Cholinergic Bronchomotor Tone and Airway Caliber in Insulin-Dependent Diabetes Mellitus

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It has been suggested that the autonomic bronchomotor tone may be altered in diabetes. In the present study, we assessed the cholinergic bronchomotor tone in 34 insulin-dependent diabetic patients and in a control group of 32 healthy subjects (group C). As an index of the intensity of cholinergic tone to the airways, we measured the increase in specific airway conductance (Gaw/VL) induced by aerosol administration of atropine sulfate. In all of the patients and normal individuals the autonomic cardiovascular activity was also evaluated by the tilting test and by the magnitude of the respiratory sinus arrhythmia (RSA). In 19 patients without symptoms of autonomic neuropathy (AN) (group D-1), the autonomic cardiovascular activity was comparable to that of group C. The other 15 patients presented with at least one symptom of AN and a depressed heart rate (HR) control when submitted to the tests of autonomic activity (group D-2). Before atropine administration, Gaw/VL was significantly higher (p<0.05) in group D-2 (2.45 ± 0.12 s⁻¹·kPa⁻¹) than in group D-1 (2.11 ± 0.10 s⁻¹·kPa⁻¹). Aerosol atropine caused a significant increase (p<0.001) in airway caliber in all three groups; however, the increase in Gaw/VL was significantly lower in group D-2 (0.25 ± 0.06 s⁻¹·kPa⁻¹) when compared with group D-1 (0.63 ± 0.09 s⁻¹·kPa⁻¹; p<0.01) and group C (0.67 ± 0.06 s⁻¹·kPa⁻¹; p<0.001). A weak but significant (p<0.02) correlation was observed between the increases in Gaw/VL provoked by atropine and the magnitude of RSA. Our findings suggest that the reduction in parasympathetic bronchomotor tone may cause an increase in basal airway caliber in diabetic patients with AN, compared to patients without AN. (Chest 1992; 101:1033-43)

AN = autonomic neuropathy; ANS = autonomic nervous system; Gaw/VL = specific airway conductance; RSA = respiratory sinus arrhythmia; VTC = thoracic gas volume

Diabetes mellitus may damage the autonomic nervous system (ANS) of virtually all organs, with clinical manifestations of dysautonomia being more common in the cardiovascular, genitourinary, gastrointestinal, and thermoregulatory systems.¹,² There is some evidence that the autonomic control of airway smooth muscle may also be altered in diabetes.³⁷

Although it is well known that the parasympathetic division of the ANS represents the dominant neural bronchoconstrictor mechanism and plays an important role in the regulation of bronchomotor tone under both normal and pathologic conditions,⁶,⁹ the amount of the airway vagal tone cannot be directly measured in man; however, the intensity of the parasympathetic control of bronchial smooth muscle tone may be estimated by measuring the increase in airway caliber induced by the administration of anticholinergic drugs.¹⁰,¹¹

In diabetic patients with autonomic neuropathy (AN), Douglas et al⁶ observed a reduced bronchodilating response to the inhalation of ipratropium bromide, while Heaton et al⁶ reported a depression of the bronchoconstrictor response to eucapnic hyperventilation with cold air. In both studies, the altered responses were attributed to a decrease in vagal activity; however, the mean baseline airway caliber was not affected in these subjects, in spite of the reduced vagally mediated smooth muscle tone.

The purpose of our study was to analyze the airway caliber under basal condition and the changes induced by cholinergic blockade with atropine sulfate in insulin-dependent diabetic patients with and without AN and in a control group of nondiabetic individuals. A quantitative study of autonomic cardiovascular activity was also carried out on the same subjects using the tilting test and the magnitude of respiratory sinus arrhythmia (RSA).

