Chronic tobacco smoke exposure increases airway sensitivity to capsaicin in awake guinea pigs

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Bergren, Dale R. Chronic tobacco smoke exposure increases airway sensitivity to capsaicin in awake guinea pigs. J Appl Physiol 90: 695–704, 2001.— Tobacco smoke (TS) exposure induces airway hyperreactivity, particularly in sensitive individuals with asthma. However, the mechanism of this airway hyperreactivity is not well understood. To investigate the relative susceptibility of atopic and nonatopic individuals to TS-induced airway hyperreactivity, we exposed ovalbumin (OA)-sensitized and nonsensitized guinea pigs to TS exposure (5 mg/l air, 30-min exposure, 7 days/wk for 120–156 days). Two similar groups exposed to compressed air served as controls. Airway reactivity was assessed as an increase in enhanced pause (Penh) units using a plethysmograph that allowed free movement of the animals. After 90 days of exposure, airway reactivity increased in OA-TS guinea pigs challenged with capsaicin, bradykinin, and neuropeptide catabolism were administered before either methacholine or histamine challenge in OA-TS guinea pigs. Chronic TS exposure enhanced neither airway reactivity to histamine or histamine nor contractility of isolated tracheal rings. In conclusion, chronic TS exposure enhances airway hyperreactivity observed after either methacholine or histamine challenge in OA-TS guinea pigs. Chronic TS exposure enhanced neither airway reactivity to histamine or histacholine nor contractility of isolated tracheal rings. In conclusion, chronic TS exposure increased airway reactivity to capsaicin and bradykinin aerosol challenge, and OA-TS guinea pigs were most susceptible to airflow dysfunction as the result of exposure to TS compared with the other groups. Increased airway reactivity to capsaicin suggests a mechanism involving neurogenic inflammation, such as increased activation of lung C fibers. Our major objective was to determine whether chronic TS exposure enhances neurogenic inflammation. Because neurogenic inflammation is mediated by C-fiber activation, chronic TS exposure may enhance C-fiber reactivity or decrease the threshold of activation. To perform this study, we selected capsaicin and bradykinin, which activate neurogenic inflammation, such as increased airway hyperreactivity to certain endogenous mediators and inhaled airway irritants (6, 8, 16, 20, 23). Chronic TS exposure increases the risk of developing dyspnea, bronchitis, and asthma in individuals who have never smoked (19). Acute, passive TS exposure induces bronchoconstriction in certain individuals with asthma (7). The mechanism by which either chronic or acute TS exposure induces airway dysfunction is poorly understood. However, this airway hyperreactibility may be partially mediated by enhanced neurogenic inflammation. Afferent C-fiber activation mediates neurogenic inflammation that induces bronchoconstriction, increased airway secretions, vasodilatation, and localized edema (5). C-fiber activation stimulates centrally mediated and local axon reflexes. The latter involves release of neuropeptides such as substance P and neuropeptide catabolism were administered before either methacholine or histamine challenge in OA-TS guinea pigs. Chronic TS exposure altered contractility. To determine whether TS-induced airway reactivity included central and local reflexes, either a cocktail of NK-receptor antagonists or inhibitors of neuropeptide catabolism were administered before either TS exposure or agonist challenge of the airways. 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mation appears to be enhanced in atopic individuals, particularly those with asthma, we also tested the hypothesis that airway sensitization acts in a synergistic manner with TS exposure to enhance neurogenic inflammation, i.e., the reflex effects that are mediated by C-fiber activation.

MATERIALS AND METHODS

Animals. The study was performed with the approval of the Creighton University Animal Use Committee. Sixty-four male Hartley guinea pigs (Harlan, Minneapolis, MN), weighing 300 g at the time of purchase, were housed in the Creighton University School of Medicine Animal Resource Facility with food and water provided ad libitum. Guinea pig weights were determined on arrival and at 30, 60, 90, and 120 days. There was no difference in mean weight gain among treatment groups.

Airway sensitization. Two days after their arrival, 28 guinea pigs were injected intraperitoneally with ovalbumin (OA; 10 μg) and aluminum hydroxide (100 mg) in distilled water (0.5 ml). A booster injection of OA (10 μg/0.5 ml distilled water) was administered 12 days later as per Andersson (1). Another 36 guinea pigs were injected with the OA vehicle and remained nonsensitized (NS) to OA. Airway reactivity to OA was determined, beginning 14 days after the last injection, by OA aerosol challenge into the lungs and was verified monthly. OA aerosol challenge (400 μg/ml, 30 s) increased airway resistance in OA-treated guinea pigs but did not increase airway resistance of vehicle-treated guinea pigs (Fig. 1).

