Exhaled Nitric Oxide and Thermally Induced Asthma

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The purpose of the present study was to determine if nitric oxide (NO) is involved in the pathogenesis of thermally induced asthma. To provide data on this issue, 10 normal and 13 asthmatic subjects performed isocapnic hyperventilation with frigid air while the fractional concentration of NO in the expirate air (F_ENO) was serially monitored with a chemiluminescence analyzer. FEV_1 was measured before and after hyperpnea. Prior to and throughout the challenge, the asthmatics had significantly larger values for F_ENO (baseline F_ENO normal, 11 ± 2 ppb; asthma, 16 ± 1; p = 0.03). Posthyperpnea, the normal subjects had little change in bronchial caliber (ΔFEV_1 baseline to 5 min posthyperpnea, −3.5 ± 1.5%; p = 0.06), whereas the patients with asthma developed significant airway obstruction (ΔFEV_1, −27.7 ± 2.9%; p = 0.0001). During hyperventilation, the volume of NO rose in both groups. The asthmatic subjects, however, generated approximately 55% more NO/min than did the normal control subjects even though their level of ventilation was approximately 66% less. In contrast to the normal subjects, NO production in the asthmatics continued into the recovery period after the challenge stopped and F_ENO rose temporally as the airflow limitation developed. These results suggest that NO plays an intimate role in the development of airway obstruction that follows hyperpnea.

Nitric oxide (NO) is a ubiquitous molecule that plays a vital role in airway and vascular physiology (1, 2). In addition to assisting in the maintenance of normal homeostasis, there is a growing body of information that this compound may be involved in the functional abnormalities seen in asthma. Asymptomatic patients with this illness have higher endogenous quantities of NO in their expired air than do normal control subjects (3, 4), and the levels rise and fall in response to events known to augment and attenuate the severity of the disease (5–7).

One situation where NO may be particularly important is in the pathogenesis of thermally induced asthma. It has been postulated that this condition derives from an aberration in the regulation of the bronchial circulation and that the cycle of airway cooling and rewarming that occurs during hyperpnea and recovery increases the permeability of a persistently inflamed vascular bed (8, 9). Because nitric oxide synthase expression has been identified in multiple elements of the tracheobronchial tree (i.e., the airway epithelium, the vascular endothelium, and the sensory nervous system) and because NO has profound effects on airway and vasomotor tone, it may be a candidate to mediate such a phenomenon (1, 2, 10). If this reasoning is valid, there should be a temporal relationship between the fractional concentration of NO in the exhaled air and the development of airflow limitation. Further, since this condition only occurs clinically in asthmatics, there should also be major differences in NO kinetics between them and normal persons. The present study was undertaken to evaluate these possibilities.

METHODS

Subjects

Ten normal control subjects (four male and six female with a mean age of 33 ± 3 SEM yr) and 13 asthmatic patients (six male and seven female with a mean age of 29 ± 2 yr) served as our subjects (Table 1). Thermally induced asthma was considered to be present if there were symptoms of airway obstruction after exertion that were associated with a decrease in FEV_1 of 15% or more. None of the subjects smoked and none had symptoms of an upper respiratory tract infection during the 6 wk preceding the study. The asthmatic participants did not use oral corticosteroids or leukotriene-modifying drugs. Five asthmatic subjects received inhaled corticosteroids. The dose was stable for a minimum of 1 mo prior to the study. All bronchodilators were withheld for 12 h or more and long-acting decongestants and antihistamine compounds were not permitted for 5 d prior to the investigation. The normal subjects were not receiving any medications. The institutional review board for human investigation approved the protocol, and all participants gave informed consent.

