Cerebrovascular reactivity and hypercapnic respiratory drive in diabetic autonomic neuropathy

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Cerebrovascular reactivity and hypercapnic respiratory drive in diabetic autonomic neuropathy. J Appl Physiol 90: 889–896, 2001.—Because abnormalities in cerebrovascular reactivity (CVR) in subjects with long-term diabetes could partly be ascribed to autonomic neuropathy and related to central chemosensitivity, CVR and the respiratory drive output during progressive hypercapnia were studied in 15 diabetic patients without (DAN−) and 30 with autonomic neuropathy (DAN+), of whom 15 had postural hypotension (PH) (DAN+PH+) and 15 did not (DAN+PH−), and in 15 control (C) subjects. During CO2 rebreathing, changes in occlusion pressure and minute ventilation were assessed, and seven subjects in each group had simultaneous measurements of the middle cerebral artery mean blood velocity (MCAV) by transcranial Doppler. The respiratory output to CO2 was greater in DAN+PH+ than in DAN+PH− and DAN− (P < 0.01), whereas a reduced chemosensitivity was found in DAN+PH− (P < 0.05 vs. C). MCAV increased linearly with the end-tidal PCO2 (PETCO2) in DAN+PH− but less than in C and DAN− (P < 0.01). In contrast, DAN+PH+ showed an exponential increment in MCAV with PETCO2, mainly >55 Torr. Thus CVR was lower in DAN+ than in C at PETCO2 <55 Torr (P < 0.01), whereas it was greater in DAN+PH+ than in DAN+PH− (P < 0.01) and DAN− (P < 0.05) at PETCO2 >55 Torr. CVR and occlusion pressure during hypercapnia were correlated only in DAN+ (r = 0.91, P < 0.001). We conclude that, in diabetic patients with autonomic neuropathy, CVR to CO2 is reduced or increased according to the severity of dysautonomy and intensity of stimulus and appears to modulate the hypercapnic respiratory drive.

A higher rate of mortality has been extensively reported in diabetic patients with autonomic neuropathy (DAN+) compared with diabetic patients without dysautonomy (43). In DAN+, most deaths occur suddenly during stressing conditions and/or sleep and have often been attributed to cardiorespiratory events (12, 28, 36). Hence, a number of studies have investigated the influence of the diabetic dysautonomy on the control of breathing (19, 24, 35, 42), leading, however, to controversial results. In fact, whereas a consistent decrease in hypoxic drive has been found in DAN+, indicating a reduced peripheral chemosensitivity in these subjects (24, 27, 34, 42), conflicting results have been reported, as far as the central chemosensitivity was concerned, as increased (27), normal (34, 35), and decreased (19, 24, 40, 42) responses of the respiratory drive to hypercapnia have been described in similar populations of DAN+. Recently, by splitting DAN+ into two groups that were characterized, according to the standard cardiovascular tests (13), by the presence of either parasympathetic and sympathetic damage with postural hypotension (PH) (DAN+PH+) or predominant parasympathetic damage without PH (DAN+PH−), we were able to show an increased response of the respiratory centers to progressive hypercapnia in the former group, whereas the opposite was found in the latter (38). These findings may suggest a modulatory effect of the sympathetic arm of the autonomic nervous system on the CO2 responsiveness of the respiratory centers that, in fact, is enhanced when the sympathetic activity is markedly decreased, as in DAN+PH+, and reduced when this is not counterbalanced, as in DAN+PH−. The operating mechanism, however, is unknown. Because abnormalities in cerebrovascular reactivity (CVR) to different stimuli have been shown in large subsets of patients with long-lasting diabetes, many of whom were young and possibly suffering from autonomic neuropathy (11), we reasoned that, besides a potential direct mechanism, the sympathetic nervous system could indirectly influence the respiratory center output by regulating the cerebral blood flow (CBF) in the presence of effective stimuli. To verify this hypothesis, the CVR to CO2 was assessed in diabetic patients with different degrees of dysautonomy, while the ventilatory and neuromuscular response of the respiratory drive to progressive hypercapnia was monitored.

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METHODS

Subjects. Forty-five male diabetic patients, 15 without (DAN–) and 30 with (DAN+) diabetic autonomic neuropathy were recruited from the Dipartimento di Medicina Interna e Scienze Endocrine e Metaboliche of University of Perugia and enrolled in the study after they had given fully informed consent. The protocol was approved by the local ethics committee and was in accordance with the Helsinki Declaration.

All patients on treatment with regular insulin before each meal and intermediate-acting insulin at bedtime were in a satisfactory metabolic control (Table 1). No drugs able to influence pulmonary or cardiovascular function were being taken by the patients.

