Inhalation Toxicology, 15:1179–1189, 2003

ISSN: 0895-8378 print / 1091-7691 online
DOI: 10.1080/08958370390229852

Taylor & Francis
healthsciences

INFLUENCE OF AGE, GENDER, AND ALLERGY STATUS ON NASAL REACTIVITY TO INHALED CHLORINE

Dennis Shusterman

Department of Medicine, University of California, San Francisco, California, USA

Mary Alice Murphy

Department of Pediatrics, University of California, San Francisco, California, USA

John Balmes

Department of Medicine, University of California, San Francisco, California, USA

Upper-respiratory-tract symptoms (nasal irritation, congestion and rhinorrhea) are prevalent complaints in so-called "problem buildings." In epidemiologic studies, symptom reporting is associated with selected subject characteristics, including younger age, female gender, and the presence of allergic rhinitis. The physiologic correlates of these differential reporting patterns, however, are largely unknown. Using dilute chlorine gas as a model upper-respiratory-tract irritant, we studied 52 otherwise healthy volunteers in a sample stratified on age (18–69 yr), gender, and allergy status. In a single-blinded crossover study, subjects had their nasal airway resistance measured preexposure, immediately postexposure, and 15 min postexposure to both filtered air and chlorine (1.0 ppm in air) for 15 min. Allergic rhinitic subjects showed a significantly greater net (chlorine minus air) congestive response at 15 min postexposure than did nonrhinitic controls ($p < .01$*). Advancing age also predicted a greater response immediately post-exposure (*$p < .01$*). No gender effect was observed. Significant interindividual variability was evident in the nasal congestive response to irritant (chlorine) provocation. The rhinitis effect was consistent with prior observations, whereas the effect of advancing age was opposite to that hypothesized.*

Upper airway irritation and associated reflex endpoints (nasal congestion and rhinorrhea) are commonly reported symptoms in both industrial workplaces and so-called "problem buildings." Water-soluble/reactive air pollutants and coarse particulate matter disproportionately impact the upper versus lower airway (Dahl, 1990). The resulting upper-respiratory-tract and eye irritation (collectively referred to as "sensory irritation") acts as a warning to reduce further exposure, in some cases preventing more serious health outcomes (U.S. PHS, 1986).

Received 30 April 2003; sent for revision 26 May 2003; accepted 30 May 2003.

For this study, the grant sponsor was NIH/NIEHS under grant R01 ES10424. We wish to acknowledge the assistance of Paula Walsh, Elizabeth Matovinovic, MSc, and Alice Tarun, PhD, in the recruitment and screening of participants for this study.

Address correspondence to Dennis Shusterman, MD, MPH, Upper Airway Biology Laboratory, 1301 So. 46th Street, Bldg. 112, Richmond, CA 94804, USA. E-mail: dennis@itsa.ucsf.edu

Self-reported sensory irritation has been documented in a number of epidemiologic field investigations carried out in buildings with perceived indoor air quality problems. Potential exposures include combustion products (environmental tobacco smoke, reentrained vehicle exhaust, combustion appliances), ozone from photocopying machines, volatile organic compounds or VOCs (cleaning products, office supplies, off-gassing from furniture and building materials, microbial sources), and extremes of temperature and humidity (Hodgson, 2002; Jaakkola et al., 1994; Junker et al., 2001; Molhave, 1992; Ten Brinke et al., 1998). Although environmental sampling has identified specific offending indoor air pollutants in only a minority of cases, consistency has emerged in symptom-reporting patterns, highlighting potential personal risk factors. Females, younger workers, and individuals with preexisting upper airway allergies (rhinitis) are more likely to report symptoms given equivalent exposures (Brasche et al., 2001; Cummings et al., 1991; Hall et al., 1993; Mendell, 1993; Menzies & Bourbeau, 1997; Stenberg & Wall, 1995). By and large, these observed patterns have been interpreted as reflecting psychosocial, rather than biological, differences between subgroups (Brasche et al., 2001; Ryan & Morrow, 1992; Stenberg & Wall, 1995). However, population variability in objective "nasal irritant sensitivity" (sensory acuity and physiologic reactivity) has been incompletely studied (Shusterman, 2002).