Materials and Methods

Subjects

We studied 34 insulin-dependent diabetic patients aged 18 to 45 y and a control group of 32 healthy individuals aged 23 to 35 y. The diabetic patients were divided into two groups on the basis of clinical manifestations of AN and the responses to the two tests of autonomic cardiovascular activity, i.e., the tilting test and the magnitude of RSA (see procedures for details). The first group of diabetic patients (group D-1) consisted of 19 subjects with no AN symptoms and with circulatory autonomic activity in the range of that observed in normal individuals previously studied in our laboratory. Mean age for group D-1 was 25 y (range, 18 to 39 y).
the second group (group D-2) consisted of 15 diabetic patients presenting with at least one symptom suggestive of AN (impotence, sweating abnormalities, postural dizziness, episodic diarrhea, and neurogenic alterations of the bladder) and depressed heart rate (HR) control when submitted to the autonomic activity tests. Mean age for group D-2 was 45 y (range, 21 to 45 y). None of the patients or normal volunteers had a recent or remote history of cardiorespiratory disease or respiratory allergy, and none had suffered an acute respiratory infection during the 3 mo that preceded the study. Serologic tests for Chagas' disease (complement fixation and immuno- fluorescence) were negative in all individuals studied. Table 1 shows the characteristics of the three groups studied. All subjects gave informed consent to participate in the study, and the protocol was approved by the ethics committee of the institution.

**Procedures**

The head-up tilting test with an inclination of 70° was performed with the aid of a tilting table that permitted rapid passive changes in body position without muscular effort on the part of the individual, as previously reported.14 The HR was monitored by continuous ECG recording (Siemens-Elema Mingraf 34). Arterial pressure was measured with a sphygmomanometer during the basal period in the supine position and then at 1-min intervals during the tilting test. After 30 min of rest in the supine position, normal and diabetic patients were submitted to rapid 70° head-up tilting and were left in this position for 5 min, when the test was terminated by returning the table to the horizontal position. Previous results from our laboratory7 and reported by others14 have shown that the increase in HR during the first 5 to 10 s of postural change essentially depends on the rapid decrease in vagal activity on the sinus node. In turn, sympathetic stimulation becomes the dominant mechanism of HR increase during the stabilization period after a few minutes in the tilted position.13

For the determination of RSA magnitude, the individuals were in the sitting position and breathing through the mouth (with the nose occluded with a nose clip) into a pneumotachograph (Fleisch No. 2) with electronically integrated flow (Hewlett-Packard model 8815-A integrator) so as to permit the measurement of tidal volume. A function generator (Hewlett-Packard 3310-A) permitted the introduction of a sine wave into the oscilloscope. This signal served as a model for the individual, who visually superimposed on it the tidal volume signal while breathing through the pneumotachograph.15 In each case the test was performed with 1-L and 2-L tidal volumes, with respiratory frequency fixed at 6 cycles per minute. Instantaneous HR (Hewlett-Packard model 8812-A cardiactachometer), ECG, and tidal volume were recorded on heat-sensitive paper (Hewlett-Packard model 7754-A recorder). The RSA magnitude is reported as the mean of the differences between maximum and minimum instantaneous HR values over six successive respiratory cycles. Previous experience in our laboratory has demonstrated the importance of the participation of the efferent vagal control as a determinant of RSA magnitude.16,17

Forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV₁) were measured using a 9-L spirometer (N.V. Godart) as recommended by the American Thoracic Society.17 The spirometric data were expressed as a percentage of predicted normal values obtained from the equations of Crapo et al.18 Thoracic gas volume (Vtg) and airway resistance (Raw) were measured by the methods of DuBois et al.19,20 using a constant-volume body plethysmograph (W.E. Collins 09001). Specific airway conductance (Gaw/Vt) was calculated by dividing the Raw reciprocal, (ie, L/Raw) by the Vtg at which the measurements were made. At least ten measurements were made per individual during each phase of the study, and the results are reported as mean values.

The changes in airway caliber induced by cholinergic blockade were inferred through the Gaw/Vt changes provoked by the aerosol administration of atropine sulfate at the concentration of 1 mg/ml.

### Table 1 — Characteristics of Groups*

<table>
<thead>
<tr>
<th>Group</th>
<th>C (Control)</th>
<th>D-1 (Diabetic without AN)</th>
<th>D-2 (Diabetic with AN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex ratio (M/F)</td>
<td>16/16</td>
<td>10/9</td>
<td>6/9</td>
</tr>
<tr>
<td>Age, yr</td>
<td>27 (23-33)</td>
<td>25 (18-39)</td>
<td>35 (21-45)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>67 (44-90)</td>
<td>60 (45-90)</td>
<td>60 (45-80)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>170 (151-189)</td>
<td>163 (150-178)</td>
<td>161 (147-179)</td>
</tr>
<tr>
<td>Duration of diabetes, yr</td>
<td>. . .</td>
<td>11 (4-28)</td>
<td>13 (5-20)</td>
</tr>
<tr>
<td>No. of smokers</td>
<td>0</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

*Data for age, weight, height, and duration of diabetes are means and range.

