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Acute Respiratory Responses of the Mouse to Chlorine

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In human subjects 15-min exposure to 0.5–1.0 ppm chlorine gas causes a nasal obstructive response in the absence of a marked sensation of irritation. The current investigation was designed to assess the response of the mouse for comparative purposes. Respiratory physiological responses were measured in female C57Bl/6J mice exposed to 0.8 to 4.0 ppm chlorine gas. Chlorine was a potent sensory irritant with an RD50 of 2.3 ppm. The gas produced airway obstruction as indicated by a concentration-dependent increase in specific airways resistance (sRaw) during the 15-min exposure. At 0.8 ppm, chlorine produced only mild sensory irritation (<20% change in breathing frequency) and a 65% increase in sRaw. Pretreatment with atropine was without effect on the obstructive response, suggesting a lack of involvement of muscarinic cholinergic pathways. Pretreatment with the sensory nerve toxin, capsaicin, dramatically reduced both the sensory irritation and obstructive responses to chlorine, suggesting the involvement of sensory nerves. Studies were also performed using the surgically isolated upper respiratory tract of the anesthetized mouse. Chlorine was efficiently scrubbed from the airstream (>97%) in that site and produced an obstructive response that was of sufficient magnitude to account for the entire response observed in the intact animal. In summary, chlorine gas produces an immediate nasal obstructive response in the mouse that appears to be similar to that in the human.

Key Words: **chlorine; upper respiratory tract; nose.**

Airborne irritants can produce multifaceted nasal responses that include the sensations of irritation, airflow obstruction, vascular congestion, mucus hypersecretion, and rhinorrhea (Baraniuk, 1994; Shusterman, 2003). These responses are apparent within minutes of the onset of exposure and are among the most prevalent complaints in indoor air (Hodgson, 2002). Although these responses are often protective, in allergic airway disease these responses, in particular the obstructive response, can be exaggerated, leading to exacerbation of symptoms and/or physiological impairment (Lundberg, 1995; Shusterman *et al*., 2003; Undem *et al*., 2000). Given the rapidity with which the obstructive response occurs, it is not likely to be mediated by cytotoxicity, but rather by direct interaction of irritants with effector cells (e.g., goblet cells, endothelium) or by release of physiologically active mediators that then act on effector cells. Nasal epithelial cells, sensory nerves, and mast cells may be the source for such mediators. The precise roles of these cellular pathways in mediating the integrated response to inspired irritants are poorly defined. The current study focuses on the role of sensory nerves in mediating the responses to an oxidant gas, chlorine.

Chlorine is a water-soluble gas that is a common occupational irritant. The gas rapidly reacts with water to form hydrochloric and hypochlorous acids, the later being a strong oxidant (Winder, 2001). It is scrubbed from the airstream with high efficiency in the human nose (Nodelman and Ultman, 1999). The acute nasal responses to chlorine in human subjects include the sensation of irritation and increased nasal airflow resistance (Shusterman *et al*., 1998, 2003a,b). At exposure concentrations of 0.5–1.0 ppm there is a mild sensation of irritation and a mild obstructive response (~15% increase in nasal flow resistance over baseline) in healthy subjects (Shusterman *et al*., 2003b). The obstructive response, but not the sensation of irritation, is more marked in subjects with allergic rhinitis. The mechanisms through which chlorine produces the obstructive response are not known; however, neither mast cell activation nor parasympathetic cholinergic muscarinic pathways appear to be involved (Shusterman *et al*., 2002, 2003a). While sensory nerves undoubtedly mediate the sensation of irritation, their role in the obstructive response is unclear.

The airways of the human and rodent are extensively innervated with sensory nerves including neuropeptide-rich C fibers and neuropeptide-poor Aδ fibers (Coleridge and Coleridge, 1984; Nielsen, 1996; Undem and Carr, 2001). In rodents, stimulation of nasal trigeminal sensory nerves initiates the "sensory irritation" response, characterized by slowed respiration due to a prolonged pause at the beginning (stage I) of expiration (Alarie, 1973; Bos *et al*., 1992; Nielsen, 1991; Schaper, 1993; Vijayaraghavan *et al*., 1993). Chlorine is a known sensory irritant (Barrow *et al*., 1977; Gagnaire *et al*., 1994). Our previous studies have shown that, in addition to acting as sensory irritants, the electrophilic vapor, acrolein, and the acidic vapor, acetic acid, also induce an immediate nasal obstructive response in the C57Bl/6J mouse (Morris *et al*., 2003). Chemical-induced

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degeneration of sensory nerves by large-dose capsaicin pretreatment diminished the responses to both vapors (Morris *et al*., 2003). Capsaicin acts through the TRPV1 receptor to cause axonal degeneration of TRPV1-expressing C fibers, presumably through an excitotoxic mechanism (Holzer, 1991; Szallasi and Blumberg, 1999). Thus, these results suggest involvement of TRPV1-expressing sensory nerve C fibers in mediating the respiratory responses to these irritants. While TRPV1-expressing nerves are involved, the TRPV1 receptor itself does not appear to play a role in mediating the responses to acrolein and acetic acid in the C57Bl/6J mouse (Symanowicz *et al*., 2004). It is not known if a similar pattern exists for the oxidant irritant chlorine.

