Rhinology, 40, 141-146, 2002

Cholinergic blockade does not alter the nasal congestive response to irritant provocation*

Dennis Shusterman[1], Mary-Alice Murphy[2], Paula Walsh[1], John R. Balmes[1]

[1] Division of Occupational and Environmental Medicine, University of California, San Francisco, U.S.A.
[2] Department of Pediatrics, University of California, San Francisco, U.S.A.

SUMMARY

Objective: To understand the mechanism underlying the nasal congestive response to irritant challenge.

Methods: We exposed 22 subjects – 8 with seasonal allergic rhinitis (SAR), 6 with perennial allergic rhinitis (PAR), and 8 normals – to chlorine (Cl_2) gas (1.0 ppm x 15 min.) by nasal CPAP mask. Control exposures (filtered air) were carried out on separate days, with counter-balancing of exposure order. Nasal airway resistance (NAR) was measured in triplicate before and after the provocation sequence using active posterior rhinomanometry. For each subject, this experiment was repeated twice, after [double-blinded] pre-treatment with: 1) ipratropium bromide (IB) 0.6% nasal spray, and 2) vehicle.

Results: As a group, allergic rhinitics (SAR + PAR) showed greater [Cl_2] exposure-related increases in NAR than did normals on placebo (vehicle) pretreatment days ($p < 0.05$). IB pre-treatment, however, did not have a systematic effect on Cl_2-induced congestion.

Conclusion: Cholinergic mechanisms do not appear to be responsible for the nasal congestive response to irritant provocation.

Key words: nasal irritation, nasal congestion, chlorine, cholinergic reflexes, ipratropium bromide

INTRODUCTION

Physical or chemical irritation of the upper respiratory tract may be associated with reflex responses, including nasal congestion and rhinorrhea (McLean et al., 1979; Widdicombe, 1990; Bascom et al., 1991; Kjaergaard et al., 1995; Shusterman et al., 1998). Experiments involving exposure of human volunteers to environmental tobacco smoke (ETS) suggest that non-allergic - including possible neurogenic - mechanisms may be responsible for the acute congestive response to chemical nasal irritation (Bascom et al., 1991). Potential neurogenic mechanisms include both central (autonomic) and local (axon) reflexes (Figure 1) (Bascom, 1992). For the irritant-induced nasal secretion, evidence is compelling that central (parasympathetic) reflexes are operative (Meltzer, 1992; Sanico and Togias, 1998). For irritant-related congestion, on the other hand, direct evidence regarding mechanism (which must act on the vascular compartment in order to affect airway caliber) is largely lacking.

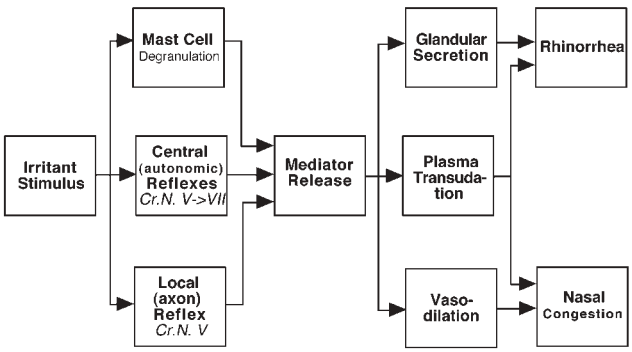

Figure 1. Potential mechanisms involved in acute reflex responses to nasal irritation (including nasal congestion and rhinorrhea).

We have previously shown that low-level chlorine (Cl_2) gas, administered by nasal mask, is an effective stimulus for nasal congestion, and that seasonal allergic rhinitic subjects are more responsive to this stimulus than are normal controls (Shusterman et al., 1998). We wished to build upon these findings in order to better understand the pathophysiology of irritant-related nasal congestion. The current study was designed to explore the role of autonomic (specifically, cholinergic) mecha-

*Received for publication: September 4, 2001; accepted: May 20, 2002

nisms in the congestive response to irritant provocation. To this end, the topical cholinergic blocker, ipratropium bromide (IB), was employed as a pharmacologic probe. IB was applied, alternating with placebo in a double-blinded manner, prior to nasal irritant provocation with Cl_2 gas, examining the effect, if any, of this pre-treatment on irritant-related nasal congestion.