Experimentally, the phenomenon of augmented nasal physiologic responsiveness to irritants in allergic rhinitics was first documented by Bascom and colleagues (1991), who measured the nasal congestive response to environmental tobacco smoke (ETS) exposure using posterior rhinomanometry. Although their study group was stratified by self-reported reactivity to ETS, their "historically sensitive" group had substantially higher rates of skin test reactivity to aeroallergens than did the "historically insensitive" group, raising the possibility that allergy status, not self-report, was the relevant biological variable. We have similarly studied the nasal congestive response to irritant provocation using dilute chlorine gas (Cl_2) in otherwise healthy volunteer subjects with and without allergic rhinitis. In a series of counterbalanced, single-blinded, crossover studies, we have found that exposure to Cl_2 (0.5–1.0 ppm × 15 min by nasal mask) produces significant increases in nasal airway resistance (NAR) in seasonal allergic rhinitic (SAR) but not nonrhinitic (NR) subjects (Shusterman et al., 1998, 2002, 2003). As our previous studies were of modest overall size (16–24 subjects) and limited age range (18–40 yr), we have hitherto been unable to examine more than one imputed susceptibility marker. Here, we study a total of 52 subjects spanning the range of 19–68 yr and having relatively symmetrical gender and allergy distributions, with the objective of studying age, gender, and allergic rhinitis status as potential markers of nasal physiologic reactivity to an inhaled irritant.

METHOD

Subject Recruitment and Screening

Subjects were recruited through posters and newspaper advertisements. Inclusion criteria were age 18–69 yr and "general good health." Exclusion

criteria were: (1) a history of asthma, (2) cigarette smoking (active or within previous 6 mo), (3) pregnancy or lactation, (4) a history of severe allergic reactions (anaphylaxis or angioedema), and (5) continuous therapy with medications having antihistaminic side effects (e.g., tricyclic antidepressants). Subjects read and signed an informed consent document approved by the Committee on Human Research of the University of California, San Francisco. Questionnaires were administered to each potential subject, who was provisionally classified as having seasonal allergic rhinitis (SAR), no rhinitis (NR), or "other" (including perennial allergic rhinitis).

Allergy skin-prick tests (to 13 regionally common aeroallergens/mixes, plus saline and histamine controls) were then administered. For purposes of this study, "seasonal allergic rhinitics" were defined as subjects with: (1) a history of seasonally occurring sneezing, nasal pruritus, rhinorrhea, postnasal drip, and/or nasal congestion, with or without known precipitants; and (2) skin-test reactivity to at least one seasonally occurring agent from the panel that corroborated the history. "Skin-test reactivity" was defined as a wheal reaction to skin-prick testing with a diameter greater than or equal to that of the histamine control. "Nonrhinitics" were defined as subjects who report, at most, infrequent nasal symptoms, without identified seasonal variation or precipitants, with significant skin test reactivity to no more than 1 agent in the panel of 13 aeroallergens, and with normal findings on anterior rhinoscopy. Prior to skin testing, subjects were asked to refrain from taking antihistamines for 72 h (hydroxyzine for 3 wk, astemizole for 12 wk).

All potential subjects for this study first participated in a study of sensory thresholds to pulsed carbon dioxide (detection) and to vapor-phase *n*-propanol (localization; Shusterman et al., in press). During the course of that study, subjects were screened for their ability to generate meaningful tracings by active posterior rhinomanometry, and 8 of 60 subjects were unable to do so, despite extensive coaching. The two study sequences were separated by at least 2 wk, and as the sensory measures were of a threshold nature, subjects experienced minimal subjective irritation and there was minimal potential for stimulus carryover or "priming."

Experimental Design and Procedures

The study design was experimental, utilizing a semirandomized crossover design comparing the effect of dilute Cl_2 gas with that of air (Figure 1). The concentration and duration of Cl_2 exposure—1.0 ppm times 15 min—is the (U.S.) occupational short-term exposure limit, and hence is of both scientific and regulatory interest. The two exposures (a week apart) employed active posterior rhinomanometry, with nasal airway resistance measured at baseline, immediately after exposure and again 15 min postexposure. On a given day, exposure was either to pure (medical grade) air or to Cl_2 (1.0 ppm) diluted in air. The order of exposure within each pair of testing dates was determined by limited randomization (i.e., with initial exposure for subjects in each gender/rhinitis/age group stratum alternating between air and Cl_2). For stratification purposes, subject age was divided into three groups: 18–34, 35–51, and 52–69 yr.

