute exposures to mainstream tobacco smoke (10, 23–25).

The consequences of chronically exposing young guinea pigs to ETS on the activity of the bronchopulmonary C fibers are unknown. However, data on the effects of prolonged exposure to mainstream smoke on changes in bronchopulmonary C-fiber reflex function may correspond to changes in C-fiber activity. The findings, all in adult animals, are somewhat disparate. Karlsson et al. (20) reported that chronic exposure to mainstream tobacco smoke (1 h twice daily for 2 wk) enhanced coughing induced by nebulized citric acid or capsaicin, suggesting that prolonged exposure to mainstream smoke may augment C-fiber activity or at least reflex function. On the other hand, Swanny et al. (33) showed that chronic exposure to mainstream tobacco smoke (over 4–8 wk) attenuated the reflex apnea and tachypnea evoked by acutely inhaled mainstream tobacco smoke. These findings suggest that C-fiber responsiveness, at least to acute inhalation of mainstream tobacco smoke, rather than being augmented, may be blunted by continuous exposure to mainstream tobacco smoke (33).

We examined the effects of chronically exposing young guinea pigs from 1 to 6 wk of life to sidestream tobacco smoke (the surrogate for ETS) on the local axon reflex and found that their isolated lungs showed a smaller increase in lung resistance (RL) in response to capsaicin injected in the pulmonary circulation than the filtered air-exposed controls did (18). These findings, like those by Swanny et al. (33), are consistent with the notion that chronic exposure to environmental or mainstream tobacco smoke can result in a blunted responsiveness of some components of the C-fiber reflex.

Still lacking, however, is direct evidence relating to C-fiber responsiveness obtained by electrophysiological recording of C-fiber afferent impulse activity. Hence, the major objective of the present study was to examine the consequences of chronic exposure of the developing organism to sidestream smoke on the responsiveness of intrapulmonary bronchopulmonary C fibers as measured by recordings of their impulse activities. Side-stream smoke was collected from the smoldering end of cigarettes, and its constituents were explicitly characterized. We determined the effects of 5 wk of sidestream smoke exposure (during the equivalent time of human childhood) on the baseline and evoked activity of bronchopulmonary C fibers in intact guinea pigs. A variety of stimuli and routes of administration were used to determine whether potential differences were due to 1) the administration route of the same stimulant, 2) the chemical nature of the stimulant, and/or 3) whether the...
fibers that responded with shorter onset latencies (16). We focused our analysis on bronchopulmonary C fibers in an in vitro preparation (14), and administered by injection into the left atrium or by changes in airway function, we examined the temporal changes in C-fiber activity were mediated secondarily to determine whether the sidestream smoke-induced pressure (ABP), and heart rate (HR) were also monitored during administration of left atrial capsaicin, nebulized capsaicin, and nebulized bradykinin. Finally, to determine whether the sidestream smoke-induced changes in C-fiber activity were mediated secondarily by changes in airway function, we examined the temporal relationship between the changes in P2r and C-fiber activity.

METHODS

All experimental protocols in this work were reviewed and approved by the Institutional Animal Care and Use Committee in compliance with the Animal Welfare Act and in accordance with Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Chronic exposure to sidestream tobacco smoke. Male Dunkin-Hartley guinea pigs (Charles River Laboratories, Raleigh, NC) were randomly assigned to a group exposed to sidestream smoke, the surrogate for ETS (n = 29), or filtered air (n = 27) for 6 h/day, 5 days/wk, from 7–9 to 41–45 days of life. During exposures the guinea pigs were housed in polycarbonate cages (41 × 20 cm cross-sectional area) with wire lids and autoclaved wood shavings for bedding. They were fed guinea pig chow and water ad libitum, including during the exposure periods. Sidestream smoke was generated by a modified ADL/II smoke-exposed system (Little, Cambridge, MA) from conditioned 1R4F cigarettes from the University of Kentucky Tobacco and Health Research Institute (Lexington, KY). Two cigarettes at a time were smoked under Federal Trade Commission conditions in a staggered fashion at a rate of 1 puff/min (35 ml, 2-s duration). The sidestream smoke was diluted with filtered air in a mixing chamber, then passed into the stainless steel-and-glass Hinners-type 0.44-m³ exposure chamber. The exposure chamber was characterized by the following parameters (means ± SD): relative humidity of 46.7 ± 12.1%, temperature of 23.8 ± 1.4°C, total suspended particulate concentration of 1.00 ± 0.08 mg/m³, carbon monoxide concentration of 6.4 ± 1.2 ppm, and nicotine concentration of 144.41 ± 90.46 µg/m³. Relative humidity and temperature were sampled continuously. Nicotine was sampled daily for 15 min twice during each 6-h exposure period. The total suspended particle concentration was sampled with the piezo-balance technique for 2 min every 0.5 h per chamber.