MATERIALS AND METHODS

Experimental design

The provocation experiment – which was repeated twice on each subject after double-blinded pretreatment with either IB or placebo – consisted of a randomized, cross-over design comparing the response of allergic rhinitic (AR) and non-rhinitic (NR) subjects to a 15-minute controlled exposure to dilute Cl_2 by nasal mask. The physiologic endpoint of interest was nasal airway resistance (NAR), measured before pretreatment, after pretreatment, immediately after exposure, and again 15 minutes after exposure. Exposures consist of either pure air (control conditions) or chlorine (1.0 ppm) diluted in air. Cl_2 and air exposures took place on separate days, roughly one week apart. The study was counterbalanced with respect to order of exposure and subject gender (Figure 2).

Subjects

Subjects were recruited through posters and newspaper advertisements. Inclusion criteria were: age 18-40 yrs. and "general good health"; exclusion criteria were: 1) a history of asthma, 2) cigarette smoking (active or within previous 6 months), 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). After completion of a screening questionnaire, subjects read and signed an informed consent document

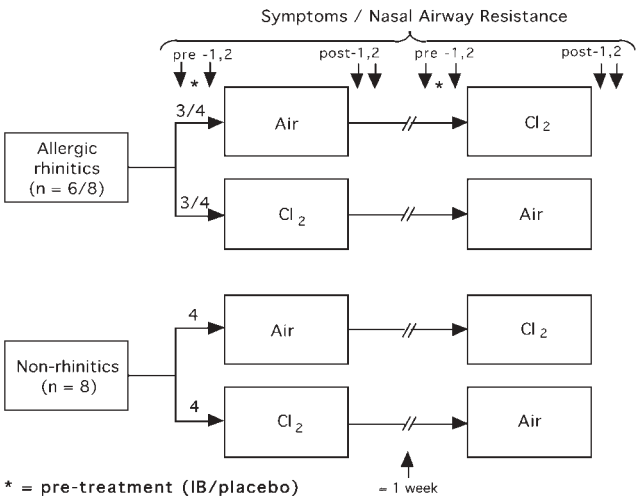

Figure 2. Counterbalanced, cross-over design of chlorine-provocation experiment. Experiment was repeated twice, on a double-blinded basis, after pre-treatment with either ipraropium bromide 0.6% (IB) or placebo (vehicle) nasal spray, 0.2 mL/nostril.

approved by the Committee on Human Research of the University of California, San Francisco. Detailed questionnaires were then administered to each potential subject, who was then provisionally classified as having seasonal allergic rhinitis (SAR), perennial allergic rhinitis (PAR), no rhinitis (NR), or "other" based upon questionnaire responses.

Allergy skin prick tests

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 pruritis, rhinorrhea, post-nasal 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" is defined as a wheal reaction to skin-prick testing with a diameter $\geq p$ the histamine control.) "Perennial allergic rhinitics" were defined as subjects with year-around symptoms who had predominant skin test reactivity to dust mites, molds, pet danders or cockroach antigen(s). "Non-rhinitics" were defined as subjects who report, at most, infrequent nasal symptoms, without identified seasonal variation or precipitants, and with significant skin test reactivity to no more than one agent in the panel of 13 aeroallergens. Prior to skin testing, subjects were asked to refrain from taking antihistamines for 72 hours (hydroxyzine for 3 weeks, astemizole for 12 weeks).

Heading? Irritant challenge

SAR subjects were tested outside of their relevant pollen season. All subjects were asked to avoid exercising, consumption of spicy foods, or use of scented cosmetics on the day of testing. In addition to the above antihistamine preclusions, subjects were asked to avoid using nasal steroids for at least 2 weeks, and nasal decongestants for at least 48 hours prior to testing. Upon arrival at the laboratory, subjects entered a climate-controlled chamber (22 ± 1°C, 40 ± 3% RH) with filtered air (activated charcoal and high-efficiency particulate). After a 15-min waiting period, baseline symptoms (nasal irritation, nasal congestion, rhinorrhea, post-nasal drip, and odor) were rated on a visual analog scale using a computer mouse (Performa 6115CD computer, Apple Computers, Cupertino, CA; LabView® software, National Instruments, Austin, TX). The scale was 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. Baseline nasal airway resistance (NAR) was then obtained in triplicate via the technique of active posterior rhinomanometry using a commercial instrument (Model NR6-2, GM Instruments, Kilwinnig, UK). The rhinomanometer was calibrated on a daily basis; the pressure channel to a tolerance of ± 3% using a Model 405 incline manometer (Airflow Developments, Inc., High Wycombe, GB), and flow to a tolerance of ± 5% with a Model 235 flow meter (Cole-

Parmer/Gilmont Instruments, Vernon Hills, IL). Finally, baseline pulmonary peak flow was obtained in triplicate using a hand-held peak flow meter (Wright Peak Flow Mini-Meter, Clement Clarke International, Ltd., UK).