Chronic TS exposure. After the initial OA injection, 18 OA-sensitized and 18 NS guinea pigs were exposed to mainstream TS from four standard 2R1 reference cigarettes (Tobacco and Health Research Institute, Lexington, KY) drawn into an exposure chamber (36 liters in volume). Exposures were 30 min/day and 7 days/wk. The concentration of TS inside the chamber increased from only 24 to 25°C. The concentration of TS inside the chamber was 5 mg/l during the exposure. Temperature inside the chamber increased from only 24 to 25°C. Animals not exposed to TS were exposed to compressed air.

Airway reactivity to ovalbumin (OA) aerosol challenge (0.04% for 30 s) in 4 experimental groups of guinea pigs (n = 10 animals/group). Airway reactivity is determined by monitoring enhanced pause (Penh) units, which are equal to pause × peak expiratory pressure/peak inspiratory pressure (see Ref. 12 for details). NS-A, nonsensitized and compressed-air-exposed group of guinea pigs; OA-A, OA-sensitized and compressed-air-exposed group; NS-TS, nonsensitized and tobacco-smoke-exposed group; OA-TS, OA-sensitized and tobacco-smoke-exposed group. Chronic TS exposure neither enhanced airway reactivity to OA challenge nor altered base-level Penh units. Increased peak Penh units vs. base-level Penh units, *P < 0.05.

Measurement of pulmonary function. Airway reactivity was determined by monitoring enhanced pause (Penh) units obtained from a plethysmograph that measures respiratory function in unanesthetized animals (Buxco Electronics, Sharon, CT). Mediator challenges by aerosol inhalation included capsaicin, bradykinin, NKA fragment 4–10, MCh, and histamine. To establish that increases in Penh units corresponded to airway reactivity in guinea pigs, MCh (200 and 400 μg/ml, 30 s) was aerosolized into a plethysmograph from which Penh units are derived (see Ref. 12) and into a two-chambered plethysmograph (Pennock box, Buxco Electronics) from which specific airway resistance (sRaw) is derived (cmH2O·s). Measurement of sRaw is a well-established measurement of airway reactivity (3). Doses were chosen from a series of experiments that induced significant, but not life-threatening, bronchoconstriction (data not shown). Airway reactivity was monitored to MCh by both methods in the same group of 16 guinea pigs. MCh was selected to study correlation between sRaw and Penh units, as it induces direct airway smooth muscle contraction. We determined that Penh units and sRaw were correlative measurements of airway reactivity (Fig. 2).

Acute TS challenge. To determine the acute effects of TS challenge on airway obstruction, guinea pigs were exposed to TS for 5 min in the exposure chamber and then transferred immediately into the plethysmograph, where airway function was monitored. Acute TS challenges were performed with no pretreatment or pretreatment with a 2-min aerosol of either the antihistamine pyrilamine or a cocktail of CP-96345 (4 × 10⁻⁴ M, a potent NK1 antagonist; Pfizer, Groton, CT) and [Tyr⁵,D-Trp⁶,⁷,⁹,Lys¹⁰]-α-NKA (5 × 10⁻⁶ M, a potent NK2 antagonist), which served to antagonize the effects of substance P and NKA, respectively. Antagonists were administered 5 min before the TS exposure.

Mediator challenge. Aerosols (1–5 μm in diameter) of the mediators were generated and delivered by an ultrasonic
nebulizer (model 65, DeVilbiss, Sommerset, PA) directly into the plethysmographs. In some experiments, denser aerosols of certain mediators were obtained by placing the guinea pigs in a small exposure chamber that produced minimal flow resistance to the nebulizer compared with the plethysmograph. The animals were then immediately transferred to the plethysmograph to monitor Penh units.

Experiments of the aerosol challenges reported in this study were conducted after 90 days of exposure to TS or A. However, exposures to TS or A continued daily throughout the duration of the study. During the course of the study, aerosol challenges were scheduled so that an effective dose of the same mediator was not delivered on consecutive days. Furthermore, aerosol challenges of capsaicin and bradykinin were separated by at least 72 h to avoid possible airway tachyphylaxis. Except where indicated, challenges were performed before TS exposure for that day to determine the chronic effects of TS exposure. However, when indicated, aerosol challenges were performed immediately after TS exposure to assess the acute effects of acute TS exposure on airway reactivity.