Bronchoprovocations

Isocapnic hyperventilation (HV) was used as a surrogate for exercise (8, 9), and it was performed at progressively increasing levels of minute ventilation ($\dot{V}_E$) while inhaling frigid air through a heat exchanger (Figure 1). Recovery took place while breathing room air. The water content of the inspirate during hyperpnea was less than 1 mg H_2O/L, which, for the purposes of this study, was considered to be zero. Each bout of hyperventilation lasted 4 min. In the asthmatic subjects, the provocation was stopped when the FEV_1 decreased ⩾ 15% from the prechallenge values. The $\dot{V}_E$ at this point was then used in the studies on NO production. The maximum interval between challenges was 2 d. Because bronchial narrowing did not develop in the normal subjects, the highest ventilation that each participant achieved was employed in subsequent trials.

The expired air was directed away from the heat exchanger into a reservoir balloon that was being constantly evacuated at a known rate through a calibrated rotameter into a dry gas meter (Figure 1) (8, 9). The subjects were coached to keep the balloon filled, and in so doing, their $\dot{V}_E$ could be controlled at any desired value. The level of ventilation was then verified directly with the dry gas meter. End-tidal CO_2 ($P_{ET_{CO_2}}$) concentrations during hyperventilation were monitored with a Nellcor N-1000 analyzer (Mallinckrodt, Inc., Kansas City, KS) and sufficient CO_2 was added to the inspiratory port of the exchanger to maintain $P_{ET_{CO_2}}$ at eucapnic levels.

Nitric Oxide Measurements

Nitric oxide was measured using published recommendations (Figure 1) (11). The subjects exhaled against a fixed resistance of 5 cm of H_2O built into the mouthpiece to exclude nasal NO (11). The fractional concentration of NO in the expired gas (F_ENO) was recorded with a chemiluminescence NO analyzer designed for physiologic measurements (CLD 77 AM; ECO Physics, Inc., Ann Arbor, MI). The system was calibrated with NO free gas and a standard NO concentration of 15 parts per million (ppm) (Praxair, Inc., Bethlehem, PA) daily before use. The linearity of the analyzer was verified with analytically certi-

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TABLE 1

BASELINE MORPHOMETRIC AND PHYSIOLOGIC DATA IN NORMAL AND IN ASTHMATIC SUBJECTS

Subject No.	Age (*yr*)	Sex	FEV_1 (*L*)	FENO*
Normal subjects				
1	42	F	4.02	18
2	44	F	2.84	3
3	31	F	3.14	6
4	37	M	4.25	5
5	40	M	4.86	24
6	29	F	3.69	9
7	25	F	2.75	15
8	29	F	3.21	10
9	23	M	3.83	13
10	32	M	4.76	6
Mean	32	6F/4M	3.74	11
SEM	3	—	0.25	2
Asthmatic subjects				
1	30	M	4.10	11
2	44	M	3.34	20
3	36	M	2.20	22
4	34	F	2.45	18
5	22	F	2.48	9
6	46	F	2.19	13
7	29	M	2.66	22
8	27	F	2.11	14
9	27	M	2.76	15
10	26	F	3.02	15
11	28	F	2.51	22
12	21	M	3.10	16
13	31	F	2.64	11
Mean	29	7F/6M	2.74	16
SEM	2	—	0.16	1

* Fractional expired concentration of nitric oxide in parts per billion.

fied gases of 0 and 200 ppb NO (Scott Specialty Gases, Inc., Plumsteadville, PA). The detection limit was 1.1 ± 0.2 ppb (coefficient of variation < 1%). Ambient NO levels were recorded at the start and end of each experiment. During the studies, samples were drawn continuously from the expired port of the mouthpiece at a rate of 300 ml/min during resting tidal breathing and hyperventilation. The output of the analyzer was fed into a time-based recorder (Omega Engineering Inc., Stamford, CT) for on-line display. The 100% response time of the instrument complex (analyzer, sample tubing, and recorder) to a square wave of NO was 320 ms. During hyperpnea, the FENO was determined from the peak phase of each breath and expressed as an average for each minute. During resting tidal breathing, the plateau was taken as the FENO. The output of NO/min ($\dot{V}_{NO}$) was calculated as the product of the FENO × $\dot{V}_E$.

