Preservation of the hypoxic drive to breathing in diabetic autonomic neuropathy

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Summary
1. Unexplained cardiorespiratory arrests have been reported in patients with diabetic autonomic neuropathy and these could be due to denervation of the carotid chemoreceptors.
2. We have studied the ventilatory response to transient hypoxia (Ve/PetO2) during exercise in 22 male diabetic patients, six with symptomatic and cardiovascular evidence to suggest diabetic autonomic neuropathy (DAN+) and 12 without these features (DAN-).
3. There was no difference in the ventilatory response to transient hypoxia between the different groups of diabetic patients (Ve/PetO2 in DAN+ patients = -0.9 ± 0.2 litre min⁻¹ kPa⁻¹; Ve/PetO2 in DAN- patients = -1.2 ± 0.6 litres min⁻¹ kPa⁻¹) even allowing for differences in the level of exercise achieved (CO₂ production in DAN+ patients = 743 ± 103 ml/min; CO₂ production in DAN- patients = 800 ± 144 ml/min). These results fell within our normal range for ventilatory response to transient hypoxia at this level of exercise.
4. The heart rate response to transient hypoxia varied within the groups but was significantly (P < 0.05) less in the patients with established diabetic autonomic neuropathy.
5. We conclude that the peripheral chemoreceptors are intact in diabetic autonomic neuropathy and that other mechanisms must be implicated in the unexpected cardiorespiratory arrests seen in these patients.

Key words: cardiorespiratory arrest, diabetes mellitus, chemosensitivity, transient hypoxia, ventilation.

Introduction
Autonomic neuropathy is recognized as an important complication of diabetes, which has a considerable morbidity and a poor prognosis [1, 2]. Abnormalities of autonomic function are widespread and involve changes especially in the cardiovascular, gastro-intestinal and genito-urinary systems, and the diagnosis can be established objectively by simple non-invasive tests of cardiovascular reflexes [3]. To date, relatively little is known about the involvement of the respiratory system, but recent reports of unexplained cardiorespiratory arrest in diabetic patients with established autonomic neuropathy [4] led us to speculate that the reflex control of ventilation may be defective in these patients. Although only 10% of the ventilatory drive at rest can be attributed to the activity of the carotid chemoreceptors, their role is more important during hypoxic exercise [5]. In man the afferent fibres from the peripheral chemoreceptors are in the glossopharyngeal nerve and might be affected in diabetic autonomic neuropathy. This study was designed to assess the function of the carotid chemoreceptors in diabetic patients with and without evidence of autonomic neuropathy.

Patients
Three groups of male diabetic patients were studied (Table 1). Twelve had no symptoms...
TABLE 1. Age, duration of diabetes and respiratory function in 22 male diabetic patients

FEV₁, Forced expiratory volume in 1s; FVC, forced vital capacity; TLC, total lung capacity measured by helium dilution; Tco, transfer factor for carbon monoxide; Sao₂, ear oxygen saturation.

<table>
<thead>
<tr>
<th>Diabetic patients</th>
<th>n</th>
<th>Age (years)</th>
<th>Duration of diabetes (years)</th>
<th>FEV₁ (1)</th>
<th>FVC (1)</th>
<th>TLC (1)</th>
<th>Tco</th>
<th>Sao₂ at rest (%)</th>
</tr>
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<tbody>
<tr>
<td><strong>Group 1</strong></td>
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<tr>
<td>Without autonomic neuropathy (DAN−)</td>
<td>12</td>
<td>45 ± 9.1</td>
<td>17.1 ± 9.4</td>
<td>3.5 ± 0.5</td>
<td>4.3 ± 0.3</td>
<td>6.8 ± 0.7</td>
<td>9.4 ± 1.7</td>
<td>96.1 ± 0.9</td>
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<tr>
<td><strong>Group 2</strong></td>
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<tr>
<td>With borderline test results (DANI)</td>
<td>4</td>
<td>46.5 ± 11.2</td>
<td>23.8 ± 9.2</td>
<td>3.9 ± 0.8</td>
<td>3.95 ± 0.9</td>
<td>7.4 ± 1.3</td>
<td>8.4 ± 0.9</td>
<td>96.9 ± 0.7</td>
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<tr>
<td><strong>Group 3</strong></td>
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<tr>
<td>With autonomic neuropathy (DAN+)</td>
<td>6</td>
<td>42 ± 8.7</td>
<td>18.2 ± 9.9</td>
<td>3.5 ± 0.5</td>
<td>4.2 ± 0.5</td>
<td>6.7 ± 1.0</td>
<td>7.7 ± 0.6</td>
<td>96.8 ± 1.0</td>
</tr>
</tbody>
</table>

TABLE 2. Cardiovascular autonomic function tests in the two abnormal diabetic groups

A, Abnormal; N, normal; B, borderline.