After rating baseline symptoms and having baseline rhinomanometry and pulmonary peak flow measurements, subjects were administered either IB or placebo (vehicle) on a double-blinded basis. Both agents were administered by aerosol spray unit (No. 34478, Pfeiffer of America, Princeton, NJ), consisting of 3 sprays of 0.067 mL each (total volume, 0.20 ± 0.01 mL/nostril). Fifteen min later, symptom rating, rhinomanometry, and pulmonary peak flow were repeated.

The investigator then stepped behind a screen and adjusted the breathing mixture for the nasal mask assembly. The chlorine dilution apparatus blended compressed medical-grade air and compressed chlorine diluted to 10 ppm in medical-grade air (Nellcor Puritan-Bennett, San Ramon, CA) in a stainless steel mixing chamber (Model FMX7311: Omega Engineering, Stamford, CT). Diluent air was pre-conditioned to 22°C and 40% RH using a Model 009700 humidifier-heater (Intertech Corporation, Bannockburn, IL). Immediately downstream from the mixing chamber was the sampling port for an electrochemical chlorine monitor (Model 1340; Interscan Corp., Chatsworth, CA), which continuously sampled the gas mixture and fed its output to a strip chart recorder (Model 1200; Linear Instruments, Inc., Irvine, CA). The gas mixture was conveyed to the subject with 2.5 cm diameter corrugated respiratory tubing, connected by T-piece to a nasal CPAP* mask (Series 3121; Respironics, Inc., Murraysville, PA), which was sized according to the individual subject. The second limb of the T-piece connected to a low-pressure scavenger system, which led to an exhaust outside of the chamber and building. The combination of a high flow rate (60 l/min) and the scavenger system allowed subjects to breathe with negligible superimposed pressure or resistance. The chlorine meter was re-calibrated on a daily basis using the [certified] contents of the chlorine cylinder as the standard.

The 15-minute exposure period via nasal mask took place on a single-blinded basis, and the order-of-presentation was subject to limited randomization (within the constraints of the counterbalanced study design). Immediately after cessation of exposure – and then again 15 minutes later – the investigator asked subjects to re-rate nasal symptoms using visual analog scales. NAR was re-measured in triplicate after each symptom rating session, and finally, pulmonary peak flow was re-assessed.

Statistics

Statistical analysis was performed using JMP (SAS Institute, Carey, NC). The hypotheses tested were: 1) that seasonal allergic rhinitic subjects would show a significantly greater increase in NAR over baseline (comparing chlorine- vs. air-exposure days); and 2) that pretreatment with IB would reduce or eliminate this difference. Data were first examined for normality, and either a paired Student's *t* or a paired non-parametric test (Wilcoxon signed-ranks) utilized as appropriate. Subjects acted as their own controls in paired group analysis of NAR changes on Cl_2 exposure vs. air days. Results were then compared for IB- vs. vehicle (placebo) pretreatmet.

* CPAP = Continuous positive airway pressure

RESULTS

A total of 22 subjects were enrolled, consisting of 8 seasonal allergic rhinitics, 6 perennial allergic rhinitics, and 8 non-rhinitic controls. Each group was evenly divided by gender. The mean age for each subgroup was 27.8, 28.0, and 29.3 years, respectively.

Individual rhinomanometry data appear in Table 1. Because of small subgroup size and power considerations, SAR and PAR subjects were combined for purposes of analysis of rhinomanometry data. For baseline NAR values, there was no significant effect of pretreatment with either IB or placebo (data not shown). As a consequence, baseline values for the NAR and symptom analyses were taken *after* pretreatment with either IB or vehicle. Nasal provocation with dilute (1.0 ppm) Cl_2 for 15 min. produced a significantly greater congestive response among allergic rhinitic (AR) subjects than among nonrhinitic controls ($p < 0.05$ for three of four testing conditions - Table 2). With the exception of a single individual (subject # 20), pretreatment with IB (0.06%; 0.2 mL/nostril) 15 min. prior to Cl_2 challenge did not significantly alter the magnitude of reflex congestion among AR subjects (treatment effect $p = 0.98$ immediately post-exposure, and $p = 0.49$ at 15 min. post-exposure).