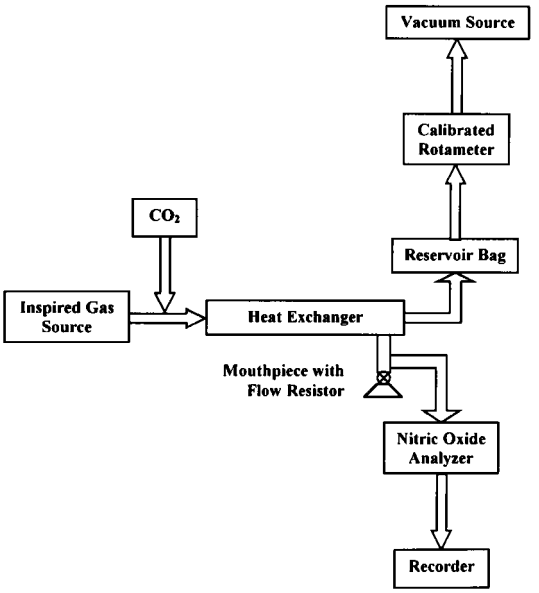

Figure 1. Schematic representation of the experimental setup.

Lung Function Measurements

Maximum forced exhalations were performed in triplicate using a waterless spirometer before and 5 min after cessation of each bout of hyperpnea. The curves with the largest FEV_1 were chosen for analysis.

Experimental Protocol

After the screening phase was completed, the subjects returned to the laboratory where the main study commenced. After 5 min of quiet tidal breathing, they were exposed to the previously determined maximum levels of ventilation for 4 min. The fractional concentration of NO in the expired air was continuously recorded before, during, and for 30 min after hyperpnea. Spirometry was measured as above.

Statistical Analysis

The data were analyzed by paired and unpaired *t* tests, and a multifactorial repeated measure analysis of variance. The $\dot{V}_{NO}$ data were logarithmically transformed to meet the assumptions of the ANOVA model. All statistical tests were two-sided, and a p value < 0.05 was considered significant.

RESULTS

The individual prechallenge values for the relevant physiologic variables are displayed in Table 1 and the mean data are

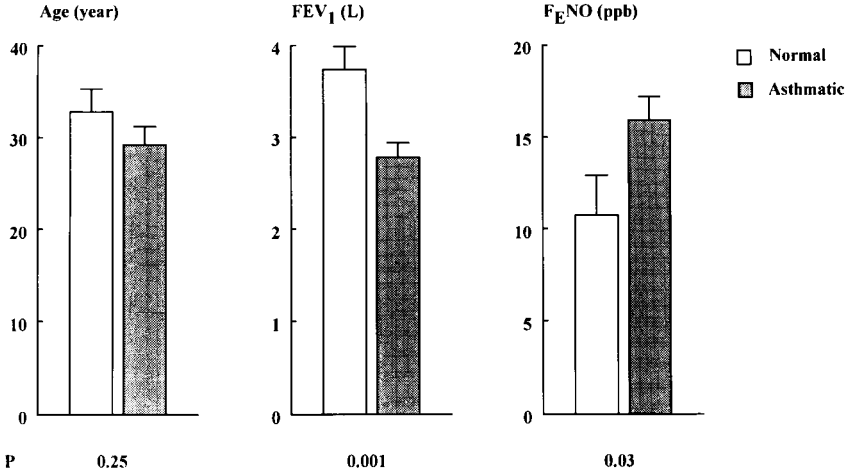

Figure 2. Comparison of baseline physiologic variables in normal and in asthmatic subjects. The heights of the bars represent mean values, and the brackets indicate one standard error of the mean. FENO = fractional concentration of expired nitric oxide. $\dot{V}_{NO}$ = nitric oxide production. The p values below each graph indicate comparisons between the normal and the asthmatic groups.

