Immediate sensory nerve-mediated respiratory responses to irritants in healthy and allergic airway-diseased mice

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Morris, John B., Peter T. Symanowicz, Joshua E. Olsen, Roger S. Thrall, Michelle M. Cloutier, and Andrea K. Hubbard. Immediate sensory nerve-mediated respiratory responses to irritants in healthy and allergic airway-diseased mice. J Appl Physiol 94: 1563–1571, 2003. First published November 27, 2002; 10.1152/japplphysiol.00572.2002.—The immediate responses of the upper respiratory tract (URT) to the irritants acrolein and acetic acid were examined in healthy and allergic airway-diseased C57Bl/6J mice. Acrolein (1.1 ppm) and acetic acid (330 ppm) vapors induced an immediate increase in flow resistance, as measured in the surgically isolated URT of urethane-anesthetized healthy animals. Acrolein, but not acetic acid, induced a small URT vasodilatory response. In awake spontaneously breathing mice, both vapors induced a prolonged pause at the start of expiration (a response mediated via stimulation of nasal trigeminal nerves) and an increase in total respiratory specific airway flow resistance, the magnitude of which was similar to that observed in the isolated URT. Both responses were significantly reduced in animals pretreated with large doses of capsaicin to defunctionalize sensory nerves, strongly suggesting a role for sensory nerves in development of these responses. The breathing pattern and/or obstructive responses were enhanced in mice with ovalbumin-induced allergic airway disease. These results suggest that the primary responses to acrolein and acetic acid vapors are altered by the concentration that produces a 50% decrease in specific airway resistance.

THE RESPIRATORY TRACT IS INNERVATED with a rich supply of sensory nerves that detect and initiate responses to inspired chemicals (1a, 30). The sensory nerve network consists of thick neurofilament expressing myelinated Aδ-fibers and thin nonmyelinated C fibers. It is believed the Aδ-fibers serve primarily a mechanosensory function, whereas C fibers are important in the detection of irritant chemicals (15, 30, 44). The distinction is not complete, however, because these functions overlap. The best-characterized sensory nerve stimulant is the plant toxin capsaicin, which acts via stimulation of the vanilloid receptor. At low doses, this toxin produces acute transient stimulation; at high doses, it causes a prolonged degeneration of sensory nerves, presumably through an excitotoxic mechanism (14, 18, 43, 48). Capsaicin exerts its effects preferentially on C fibers, which express high levels of the vanilloid receptor. However, a subset of Aδ-fibers may be responsive to this toxin as well (20, 23, 36, 48). C fibers can be separated into two types on the basis of lectin binding: IB4+ and IB4− fibers. The former express large amounts of neuropeptides, including substance P and calcitonin gene-related peptide; the latter do not (40).

Stimulation of respiratory sensory nerves produces centrally mediated afferent reflex responses and local tissue responses (1a, 4–7, 15, 30). The afferent reflex responses include cough and changes in breathing patterns. By definition, “sensory irritants” are compounds that stimulate nasal trigeminal C fibers and decrease breathing rate by inducing a prolonged pause at the start of expiration (for reviews of sensory irritation mechanisms, see Refs. 1a, 9, and 30). This reflex change is blocked by trigeminal nerve ligation and reproduced by trigeminal ganglion stimulation (1a). The sensory irritation response is particularly marked in mice. The potency of sensory irritants is quantified by the concentration that produces a 50% decrease in breathing rate (RD50) (1a). Capsaicin is a potent sensory irritant (1a, 30); in our hands a 50% reduction in breathing frequency in the mouse is induced by exposure to capsaicin at ~0.2 μg/l.

Stimulation of C fibers can also cause local responses, including vasodilation, neurogenic edema, mucus secretion, and/or airway obstruction, which are mediated, in part, by release of neuropeptides via antidromal stimulation (4–7, 32). Presumably the neuropeptide-rich IB4+ fibers (40) are most important in these responses. Activation of parasympathetic efferents may also play a role in the mucus secretory and airway obstructive responses (4, 5).