Mediator antagonism. Antagonists were administered by aerosol generated by nebulizers (described above) and delivered into exposure chambers. Pyrilamine aerosol administration (2% for 2 min) served as an H1-receptor antagonist. Lidocaine (1–5%, 2 min) and atropine (2%, 1 min) aerosol administration served to attenuate the effects of the afferent and efferent central cardiopulmonary defense reflex, respectively. A cocktail of CP-96345 (4 × 10⁻⁷ M, a potent NK1 antagonist; Pfizer) and [Tyr₅,D-Trp₆,7,9,Lys₁₀] substance P (4 × 10⁻⁷ M, a potent NK₂ antagonist) served to antagonize the action of substance P and NKA, respectively. Phosphoramidon (10⁻⁴ M, 1 min) served to antagonize the action of neutral endopeptidase. The guinea pigs were then transferred to the plethysmographs or exposure chambers for the acute TS challenges 5 min after administration of the antagonists.

Surgical preparations. After 120–156 days of exposure to either TS or A, guinea pigs were anesthetized with pentobarbital (50 mg/kg ip; Astra, Arcadia, CA). After induction of a deep surgical stage of anesthesia, guinea pigs were placed in the supine position on an operating table with a heating pad set to maintain normal body temperature (Gaymar, Orchard Park, NY). The trachea was cannulated and connected to a ventilator (rodent ventilator model 683, Harvard Apparatus, Holliston, MA). The ventilator was set at 70 cycles/min and ventilator (rodent ventilator model 683, Harvard Apparatus, Holliston, MA). The ventilator was set to maintain normal body temperature (Gaymar, Orchard Park, NY). The trachea was cannulated and connected to a ventilator (rodent ventilator model 683, Harvard Apparatus, Holliston, MA). The ventilator was set at 70 cycles/min and ventilator (rodent ventilator model 683, Harvard Apparatus, Holliston, MA). The ventilator was set to maintain normal body temperature (Gaymar, Orchard Park, NY). The trachea was cannulated and connected to a ventilator (rodent ventilator model 683, Harvard Apparatus, Holliston, MA). The ventilator was set at 70 cycles/min. The trachea was cannulated and connected to a ventilator (rodent ventilator model 683, Harvard Apparatus, Holliston, MA). The ventilator was set to maintain normal body temperature (Gaymar, Orchard Park, NY). The trachea was cannulated and connected to a ventilator (rodent ventilator model 683, Harvard Apparatus, Holliston, MA). The ventilator was set at 70 cycles/min. The trachea was cannulated and connected to a ventilator (rodent ventilator model 683, Harvard Apparatus, Holliston, MA). The ventilator was set to maintain normal body temperature (Gaymar, Orchard Park, NY). The trachea was cannulated and connected to a ventilator (rodent ventilator model 683, Harvard Apparatus, Holliston, MA). The ventilator was set at 70 cycles/min.

Perfused lung preparation. The lung perfusate was Krebs-Henseleit solution (pH 7.4, 37°C, bubbled with 95% O₂ and 5% CO₂) that contained thiorphan and captopril (both 10⁻⁶ M) to inhibit neutral endopeptidase and angiotensin-converting enzyme that catabolize neuropeptides. The lungs were perfused at 10 ml/min. Five-minute samples (50 ml/sample) were collected before and during acute TS exposure. Four pulmonary effluent samples were collected. The first fraction was discarded. Thereafter, a 5-min fraction was collected as the base-level substance P release. During the next 5-min collection period, TS was introduced into the intake valve of the respirator at a ratio of 1 part smoke to 10 parts room air. A last sample was then collected over 5 min during undiluted TS delivery. Polypropylene vessels placed in an ice bath served as the collecting containers for the pulmonary effluent samples. The effluent samples were acidified with acetic acid to a pH of 3.0 ± 0.1 to minimize peptide loss.

Enzyme immunoassay. The substance P content of the lung perfusate samples was analyzed by enzyme immunoassay (Peninsula Laboratories, Belmont, CA). Procedures for substance P analysis were in accordance with the manufacturer’s instructions. Substance P analysis of lung perfusate samples in duplicate was performed by placing the immunoplate containing the wells of samples on a microtiter plate reader for analysis (model 3500, Bio-Rad, Hercules, CA).