The aim of the current study was to characterize the response to inhaled chlorine gas in the mouse, using exposure protocols analogous to those used for the human. The rationale was twofold. Our previous studies in the mouse included both acidic and electrophilic irritants. Since chlorine has oxidant properties, studies of this irritant provide an opportunity to compare and contrast reflex responses to acidic and electrophilic irritants to an oxidant irritant. Second, the nasal responses to chlorine are well characterized in the human. Characterization of the responses in mice, using analogous exposure protocols, allows the opportunity for an evaluation of potential species differences in nasal reflex responses. Reflex responses were assessed noninvasively in spontaneously breathing C57Bl/6J mice by double plethysmography to match the methodology and mouse strain used in our previous studies on acrolein and acetic acid (Morris *et al*., 2003). In this method, ventilatory parameters are measured directly by pnemotachographs in both the nasal and thoracic chambers of a double plethysmograph, and the phase lag between the two chambers is used to calculate specific airways resistance (sRaw). The theoretical basis for this methodology has long been understood (Pennock *et al*., 1979).

Concentration-response studies were performed. The role of parasympathetic nervous system stimulation and cholinergic muscarinic pathways in mediating the obstructive response (Baraniuk, 1994) was assessed by pretreatment with atropine. The role of TRPV1-expressing sensory nerves was assessed by pretreatment of mice with capsaicin. The responses of the isolated upper respiratory tract (URT) of anesthetized mice were also assessed, as well as the capacity of that site to scrub chlorine from the inspired airstream. Finally, mice were exposed to sodium hypochlorite aerosol to determine if it produced responses similar to those to chlorine gas.

MATERIALS AND METHODS

Animals and drug treatments. Chlorine gas concentration-response, capsaicin-pretreatment, sodium hypochlorite aerosol, and isolated URT studies were performed. Female C57Bl/6J mice were used in all studies and were obtained from Jackson Laboratories (Bar Harbor, ME). Animals were housed over hard wood bedding in animal rooms maintained at 22–25°C with a 12 h light-dark cycle (lights on at 6:30 A.M.). Animals weighed between 18 and 25 g and were 7–15 weeks of age. Food (Purina Rodent Chow) and tap water were provided *ad libitum.* All protocols were approved by the University of Connecticut IACUC committee. Animals were acclimated at least 2 weeks prior to use. When administered, atropine (1.25 mg/ml in saline) was given at a dosage of 5 mg/kg ip 30 min prior to irritant exposure. (In our hands, this dosage inhibited the obstructive response to inspired methacholine aerosol, Morris *et al*., 2003). Control mice received saline injection. For capsaicin pretreatment (Morris *et al*., 2003) mice received the toxin at an initial dosage of 25 mg/kg, followed by 75 mg/kg one day later (5 and 15 ml/kg of 5 mg/ml capsaicin sc, dissolved in 1:1:8 ethanol:Tween80:saline, respectively). Immediately prior to capsaicin injections, animals were first anesthetized with avertin (250 mg/kg, ip) and then treated with 10 mg/kg theophylline (sc, 5 mg/ml in distilled water) and 0.1 mg/kg terbutaline (ip, 0.05 mg/ml in saline) to minimize the acute respiratory effects of the capsaicin. Control mice received drugs and capsaicin vehicle injections. All drugs were obtained from Sigma/Aldrich (St. Louis, Mo).

Intact mice studies. Spontaneously breathing mice were exposed and respiratory responses monitored in a Buxco double plethysmograph (Buxco, Inc. Sharon, CT) using the Buxco noninvasive airway mechanics software. Animals were restrained in the double plethysmograph but were not anesthetized. Irritant laden air was drawn from a mixing tube (see below) and into the head space side of the double plethysmograph at a flow rate of 0.6 l/min. Three responses were monitored: breathing rate, early (stage I) expiratory pause duration, and specific airway resistance (sRaw). After a >10-min acclimatization period, a period clean air exposure baseline of 10 min commenced, followed by 15-min exposure to irritant. Breathing parameters were collected during the baseline and exposure periods. One-minute average values were recorded, and the peak responses (1 min averages) were used for statistical analysis. Breathing frequency was expressed as percent of baseline as per standard protocols (Alarie, 1981; ASTM, 1984). Absolute values for expiratory pause duration (ms) were used. The sRaw (cm H_2O s) response for each animal was normalized to its average baseline value and then expressed as percent increase over baseline.