Previous studies in our laboratory were aimed at characterizing upper respiratory tract (URT) sensory
nerve-mediated responses to inspired irritants in the rat and included measures of afferent reflex responses (changes in breathing pattern) and local tissue responses, including neurogenic edema, airway obstruction, and mucus secretion (26, 28, 39). The URT is defined as all portions of the respiratory tract anterior to and including the larynx.) These studies have examined the effects of two reactive electrophilic vapors, acrolein and ethyl acrylate, and two vapors for which tissue acidification is believed to be important, acetic acid vapor and acetaldehyde. (Acetaldehyde is metabolized in nasal tissues to acetic acid via aldehyde dehydrogenase, and this is believed to be a critical step in its nasal toxicity (24, 38, 39).) Of the responses measured, the most sensitive for all four vapors was vasodilation. The vasodilatory response to every vapor was greatly reduced and/or abolished in animals pretreated with capsaicin, confirming a key role for sensory nerves. The precise mediators involved in this response are not known; however, preliminary studies suggest a role for calcitonin gene-related peptide and nitric oxide (27–29).

The aims of the present study were twofold: 1) to characterize the response of the mouse nose to acrolein and acetic acid vapor to allow for comparison with our laboratory’s earlier rat studies (26, 28, 29, 39) and 2) to compare responses in healthy and allergic airway-diseased mice. The initial experiments relied on methodologies identical to those used in our previous rat studies to facilitate comparisons. Toward these ends, the URT of the urethane-anesthetized mouse was isolated by tracheostomy and insertion of an endotracheal tube. The mouse was then placed in a nose-only inhalation chamber, and irritant-laden air was drawn continuously through the isolated URT at a constant flow rate (25 ml/min) for 50 min. Chamber air also contained acetone vapor. Uptake of acetone vapor was monitored throughout exposure to provide a continuous measure of URT perfusion rates (11, 28, 29, 35). The pressure drop across the URT was also measured during exposure to assess airway flow resistance throughout the exposure. Before exposure, animals were injected with Evans blue, a dye that binds to albumin. Dye content in nasal tissues and nasal airway lavage fluid (obtained immediately after exposure) provides a measure of plasma protein leakage/secretion into these compartments (28, 29, 31, 39). The same technique was used to quantitate URT uptake efficiency of acrolein and acetic acid vapors.

Initial studies on the mouse isolated URT revealed that the most sensitive response to acrolein and acetic acid vapors was increased flow resistance, rather than vasodilation, as observed in the rat. Because airway obstruction can be measured noninvasively by plethysmography, subsequent studies were performed to assess irritant-induced airway obstruction and breathing pattern changes in intact spontaneously breathing mice. The role for sensory nerves in the responses was examined by pretreatment with capsaicin to defunctionalize sensory nerves (18, 43). In addition, the effects of atropine were examined to provide information on the potential role of cholinergic mechanisms. Some studies have suggested that humans with allergic airway disease, i.e., rhinitis and/or asthma, may have heightened sensitivity to inspired irritants (33, 46). Indeed, subjects with asthma develop an airway obstructive response to sulfuric acid aerosol at concentrations to which healthy subjects are unresponsive (46).

Therefore, in mice with ovalbumin (OVA)-induced allergic airway disease, the effects of acetic acid and acrolein were also examined using the model developed previously in our laboratories (50) to determine whether a similar phenomenon could be demonstrated in this animal model.

METHODS

Animals and chemicals. Male or female C57Bl/6J mice (Jackson Laboratories, Bar Harbor, ME) were housed over hardwood bedding in animal rooms maintained at 22–25°C with a 12:12-h light-dark cycle (lights on at 6:30 AM). Animals weighed 18–25 g and were 10–18 wk of age. Food (Harlan Laboratory Chow) and tap water were provided ad libitum. Animals were acclimated for ≥2 wk before use. All chemicals were obtained from Sigma Aldrich (St. Louis, MO) unless otherwise indicated.

Irritant atmosphere generation and analysis. Chamber atmospheres were generated with a syringe-pump system. Aqueous solutions of acrolein or acetic acid and/or acetone (Fisher Scientific, Springfield, NJ) were prepared daily and fed into a heated tube maintained at 60°C. Air (5 l/min) was passed through the tube and into the exposure chamber. The generation system was operated for ≥45 min before use to allow for equilibration. Chamber concentrations were controlled by changing the irritant concentrations in the aqueous generation fluids. Isolated URT exposure took place in a nose-only chamber. Exposures to intact mice took place in the Buxco double plethysmograph (see below).