The aerosol was generated by a nebulizer (De Vilbiss 645) connected to a compressed air source with a flow of 8 L/min. A hand-operated valve permitted nebulization to occur only during the inspiratory phase of the respiratory cycle. The aerosol was inhaled through the mouth during slow and deep inspirations initiated at functional residual capacity (FRC) and lasting approximately 4 s. In each case the nebulized volume was 1 ml, equivalent to 1 mg of atropine sulfate, a sufficient dose to provoke bronchodilatation in normal individuals.18 The Vtg and Raw measurements were repeated at 10 to 20 min after the end of nebulization.

### Statistical Analysis

The data are expressed as the mean ± SE. Analysis of variance (ANOVA) was performed to test for differences of the data among the groups. If a significant difference was detected, then the t-test analysis was performed to determine the level of statistical significance of the differences between two groups. Also, the paired t-test was used to analyze the changes in Gaw/Vt induced by atropine within each group of subjects. The correlation coefficient was calculated by means of linear regression analysis to determine whether any correlation existed between the changes in Gaw/Vt provoked by atropine and the changes in HR during the autonomic tests. Values of p<0.05 were taken as significant.

### RESULTS

#### Autonomic Cardiovascular Activity

The results obtained during the study of the autonomic cardiovascular activity are given in Table 2. In the resting supine position the patients of group D-2,

### Table 2 — Results of Autonomic Cardiovascular Evaluation Using Tilting Test and Magnitude of RSA*

<table>
<thead>
<tr>
<th>Data and Test</th>
<th>Group C</th>
<th>Group D-1</th>
<th>Group D-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting supine HR, beats per min</td>
<td>67.7±1.6</td>
<td>71.1±1.9</td>
<td>86.5±3.5†‡</td>
</tr>
<tr>
<td>ΔHR, beats per min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tilting test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tilting, 5 s</td>
<td>12.1±1.3</td>
<td>12.6±1.5</td>
<td>2.7±1.0‡</td>
</tr>
<tr>
<td>Tilting, 10 s</td>
<td>13.3±1.6</td>
<td>13.8±2.4</td>
<td>3.8±1.3§</td>
</tr>
<tr>
<td>Tilting, 5 min</td>
<td>14.3±1.3</td>
<td>17.8±2.2</td>
<td>11.3±2.5</td>
</tr>
<tr>
<td>RSA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-L Vtg</td>
<td>16.9±1.1</td>
<td>16.7±1.4</td>
<td>5.9±1.8§</td>
</tr>
<tr>
<td>2-L Vtg</td>
<td>23.6±1.1</td>
<td>19.5±1.9</td>
<td>6.3±1.1‡</td>
</tr>
</tbody>
</table>

*Values are means ± SE.

†p<0.001 compared with group C.
‡p<0.001 compared with group D-1.
§p<0.05 compared with group D-2.
As compared with the other two groups, had significantly higher values of HR (p<0.001). The increase in HR at 5 s of tilting test was significantly lower in group D-2 than in group C (p<0.001) and group D-1 (p<0.001). Similar HR results were observed at 10 s of the tilting period, with smaller variation in group D-2 than in group C (p<0.001) and group D-1 (p<0.005). No significant differences among groups were observed in HR increases at the end of 5 min of tilting.

Basal systemic arterial pressure (both systolic and diastolic [S/D]) in the supine position was significantly higher in group D-2 (139±9/92±5 mm Hg) than in group C (111±2/73±1.3 mm Hg) (p<0.001) and in group D-1 (118±4/77±3 mm Hg) (p<0.05), but no significant differences in arterial pressure variations were observed among the three groups during the tilting test. Only one patient of group D-2 had to interrupt the test before the fifth minute due to symptomatic postural hypotension.

The magnitude of RSA (ΔHR) with 1-L and 2-L tidal volume was significantly lower in group D-2 as compared with groups D-1 (p<0.001) and C (p<0.001).

