documented in a murine model in which animals are presensitized to ovalbumin before challenge with acetic acid. Interestingly, in this model, enhanced responsiveness to acrolein vapor is not observed (Morris et al., 2003). We were interested in knowing whether the augmented responsiveness to Cl_2 provocation observed in humans with SAR generalized to a chemically distinct stimulus, acetic acid. The ultimate goal of this line of research is to develop a tandem human–murine mechanistic model for the nasal obstructive response to irritants belonging to various chemical classes.

METHOD

Subject Recruitment and Screening

Based on effect sizes observed in our chlorine experiments, 16 subjects (8 each, seasonal allergic rhinitic and nonrhinitic) were recruited. Recruitment occurred 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 16 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 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 one agent in the panel of 16 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). All potential subjects for this study first were screened for their ability to generate meaningful tracings by active posterior rhinomanometry.

Experimental Design and Procedures

The study design was experimental, utilizing a semirandomized crossover design comparing the effect of dilute acetic acid vapor with that of air. The concentration and duration of acetic acid exposure—15 ppm for 15 min —is the (U.S.) occupational short-term exposure limit, and hence is of both scientific and regulatory interest. The two exposures (1 wk apart) employed active posterior rhinomanometry, with nasal airway resistance measured in triplicate at baseline, immediately after, and again at 15 min postexposure. On a given day, exposure was either to pure (medical grade) air or to acetic acid vapor (15 ppm) diluted in air. The order of exposure within each pair of testing dates was counterbalanced (i.e., with initial exposure for subjects in each gender/rhinitis group alternating between air and acetic acid).

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. Further, subjects reporting symptoms consistent with an upper-respiratory-tract infection were rescheduled at least 2 wk post symptom resolution. Upon arrival at the laboratory, subjects entered a climate-controlled chamber ($22 \pm 1^{\circ}$C; $40 \pm 3\%$ relative humidity) with filtered air (activated charcoal and high-efficiency particulate). After a 15-min waiting period, baseline symptoms (nasal irritation, congestion,

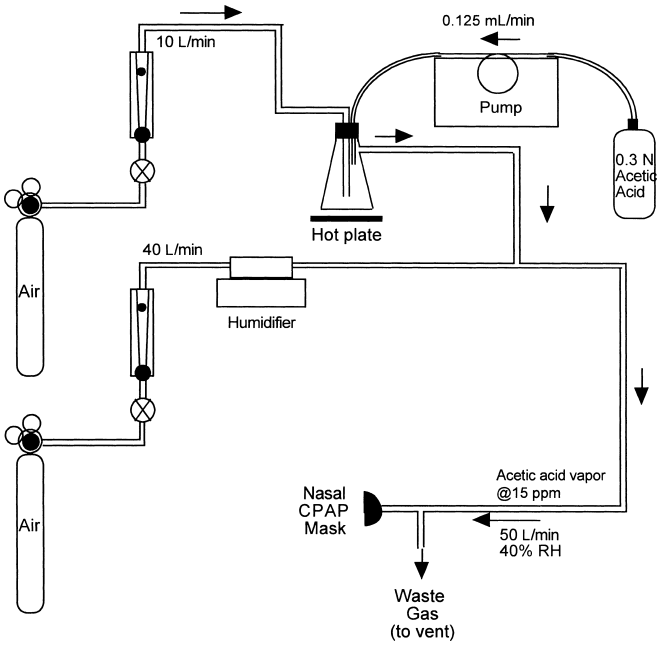

FIG. 1. Acetic acid vapor generation apparatus. Acetic acid (0.3 *N*) was metered via peristaltic pump into a heated Erlenmeyer flask at a rate of 0.125 ml/min, with the flask being flushed with filtered air at the rate of 10 L/min. The output of this vaporizer apparatus was mixed with 40 L/min of air prehumidified to 40% RH, to achieve a final target acetic acid concentration of 15 ppm.