Autonomic neuropathy was assessed by the following classic cardiovascular tests: 1) heart rate (HR) variation to deep breathing; 2) HR response to Valsalva maneuver; 3) HR response from lying down to standing; 4) postural fall in systolic blood pressure; and 5) diastolic blood pressure rise to sustained handgrip (13). According to the literature (2), each test result was scored 0 if normal, 1 if borderline, and 2 if abnormal. The diabetic patients were considered suffering from autonomic neuropathy only if the total score was ≥4. Fifteen of 30 DAN+ patients had predominant parasympathetic involvement of the autonomic nervous system, that is, an impairment of at least two tests of deep breathing, Valsalva maneuver, and lying down to standing, but a normal increase in diastolic blood pressure to sustained handgrip and lack of PH, and we referred to them as DAN+PH–. The remaining 15 DAN+ exhibited severe autonomic neuropathy, that is, parasympathetic damage associated with an overt sympathetic damage as reflected by PH, i.e., an upright fall of systolic blood pressure >30 mmHg and impaired response to sustained handgrip, i.e., an increment of diastolic blood pressure <16 mmHg, and we referred to them as DAN+PH+. (4, 5, 29). One patient in the DAN+ group, four in the DAN+ PH+ group, and nine in the DAN+PH+ group had pro/pre proliferative retinopathy; two DAN+PH+ patients had proliferative retinopathy. The remaining patients had no proliferative or background retinopathy. Two subjects in the DAN– group, seven in the DAN+PH– group, and 10 in the DAN+PH+ group had microalbuminuria (overnight urinary protein excretion of 20–200 μg/min); one subject in the DAN+PH– group and four subjects in the DAN+PH+ group had proteinuria (<2 g/24 h), but none of them had serum creatinine >1.5 mg/dl. Two patients in the DAN+PH+ group had symptoms related to autonomic dysfunction, that is, nocturnal watery diarrhea and gustatory sweating, respectively.

All patients had to be normotensive, without evidence or history of ischemic heart and cerebrovascular disease. Duplex scans of the carotid arteries had to show no or only mild (<30%) stenosis of the internal carotid artery. None of the patients studied had symptoms or signs of endocrine or metabolic disease other than diabetes. No respiratory symptoms were observed or reported at the time of the study. Fifteen male normal subjects recruited from the University staff were studied as the control (C) group (Table 1).

Study design. All patients and C subjects underwent pulmonary function tests including spirometry, flow/volume curves, determination of lung volumes (by multiple-breath nitrogen washout technique), and measurements of maximal inspiratory (MIP) and expiratory mouth pressures (MEP). All tests were performed with subjects in the sitting position, wearing a nose clip, and breathing through a mouthpiece connected to a computerized measuring system (MedGraphics 1070; Medical Graphics, St. Paul, MN). The predicted values for volumes and flows were those proposed by the European Community for Coal and Steel (31). Measurements of MIP and MEP, sustained for at least 1 s, which could not differ by >5%, were obtained in triplicate at functional residual capacity by a differential pressure transducer (±300 cmH2O; Validyne, Northridge, CA). Subjects were comfortably seated, wearing a nose clip, and performed maximal inspiratory and expiratory efforts against an obstructed mouthpiece with a small leak (internal diameter ~2 mm) during inspiration to prevent the subjects from generating pressures with their facial muscles. The mean of the two best efforts was considered for analysis. Predicted values for MIP and MEP were those proposed by Cook et al. (7). Minute ventilation (VE), tidal volume (VT), and respiratory rate (RR)