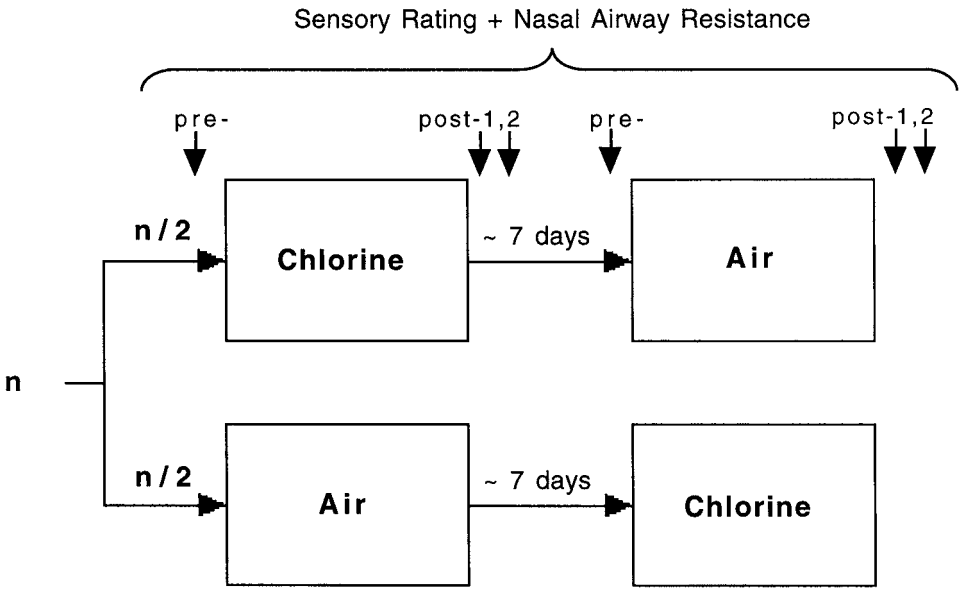

FIGURE 1. Counterbalanced, crossover design of exposure sequence. For each age, gender, and rhinitis stratum of 4–6 (*n*) subjects, half of the subjects (*n*/2) underwent exposure in each sequence.

SAR subjects were tested out of their relevant pollen season. All subjects were asked to avoid exercising, consumption of spicy foods, and use of scented cosmetics on the day of testing. In addition to antihistamine preclusions (as specified for skin-prick testing), subjects were asked to avoid using nasal steroids for at least 2 wk, and nasal decongestants for at least 48 h prior to testing. Upon arrival at the laboratory, subjects entered a climate-controlled chamber (22 ± 1°C; 40 ± 3% relative humidity [RH]) with filtered air (activated charcoal and high-efficiency particulate). After a 15-min waiting period, baseline symptoms (nasal irritation, congestion, rhinorrhea, postnasal drip, and odor) were rated on computer-based visual analog scales or VAS (LabView software, National Instruments, Austin, TX). The scales were indexed at equal intervals with the words "none," "slight," "moderate," "strong," "very strong," and "overpowering," corresponding to the numerical range of 0.00 to 5.00. Symptom rating was repeated (times three) at 5-min intervals during exposure, and again at 15 min postexposure.

Cl_2 or air was administered on a single-blinded basis for a period of 15 min. Cl_2 was diluted in medical-grade air, which was preconditioned to 22°C and 40% RH (verified using the same temperature/humidity recorder employed in the chamber itself); the dilution apparatus has previously been described in detail (Shusterman et al., 1998). Cl_2 and air were supplied by Puritan Medical Products (Hayward, CA). Cl_2 concentrations were measured in real time using an electrochemical monitor (model 1340; Interscan Corp., Chatsworth, CA). The meter was recalibrated on a daily basis using the cer-

tified contents of the Cl_2 cylinder as the standard. The gas mixture was administered to the subject through a nasal CPAP mask (series 3121; Respironics, Inc., Murraysville, PA), which was sized according to the individual subject. The combination of a high flow rate (60 L/min) and a low-pressure gas scavenging system allowed subjects to breathe with negligible superimposed pressure or resistance.

Nasal airway resistance for each testing condition was taken as the mean of three values, as ascertained by active posterior rhinomanometry using a commercial instrument (model NR6-2, GM Instruments, Kilwinnig, UK). NAR was calculated using the pressure-cutoff method (75 Pa), and was obtained at baseline, immediately postexposure, and 15 min postexposure on both Cl_2 and air days. Calibration procedures were employed as previously detailed (Shusterman et al., 1998).

