Effect of Short-Term, Low-Level Nitrogen Dioxide Exposure on Bronchial Sensitivity of Asthmatic Patients

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Abstract Our purpose was to determine whether exposure to a realistic concentration of nitrogen dioxide (NO_2) could increase the bronchial sensitivity of asthmatic patients to bronchoconstrictor agents. We established dose-response curves for changes in specific airway resistance (SR_{aw}) in response to aerosolized carbachol in 20 asthmatics after each had spent 1 h in an exposure chamber breathing on one occasion unpolluted air and on a separate occasion 0.1 ppm NO_2: sequence of exposures to unpolluted air and to low levels of NO_2 were randomized in a single-blind fashion. NO_2 induced a slight but significant increase in initial SR_{aw} and enhanced the bronchoconstrictor effect of carbachol in 13 subjects: curves were shifted to the left and the mean dose of carbachol producing a twofold increase in initial SR_{aw} was decreased from 0.66 mg to 0.36 mg ($P < 0.001$). In contrast, NO_2 neither modified the initial SR_{aw} nor the bronchoconstrictor effect of carbachol in seven subjects. In 4 out of the 20 subjects, exposure to a higher concentration of NO_2 (0.2 ppm) yielded variable results.

Potentiation of the carbachol bronchoconstrictor response by NO_2 could not be related to any physical or clinical characteristics of the subjects tested. Although the mechanisms underlying the NO_2 effect remain controversial, the present results demonstrate that very low levels of NO_2 can adversely affect some asthmatics.

INTRODUCTION

Epidemiological studies have suggested there is a relationship between air pollution and the prevalence and severity of asthma as well as chronic pulmonary diseases in general (for review see references 1–4). However, the role of air pollution is still questioned since in experiments with controlled exposures, air pollutants exert a detectable effect on lung function only at concentrations that exceed those commonly observed in urban polluted atmosphere. To our knowledge very few of these laboratory studies (5–7) have been performed with asthmatics. These few have demonstrated that asthmatics reacted to smaller doses of pollutants than normal subjects, but the doses used were still higher than those usually encountered in the atmosphere.

In this study we have investigated the direct bronchomotor effect of realistic concentrations of nitrogen dioxide (NO_2), one of the major air pollutants, in a group of asthmatics. We have also measured the bronchial sensitivity to carbachol before and after NO_2 exposure in order to establish whether NO_2 could make the airways "hyperreactive."

METHODS

Subjects. 20 asthmatics volunteered for this study (NO_2 group, Table I). All were outpatients, suffering from slight to mild asthma. They were studied during symptom-free periods and received no symptomatic medication for at least 24 h beforehand. None of these subjects was undergoing long-term steroid therapy and all of them lived in an urban area.

Airway resistance (R_{aw})[1] measurements. We recorded simultaneously R_{aw} and thoracic gas volume (TGV; 8) with a constant volume body plethysmograph (DR-8 amplifier-recorder, Electronics for Medicine, Inc., White Plains, N. Y.). The subject panted at a frequency of 2 cycles/s and a flow rate of 0.5 liter/s (9). The results were expressed as specific airway resistance, $SR_{aw} = R_{aw} \times TGV$, (expressed in centimeters of water × second) which is preferable to the use of specific airway conductance (10).

NO_2 exposure. Cylinders of 0.01% NO_2 in nitrogen were obtained commercially as the source of NO_2 (Compagnie Française des Produits Oxygénés, Paris). A volume of gas, calculated to give a concentration of NO_2 approximatively

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[1] *Abbreviations used in this paper:* D_{100}, the dose of carbachol causing a 100% increase of initial SR_{aw}; R_{aw}, airway resistance; SR_{aw}, specific R_{aw}; TGV, thoracic gas volume.