TABLE 2

INSPIRED TEMPERATURES, MINUTE VENTILATIONS, AND CHANGES IN LUNG FUNCTION IN NORMAL AND IN ASTHMATIC SUBJECTS WITH HYPERPNEA

Subject No.	Ti (°C)	$\dot{V}_E$ (*L/min*)	ΔFEV_1
Normal subjects			
1	−2.6	90	−0.3
2	−1.0	60	−6.0
3	−2.6	80	−1.3
4	−3.4	80	1.8
5	−35.0	100	−5.3
6	−28.1	80	−12.0
7	−38.0	80	−4.0
8	−34.2	80	−7.2
9	−19.8	80	−3.9
10	−16.7	100	3.4
Mean	−18.1	83	−3.5
SEM	5.0	4	1.5
Asthmatic subjects			
1	−1.7	60	−31.0
2	−1.7	80	−15.3
3	−3.4	80	−16.8
4	−0.5	60	−23.7
5	−2.1	80	−28.2
6	−35.7	80	−23.3
7	−26.6	20	−34.6
8	−32.0	60	−21.3
9	−35.0	40	−28.6
10	−30.4	60	−19.9
11	−40.3	40	−44.2
12	−26.6	20	−49.7
13	−22.8	40	−23.5
Mean	−19.9	55	−27.7
SEM	4.5	7	2.9

Definition of abbreviations: Ti = temperature of the inspirate in °C; $\dot{V}_E$ = minute ventilation during hyperpnea in L/min; ΔFEV_1 = percent change in the one-second forced expiratory volume.

compared in Figure 2. The average FEV_1 was significantly larger in the normal control subjects than in the asthmatics (3.75 ± 0.25 L [107 ± 4% of predicted] versus 2.74 ± 0.16 L [81 ± 5% of predicted], p = 0.001). Conversely, the asthmatics had higher values for the FeNO (asthma, 16 ± 1; normal, 11 ± 2 ppb; p = 0.03).

During hyperventilation, the mean temperature of the inspired air ranged between −18 ± 5 and −20 ± 5° C (p = 0.78) (Table 2 and Figure 3). Minute ventilations averaged 83.0 ± 3.9 L/min in the normal subjects and 55.4 ± 6.3 L/min in the asthmatic group, respectively (p = 0.002). As expected, the normal subjects had little change in bronchial caliber postchallenge (ΔFEV_1 baseline to 5 min posthyperpnea, 3.5 ± 1.5%; p = 0.06), whereas the asthmatic subjects developed significant airway obstruction (ΔFEV_1, −27.7 ± 2.9%; p = 0.0001). The difference between groups was highly significant (p < 0.001).

The pattern of NO kinetics that accompanied the thermal provocations is presented in Figure 4. The individual values for FeNO are shown in Table 3. In the normal control subjects, $\dot{V}_E$ rose from its resting level to its maximum in 1 min, remained constant until hyperpnea ceased, and then fell sharply towards its prechallenge value (Figure 4A). Although the magnitude of ventilation was less in the asthmatics, the overall pattern of events was similar. There were no between-group differences in $\dot{V}_E$ during the recovery period. The respiratory rates were similar before, during, and after hyperpnea (before: normal, 15 ± 1 breaths/min; asthma, 16 ± 1; p = 0.46; during: normal, 50 ± 2; asthma, 45 ± 3; p = 0.09; after: normal, 16 ± 2; asthma, 17 ± 1; p = 0.50).

The FeNO was significantly higher in the asthmatics at all time points (p < 0.05 for all comparisons) (Figure 4B). When the asthmatic subjects hyperventilated, the FeNO decreased and then rose rapidly when the challenge stopped. By the second minute of the recovery period, it reached baseline values (p = 0.63) and exceeded them at 5 min (p = 0.02). Ten minutes after the challenge, FeNO returned to the levels seen before the provocation. The FeNO also fell during hyperventilation in the normal control subjects, but the posthyperpnea overshoot did not occur (p < 0.001 versus asthma). Instead, the levels rapidly returned to baseline and remained there.

The changes in $\dot{V}_{NO}$ are presented in panel C of Figure 4. Even though $\dot{V}_E$ was approximately 66% less in the asthmatic subjects than in the normal subjects, they exhaled approximately 55% more NO during each minute of hyperpnea (p < 0.001). The increased production in the recovery period in the asthmatics is also reflected in the much slower rate of decline in $\dot{V}_{NO}$ for minutes 1 through 5 (minute 1 asthmatic versus normal, p = 0.006; minute 5, p = 0.0001).