Experimental procedures. Each guinea pig was anesthetized with an injection of urethane (1.8 g/kg ip) and then given supplemental doses of pentobarbital sodium (4 mg/kg iv) about every hour as needed. Adequacy of anesthesia was assessed by a paw-pinching test, in which the hindlimb paw was pinched and the animal was monitored for a hindlimb flinch or withdrawal and/or a sudden fluctuation in ABP or HR. Catheters were introduced into the jugular vein for administering fluids and drugs and into the carotid artery for monitoring ABP and withdrawing samples for arterial blood gases. The trachea was cannulated, and a catheter was connected to a side port of the endotracheal tube to monitor P2r. Each guinea pig was prepared with bilateral pneumothoraces by incisions made in the chest wall and mechanically ventilated with oxygen-enriched humidified air with a tidal volume of 8 ml/kg. The ventilator rate was set initially at 65–75 breaths/min, and the positive end-expiratory pressure was set at 2 cmH2O. Arterial blood gases and pH were maintained so that the pH was between 7.35 and 7.45 and the arterial PCO2 was between 35 and 45 Torr by adjusting ventilator rate and by infusing sodium bicarbonate. After tracheal intubation, each animal was paralyzed with gallamine (3 mg/kg iv) every hour as needed. The pericardium was opened, and a cannula (0.58 mm ID) prefilled with capsaicin (2.5 µg/ml) was inserted into the left atrium through the left atrial appendage. Body temperature was maintained at 37 ± 1°C by means of a servo-controlled water blanket.

For recording C-fiber activity, the left cervical vagus nerve was separated from the carotid artery and transected below the nodose ganglion, and its caudal end was placed on a dissecting platform in a pool of mineral oil. In most experiments (90%), recordings were only made from the left vagus, and the contralateral vagus was left intact. In 10% of the experiments, recordings were made from the right vagus nerve, and both vagi were transected. The nerve was desheathed with the aid of a dissecting microscope, then a small filament was isolated, and afferent nerve activity was recorded via a monopolar silver hook electrode. A nerve bundle containing a suspected C fiber was split so that the fiber was the only active fiber discernible or in which the signal-to-noise ratio was sufficient to differentiate its activity from the noise by use of a window discriminator. The signal recorded via the electrode was amplified and fed in parallel to an oscilloscope, thermal chart recorder, audio monitor, and digital tape recorder with a sampling rate of 11 kHz per channel for off-line analysis.

Suspected C fibers were preliminarily identified as irregularly, sparsely firing fibers that did not discharge faithfully and robustly with lung inflation imposed by the ventilator (eliminating slowly adapting receptors) and that did not rapidly adapt to fast-rising then maintained (22 cmH2O) lung hyperinflation (eliminating rapidly adapting receptors) (21). Fibers that were silent with normal ventilation but were activated with hyperinflation maneuvers without rapid adaptation were also suspected to be C fibers. Once a suspected C fiber was identified, its conduction velocity was measured. To measure the conduction velocity, first, we preliminarily identified the receptive field in the hilum or the lung by gentle probing with a saline-soaked cotton-tip applicator. Then the stimulating electrode was positioned on the receptive field, and a field stimulus was applied by delivering rectangular constant-current pulses (1-ms duration, 0.3- to 3.0-mA intensity) generated by a pulse generator and a stimulus-isolation unit. If stimulating the receptive field or the hilum or lung did not stimulate the fiber, we did not study the fiber further. Thus only intrapulmonary C fibers were studied. The distance from the receptive field to the recording electrode was measured. The conduction time was determined as the time between the stimulus artifact, which triggered the oscilloscope trace, and the onset of the action potential. The conduction velocity was calculated by dividing the distance by the time. To confirm the preliminary localization of the C fibers, at the end of the experiment the receptive field of the lungs for individual fibers was again systematically explored by pressing the lungs with a saline-soaked cotton-tipped
Applicator. Only C fibers that were located in the lungs and with conduction velocities <2.0 m/s were reported (7).

For measurement of pulmonary function, a differential pressure transducer (model DP45-22, Valdyne, Northridge, CA) measured transpulmonary pressure and a Fleisch no. 0000 pneumotachograph measured airflow via a second pressure transducer (model DP103-10, Valdyne). All voltages were passed through carrier demodulators (model CD15, Valdyne) into a Modular Instruments data-acquisition system (model M100, Malvern, PA) by which Cdyn and Rl were calculated by using the method of Amdor and Mead (1). Prr, ABP, and HR were also recorded through the modular instruments system. Values averaged over 5 s were used.

Drugs were nebulized through an extra circuit, which bypassed the pneumotachograph. A microcatst ultrasonic nebulizer (Caire, Littleton, CO) was connected in series with the inspiratory line from the ventilator so that during each ventilatory cycle air was drawn through the nebulizer. The time lag of the nebulizer between the onset and initial appearance of a bubble indicating presence of drug was 9 s. The circuit was made so that the changes of pulmonary function from the normal circuit were minimal. Cdyn and Rl were not measured when the nebulization circuit was in place because of bypassing the pneumotachograph.