Isolated URT studies. The experimental methodology for isolated URT exposures has been described in detail (Morris, 1999). Mice were anesthetized with urethane (1.3 g/kg, ip). After the onset of anesthesia the trachea was isolated, incised, and an endotracheal tube was inserted in an anterior direction until its tip lay at the larynx. The animal's nose was snuggly placed in a mixing tube into which chlorine was generated (see below). The animal was in a supine position, and air from the mixing tube was drawn through the isolated URT at a constant flow rate of 25 ml/min for 5 min. (This was the highest flow rate that could be easily maintained.) To mimic the intact animal studies, exposures were to clean filtered air for a 10-min baseline period followed by a 15-min exposure to chlorine. The tracheotomized animals respired room air during the exposure, thus only the URT was directly exposed to chlorine.

URT flow resistance was monitored throughout exposure. Toward this end, the endotracheal tube contained a T, one side of which was connected to a Validyne DP45 differential pressure transducer (Validyne, Northridge, CA) for measurement of URT pressure drop at 2-min intervals. Flow resistance was obtained by dividing the pressure drop by the flow rate (25 ml/min). The other side of the T was connected to a chlorine sample train (see below) to allow for analysis of chlorine content in air exiting the URT. Comparison of chlorine concentration in this sample to that in the mixing tube allowed for calculation of URT uptake efficiency. This methodology has been described in detail (Morris, 1999).

Inhalation atmosphere generation and analysis. Both chlorine gas and sodium hypochlorite aerosol exposures were performed. In both cases, the atmospheres were generated into a PVC mixing tube. Chlorine atmospheres were generated by metering the output of a compressed gas cylinder containing 500 ppm chlorine in nitrogen (Matheson Tri-Gas, Montgomery, PA). Sodium hypochlorite aerosols were generated by nebulizing 1% sodium hypochlorite in Krebs-Ringer buffer (pH adjusted to 9.0 to minimize chlorine outgassing) with a Lovelace Nebulizer (In-Tox Products, Albuquerque, NM). Control animals for the hypochlorite studies were exposed to nebulized Krebs-Ringer buffer (pH 9.0). Total airflow rates in the mixing tube ranged between 2 and 5 l/min, depending on the exposure concentration. Exposures to spontaneously breathing mice were performed in the Buxco double plethysmograph, with

mixing tube air being drawn into the headspace of the plethysmograph as indicated above. For isolated URT exposure, the anesthetized animal's nose was placed directly into the mixing tube through a tightly fitting hole.

For analysis, air samples were drawn during the exposure from the plethysmograph headspace (intact animal exposures) at a flow rate of 100 ml/min. For isolated URT exposures, air samples were drawn directly from the mixing tube immediately before and after the animal exposure at a flow rate of 25 ml/min. For isolated URT exposure, air was drawn through the URT at 25 ml/min, with airborne chlorine analysis being performed on that air sample (Morris, 1999). All air samples were passed through two midget impingers in series, each containing 10 ml of 1 mM sodium hydroxide, and the sample line was rinsed following collection with 1 ml of 1 mM sodium hydroxide. The fluid was then analyzed spectrophotometrically for total chlorine content by the EPA standard 4500-Cl G colorimetric N,N-diethy-*p*-phenylendiamine methodology (PPD-2, HF Scientific, Ft Meyers, FL). More than 95% of the chlorine that was collected was present in the first impinger, indicating the high collection efficiency of the sample train. For hypochlorite aerosol studies, air samples were drawn through a 0.2-μm pore polyethersulfone filter. The collected material was eluted with 1 mM sodium hydroxide and analyzed spectrophotometrically as described above.

Statistical analysis. Data are reported as mean ± SD and were compared among groups by ANOVA followed by Newman-Keuls test. Repeated measures ANOVA tests were performed on the URT resistance and intact animal sRaw data to assess the time course of the response. RD50 values were calculated by log linear regression of breathing frequency versus exposure concentration data as described by Alarie (1973). The RD50 value represents the concentration which produces a 50% reduction in breathing frequency (Alarie, 1981; ASTM, 1984). A p value ≤ 0.05 was required for significance. Statistical calculations were performed with Statistica software (Stat Soft, Tulsa, OK).

RESULTS

Dose-Response, Characterization Studies

To examine concentration response relationships mice were exposed to 1, 2, 3, and 4 ppm chlorine. The measured exposure concentrations averaged 0.8, 2.0, 3.1, and 3.8 ppm, respectively. During exposure to chlorine gas, mice exhibited decreased breathing frequency, a pause at the start of each expiration (i.e., stage I, Vijayaraghavan *et al*., 1993), and increased sRaw. All responses developed gradually throughout the 15-min exposure (the time course is described below). As per standard methodologies for sensory irritation (Alarie, 1981; ASTM, 1984), the maximal responses (1 min average) were calculated and are shown in Table 1. As can be seen, all responses were concentration dependent ($p < 0.001$ ANOVA, for each response). The RD50 calculated from these data is 2.3 ppm. In the 3.8 ppm group the responses were large; for example, breathing frequency was decreased to 30% of baseline, expiratory pause increased from a baseline of 9 ms to a maximum of ~500 ms, and sRaw was increased almost 200% over baseline. At the low-exposure concentration (0.8 ppm), there was a significant decrease in respiratory rate (to ~80% of baseline), but only a small (compared to the higher chlorine concentration groups) increase in expiratory pause duration (from baseline of 9 ms to a maximum of 20 ms). Minute ventilation rates were decreased as breathing frequency decreased. Minute ventilations rates during exposure averaged 51, 40, 38, and 33 ml/min in the 0.8, 2, 3.1 and 3.8 ppm groups, respectively, compared to an average baseline value of 59 ml/min. The average inspired dosage rate for chlorine (calculated from the product of the inspired concentration and average minute ventilation rate) averaged 1.8, 4.1, 5.0, and 5.8 nmol/min in these groups, respectively.