There were no significant differences in the concentration of NO in the ambient air from the beginning to the end of any challenge (grand mean: before, 16 ± 4 ppb; after, 15 ± 4 ppb; p = 0.57; n = 21) or between the asthmatic and normal groups (prechallenge, p = 0.30; postchallenge, p = 0.30).

DISCUSSION

The results of the present study demonstrate that NO appears to play an important role in the pathogenesis of thermally induced asthma. The asthmatic subjects exhale almost twice as

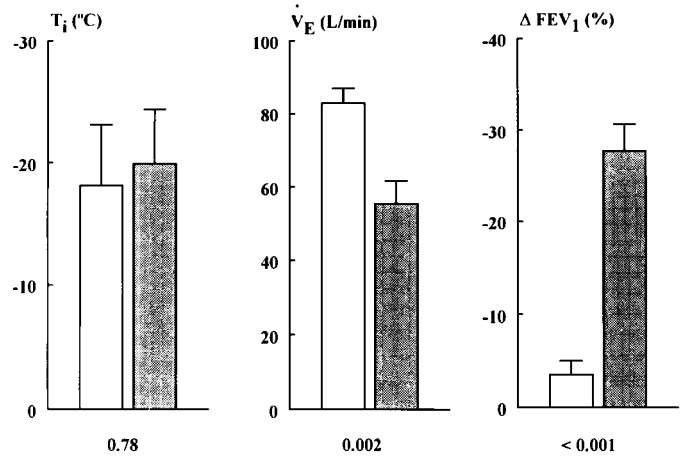

Figure 3. Inspired temperatures (Ti), minute ventilations ($\dot{V}_E$) and lung function in normal and in asthmatic subjects. The heights of the bars represent mean values and the brackets indicate one standard error of the mean. ΔFEV_1 = the postchallenge decrement from baseline FEV_1. The p values below each graph indicate comparisons between the normal and the asthmatic groups.

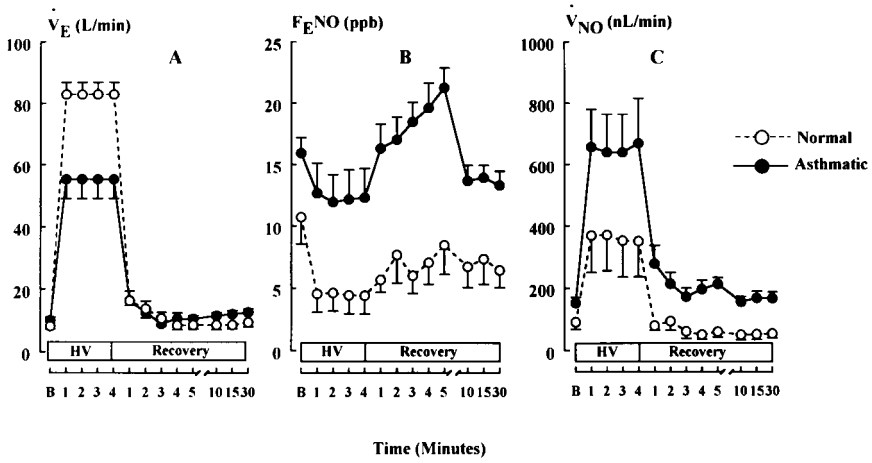

Figure 4. Minute ventilation (*panel A*) and measurements of exhaled nitric oxide (*panels B* and *C*) in normal and in asthmatic subjects before, during, and after hyperpnea. The data points represent mean values and the brackets one standard error of the mean. $\dot{V}_E$ = minute ventilation. FENO = fractional concentration of expired nitric oxide. $\dot{V}_{NO}$ = nitric oxide production. B indicates the baseline data. The rectangles marked HV indicate the 4 min spent hyperventilating. The adjacent rectangles indicate the time points at which observations were made in the recovery period.

much NO during hyperpnea for each minute of ventilation as do the normal subjects indicating that more of the molecule is generated and/or released during airway cooling. Equally importantly, unlike the control subjects, increased production of NO continues in the asthmatics as airflow limitation develops. Because the exhaled concentration of NO does not rise after the acute exposure to antigen (12) or other stimuli thought to be important in asthma pathogenesis (13), our findings strongly suggest that this compound may be one of the biochemical signals by which thermal events are translated into airway narrowing.