The dose of capsaicin and bradykinin, time intervals, and sequence of trials were determined in pilot studies. Specifically, we used the lowest dose 1) that increased C-fiber activity by ≥50% or, if the fiber was quiescent, by at least one action potential per second in the 6-s period, during which the peak response was measured, 2) that resulted in no tachyphylaxis during repeated testing on C-fiber activities within 30 min, and 3) from which pulmonary function recovered within 30 min. For left atrial capsaicin, doses of 0.5 and 1 µg/kg were tested: 0.5 µg/kg resulted in no tachyphylaxis when administered in 30-min intervals, whereas for >1 h after a 1 µg/kg dose there was no response or a much attenuated response to subsequent doses of 0.5 or 1 µg/kg; in addition, pulmonary function failed to recover for up to 1 h. For this reason, we did not attempt to test doses >0.5 µg/kg during the protocol. If a C fiber failed to respond to 0.5 µg/kg left atrial capsaicin, that fiber was still subjected to the entire protocol of nebulized capsaicin. At the end of the protocol, the fiber was tested with 1.0 µg/kg left atrial capsaicin to determine whether a larger dose stimulated it. No fibers that failed to respond to 0.5 µg/kg were stimulated by 1.0 µg/kg. For nebulized capsaicin, from doses of 0.8 × 10⁻⁴ and 1.6 × 10⁻⁴ M delivered for 51 or 21 s, the larger dose delivered over 21 s was used. For nebulized bradykinin, we initially tested 9 × 10⁻⁵ M used previously (2). This dose failed to stimulate C fibers and so was increased by half-logos to an effective dose (3 × 10⁻³ M) for 21 s.

Experimental protocol. The investigators were blinded as to the exposure treatment of the 56 animals studied. Once a C fiber was identified, it was tested for its responses to 1) lung hyperinflation (22 cmH₂O), 2) left atrial injection of capsaicin (0.5 µg/kg bolus), 3) intralung bradykinin (3 × 10⁻³ M, 21 s), and 4) intralung capsaicin (1.6 × 10⁻¹ M, 21 s).

Data collected included C-fiber activity, Cdyn, Rl, Prr, ABP, and HR, except during nebulization, when only C-fiber activity, Prr, ABP, and HR were recorded. At the beginning of the protocol, data were collected over a 2-min baseline period, then the lung was hyperinfated for 3–5 s. After another 2-min baseline period, capsaicin was injected into the left atrium. After capsaicin, the lungs were hyperinflated every 15 min to maintain a constant lung volume history and to reverse any atelecstasy that may have occurred. The hyperinflation was repeated until Cdyn returned to baseline. After a 30-min interval, data were collected over a 2-min baseline period for Cdyn and Rl with the normal ventilator circuit and then over a 2-min baseline period for C-fiber activity, Prr, ABP, and HR with the nebulization circuit. After these two 2-min intervals, bradykinin was nebulized for 21 s. The circuit was switched to the normal circuit immediately after the nebulization. After a 30-min interval, capsaicin was nebulized for 21 s with use of the same nebulization protocol. If a C fiber was quiescent at the end of the protocol, the lungs were hyperinflated or capsaicin was again injected into the left atrium to ensure that the fiber was still present and viable. Animals were killed after the experiment by a lethal injection of pentobarbital sodium.

Pharmacological agents. A stock solution of capsaicin (1 × 10⁻² M; Sigma Chemical, St. Louis, MO) was prepared in a vehicle of 10% Tween 80, 10% ethanol, and 80% saline. A stock solution of bradykinin (1 × 10⁻² M; Sigma Chemical) was prepared in saline. All drugs were kept frozen, and the desired concentration was prepared from concentrated stock solutions on the day of the experiment by dilution in saline.

Data analysis. The impulse activity of C fibers was analyzed by using EGAA/Computerscope software (RC Electronics, Goleta, CA) for each 1-s interval. The baseline activity, expressed as impulses per second, was determined over a 2-min period, and the evoked activity, expressed as impulses per second, was determined for the first 15 s immediately after left atrial capsaicin or 21 s during inhaled bradyginin or capsaicin. The peak C-fiber response was defined as the average number of impulses per second during the most active 6 s of the initial 15 s after left atrial capsaicin or 21 s during inhaled bradykinin or capsaicin. For individual fibers, a positive response was designated as a peak response of a ≥50% increase over baseline activity or at least one action potential per second evoked when the baseline activity for 2 min was zero. The onset latency for the increase in C-fiber activity was defined as the first detectable increase after the injection. On the basis of the bimodal distribution of the onset latencies of the responses, we categorized all C fibers with a response onset of ≤5 s to left atrial capsaicin as early-responding C fibers to ensure that bronchial C fibers were studied. Those responding with latencies of >5 s were defined as late-responding fibers.

