Role of tachykinins in airway responses to ozone in rats

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Role of tachykinins in airway responses to ozone in rats. J. Appl. Physiol. 85(2): 442–450, 1998.—Previous studies that used neonatal capsaicin (Cap) treatment to ablate C fibers indicate that C fibers act to inhibit lung damage and airway hyperresponsiveness after ozone (O₃) exposure in rats. The purpose of this study was to determine 1) the role of tachykinins in these protective effects and 2) whether differences in minute ventilation (Ve) during O₃ exposure might account for the effect of Cap. In the first study, male Sprague-Dawley rats were exposed to 1 part/million O₃ or air for 3 h. Four hours later, a bronchoalveolar lavage (BAL) was performed or airway responsiveness was measured. Rats were treated with CP-99994 and SR-48968, selective neurokinin-1- and -2-receptor antagonists, respectively, or with vehicle (Veh). O₃ caused an increase in the number of neutrophils recovered from BAL fluid in both the Veh-treated and tachykinin-receptor antagonist (TKRA)-treated rats, but the number of neutrophils was approximately twofold greater in the TKRA-treated rats. In contrast, TKRA treatment had no effect on baseline pulmonary mechanics or airway responsiveness. After O₃ exposure, the number of neutrophils in BAL fluid was also greater in Cap- than in Veh-treated rats. O₃ reduced Ve in both Veh- and Cap-treated rats, but the response was greater (reduction of 44.7 ± 3.7 vs. 27.8 ± 6.8%) and occurred earlier (10 vs. 70 min) in Cap- than in Veh-treated rats (P < 0.02). These results suggest that tachykinins mediate protective effects of C fibers against O₃-induced lung inflammation. The results also indicate that the more pronounced effect of O₃ on BAL neutrophils in Cap-treated rats is not the result of a greater inhaled dose of O₃ resulting from greater Ve.

C FIBERS, a class of unmyelinated sensory neurons, innervate the lungs and airways of many species, including humans. The terminal ramifications of these neurons are found underneath and between epithelial cells, around blood vessels, and near airway smooth muscle and mucous glands (2, 24). In response to stimulation, the tachykinins, substance P (SP) and neurokinin A (NKA), are released from the peripheral endings of these neurons. The biological effects of these peptides in the lung and airways are diverse. Tachykinins can induce bronchoconstriction, increase microcirculation, cause mucous secretion, and increase vascular permeability (19). Stimulation of C fibers also causes apnea, followed by rapid, shallow breathing (33). C fibers are stimulated by various inhaled irritants including ozone (O₃) (4) as well as by many endogenous inflammatory mediators (19). In addition, SP is released into the airway lavage fluid of humans exposed to O₃ (12).

Neonatal capsaicin (Cap) pretreatment causes degeneration of C fibers and decreases neuropeptide content in the lungs and airways (14, 27). Our laboratory (15) has previously reported that, in Sprague-Dawley rats treated neonatally with Cap, airway hyperresponsiveness to inhaled methacholine is induced by exposure to concentrations of O₃, which do not affect responsiveness in control rats. Neonatal Cap treatment also results in more airway epithelial injury and more inflammation after O₃ exposure, as determined by pathology and bronchoalveolar lavage (BAL) in Wistar rats (37). Taken together, these results suggest that C fibers protect the airways during exposure to O₃, minimizing airway injury and inflammation and inhibiting the development of airway hyperresponsiveness. C fibers have also been shown to play a protective role in the responses to other inhaled irritants such as sulfur dioxide (22, 23), hydrogen sulfide (30), and acrolein (41).