Air samples were drawn from the nose-only chamber or plethysmograph during exposure to determine exposure concentration using methods employed in our laboratory’s previous studies (28, 29, 39). For acrolein or acetone analysis, air was drawn through a gas-sampling valve attached to a gas chromatograph (model 3600, Varian, Sugar Land, TX) with flame ionization detection. A 15-m DB-WAX column (J & W Scientific, Folsom, CA) was used with a column temperature of 33°C and a carrier gas (N₂) flow rate of 30 ml/min. To measure acetic acid concentration, chamber air was drawn through two impingers in series, each containing 10 ml of distilled water (100 ml/min flow rate). The concentration of acetate in each impinger was then determined via HPLC with ultraviolet detection at 210 nm (model 2510, Varian). (The second impinger collected 20-fold less acetic acid than the first, indicating the high collection efficiency of the system.) Analysis was performed using a mobile phase consisting of 5:95 vol/vol acetonitrile-0.1% H₃PO₄ at a flow rate of 1.3 ml/min. The retention time for the acetate peak was ~2.1 min.

Isolated URT exposures. Initial studies focused on characterizing the responses of the isolated URT and included measures of perfusion rate (as assessed by acetone vapor uptake), flow resistance, plasma protein extravasation (tissue Evans blue dye content), and mucus secretion (lavage Evans blue dye content). Control mice were exposed to clean air. Nominal exposure concentrations for acrolein and acetic acid were 1.2 and 300 ppm, respectively. These exposure concentrations were estimated to approximate the RD₅₀.
based on pilot studies. Our laboratory’s previous studies on the rat indicated that local tissue responses were easily demonstrable at RD50 of each irritant (26, 28, 29, 39).

The experimental methodology has been described in detail (28, 29). Mice were anesthetized with urethane (1.3 g/kg ip). After the onset of anesthesia, the trachea was isolated and incised and an endotracheal tube was inserted in a cephalad direction until its tip lay at the larynx. Evans blue dye (30 mg/kg) was injected into the tail vein. The animal was then placed in the nose-only chamber in a supine position, and chamber air was drawn through the isolated URT at a constant flow rate of 25 ml/min for 50 min, which was the highest flow rate that could be easily maintained. The tracheotomized animals respired room air during the exposure; thus only the URT was directly exposed to the irritant vapors.

Chamber temperature was maintained at ~39°C, and water content was >33 mg/l, corresponding to >75% relative humidity at 37°C. These conditions were necessary to prevent nasal dehydration. The chamber walls were heated to prevent condensation. Immediately after exposure, the animal was decapitated, the skull was split sagittally, and nasal tissues were collected and digested in 3 ml of formamide. The Evans blue dye content of the lavage and tissue was determined fluorometrically as described previously (28, 31).

URT acetone vapor uptake was monitored during exposure as a measure of URT perfusion rates by the methods used in our laboratory’s previous studies (26, 28, 31). Briefly, vapor concentrations in chamber air (Cch) and in air that had been drawn through the isolated URT (Cneo) were measured by gas chromatography as described above. Cch was measured immediately before and immediately after the 50-min exposure. The ratio of the before and after samples averaged 99.9 ± 4.3%, indicating that exposure concentration remained constant. The gas-sampling valve injected 0.5-ml samples into the gas chromatograph at 3-min intervals to provide continuous monitoring of Cch during exposure. Acetone uptake rates were calculated from the ratio of Cch to Cneo and normalized to an inspired concentration of 44 ppm (100 μg/l).

URT flow resistance was also monitored throughout exposure. Toward this end, the endotracheal tube contained a “T,” one side of which was connected to a differential pressure transducer (model DP45, Validyne, Northridge, CA) for measurement of URT pressure drop at 5-min intervals. Flow resistance was obtained by dividing the pressure drop by the flow rate (25 ml/min).

In a separate group of mice, URT uptake efficiencies of acrolein and acetic acid vapors were measured. Animals were exposed as described above, except acetone vapor was not present. Irritant-laden air was drawn through the URT for 50 min (flow rate 25 ml/min), and irritant concentration was measured in the URT exiting air (Cneo). Acrolein concentration was measured in the URT exiting air by gas chromatography (see above). For acetic acid, URT exiting air was drawn through two midget impingers in series, each containing 5 ml of distilled water, and the impinger was analyzed for acetic acid content by HPLC, as described above. The uptake efficiency was calculated from the inspired (chamber) air concentration (Cin) and the URT exiting air concentration (Cneo) and expressed as a percentage. This methodology has been described in detail (25).