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Valsalva manoeuvre (Valsalva ratio)</th>
<th>Lying to standing heart rate response (30:15 ratio)</th>
<th>Heart rate variation during deep breathing (max.-min. heart rate: beats/min)</th>
<th>Postural fall in blood pressure (fall in systolic BP:mmHg)</th>
<th>Blood pressure response to sustained handgrip (rise in diastolic BP:mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 2</strong> (DANI)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1</td>
<td>1-10 (A)</td>
<td>0.97 (A)</td>
<td>-</td>
<td>10 (N)</td>
<td>30 (N)</td>
</tr>
<tr>
<td>2</td>
<td>1-20 (B)</td>
<td>0.97 (A)</td>
<td>-</td>
<td>15 (B)</td>
<td>23 (N)</td>
</tr>
<tr>
<td>3</td>
<td>1-15 (B)</td>
<td>1.04 (N)</td>
<td>11 (B)</td>
<td>+5 (N)</td>
<td>38 (N)</td>
</tr>
<tr>
<td>4</td>
<td>1-10 (A)</td>
<td>1.05 (N)</td>
<td>11 (B)</td>
<td>10 (N)</td>
<td>30 (N)</td>
</tr>
<tr>
<td><strong>Group 3</strong> (DAN+)</td>
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<tr>
<td>1</td>
<td>1-02 (A)</td>
<td>0.93 (A)</td>
<td>-</td>
<td>60 (A)</td>
<td>5 (A)</td>
</tr>
<tr>
<td>2</td>
<td>1-07 (A)</td>
<td>0.94 (A)</td>
<td>-</td>
<td>40 (A)</td>
<td>53 (N)</td>
</tr>
<tr>
<td>3</td>
<td>1-13 (B)</td>
<td>1.00 (A)</td>
<td>-</td>
<td>30 (A)</td>
<td>5 (A)</td>
</tr>
<tr>
<td>4</td>
<td>1-00 (A)</td>
<td>1.00 (A)</td>
<td>-</td>
<td>44 (A)</td>
<td>19 (N)</td>
</tr>
<tr>
<td>5</td>
<td>1-25 (N)</td>
<td>0.91 (A)</td>
<td>-</td>
<td>20 (B)</td>
<td>2 (A)</td>
</tr>
<tr>
<td>6</td>
<td>1-04 (A)</td>
<td>1.00 (A)</td>
<td>-</td>
<td>15 (B)</td>
<td>10 (A)</td>
</tr>
</tbody>
</table>

Referable to the autonomic nervous system or abnormality of cardiovascular reflexes, as judged by normal heart rate responses to the Valsalva manoeuvre and standing, and normal blood pressure responses to sustained handgrip and to standing (group 1, DAN−). Four patients, although without symptoms of autonomic neuropathy, nevertheless showed abnormalities suggesting cardiac parasympathetic damage (group 2, DANI). The remaining six patients had two or more of the following symptoms: impotence, faintness on standing, intermittent 'diabetic' diarrhoea, sweating abnormalities, hypo-glycaemia unawareness, and gastric fullness, and abnormalities of cardiovascular reflexes suggesting both parasympathetic and more widespread sympathetic damage (group 3, DAN+). Results of the cardiovascular autonomic function tests are given in Table 1. None of the patients had an elevated blood urea, although six of the ten patients with established or borderline abnormalities of autonomic function had advanced diabetic retinopathy and peripheral neuropathy. These complications were present in only one patient with normal results for autonomic function tests. All patients were insulin dependent and clinically stable at the time of the study and none complained of exertional chest pain or had any abnormality in a 12-lead ECG. One patient had intermittent claudication but this did not prevent him from carrying out the exercise study.

Methods

Autonomic function testing

Four tests of cardiovascular autonomic function were performed in all subjects. A fifth, the heart rate response to deep breathing, was
performed in only two subjects in group 1 and two in group 2. The heart rate responses to the Valsalva manoeuvre, standing up and deep breathing assess the integrity of the cardiac parasympathetic pathways; the other two tests, the blood pressure responses to standing and sustained handgrip, give abnormal results only if there is more widespread sympathetic damage. Subjects were described as having cardiac parasympathetic damage if one or more of the first three tests were abnormal or two borderline, and as having additional sympathetic damage if both of the sympathetic tests were also abnormal. We have previously published technical details of the test and the normal reference ranges [2, 3].