Table 1. Clinical features of the subjects

<table>
<thead>
<tr>
<th>Subjects, no.</th>
<th>C</th>
<th>DAN–</th>
<th>DAN+PH–</th>
<th>DAN+PH+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>15</td>
<td>44.1 ± 3.2</td>
<td>46.3 ± 2.7</td>
<td>46.3 ± 2.5</td>
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<tr>
<td>BMI, kg/m²</td>
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<td>24.0 ± 1.0</td>
<td>24.0 ± 0.5</td>
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<tr>
<td>Smoking habit, pack-yr</td>
<td>9.5 ± 2.3</td>
<td>12.0 ± 4.2</td>
<td>17.1 ± 4.7</td>
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<tr>
<td>Type 1/2 diabetes, no.</td>
<td>11/4</td>
<td>10/5</td>
<td>10/5</td>
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<tr>
<td>Duration of diabetes, yr</td>
<td>16.1 ± 1.8</td>
<td>17.9 ± 3.2</td>
<td>18.5 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>Autonomic neuropathy score</td>
<td>0.3 ± 0.1</td>
<td>4.9 ± 0.3*</td>
<td>7.6 ± 0.2**†</td>
<td></td>
</tr>
<tr>
<td>Hba1c, %</td>
<td>7.3 ± 0.2</td>
<td>7.5 ± 0.2</td>
<td>7.9 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Retinopathy</td>
<td>7</td>
<td>8</td>
<td>3</td>
<td></td>
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<td>Background</td>
<td>1</td>
<td>4</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Pre-proliferative</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proliferative</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nephropathy</td>
<td>2</td>
<td>7</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>46 ± 3.2</td>
<td>36 ± 2.8‡</td>
<td>24 ± 3.2*</td>
<td></td>
</tr>
<tr>
<td>Proteinuria</td>
<td>320 ± 21</td>
<td>317 ± 26</td>
<td>231 ± 15§</td>
<td></td>
</tr>
<tr>
<td>Plasma glucose, mg/dl</td>
<td>186 ± 12</td>
<td>189 ± 10</td>
<td>180 ± 24</td>
<td></td>
</tr>
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</table>

Values are means ± SE. DAN–, subjects without autonomic neuropathy; DAN+PH–, subjects with diabetic autonomic neuropathy without postural hypotension; DAN+PH+, subjects with severe autonomic neuropathy including postural hypotension; C, control subjects; BMI, body mass index; Hba1c, Hb A1 glycosylate. Hba1c values in nondiabetic subjects are 3.8–5.5%. Plasma glucose values are before the CO2 rebreathing. Significant difference: *P < 0.001 vs. DAN–; †P < 0.001 vs. DAN+PH–; ‡P < 0.05 vs. DAN–; §P < 0.01 vs. DAN– and DAN+PH–.
were measured at rest from the time-integrated flow signal with the subjects breathing room air in the seated position through a two-way, non-rebreathing, balloon shutter occlusion valve (Hans-Rudolph, Kansas City, MO). Randomly, during each four to eight breaths during expiration, the inspiratory line was silently closed by automatically inflating the balloon with a computer-supported pneumatic system (respiratory pressure module MedGraphics, Medical Graphics). The mouth pressure was measured during the following occluded inspiration at a side port on the occlusion valve, which was connected to a pressure transducer (+150 cmH2O; Validyne) through a noncompliant polyethylene catheter (internal diameter 1.4 mm; length 95 cm). The value of mouth pressure with occluded airways, calculated 100 ms after the beginning of the inspiration (P0.1), was displayed by the computer (respiratory pressure module MedGraphics, Medical Graphics) (41). Baseline P0.1 was obtained as a mean of at least eight values of occlusion pressure, after the lowest and the highest were rejected. CO2 was continuously sampled at least eight values of occlusion pressure, and, only in diabetic patients in the morning, venous blood samples were obtained by means of an ear pulse oximeter (Biox 3700, Criticon). The MIP and MEP were obtained after the occlusion maneuver, and V˙E, V T, and RR were calculated from the four breaths preceding each occlusion. For each rebreathing test, V˙E and P0.1 were plotted against PETCO2, and data were fitted according to the least squares method. The slopes and intercepts of the linear regression were computed. The coefficient of correlation was >0.97 in all subjects. At a flow rate of 1 l/s, the resistance of the rebreathing circuit was 1.1 cmH2O l−1·s−1.

Baseline HR and arterial hemoglobin oxygen saturation were obtained by means of an ear pulse oximeter (Biox 3700, Ohmeda, Boulder, CO), and systolic and diastolic arterial blood pressures were obtained by using a sphygmanometer in all subjects when they were sitting.

In each diabetic group, seven subjects with similar clinical and functional characteristics and seven matched C subjects underwent measurements of mean blood velocity in the middle cerebral artery (MCAV) by ultrasound transcranial Doppler using a Multidop (ESAOTE, Firenze, Italy), both in basal conditions and during rebreathing. After the middle cerebral artery was insonated at a depth of 50 mm, on the temporal window, by using a hand-held 2-MHz probe, MCAV was continuously assessed and measured at each occlusion maneuver for the analysis. The CVR, which is the increase in MCAV expressed as a percentage of baseline MCAV (14), was also computed at different values of PETCO2 (45, 50, 55, and 60 Torr) and at peak.

In all instances, positioning of the probe and MCAV measurements were made by the same operator (C. Fiorani).

Simultaneously, in these subjects, HR, arterial blood pressure, and, only in diabetic patients in the morning, venous blood samples to measure catecholamine plasma levels were obtained under basal conditions and during the rebreathing test at 55 Torr and at peak value of PETCO2.