Effect of pharmacological pretreatment. The next experiment was aimed at determining the effects of capsaicin or atropine pretreatment on the responses to acrolein and acetic acid vapors. Nominal exposure concentrations were 1.2 and 300 ppm, respectively. In initial studies, capsaicin was administered at the dose used in our previous rat studies (50 mg/kg). This dose was lethal in the mouse. Therefore, it was decided to split the capsaicin dose and to perform a capsaicin dose-response study. All capsaicin-treated mice received the toxin at 25 mg/kg on day 1, and on day 2 the animals received the toxin at 25, 50, or 75 mg/kg. For treatment, animals were first anesthetized with tribromoethanol (Avertin) and then treated with theophylline (10 mg/ml sc in 10:90 vol/vol ethano-

Nasal reflex responses to acrolein and acetic acid

Effect of OVA-induced allergic airway disease. To examine the responses of mice with allergic airway disease, the OVA treatment protocol of Yamouyiani et al. (50) was used. This model has been extensively characterized in our previous studies (34, 49, 50). Briefly, animals received three weekly intraperitoneal injections of 25 μg of OVA (grade V), adsorbed to 2 mg of aluminum hydroxide. One week after the last injection, animals were exposed for 1 h/day to aerosolized OVA. Exposures took place in a directed-airflow nose-only inhalation chamber (CH Technologies). Atmospheres were generated by nebulization (Lovelace Nebulizer, In-Tox Products, Albuquerque, NM) of 1% OVA in saline. Aerosol concentration averaged ~20 mg/m3 (~1.6-μm mass median aerodynamic diameter). Control animals received no OVA exposures. Chamber temperatures were 22–25°C, and relative humidity was 20–40%.

To assess irritant sensitivity, animals were exposed to irritant fluid without concurrent monitoring of breathing parameters by a protocol identical to that used in the capsaicin studies. In each animal, irritant sensitivity was examined 24 h after the last OVA aerosol exposure. Two time points in the OVA model were selected. Animals were examined 24 h after the third daily OVA aerosol challenge expo-
pressure (OVA-d3) to examine a time when the airway inflammation is in its early stages. Another group of animals was examined 24 h after the 7th–10th daily OVA aerosol challenge exposure (OVA-d7–10) to examine a time when the inflammation was fully developed (34, 50). As in the capsaicin studies, the peak response (1-min averages) during the exposure period was used for statistical analysis.

Statistical analysis. Values are means ± SD and were compared among groups by one- or two-factor ANOVA followed by Newman-Keuls test. Animal groups consisted of three to eight animals. Repeated-measures ANOVA was performed on the URT uptake and resistance data. RD_{50} values were calculated by logarithmic linear regression of breathing frequency vs. exposure concentration data (1a). P < 0.05 was required for significance. Statistical calculations were performed with Statistica software (Stat Soft, Tulsa, OK).

RESULTS

Isolated URT studies. Measured exposure concentrations averaged 1.1 and 330 ppm for acrolein and acetic acid, respectively. The effects of the irritants on acetone vapor uptake are shown in Fig. 1. Repeated-measures ANOVA revealed that acrolein produced a significant increase in acetone uptake rate (P < 0.05) over the levels in air-exposed control mice. An effect of exposure time (the repeated measure) was not detected (P > 0.05). Uptake rates were increased from ~4 to 5 μg/min by this irritant. Although a transient increase may have occurred, acetic acid was without statistically significant effect on uptake. URT flow resistance during exposure to these irritants is shown in Fig. 2. Both vapors produced a rapid and statistically significant increase in URT flow resistance (P < 0.05, repeated-measures ANOVA). An effect of exposure time was not detected (P > 0.05), suggesting that this was an immediate and sustained response to the irritants.

Flow resistance increased from control values of ~2 to ~5 cmH2O·ml⁻¹·s. Nasal tissue and nasal lavage Evans blue dye content in control and irritant-exposed animals are shown in Fig. 3. Values were similar in control and exposed animals (P > 0.05, ANOVA), suggesting that the irritants did not induce significant plasma protein extravasation and/or mucus secretion during exposure.

The URT uptake efficiency studies revealed that acrolein and acetic acid were scrubbed from the airstream with high efficiency in the URT. Acrolein exposure concentration (C_{in}) averaged 1.2 ppm in this study; acrolein vapor was not detected in URT exiting

![Fig. 1. Upper respiratory tract (URT) acetone uptake rates during exposure to clean air (control), 1.1 ppm acrolein, or 330 ppm acetic acid. SD bars are omitted for the sake of clarity; SD averaged ~0.5 μg/min. Acrolein and acetic acid data are displaced slightly to the left and right, respectively, for clarity. Repeated-measures ANOVA revealed no statistical effect of exposure time, a significant difference between exposure groups (P < 0.05), and no statistical interaction between exposure group and time. Newman-Keuls test revealed that flow resistance in the acrolein and acetic acid groups was significantly elevated over control levels (P < 0.05). Each group consisted of 9–12 animals.](image1)