TABLE I

Physical and Clinical Characteristics of the Asthmatics in the NO_2 Group

Subject	Age (yr) and sex	Intrinsic (I) or extrinsic (E) asthma	Duration of asthma	Severity of asthma*	Cigarettes per day
			yr		
1	27 M	E (house dust)	6	1	10
2	44 F	I	12	1	0
3	19 F	E (grass pollen)	3	2	6–10
4	20 M	I	<1	1	50
5	40 F	E (grass pollen)	24	1	0
6	27 M	E (house dust)	2	2	0
7	35 M	E (house dust)	4	2	0
8	40 M	E (grass pollen)	12	2	13
9	20 M	E (weeds pollen)	14	1	10
10	24 M	E (house dust)	9	1	0
11	25 F	E (house dust)	2	2	0
12	18 M	E (house dust)	6	1	10
13	16 F	E (house dust)	<1	1	0
14	25 M	I	2	2	0
15	22 M	E (house dust)	21	1	0
16	37 F	I	12	2	0
17	17 M	E (grass pollen)	4	1	0
18	15 M	E (moulds)	2	1	0
19	31 F	E (grass pollen)	5	1	0
20	25 M	E (house dust)	5	1	0

* Grade 1, less than 8 days of dyspnea yearly; grade 2, less than 60 and more than 8 days of dyspnea yearly.

over 200 $\mu g/m^3$ was allowed to flow into an airtight exposure room. Actual NO_2 concentrations were measured by sampling room air with a pump during 15-min intervals with the method of Saltzman (11). The air sampler was close to the subject's face and a fan was used to circulate air within the room. The average concentration (mean±SE for 20 experiments) during the first 15-min interval was 246±18 $\mu g/m^3$ and decreased regularly: −10% for the second interval, −9% for the third interval, and −16% for the fourth interval. The average concentration during the hour exposure was 210 $\mu g/m^3$, which is approximatively 0.1 ppm. In some cases a higher dose of NO_2 was used: average concentration of 488 $\mu g/m^3$ (slightly over 0.2 ppm). None of the subjects reported having detected a particular odor due to the gas.

Carbachol dose-response curves. After measurement of basal SR_{aw} (mean of five determinations) a dose-response curve was established for each subject by using a 0.1% (wt/vol) nebulized solution of carbachol (Merck A.G. Inc., Darmstadt, West Germany) in 0.9% saline and changes in SR_{aw} as an index of response. An aerosolizer (Gauthier, Paris; particle size of 0.1–5 μm) delivering 0.0232 mg of carbachol base per liter of air, was used to fill a spirometer bell with fresh aerosol. A two-way valve allowed inspiration from the spirometer and expiration outside the room. The subject was instructed to make from one to five inspirations of a fixed volume of aerosol (860 ml) and to hold his breath for 4 s after each inspiration to ensure a large particle retention (12). The carbachol inhalation of one to five 860-ml volumes represented a quantity of carbachol base varying from 0.02 to 0.1 mg. Because of inaccuracy inherent in any aerosol inhalation technique, the doses of carbachol actually deposited in the airways are probably different from those administered. However, since the inhalation technique was standardized, the error was thought to be constant throughout the different tests. After each carbachol inhalation SR_{aw} was measured (mean of three determinations). The sequence—filling the spirometer with fresh aerosol, carbachol inhalation, and SR_{aw} determination—lasted about 2–4 min and was repeated until at least a 100% increase of initial SR_{aw} was obtained. This procedure yielded a gradual increase in SR_{aw}, and the observer could easily modulate the intensity of the bronchial response by adjusting the magnitude of the carbachol inhalation. Since the progressive increase of SR_{aw} with repetitive carbachol inhalations was not interrupted by allowing a return to base-line values between each carbachol inhalation and since carbachol is not metabolized by acetylcholinesterase, the dose-response curves obtained in this way were considered to be of the cumulative type (13).

Experimental protocols. Each subject was tested according to two different randomized protocols, between 2 and 6 p.m. on two separate days, with a 1-wk interval. Each test was run as follows: After determination of basal SR_{aw} the subject was taken to the exposure room. The subject was left free to breathe either through the nose or mouth and remained seated in the room for 1 h. Then, new determinations of SR_{aw} were made and carbachol dose-response curves were obtained as described. At one occasion, NO_2 was present (NO_2 test) in the air within the exposure room, whereas for the control test performed on a separate day, it was absent. The subject was unaware of the presence or absence of NO_2. All 20 subjects of the NO_2 group had a control test and a NO_2 test with 0.1 ppm NO_2. Subject 16 had two control tests (3-mo interval) and two 0.1 ppm NO_2 tests (separated from the first control test

