

Priming of the nasal mucosa by ragweed extract or by an irritant (ammonia)

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Confirming previous work by Connell, it was shown that challenging nine subjects intranasally on successive days with short ragweed extract (SRW) resulted in a priming effect manifested by significantly augmented increases in nasal airways resistance as well as in increasing subjective symptoms. Priming was reproducible and transient. In contrast, significant priming to an irritant, ammonia gas (NH₃), could not be unequivocally demonstrated over a similar period of time in these same subjects under the conditions employed. Also, priming these subjects with SRW extract failed to enhance responsiveness to NH₃.

In 1960 Connell¹ reported that the nasal mucosa becomes hyperresponsive to antigen challenge on consecutive days. This increased responsiveness was termed "priming effect on the end organ," and in a subsequent, intensive study of two individuals, Connell found that the heightened responsiveness was a local phenomenon that was not antigen specific.^{2, 3} The nature and extent of "priming" has not received the additional study that it appears to merit. It may be relevant to such clinical problems as persisting hay fever symptoms after the pollen count has fallen almost to zero, apparent intolerance to a variety of irritants during active allergic rhinitis, and difficulties in assessing patterns of symptoms in patients with multiple sensitivities. In addition, better knowledge of priming becomes increasingly important as intranasal challenge testing and intranasal immunotherapy become more extensively employed: it may underlie apparent inconsistencies in dose-response data relating to nasal allergy and affect patients' tolerance of intranasal immunotherapy. Accordingly, we sought (1) to confirm the occurrence of nasal priming with an allergen; (2) to determine if priming could be elicited

with an irritant such as ammonia gas; and (3) to ascertain whether priming with an allergen simultaneously heightens responsiveness to an irritant.

MATERIALS AND METHODS

As in our previous nasal studies,⁴ volunteer subjects were screened by a questionnaire, examination, spirometry, nasal smear for eosinophils, and a battery of 19 prick and six intracutaneous tests. Nine volunteers (six female and three male, aged 19 to 35 yr) employed in this study were considered to have ragweed hay fever by virtue of their characteristic histories of allergic rhinitis with seasonal exacerbation from late August through mid-September, as well as 3+ or 4+ prick-test reactions to ragweed pollen extract. Hay fever symptoms had been present for at least the 2 previous years, and all except one had not received immunotherapy during the previous 3 yr. Other aeroallergens elicited occasional positive skin-test reactions but were not significant by history. All volunteers had been symptom free for at least several weeks before being studied, and no one was taking medications known to influence skin or mucosal tests. No subject had nasal polyps or major nasal airway obstruction due to fixed anatomical abnormalities.

Isotonic phosphate-buffered saline (PBS), pH 7.4, was employed as the control solution (without phenol) in the ragweed priming experiments. Seven concentrations of aqueous short ragweed extract (SRW) ranging in threefold increments from 0.14 to 100 µg protein N/ml were prepared each test day from previously frozen aliquots of a 100-µg protein N/ml stock solution. The ragweed antigen E content assayed by a radioallergosorbent test (RAST) inhibition technique was 16.2 µg AgE/ml in the 100 µg protein N/ml solution.

Aerosol challenges were delivered from a DeVilbiss No.

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251 atomizer activated by compressed air at a pressure of 6 psi. The volume delivered ($0.1 \pm 10\%$ per spray) was controlled by a solenoid timing unit regulating the compressed air cylinder.⁴

Ammonia gas (99.5 ± 6.4 ppm) for challenge was delivered from an ammonia cylinder (Matheson Gas Co., Joliet, Ill.) at a pressure of 6 psi. The rated NH_3 concentration was verified in our laboratory by passing the gas into 0.1 N H_2SO_4 followed by analysis with Nessler's reagent against an NH_4Cl standard. Under the conditions employed flow was found to be 8.5 L/min as measured by a Fischer-Porter rotameter. This flow rate of 100 ppm delivered $0.55 \mu\text{M}$ NH_3 every 10 sec. Compressed air (Liquid Carbonic Corp., Chicago, Ill.) delivered at 6 psi was employed as the control gas in the NH_3 priming experiments.