Total and differential cell counts of lung lavage fluid. After collection of the last perfusate fraction, the lungs were lavaged with Hanks’ balanced salt solution (pH 7.4, 20 ml in 5 ml aliquots) through the tracheal cannula. The lavage fluid was then centrifuged at 2,000 rpm at 4°C. The supernatant was discarded, and the cell pellet was resuspended in 3 ml of Hanks’ solution in preparation for total cell counts and differential cell counts. Total cell counts were determined by using a Neubauer chamber (American Optical, Southbridge, MA) after adding several drops of LeukoStat I and II stain (Leukostat, Fisher Diagnostics, Fair Lawn, NJ) to the cell suspension. Differential cell counts of 10-μl aliquots of the cell suspension were determined from slides placed in a cytocentrifuge set at 1,000 rpm for 6 min (Shandon Lipshaw, Pittsburgh, PA). The cells on the slides were fixed and stained with the LeukoStat, according to the manufacturer’s instructions, and 300 cells/slide were counted under ×400 magnification. Cells were identified as mononuclear cells, eosinophils, and neutrophils. The absolute cell numbers and the percentage of each cell type were calculated.

Agents. All agents were purchased from Sigma Chemical (St. Louis, MO), except when stated otherwise in the text.

Results. Airway response to acute TS exposure. Acute TS was observed to induce dyspnea and cyanosis in various guinea pigs during the course of the study. Dyspnea during TS exposure was evidenced visibly by an increased effort to breath and cyanotic coloring of the eyes. Twenty-one different guinea pigs experiencing dyspnea and cyanosis during exposure were transferred to the plethysmograph. Base-level Penh units were determined previously in that same day. Penh units were monitored for 1 min immediately after the TS exposure. Thereafter, an aerosol of terbutaline (1%, 30 s) was introduced into the plethysmograph. Penh

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units were then monitored for 2 min. This acute TS exposure increased airway resistance in those guinea pigs exposed to TS. The averaged base-level Penh unit from earlier in the day was 0.26 ± 0.02. The averaged Penh unit after acute TS challenge was 1.46 ± 0.17 before terbutaline treatment, which then fell to 0.44 ± 0.04 and 0.30 ± 0.02 Penh units during minutes 1 and 2, respectively, after the terbutaline treatment (P < 0.0001). Both eupnea and normal eye color returned with a decrease in Penh units.

To gain insight into the mechanism of airway reactivity to TS exposure, OA-TS guinea pigs were acutely challenged with TS before and after administration of either an H1 antagonist or the cocktail of neuropeptide antagonists. Penh units were monitored for 1 min after a 5-min TS exposure with no pretreatment and with pretreatment of either pyrilamine or the cocktail of CP-96345 and [Tyr5,D-Trp6,7,9,Lys10]-α-NKA. Both the antihistamine and the cocktail of NK antagonists attenuated the effect of TS (Fig. 3).

Airway response to capsaicin, bradykinin, and NKA fragment 4–10 after chronic exposure to TS. Because the cocktail of neuropeptide antagonists attenuated the bronchoconstriction induced by acute TS exposure and because neuropeptides are released from C fibers, the lungs were then challenged with agents known to activate C fibers as well as a neuropeptide analog. In an initial series of experiments, capsaicin aerosol challenge (10 μg/ml, 30 s) did not increase Penh units above base level in NS guinea pigs exposed to A, OA-sensitized guinea pigs exposed to A, or NS guinea pigs exposed to TS. However, in OA-sensitized guinea pigs exposed to TS, Penh units increased from 0.27 ± 0.07 to 0.80 ± 0.16 (P < 0.0001).