TABLE II

Physical and Clinical Characteristics of the Asthmatics of the Control Group

Subject	Age (*yr*) and sex	Intrinsic (I) or extrinsic (E) asthma	Duration of asthma	Severity of asthma*	Cigarettes per day	D_{100} First control	D_{100} Second control
			yr			*mg*	
5		See Table I.				0.24	0.24
16						0.94	0.78
21	26 M	E (house dust)	20	2	0	0.078	0.092
22	16 M	E (house dust)	12	1	0	0.10	0.11
23	37 M	I	4	1	0	0.15	0.17
24	25 F	I	3	1	0	0.34	0.31
25	41 F	E (grass pollen)	14	1	0	0.35	0.34
26	33 M	E (house dust)	4	2	0	0.31	0.38
27	19 F	I	<1	1	20	0.68	0.68
28	24 M	I	2	1	15	1.27	1.57
29	64 M	I	16	2	0	0.13	0.13
30	20 F	E (house dust, cat dander)	2	1	0	0.22	0.21

* Grade 1, less than 8 days of dyspnea yearly; grade 2, less than 60 and more than 8 days of dyspnea yearly.

by 1 and 2 wk, respectively). Four subjects (subjects 2, 8, 13, 20), in addition to the 0.1 ppm NO_2 test, underwent a test using 0.2 ppm NO_2. In these cases, the order of the tests was also randomized.

With each subject serving as his own control, we compared the carbachol dose-response curves obtained on the control test to those obtained on the NO_2 test to determine if NO_2 changed the bronchial sensitivity to carbachol. To quantify the results we calculated from the curves the doses of carbachol causing a 100% increase of initial SR_{aw} (D_{100}).

Reproducibility of carbachol dose-response curves. To assess the spontaneous variability of carbachol dose-response curves we tested another group of 10 asthmatics (control group) with clinical histories and functional values similar to those of the subjects in the NO_2 group. Two control carbachol dose-response curves were performed at a 1-wk interval and the D_{100} values were calculated from the curves. In addition, two subjects (subjects 5 and 16) of the preceding group had two control tests. Table II shows that the D_{100} was reproducible and that no systematic error appeared attributable to repetition of the procedure.

RESULTS

Individual results are shown in Fig. 1. As expected, the bronchial sensitivity to carbachol determined in the control test was variable among the individuals examined. Exposure to 0.1 ppm of NO_2 markedly increased the basal value of SR_{aw} in only three subjects (subjects 3, 6, and 16). In others, SR_{aw} was marginally increased, if at all. The effect of 0.1 ppm of NO_2 on the bronchial sensitivity to carbachol was also variable. In some subjects no clear change could be detected whereas in others the effect of carbachol on SR_{aw} was enhanced. Dose-response curves were shifted to the left with a resulting decrease in the D_{100}, and the slopes were usually steeper than the slopes of control dose-response curves. In subject 16, the enhancement of the carbachol effect by 0.1 ppm NO_2 was reproduced on two occasions. When a concentration of 0.2 ppm of NO_2 was used it appears from Fig. 1 that this higher dose was (*a*) no more effective than 0.1 ppm in increasing the carbachol effect in subjects 2 and 8, (*b*) as effective as 0.1 ppm in subject 13, and (*c*) more effective in subject 20, in whom the D_{100} was reduced from 0.94 to 0.42 mg. Exposure to NO_2 did not change the TGV. Since the degree of bronchial obstruction produced by carbachol inhalation was similar before and after NO_2 the accompanying increase in TGV was also similar in both occasions.

Fig. 2 shows the changes in D_{100}, for each subject, observed in this group of asthmatics (NO_2 group) after exposure to 0.1 ppm NO_2 as compared to the spontaneous changes observed in the control group. For the NO_2 group, "test 1" refers to the D_{100} before NO_2 and "test 2" to the D_{100} after NO_2. For the control group, the largest D_{100} observed was chosen as test 1 and the smallest value as test 2 since the expected change in D_{100} after NO_2 exposure was a decrease. It appears from the figure that the spontaneous decreases in D_{100} observed on two different tests in the control group were smaller than 20%. Seven asthmatics of the NO_2 group, being inside this 20% limit, were classified as "NO_2-nonresponders" (subjects 2, 5, 7–9, 11, and 12), whereas 13 subjects having a decrease of more than 20% in D_{100} after NO_2 exposure were classified as "NO_2-responders" (subjects 1, 3, 4, 6, 10, and 13-20).