Data Analysis

NAR values were normalized to baseline measurements for each experimental session, yielding the metric "Δ% NAR" [from baseline] for each of two time points (immediately postexposure and 15 min postexposure) on each of two (Cl_2 and air) experimental conditions. The difference between the 2 days was further summarized as a net measure (Cl_2 minus air day): "Δ% NAR (Net)." Data were examined for normality and then analyzed by analysis of variance (ANOVA) if this condition was satisfied. The hypotheses to be tested were: (1) SAR subjects would show significantly greater Cl_2-related increases in NAR over baseline ["Δ% NAR (Net)"] than would NR controls, (2) younger subjects would show significantly greater increases in NAR than older subjects, and (3) females would show significantly greater increases in NAR than males.

RESULTS

The demographic and health characteristics of the 52 participants are summarized in Table 1. Subject ages ranged from 19 to 68 yr, with 24 males (46%) and 27 allergic rhinitics (52%). Seventeen subjects were recruited in the 18–34 yr age range, 18 in the 35–51 yr range, and 17 in the 52–69 yr range. Overall, 15 of 28 females were allergic rhinitic (54%) compared with 12 of 24 males (50%). The mean ages of males and females were similar, as

TABLE 1. Characteristics of subjects

Characteristic	Status (number)	Age: mean ± SD (yr)
Gender	Male (24)	40.9 ± 14.8
	Female (28)	41.8 ± 14.2
Allergic rhinitis	Yes (27)	40.9 ± 13.6
	No (25)	41.8 ± 15.3
Combined	(52)	41.3 ± 14.3

were the mean ages of allergic rhinitics and nonrhinitics (Table 1; $p > .80$). Given this nearly balanced study sample, study power could be maximized in subsequent analysis by utilizing univariate models.

The exposure metric, Δ% NAR (Net), was normally distributed, both immediately postexposure and 15 min postexposure, and thus was analyzed by ANOVA. Results by age, gender, and rhinitis status, both immediately postexposure and 15 min postexposure, appear in Figure 2. At 15 min postexposure, SAR subjects showed a significantly greater congestive response to Cl_2 (compared to air) than did nonallergic controls ($p < .01$). An enhanced response to Cl_2 was also seen as a function of age, although the effect was opposite of that predicted (i.e., older subjects congested more than younger; $p < .01$ immediately postexposure). Gender did not significantly predict the congestive response to Cl_2 at either sampling time, although there was a nonsignificant trend ($p = .15$) toward greater Δ% NAR (Net) in males than females at 15 min postexposure.

Subjectively rated nasal irritation, congestion, rhinorrhea, postnasal drip, and odor were all quite modest in this experiment. The mean peak VAS ratings for odor and irritation during exposure, for example, were less than "slight" (i.e., <1 on a scale of 0–5), and showed the predicted time course of response of adaptation for odor and temporal integration for irritation (Figure 3). There was no significant differential symptom rating by rhinitis status, age, or gender, and the remaining symptoms (congestion, rhinorrhea, postnasal drip) were of even lower magnitude (data not shown).