The time course of the sRaw response is depicted in Figure 1. For each exposure group, repeated measures ANOVA was performed individually to compare baseline and exposure

TABLE 1
Concentration Response Relationships for Responses to Chlorine in the Mouse

	Respiratory frequency (% baseline)[a]	Expiratory pause (ms)[a]	sRaw (% increase over baseline)[a]
0.8 ppm	80 ± 7[b]	20 ± 9[b]	64 ± 42[b]
2.0 ppm	62 ± 12[c]	125 ± 108[c]	92 ± 41[b]
3.1 ppm	41 ± 5[d]	332 ± 131[d]	154 ± 46[c]
3.8 ppm	30 ± 8[d]	522 ± 233[d]	185 ± 39[c]

Note. Data presented as mean ± SD, with 5–9 animals per group. Measured exposure concentrations in the 1, 2, 3, and 4 ppm groups averaged 0.8, 2.0, 3.1, and 3.8 ppm, respectively. Baseline minute ventilation averaged 59 ± 7 ml/min with corresponding average chlorine dosage rates for the 0.8, 2.0, 3.1, and 3.8 ppm groups averaging 1.8, 4.1,5.0 and 5.8 nmol/min, respectively.

[a]Baseline values averaged: breathing frequency = 293 ± 26 breaths/min, expiratory pause duration = 9 ± 3 ms, sRaw = 1.72 ± 0.21 cm H_20•s. During exposure all measures in all exposure groups were significantly different from baseline ($p < 0.05$).

[b,c,d]Data were compared by ANOVA, followed by Neuman-Keuls test. Groups with differing superscripts differ at the $p < 0.05$ level.

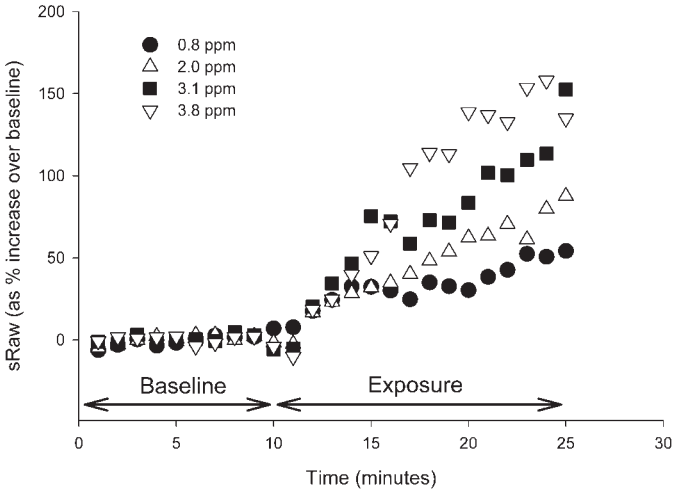

FIG. 1. Shown is sRaw (as percent increase over baseline) during the 10-min baseline and subsequent 15-min exposure periods in animals exposed to 0.8, 2.0, 3.1, or 3.8 ppm chlorine. Standard deviations are omitted for the sake of clarity and averaged approximately ±40%. Groups contained five to nine mice. Repeated measures ANOVA of sRaw values during exposure revealed a significant effect of exposure concentration ($p < 0.001$), time (the repeated measure, $p < 0.001$), and an interaction between exposure concentration and time ($p < 0.005$).

sRaw values. In all exposure concentration groups, sRaw was increased significantly over baseline during the exposure. Responses among exposure concentration groups were compared to each other by repeated measures ANOVA of the data obtained during exposure. This analysis revealed a significant effect of exposure concentration ($p < 0.001$), an effect of exposure time (the repeated measure, $p < 0.001$), and a statistical interaction between these factors ($p < 0.005$). A group of mice was pretreated with atropine prior to 3.1 ppm chlorine exposure to assess the potential role of parasympathetic cholinergic activation in mediating the obstructive response. The time course of the sRaw response in these animals was similar to that in the 3.1 ppm group. The peak sRaw value in this group averaged 140 ± 30% of control, a value similar to that in the 3.1 ppm group (Table 1).