Table III shows the average effect of NO_2 exposure on initial SR_{aw} and on control D_{100} in the NO_2 group.

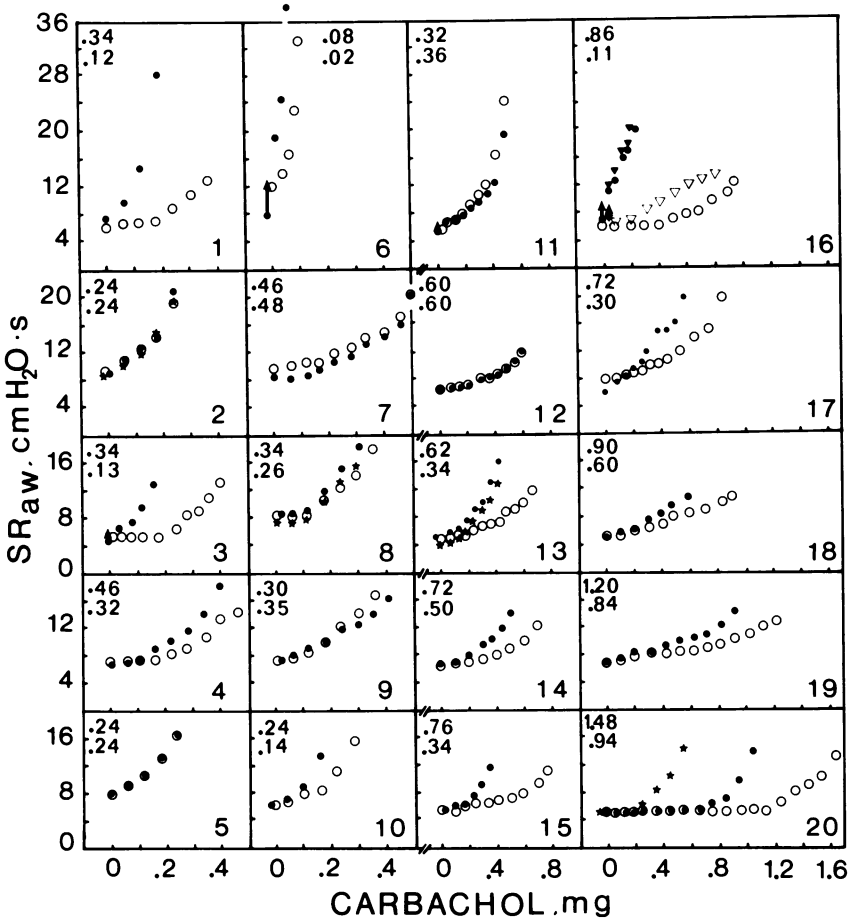

FIGURE 1 Effect of 1-h exposure to NO_2 on the dose-response curves established in 20 asthmatic patients obtained by using cumulative doses of inhaled carbachol aerosol (abscissa) and measuring SR_{aw} (ordinate) as an index of the response. Open symbols, control determinations; closed symbols, determinations after exposure to NO_2; circles and triangles, 0.1 ppm NO_2; stars, 0.2 ppm NO_2. Each panel shows the result for one individual. The number in the right lower corner identifies the subject. The two numbers in the left upper corner indicate the dose of carbachol, calculated from the curves and expressed in milligrams, which causes a 100% increase of initial SR_{aw} (D_{100}); the upper number refers to control D_{100}; the number below refers to D_{100} after exposure to 0.1 ppm NO_2. The arrows indicate the changes in basal SR_{aw} value observed in some subjects after exposure to NO_2.