Blood samples for determination of the plasma epinephrine and norepinephrine, drawn throughout an 18-gauge Teflon catheter inserted into a superficial vein of the forearm, were immediately placed on ice until centrifugation and storage of plasma at −20°C could be performed. Later analysis was carried out by using the HPLC method (18).

Plasma glucose concentration was measured at baseline and at the end of the rebreathing test in all diabetic patients by means of a Beckman glucose analyzer (Beckman Instruments, Palo Alto, CA). The rebreathing test and ultrasound transcranial Doppler were performed twice, in the morning between 1100 and 1130 and in the afternoon between 1600 and 1630. The values of MCAV, CVR, and slopes of the linear relationship of P0.1 against PETCO2 (ΔP0.1/ΔPETCO2) and of V˙E against PETCO2 (ΔV˙E/ΔPETCO2) averaged from the two tests were considered for the analysis.

Statistical analysis. Comparison of the four groups was performed by using the ANOVA, and orthogonal comparisons were done by adopting the two-tailed unpaired Student’s t-test, corrected according to Bonferroni, when allowed by ANOVA. If no assumption about the scatter of the data could be made, the groups were compared by adopting the Kruskal-Wallis test, and, when a significant difference was found, multiple comparisons were performed to compute the difference of the rank’s means. Linear correlations were calculated by means of the least squares method. A P value < 0.05 was considered as significant. Data are expressed as means ± SE.

RESULTS

Pulmonary function tests. Lung volumes, indexes derived from the flow/volume curve, MIP, and MEP are listed in Table 2. The pulmonary function parameters were within the normal range in all the groups. Vital capacity was higher in C and DAN− than in both DAN+ groups (P < 0.05). Total lung capacity, inspiratory capacity, and forced expiratory volume in the first second were higher in C than in both DAN+ groups (P < 0.01), and inspiratory capacity and forced expiratory volume in the first second were higher in DAN− than in DAN+ PH+ (P < 0.05). Total lung capacity was higher in DAN− than in DAN+ PH− (P < 0.05). The other parameters of lung function did not differ between C and diabetic patients. MIP and MEP were not.

Table 2. Baseline pulmonary function data and gas exchange parameters

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>DAN−</th>
<th>DAN+ PH−</th>
<th>DAN+ PH+</th>
</tr>
</thead>
<tbody>
<tr>
<td>VC, %pred</td>
<td>119 ± 2</td>
<td>112 ± 4</td>
<td>102 ± 3*</td>
<td>97 ± 2*</td>
</tr>
<tr>
<td>IC, %pred</td>
<td>115 ± 3</td>
<td>110 ± 5</td>
<td>100 ± 4*</td>
<td>91 ± 3*</td>
</tr>
<tr>
<td>FR, %pred</td>
<td>110 ± 7</td>
<td>103 ± 6</td>
<td>93 ± 6</td>
<td>112 ± 9</td>
</tr>
<tr>
<td>RV, %pred</td>
<td>111 ± 4</td>
<td>107 ± 6</td>
<td>95 ± 6</td>
<td>120 ± 10</td>
</tr>
<tr>
<td>TLC, %pred</td>
<td>113 ± 2</td>
<td>107 ± 4</td>
<td>96 ± 4*</td>
<td>100 ± 4*</td>
</tr>
<tr>
<td>FEV1, %pred</td>
<td>110 ± 3</td>
<td>103 ± 5</td>
<td>93 ± 4</td>
<td>95 ± 2*</td>
</tr>
<tr>
<td>FEV1/FVC, %</td>
<td>81 ± 1</td>
<td>82 ± 1</td>
<td>83 ± 1</td>
<td>81 ± 1</td>
</tr>
<tr>
<td>FEF25–75, %pred</td>
<td>95 ± 4</td>
<td>90 ± 6</td>
<td>91 ± 5</td>
<td>81 ± 1</td>
</tr>
<tr>
<td>MIP, cmH2O</td>
<td>102 ± 4</td>
<td>103 ± 9</td>
<td>102 ± 9</td>
<td>92 ± 7</td>
</tr>
<tr>
<td>MEP, cmH2O</td>
<td>94 ± 3</td>
<td>112 ± 10</td>
<td>106 ± 8</td>
<td>99 ± 7</td>
</tr>
<tr>
<td>MEP, %pred</td>
<td>116 ± 10</td>
<td>132 ± 12</td>
<td>124 ± 14</td>
<td>103 ± 14</td>
</tr>
<tr>
<td>SaO2, %</td>
<td>98.3 ± 0.2</td>
<td>98.2 ± 0.4</td>
<td>98.1 ± 0.3</td>
<td>98.3 ± 0.2</td>
</tr>
<tr>
<td>PETCO2, Torr</td>
<td>38.2 ± 0.7</td>
<td>38.7 ± 0.5</td>
<td>38.0 ± 0.7</td>
<td>38.7 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. %pred, percentage of predicted values; VC, vital capacity; IC, inspiratory capacity; FRC, functional residual capacity; RV, residual volume; TLC, total lung capacity; FEV1, forced expiratory volume in the first second; FVC, forced expiratory volume; FEF25–75, forced expiratory flow between 25 and 75% of forced vital capacity ratio; MIP, maximal inspiratory pressure; MEP, maximal expiratory pressure; SaO2, oxygen saturation; PETCO2, end-tidal CO2 partial pressure. Significant difference: *P < 0.01 vs. C; †P < 0.01 vs. DAN−; ‡P < 0.05 vs. DAN−.
significantly different among the groups, both as absolute value and as percentage of predicted. Baseline PETCO₂ and arterial hemoglobin oxygen saturation were normal and nearly identical in all groups (Table 2).