The peak increase in Prr and the peak decrease in ABP or HR were defined as the 5-s bins with the biggest change within the initial 15 s after left atrial capsaicin or 21 s during inhaled bradykinin or capsaicin. Because during the nebulization period for bradykinin or capsaicin Cdyn and Rl were not measured (because of bypassing the pneumotachograph) and to exclude the possibility that even minimum transient changes in Cdyn or Rl associated with switching from the nebulization to the normal ventilatory circuit did not confound the results, we defined the changes in Cdyn and Rl as the average of three 5-s bins immediately after switching from the nebulization to the normal ventilatory circuit from the 2-min baseline measurement of Cdyn and Rl. To determine whether the evoked peak response of C-fiber activity, Prr, Cdyn, Rl, ABP, or HR was significantly different from the 2-min baseline values within the sidestream smoke or the filtered air-exposed groups, a paired t-test was used. To determine whether the evoked change from the baseline value to the peak response for C-fiber activity, Cdyn, Rl, Prr, ABP, and HR was significantly different in animals in the filtered air-exposed vs. the sidestream smoke-exposed group, an unpaired t-test was used. Body weights, blood gases, and conduction velocities were compared with an unpaired t-test.
Statistical significance was claimed when the probability of a type I error was <0.05. Values are means ± SE, unless otherwise indicated.

RESULTS

A total of 55 C fibers were recorded in 39 guinea pigs: 16 were exposed to filtered air and 23 to sidestream smoke. At the commencement of the experiments, the weights and arterial blood gases were not different between the two groups (Table 1). All C fibers reported were located in the lung: 4 in the cranial lobe, 2 in the middle lobe, and 49 in the caudal lobe. Conduction velocity was obtained for every C fiber; the average conduction velocity was 1.17 ± 0.06 m/s (range 0.70–1.67 m/s) for fibers in the filtered air-exposed control group (n = 24) and 1.18 ± 0.05 m/s (range 0.78–1.87 m/s) for fibers in the sidestream smoke-exposed group (n = 31, P = 0.45).

C-fiber responses. Exposing young guinea pigs to sidestream tobacco smoke throughout the equivalent period of human childhood augmented C-fiber responsiveness to left atrial injection of capsaicin and to lung hyperinflation, but not to inhaled capsaicin or bradykinin. Sidestream smoke exposure had no effect on the baseline activity of the C fibers.

An example of the response of a C fiber from a filtered air-exposed guinea pig is shown in Fig. 1. The fiber was insensitive to hyperinflation (Fig. 1B) but was activated by left atrial injection of capsaicin, nebulized capsaicin, and nebulized bradykinin (Fig. 1, C–E). An example of the response of a C fiber recorded from a sidestream smoke-exposed animal is shown in Fig. 2. The fiber was sensitive to hyperinflation (Fig. 2B) and was also activated by all the drug challenges (Fig. 2, C–E). This fiber was excited by left atrial capsaicin to a greater degree than was the C fiber from the filtered air-exposed guinea pig (Figs. 1C and 2C).

The grouped data for the C-fiber responses to left atrial capsaicin injection are shown in Fig. 3. Sidestream smoke exposure augmented the responsiveness of all left atrial capsaicin-sensitive C fibers taken as a group (Fig. 3A). The increase in activity over baseline in the filtered air-exposed group (n = 21, 2.5 ± 0.49 impulses/s) was nearly doubled in the sidestream smoke-exposed group (n = 25, 4.5 ± 0.97 impulses/s, P = 0.047).

The C fibers were divided into two groups on the basis of response onset latencies to left atrial capsaicin, which were bimodally distributed into ≤5 s (with a mean onset latency of 3.31 ± 0.35 s in the filtered air-exposed group and 2.50 ± 0.26 s in the sidestream smoke-exposed group) and >5 s (with a mean onset latency of 8.20 ± 0.80 s in the filtered air-exposed group and 7.86 ± 0.60 s in the sidestream smoke-exposed group). We categorized all C fibers with a response onset of ≤5 s in the early-responding group to ensure that bronchial C fibers were studied and focused the analysis on those fibers. Of the entire group of left atrial capsaicin-sensitive C fibers, 34 fibers (n = 16 in the filtered air-exposed group and n = 18 in the sidestream smoke-exposed group) were categorized as early responders. The augmentative effect of sidestream smoke exposure of the entire group resided in the early-responding C fibers (Fig. 3B). The increase in activity was 2.72 ± 0.62 impulses/s in the filtered air-exposed group vs. 5.67 ± 1.24 impulses/s in the sidestream smoke-exposed group (P = 0.02). Sidestream smoke exposure had no effect on the responsiveness of the late responders to left atrial capsaicin (Fig. 3C).

The grouped data for the magnitude of the responses to hyperinflation are shown in Fig. 4. For all C fibers responsive to left atrial capsaicin, the hyperinflation-induced increase in activity was sevenfold higher than in the filtered air-exposed group (0.40 ± 0.14 vs. 0.04 ± 0.14 impulses/s, P = 0.03; Fig. 4A). The magnitude of the hyperinflation-induced increase in activity in the early responders to left atrial capsaicin was 0.002 ± 0.13 impulses/s in the filtered air-exposed group vs. 0.35 ± 0.14 impulses/s in the sidestream smoke-exposed group (P = 0.04; Fig. 4B). Sidestream smoke exposure had no statistically significant effect on the responsiveness to hyperinflation in the late responders to left atrial capsaicin (Fig. 4C).