![Fig. 2. URT flow resistance (mean ± SD) during exposure to clean air (control), 1.1 ppm acrolein, or 330 ppm acetic acid. Acrolein and acetic acid data are displaced slightly to the left and right, respectively, for clarity. Repeated-measures ANOVA revealed no statistical effect of exposure time, a significant difference between exposure groups (P < 0.05), and no statistical interaction between exposure group and time. Newman-Keuls test revealed that flow resistance in the acrolein and acetic acid groups was significantly elevated over control levels (P < 0.05). Each group consisted of 9–12 animals.](image2)

![Fig. 3. Evans blue dye content in nasal tissue (μg) and nasal lavage (μg/ml) in control (air-exposed) mice and mice exposed to 1.1 ppm acrolein and 330 ppm acetic acid. Values are means ± SD. ANOVA detected no significant differences among exposure groups. Each group consisted of 9–12 animals.](image3)
Air. On the basis of the limit of detection of the gas chromatographic technique of 0.1 ppm, URT uptake efficiencies were estimated to be >92%. Acetic acid exposure concentration averaged 285 ppm in this study; the concentration in URT exiting air averaged 9 ± 10 ppm; URT uptake efficiency averaged 97 ± 4%.

**Pharmacological pretreatment studies.** Exposure concentrations for acrolein and acetic acid were similar to those in the isolated URT study, averaging 1.3 and 290 ppm, respectively. There were no indications of sneezing or water rhinorrhea during exposure, nor did the animals demonstrate avoidance behavior. Baseline breathing frequency, expiratory pause duration, and sRaw averaged 301 ± 23 breaths/min, 9.6 ± 2.0 ms, 2.5 ± 0.23 cmH2O·s, respectively. Both vapors caused a significant decrease in respiratory rate, a significant expiratory pause, and a significant increase in sRaw (Table 1). The increases in respiratory resistance in absolute terms (cmH2O·mol⁻¹·s) are not known, inasmuch as the plethysmographic technique provides a measure of sRaw, not true resistance. With the use of an estimated functional residual capacity for the mouse of 0.5 ml, the increase in resistance induced by acrolein and acetic acid is estimated to be 1.2 and 4.2 cmH2O·mol⁻¹·s, respectively. This should be viewed as a rough estimate, because it is not known whether either irritant altered functional residual capacity. However, these values are similar to the flow resistance changes observed in the isolated URT (Fig. 2).

For each response measure (respiratory rate, expiratory pause duration, and increase in sRaw; Table 1), data were analyzed by two-factor ANOVA, with the factors being irritant (acrolein vs. acetic acid) and capsaicin dose (25 + 25, 25 + 50, and 25 + 75 mg/kg). Regardless of the measure, the two-factor ANOVA revealed a significant difference between vapors, with acetic acid producing larger responses, and a significant effect of capsaicin pretreatment, with significantly lower responses in the 20 + 50 and 20 + 75 groups than in the vehicle control or 25 + 25 group (Newman-Keuls test). A significant interaction between irritant and capsaicin dose was not detected, indicating that capsaicin exerted statistically similar effects on the responses to both irritants.

Atropine was without statistically significant effect (P > 0.05, ANOVA) on the responses to 1.4 ppm acrolein or 260 ppm acetic acid. The respiratory rate, expiratory pause, and increase in sRaw during acetic acid exposure averaged 60 ± 12% of baseline, 224 ± 91 ms, and 0.82 ± 0.18 cmH2O·s, respectively, in vehicle-treated mice compared with 55 ± 7% of baseline, 244 ± 47 ms, and 0.92 ± 0.78 cmH2O·s, respectively, in atropine-pretreated mice. For acrolein, these values averaged 58 ± 18% of baseline, 215 ± 124 ms, and 1.48 ± 0.82 cmH2O·s, respectively, in vehicle-treated mice compared with 49 ± 4% of baseline, 268 ± 52 ms, and 1.00 ± 0.86 cmH2O·s, respectively, in atropine-pretreated mice.