Nasal airway resistance (NAR) was measured by posterior rhinometry as reported previously.^{5, 6} Briefly, nasal air flow was measured through a pneumotachograph attached to a mask covering the face. The pressure drop across the nose was estimated as the difference between the pressure in the oropharynx, sampled through a catheter held between the lips, and that simultaneously in the mask external to the nares. Using two differential pressure transducers, the pressure drop was displayed on the x-axis and flow on the y-axis of a calibrated Hewlett-Packard oscilloscope. NAR in centimeters H_2O /liter/second was calculated from the slope of this pressure-flow curve near 0 flow in expiration.

The data were analyzed in terms of change from daily baseline NAR for each dose of SRW or NH_3 on each test day. Postpriming response was compared with prepriming response by Student's paired t test. The trend in responsiveness was also analyzed by a two-sided sign test. A p value < 0.05 was considered statistically significant.

Experimental protocol

Ragweed priming to ragweed. Following a 30-min period of acclimatization of seated subjects to the temperature and humidity of the test room, NAR measurements were recorded eight times during a 15-min control period, and the mean of these values was used as the subjects' baseline NAR for that day. Then, in "single-blind" fashion, nasal challenges were initiated by aerosolization of 0.1 ml of PBS into each nostril, and NAR measurements were repeated every minute for 5 min and every 2 min for the succeeding 10 min. Following this, $0.14 \mu\text{g}$ protein N/ml SRW extract was administered in the same volume (i.e., 0.1 ml in each nostril) with subsequent NAR measurements at the same intervals as after PBS challenge. Additional test challenges with incremental threefold concentrations of SRW were made at 15-min intervals until NAR exceeded baseline by 200% or the maximal strength, i.e., $100 \mu\text{g}$ protein N/ml, of SRW aerosol was administered. Subjects were tested at approximately the same time of day on days 1, 2, 3, 4, 8, and 15 of a test sequence outside of the ragweed season.

NH_3 priming to NH_3 . At least 1 mo after completion of SRW priming, the subjects returned for testing with NH_3 according to a previously described procedure.⁷ After the initial period of acclimatization and measurement of

baseline NAR, the subjects received NH_3 at a concentration of 100 ppm (in air) into each nostril for a 5-sec period. This was accomplished by directing the gas from the storage tank at 6 psi into the orifice of one nostril while the subject voluntarily suspended respiration. After briefly shutting off the gas flow, NH_3 was introduced similarly into the opposite nostril. Then NAR was measured over a 15-min period as described previously. Subsequently, both nostrils received 10-, 15-, and 20-sec exposures to NH_3 at successive 15-min intervals. The final test in each series was a control challenge with compressed air delivered into each nostril for 20 sec at 6 psi. Subjects were tested in this manner on days 1, 2, 3, 4, and 8 of the NH_3 test sequence.

SRW priming to NH_3 . Approximately 1 yr after the SRW and NH_3 priming experiments the subjects returned for SRW priming to NH_3 . The first NH_3 challenge was given approximately 1 wk prior to 4 consecutive days of SRW priming. NH_3 challenge was repeated the day following SRW priming, i.e., day 5, and then again 1 wk later on day 12.

RESULTS

SRW priming to SRW

During sequential SRW exposures, the increase over mean baseline value of NAR after PBS control challenge was consistently less than 50%, and neither this response nor the baseline NAR changed significantly over the entire test period. However, the nine subjects did exhibit increasing NAR responsiveness and subjective worsening of allergic rhinitis symptoms (sneezing, rhinorrhea, nasal and oropharyngeal pruritus) over the 4-day challenge period at each test-dose level. Several made the unsolicited comment that they were more symptomatic with each successive testing session, and symptoms appeared more rapidly after challenge each day. However, none of the subjects manifested shortness of breath, chest tightness, or overt wheezing. By day 3, a statistically significant increase in NAR response was seen in the five subjects who received $1.23 \mu\text{g}$ protein N/ml SRW ($p < 0.05$ by paired Student's t test). By day 4, the mean response to $0.14 \mu\text{g}$ protein N/ml was 79% greater than day 1 ($p < 0.01$), while the response to $1.23 \mu\text{g}$ protein N/ml was 107% greater ($p < 0.05$) (Table I). Evaluation of higher doses was impossible as most subjects exceeded a 200% increase in NAR at $1.23 \mu\text{g}$ protein N/ml and consequently concluded the test at that dose.