Because airway reactivity to capsaicin was enhanced in OA-TS guinea pigs, the effect of capsaicin challenge was further investigated in this group. Capsaicin aerosol challenge increased Penh units in OA-TS guinea pigs in a dose-related manner. These same doses of capsaicin aerosol had little effect on NS-A guinea pigs (Fig. 4). To investigate the contribution of the central reflex arc of capsaicin-induced airway reactivity, NS-TS guinea pigs were pretreated with atropine, and OA-TS guinea pigs were pretreated with either atropine or lidocaine, before capsaicin aerosol challenge. Neither pretreatment with atropine nor lidocaine attenuated the effects of capsaicin on airway reactivity (Fig. 5). We also investigated the contribution of the axon reflex to capsaicin-induced airway reactivity in OA-TS guinea pigs after pretreatment with atropine (2% for 2 min) and lidocaine (Lido; for 2 min) in OA-TS guinea pigs (n = 16) (B). Base; base level. Neither agent attenuated airway reactivity to capsaicin. Penh units increased above base level, *P < 0.05.
OA-TS guinea pigs. Capsaicin aerosol challenge was repeated before and after administration of phosphoramidon or a NK1- and NK2-antagonist cocktail of [Tyr\(^6\),p-Trp\(^6,7,9\),Lys\(^10\)]-o-NKA and CP-96345. Phosphoramidon administration did not enhance the effects of capsaicin, but the NK-antagonist cocktail did attenuate the effects of capsaicin in these animals (Fig. 6). Capsaicin aerosol challenges to NS-A guinea pigs were also performed before and after administration of phosphoramidon (10\(^{-4}\) M, 60 s). Before phosphoramidon administration, capsaicin aerosol challenge did not statistically change Penh units from 0.29 ± 0.02 to 0.34 ± 0.03 (nonsignificant, n = 8). However, after phosphoramidon administration, capsaicin aerosol increased Penh units from 0.25 ± 0.03 to 0.43 ± 0.05 (P = 0.04). Although airway reactivity to capsaicin increased after the phosphoramidon administration, it was not nearly to the capsaicin level of the TS-exposed guinea pigs.

Whereas capsaicin is an exogenous agent that activates C fibers, bradykinin is an endogenous agent that activates C fibers. Capsaicin and bradykinin act on different membrane receptors to induce C-fiber activation (9). It is possible that the mechanism of enhanced TS-induced airway reactivity may or may not be selective for capsaicin. Therefore, we challenged the airways of three groups of guinea pigs with bradykinin (1 × 10\(^{-4}\) M, 30 s). Bradykinin aerosol challenge increased airway obstruction in guinea pigs chronically exposed to TS but had little effect on airways of guinea pigs not exposed to TS (Fig. 7). Although peak Penh units were numerically higher in the OA-TS group than in the NS-TS group, there was no demonstrable separation between the two groups.

To determine whether TS exposure increases airway reactivity to tachykinins, guinea pigs were challenged with aerosols of NKA fragment 4–10 (8 × 10\(^{-5}\) M, 1 min). NKA fragment 4–10 aerosol increased Penh units in all three groups of guinea pigs; however, airway reactivity was greater in the TS-exposed groups (Fig. 8). NKA is metabolized by neutral endopeptidase, and TS is known to inhibit neutral endopeptidase (8). Therefore, the NKA fragment 4–10 challenge was repeated in two nonsensitized guinea pigs not exposed to TS after treatment with phosphoramidon. Penh units began to increase at ~2 min after the aerosol challenge of the NKA fragment 4–10. Both animals were removed from the plethysmographs and treated with 2% isoproterenol aerosol. Airway distress continued to worsen. Epinephrine was intraperitoneally administered with no relief in airway distress. Each animal subsequently died because of the combination of phosphoramidon and NKA fragment 4–10 treatment.

Histamine and MCh aerosol challenge. To determine whether TS exposure induces generalized airway hyperirritability, the airways were challenged with two agents known to act directly on airway smooth muscle. Guinea pigs were challenged with histamine and MCh 18–24 h after the last TS exposure. Histamine aerosol challenge of 40 \(\mu\)g/ml increased Penh units in all four experimental groups, but there was no statistical separation among the four experimental groups (data not shown). Thereafter, in a second series of experiments, airway reactivity to several doses of histamine and MCh was determined in NS-A and OA-TS guinea pigs. Again airway reactivity to neither histamine nor MCh
was enhanced by either chronic TS exposure or OA sensitization (Fig. 9, A and B, respectively).

Acute TS exposure also failed to enhance histamine airway reactivity (Fig. 10). Airway reactivity to MCh before and after acute TS exposure was determined in one series of experiments. In NS-TS guinea pigs, Penh units were $0.38 \pm 0.02$ before and $0.61 \pm 0.03$ after MCh aerosol challenge (100 μg/ml, 30 s). Within 15 min of a 5-min TS exposure, peak Penh units were $0.33 \pm 0.03$ before and $0.60 \pm 0.07$ after the MCh aerosol challenge. However, acute TS exposure increased the sensitivity of OA-sensitized guinea pigs to capsaicin. Capsaicin aerosol challenge (10 μg/ml, 30 s) 18–24 h after the last TS exposure increased Penh units from $0.32 \pm 0.01$ to $0.63 \pm 0.06$. Capsaicin administered within 10 min of acute TS exposure increased Penh units from $0.30 \pm 0.01$ to $0.95 \pm 0.10$ ($P < 0.05$ for peak Penh units).