The grouped data showing the lack of effect of sidestream smoke exposure on lung C-fiber responsiveness to inhaled capsaicin or inhaled bradykinin are shown in Fig. 5. In the filtered air-exposed control group, nebulized capsaicin was tested in 13 C fibers that were responsive to left atrial capsaicin, 10 of which were early responders; 7 of those 10 responded to nebulized capsaicin. In the sidestream smoke-exposed group, nebulized capsaicin was tested in 16 C fibers responsive to left atrial capsaicin, 13 of which were early responders; 11 of those 13 responded to nebulized capsaicin. As shown in Fig. 5A in the early-responding left atrial capsaicin-sensitive C fibers, the increase in nebulized capsaicin C-fiber activity was the same in the filtered air-exposed controls (n = 10) and in the sidestream smoke-exposed (n = 13) animals (P = 0.46). Sidestream smoke exposure had no effect on C-fiber responsiveness to nebulized capsaicin, regardless of the sensitivity of the fiber to left atrial capsaicin or the onset latency of the response to left atrial capsaicin.

As was the case with the nebulized capsaicin-induced responses, sidestream smoke exposure had no effect on the responsiveness to nebulized bradykinin, regardless of the sensitivity or onset latency of the C-fiber responses to left atrial capsaicin. In the filtered air-exposed control group, nebulized bradykinin was tested

Table 1. Weight and blood gases for animals in filtered air- and sidestream smoke-exposed groups

<table>
<thead>
<tr>
<th></th>
<th>FA (n = 16)</th>
<th>SS (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, g</td>
<td>453 ± 9</td>
<td>467 ± 11</td>
</tr>
<tr>
<td>Pao2, Torr</td>
<td>251 ± 16</td>
<td>259 ± 13</td>
</tr>
<tr>
<td>Paco2, Torr</td>
<td>39.9 ± 0.7</td>
<td>38.7 ± 0.7</td>
</tr>
<tr>
<td>pH</td>
<td>7.38 ± 0.01</td>
<td>7.39 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. FA, filtered air; SS, sidestream smoke; Pao2, arterial PO2; Paco2, arterial PCO2.
in 14 C fibers that were responsive to left atrial capsaicin, 11 of which were early responders; 9 of those 11 responded to nebulized bradykinin. In the sidestream smoke-exposed group, nebulized bradykinin was tested in 18 C fibers responsive to left atrial capsaicin, 13 of which were early responders; 9 of those 13 responded to nebulized bradykinin. As shown in Fig. 5B in the early-responding C fibers, the nebulized bradykinin-induced increase in activity was the same in the filtered air- and sidestream smoke-exposed animals (n = 11 and 13, respectively, P = 0.43).

Pulmonary function and cardiovascular responses. In these guinea pigs, only one vagus was intact, thereby most likely interfering with the full expression of the reflex airway responses. Under these experimental conditions, sidestream smoke exposure did not alter the airway responses to left atrial capsaicin injection, inhaled capsaicin, or inhaled bradykinin (Fig. 6). Left atrial and nebulized capsaicin produced statistically significant increases in P\(_{\text{tr}}\) and R\(_{\text{l}}\) and decreases in C\(_{\text{dyn}}\) that were the same in the filtered air- and sidestream smoke-exposed groups. Nebulized bradykinin produced significant increases in P\(_{\text{tr}}\) and decreases in C\(_{\text{dyn}}\); however, the trend for an increase in R\(_{\text{l}}\) did not reach statistical significance. There were no statistically significant differences in the baseline values for P\(_{\text{tr}}\), R\(_{\text{l}}\), or C\(_{\text{dyn}}\) in the filtered air- and sidestream smoke-exposed groups.
Fig. 2. Effects of hyperinflation (B), left atrial capsaicin (C), nebulized capsaicin (D), and nebulized bradykinin (E) on C-fiber action potentials, ABP, and Ptr from a guinea pig exposed to sidestream smoke from 1 to 6 wk of life. A: stimulus artifact (●) and evoked action potential used to determine conduction velocity (1.87 m/s). ▲, Time of bolus injection or onset of aerosolization.

Fig. 3. Grouped data showing effect of sidestream smoke (SS) exposure compared with filtered air (FA) exposure on increases in C-fiber activity evoked by left atrial capsaicin. A: sidestream smoke exposure significantly increased grouped responses of all C fibers that were sensitive to left atrial capsaicin. Increased responsiveness occurred in subgroup of early-responding C fibers to left atrial capsaicin (B) but not in late-responding fibers (C). *P < 0.05.
Fig. 4. Grouped data showing effect of sidestream smoke exposure compared with filtered air exposure on hyperinflation-induced increases in C-fiber activity. A: sidestream smoke significantly increased grouped responses to hyperinflation of C fibers that responded to left atrial capsaicin (A) and of subgroup of C fibers that were early responders to left atrial capsaicin (B) but had no statistically significant effect on responses to hyperinflation in C fibers that were late responders to left atrial capsaicin (C). *P < 0.05.