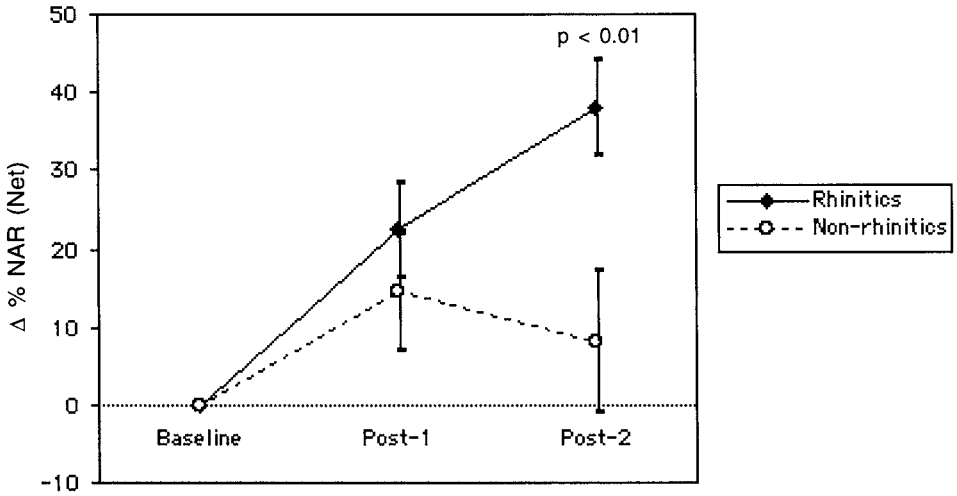

FIGURE 2. Δ% NAR (Net) measured immediately (Post-1) and 15 min (Post-2) after (Cl_2 minus air) exposure, as a function of (a) rhinitis status, (b) age, and (c) gender. Δ% NAR (Net) increased significantly with age immediately postexposure ($p < .01$) and was significantly greater in allergic rhinitics than in nonrhinitics 15 min postexposure ($p < .01$). The difference between males and females at 15 min postexposure was not statistically significant ($p = .15$).

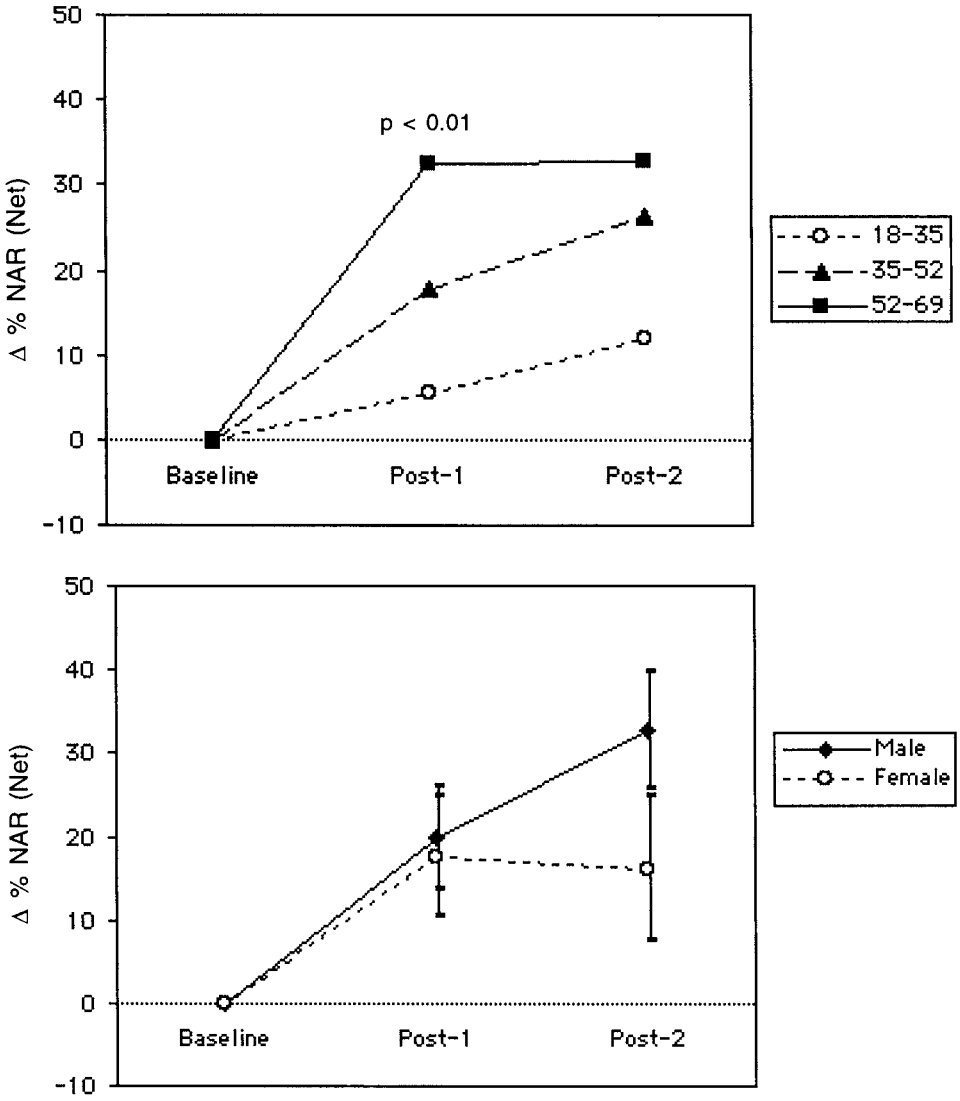

FIGURE 2. (*Continued*).