At the conclusion of the study, tracheal rings were challenged with histamine, MCh, and capsaicin to determine whether airway smooth muscle contractility was affected by chronic TS exposure. Both histamine and MCh increased ring tension in a dose-related manner, but again there was no statistical separation among treatment groups (Fig. 11, A and B). An increase in the dose-tension relationship in response to capsaicin administration occurred only in the tracheal rings from the OA-sensitized guinea pig exposed to TS (Fig. 11C).

**Substance P content of lung perfusate fractions.** Because TS exposure enhanced airway reactivity to agents that activate C fibers, we investigated whether chronic TS exposure enhanced neuropeptide release during acute TS challenge. Acute TS challenge increased the spillover of SP into lung perfusate samples in the OA-sensitized guinea pigs chronically exposed to TS. Substance P increased from $11.3 \pm 3.5$ to $60.2 \pm 7.9$ fmol/fraction during the 5-min 10% TS challenge ($P < 0.0005$). In the other three groups, SP content of lung perfusate fluid during acute TS challenge was unchanged. However, in NS-A guinea pigs, acute TS challenge increased SP content from $9.8 \pm 5.6$ to $32.1 \pm 13.1$ fmol/fraction ($P = 0.07$).

**Total and differential cell counts of lung lavage fluid.** Further confirmation of OA sensitization was provided at the end of the study by analysis of lung lavage fluid for total and differential cell counts. Total cell counts of the OA-sensitized guinea pigs were nearly four times that of the nonsensitized group exposed to A (Fig. 12). The increase in eosinophils in both OA-sensitized groups was similar. The total number of cells recovered from NS-TS guinea pigs was double that from NS-A guinea pigs. No difference in the percentage of cell types occurred between the two nonsensitized groups; however, the number of eosinophils increased in NS-TS guinea pigs (10.4 ± 3.4 × 10^5 cells) vs. the NS-A guinea pigs (5.2 ± 0.8 × 10^5 cells, $P < 0.02$).

**DISCUSSION**

The present study demonstrates that chronic TS exposure induces airway hyperirritability in guinea pigs, which is further enhanced by OA sensitization. Because the enhanced airway hyperirritability was determined to occur with agents that selectively activate C fibers, the mechanism of airway hyperirritability induced by TS probably involves enhanced C-fiber reactivity. C-fiber activation induces neurogenic reflexes such as airway smooth muscle contraction, in-
creased airway secretion, and edema formation through both central and local axon reflex arcs (2, 5). Guinea pig airway responsiveness to capsaicin challenge as measured by sRaw is unaltered by administration of either atropine or bilateral vagotomy (3), suggesting that the axon reflex alone induces intense airway obstruction. In the present study, neither lidocaine nor atropine attenuated the effects of capsaicin aerosol challenge on airway resistance, again suggesting that local rather than central mechanisms dominate airway reactivity to capsaicin challenge. However, airway hyperirritability due to an enhancement of the central reflex pathway cannot be discounted. Since the submission of this study, another study has been published that is largely in agreement with the results of this study (27).

**Acute TS challenge vs. chronic TS exposure.** Acute TS exposure induced bronchoconstriction that was readily reversed by terbutaline. Both histamine and neuropeptides contribute to the airway reactivity induced by acute TS exposure because either pyrilamine or the cocktail of neuropeptide antagonists attenuated airway reactivity. On the other hand, chronic TS exposure induced enhanced airway reactivity to agents that activate C fibers. Furthermore, enhanced airway hyperirritability to neither histamine nor MCh was demonstrable in TS-exposed guinea pigs. Although chronic TS exposure induces selective enhanced airway reactivity, baseline Penh units were similar in all experimental groups of guinea pigs.