One aim of this study was to determine whether tachykinins were important in mediating these protective effects of C fibers against O₃-induced airway inflammation and airway hyperresponsiveness in rats. To this end, we treated rats with tachykinin-receptor antagonists (TKRAs) or the vehicle (Veh) used to dissolve them, exposed them to O₃ or air, and then either performed a BAL or instrumented them for the measurement of pulmonary mechanics and responsiveness to inhaled aerosolized methacholine. The TKRAs, CP-99994 and SR-48968, which possess high potency and selectivity in blocking the neurokinin-1 (NK₁) and -2 (NK₂) receptors, respectively (8, 26), were employed. Because our experiments were performed in Sprague-Dawley rats, whereas those of Sterner-Kock et al. (37) were performed in Wistar rats, and because differences in responses to tachykinins and to O₃ have been observed among strains of rat (9, 16, 39, 42), we also examined the effect of neonatal Cap treatment on O₃-induced alterations in BAL cells and protein and confirmed the results of Sterner-Kock et al. (37) indicating that ablation of C fibers causes more lung injury and inflammation after O₃ inhalation.

One explanation for the greater inflammatory response of the Cap-treated rats is that a larger dose of O₃ is delivered to the lungs of these rats despite a similar O₃ concentration. The dose of O₃ delivered to the lungs is a function of both O₃ concentration and minute ventilation (Ve) (47). During O₃ exposure, rats decrease their Ve (1, 25). Because O₃ stimulates C fibers and stimulation of C fibers alters Ve (4, 33), it is possible that C fibers are the sensory arm of O₃-induced changes...
in \( V_e \). If so, one might expect \( V_e \) to be reduced during \( O_3 \) exposure in normal rats, but not in Cap-treated rats lacking C fibers, leading to an overall greater dose of \( O_3 \) in the Cap-treated rats. To test this hypothesis, we measured \( V_e \) and its components, tidal volume (\( V_t \)) and breathing frequency (\( f \)), during exposure of awake Veh- and Cap-treated rats to \( O_3 \) or air.

**METHODS**

Animals. This study protocol was approved by the Harvard Medical Area Standing Committee on Animals. For the studies with TKRAs, pathogen-free Sprague-Dawley rats were obtained from Charles River Breeding Laboratories (Wilmington, MA) and were housed in group cages. For the study with neonatal Cap treatment, pregnant female Sprague-Dawley rats were purchased from Charles River and were housed individually. In each litter, rats were treated either with 50 mg/kg of Cap, dissolved in a mixture of 10% Tween 80 and 10% ethanol in saline, or with an equivalent amount of Veh subcutaneously 2 or 3 days after their birth. During Cap treatment, rats were anesthetized with halothane. The rats were weaned at 1 mo of age, and males and females were separated.

Verification of Cap desensitization. Our laboratory has previously reported that neonatal Cap pretreatment performed in this manner decreases tachykinins in lung and other tissues (15, 23). To confirm a decrease in tachykinins in these rats, six Veh- and six Cap-pretreated rats from the same cohort used for measurements of ventilatory responses to \( O_3 \) were killed with an overdose of pentobarbital sodium, and the lungs were excised. The lungs were immediately frozen in liquid nitrogen and stored at −70°C until extraction and assay for SP content by an enzyme-linked immunosorbent assay. SP was extracted from the lungs as previously described (18). The enzyme-linked immunosorbent assay for SP was performed by using methods described by Folkesson et al. (10). The antibody for SP was purchased from Peninsula Laboratories (Belmont, CA). The lower limit of detection of this assay was 3 fmol of SP, and the assay has <0.01% cross-reactivity with NKA (18). Cap pretreatment significantly reduced the SP content of the lungs from 2.83 ± 0.5 to 1.75 ± 0.05 fmol/g tissue (\( P < 0.001 \)).

\( O_3 \) exposure. Exposure to \( O_3 \) or filtered room air was conducted in a stainless steel chamber with a Plexiglas door on the front (−145 liters in volume). \( O_3 \) was generated by passing dry 100% \( O_3 \) through ultraviolet light and mixing it with filtered room air in the chamber. Chamber atmosphere was drawn continuously via a sampling port, and \( O_3 \) concentration was measured by an \( O_3 \) chemiluminescent analyzer (model 49, Thermoelectron Instruments, Hopkinton, MA), which was calibrated by an ultraviolet photometric \( O_3 \) calibrator (model 49PS, Thermoelectron Instruments).