Control of breathing. The baseline values of P₀.₃, Vₑ, Vᵗ, and RR were similar in all groups (Table 3). At the end of the rebreathing test, the peak value of PETCO₂ was not different among the groups, amounting to 65 ± 1 Torr for the C subjects and 65 ± 1, 64 ± 1, and 62 ± 1 Torr for DAN⁻, DAN⁺PH⁻, and DAN⁺PH⁺, respectively. The slope of the linear relationship of ΔP₀.₃/ΔPETCO₂, which was 0.45 ± 0.04 cmH₂O/Torr in C subjects, was significantly higher in DAN⁺PH⁺ (0.62 ± 0.05 cmH₂O/Torr) than in DAN⁻ (0.36 ± 0.04 cmH₂O/Torr; P < 0.01) and DAN⁺PH⁻ (0.28 ± 0.03 cmH₂O/Torr; P < 0.01). Moreover, the ΔP₀.₃/ΔPETCO₂ exhibited by DAN⁺PH⁻ was significantly lower than that shown by C subjects (P < 0.05) (Table 3).

The slope of the linear relationship ΔVₑ/ΔPETCO₂ amounted to 3.49 ± 0.51 l·min⁻¹·Torr⁻¹ in the DAN⁺PH⁺ group and was higher than that in the DAN⁺PH⁻ (2.01 ± 0.18 l·min⁻¹·Torr⁻¹; P < 0.05) and DAN⁻ groups (2.43 ± 0.26 l·min⁻¹·Torr⁻¹; not significant). ΔVₑ/ΔPETCO₂ was 3.31 ± 0.21 l·min⁻¹·Torr⁻¹ in the C group and was significantly higher (P < 0.01) than that in the DAN⁻ group (Table 3). The individual values of the ΔP₀.₃/ΔPETCO₂ and ΔVₑ/ΔPETCO₂ slopes are shown for the diabetic groups and C subjects in Fig. 1. The correlation coefficient of the identity scatterplot of the ΔP₀.₃/ΔPETCO₂ slope values between the two CO₂ rebreathing tests was 0.91 (P < 0.001).

MCAV and CVR. Baseline MCAV did not differ among the groups, amounting to 60.3 ± 4.2 cm/s in DAN⁻, 58.1 ± 4.8 cm/s in DAN⁺PH⁻, 63.4 ± 2.4 cm/s in DAN⁺PH⁺, and 67.3 ± 2.7 cm/s in C, although it was slightly lower in diabetic patients than in C subjects. The increment in MCAV due to progressively increasing hypercapnia was different among the groups (P < 0.001). In fact, at PETCO₂ of 50 Torr, MCAV was higher in C (87.6 ± 4.5 cm/s) than in DAN⁻, DAN⁺PH⁻, and DAN⁺PH⁺ (72.6 ± 3.4 cm/s, P < 0.05; 67.2 ± 6.2 cm/s, P < 0.01; and 73.7 ± 3.4 cm/s, P < 0.05, respectively), whereas at PETCO₂ of 60 Torr MCAV was higher in DAN⁺PH⁺ (137.8 ± 10.7 cm/s) than in DAN⁻ (96.4 ± 5.8 cm/s; P < 0.01) and DAN⁺PH⁻ (84.9 ± 9.0 cm/s; P < 0.01). At this value of PETCO₂, MCAV in DAN⁺PH⁻ was significantly lower than that shown by C subjects (P < 0.001 vs. C; ‡P < 0.01 vs. DAN⁻ and DAN⁺PH⁻; §P < 0.01 vs. DAN⁺PH⁻). Thus the relationship between MCAV and PETCO₂ was essentially linear in DAN⁻, DAN⁺PH⁻, and C was exponential in DAN⁺PH⁺ because of the progressively greater increase in MCAV at PETCO₂ values of 55 Torr (Fig. 2).
the CO₂ rebreathing test compared with baseline in compared with DAN.