Fig. 5. Group data showing lack of effect of sidestream smoke exposure compared with filtered air exposure on responsiveness to nebulized capsaicin (A) or nebulized bradykinin (B) in C fibers that were early responders to left atrial capsaicin.

Fig. 6. Group data showing effect of sidestream smoke exposure compared with filtered air exposure on evoked changes in P}_{\text{tr}}, lung resistance (R}_{L}), and dynamic compliance (C_{\text{dyn}}). Sidestream smoke exposure did not change baseline lung function or responses to left atrial capsaicin, nebulized capsaicin, or nebulized bradykinin.
Sidestream smoke exposure also had no effect on the cardiovascular responses to left atrial capsaicin, neb-
lized capsaicin, or nebulized bradykinin.

Time courses of the changes in C-fiber activity and Ptr. The time course of the effects of left atrial capsaicin injection, inhaled bradykinin, and inhaled capsaicin on C-fiber activity superimposed on the associated Ptr 15 s before and 30 s after the onset of the interventions is shown in Fig. 7. Ptr was averaged over 5-s intervals, and C-fiber activity was averaged over 1-s intervals.

For left atrial capsaicin injection, we focused on the early responders, since the major sidestream smoke effect was in this group. In the filtered air-exposed group, C-fiber activity showed a small increase immediately after injection that peaked within 1 s and then at 5 s, returning to near baseline at 10–15 s. In the sidestream smoke-exposed group, C-fiber activity began to increase within 1 s of injection, was augmented over 3–5 s, and then began to wane, returning to near control level within 10–15 s (Fig. 7A). In filtered air- and sidestream smoke-exposed groups, Ptr began to increase immediately after injection and continued to increase over 15–20 s after C-fiber activity had returned to near baseline values.

For the 21 s of capsaicin inhalation, C-fiber activity from the filtered air- and sidestream smoke-exposed groups increased immediately after the onset of inhalation, continued to increase for 9–15 s, and then began to decline, returning to near control level after the inhalation was stopped (Fig. 7B). The peak increase in C-fiber activity from the filtered air- and sidestream smoke-exposed animals occurred at 6–9 and 12–14 s after the onset of inhalation, respectively. In filtered air- and sidestream smoke-exposed groups, Ptr began to increase immediately after the onset of inhalation and continued to increase (~25%) after the inhalation ended, while C-fiber activity was returning to near baseline level.

For inhaled bradykinin, there was no clear temporal relationship between C-fiber activity and Ptr in the filtered air- or the sidestream smoke-exposed group (Fig. 7C).

Fig. 7. Time course of effect of left atrial capsaicin (A), nebulized capsaicin (B), and nebulized bradykinin (C) on C-fiber activity and Ptr in animals exposed to filtered air or sidestream smoke. For left atrial capsaicin, only C fibers responding within 5 s are shown. Left atrial capsaicin was injected at time 0. For nebulized capsaicin and nebulized bradykinin, aerosol reached animal at time 0 and continued for 21 s. For left atrial capsaicin and nebulized capsaicin, Ptr and C-fiber activity began to increase together, but C-fiber activity returned to near baseline while Pτr continued to increase. For nebulized bradykinin, there was no clear temporal relationship between Pτr and C-fiber activity.
DISCUSSION

This study provides evidence from electrophysiological recordings of bronchopulmonary C-fiber impulse activities that exposure to sidestream tobacco smoke (the surrogate for ETS) for 5 wk in young guinea pigs augments the responsiveness of C fibers to two stimuli: the chemical stimulus of left atrial capsaicin injection and the mechanical stimulus of lung hyperinflation. By contrast, the exposure had no effect on C-fiber responsiveness to inhaled capsaicin, bradykinin, or baseline activity.

All C fibers were identified by localization in the lung and by a conduction velocity of $<2.0 \text{ m/s}$ (8). Only 15% of the fibers were insensitive to left atrial capsaicin, failing to respond to 0.5 or 1.0 µg/kg. It is possible that, with still higher doses, the other C fibers may have been stimulated but nonetheless would have been considered relatively insensitive to left atrial capsaicin. The physiological relevance of capsaicin-insensitive C fibers is unclear. The capsaicin-sensitive lung C fibers are thought to protect the lungs from noxious irritants via central and local axon reflexes by eliciting defensive responses: airway responses of bronchoconstriction, mucus secretion, and microvascular leak; breathing responses of apnea, rapid shallow breathing, and cough; and cardiovascular responses of hypotension and bradycardia (8).