**Concentration response in healthy and allergic airway-diseased mice.** The effect of acrolein exposure on breathing frequency in naive and OVA-challenged animals is shown in Fig. 4. Acrolein exposure concentrations were 0.3, 1.6, and 3.9 ppm. A pause during expiration was observed in the irritant-exposed animals. Two-factor ANOVA revealed a significant difference between exposure concentrations (P < 0.001), a significant difference between OVA groups (P < 0.01), and no interaction between factors. Newman-Keuls test revealed that the response in the naive and OVA-d3 animals were statistically similar and that the response was greater in the OVA-d7–10 than in the naive or OVA-d3 animals. RD50 values were 1.59, 1.44, and 0.82 ppm in naive, OVA-d3, and OVA-d7–10 animals, respectively (log-linear regression). Acrolein also induced an increase in sRaw, with the increase over baseline averaging 0.29 ± 0.08, 2.03 ± 0.21, and 1.95 ±

Table 1. Respiratory tract responses to acrolein and acetic acid vapors in normal and capsaicin-pretreated mice

<table>
<thead>
<tr>
<th>Respiratory rate, %baseline</th>
<th>Vehicle</th>
<th>25 + 25 mg/kg</th>
<th>25 + 50 mg/kg</th>
<th>25 + 75 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrolein</td>
<td>55 ± 4</td>
<td>63 ± 13</td>
<td>77 ± 15†</td>
<td>85 ± 11†</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>47 ± 6</td>
<td>53 ± 9</td>
<td>71 ± 14†</td>
<td>76 ± 6†</td>
</tr>
<tr>
<td>Expiratory pause, ms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acrolein</td>
<td>202 ± 66</td>
<td>155 ± 71*</td>
<td>64 ± 94†</td>
<td>11 ± 9†</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>321 ± 39</td>
<td>250 ± 38*</td>
<td>159 ± 33†</td>
<td>119 ± 27†</td>
</tr>
<tr>
<td>sRaw, cmH2O·s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acrolein</td>
<td>0.56 ± 0.34</td>
<td>0.58 ± 0.48</td>
<td>0.13 ± 0.23*</td>
<td>0.09 ± 0.15*</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>2.06 ± 0.70</td>
<td>1.47 ± 0.55</td>
<td>1.08 ± 0.29*</td>
<td>0.89 ± 0.15*</td>
</tr>
</tbody>
</table>

Values are means ± SD of 4–6 mice. Expiratory pause and specific airway resistance (sRaw) are expressed as increase over baseline. Animals were exposed in a double plethysmograph to 1.3 ppm acrolein or 290 ppm acetic acid, and responses were monitored during the 10-min exposure. Animals were pretreated on 2 consecutive days with vehicle or capsaicin at 25 mg/kg followed by 25 mg/kg, 25 mg/kg followed by 50 mg/kg, or 25 mg/kg followed by 75 mg/kg and exposed to acrolein and acetic acid vapor 1 and 2 wk later, respectively. Baseline values for respiratory rate, expiratory pause, and sRaw averaged 301 ± 23 breaths/min, 9.6 ± 2.0 ms, 2.5 ± 0.23 cmH2O·s, respectively. In vehicle-treated animals, respiratory rates were significantly >100% of baseline, and increases in expiratory pause duration and sRaw were significantly > 0 (P < 0.05, t-test). Data were analyzed by 2-factor ANOVA, with factors being irritant (acrolein or acetic acid) or capsaicin dose. A significant difference between irritants was detected for each measure (P < 0.002 in all cases). A significant difference between capsaicin dose groups was also detected for each measure (P < 0.02 in all cases). *Statistically different from vehicle; † statistically different from 25 + 25 mg/kg capsaicin (P < 0.05, Newman-Keuls test).
0.40 cmH2O·s in the 0.3, 1.6, and 3.9 ppm exposure groups (P < 0.05, ANOVA). (Baseline sRaw values were similar in naive and OVA groups.) The sRaw response observed in this study was greater than that observed in the capsaicin study (Table 1). The reasons are unclear. The sRaw response was not increased over that of naive animals in the OVA-d3 or OVA-d7–10 groups (P > 0.05, 2-factor ANOVA). In the OVA-d7–10 group, the increase in sRaw averaged 0.5 and 1.9 cmH2O·s at 0.3 and 1.6 ppm, respectively.