norepinephrine was significantly lower (plasma catecholamines, and the final level of plasma

PH 1 found in these two groups at the end of the rebreathing and a smaller increase in mean blood pressure was

increase linearly with PETCO₂ in C, DAN PH 1 PH 2

MCAV is lower in DAN PH 1 PH 2 DAN PH 2 PH 1, as well as in DAN PH− and DAN PH+ at lower levels of PETCO₂ with respect to C, whereas it is significantly increased in DAN PH+, compared with the other diabetic groups, at higher levels of PETCO₂. Data are means ± SE. Significant difference: *P < 0.05, **P < 0.01 vs. C; †P < 0.01 vs. DAN+PH+.

As a result, at PETCO₂ of 50 Torr, CVR was higher in C (32.6 ± 1.4%) than in DAN−, DAN+PH−, and DAN+PH+ (21.9 ± 5.6%, P < 0.05; 15.4 ± 1.3%, P < 0.01; and 16.9 ± 3.7%, P < 0.01, respectively). Conversely, at PETCO₂ of 60 Torr, CVR was higher in DAN+PH+ (111.2 ± 17.7%) compared with DAN− (59.6 ± 5.1%; P < 0.05), DAN+PH− (44.7 ± 5.1%; P < 0.01), and, although not significantly, also C (64.0 ± 5.1%; P = 0.09) (Fig. 3).

The coefficient correlation of the identity scatterplot of the CVR values computed at PETCO₂ of 60 Torr between the two transcranial Doppler measurements of MCAV was 0.67 (P < 0.01).

Catecholamines, HR, and arterial blood pressure. The values of plasma norepinephrine and epinephrine, HR, and mean arterial blood pressure in basal conditions and at the end of the rebreathing test for those diabetic patients and C subjects who performed ultrasound transcranial Doppler are listed in Table 4. The HR was unchanged in DAN+PH− and DAN+PH+, and a smaller increase in mean blood pressure was found in these two groups at the end of the rebreathing test. DAN+PH+ exhibited the lowest increment in plasma catecholamines, and the final level of plasma norepinephrine was significantly lower (P < 0.01), compared with DAN−.

The plasma glucose was not different at the end of the CO₂ rebreathing test compared with baseline in each diabetic group.

DISCUSSION

The main findings of the study are that 1) diabetic patients have an impaired CVR (less increase in CBF) with respect to C subjects at PETCO₂ of <55 Torr (i.e., facing with stronger vasodilating stimuli); 3) DAN+PH+ show an increased hypercapnic respiratory drive that is greater than that observed in DAN+PH− and DAN−; 4) DAN+PH− are characterized by the lowest CVR and gain of the respiratory center output in response to CO₂.

To assess CVR to hypercapnia, we measured blood flow velocity in large cerebral arteries by means of ultrasound transcranial Doppler. This method has been widely adopted (14, 20, 22) instead of determination of regional CBF by single-photon emission computerized tomography or by positron emission tomography to evaluate cerebral vasoreactivity or detect abnormalities of cerebral hemodynamics (9, 10, 37). In fact, changes in velocity and regional CBF have been shown to correlate closely when transcranial Doppler was used simultaneously with these more expensive and technically complex methods.

In the present study, transcranial Doppler ultrasonography was used during progressive hypercapnia, assuming that changes in blood velocity parallel changes in CBF under these conditions also. Because, in normal subjects, the cross-sectional surface area of the basal cerebral arteries is not affected by changes in arterial Pco₂ (1), this assumption is valid for C, but we are not aware of whether this has been demonstrated in DAN+. However, if the increment in arterial Pco₂, which is a powerful vasodilating stimulus, had increased the cross-sectional surface area of the middle cerebral artery, especially in DAN+PH+, then the increase in MCAV that we recorded in these subjects
could have only underestimated the increase in their CBF.

Although some studies performed with the single-photon emission computerized tomography technique have suggested a reduced resting CBF in diabetic patients compared with age-matched C subjects, the lack of significant difference in baseline MCAV between our diabetic and C groups does agree with that reported in several previous works, showing, on average, no reduction either in resting MCAV (14) or in basal global and regional CBF in diabetic patients compared with C subjects (11, 25, 33).

The reduced CVR found in our diabetic patients is in line with the earlier reports showing less increase in CBF after the administration of either intravenous acetazolamide or a given, relatively low percentage of inspiratory CO₂ (5%) in general populations of patients with long-term diabetes (11, 14).