The C fibers in the airways and lungs have been categorized as bronchial or pulmonary C fibers on the basis of their blood supply (bronchial vs. pulmonary circulation). Pulmonary C fibers have been distinguished from bronchial C fibers by rapid-onset response to right atrial or pulmonary circulation injections ($0.3-4.0 \text{ s}$) and a slower-onset, a smaller, or no response to left atrial or bronchial circulation injections in dogs, cats, and rats (6, 8, 17). Such criteria are somewhat tenuous in distinguishing bronchial from pulmonary C fibers in small animals because of the fast circulation time and presence of anastomoses (28). The presence of C fibers accessible from both circulations with similar onset latencies has also been reported in cats (11), suggesting some uncertainties for the general criteria for segregating bronchial and pulmonary C fibers. We did not attempt to compare the responsiveness to left vs. right atrial injections of capsaicin and categorized the C fibers as bronchopulmonary. We included all C fibers with an response onset of $\leq 5 \text{ s}$ in the early-responding group to ensure that bronchial C fibers were studied and focused the analysis on those fibers. C fibers with response onset of $>5 \text{ s}$ were classified as late responders.

Of consideration is why chronic sidestream smoke exposure augmented the bronchopulmonary C-fiber responsiveness specifically to left atrial capsaicin and to lung hyperinflation but not to aerosolized capsaicin or bradykinin. First, why did the exposure augment the responsiveness to intravascular but not to aerosolized capsaicin? One hypothesis is that sidestream smoke exposure caused all C fibers to undergo the same changes in excitability, making them generally more responsive, but in addition caused other changes that limited the accessibility, specifically, to inhaled stimuli. The changes in excitability may have been due to changes in membrane properties or in transduction processes or indirectly caused by increases in microvascular leak. Regarding changes in membrane properties, in primary sensory neuron cell bodies in the nodose ganglia, allergen exposure has been shown to cause membrane depolarization, changes in resting membrane conductance, inhibition of inward rectifying currents, and blockade of a slow afterhyperpolarization current, all of which can render the nerve more sensitive to stimuli (34). It has also been shown that even subtle increases in fluid leak can also stimulate lung C fibers (19). However, prolonged exposures to injurious agents can also increase the diffusion barrier across the airways, limiting the accessibility to inhaled agents. Specifically, chronic exposure to mainstream tobacco smoke results in airway structural changes, including airway epithelial hypertrophy and hyperplasia (5) and mucus hypersecretion (27); moreover, exposure to maternal cigarette smoking has been recently linked to increased airway wall thickness in children (26). All these changes could result in a relatively diminished accessibility of C fibers to inhaled stimuli. It follows that an overall increase in C-fiber excitability from the sidestream smoke exposure might be counteracted or masked by the limited accessibility by aerosolized agents. In addition to the possibility of a greater diffusion barrier, sidestream smoke exposure may have...
prevented the arborization of the developing C fibers into the epithelial layer, again limiting the accessibility of inhaled stimulants to the nerve endings. This possibility is consistent with previous studies in which chemical irritants have been shown to cause profound neuroanatomic remodeling of nerve arbors (31). In either case, increased diffusion barrier and/or disrupted arborization in the epithelial layer, the C-fiber response to left atrial capsaicin in the sidestream smoke-exposed group would be expected to exceed the response to aerosolized capsaicin, whereas no such difference would be expected in the filtered air control group. In the sidestream smoke-exposed animals, for the same C-fibers that were excited by both left atrial capsaicin (with a short onset latency) and nebulized capsaicin (n = 11), the response to left atrial capsaicin (8.2 ± 0.11 impulses/s) was twice the response to nebulized capsaicin (4.1 ± 1.9 impulses/s, P = 0.05). By contrast, in the filtered air-exposed animals the average responses of the same fibers to left atrial and aerosolized capsaicin (n = 9) were not different (3.0 ± 0.7 and 4.5 ± 3.2 impulses/s, respectively). These offsetting effects of sidestream smoke exposure would also explain the results obtained with the nebulized bradykinin.

An alternative possibility is that sidestream smoke exposure simply increased the accessibility to agents given intravascularly, perhaps by increasing microvascular permeability. This possibility seems less likely, since the exposure also increased the responsiveness of C fibers to the mechanical stimulus of lung hyperinflation.

The mechanism whereby sidestream smoke exposure augmented C-fiber responsiveness to lung hyperinflation is not known. However, the finding that the exposure increased the responsiveness to two different stimuli, one chemical and one mechanical, suggests the induction of some neuroplastic changes in the nerve endings or in transduction processes that resulted in an overall increase in excitability.

Of related interest was that the increased excitability to the chemical and mechanical stimulant resided in the early-responding C fibers, suggesting that the late responders were less susceptible or accessible to the effects of inhaled sidestream smoke. The late responders differed also in that fewer of them were recorded (5 of 21 in the filtered air- and 3 of 25 in the sidestream smoke-exposed group), raising the possibility that they were more difficult to isolate or were fewer in number. Their conduction velocities were not different from those of the early-responding C fibers, suggesting a similar fiber size, but their responses to left atrial capsaicin were small, raising the possibility that the drug was diluted by the time it reached those fibers.

The delayed responses of the late responders to left atrial capsaicin may be a direct effect, if they are indeed pulmonary C fibers supplied by the pulmonary circulation, or an indirect effect, by the local release of tachykinins and the subsequent increase in mucus secretion, microvascular leak, or bronchoconstriction. That the response was secondary to local changes in the lungs is suggested by the time course of the late-responding C fibers to left atrial capsaicin, which paralleled the increase and decrease in Ptr (Fig. 8).