Experimental protocol. Two different studies were performed. The purpose of the first study (TKRA) was to determine whether tachykinins act to limit airway injury and inflammation induced by \( O_3 \) and thereby limit the degree of airway hyperresponsiveness induced by \( O_3 \). In this study, rats were treated with CP-99994 (0.75 mg/kg ip) or SR-48968 (0.75 mg/kg ip) or with Veh 15 min before exposure to filtered air or \( O_3 \). The rats were then exposed to air or \( O_3 \) (1 part/million ppm), for 3 h and were retreated with the same doses of CP-99994 and SR-48968 or the Veh immediately after the exposure to ensure continued receptor blockade. These doses of TKRAs were chosen based on reports that indicated their effectiveness in inhibiting responses to SP and NKA in rats and guinea pigs (8, 13, 26, 36). Four hours after the air or \( O_3 \) exposure had ended, rats were killed, and BAL was performed. Alternatively, 2 h after exposure, rats were anesthetized for the measurement of airway responsiveness to inhaled methacholine, followed by administration of intravenous SP to confirm the efficacy of the antagonists. The time required to anesthetize the animals, perform the necessary surgery, instrument them, and perform the measurements of responsiveness was such that the measurements of the steep part of the dose-response curve coincided in time with BAL performed in the other animals.

The purpose of the second study was to confirm, in Sprague-Dawley rats, reports about Wistar rats indicating that Cap-treated rats that lack C fibers have increased airway injury and airway inflammation after \( O_3 \) exposure (37). We also sought to determine whether differences in \( V_e \) during \( O_3 \) exposure might contribute to these enhanced responses. Two cohorts of rats were used in this study (Cap). In the first cohort, adult rats with or without neonatal Cap treatment were exposed either to 1 ppm \( O_3 \) or to filtered air for 3 h. To evaluate inflammatory changes induced by \( O_3 \), BAL was performed 4 h after the end of \( O_3 \) exposure. In the second cohort, adult rats treated neonatally with either Cap or Veh were placed in head-out restraining tubes that served as body plethysmographs. Once the animal was in the tube, the cranial end of the tube was inserted into a port in the door of the \( O_3 \)-exposure chamber, so that animals breathed air from within the chamber. We then monitored \( V_e \) for 45 min before \( O_3 \) was introduced into the chamber. \( O_3 \) exposures (2 ppm) were for 90 min followed by a 45- to 90-min recovery period during which we continued to monitor \( V_e \). For these experiments, the washin of \( O_3 \) was such that 2 ppm was reached within 5 min of initiating the exposure. The washout was such that \( O_3 \) had decreased to <0.02 ppm within 5 min after the \( O_3 \) flow to the chamber was stopped. Other Cap- and Veh-treated rats were treated identically but were exposed to filtered air only.

Measurements of pulmonary mechanics and airway responsiveness to inhaled aerosolized methacholine were performed as previously described (15). Briefly, rats were anesthetized with pentobarbital sodium (64.8 mg/kg ip), tracheostomized with a tubing adapter (14 gauge, 1.65-mm ID), and mechanically ventilated with a rodent ventilator (Harvard Apparatus, Natick, MA) at 8 ml/kg and 60 breaths/min. End-expiratory pressure was maintained at 3.5 cmH\(_2\)O throughout the experiment. The left carotid and right jugular vein were catheterized for blood pressure measurement and drug administration, respectively. The chest wall was opened widely to expose the lungs to atmospheric pressure, and the rat was then placed in a body plethysmograph. Plethysmograph pressure relative to a reference bottle of a similar size was measured with a differential pressure transducer (model LCVR, Celesco, Canoga Park, CA) and used as a volume signal. Flow was calculated by electrical differentiation of the volume signal. Transpulmonary pressure was obtained with a second pressure transducer (Celesco) placed between a side tap in a tracheal cannula and the inside of the plethysmograph. All three signals were recorded on a strip chart (model RS65P, General Scanning, Watertown, MA) and fed to a computer for calculation of dynamic compliance (Cdyn) and pulmonary conductance (GL), according to the method of Von Neergard and Wirz (44, 45).