Ventilatory response to transient hypoxia

Respiratory studies were performed at rest, and during modest level treadmill walking for 20 min, designed to produce an oxygen uptake ($V_{O_2}$) of between 600 and 1200 ml/min. While the subject breathed through a modified Otis McKerrow valve, the expired gas passed through a pneumotachygraph generating a signal for tidal volume ($V_t$) and frequency ($f$), from which the instantaneous minute ventilation ($V_E$ inst. = $V_t \times f$) could be calculated. The expired gas then passed into a mixing chamber and a dry gas meter (Parkinson Cowan CD4) from which gas was sampled for off-line analysis of oxygen uptake and CO$_2$ production ($V_{CO_2}$). The end-tidal partial pressures of oxygen (PETO$_2$) and CO$_2$ (PETO$_{CO_2}$) of each breath were measured by a mass spectrometer (Varian M3), previously calibrated with six standard gas mixtures. End-tidal gas tensions were displayed on an oscilloscope and a satisfactory end-tidal plateau was obtained both at rest and during exercise. The arterial oxygen saturation was measured continuously by a Hewlett-Packard HP 47201A ear oximeter. The ECG was recorded throughout from three standard chest leads and the ECG, ear oxygen saturation, $V_t$, $f$ and end-tidal gas pressures were recorded on-line by a PDP11/40 computer, which produced a visual display of these variables every five breaths. The inspired gas could be changed for up to three breaths from air to pure nitrogen without the subject’s knowledge, and the subsequent breath-by-breath ventilatory response was then related to this transient hypoxic stimulus by a computer method which allows for the delay between the stimulus (PETO$_2$) and the response ($V_E$ inst.) as we have previously described [6].

The response to transient hypoxia ($V_E$ inst.) lags behind the stimulus (PETO$_2$) due to the circulation time from pulmonary capillary to carotid body. We treat each recorded breath as a separate data point and our analysis includes five breaths before and 10 breaths after the transient hypoxic stimulus. We relate stimulus to response and obtain a correlation coefficient ($r$) between these variables by the least squares method. The calculation is then repeated with different assumed values for the delay between stimulus and response (from $-5$ to $+10$ breaths). We then assume that the correct value for the physiological delay is that which yields the highest value of $r$, and it is the slope of this regression that we use as our index of hypoxic responsiveness.

Protocol

Earlier studies on patients with familial dysautonomia [7] described serious adverse effects including convulsions and syncope after prolonged hypoxia. In view of this, a cautious approach was employed in this study with progressive increments in the hypoxic stimulus at rest and during exercise until we were certain that a satisfactory stimulus could be given safely, as described below. Each subject was studied initially at rest, and after measurement of oxygen uptake and CO$_2$ production was given one breath of nitrogen. If no untoward effects occurred he was then given two and subsequently three breaths of nitrogen, at 60 breath intervals. This procedure was repeated during the exercise, the patient being carefully observed for side effects. After a minimum of 20 min rest the walk was repeated, with metabolic measurements being made after 7 min walking, and then repeated after exposure to three breaths of nitrogen on six separate occasions during the remaining 13 min of the walk. Previous studies had shown that the ventilatory response to transient hypoxia at rest is less reproducible than transient hypoxia on exercise, and that the hypoxic response to three consecutive breaths of nitrogen (as measured by PETO$_2$) gave a more consistent stimulus than either one or two breaths [8].

Analysis of results

The initial studies were used to acclimatize the subjects to the equipment and to ensure that there were no adverse effects. We have analysed only those responses obtained during the second period of exercise. The results are expressed as the slope of the $V_E$ inst./PETO$_2$ relationship in 1 min$^{-1}$ kPa$^{-1}$ and have been compared with the results from our previous studies of 41 healthy mine rescue workers exercising at similar but
slightly higher oxygen uptakes (mean $\dot{V}O_2$ 1198 ± 158 ml/min), and of similar age (mean age 34.5 ± 6.5 years) but with no respiratory disease or neurological abnormalities [9]. Each slope represents the pooled results of the six occasions on which that subject was exposed to three breaths of nitrogen during the walk, all the data from the six nitrogen transients being combined and analysed as described above.

In these studies the heart rate was recorded on a breath-by-breath basis, each value representing the mean heart rate of the preceding six beats, thus giving a general trend of response rather than the R-R interval at the time of that breath.

Statistical analysis between and within groups was made with the Wilcoxon rank sum test, without assuming a normal distribution of the measured variables. Values are given as means ± SD unless otherwise indicated.