**Pulmonary Function and Airway Response to Atropine**

The mean value of FEV₁ (percent of predicted) was greater in group C (92.3±2.0 percent of predicted) than in group D-1 (80.9±2.6 percent of predicted; p<0.01) and in group D-2 (79.3±3.6 percent of predicted; p<0.01); however, no significant differences among groups were observed in the FEV₁/FVC expressed as a percentage of the predicted normal value (99.1±1.5 in group C, 95.0±2.0 in group D-1, and 97.0±1.6 in group D-2).

Mean basal Gaw/VL (Fig 1) was significantly higher in group D-2 (2.48±0.12 s⁻¹·kPa⁻¹) than in group D-1 (2.11±0.10 s⁻¹·kPa⁻¹; p<0.05), but the difference in relation to group C (2.34±0.08 s⁻¹·kPa⁻¹) was nonsignificant.

Aerosol administration of atropine sulfate (Fig 1 and 2) caused a significant increase (p<0.001) in Gaw/VL in all three groups, but the magnitude of the Gaw/VL increase (ΔGaw/VL) was much lower in group D-2 (0.27±0.05 s⁻¹·kPa⁻¹) than in group D-1 (0.63±0.14 s⁻¹·kPa⁻¹; p<0.01) and in group C (0.67±0.06 s⁻¹·kPa⁻¹; p<0.01).

After atropine, there were no significant differences of mean Gaw/VL values among groups (3.01±0.12 s⁻¹·kPa⁻¹ in group C, 2.74±0.14 s⁻¹·kPa⁻¹ in group D-1, and 2.74±0.14 s⁻¹·kPa⁻¹ in group D-2; Fig 1).
Airway Response to Atropine and Autonomic Cardiovascular Activity

A weak but significant correlation \( (r = 0.30; \ p < 0.02) \) was obtained between the individual values of \( \Delta G_{aw/Vl} \) after atropine aerosol and the RSA magnitude (2-L tidal volume) for all of the subjects included in the three groups (Fig 3). No significant correlation was observed between \( \Delta G_{aw/Vl} \) and the HR responses to the tilting test.

Discussion

On the basis of clinical and functional criteria used in the present study, the diabetic patients were divided into two groups in terms of ANS involvement. Patients of group D-1, with no clinical manifestation of AN, behaved like group C in terms of circulatory responses to the tilting test and RSA magnitude. On the other hand, group D-2 of patients with AN symptoms showed intense depression of the parasympathetic autonomic control of the heart, as judged by the higher HR observed under basal conditions in the supine position, by the lower initial variation in HR during the tilting test, and by the lower magnitude of RSA. Results reported by different laboratories have pointed out the importance of the integrity of vagal efferent control for the initial increases in HR after a change in posture \cite{13,14,32} and for the magnitude of HR variation with the respiratory movements \cite{15,16,33,24}. The patients in group D-2 were on average older than the individuals of the other two groups, and there is evidence that the reflex autonomic control of HR normally declines with aging \cite{2,25-28}; however, the vagal autonomic depression observed in group D-2 was too intense to be explained on the basis of the slightly more advanced age of these patients \cite{29,27}.

Analysis of the time course of HR variation during the tilting test in normal individuals demonstrates that while the initial tachycardia depends on suppression of vagal tone, the maintenance of an elevated HR during the equilibration period after 5 min in the tilted position is related to sympathetic stimulation \cite{13}. These results show that at the fifth minute of the tilting test (ie, during the equilibrium phase), the mean increase in HR in the two diabetic groups and in the control group of nondiabetic subjects did not differ from one another. Furthermore, the increases in HR observed in the three groups were close to the values reported by Hainsworth and Al-Shammas \cite{29} for normal individuals submitted to the tilting test with 60° inclination. On this basis, these results suggest that even in the patients with dysfunction of the parasympathetic control of the heart (group D-2), there was sufficient sympathetic activity to maintain a normal HR increase during the equilibrium phase of the passive tilting. Thus, the maintenance of normal arterial pressure levels during the tilting test in all patients except one demonstrates the preservation of the sympathetic efferent mechanism of peripheral vascular resistance regulation. The fact that in this group of diabetic patients the sympathetic function was relatively preserved may be related to the criteria for selection. The patients investigated in our study were in a stable metabolic condition, without limitations in their daily activities. It has been previously demonstrated that in diabetes mellitus, the tests that mainly reflect the parasympathetic function of the circulatory system yield abnormal results at a higher frequency than the tests of sympathetic activity \cite{26-31}; however, it is not known whether these results represent an earlier involvement of the parasympathetic system in the natural history of diabetes or are simply due to a lower sensitivity of the tests of the sympathetic system \cite{2}.