TABLE 1
Subject demographics

Trait	Subgroup	Number	Age (yr), mean (range)	Number of positive skin tests, mean (range)
Allergic rhinitis	Yes	$n = 8$	39.9 (22–63)	3.6 (3–5)
	No	$n = 8$	39.3 (21–56)	0.4 (0–1)
Gender	Male	$n = 8$	39.6 (21–57)	
	Female	$n = 8$	39.5 (26–63)	
Combined		$n = 16$	39.6 (21–63)	

rhinorrhea, postnasal drip, and odor) were rated on computer-based visual analog scale 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 (three times) at 5-min intervals during exposure, and again 15 min postexposure.

Acetic acid vapor or air was administered on a single-blinded basis* for a period of 15 min. Acetic acid was vaporized in a closed, heated flask, with the vapor output diluted in medical-grade air, which was preconditioned to 22°C and 40% relative humidity (RH) (Figure 1). Acetic acid was supplied by Sigma-Aldrich (St. Louis, MO), and medical-grade air by Puritan Medical Products (Hayward, CA). Acetic acid vapor concentrations were measured at the beginning and end of each exposure session using colorimetric indicator tubes (Drager Safety AG & Co., Lubek, Germany). 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 (50 L/min) and a low-pressure gas scavenging system allowed subjects to breathe with negligible superimposed pressure or resistance.

Nasal airway resistance (NAR) 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 acetic acid 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 "NAR/baseline" for each of two time points (immediately post exposure and 15 min postexposure) on each of two (acetic acid and air) experimental conditions; 1.00 plus the difference between the (acetic acid minus air) days was further summarized as the metric "Net [NAR/baseline]." This metric was examined for normality, and if necessary, log-transformed and then analyzed by rhinitis status utilizing analysis of variance (ANOVA) on JMP (SAS Institute, Cary, NC). The hypothesis to be tested was that SAR subjects would show significantly greater acetic acid-related increases in NAR over baseline (i.e., greater Net NAR/baseline) than would controls.

*Subjects were not informed of the testing condition before each session; however, all subjects were able to correctly identify the acetic acid and air exposure days at the conclusion of testing.

RESULTS

The demographic and health characteristics of the 16 participants are summarized in Table 1. Subjects ranged in age from 21 to 63 yr, with equal numbers of males and females, each gender subgroup consisting, in turn, of equal numbers of allergic rhinitics and controls. The mean age of males and females were similar, as were the mean age of allergic rhinitics and

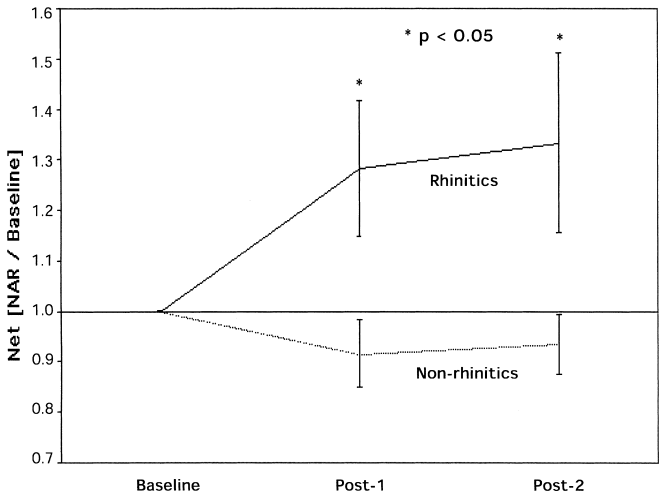

FIG. 2. Mean net [nasal airway resistance/baseline] before, immediately after, and 15 min after exposure to acetic acid vapor (15 ppm for 15 min). Net changes in NAR were significantly greater in the seasonal allergic rhinitic than in the nonallergic control groups, both immediately after and 15 min after exposure.

nonrhinitics. Allergic rhinitics showed 3–5 positive skin tests (mean, 3.6), whereas nonrhinitic controls showed 0–1 (mean, 0.4). With regard to exposures, mean acetic acid levels, taken as the average of values obtained at the beginning and end of each acetic acid exposure run, were 13.9 + 0.36 (SEM) ppm for the SAR group and 14.2 ± 0.57 ppm for the NR group ($p = .65$).