Airway hyperirritability after acute TS exposure to histamine or cholinergic agonists has been reported by some (22, 23) but not by others (16). The studies differ in mediator dose, time of mediator challenge after TS exposure, route of mediator challenge, and the method by which airway reactivity is determined. In a TS exposure study 2 wk in duration, Karlsson et al. (16) measured time to bronchoconstriction as an abrupt development of slow and labored breathing. In that study, TS exposure failed to induce airway hyperirritability to histamine challenge. Similarly, the Penh units in the present study are based on breathing patterns as well as pressures, and we also did not observe TS-induced airway hyperirritability to histamine or MCh challenge. In contrast, Omini et al. (23) reported increased airway reactivity to histamine after acute TS exposure, but it was associated only with an increase in reactivity of parenchymal strips and not tracheal smooth muscle preparations. We also observed no increase in reactivity to histamine in isolated tracheal rings from guinea pigs exposed to TS vs. A. If increased airway reactivity to histamine occurs preferentially in small airways, then it is possible that measurements of airway resistance failed to detect small airway dysfunction.

Time of mediator challenge after TS exposure is an important factor affecting airway hyperirritability. Hulbert et al. (14) demonstrated that TS induces airway hyperirritability to histamine challenge at 30 min postexposure but not after 6 or 24 h. This is similar to what we...
observed. Daffonchio et al. (6) determined that airway reactivity to acetylcholine was demonstrable 5–50 min after the TS exposure; however, airway reactivity to acetylcholine was obviously decreasing during this time. In the present study when histamine, MCh, or capsaicin challenge was conducted before the day’s TS exposure, prolonged airway hyperreactivity was observed only with capsaicin challenge.

Mechanisms of enhanced airway reactivity resulting from chronic TS exposure. Chronic TS exposure may enhance airway reactivity to capsaicin and bradykinin challenge after C-fiber activation through central and/or local “axon” reflexes. In both the present and previous study (3), guinea pig airway responsiveness to capsaicin challenge was unaltered by administration of atropine, lidocaine, or bilateral vagotomy. These results suggest that the axon reflex alone can induce intense airway obstruction in this species. However, TS-induced airway hyperirritability due to an enhancement of the central reflex pathway is likely to occur. It is not known whether the threshold or reactivity of the central and the axon reflex is the same in either healthy or sensitized airways.

Chronic TS exposure may enhance the axon reflex by several mechanisms. TS exposure decreases neuropeptide metabolism by inhibiting the action of neutral endopeptidase (8). Our data are in agreement with those of Dusser et al. (8). Chronic TS exposure increased airway reactivity to the NK-receptor agonist NKA fragment 4–10 compared with guinea pigs not exposed to TS. Treatment with the neutral endopeptidase inhibitor duplicated qualitatively the effect of TS exposure. Our chronic TS exposure probably did not totally block neutral endopeptidase because the reaction in these animals was less severe than in guinea pigs pretreated with phosphoramidon.

Inhibition of neutral endopeptidase appears to be only part of the mechanism of TS-induced airway hyperirritability. Data from the present study suggest that TS exposure may also enhance the axon reflex by enhancing release of tachykinins. Our lung perfusate contained a cocktail of captopril and thiorphan, which antagonizes angiotensin-convertase enzyme and neutral endopeptidase, respectively. If the effects of TS exposure were limited to inhibition of neutral endopeptidase, then the perfusate from all experimental groups should have been similar in substance P content. These results imply enhanced release and possible enhanced content of tachykinin in guinea pig lungs chronically exposed to TS. In addition, airway reactivity to capsaicin was only modestly enhanced in nonsensitized guinea pigs exposed to air after phosphoramidon administration. If the mechanism of action of chronic TS exposure is due solely to inhibition of neutral endopeptidase, then airway reactivity to capsaicin in guinea pigs treated with phosphoramidon should have been similar to airway reactivity of guinea pigs chronically exposed to TS. Therefore, chronic TS exposure may increase the lung’s content of tachykinins as well as its release. Because the connection between the pulmonary and bronchial circulations is extensive in guinea pigs (24), the substance P in the lung perfusate samples could have originated from both bronchial and pulmonary C fibers.