In the NO_2-nonresponders group statistical analysis showed that the initial value of SR_{aw} was not significantly different between the two tests and that exposure to 0.1 ppm of NO_2 did not significantly change the initial value of SR_{aw} nor the bronchial sensitivity to carbachol, expressed as D_{100}. In the NO_2-responders group it appeared that the inital value of SR_{aw} was also similar for the two tests and that exposure to 0.1 ppm of NO_2 slightly, but significantly, increased the pre-NO_2-exposure value of SR_{aw}. After NO_2, D_{100} was significantly reduced (45% decrease). From the comparison between responder and nonresponder groups it is apparent that the initial value of SR_{aw}, while slightly higher in the NO_2-nonresponder group was not significantly different from the value observed in the NO_2-responder group for the control tests. In contrast, the initial value of SR_{aw} was significantly different in the two groups for the NO_2 test. This difference may be attributable to subject 6, who had a much lower value of initial SR_{aw} on the NO_2 test than on the control test. The mean control D_{100} was lower in the NO_2-nonresponder group than in the NO_2-responder although the difference was not significant. Thus, comparisons between the two groups showed that the NO_2-nonresponders had initially a more severe airway obstruction and were more sensitive to carbachol than the NO_2-responders. No obvious differ-

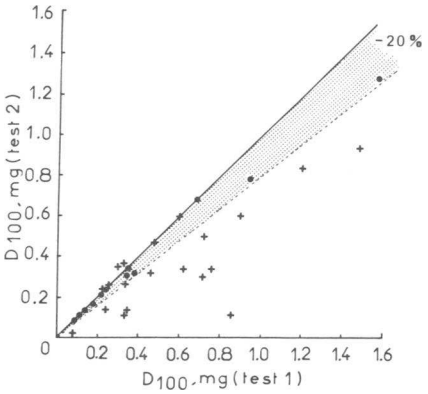

FIGURE 2 Changes in D_{100} in the NO_2 group (20 asthmatics, crosses) as compared to the changes spontaneously observed in the control group (12 asthmatics, closed circles). For the NO_2 group, test 1 represents the control determination of D_{100} and test 2, the determination of D_{100} after 1 h exposure to 0.1 ppm NO_2. For the control group, in which two control determinations of D_{100} were made, test 1 represents the highest value and test 2, the lowest value of D_{100}. The solid line indicates the line of identity and the dotted line, a 20% decrease of test 1 D_{100} as compared to test 2 D_{100}.

ences appeared between the two groups as regard to their physical characteristics (age, sex), the clinical history (duration, severity of asthma), the etiology of asthma (extrinsic or intrinsic), and the smoking history of the subjects tested.

DISCUSSION

The present results demonstrate that, in some asthmatics, exposure to low concentrations of NO_2 causes a moderate bronchial obstruction and markedly increases their bronchial sensitivity to a bronchoconstrictor agent. Such an increase in bronchial sensitivity has been reported in animals with larger doses of NO_2 or other pollutants (14-15).

So far the mechanism underlying this phenomenon is unclear and several hypotheses are conceivable. R_{aw} is mostly determined by bronchial caliber, which in turn is determined by both bronchial factors and extrabronchial factors such as elastic recoil forces. NO_2 could, therefore, alter one of these elements with the same resulting effect. NO_2 has been reported as causing emphysema (see references 2 and 3) and consequently, a change in lung elastic recoil. However, this is unlikely to have happened in our study since these changes appeared after long-term exposure. Short-term exposure to NO_2 could increase the bronchial tone by releasing histamine, as suggested by Nieding and Krekeler (16), or by stimulating the lung irritant receptors (17). Vagally mediated bronchoconstriction has been demonstrated for SO_2 (18), and the irritant receptors are thought to be hypersensitive in asthma (5). Such an increase in bronchial tone by NO_2 would explain the enhanced effect of carbachol since interaction between a bronchoconstrictor agent and increased airway tone would result in a potentiation of the effect of the bronchoconstrictor agent (19).