The flow resistance response of the isolated URT to chlorine is shown in Figure 2. In this experiment the exposure concentration averaged 4.8 ppm, corresponding to an inspired dosage rate of 4.7 nmol/min. (The average inspired dosage rate of chlorine in the 3.1 ppm group of the concentration-response study was 5.0 nmol/min [see above].) URT flow resistance increased steadily throughout the 15-min isolated URT exposure to a level greater than two-fold over the baseline value of 3.1 cm H_2O/ml/s. The URT flow resistances at the end of exposure were significantly greater than during the baseline (repeated measures ANOVA followed by Newman-Keuls test). During the last 5 min of exposure, URT flow resistance averaged 6.0–6.9 cm H_2O/ml/s. In the 3.1 ppm group of the concentration-response study the total airways resistance (obtained by correcting the sRaw by the FRC of 0.5 ml, Lai, 1992) was increased from a baseline level of 3.4 cm H_2O/ml/s to a level of 6.0–7.2 cm H_20/ml/s during the last 5 min of exposure (see Fig. 1). Although this is a rough estimate of total airways resistance, a concordance between the isolated URT and intact animal responses is apparent. Chlorine uptake efficiency in the isolated URT was also measured. Chlorine was scrubbed with great efficiency in the URT, with uptake efficiency averaging 97.5 ± 1.0% (mean ± SD, $n = 5$) at the flow rate of 25 ml/min.

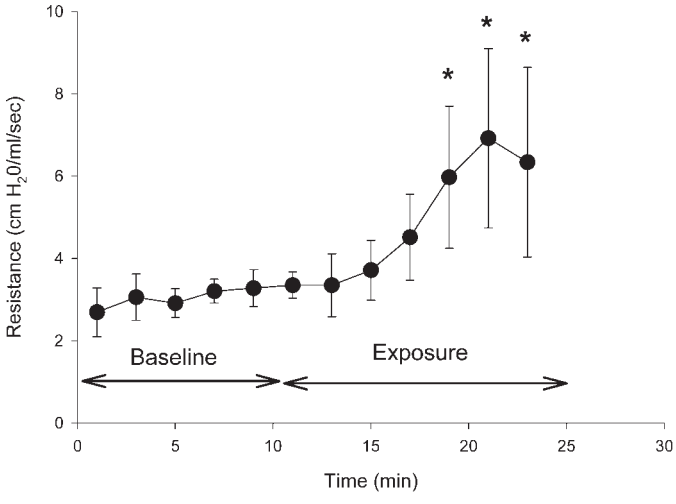

FIG. 2. URT flow resistance during the 10-min baseline and subsequent 15-min exposure periods in animals exposed to 5 nmol/min chlorine. Closed circles represent URT flow resistance (Rurt) as measured in isolated URT exposures (4.8 ppm inspired chlorine concentration, see text for details). Data are shown as mean ± SD. Groups contained five to nine mice. Repeated measures ANOVA of the isolated URT data revealed Rurt was increased over baseline levels during the last 6 min of exposure ($p < 0.05$).

Capsaicin-Pretreatment Studies

Shown in Figures 3A, 3B, and 3C are the breathing frequency, expiratory pause, and sRaw responses to 3.9 ppm chlorine in vehicle- and capsaicin-pretreated animals. Data were analyzed by one-factor (vehicle vs. capsaicin) repeated measures (time) ANOVA, because capsaicin pretreatment may alter both the magnitude and the time course of the responses to chlorine. Breathing frequency, expiratory pause duration, and sRaw were all altered from baseline levels during exposure in both vehicle and capsaicin-pretreated mice. Responses between groups during the exposure period itself were compared; for all three responses a significant difference between vehicle- and capsaicin pretreated animals was detected ($p < 0.001$ in all cases). In addition, for all three responses, significant effects of time (the repeated measure $p < 0.0001$) and significant statistical interactions between pretreatment group and time were detected ($p < 0.0001$). The expiratory pause response was virtually abolished by the capsaicin pretreatment; the breathing frequency and sRaw responses were diminished, but to a lesser degree, by the toxin. In the last 2 min of the exposure the capsaicin-pretreated mice demonstrated a reduced breathing frequency without an enhanced respiratory pause. Specifically, in the capsaicin-pretreated mice the expiratory pause duration averaged 14 ± 15 ms at the end of exposure, compared to 13 ± 4 ms during baseline, but breathing frequency averaged significantly below baseline (80 ± 8% of baseline at the end of exposure).

Hypochlorite Studies

To examine the potential role of the hypochlorite ion (i.e., chlorine oxidizing activity) in inducing respiratory responses, animals were exposed to sodium hypochlorite or Krebs Ringer buffer aerosol as a control. Exposure concentration averaged 25 nmol hypochlorite/l air. (At a concentration of 0.8 ppm, the airborne chlorine concentration is 33 nmol/l.) Air samples were drawn from the mixing tube and particle size determined with a Mercer impactor. Particle size averaged 2.5 μm (MMAD) with $\sigma_g = 2.7$. Particles of this size deposit with high efficiency (~70%) in the mouse URT (Raabe *et al.*, 1988). During hypochlorite aerosol exposure, animals exhibited diminished breathing frequencies compared to vehicle aerosol control (minimal 1 min average of 63 ± 5% vs. 85 ± 5% of baseline, $n = 4$–5, $p < 0.001$, *t*-test) and a prolonged expiratory pause (maximal 1 min average 130 ± 72 vs. 31 ± 15 ms, $p < 0.01$, *t*-test).