Synergistic effect of chronic TS exposure and airway OA sensitization. Poorly understood is the synergistic effect of TS exposure and airway sensitization. Certain asthmatic individuals are hypersensitive to TS (7). Airway hyperirritability in atopic individuals is accompanied by an influx into the lungs of polymorphonuclear leukocytes, predominantly eosinophils (10, 15). Increased numbers of eosinophils in the bronchoalveolar lavage of OA-sensitized guinea pigs were confirmed in the present study. Studies in antigen-sensitized guinea pigs suggest that infiltration of eosinophils from the circulation into the lungs may be a necessary factor in the development of airway hyperirritability (17, 26). Eosinophils release agents that may induce bronchial hyperirritability, such as substance P, major basic protein, eosinophil-derived neurotoxin, and eosinophil cationic peptide (11, 26). However, eosinophil influx into the lungs alone did not account for the increased airway hyperirritability to capsaicin aerosol challenge in the present study, because OA-sensitized guinea pigs exposed to A were not as sensitive to capsaicin as were OA-sensitized guinea pigs exposed to TS. The mechanism, however, remains to be determined. It is possible that chronic TS exposure affects the activation threshold of these cells to release bronchoactive agents. Unknown is what direct action capsaicin or TS exposure has on eosinophil activation. The possibility remains that airway hyperirritability induced by chronic TS exposure may in part result from enhanced activation of eosinophils.

Interestingly, chronic TS exposure produced no statistical difference in airway reactivity to OA aerosol challenge between OA-sensitized guinea pigs exposed to TS and those exposed to A. This is perhaps an unexpected result, considering the differing airway reactivity observed with capsaicin challenge. If neuropeptides play a significant role in the airway response to antigen challenge, then increased airway reactivity to OA challenge would be expected in airways that are hyperirritable to capsaicin. The immediate phase of the allergic reaction is dominated by mast cell degranulation. The dominant mediators released are histamine and newly synthesized mediators such as the prostaglandins and leukotrienes. Airway reactivity in the present study was monitored for 5 min after the antigen challenge. The extreme sensitivity of guinea pig airways to histamine dominates the time frame in which we monitored airway function after OA challenge. We observed that pyrilamine greatly attenuated the effects of OA aerosol challenge in the present study, although the cocktail of NK1 and NK2 antagonists also had a protective effect. Because we did not observe changes in histamine airway reactivity among groups, it is reasonable that we also did not observe changes in OA airway reactivity among groups.

Airway function and its measurement with Penh units. Airway reactivity was determined in vivo as an increase in Penh units obtained from an “unrestrained”
whole body plethysmograph. Penh units are the product of the ratio of early and late expiratory phases (ventilatory pause) and the ratio of peak expiratory pressure to peak inspiratory pressure. Our studies correlating Penh units to sRaw during MCh challenge indicate that Penh units are valid indicators of bronchoconstriction. Similar results are reported by Hamelmann et al. (12). Chong et al. (4) established a correlation of Penh units and sRaw in guinea pigs during histamine challenge. Other observations in our laboratory support correlation between the Penh unit and sRaw. Increased Penh units always accompanied dyspnea and cyanosis. In addition, the increase in Penh units coincided with the time course of the expected increased airway resistance induced by capsaicin and histamine, as well as OA antigen challenges. For example, the effect of histamine on airway resistance was rapid, whereas the effect of OA antigen was slower. In addition, vehicle administration or OA-antigen challenge of nonsensitized guinea pigs did not increase Penh units. Finally, increased Penh units produced by TS, capsaicin, histamine, and MCh were all rapidly reversed by $\beta_2$-adrenergic agonist administration. This suggests that airway resistance induced by airway smooth muscle contraction and Penh units is highly correlated. However, there are several concerns about interpretation and quantification of Penh units that remain to be clarified (21).

In conclusion, chronic TS exposure can induce airway hyperresponsiveness that appears to be mediated by C-fiber activation. This airway hyperresponsiveness can be further enhanced through airway sensitization as observed in the OA-sensitized guinea pig. The presence of airway eosinophilia alone did not account for the airway hyperresponsiveness to capsaicin. Although both the central reflex arc and axon reflex are involved in neurogenic inflammation, the local reflex mechanism appears to dominate bronchoconstriction. The results of the present study support a mechanism of airway sensitization through enhanced neuropeptide action after chronic exposure to TS. An enhanced effect of neuropeptides might occur through C-fiber sensitization. Chronic TS exposure could decrease the threshold of either or both the central reflex arc and the local or axon reflex. Chronic TS exposure might also result in enhanced neuropeptide synthesis within C fibers. A probable mechanism of enhanced action of neuropeptides after their release is the decrease in catabolism of the peptides, as Dusser et al. (8) determined that TS inhibits neutral endopeptidase. Therefore, the increased airway reactivity to capsaicin observed in this study may be the result of several factors.

REFERENCES