The effect of acetic acid vapor on breathing frequency in naive and OVA-treated animals is shown in Fig. 5. Acetic acid exposure concentrations averaged 82, 340, and 660 ppm. A pause during expiration was observed in the irritant-exposed animals. Two-factor ANOVA revealed a significant difference between exposure concentrations (P < 0.001), a significant difference between OVA groups (P < 0.01), and no significant interaction between factors. Newman-Keuls test revealed that the responses in the naive and OVA-d3 animals were statistically similar and that the response was greater in the OVA-d7–10 than in the naive or OVA-d3 animals. RD50 values were 239, 247, and 136 ppm in naive, OVA-d3, and OVA-d7–10 animals, respectively (log-linear regression). Acetic acid vapor also induced airway obstruction, as indicated by a dose-dependent increase in sRaw (Fig. 6). (Baseline sRaw values were similar in naive and OVA groups.) Two-factor ANOVA revealed a significant difference between exposure concentrations (P < 0.001), a significant effect of OVA treatment (P < 0.01), and no significant interaction between factors. Newman-Keuls test revealed that the response was significantly greater in the OVA-d7–10 group than in the naive or OVA-d3 group (which were statistically similar). Interestingly, in the 80-ppm groups, acetic acid did not significantly increase sRaw over baseline (mean change 0.3 ± 0.2 cmH2O·s) in naive animals, whereas in the OVA-d7–10 animals...
this exposure level produced a significant increase in sRaw (mean change 0.73 ± 0.27 cmH₂O·s⁻¹). The design of the experiment was based on use of identical exposure concentrations in the naive and OVA-treated mice. Future experiments would be needed to establish the threshold for acetic acid-induced effects in the OVA-treated animals.

DISCUSSION

These results elucidate similarities and dissimilarities in the URT responses of the rat and mouse to two irritants: acrolein and acetic acid. Both vapors produced a sensory irritation response in both species, a response known to be directly mediated via nasal trigeminal nerve stimulation (1a, 30). As has been observed for many vapors (9), the RD₅₀ was much lower in the mouse than in the rat for acrolein (1.59 ppm (Fig. 3) vs. 6 ppm (3)) and acetic acid (329 ppm (Fig. 4) vs. 1,040 ppm (39)). The reasons for the difference are not known. In the rat, the most sensitive URT response to either irritant was vasodilation (28, 29, 39). In this species, the response was quite marked, as indicated by a twofold or greater increase in acetone uptake rates. A small vasodilatory response (20% increase in acetone uptake) was observed in acrolein-exposed mice (Fig. 1) but was not evident in acetic acid-exposed mice. (It was not possible to examine the effect of higher acetic acid exposure concentrations because of URT obstruction.) These results suggest that the mouse is capable of mounting a vasodilatory response to irritants, but the response is much smaller than in the rat nose. In contrast, a marked URT obstructive response was induced in the mouse by both irritants. A sensory nerve-mediated obstructive response to acrolein (but not acetic acid) occurs in the rat nose (28, 29, 39), but it is delayed in onset and of smaller magnitude than that observed in the mouse. Thus the principal URT response to these irritants appears to be vasodilation in the rat and obstruction in the mouse. The range of sensory nerve responses induced in the human by specific irritant classes (e.g., electrophiles vs. acids) has not been well defined.

The breathing frequency and obstructive responses were significantly attenuated by capsaicin pretreatment, providing strong evidence that sensory nerves were involved in their elicitation. Moreover, because capsaicin acts through the vanilloid receptor (12, 18, 43, 48), these results suggest that the responses were mediated by vanilloid receptor-expressing C fibers. Whether either vapor selectively stimulates IB₄⁺ or IB₄⁻ nerves is not known. Future studies are needed to determine why sensory nerve stimulation induces a vasodilatory response in the rat vs. an obstructive response in the mouse.

The present study included measures in the isolated URT of anesthetized mice and in intact spontaneously breathing conscious mice. Similar obstructive responses were observed in both paradigms. Although precise comparisons cannot be made, the magnitude of the obstructive response in the URT was sufficient to account for the entire response observed in the intact animal studies. This suggests that the URT is a significant contributor to the obstructive response observed in intact animals. Because the uptake efficiency of acrolein and acetic acid vapors in the URT exceeded 90%, this is, perhaps, not unsurprising. The URT also appears to be a significant contributor to the respiratory tract obstructive response to methacholine aerosol (41). Upper airway obstruction may be due to vascular congestion and/or excessive mucus secretion. For acrolein and acetic acid, the former seems more likely, because the response was immediate and not blocked by atropine and nasal lavage Evans blue dye content was not increased by the irritants (Fig. 3).