Subjectively, subjects reported modest increases in odor and nasal irritation ratings from baseline to post-chlorine exposure, regardless of diagnostic subgroup or pre-treatment status (Table 3). Pooling data across subjects, significant increases in self-rated odor and irritation were apparent immediately post-exposure for both IB and placebo pretreatment days. There were no significant changes in self-rated nasal congestion post-exposure. Interestingly, there was a trend toward *decreasing* rhinorrhea and post-nasal drip ratings after either Cl_2 or air provocation, unrelated to pre-treatment status (i.e., IB vs. placebo). No systematic changes in pulmonary peak flow were observed after either Cl_2 or air exposure (data not shown).

DISCUSSION

Reflex nasal congestion has been documented in response to irritant provocation with ammonia (NH_3), sidestream tobacco smoke (STS), Cl_2, and mixed volatile organic compounds (VOCs) (McLean et al., 1979; Bascom et al., 1991; Kjaergaard et al., 1995; Shusterman et al., 1998). Given the lack of markers of mast cell degranulation noted after STS provocation, Bascom (1992) hypothesized that neurogenic mechanisms – including central (autonomic) and local (axon) reflexes – may be responsible for the congestive response to nasal irritation. Direct evidence on this issue is largely lacking. With regard to central reflexes, however, McLean and colleagues (1979) pre-

Table 1. Individual Rhinomanometry Data (Pa/L/s).

				NAR: Chlorine Trial			NAR: Air Trial		
Subject No.	Rhinitis	Gender	Treatment	Baseline	Post-1	Post-2	Baseline	Post-1	Post-2
1	SAR	Female	IB	275	229	213	294	260	232
			Placebo	379	319	358	334	252	261
2	SAR	Male	IB	259	264	360	311	307	325
			Placebo	314	380	356	265	311	311
3	PAR	Male	IB	231	251	264	258	261	203
			Placebo	178	209	229	211	221	195
4	SAR	Female	IB	196	232	253	205	237	246
			Placebo	305	349	499	242	266	259
5	PAR	Female	IB	308	273	287	283	319	337
			Placebo	231	240	207	234	203	235
6	SAR	Male	IB	465	705	774	354	379	418
			Placebo	452	532	501	300	352	295
7	NR	Female	IB	340	300	293	284	250	247
			Placebo	319	288	236	336	284	325
8	NR	Female	IB	161	162	192	186	173	225
			Placebo	186	196	214	205	202	222
9	SAR	Male	IB	139	127	121	137	144	144
			Placebo	266	197	197	205	182	163
10	SAR	Female	IB	257	250	255	264	280	255
			Placebo	303	304	295	301	253	240
11	PAR	Female	IB	340	658	496	522	521	504
			Placebo	469	1015	750	439	385	428
12	NR	Male	IB	228	234	222	250	274	255
			Placebo	266	237	218	206	234	219
13	NR	Male	IB	200	206	221	206	182	197
			Placebo	189	200	201	186	191	179
14	NR	Male	IB	274	284	325	262	319	327
			Placebo	225	242	261	211	234	257
15	NR	Male	IB	284	274	329	194	199	318
			Placebo	207	255	277	222	292	327
16	NR	Female	IB	173	164	178	168	180	167
			Placebo	124	130	141	156	160	166
17	NR	Female	IB	236	226	278	252	223	241
			Placebo	228	269	260	218	267	298
18	PAR	Male	IB	251	274	293	238	229	231
			Placebo	236	218	225	257	259	254
19	PAR	Female	IB	186	193	224	204	197	197
			Placebo	190	197	184	191	206	207
20	SAR	Male	IB	496	562	795	374	465	412
			Placebo	447	1035	3402	728	1098	1038
21	SAR	Female	IB	226	347	384	336	367	355
			Placebo	264	314	294	339	373	335
22	PAR	Male	IB	272	808	736	269	327	265
			Placebo	322	448	341	453	485	419

SAR = Seasonal allergic rhinitic
PAR = Perennial allergic rhinitic
NR = Non-rhinitic

Post-1 = Immediately post-exposure
Post-2 = 15 min post-exposure

treated subjects with atropine (a cholinergic blocker) prior to NH_3 challenge, and did *not* note a systematic effect on irritant-related congestion.

In this experiment, we again found differential sensitivity to irritant (Cl_2) provocation among allergic rhinitic vs. non-rhinitic subjects. Specifically, rhinitics alone showed significant congestive response, both immediately – and 15 min. after – irritant provocation. Pre-treatment with IB did not materially alter this response. This suggests that any parasympathetic reflex response elicited by Cl_2 exposure did not have a significant effect upon the vascular compartment of the nose (which

Table 2. Mean Change in Nasal Airway Resistance, Chlorine Minus Air Trial (Pa/L/s ± SEM).