Airway responsiveness was measured as follows. A breath equal to three times \( V_t \) was given to provide a constant volume history. Baseline measurements of \( GL \) and Cdyn were obtained 1 min later. Animals were then given 20 tidal breaths of aerosolized saline generated in an acorn nebulizer. A flow rate of 4.5 l/min was used to generate the aerosol. \( GL \)
RESULTS

TKRA study. To determine whether tachykinins released from C fibers act to limit O₃-induced airway injury and inflammation, we examined the effect of TKRAs on BAL cells and protein in O₃-exposed animals. The profile of cells recovered from BAL was affected by both O₃ exposure and TKRA treatment (Table 1). Analysis of variance indicated significant differences across the groups in the number of BAL neutrophils and ciliated epithelial cells and the BAL protein concentration, whereas total cell numbers, lymphocytes, and macrophages were unchanged. Treatment with TKRAs did not affect either the BAL cell profile or the BAL protein concentration in air-exposed rats. O₃ exposure increased the number of neutrophils in both the Veh- and the TKRA-treated rats, compared with air-exposed controls. However, the number of neutrophils recovered from the TKRA-treated rats exposed to O₃ was significantly greater than that from the Veh-treated rats exposed to O₃. O₃ exposure also increased BAL epithelial cells and BAL protein concentration in both the Veh- and the TKRA-treated rats, compared with air-exposed controls (Table 1). However, in contrast to the effect of TKRA on BAL neutrophils,

Table 1. Effects of O₃ and inhibition of tachykinin receptors on no. of cells and total protein concentration in bronchoalveolar lavage fluid

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Treatment</th>
<th>Body Wt, g</th>
<th>Total Cells</th>
<th>Macrophages</th>
<th>Neutrophils*</th>
<th>Lymphocytes</th>
<th>Epithelial Cells*</th>
<th>Total Protein,* mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>Vehide</td>
<td>301 ± 3</td>
<td>12.8 ± 0.42</td>
<td>12.3 ± 0.48</td>
<td>0.07 ± 0.02</td>
<td>0.22 ± 0.04</td>
<td>0.06 ± 0.01</td>
<td>0.31 ± 0.02</td>
</tr>
<tr>
<td>Air</td>
<td>TKRA</td>
<td>302 ± 3</td>
<td>11.4 ± 0.55</td>
<td>11.0 ± 0.54</td>
<td>0.07 ± 0.01</td>
<td>0.18 ± 0.02</td>
<td>0.04 ± 0.01</td>
<td>0.34 ± 0.04</td>
</tr>
<tr>
<td>O₃</td>
<td>Vehide</td>
<td>301 ± 3</td>
<td>12.0 ± 0.64</td>
<td>11.0 ± 0.67</td>
<td>0.55 ± 0.071</td>
<td>0.24 ± 0.03</td>
<td>0.17 ± 0.03</td>
<td>0.51 ± 0.03</td>
</tr>
<tr>
<td>O₃</td>
<td>TKRA</td>
<td>303 ± 4</td>
<td>14.0 ± 0.17</td>
<td>12.5 ± 0.94</td>
<td>1.07 ± 0.13</td>
<td>0.23 ± 0.04</td>
<td>0.27 ± 0.05</td>
<td>0.51 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 animals. Body weight of animal on day of experiment is shown. TKRA, tachykinin-receptor antagonist. Cells are expressed as no. of cells in bronchoalveolar lavage fluid × 10⁶. *P < 0.001 by ANOVA; †P < 0.02, ozone (O₃)-exposed, vehicle-treated group compared with air-exposed groups (vehicle and TKRA combined); ‡P < 0.02, O₃-exposed, TKRA-treated group compared with air-exposed groups; §P < 0.02 compared with O₃/vehicle.
there was no difference in BAL epithelial cells or BAL protein concentration between the TKRA-treated rats exposed to O₃ and the Veh-treated rats exposed to O₃.

We also measured pulmonary mechanics and airway responsiveness to inhaled aerosolized methacholine in TKRA- and Veh-treated rats exposed to air or O₃. Exposure to O₃ did not alter baseline airway mechanics or induce airway hyperresponsiveness to inhaled methacholine in either the Veh- or TKRA-treated rats (Table 2).