All patients gave their informed consent to these studies, which had previously been approved by the Hospital Ethical Committee.

### Results

The level of exercise attained varied among the subjects, $\dot{V}O_2$ ranging from 630 ml/min to 1147 ml/min, but there were no significant differences between the groups either in terms of $\dot{V}O_2$ or $\dot{V}CO_2$ (Table 3). The ventilatory response to hypoxia also varied considerably from -0.27 to -2.37 litres min⁻¹ kPa⁻¹. In the subjects with diabetic autonomic neuropathy (DAN+) the ventilatory response to hypoxia, expressed as $\dot{V}e$/Peto, had a mean value of -0.95 litres min⁻¹ kPa⁻¹, whereas in those without evidence of autonomic neuropathy (DAN-) the $\dot{V}e$/Peto, mean value was -1.2 litres min⁻¹ kPa⁻¹, the borderline subjects (DANI) having a mean $\dot{V}e$/Peto, of -1.2. Comparison with the previous results obtained in normal healthy subjects showed no significant difference between any of the groups of patients with diabetes and our normal subjects (Fig. 1). There was no relationship between the level of exercise, expressed as $\dot{V}O_2$ or $\dot{V}CO_2$, and the ventilatory response to hypoxia in the abnormal, normal or borderline subjects. Moreover the physiological delay between the hypoxic stimulus and the ventilatory response was similar in each group (DAN+ 2.2 ± 0.4; DANI 2.4 ± 0.6; DAN- 2.0 ± 1.5).

The mean resting heart rate in the patients with autonomic neuropathy was 97.3 ± 12.6 beats/min as compared with 87.9 ± 11.6 beats/min in those patients without autonomic neuropathy. There was no significant difference in heart rate between these groups on exercise (DAN+ = 109.3 ± 6.5 beats/min; DAN- = 107.4 ± 10.1 beats/min). The resting heart rate failed to increase on exercise in three of the six patients with unequivocally abnormal cardiovascular reflexes but rose significantly in the other three and in all the DAN- patients. The response of the heart rate to transient hypoxia was assessed in a similar fashion to the ventilatory response, with a computer method to allow for the delay between stimulus (Peto,) and change in heart rate. The response to hypoxia varied considerably in each group, the increase in heart rate per kPa fall in oxygen pressure ranging from 0.01 to 0.6 beat min⁻¹ kPa⁻¹ in the DAN+ patients and 0.3 to 1.1 beat min⁻¹ kPa⁻¹ in the DAN- patients, the difference between the groups reaching statistical significance at the 5% level. There was no

### Table 3. Oxygen uptake and carbon dioxide production at rest and during exercise, and ventilatory and heart rate responses to hypoxia in the three groups of diabetic patients

<table>
<thead>
<tr>
<th>Diabetic patients</th>
<th>At rest (ml/min)</th>
<th>During exercise (ml/min)</th>
<th>$\dot{V}e$/Peto₂</th>
<th>Heart rate (beats/min)</th>
<th>HR/Peto₂ (beats min⁻¹ kPa⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Without autonomic neuropathy (DAN-)</td>
<td>330 ± 42</td>
<td>916 ± 312</td>
<td>144</td>
<td>-1.20</td>
<td>87.9 ± 11.6</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>With borderline results (DANI)</td>
<td>320 ± 29</td>
<td>946 ± 40</td>
<td>98</td>
<td>-1.20</td>
<td>92.7 ± 20.7</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
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<td></td>
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<tr>
<td>With autonomic neuropathy (DAN+)</td>
<td>315 ± 77</td>
<td>894 ± 186</td>
<td>103</td>
<td>-0.95</td>
<td>97.3 ± 12.6</td>
</tr>
</tbody>
</table>

$\dot{V}O_2$, Oxygen uptake; $\dot{V}CO_2$, CO₂ production; $\dot{V}e$/Peto₂ and heart rate (HR)/Peto₂; values are given as means ± SD or ranges.
Hypoxic drive in autonomic neuropathy

**FIG. 1.** Ventilatory response to transient hypoxia in 41 healthy mine rescue workers and 12 diabetic patients without autonomic neuropathy (DAN–, group 1) and six diabetic patients with autonomic neuropathy (DAN+, group 3). Hypoxic drive is the slope $V/E_{PAO_2}$.

significant correlation between the heart rate response to hypoxia and the ventilatory response to hypoxia but those patients with diabetic autonomic neuropathy who showed least increase in heart rate during exercise also showed small heart rate responses to transient hypoxia. The delay between the hypoxic stimulus and the heart rate response was greater in the diabetic patients with normal autonomic function (DAN 7.2 ± 2.0).