An alternative method of data analysis, which provides a graphic representation of the results, is based on changes in the SRW concentration required to produce greater than a 200% increase in NAR.⁸ As seen in Fig. 1, there is a progressive shift towards a decreased threshold of reactivity from day 1 through day 4 with a return towards the original threshold on days 8 and 15. The increased reactivity at day 4 was sig-

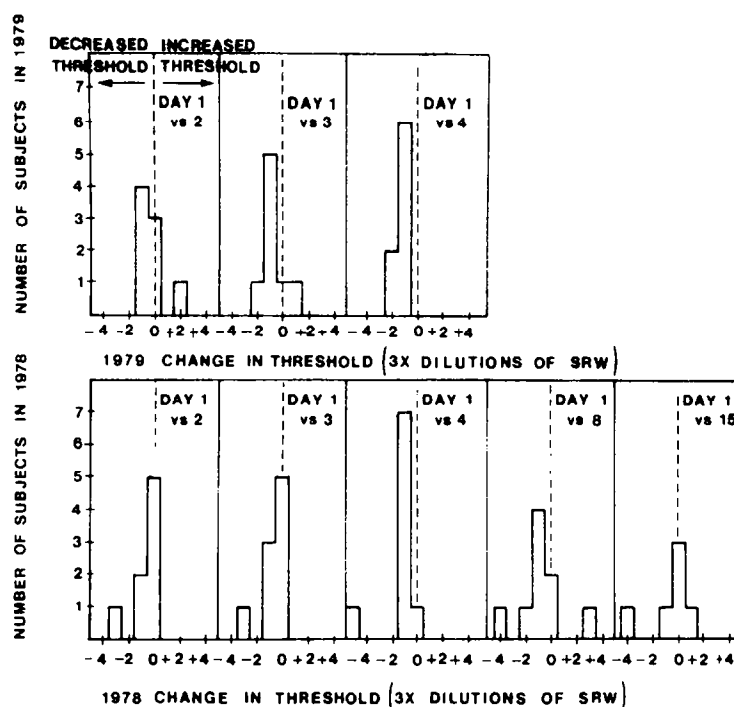


FIG. 1. Changes in dose of short ragweed (SRW) extract required to produce a threshold response (200% increase in nasal airways resistance [NAR]) determined by intranasal administration of threefold allergen dilutions at 15-min intervals. Lower panels show results obtained in 1978 and the upper panels show results obtained in 1979 in conjunction with attempted priming to NH_3 with SRW (see text, phase III). By two-sided sign test, the change in sensitivity between day 1 and day 4 was significant both in 1978 ($p = 0.01$) and 1979 ($p = 0.01$).

TABLE I. SRW priming to SRW: Change in NAR response on first vs fourth challenge day

	Baseline	PBS	SRW aerosol (μg protein N/ml)		
			0.14	0.41	1.23
No. of subjects tested	9	9	9	7	3
Mean Δ NAR (day 4 minus day 1)	-0.12	11.2%	79.0%	182.9%	107.0%
SD	± 1.71	$\pm 35.2\%$	$\pm 62.8\%$	$\pm 239.6\%$	$\pm 29.0\%$
p	>0.20	>0.20	<0.01	<0.10	<0.05

SRW: Short ragweed; NAR: Nasal airways resistance; PBS: phosphate-buffered saline.

nificant ($p = 0.01$ by sign test), and the lone subject who required the same threshold dose ($1.23 \mu\text{g}$ protein N/ml) responded with a 314% NAR increase on day 4 as compared with 209% on day 1. No subject was less responsive after 4 consecutive days of SRW challenge. Although the shift in threshold was not significant at day 8 or 15, on day 8 the reactivity at the lowest challenge dose ($0.14 \mu\text{g}$ protein N/ml) was still 103% greater than on day 1 (<0.05 by paired Student's t test).