DISCUSSION AND CONCLUSIONS

In this sample, we found that allergic rhinitis predicts a greater congestive response to irritant provocation (i.e., increases in NAR measured at 15 min postexposure), similar to findings in our earlier studies (Shusterman et al., 1998, 2002, 2003). In addition, we found that more advanced age predicted a more marked congestive response, as measured immediately postexposure. Finally, gender did not emerge as a significant predictor of physiologic re-

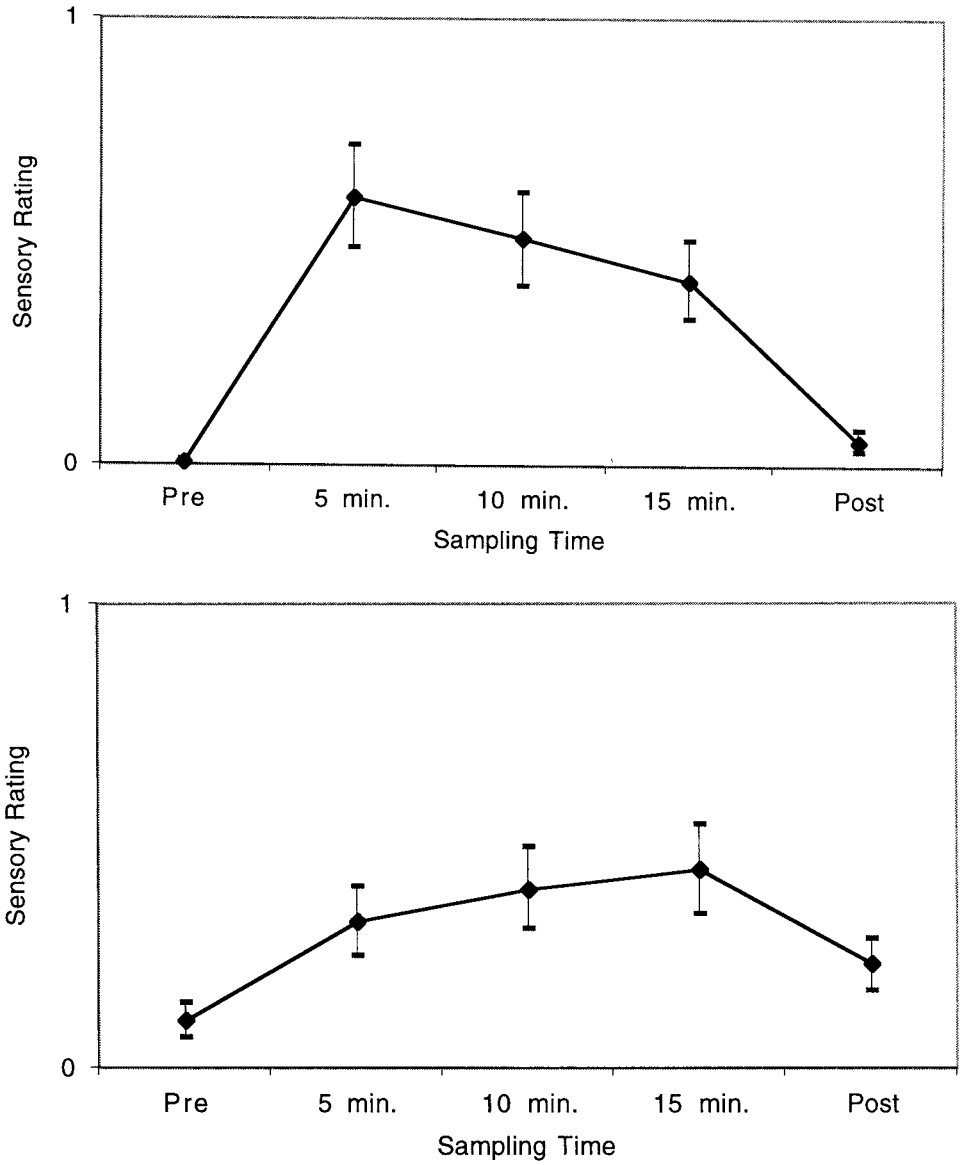

FIGURE 3. Subjective rating by visual analog scale of Cl_2: (a) odor intensity, and (b) nasal irritation, preexposure, at 5-min intervals during exposure, and 15 min postexposure (all subjects combined). Symptom/sensation ratings were modest and followed predicted temporal trends (i.e., decrease—or adaptation—for odor, and increase—or temporal integration—for irritation). Scale was 0–5 where: 0 = "none," 1 = "slight," 2 = "moderate," 3 = "strong," 4 = "very strong," and 5 = "overpowering."

activity in this study sample. The observed changes in NAR occurred with very modest subjective nasal irritation.