Our data, however, show that autonomic neuropathy might play a role in further decreasing CVR in diabetic patients. Indeed, the average slope of the MCAV/\(P_{\text{ETCO}_2}\) linear relationship, computed between 45- and 55-Torr \(P_{\text{ETCO}_2}\), to avoid the dog-leg phenomenon exhibited by these subjects (Fig. 2), is lower in DAN+PH⁻ (1.84 ± 0.17 cm²·s⁻¹·Torr⁻¹; \(P < 0.01\)) and also in DAN+PH⁺ (1.92 ± 0.19 cm²·s⁻¹·Torr⁻¹; \(P < 0.05\)) than in DAN⁻ (2.76 ± 0.29 cm²·s⁻¹·Torr⁻¹). Although this difference was not large enough to induce a significantly lesser CVR in DAN+ than in DAN⁻ at 50-Torr \(P_{\text{ETCO}_2}\) (Fig. 3), nevertheless, it may suggest a cerebral vasoregulatory action of the autonomic nervous system, possibly cholinergic, which is altered in DAN+.

Therefore, besides microangiopathic changes of the brain resistance arterioles, hemorheological changes (14, 23), and presence of vasoactive substances (14, 23), autonomic neuropathy could be another cause of reduced reactivity of the cerebral vessels to physiological stimuli in diabetes.

At the same time, a subset of DAN+, namely the DAN+PH⁻ group, exhibited a reduced, neuromuscular, and ventilatory output of the respiratory drive in response to progressive hypercapnia, reflecting a significantly decreased central chemosensitivity.

However, compared with DAN+PH⁻ and DAN⁻, and even with C, DAN+PH⁺ showed a markedly greater increase in MCAV at higher values of \(P_{\text{ETCO}_2}\) (Fig. 2) and an enhanced hypercapnic respiratory drive (Fig. 1). This late, paradoxical increase in CVR does imply an exaggerated vasodilation to severe hypercapnia in diabetic patients with advanced dysautonomia, strongly supporting the idea that, in the presence of stressing stimuli, the sympathetic nervous system may play a role in limiting the increase of the CBF, as well as in modulating the output of the respiratory centers to hypercapnia.

Because physiological activation or electrical stimulation of sympathetic nerves in animals has been shown to induce moderate cerebral vasoconstriction and regulate CBF variations (16, 17, 30), direct inhibitory action of the sympathetic nervous system on the cerebral vascular bed in the face of strong vasodilating stimuli may actually be envisaged in humans. Moreover, alterations of adrenergic and cholinergic innervation of brain arterioles have been described in experimental diabetes (14).

Also, the responsiveness of the central drive to CO₂ could be directly modulated by the sympathetic nervous system. Indeed, pulmonary sympathetic afferents have been found in dogs and monkeys, and the stimulation of nerves carrying these fibers was able to inhibit the phrenic discharge in these anesthetized animals (21). In addition, in the absence of catecholamine-mediated peripheral effect, central release of norepinephrine at respiratory-related units in the central nervous system has been shown to inhibit ventilatory output through an \(\alpha\)-receptor-mediated action (3, 6).

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**Table 4. Heart rate, blood pressure, and catecholamines before (baseline) and at the end (peak) of CO₂ rebreathing**

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>DAN⁻</th>
<th>DAN+PH⁻</th>
<th>DAN+PH⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>73 ± 2.9</td>
<td>70 ± 2.8</td>
<td>100 ± 3.9*</td>
<td>89 ± 4.9*</td>
</tr>
<tr>
<td>MBP, mmHg</td>
<td>103 ± 2.0</td>
<td>106 ± 1.9</td>
<td>106 ± 2.7</td>
<td>102 ± 2.8</td>
</tr>
<tr>
<td>Norepinephrine, pg/ml</td>
<td>293 ± 36</td>
<td>251 ± 26</td>
<td>199 ± 15</td>
<td>334 ± 33</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>42 ± 2.7</td>
<td>37 ± 3.2</td>
<td>26 ± 3.9</td>
<td>63 ± 6.1</td>
</tr>
<tr>
<td>Peak</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>88 ± 4.8</td>
<td>85 ± 5.9</td>
<td>105 ± 3.7</td>
<td>96 ± 4.4</td>
</tr>
<tr>
<td>MBP, mmHg</td>
<td>137 ± 3.5</td>
<td>139 ± 5.1</td>
<td>134 ± 2.7</td>
<td>129 ± 2.9</td>
</tr>
<tr>
<td>Norepinephrine, pg/ml</td>
<td>307 ± 34</td>
<td>294 ± 22</td>
<td>294 ± 22</td>
<td>294 ± 22</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>63 ± 6.1</td>
<td>48 ± 9.5</td>
<td>38 ± 3.1</td>
<td>38 ± 3.1</td>
</tr>
</tbody>
</table>

Values are means ± SE for \(n = 7\) subjects/group. HR, heart rate; MBP, mean arterial blood pressure. Significant difference: *\(P < 0.01\) vs. C and DAN⁻; †\(P < 0.05\) vs. DAN⁻.