NH_3 priming to NH_3

During attempted NH_3 priming, neither the baseline NAR nor the response to the compressed air control showed a statistically significant change. The

eight subjects (one had dropped out due to unrelated illness) exhibited some trend toward increased NAR responsiveness, which, however, did not achieve statistical significance (Table II). At 20 sec NH_3 /nostril, six subjects were more reactive by day 4 while two were less reactive (two-sided sign test, $p > 0.05$). The subjects complained of nasal congestion but had no rhinorrhea, sneezing, or pruritus; the level of subjective symptoms at each dosage level did not change over the test period. After the 3-day hiatus in NH_3 challenges, reactivity on day 8 again showed no significant increase in NAR responsiveness except after 20-sec exposure ($p < 0.05$), but the number of subjects who were more reactive was not significantly increased (two-sided sign test, $p > 0.05$).

TABLE II. NH₃ priming to NH₃: Change in NAR response first vs fourth challenge day

	Baseline NAR (cm H ₂ O/L/sec)	Ammonia				Compressed air
		5 sec	10 sec	15 sec	20 sec	20 sec
No. of subjects tested	8	8	8	8	8	8
Mean Δ NAR (day 4 minus day 1)	-0.58	3.0%	9.5%	25.6%	42.4%	3.6%
SD	± 0.76	$\pm 18.7\%$	$\pm 23.7\%$	$\pm 52.7\%$	$\pm 64.2\%$	$\pm 34.1\%$
p	>0.05	>0.20	>0.20	>0.10	>0.10	>0.20

NAR: Nasal airways resistance.

TABLE III. SRW priming to NH₃: Change in NAR response to NH₃ before vs after SRW priming

	Baseline NAR (cm H ₂ O/L/sec)	Ammonia				Compressed air
		5 sec	10 sec	15 sec	20 sec	20 sec
No. of subjects tested	8	8	8	8	8	8
Mean Δ NAR	0.63	18%	-11.5%	2.9%	-47.2%	3.2%
SD	± 1.70	$\pm 37.4\%$	$\pm 70.2\%$	$\pm 98.2\%$	$\pm 102.6\%$	$\pm 35.8\%$
p	>0.20	>0.20	>0.20	>0.20	>0.20	>0.20

SRW: Short ragweed; NAR: nasal airways resistance.

SRW priming to NH₃

The nine subjects responded similarly during the 4 days of SRW priming as in phase I, i.e., baseline NAR and PBS control reactivity were unchanged while NAR reactivity and subjective symptomatology increased from day 1 to day 4 (Fig. 1). The increase following 0.14 and 0.41 μ g protein N/ml on day 4 was significant ($p < 0.05$ and $p < 0.02$, respectively), and the change in threshold response on this date also was significant ($p = 0.01$ by two-sided sign test). In short, the 1979 results were in close agreement with the SRW priming data on the same subjects 1 yr earlier (Fig. 1). On the other hand, analysis of the change in responsiveness to NH₃ following SRW priming reveals that there was neither increased nor decreased sensitivity (Table III) compared with pre-SRW values elicited by NH₃.

DISCUSSION

The priming experiments with SRW confirm and extend the earlier observations of Connell¹⁻³; this phenomenon is reproducible, transient, and can be documented by significant changes in NAR response in addition to increasingly severe subjective symptoms following daily out-of-season exposure of sensitive subjects to SRW extract. Priming occurred in the absence of changes in baseline NAR or in responses to control PBS aerosols. In the present work priming was evoked with relatively large amounts of SRW extract aerosols, in contrast to Connell's experiments

utilizing small numbers of defatted SRW pollen grains.¹ The latter approach is more relevant to explaining the persistence of hay fever symptoms during waning pollen exposures toward the end of the season, though the recently observed *increased* nasal threshold reported by Schumacher and Pain a few weeks following the grass pollen season suggests that the priming effect from natural pollen exposures also is short-lived.⁸ On the other hand, our data imply that extraseasonal nasal challenge tests with extracts of allergens may lead to fallacious results if performed daily. Also, current attempts at intranasal immunotherapy may lead to aggravation of symptoms due to the priming effect if the treatment is given at frequent intervals. Indeed, in a recent trial of intranasal immunotherapy carried out by us with a polymerized ragweed extract, there was increased nasal reactivity following the preseasonal course of treatment, very likely representing a priming effect.⁹ The *decreased* nasal responsiveness reported by Taylor and Shivalkar¹⁰ followed a program in which grass pollen extract aerosols were administered only at 2- to 3-day intervals. In short, daily large doses of intranasal allergen are apt to produce priming, while less frequent administration of smaller doses may be associated with increased tolerance to an allergen.