This study represents an extension of our previous descriptive work on Cl_2-induced nasal congestion, with the upper age limit for recruitment being extended from 40 to 69 yr, and with an overall study size (52) sufficiently

large to examine more than one susceptibility marker. By employing targeted recruiting into age/gender/allergy strata, we were able to construct a sufficiently balanced study sample to permit optimization of statistical power using univariate statistical methods (i.e., ANOVA). Further, order of exposure was counterbalanced within strata, effectively eliminating stimulus order or stimulus carryover as potential confounders. Although "single blinding" of exposure is only partially effective with an odorous air pollutant, suprathreshold ratings of both odor and irritation were quite modest, and roughly one of four subjects were unable to correctly identify Cl_2 versus air exposure days.

Of our two significant physiologic findings, the positive correlation between age and congestive response to Cl_2 was contrary to that predicted by epidemiologic reporting patterns. This finding is surprising in light of the clinical assumption that rates of "atrophic rhinitis" increase with age and that this condition is accompanied by impaired vascular reactivity (Bende, 1985). Further, these findings may signal a "disconnect" between sensory and physiologic data. Specifically, in a largely overlapping cohort of 60 subjects, we found that older age predicted *higher* irritant detection thresholds (i.e., *less* "sensitivity"), utilizing carbon dioxide and *n*-propanol as test compounds (Shusterman et al., in press). Another difference between sensory and physiologic endpoints in these two studies included the finding of significantly lower irritant (CO_2) detection thresholds in female subjects, but no gender difference in the congestive response to Cl_2.

The mechanism(s) involved in the nasal congestive response to irritant provocation are unclear. Despite the link between physiologic reactivity to irritants and nasal allergy status, mast-cell degranulation seems unlikely in light of nasal lavage findings after provocation with both environmental tobacco smoke (Bascom et al., 1991) and Cl_2 (Shusterman et al., 2003). Further, cholinergic parasympathetic reflexes seem to have variable role in this phenomenon, based on studies in which cholinergic blockers were administered prior to provocation with ammonia (McLean et al., 1979) versus Cl_2 (Shusterman et al., 2002). Interest has been expressed in the potential involvement of vasoactive neuropeptides released by sensory nerves (Bascom, 1992). However, a recent study examining the congestive response to a hyperosmolar irritant stimulus found evidence for neither mast-cell degranulation nor neuropeptide (substance P) release (Koskela et al., 2000). Should airway sensory nerves prove to be involved in irritant-induced nasal congestion, however, the logical differential response mechanism for allergic rhinitics would be so-called "neuromodulation" (Undem et al., 2000).

Notwithstanding ambiguities surrounding the mechanism(s) of irritant-related nasal congestion, the finding of differential nasal responsiveness to irritant air pollutants is one that should be kept in mind by clinicians, risk assessors, and public health practitioners. The common practice of ascribing differential upper-airway symptom reporting to psychosocial factors alone is one that should be tempered in light of human experimental data demonstrating heterogeneity of response in objective tests of sensory acuity and physiologic reactivity. Studies such as these may have further value by allowing substitution of empirical data for default assumptions regarding interindi-

vidual variability when conducting risk assessments including the upper-airway impact of irritant chemicals.

REFERENCES

Bascom, R. 1992. Differential responsiveness to irritant mixtures: Possible mechanisms. *Ann. NY Acad. Sci.* 641:225–247.

Bascom, R., Kulle, T., Kagey-Sobotka, A., and Proud, D. 1991. Upper respiratory tract environmental tobacco smoke sensitivity. *Am. Rev. Respir. Dis.* 143:1304–1311.

Bende, M. 1985. Nasal mucosal blood flow in atrophic rhinitis. *ORL J. Otorhinolaryngol. Relat. Spec.* 47(4):216–219.

Brasche, S., Bullinger, M., Morfeld, M., Gebhardt, H. J., and Bischof, W. 2001. Why do women suffer from sick building syndrome more often than men?—Subjective higher sensitivity versus objective causes. *Indoor Air* 11(4):217–222.