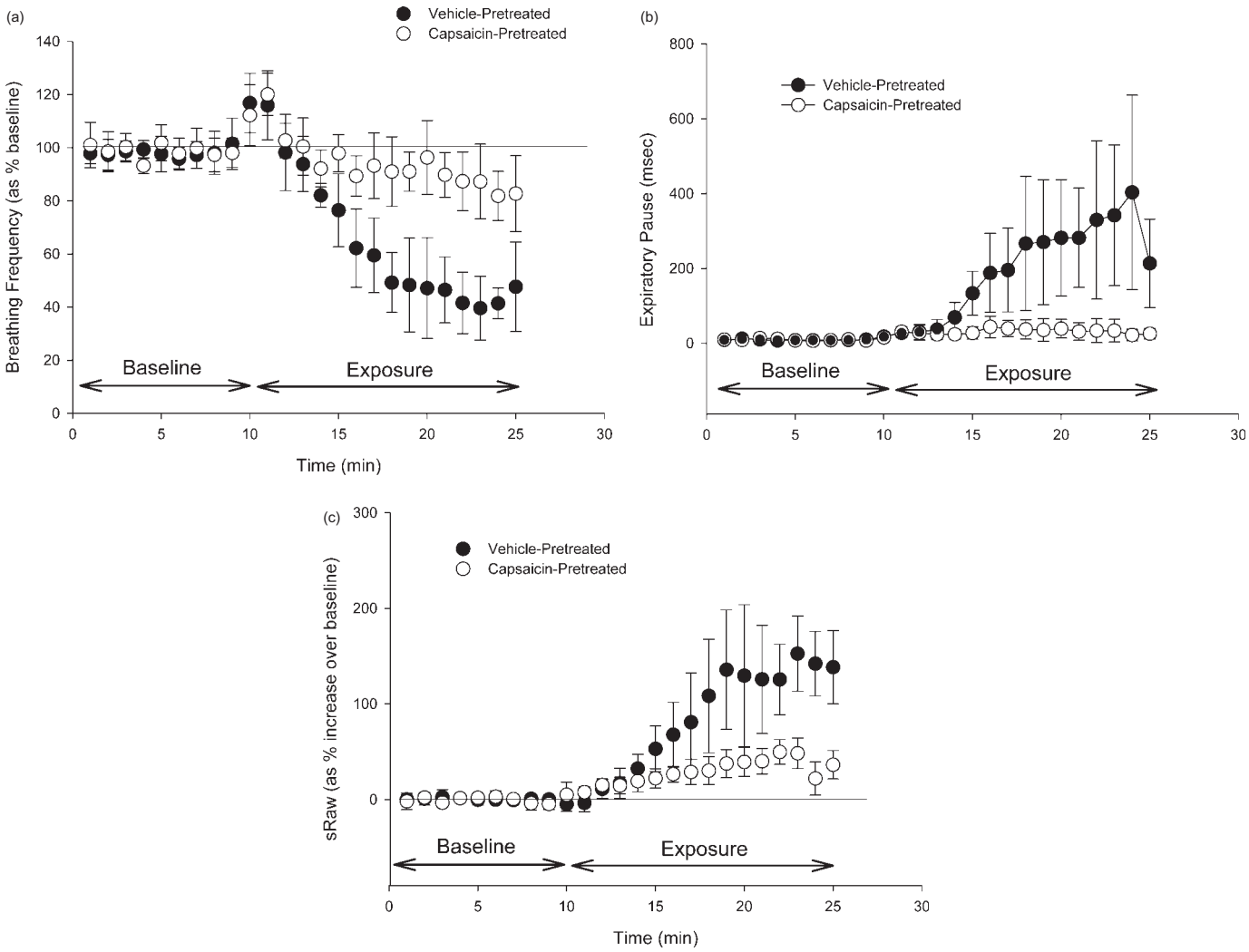

FIG. 3. Shown are breathing frequency (A), expiratory pause duration (B), and sRaw (C) during the 10-min baseline and subsequent 15-min exposure to 3.9 ppm chlorine. Breathing frequency data are presented as percent of baseline (baseline frequency averaged 319 breaths per min); expiratory pause duration data are presented in ms, and sRaw data are presented as percent increase over baseline (baseline sRaw averaged 1.6 $cmH_2O \cdot s$). All data are shown as mean ± SD. Groups contained seven to eight animals. For each measure, breathing frequency, expiratory pause duration, and sRaw, statistical analysis of the data collected during the exposure by repeated measures ANOVA revealed a significant difference between vehicle- and capsaicin-pretreated groups ($p < 0.0001$), a significant effect of exposure time ($p < 0.0001$), and a statistical interaction between pretreatment groups and exposure time ($p < 0.0001$). See text for details of the statistical analysis.