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**Fig. 4. Relationship between individual values of CVR (CVR₆₀) and \(P_{\text{O}_2}\) (\(\bar{P}_{\text{O}_2}\)₆₀) measured at \(P_{\text{ETCO}_2}\) of 60 Torr during CO₂ rebreathing in DAN+ groups. The lesser CVR exhibited by DAN+PH⁻ is associated with lower values of \(P_{\text{O}_2}\) whereas the opposite occurs in DAN+PH⁺.**
Accordingly, greater CVR and enhanced hypercapnic respiratory drive shown by DAN+PH+ could be seen as independent, different expressions of the same phenomenon represented by the defective central modulation of the sympathetic autonomic nervous system, whereas the opposite could be invoked in DAN+PH−.

However, a close, direct correlation between CVR, reflecting the increment of CBF, and the P0,1 values (or the ΔP0,1/ΔPETCO2 slope), reflecting the response of the respiratory centers, is present during progressive hypercapnia in DAN+ (Fig. 4). Such a strong relationship tends to suggest a causal link between these two phenomena, possibly indicating that a greater or lesser CO2 load carried out on the central chemoceptors as a consequence of an excessive or reduced vasodilation in the presence of increasing circulatory CO2 levels can markedly influence the output of the respiratory centers to CO2. In other words, the hypercapnic central inspiratory drive, enhanced in DAN+PH+ and depressed in DAN+PH−, would be an expected, normal response toward a different acidic stimulus and would be relatively increased in DAN+PH+ or reduced in DAN+PH−, due, in both instances, to an impaired CVR.

No correlation was found between CVR and the ΔP0,1/ΔPETCO2 slope or the P0,1 values during CO2 rebreathing in DAN− and C.

It should be noted, however, that the vascular responses to progressive hypercapnia were assessed in the forebrain circulation (middle cerebral artery) rather than in the hindbrain circulation, where, because of the lesser sympathetic innervation to blood vessels, the changes in CVR could be quantitatively different and more crucial in influencing the activity of the central chemoceptors, especially in these subjects.

The results of the present study differ to some extent from those of other investigations carried out in patients with long-term diabetes and also in diabetic patients with disturbed autonomic innervation, in whom a preserved or slightly impaired CVR to administration of acetazolamide (33), CO2 inhalation (15), or blood pressure changes (8) was found, suggesting a minor role of the autonomic neuropathy in the cerebrovascular dysregulation in diabetes. Selection of patients, different techniques, and stimuli could explain the discrepancies. In fact, in obvious contrast to the aforementioned reports, in the present study the diabetic patients were carefully matched and categorized according to the presence and severity of the autonomic neuropathy, and their change in MCAV to hypercapnia was explored at different levels of CO2 by progressively increasing the magnitude of the stimulus.

These findings are clinically relevant because diabetic patients with dysautonomy might be at particular risk for developing cerebrovascular disease. Both the response to hypotension and the ability to increase CBF to cope with increasing metabolic demand could be further affected if the cerebral vasodilating response is further compromised by the presence of autonomic neuropathy. Moreover, in diabetic patients with more advanced dysautonomy, the excessive CVR after intense vasodilating stimuli might also be a negative event. For instance, in the presence of cerebral transient ischemia, it could allow an increased luxury flow in collaterally perfused vascular territories and thus divert blood flow from the ischemic area, thus accelerating the course of cerebral ischemia. This phenomenon might contribute to the greater prevalence of thrombotic stroke than transient ischemic attack in patients with diabetes, compared with the general population (23).

Finally, a possible explanation of the controversial results on the control of breathing in diabetic patients with autonomic neuropathy is given. In fact, a double response of the respiratory drive to hypercapnia is clearly shown by DAN+, depressed in DAN+PH−, and enhanced in DAN+PH+. This finding suggests a modulatory effect, likely mediated through the CBF regulation and exerted by the sympathetic nervous system on the central chemosensitivity, which, consequently, is either reduced or augmented in these patients.

In conclusion, the presence and severity of autonomic neuropathy does affect the CVR to CO2 and influence the hypercapnic drive to breathing in diabetic patients.

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