To confirm the efficacy of the TKRA treatment, after the measurements of airway responsiveness to methacholine were taken, changes in blood pressure induced by SP were assessed (Table 3). Infusion of SP caused a triphasic blood pressure change as reported by others (35): an immediate fall phase, recovery within 1 min, and, at higher doses, an extended secondary decrease. The lowest mean blood pressure observed in the first phase is reported. Intravenous injection of 10⁻¹² mol/kg of SP did not alter blood pressure in either the Veh- or the TKRA-treated groups. In the Veh-treated animals, 10⁻¹¹ mol/kg SP caused a significant decrease in blood pressure. In the TKRA-treated groups, the same dose of SP had no effect (P < 0.05 compared with the Veh group). At 10⁻¹⁰ mol/kg, blood pressure fell in both groups, but the decrease was greater in the TKRA-treated than in the TKRA-group treated.

Cap study. To verify a protective role for C fibers in the airway inflammation and injury induced by O₃ exposure in Sprague-Dawley rats, as had previously been demonstrated in Wistar rats (37), adult rats treated neonatally with Cap or the Veh used to dissolve Cap were exposed to air or O₂ and a BAL was performed. The results are summarized in Table 4. Analysis of variance indicated a statistically significant difference across groups in the numbers of neutrophils, lymphocytes, and ciliated epithelial cells and in total protein. Follow-up analysis indicated that, compared with air-exposed controls, the numbers of neutrophils and ciliated epithelial cells were increased significantly in both the O₃-exposed, Cap-treated group and the O₃-veh, Veh-exposed group. As observed with TKRA treatment, the number of neutrophils in BAL fluid recovered from Cap-treated animals exposed to O₃ was significantly greater than was the number of neutrophils recovered from BAL of Veh-treated animals exposed to O₃. Compared with air-exposed animals, the total protein concentration was elevated in the O₃-exposed, Cap-treated group but not in the Veh-treated group.

To determine whether the effective dose of O₃ delivered to the lungs might be different in Cap- and Veh-treated rats, we measured Vₑ and examined the pattern of breathing during air or O₃ exposure. Table 5 shows mean baseline ventilatory parameters measured during the 20 min immediately before O₃ (or air) exposure (after the animals were allowed 25 min to settle in the restraining tubes). No significant differences between Cap- and Veh-treated rats were observed.

In Cap- and Veh-treated rats exposed to filtered air, there was substantial minute-to-minute variability in Vₑ, but no significant trend over time in either group and no significant effect of Cap treatment (Fig. 1). Similarly, no significant changes in V₉, f, Tᵢ, or Tₑ were observed with time in air-exposed animals (data not shown).

Figure 2 shows the effect of a 90-min O₃ exposure on ventilatory parameters in O₃-exposed rats. O₃ caused a decrease in Vₑ in both Veh- and Cap-treated rats, but there was a significant effect of Cap treatment on this response (P < 0.018). In Cap-treated rats, a significant decrease in Vₑ occurred within 10 min of O₃ exposure, whereas a significant decrease in Vₑ was not observed in the Veh-treated animals until after 70 min of O₃ exposure.

In both groups, the change in Vₑ was primarily the result of a decrease in V₉ (Fig. 2), and, again, there was a significant effect of Cap treatment on this change (P < 0.012). Vₑ decreased earlier in the Cap-treated rats (30 min) than in the Veh-treated controls (70 min). There was substantial variability in the f response to O₃ exposure among rats. Some rats showed an increase in f, whereas others showed a decrease. For Veh-treated rats, the mean effect was no net change over time, whereas for the Cap-treated rats, there was a statistically significant decrease in f unexpectedly.