The magnitudes of these responses were greater than that produced by 0.8 ppm chlorine (see Table 1). The time course of the sRaw response to the aerosol is shown in Figure 4. Repeated measures ANOVA of the data obtained during the exposure revealed a significant effect of exposure group (hypochlorite vs. buffer aerosol, $p < 0.05$), an effect of time (the repeated measure, $p < 0.001$), and interaction between exposure group and time ($p < 0.001$). In the buffer control animals, sRaw did not increase during exposure, remaining within 10% of baseline at all times ($p > 0.05$, repeated measures ANOVA). In the hypochlorite-exposed animals, sRaw increased to approximately 80% over baseline by the end of exposure ($p < 0.05$, repeated measures ANOVA). The magnitude of the sRaw response to 0.8 ppm, an approximately equimolar concentration, is shown for comparative purposes and was similar to that to the hypochlorite aerosol.

DISCUSSION

Chlorine is a potent sensory irritant and induces an immediate obstructive response in mouse. In our hands an RD50 of 2.3 ppm was observed. This is lower than the previously reported values of 3.5 ppm in the OF1 mouse (Gagnaire *et al*., 1994) and 9.3 ppm for Swiss Webster mice (Barrow *et al*., 1977). The differing RD50 values may represent strain differences. Since chlorine reacts with water to produce hydrochloric and hypochlorous acids, it may initiate reflex responses via acidic and/or an oxidant mechanisms. It has previously been reported that the RD50 for

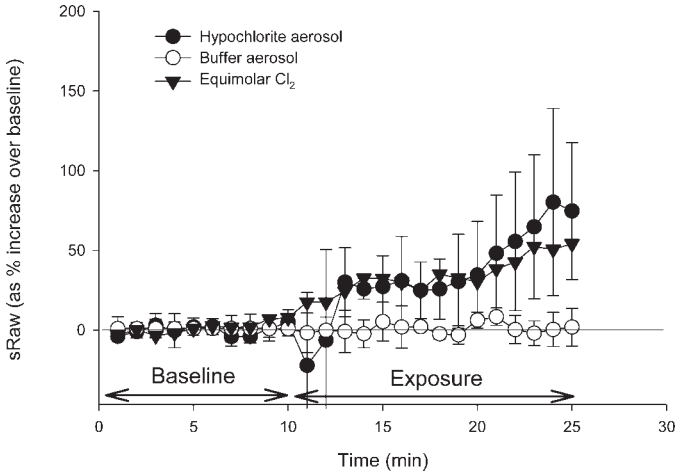

FIG. 4. Shown is sRaw (as percent increase over baseline) during the 10-min baseline and subsequent 15-min exposure periods in animals exposed to sodium hypochlorite aerosol, buffer aerosol, or 0.8 ppm chlorine (from Fig. 1). Data are presented as mean ± SD; there were four or five animals in the aerosol groups and nine animals in the 0.8 ppm chlorine group. Repeated measures ANOVA revealed a difference among exposure groups ($p < 0.05$), a significant effect of time (the repeated measure, $p < 0.001$), and a statistical interaction between groups and time ($p < 0.001$). See text for details of the statistical analysis.

chlorine is 9.3 ppm for Swiss-Webster mice compared to a value of 309 ppm for hydrochloric acid in the same strain (Barrow *et al*., 1977). That chlorine is approximately 30-fold more potent as a sensory irritant than hydrochloric acid (Barrow *et al*., 1977) suggests the oxidant rather than acidic properties are more important relative to sensory irritation. The results of the current study support this conclusion with respect to both the sensory irritation and obstructive responses. An RD50 of 2.3 ppm was observed in the C5Bl/6 J mouse in the current study compared to 239 ppm for acetic acid in the same strain (Morris *et al*., 2003). (Both acetic and hydrochloric acids are essentially totally ionized at pH > 6.0; thus the acetic acid response would be expected to be similar to that to hydrochloric acid.) A significant increase in sRaw was observed in mice exposed to 0.8 ppm chlorine, yet not change in sRaw was observed in mice exposed to 80 ppm acetic acid vapor (Morris *et al*., 2003). Thus, based on relative potencies, it seems unlikely that sufficient amounts of acid are produced by chlorine to produce either of the observed responses. Moreover, the sensory irritation and obstructive responses produced by hypochlorite aerosol are similar to those produced by chlorine (Fig. 4), suggesting the oxidant properties alone are sufficient to account for the observed responses.