The enhancement of bronchial sensitivity by NO_2 was variable among individuals in the NO_2-responder group. This NO_2 effect seems reproducible as observed in subject 16. The effect of NO_2 does not appear to be necessarily related to the dose since a dose-effect relationship

TABLE III

Effect of 1-h Exposure to 0.1 ppm of NO_2 on the SR_{aw} and the Bronchial Sensitivity to Inhaled Carbachol Expressed as D_{100} of a Group of 20 Asthmatics

Subjects	Control		After 0.1 ppm NO_2			Decrease in D_{100}
	Initial SR_{aw}	D_{100}	Initial SR_{aw}	SR_{aw} afer NO_2	D_{100}	
	cm H_2O × s	*mg*	*cm H_2O × s*		*mg*	*% of control*
NO_2-nonresponders (n = 7)	7.9±0.6 (*a*)	0.36±0.05 (*b*)	7.7±0.5 (*c*)	8.0±0.4 (*d*)	0.35±0.05 (*e*)	2
NO_2-responders (n = 13)	6.6±0.5 (1)	0.66±0.10 (2)	6.0±0.2 (3)	6.9±0.5 (4)	0.36±0.07 (5)	45
		t	*P*		*t*	*P*
Horizontal comparisons made with Student's paired *t* test	(*a*) vs. (*c*)	0.79	>0.05	(1) vs. (3)	1.34	>0.05
	(*c*) vs. (*d*)	0.70	>0.05	(3) vs. (4)	2.30	<0.05
	(*b*) vs. (*e*)	0.08	>0.05	(2) vs. (5)	5.93	<0.001
Vertical comparisons made with Student's *t* test	(*a*) vs. (1)	1.54	>0.05	(*c*) vs. (3)	3.08	<0.01
	(*b*) vs. (2)	1.96	>0.05	(*e*) vs. (5)	0.08	>0.05

Mean values±SE. The subjects having a decrease in D_{100} after NO_2 of more or less than 20% are classified as NO_2-responders or NO_2-nonresponders, respectively. The level of statistical significance was chosen at $P < 0.05$.

was observed only in one out of two subjects. This could be explained if the effect of NO_2 is of the "all or none" type, i.e., increasing the dose has no effect until a certain threshold is reached where the effect occurs. Then, a further increase in dose yields no further effect until a second threshold is reached and so on. If the thresholds are variable among individuals one might think that the second critical threshold was reached only in one of the two subjects (subject 20).

It is also unclear why some asthmatics responded to NO_2 and some others did not. Asthmatics have variable sites of airway obstruction, either central or peripheral (20), although the SR_{aw} technique that we used reflects primarily changes in central airways. It is thus possible that any peripheral airway effect by NO_2 was missed. However, we could not use other techniques to detect peripheral obstruction since they are either too complex for use with carbachol challenge (e.g., frequency dependence of compliance) or require maximum respiratory maneuvers (flow volume curves, closing volume) which modify the bronchial sensitivity of asthmatics (21). Differences in NO_2 sensitivity between asthmatics could also be due to intrinsic individual variations in bronchial responses. Indeed such variations in NO_2 effect were reported in normal subjects (22). If our previous hypothesis concerning critical threshold of NO_2 is correct, we can assume that the first critical threshold was reached neither with 0.1 ppm of NO_2 for the NO_2-nonresponders (seven subjects) nor with 0.2 ppm of NO_2 in two subjects of this group. However, an alternative explanation is to consider that the NO_2-nonresponders are not NO_2 insensitive but rather NO_2 hypersensitive. Thus, these subjects were exposed to urban concentrations of NO_2 and one can assume they had already reached the first threshold and were already "carbachol sensitized." In these conditions it is possible that exposure to 0.1 ppm of NO_2 had no further effect if the second threshold of sensitization was not reached. This hypothesis is supported by the fact that the NO_2-nonresponders were, on average, more obstructive and more sensitive to carbachol on the control test than the NO_2-responders.

Considering the practical consequences of our findings, we suggest that the incidence and severity of asthmatic attacks would be higher in areas with a polluted atmosphere, at least for some very sensitive subjects. The concentrations of NO_2 that we used are encountered in many cities, mainly as a secondary product of car emissions. However, it is difficult to establish permissible threshold limit values since the sensitivity to NO_2 varies among individuals. In addition, indoor exposure to NO_2 produced by gas heaters and gas stoves may be more detrimental for many asthmatics than outdoor exposure. Furthermore, since one of the major sources of NO_2 is cigarette smoke, it is interesting to notice that some asthmatics are smokers. However, it is known that the effects of cigarette smoke on airways are variable and complex (23) and it is possible that cigarette smoking has some acute favorable effects on airways, such as adrenergic stimulation, (24) which could balance the unfavorable ones.

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