### Table 2. Effects of O₃ and inhibition of tachykinin receptors on airway responsiveness to inhaled methacholine

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Treatment</th>
<th>Body Wt, g</th>
<th>Baseline G_l, cmH₂O·ml⁻¹·s⁻¹</th>
<th>log ED₅₀G₁, mg/ml</th>
<th>Baseline Cdyn, mln/cmH₂O</th>
<th>log ED₅₀Cdyn, mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>Vehicle</td>
<td>317 ± 8</td>
<td>9.69 ± 0.22</td>
<td>0.64 ± 0.20</td>
<td>0.546 ± 0.027</td>
<td>0.80 ± 0.11</td>
</tr>
<tr>
<td>Air</td>
<td>TKRA</td>
<td>327 ± 7</td>
<td>9.85 ± 0.12</td>
<td>0.76 ± 0.15</td>
<td>0.542 ± 0.020</td>
<td>0.72 ± 0.17</td>
</tr>
<tr>
<td>O₃</td>
<td>Vehicle</td>
<td>314 ± 9</td>
<td>9.51 ± 0.30</td>
<td>0.66 ± 0.05</td>
<td>0.581 ± 0.028</td>
<td>0.67 ± 0.03</td>
</tr>
<tr>
<td>O₃</td>
<td>TKRA</td>
<td>327 ± 8</td>
<td>9.72 ± 0.32</td>
<td>0.71 ± 0.08</td>
<td>0.593 ± 0.013</td>
<td>0.74 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 4 animals for air-exposed and 6 for O₃-exposed groups. G₁, pulmonary conductance; Cdyn, dynamic compliance; ED₅₀G₁, and ED₅₀Cdyn, inhaled methacholine dose required to decrease G₁ and Cdyn, respectively, by 50% of their baseline values. No statistically significant difference was detected.
Table 4. Effects of O₃ and C-fiber ablation on no. of cells and total protein concentration in bronchoalveolar lavage fluid

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Treatment</th>
<th>Body Wt, g</th>
<th>Total Cells</th>
<th>Macrophages</th>
<th>Neutrophils*</th>
<th>Lymphocytes*</th>
<th>Epithelial Cells*</th>
<th>Total Protein,* mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>Vehicle</td>
<td>498±5</td>
<td>11.1±1.1</td>
<td>10.7±1.1</td>
<td>0.07±0.03</td>
<td>0.34±0.05</td>
<td>0.05±0.01</td>
<td>0.30±0.05</td>
</tr>
<tr>
<td>Air</td>
<td>Capsaicin</td>
<td>495±5</td>
<td>13.6±2.0</td>
<td>13.0±2.0</td>
<td>0.09±0.03</td>
<td>0.51±0.06</td>
<td>0.03±0.01</td>
<td>0.29±0.02</td>
</tr>
<tr>
<td>O₃</td>
<td>Vehicle</td>
<td>492±7</td>
<td>11.9±1.7</td>
<td>10.7±1.6</td>
<td>0.52±0.17†</td>
<td>0.55±0.14</td>
<td>0.10±0.01†</td>
<td>0.45±0.07</td>
</tr>
<tr>
<td>O₃</td>
<td>Capsaicin</td>
<td>493±6</td>
<td>15.0±3.3</td>
<td>11.4±3.0</td>
<td>1.39±0.27§</td>
<td>2.08±0.25§</td>
<td>0.13±0.04§</td>
<td>0.59±0.07§</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 animals for air-exposed and 6 animals for O₃-exposed groups. Cells are expressed as no. of cells in bronchoalveolar lavage fluid x 10⁶. *P < 0.05 comparing O₃-exposed, vehicle-treated group with air-exposed groups (vehicle and capsaicin combined, n = 16); †P < 0.02 comparing O₃-exposed, capsaicin-treated group with air-exposed groups; §P < 0.02 comparing O₃-exposed, capsaicin-treated group with O₃-exposed, vehicle-treated group.

after O₃ exposure but not at any other time point (Fig. 2). Overall, there was no significant effect of Cap treatment on f. Even though f was not altered by O₃ exposure, the timing of the breath was altered. In particular, Ti increased in the first 10 min of O₃ exposure and then decreased to values well below baseline over the next 60 min (Fig. 2). In contrast, Te increased over the course of O₃ exposure (Fig. 2), although the changes were much more variable among animals. There were no statistically significant differences between Cap- and Veh-treated animals in the changes in Ti and Te induced by O₃ exposure.