Connell reported that biopsy specimens of the primed nasal mucosa showed multiple abnormalities including disruption of the basement membrane, decreased density of the ground substance and collagen,

eosinophilia, histiocytosis, an infiltrate of reticular cells and at times large foamy cells.¹¹ Although it is difficult to interpret these multiple abnormalities, data indicating the migration of mast cells into the nasal epithelium and basophils into the nasal secretions during natural pollen exposures, as recently reported by Hastie et al.,¹² would provide an explanation for priming if these changes also occur during experimental nasal challenges with allergens. Increased numbers of mast cells and basophils also could explain Connell's finding that priming is immunologically nonspecific. Connell suggested that disruption of the basement membrane also may play a pathogenetic role in permitting antigen to gain access to sensitized cells. However, local antibody formation is unlikely to be a significant factor because priming is rapid, transient, and immunologically nonspecific.

We had previously selected NH_3 as an irritant for nasal testing in view of its known chemical structure, capacity to be readily assayed, high water solubility (resulting in predominant upper airway removal), and well-defined clinical toxicology.⁷ Exposures up to 100 ppm for 30 sec in each nostril were felt to be quite acceptable from the standpoint of systemic toxicity when compared with the National Institute for Occupational Safety and Health's recommended standard of 50 ppm as a ceiling not to be exceeded for any prolonged period during an 8-hr workday. In the present work all subjects showed significant increases in NAR during both the first and subsequent days of challenging with NH_3 , these objective changes being regularly accompanied by subjective nasal congestion. However, the median increase in NAR after the 20-sec exposure to 100 ppm NH_3 in each nostril on the first test day was 61%, as compared with over a 200% increase in NAR being elicited with SRW. Therefore, it can not be determined from these data whether the failure to obtain significant priming with NH_3 reflects a qualitative difference between the capacity of allergens and irritants to produce priming or whether the results are due simply to quantitative differences in the challenging stimulus. The former possibility merits serious consideration in view of evidence indicating different mechanisms playing a role in allergen- and irritant-induced increases in NAR; for example, atropine but not chlorpheniramine inhibits the effects of NH_3 on NAR, while the converse is true of allergen-induced effects.⁷ It also may be relevant that the effects of NH_3 are more transient than those following pollen extract exposures, the former often disappearing in 5 min. Such observations also are in accord with the hypothesis that irritant effects are mediated through parasympathetic reflexes, while allergens may primarily induce mediator secretion in

the nose.⁷ In any case, the data clearly show that priming is not readily achieved by an irritant in concentrations sufficient to produce an easily discernible effect. Whether other irritants would produce similar results is conjectural at this time. Sulfur dioxide is known to increase NAR^{13, 14} but appears not to have been evaluated for a priming effect. Inert particulates, such as plastic dust containing carbon black,¹⁵ do not increase NAR in healthy subjects.

The failure of SRW exposures to prime the nasal mucosa for responsiveness to NH_3 may seem rather surprising in view of common clinical observations that patients notice increased nasal reactivity to a variety of irritants, such as cigarette smoke and industrial fumes, during their hay fever season. However, it should be noted that these clinical observations pertain to the simultaneous occurrence of allergic symptoms and irritant exposures, whereas in the experimental situation reported here priming for NH_3 reactivity was tested for (day 5) at a time when the previous symptoms from SRW exposure had subsided, baseline NAR and response to PBS both being at their initial levels observed on day 1. This is in contrast to the situation during the pollen season when Schumacher and Pain noted that increased nasal responses to pollen extracts were associated with increased baseline NAR and responsiveness to PBS.⁸ In addition, the previously noted dissimilarities in the mechanisms whereby allergens and irritants affect NAR may preclude one type of agent from priming for responsiveness to the other. However, the possibility can not be excluded that repeated extraseasonal pollen exposures might prime the nasal mucosa to a more powerful irritant stimulus or to a different type of irritant.

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