Irritant-induced breathing frequency and/or obstructive responses were enhanced in animals with OVA-induced allergic airway disease on days 7–10 but not on day 3. The mechanism for the enhanced sensory irritation response in allergic animals is not known. It is possible that there is enhanced deposition or uptake of the inspired irritants in the nasal tissues and increased delivery of irritant to the trigeminal nerve endings in the diseased animals. However, this seems unlikely, because URT uptake efficiencies for these vapors were >90% in the healthy animals. The time course for the irritant sensitivity appears to correlate with the airway inflammation, inasmuch as the eosinophilic infiltration is not fully developed in this model until days 7–10 (21, 34, 50). In this model, airway hyperresponsiveness to methacholine is demonstrable at day 3, as measured by pulmonary resistance, gas trapping, or enhanced pause (34, 49). Moreover, our recent studies have shown an enhanced URT obstructive response to methacholine on day 3 (41). In contrast, irritant sensitivity is not apparent until days 7–10 in this model, suggesting that irritant sensitivity and methacholine hyperresponsiveness are mediated via different mechanisms.

The precise nature of the alterations in the sensitivity to the irritants that was observed in the OVA-treated animals was irritant specific. Specifically, although the breathing pattern response was enhanced for both irritants, the obstructive response was enhanced for only acetic acid, not acrolein. If a single mechanism were responsible, it would be anticipated that breathing frequency and airway obstructive responses would demonstrate similar modulations for both vapors in the OVA-treated animals. That this was not the case suggests involvement of multiple pathways or mechanisms. Because alteration in breathing pattern is a neurally mediated afferent reflex response (1a, 30), it is likely that the enhanced responsiveness reflects an alteration in neural sensitivity and/or neural integration in the central nervous system (45), rather than an increased local release of neuropeptides. It is likely that acidic irritants stimulate sensory nerves through the vanilloid receptor (12, 13), and the capsaicin pretreatment studies indicate a role for vanilloid receptor-expressing nerves in the response. [The responses to acetic acid were not likely to be due to the acetate, inasmuch as the response to sodium acrylate is not significant to the acetate, inasmuch as the response to sodium acrylate is not significant.]

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acetate aerosol (1,000 μg/l) was quite mild and no greater than the response to equimolar hypertonic saline aerosol (data not shown). Acrolein may activate the vanilloid receptor as well (43). Perhaps there is an alteration in vanilloid receptor expression and/or function in this model of allergic airway disease. Reflex (sneeze) and local ( plasma protein extravasation) responses to the vanilloid receptor agonist capsaicin are enhanced in human subjects with rhinitis (33). Nerve growth factor (NGF) expression is enhanced in allergic airway inflammation (10, 33), and NGF has been shown to influence vanilloid receptor expression (19, 23, 47).

The airway obstructive response to acetic acid, but not acrolein, vapor was enhanced in the OVA-treated animals. Because the sensory irritation response to both vapors is enhanced, it is not likely that heightened obstructive response to acetic acid is not simply due to enhanced neural sensitivity and/or neural integration. It has recently been shown that the airway obstructive response to the irritant capsaicin is enhanced in a guinea pig allergic airway disease model (8) and that the nasal mucus secretory response to capsaicin is enhanced in human subjects with rhinitis (33). Thus the effects observed in the present study do not appear to be specific to the mouse. Sensory nerve-dependent obstruction in rodents may be mediated by axonal neuropeptide release (4, 5, 22, 37). Recent data suggest an increased expression of neuropeptides in airway sensory nerves in allergic inflammation, perhaps due to enhanced NGF release (17, 19, 33). This may reflect elevated expression in Aδ-fibers (19). However, enhanced neuropeptide-mediated responses may also result from a variety of extraneuronal mechanisms, including alterations in neuropeptide receptor expression and/or inhibition of neuropeptide catabolism via peptidases (1, 5, 32, 45). Perhaps the enhanced breathing frequency response is due to increased vanilloid receptor expression and the enhanced obstructive response is due to nerve type-specific (Aδ vs. C fiber or IB4+ vs. IB4-) increases in neuropeptide expression. Future studies are needed to clarify these possibilities.

Irrespective of the precise mechanisms, the responses to acetic acid in the mouse model appear to be similar to those observed in the human. Specifically, patients with asthma have a reduced threshold for sulfuric acid-induced airway obstruction (46), and mice with OVA-induced allergic disease respond to 80 ppm acetic acid, a concentration that is without effect in the control mice. It is not known whether the responses to high concentrations of irritants in animal models are reflective of those of humans exposed to lower concentrations; however, this murine model may provide a useful tool for investigating potential mechanisms of induction of irritant sensitivity by allergic airway disease.

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REFERENCES


20. Hunter DD and Undem BJ. Identification and substance P content of vagal afferent neurons innervating the epithelium of...