Table 5. Effect of capsaicin on baseline ventilatory parameters

<table>
<thead>
<tr>
<th></th>
<th>Ve, ml/min</th>
<th>Vr, ml</th>
<th>f, breaths/min</th>
<th>Ti, s</th>
<th>Te, s</th>
<th>Weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>205.5±17.3</td>
<td>1.58±0.13</td>
<td>135.8±9.1</td>
<td>0.257±0.026</td>
<td>0.264±0.024</td>
<td>328.1±7.1</td>
</tr>
<tr>
<td>Capsaicin</td>
<td>201.1±17.6</td>
<td>1.53±0.13</td>
<td>132.8±9.3</td>
<td>0.219±0.026</td>
<td>0.274±0.024</td>
<td>321.4±6.86</td>
</tr>
</tbody>
</table>

Values are means ± SE of data from 11 vehicle- and 11 capsaicin-treated rats. Minute ventilation (Ve), tidal volume (Vr), breathing frequency (f), inspiratory time (Ti), and expiratory time (Te) were measured in each animal as the mean values over 20-min period immediately preceding O₃ (or air) exposure.
TNF-α is a cryogenic factor (21) and could be a mediator in airway responses to O₃, suggesting that formation and release of certain chemical mediators may be necessary to evoke changes in V˙E. For example, O₃ exposure causes increased tumor necrosis factor-α (TNF-α) expression in alveolar macrophages (28). TNF-α is a cryogenic factor (21) and could contribute to the decreases in core body temperature and O₂ consumption observed.

It is not apparent why V˙E decreases more in the Cap-treated rats, but one possibility is that increased inflammation and injury, caused by loss of protective effects of tachykinins released from the peripheral ends of the neurons, result in increased generation of mediators such as TNF-α. Our laboratory (15) has previously reported that the decreased core body temperature and heart rate that occur in response to O₃ are not different in Cap- and Veh-treated rats, suggesting that metabolic rate decreases to the same extent in both groups. However, De Sanctis et al. (5) have reported a decreased ventilatory response to hypoxic gas mixtures in Cap-treated rats, probably resulting from loss of tachykinins in the neurons subtending the carotid bodies. It is possible that loss of responsiveness to hypoxia allows the Cap-treated animals to decrease V˙E to a greater extent than O₂ consumption.

O₃ causes damage to the airway epithelium including vacuolation, sloughing, and necrosis (37), and there is reason to believe that tachykinin effects on the airway epithelium could mediate the protective role of these peptides during O₃ exposure. Baluk et al. (2) reported that 85% of SP-immunoreactive sensory axons in the rat respiratory tract are located in close proximity to the epithelium, and Sertl et al. (34) showed that SP receptors were concentrated in the epithelium of the central airways in the rat lung. Tachykinins stimulate epithelial cell migration and proliferation (17). Finally, in cultured bronchial epithelial cells, pretreatment with SP decreases permeability to mannitol, a marker of tight junction permeability. Furthermore, O₃-induced increases in epithelial permeability were attenuated in cells treated with SP (48). These data suggest that SP maintains epithelial cell integrity otherwise damaged by O₃. Weakened epithelial integrity caused by O₃ in the absence of tachykinins could reflect enhanced O₃-induced injury and result in the generation of increased neutrophil chemoattractant factors.

Tachykinins also have effects on airway blood flow and mucous secretion that may protect the airways from O₃-induced damage. In rat airways, injection of SP increases microvascular blood flow through NK₁ receptors (29), and mucosal application of SP stimulates mucous secretion in an isolated trachea preparation (46). Increased blood flow to airway mucosa could facilitate removal of O₃ from the airway surface, and increased mucous secretion could contribute to the protection by covering the epithelium and reducing the concentration of O₃ that actually reaches the lung surface.