We are aware that others have sought such a phenomenon and have been unsuccessful in recording it. Scollo and colleagues (14) could not find any differences in expired NO after exercise in asthmatic children, but these investigators used noncontinuous sampling methods that may have caused them to miss what they were seeking. As shown in Figure 4, the changes in FENO develop in the first few minutes of the recovery period and then rapidly dissipate. Although it is possible that our observations on NO dynamics may be an epiphenomenon and not causally linked to the airway obstruction, we do not believe this to be the case. Preliminary data indicate that blockade of inducible nitric oxide synthase with inhaled N^G-monomethyl-L-arginine attenuates the obstructive response to cold air hyperpnea (15).

The site of release of NO in thermally induced asthma is unknown. According to current models, exhaled NO is derived from a combination of alveolar convection and diffusion from the airway wall (16). In situations of high airflows, the concentration of FENO falls and $\dot{V}_{NO}$ rises because the local gradient from the bronchial wall increases. Because thermally induced asthma is a condition that originates in the tracheobronchial tree and does not involve the pulmonary circulation or lung parenchyma (8, 9, 17, 18), the initiating sequence must transpire in the bronchi with the onset of cooling (8). Nitric oxide can evolve from the airway epithelium, the endothelium of the bronchial circulation, the nonadrenergic noncholinergic nervous system (NANC), infiltration of the airways with inflammatory cells, and/or release from stores (1–6, 10). Although each of these sources can theoretically be involved in our experiment, there are limited data to implicate several of them. For example, the rapidity of the time course of the biochemical and obstructive responses favors local release but makes cellular influx an unlikely candidate. Some of the neurotransmitters of the NANC system such as tachykinins have been implicated in hyperpnea-induced obstruction in guinea pigs (19), but it is unclear if they are important in humans. Inactivation of neutral endopeptidase attenuates the airway narrowing that follows hyperpnea in asthmatics even though tachykinin activity is potentially upregulated (20). Finally, because of the temporal association between the increase in NO in the recovery period and the thermal events transpiring then (8, 9), it is tempting to speculate that this compound derives directly from alterations in the hypertrophic porous bronchial microcirculation characteristic of asthma (21). However, this too is not very likely given the reactivity of NO. One would anticipate that most of the compound generated in the endo-

TABLE 3

EXHALED NITRIC OXIDE LEVELS AT BASELINE AND CHANGES WITH MAXIMAL HYPERVENTILATION*

		Hyperventilation				Recovery				
Subject No.	Baseline	1	2	3	4	1	2	3	4	5
Normal subjects										
1	18	6	5	4	4	6	9	12	16	16
2	3	4	4	5	5	3	5	4	5	5
3	6	16	16	16	16	7	7	7	7	7
4	5	6	6	6	6	4	4	3	5	5
5	24	2	2	2	2	11	25	13	15	24
6	9	2	2	2	2	3	1	1	1	1
7	15	2	3	3	3	10	9	2	2	2
8	10	3	4	4	4	7	9	10	9	11
9	13	3	4	4	4	4	6	7	8	9
10	6	2	1	1	1	3	2	2	3	4
Mean	11	5	5	4	4	6	8	6	7	8
SEM	2	1	1	1	1	1	2	1	2	2
Asthmatic subjects										
1	11	8	8	8	8	13	16	16	15	16
2	20	7	6	6	6	20	21	20	22	26
3	22	18	18	18	24	24	23	22	22	21
4	18	16	16	16	16	23	20	22	22	23
5	9	13	15	13	15	32	34	32	34	32
6	13	6	6	6	6	10	11	12	18	23
7	22	6	6	6	6	18	15	19	31	25
8	14	8	7	7	6	10	11	17	16	27
9	15	35	32	35	32	18	16	16	16	20
10	15	5	6	6	5	8	13	17	11	12
11	22	6	6	6	8	13	13	10	12	18
12	16	20	14	16	14	14	19	23	25	20
13	11	17	17	16	16	11	11	15	14	15
Mean	16	13	12	12	12	16	17	19	20	21
SEM	1	3	2	3	2	2	2	2	2	2