Rhinomanometric results by rhinitis status, both immediately post- and 15 min postexposure, appear in Table 2 and Figure 2. In SAR subjects, exposure to acetic acid at 15 ppm (vs. air) produced a mean Net [NAR/baseline] of 1.28 immediately postexposure and 1.33 at 15 min postexposure; the corresponding values for NR controls were 0.92 and 0.93, respectively. After log transformation, the mean $\log_e$ Net [NAR/baseline] was 0.22 for SAR subjects and −0.11 for NR subjects immediately postexposure ($p < .05$); the corresponding values 15 min postexposure were 0.24 and −0.08 ($p < .05$).

Subjectively rated nasal irritation, congestion, rhinorrhea, postnasal drip, and odor were all quite modest in this experiment. Exposure to acetic acid at 15 ppm produced net (AA-air) subjective ratings of odor in the slight-to-moderate range, and nasal irritation in the slight range (Figure 3, pooled data). Subjective nasal congestion, rhinorrhea, and postnasal drip did not differ significantly between exposure conditions, and there were no significant differences in symptom rating by rhinitis status (data not shown).

DISCUSSION AND CONCLUSIONS

In our sample, seasonal allergic rhinitic subjects, as compared to nonrhinitic controls, showed a significant nasal obstructive response to acetic acid vapor exposure (15 ppm × 15 min), both immediately after and 15 min postexposure ($p < .05$ for both). This occurred with subjective nasal irritation, on average, in the "slight" range, and with odor ratings being "slight to moderate." This differential nasal obstructive response by rhinitis status is similar to findings in our earlier studies employing Cl_2 as a provocation agent, in which there were even more modest subjective symptoms (Shusterman et al., 1998, 2002, 2003b, 2003c).

TABLE 2
Nasal airway resistance measurements: Crude values and Net [NAR/baseline]

	Rhinitics				Nonrhinitics			
	Subject	Baseline	Post-1	Post-2	Subject	Baseline	Post-1	Post-2
Air	2	229	259	251	1	358	362	362
Acetic acid		266	305	289		311	306	300
Net [NAR/baseline]		1.00	1.02	0.99		1.00	0.97	0.95
Air	6	234	248	252	3	229	236	230
Acetic acid		204	218	249		162	156	186
Net [NAR/baseline]		1.00	1.02	1.15		1.00	0.93	1.14
Air	7	299	270	284	4	283	293	301
Acetic acid		264	295	297		268	295	291
Net [NAR/baseline]		1.00	1.22	1.17		1.00	1.07	1.03
Air	8	340	340	339	5	162	216	220
Acetic acid		262	394	322		192	200	211
Net [NAR/baseline]		1.00	1.50	1.23		1.00	0.71	0.74
Air	9	274	240	262	13	174	208	205
Acetic acid		220	231	244		186	201	192
Net [NAR/baseline]		1.00	1.17	1.15		1.00	0.89	0.85
Air	10	294	304	328	14	245	322	327
Acetic acid		279	329	377		251	231	252
Net [NAR/baseline]		1.00	1.14	1.23		1.00	0.61	0.67
Air	11	107	118	110	15	150	167	193
Acetic acid		112	129	136		142	150	175
Net [NAR/baseline]		1.00	1.05	1.18		1.00	0.94	0.95
Air	12	440	416	382	16	176	209	217
Acetic acid		316	661	768		176	244	241
Net [NAR/baseline]		1.00	2.15	2.57		1.00	1.20	1.14

Note. All crude values represent the mean of three measurements. Net [NAR/baseline] = 1.00 + proportional change from baseline, acetic acid minus air trial.