**Discussion**

In 1976 we described two patients with established diabetic autonomic neuropathy who died unexpectedly, but with no post-mortem evidence of myocardial infarction or marked atheroma [1]. There have also been other reports of patients with autonomic neuropathy who have had cardiorespiratory arrests which were usually during anaesthesia or hypoxia from other causes and which were usually reversible [4]. Little is known of the ventilatory response to hypoxia in patients with acquired autonomic neuropathy but abnormal ventilatory responses have been recorded in familial autonomic dysautonomia (Riley Day syndrome) [10] and in one patient with diabetes but with the use of a methodology different from ours [11]. Hypoxia stimulates ventilation by activating peripheral chemoreceptors (principally the carotid chemoreceptors in man), which are innervated by the myelinated afferent nerve fibres running in a branch of the glossopharyngeal cranial nerve IX. Our study sought for evidence that in diabetic autonomic neuropathy the afferent nerve supply might be involved and could thus account for these unexpected deaths which appear to be associated with clinical hypoxia.

We studied the diabetic patients with and without abnormal cardiovascular reflexes using transient hypoxia during exercise, which is considered to be a relatively specific stimulus to the carotid chemoreceptors. However, we failed to demonstrate that the response to this stimulus was impaired in diabetic patients with autonomic neuropathy. Although the level of exercise achieved can affect the response to hypoxia [12] there was no evidence in our study that differences in the level of exercise were masking differences in sensitivity to hypoxia among the subjects. Neither the patient's age nor duration of diabetes, nor the presence of other complications of diabetes, was related to the magnitude of the hypoxic drive in these patients. As the patients had to exercise for 20 min for these measurements to be made some patients with severe autonomic neuropathy were excluded. However, in all the patients in the DAN+ group there were definite symptoms and unequivocal abnormalities in the cardiovascular reflex tests, which we would regard as being typical of autonomic neuropathy. The heart rate response to exercise in hypoxia showed considerable variation among the diabetic patients, the autonomic neuropathy group having a higher resting pulse rate [13] with
less change both during exercise and hypoxia. There was a wide overlap between the groups, and some patients with autonomic neuropathy had a heart rate which was unresponsive to physiological stimuli but nevertheless showed a significant increase after hypoxia.

Our finding of an intact peripheral chemoreceptor response to transient hypoxia fails to explain the observed cardiorespiratory arrests occurring in patients with autonomic neuropathy. Edelman and colleagues [10] reported that patients with familial dysautonomia had a blunted CO₂ response in hypoxia and when breathing CO₂ in a hypoxic background their ventilation fell instead of rising. However, 100% oxygen produced apnoeic episodes of up to 56 s in these patients, a finding similar to that reported for two patients described by Page & Watkins [4]. The ventilatory response to transient hypoxia (3 min duration) was normal in familial dysautonomia but the marked reduction in ventilation after exposure to 100% oxygen, which reduces the discharge from the carotid chemoreceptors, could imply that these patients were more than usually dependent on this chemoreceptor input to maintain the resting ventilation. More recently reduced CO₂ responsiveness during hyperoxic and hypoxic CO₂ re-breathing has been described [14] in patients with diabetic neuropathy, suggesting an abnormal central control of respiration. Centrally induced apnoea may not respond to chemoreceptor discharge [15] and can itself be induced by tracheal stimulation in patients with a disordered sympathetic nervous system [16].

The reasons for abnormal central control of respiration are unclear but the control of cerebral blood flow is known to be abnormal in diabetic patients [17], who show a lack of normal cerebral vasodilatation produced by an increased arterial PCO₂. Hypoxia is a potent stimulus to cerebral vasodilatation and, if this fails to occur, hypoxia depresses ventilation centrally. Cerebral autoregulation in response to hypoxia in diabetic patients has, however, not so far been studied in detail.

The carotid chemoreceptors thus appear to function normally in diabetic patients with autonomic neuropathy, but several other factors singly or in combination might lead to cardiorespiratory arrests observed in such patients. An abnormal cerebrovascular response to hypoxia could cause central respiratory depression, which, uncorrected by the otherwise intact chemoreceptors, could progress to apnoea. Alternatively, or together with the above, tracheal stimulation during the introduction of an endotracheal tube in the presence of an abnormal sympathetic nervous system could produce apnoea and severe bradycardia. Whether other causes of hypoxia, such as transient hypoxaemia during sleep described in normal subjects [18] and in patients with chronic bronchitis and emphysema [19], contribute to these episodes of cardiorespiratory arrest remains to be determined.

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References