* The nitric oxide concentrations are expressed in parts per billion. The numbers 1–4 and 1–5 indicate the average FeNO values obtained at each time point during hyperventilation and recovery, respectively.

thelium would be bound to hemoglobin and be carried away rather than leaking into the airway lumen.

One reasonable postulate is that NO is normally released from the epithelium as a constituent in the overall regulation of respiratory heat exchange. Both inducible and constitutive forms of nitric oxide synthase are expressed there and it is known that hyperpnea promotes a series of synchronized physiologic events that work to minimize tissue damage (9). Whenever ventilation rises, the temperature of the airways fall (8, 9, 22), bronchial blood flow increases in proportion to the cooling (23) and NO production is simultaneous augmented (24–26). Given the profound effects of NO on arteriolar tone, it is may be that it is the factor that links these phenomena. The amplified generation of NO that we, and others (24–26), have recorded with hyperpnea may cause blood flow to rise so that the heat lost to the environment during respiration can be resupplied dynamically from regional sources to prevent temperatures from falling to critical levels (9). Such a physiologic sequence has already been demonstrated in the nose (27). It may be that the increased production associated with asthma overwhelms local control mechanisms or that there is a dysregulation in end-organ responsiveness, or both.

We do not believe that our findings result from technical issues. Previous studies have shown that the degree of obstruction progressively increases from the end of hyperpnea and reaches a maximum 5 to 10 min after the challenge is over (9). From published data, we would have expected the NO levels to have either not changed (13) or to have fallen (28) with bronchoconstriction and not to have risen. Isocapnic hyperventilation was chosen instead of exercise for simplicity of design. It is far easier to generate stimulus-response relationships with this approach and then apply the precise provocation required to achieve any desired decrement in lung function. It has been well documented that voluntary hyperventilation produces the exact same thermal events as exercise when ventilation and temperature are matched (9). The use of inhaled steroids by five of the asthmatics may have reduced airway hyperresponsiveness and lowered the absolute level of exhaled NO in these subjects prechallenge (29), but it would not have had any impact on what transpired during and after the provocations. Paired comparisons were made, and the screening and challenge studies followed each other in short order without any alterations in either experimental design or steroid dose.

There is no consensus on the effects of ambient NO on F_{ENO} (30, 31). Some studies suggest that levels in excess of 20 ppb will result in linear increase in the expired concentrations (30), whereas others report that high levels decrease NO synthesis in the airway (31). Neither event was operational in the present study. Ambient concentrations were less the above limits and there were no differences between the asthmatics and the normal subjects.

Exhaled NO has a complex waveform, and a number of approaches have been proposed to analyze it (11, 16). We appreciate that the relatively slow response time of our instrument complex introduced a distortion in the recording that underestimated the true levels of this gas in the exhaled air. It also influenced the computations of V_{NO} (16). However, since we were not interested in the absolute values for these variables per se, but rather the changes associated with the bronchoprovocations, our observations are qualitatively valid. The imprecision would have been maximal during hyperpnea and, because the respiratory patterns were similar, it would have applied equally to the trials in both normal and asthmatic subjects. The point remains that F_{ENO} rose posthyperpnea during the development of obstruction in the asthmatics and did not change in the normal subjects at a time where the above extraneous events were minimal. The subjects in both groups were breathing at resting levels with identical tidal volumes, frequencies, and $\dot{V}_E$.

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