Subgroup	Pretreatment	Baseline to Post-1	Baseline to Post-2
Allergic rhinitic	Ipratropium Bromide	+ 73.4 ± 41.8	+ 105.6 ± 143.3 *
	Placebo (vehicle)	+ 75.3 ± 43.6 *	+ 238.6 ± 186.6 *
Non-rhinitic	Ipratropium Bromide	- 5.5 ± 8.9	- 4.1 ± 13.6
	Placebo (vehicle)	- 6.4 ± 8.6	- 23.6 ± 12.4

"Post-1" = Immediately post-exposure; "Post-2" = 15 min. post-exposure
* $p < 0.05$, baseline to post-exposure, rhinitics vs. controls

is believed to be responsible for nasal congestion) (Widdicombe, 1990). Objective congestion, on the other hand, occurred with modest subjective nasal irritation, suggesting that the reflex mechanism involved is sensitive to relatively low-level irritant stimulation.

Our findings are consistent with previous work, including both agonist and antagonist studies, suggesting that cholinergic stimulation affects nasal glandular secretion to a greater degree than it does vascular function and upper airway caliber (McLean et al, 1979; Gerth van Wijk and Dieges, 1994). The specific mechanism of irritant-induced nasal congestion is unknown, but based upon work to-date, neither parasympathetic reflexes nor mast cell degranulation appear to be likely explanations. Another candidate mechanism involves the axon reflex, with release of vasoactive peptides (including substance P) locally. Our future studies will address this possibility in a more direct fashion.

ACKNOWLEDGEMENTS

This work was supported by the National Institute on Deafness and Other Communication Disorders (DC K23 00121) and the National Institute of Environmental Health Sciences (ES R01 10424). Funding for formulation of ipratropium bromide and placebo was received in the form of an unrestricted gift from Boehringer-Ingelheim Pharmaceuticals. Aerosol spray delivery units were kindly provided by Pfeiffer of America. The authors also wish to thank Joanne Whitney of the UCSF Drug Product Services Laboratory for her expert technical assistance.

Table 3. Mean Chlorine-related Symptoms (0 to 5 scale, immediately post-exposure) by Diagnostic Subgroup and Pre-treatment Status.

Subgroup	**Pre-Treatment**	**Rating Time**	**Odor**	**Irritation**	**Congestion**	**Rhinorrhea**	**Post-Nasal Drip**
Seasonal Allergic Rhinitic (n = 8)	IB	Pre-	0.05	0.10	0.36	0.57	1.26
		Post-	1.48**	0.36	0.64	0.10	0.48*
	Placebo	Pre-	0.12	0.34	0.84	0.59	1.05
		Post-	0.95*	0.44	1.02	0.15	0.35*
Perennial Allergic Rhinitic (n = 6)	IB	Pre-	0.44	0.36	0.62	0.34	0.61
		Post-	1.53	1.00	1.05	0.12	0.37
	Placebo	Pre-	0.05	0.85	1.20	0.75	0.95
		Post-	1.43*	1.07	1.24	0.19	0.27
Non-rhinitic (n = 8)	IB	Pre-	0.01	0.23	0.28	0.04	0.61
		Post-	1.33**	0.71*	0.03	0.04	0.20
	Placebo	Pre-	0.01	0.17	0.19	0.01	0.29
		Post-	1.30**	0.55*	0.32	0.01	0.21
COMBINED SUBGROUPS	IB	Pre-	0.14	0.22	0.40	0.31	0.85
		Post-	1.44***	0.66***	0.53	0.08*	0.35**
	Placebo	Pre-	0.06	0.33	0.70	0.42	0.75
		Post-	1.21***	0.57**	0.83	0.11	0.28**
COMBINED SUBGROUPS + PRETREATMENT GROUPS		Pre-	0.10	0.27	0.55	0.37	0.80
		Post-	1.32***	0.61***	0.68	0.10**	0.31***

* $p < 0.05$ (pre- to post-exposure)
** $p < 0.01$
*** $p < 0.001$

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Dennis Shusterman, MD, MPH
Upper Airway Biology Laboratory
University of California, San Francisco
1301 So. 46th Street, Bldg. 112
Richmond, CA 94804
USA

Tel: +1-510-231-9551
Fax: +1-510-231-5636
E-mail: dennis@itsa.ucsf.edu