Exposure to O₃ (1 ppm for 3 h) did not cause any change in airway responsiveness to inhaled aerosolized methacholine in Veh (control)-treated rats (Table 2). These results are similar to reports by our laboratory and others of rats of the same strain exposed to the same concentrations of O₃ (15, 39) and measured at the same point in time after O₃ exposure, although airway hyperresponsiveness can be induced in rats with higher concentrations of O₃ or in other strains (9, 42). However, our laboratory (15) has previously reported that, even though O₃ exposure did not induce an increase in airway responsiveness in Veh-treated rats, the same concentration and time of O₃ exposure did increase responsiveness in Cap-treated rats. In contrast, combined inhibition of NK₁ and NK₂ receptors throughout O₃ exposure and methacholine challenge did not result in airway hyperresponsiveness (Table 2). These results suggest that tachykinins per se are not involved in the development of O₃-induced hyperresponsiveness in rats, even though C fibers are. It is unlikely that the lack of effect of the TKRAs on airway hyperresponsiveness...
reflects inadequate receptor antagonism. Treatment with CP-99994 and SR-48968 caused a log shift in the dose of SP required to elicit a change in blood pressure (Table 3). The observation that TKRA treatment also altered the BAL cell profile (Table 1) further supports the efficacy of the receptor antagonists. One possible explanation for the augmentation of airway responsiveness by Cap pretreatment but not by tachykinin-receptor inhibition is that substances other than tachykinins present in C fibers are important. Possible candidates are calcitonin gene-related peptide and nitric oxide (NO). Both are colocalized with tachykinins in C fibers and act as bronchodilators (3, 7, 11). Absence of these potent bronchodilators coreleased from C-fiber endings on O3 stimulation might be responsible for augmented airway responsiveness in Cap-pretreated rats.

There were also differences between TKRA treatment and neonatal Cap treatment in the effects on O3-induced BAL protein changes. BAL protein concentration was significantly increased in O3-exposed, Cap-treated rats compared with Veh-treated rats, but TKRAs did not alter the effects of O3 on BAL protein, despite significant increases in BAL neutrophils (Table 1). These results suggest that substances other than tachykinins present in C fibers may act to counteract the O3-induced changes in vascular permeability that lead to changes in protein. NO has been reported to maintain pulmonary vascular endothelial integrity (11, 20), and, in guinea pig airways, inhibition of endogenous NO resulted in enhancement of vagally induced plasma albumin leakage (20). Lack of the endogenous NO present in C-fiber endings may thus contribute to the increased plasma protein leakage induced by O3 exposure in Cap-treated animals.

There is evidence of species differences in the effect of C fibers on O3-induced airway responsiveness. In guinea pigs, depletion of neuropeptides by Cap treatment attenuates O3-induced airway hyperresponsiveness (38). In rats, Cap pretreatment promotes airway hyperresponsiveness after O3 exposure (15). One potential explanation for this species difference is the differing bronchoconstrictor potency of tachykinins. In guinea pigs, SP and NKA are both potent bronchoconstrictors (19), whereas, in rats, depending on the strain, the tachykinins either have a weak constrictor potential (16) or actually cause dilation (6). In contrast, effects of Cap pretreatment on lung injury induced by O3 appear

Fig. 2. Effects of ozone (2 parts/million) on V̇e, breathing frequency (f), tidal volume (Vt), inspiratory time (Ti), and expiratory time (Te). Results are expressed as %changes from baseline values (mean values in 20-min period before ozone exposure) and are means ± SE of data from 8 Veh-treated (○) and 8 Cap-treated (■) rats. Compared with baseline: *P < 0.05; **P < 0.01.
to be similar in the two species. Both in this study in rats (Table 4) and in the study of Tepper et al. (38) in guinea pigs, increased protein in BAL fluid was observed in Cap-treated animals compared with Veh-treated controls. Similar results were obtained by Sterner-Kock et al. (37). The accumulation of protein in BAL has been shown to be a sensitive indicator of O₃-induced pulmonary inflammation.

The least part of the protective effects of these afferents because V˙E was decreased in Cap-treated rats relative to Veh-treated controls during O₃ exposure. Together, these results support the hypothesis that tachykinins released from peripheral ending of C fibers mediate at least part of the protective effects of these afferents against O₃-induced pulmonary inflammation.

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