To optimize the experimental conditions for examining the consequences of chronic sidestream smoke exposure in bronchopulmonary C-fiber impulse activity, the guinea pigs were ipsilaterally vagotomized, which compromised the full expression of the airway responses: the increase in Ptr, the decrease in Cdyn, and the increase in RL. Thus, although sidestream smoke exposure did not augment the airway responses to C-fiber stimulation under these conditions of partial vagotomy, no conclusions can be drawn regarding the effect of sidestream smoke exposure on the reflex airway responses in animals with both vagi intact.

In general, nebulized capsaicin produced larger increases in RL and decreases in Cdyn than left atrial capsaicin in the filtered air-exposed control and sidestream smoke-exposed animals. This may be associated with the route of administration (Fig. 7). With the bolus left atrial injections, capsaicin evoked a robust peak in C-fiber activity followed by a rapid return to baseline. By contrast, the 21-s period of capsaicin nebulization resulted in a broad and more sustained response, which may have had a greater cumulative effect on pulmonary function. Regardless of whether capsaicin was injected into the left atrium or nebulized, the C-fiber activity initially increased to a greater extent and began to return to baseline while Ptr continued to increase. This temporal relationship suggests that the capsaicin-induced increase in C-fiber activity contributed to the changes in Ptr. By contrast, the bradykinin-induced increases in C-fiber activity and in Ptr were quite small, and no direct temporal relationship was apparent.

Although this is the first direct recording of the afferent activity of C fibers from guinea pigs chronically exposed to sidestream smoke, there are related data on the behavioral reflex responses with similar exposure periods to mainstream or sidestream smoke. Karlsson et al. (20) found that chronic mainstream tobacco smoke exposure increased the frequency of capsaicin- or citric acid-induced cough but not of capsaicin- or citric acid-induced bronchoconstrictor responses. One interpretation of those data is that although cough (elicited via the central reflex) was augmented, the bronchoconstrictor response (involving both the central and local axon reflexes) was not. Using an isolated perfused lung preparation to focus on the axon reflex, we found that sidestream smoke exposure (the same exposure protocol in guinea pigs the same age as those in the present study) resulted in smaller capsaicin-induced increases in RL than in the filtered air-exposed lungs (18), suggesting that the local reflex-mediated bronchoconstrictor response was diminished. Taken together, these previous findings of Karlsson et al. and Joad et al. (18), coupled with the present findings, suggest that chronic tobacco smoke exposure may downregulate local axon reflex components (perhaps minimizing the deleterious effects of local inflammation) and upregulate central reflex components (aug-
menting changes in cough frequency as a defensive mechanism).

At first glance, the present findings may appear to be at odds with those of Swanny et al. (33), who observed that chronic exposure to mainstream tobacco smoke attenuated the C-fiber reflex changes in breathing pattern of apnea and tachypnea evoked by acutely inhaled mainstream tobacco smoke. However, the decreased responsiveness to acutely inhaled mainstream tobacco smoke may reflect a desensitization to some component of tobacco smoke, such as nicotine. This possibility is also suggested by results of the study of Karlsson et al. (20), in which previous exposure to mainstream tobacco smoke augmented the responses to capsaicin and citric acid but not to mainstream smoke, and by our previous results with rapidly adapting receptors in which exposure to sidestream smoke sensitized the rapidly adapting receptors to substance P (4) but desensitized them to acute inhibition of mainstream tobacco smoke (3). Thus prolonged exposure to mainstream or sidestream tobacco smoke may sensitize vagal sensory fibers to a variety of chemical stimulants while desensitizing them to acute inhibition of mainstream tobacco smoke.

The sensitization of lung sensory C fibers is not restricted to chronic exposure to environmental or mainstream tobacco smoke. Ho and Lee (16) recently demonstrated that a relatively acute exposure of 3 h to another environmental toxicant, ozone, sensitized pulmonary C fibers in rats. Our results are strikingly similar to theirs, in that the exposure sensitized the lung C fibers to intravascular administration of capsaicin and to lung hyperinflation.

These studies were performed in young guinea pigs because of the increased prevalence of respiratory symptoms in children chronically exposed to ETS. Whether similar exposure of the adult guinea pig to sidestream smoke renders the lung C fibers more responsive to certain stimuli is unknown. Like the human, the guinea pig shows advanced development of lung function and morphology at birth (30). The age of puberty in guinea pigs is 35–70 days, and the maximum life span is ~7 yr (37). The guinea pigs were exposed to sidestream tobacco smoke from 7 to 41 to 45 days of life. Thus these guinea pigs were exposed during the equivalent of human childhood and were tested during the equivalent period of human adolescence.

In conclusion, the findings that chronic exposure to sidestream smoke exaggerated the responsiveness of lung C fibers may provide electrophysiological evidence linking increased respiratory symptoms in children to ETS exposure. The augmented responsiveness to lung hyperinflation, in particular, may explain the increased airway responses with exercise in children exposed to ETS (15).

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