The breathing frequency responses observed in the intact animal studies were likely to be nasal in origin. Chlorine induced a prolonged pause at the start of each expiration. Sensory irritation (diminished breathing frequency due to a pause at the beginning of expiration) is known to be mediated by nasal trigeminal nerve stimulation (Alarie, 1973; Nielsen, 1991) and known to be a response of the mouse to chlorine (Barrow *et al*., 1977; Gagnaire *et al*., 1994). The chlorine-induced obstructive response, on the other hand, could be either upper or lower airway in origin. Chlorine deposits with high efficiency in the rodent nose (viz. 97.5% at the flow rate used in this study). While lower uptake efficiencies will undoubtedly occur at higher, more physiologically relevant inspiratory flow rates, it is likely that the URT receives the highest delivered dosage rate during inhalation exposure, a result consistent with the induction of the nasal sensory irritation response. In this regard, the regional dosimetry of chlorine appears similar in the nose as the human, in which >95% nasal uptake has been observed (Nodelman and Ultman, 1999). The results of the isolated URT studies further show that chlorine induces an obstructive response in that site (Fig. 2). Importantly, the magnitude of the obstructive response was sufficiently great to account for the sRaw response in the intact spontaneously breathing animal. While not excluding the possibility of some contribution from the lower airways, this comparison, coupled with the efficient scrubbing in the nose, provides strong evidence that the obstructive response observed in the intact animal is nasal in origin The oxidant gas chlorine appears similar to the electrophilic vapor acrolein and the acid vapor acetic acid. These latter two vapors also deposit with high efficiency in the nose of the mouse and also induce immediate sensory irritation and nasal obstructive responses (Morris *et al*., 2003).

In healthy human subjects, 0.5–1 ppm chlorine induces a slight sensation of irritation (1 out of a scale of 5) and a demonstrable nasal obstructive response (15–20% increase in nasal airway resistance, Shusterman *et al*., 1998, 2003a,b). The responses of the mouse to 0.8 ppm appear to be analogous, being characterized by a small degree of sensory irritation, as indicated by a minimal change in breathing pattern, coupled with a demonstrable obstructive response. The responses observed at 0.8 ppm in the mouse (breathing rate reduced to 80% baseline, expiratory pause increased to 20 ms, Table 1) are quite small compared to the marked changes in these parameters that can be induced by 3.8 ppm chlorine (breathing rate to 30% baseline, expiratory pause to 500 ms). Similarly, the obstructive response to 0.8 ppm chlorine in the mouse (sRaw increase of ~60%) was demonstrable but much lower than the apparent maximal response observed at 3.8 ppm (sRaw increase of ~200%). Differences in nasal anatomy and dimensions preclude precise comparisons of the magnitude of obstructive responses across species, but in both the human and mouse, chlorine at concentrations of 0.5–1.0 ppm produced submaximal irritation and submaximal obstructive responses, suggesting a similarity of concentration response relationships in both species.

In mice, parasympathetic activation does not appear to play a role in mediating the obstructive response, as indicated by the lack of effect of atropine. Similar results have been obtained in the human (viz, the cholinergic antagonist ipratroprium bromide was without effect on the chlorine-induced obstructive response) (Shusterman *et al*., 2002). As evidence by the sensation of irritation in the human and the sensory irritation

response in the mouse, it is known that chlorine stimulates nasal sensory nerves, but the precise role of these nerves in mediating the nasal obstructive response to chlorine is not known. The current results suggest TRPV1-expressing sensory nerves are an important response pathway in the mouse, as evidenced by the significantly smaller response to chlorine that was observed in capsaicin-pretreated animals (Fig. 2).

The capsaicin pretreatment studies reveal some potentially interesting interrelationships in the responses to chlorine. This toxicant acts through the TRPV1 receptor to induce degeneration in neurons in which it is expressed (Holzer, 1991; Szallasi and Blumberg, 1999). This receptor is expressed in C fibers and, perhaps, a small subset of Aδ fibers. The expiratory pause response to chlorine was dramatically reduced in capsaicin-pretreated mice, suggesting that this central nervous system-mediated response is mediated predominantly through C fibers. Interestingly, a slightly decreased respiration rate (to 80% of baseline) was observed in capsaicin-pretreated mice at the end of exposure even though there was no significant change in expiratory pause duration at this time. This suggests that mechanisms in addition to sensory irritation may play a role in decreasing respiration rate. Perhaps, in a manner analogous to lower airway constriction (Vijayaraghavan *et al*., 1993), respiratory frequency is reduced in compensation for the increased upper airway flow resistance.

Although the expiratory pause duration was virtually absent in capsaicin-pretreated mice, a significant obstructive response was still observed. Perhaps the obstructive response is mediated, in part, via stimulation of non-TRPV1 expressing Aδ fibers, which are resistant to capsaicin. Alternatively, since it is likely that not all C-fibers are destroyed by the capsaicin pretreatment, it is possible that stimulation of a small number of fibers is sufficient to cause obstruction, whereas a stimulation of a larger number is needed for the centrally mediated sensory irritation response. In this regard, it is interesting to note that in rats irritants induce sensory nerve-stimulated vasodilation at concentrations that do not cause demonstrable sensory irritation (as shown by decreased breathing, Morris *et al*., 1999). Finally, it is possible that the obstructive response is mediated in part through nonneuronal pathways, perhaps by release of mediators from epithelial or other nasal mucosal cells. Future studies needed to resolve these possibilities.

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