Cummings, K. M., Zaki, A., and Markello, S. 1991. Variation in sensitivity to environmental tobacco smoke among adult non-smokers. *Int. J. Epidemiol.* 20:121–125.

Dahl, A. R. 1990. Dose concepts for inhaled vapors and gases. *Toxicol. Appl. Pharmacol.* 103:185–197.

Hall, H. I., Leaderer, B. P., Cain, W. S., and Fidler, A. T. 1993. Personal risk factors associated with mucosal symptom prevalence in office workers. *Indoor Air* 3:206–209.

Hodgson, M. 2002. Indoor environmental exposures and symptoms. *Environ. Health Perspect.* 110 (suppl.)4:663–667.

Jaakkola, J. J. K., Tuomaala, P., and Seppanen, O. 1994. Textile wall materials and sick building syndrome. *Arch. Environ. Health* 49(3):175–181.

Junker, M. H., Danuser, B., Monn, C., and Koller, T. 2001. Acute sensory responses of nonsmokers at very low environmental tobacco smoke concentrations in controlled laboratory settings. *Environ. Health Perspect.* 109(10):1045–1052.

Koskela, H., Di Sciascio, M. B., Anderson, S. D., Andersson, M., Chan, H. K., Gadalla, S., and Katelaris, C. 2000. Nasal hyperosmolar challenge with a dry powder of mannitol in patients with allergic rhinitis. Evidence for epithelial cell involvement. *Clin. Exp. Allergy* 30(11):1627–1636.

McLean, J. A., Mathews, K. P., Solomon, W. R., Brayton, P. R., and Bayne, N. K. 1979. Effect of ammonia on nasal resistance in atopic and nonatopic subjects. *Ann. Otol. Rhinol. Laryngol.* 88:228–234.

Mendell, M. J. 1993. Non-specific symptoms in office workers: A review and summary of the epidemiologic literature. *Indoor Air* 3:227–236.

Menzies, D., and Bourbeau, J. 1997. Building-related illness. *N. Engl. J. Med.* 337(21):1524–1531.

Molhave, L. 1992. Volatile organic compounds and the sick building syndrome. In *Environmental toxicants: Human exposures and their health effects,* ed. M. Lippmann, pp. 633–646. New York: Van Nostrand.

Ryan, C. M., and Morrow, L. A. 1992. Dysfunctional buildings or dysfunctional people: An examination of the sick building syndrome and allied disorders. *J. Consult. Clin. Psychol.* 60(2):220–224.

Shusterman, D. 2002. Individual factors in nasal chemesthesis. *Chem. Senses* 27:551–564.

Shusterman, D. J., Murphy, M. A., and Balmes, J. R. 1998. Subjects with seasonal allergic rhinitis and nonrhinitic subjects react differentially to nasal provocation with chlorine gas. *J. Allergy Clin. Immunol.* 101(6 Pt 1):732–740.

Shusterman, D., Murphy, M. A., Walsh, P., and Balmes, J. R. 2002. Cholinergic blockade does not alter the nasal congestive response to irritant provocation. *Rhinology* 40(3):141–146.

Shusterman, D., Balmes, J. R., Avila, P. C., Murphy, M. A., and Matovinovic, E. 2003. Chlorine inhalation produces nasal congestion in allergic rhinitics without mast cell degranulation. *Eur. Respir. J.* 21:652–657.

Shusterman, D., Murphy, M. A., and Balmes, J. 2003. Differences in nasal irritant sensitivity by age, gender, and allergic rhinitis status. *Int. Arch. Occup. Environ. Health*., published online 12 August 2003.

Stenberg. B., and Wall, S. 1995. Why do women report "sick building symptoms" more often than men? *Soc. Sci. Med.* 40(4):491–502.

Ten Brinke, J., Selvin, S., Hodgson, A. T., Fisk, W. J., Mendell, M. J., Koshland, C. P., and Daisey, J. M. 1998. Development of new volatile organic compound (VOC) exposure metrics and their relationship to "sick building syndrome" symptoms. *Indoor Air* 8:140–152.

Undem, B. J., Kajekar, R., Hunter, D. D., and Myers, A. C. 2000. Neural integration and allergic disease. *J. Allergy Clin. Immunol*. 106(5 suppl.):S213–220.

U.S. Public Health Service. 1986. *The health consequences of involuntary smoking: A report of the Surgeon General*. Washington, DC: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Center for Health, Promotion and Education, Office on Smoking and Health.