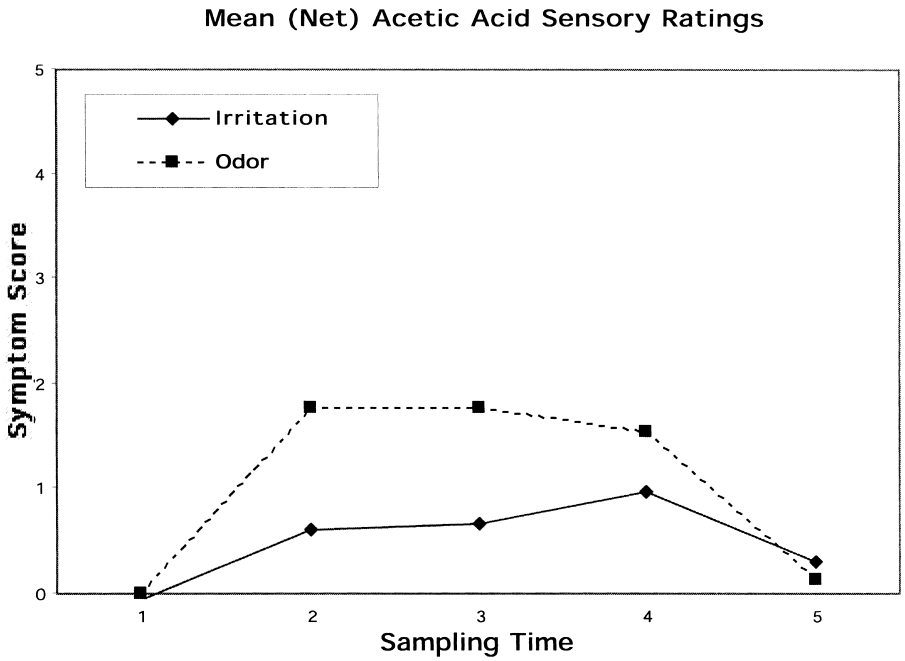

FIG. 3. Mean sensory ratings: (1) before, (2–4) during (at 5-min intervals), and (5) 15 min after exposure to acetic acid vapor (15 ppm for 15 min). Data are pooled across allergic rhinitic and nonrhinitic (control) subjects. Rating scale: 0 = none; 1 = slight; 2 = moderate; 3 = strong; 4 = very strong; 5 = overpowering.

One of us (JM) has studied the analogous response in experimental animals, using a murine model. Utilizing plethysmography in spontaneously breathing C57Bl/6J mice, as well as in preparations involving the isolated upper airway in anesthetized and intubated animals, a similar upper airway obstructive response to both acetic acid and acrolein vapors has been observed (Morris et al., 2003). Further, augmentation of the response to acetic acid (but not acrolein) vapor was apparent after airway sensitization to ovalbumin (Morris et al., 2003). Thus, a strong empirical—if not mechanistic—parallel exists between human and animal models of irritant-induced nasal airflow obstruction.

The fact that allergy-induced differential responsiveness in mice occurs with acetic acid but not acrolein suggests that the mechanism(s) involved in the nasal obstructive response may be at least partially specific to different classes of chemical irritants (i.e., acids vs. electrophiles). In our laboratories, both acetic acid vapor and chlorine gas have been studied in human and murine systems and were found to produce airflow obstruction. Elsewhere, nasal airflow obstruction has been demonstrated after controlled human exposures to sidestream tobacco smoke (Bascom et al., 1991) as well as in rodent studies of woodsmoke (Ho & Kou, 2002).

An obvious advantage of calibrating human and murine responses against one another is the potential ability to explore pathophysiologic mechanisms in the murine model using genetically manipulated organisms and/or pharmacologic agents not approved for use in humans. The importance of irritant-induced upper airway mucosal swelling goes beyond nasal airflow obstruction per se, as both otitis media and sinusitis include in their pathophysiologic chain the occlusion of ostia by mucosal swelling resulting from allergic, infectious, or toxic insults. We hope to further pursue this tandem model of irritant-induced nasal airflow obstruction in the future.

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