Dysfunction of the autonomic control of the heart such as that observed in our patients has been exhaustively demonstrated in other studies conducted on diabetic patients \cite{1,2,30-33}. In Chagas' disease, which is endemic in many South American countries, damage of the intracardiac parasympathetic ganglion cells also provokes intense depression of the vagal regulation of the heart \cite{15,37-40}; however, the negative results of specific serologic tests obtained for the present patients permitted us to exclude the concomitant presence of Chagas' heart disease.

In man, vagal blockade with intravenous atropine is associated with an increase in the anatomic dead space \cite{41}, a fall in airway resistance \cite{11,42} and a reduction of lung recoil pressure \cite{11,42,43} thus suggesting dilatation of peripheral and larger airways and of alveolar ducts. On the other hand, when vagal blockade is induced by aerosol application of atropine sulfate or ipratropium bromide, the resulting bronchodilatation is not associated with significant changes in the static pressure-volume curves of the lungs, thus suggesting a preferential action on the large airways \cite{10,11,44}. In the
present study, aerosol administration of atropine sulfate was accompanied by a significant increase in Gow/Vl in the three groups, indicating that basal parasympathetic activity on the airways was present even in the diabetic patients; however, the bronchodilatation produced by atropine was much lower in the diabetic patients with AN when compared to the other groups. This result is similar to that observed by Douglas et al.\(^3\) with the administration of ipratropium bromide to diabetic patients and demonstrates that the reduction in parasympathetic tone of the airways is one of the various functional abnormalities associated with autonomic nervous control impairment in these patients. The lower degree of parasympathetic activity on bronchial smooth muscle should induce an increase in basal airway caliber; and, in fact, this occurred, since group D-2 had a higher basal Gow/Vl value than group D-1. In this respect, our results differ from those reported by Douglas et al.\(^3\) and Heaton et al.\(^4\) who did not find significant differences in mean basal Gow/Vl values in small groups of diabetic patients with and without AN. The reasons for this discrepancy are unknown. The mean basal Gow/Vl value of our group C was slightly higher than that of group D-1 and lower than that of group D-2, but the differences were not significant; however, when the results of Gow/Vl measurements on diabetic patients are analyzed, it should be remembered that other mechanical factors in addition to bronchomotor tone also affect the caliber of intrathoracic airways. Thus, for the same degree of bronchomotor tone, the caliber of the intrathoracic airways is directly proportional to the lung elastic recoil.\(^4\) We did not study the pressure-volume relationships in our patients, but the results obtained by Schuyler et al.\(^3\) and Sandler et al.\(^4\) for insulin-dependent diabetic subjects showed a decrease in lung elastic recoil measured in volumes below the total lung capacity (TLC). If the same situation occurs in our patients, then the basal Gow/Vl values of these patients may have been lower or higher than those of nondiabetic individuals, depending on the higher or lower intensity of vagal tone on airway smooth muscle, respectively. Group D-1 showed greater interindividual variability of Gow/Vl changes than groups D-2 and C, thus suggesting that group D-1 is less homogeneous than the other two groups with respect to the intensity of vagal regulation of the airways.

Contrary to the parasympathetic nerve supply, the sympathetic innervation of the airways is much less dense;\(^9\) thus, it is unlikely that the differences in Gow/Vl observed in the two diabetic groups represent differential damage to the efferent sympathetic pathways. Furthermore, on the basis of the tilting test, the two diabetic groups showed comparable degrees of adrenergic participation in HR and arterial pressure control, and a similar situation may occur in the sympathetic control of the airways.

Another finding was that the cholinergic tone to the airways, as inferred by the increment in Gow/Vl following atropine sulfate, correlated significantly (albeit weakly) with vagal autonomic activity evaluated by the magnitude of RSA.

In conclusion, this study confirms observations reported by others indicating that the parasympathetic regulation of airway caliber may be damaged in diabetes mellitus, characterizing a bronchomotor dysautonomia. In these cases, there is a correlation between bronchial dysautonomia and cardiac dysautonomia, the latter demonstrated by quantitative analysis of the reflex HR responses.

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