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Integrated cardiovascular/respiratory control in type 1 diabetes evidences functional imbalance: Possible role of hypoxia

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ABSTRACT

Background: Cardiovascular (baroreflex) and respiratory (chemoreflex) control mechanisms were studied separately in diabetes, but their reciprocal interaction (well known for diseases like heart failure) had never been comprehensively assessed. We hypothesized that prevalent autonomic neuropathy would depress both reflexes, whereas prevalent autonomic imbalance through sympathetic activation would depress the baroreflex but enhance the chemoreflexes.
Methods: In 46 type-1 diabetic subjects (7.0 ± 0.9 year duration) and 103 age-matched controls we measured the baroreflex (average of 7 methods), and the chemoreflexes, (hypercapnic: ventilation/carbon dioxide slope during hyperoxic progressive hypercapnia; hypoxic: ventilation/oxygen saturation slope during normocapnic progressive hypoxia). Autonomic dysfunction was evaluated by cardiovascular reflex tests.
Results: Resting oxygen saturation and baroreflex sensitivity were reduced in the diabetic group, whereas the hypercapnic chemoreflex was significantly increased in the entire diabetic group. Despite lower oxygen saturation the hypoxic chemoreflex showed a trend toward a depression in the diabetic group.
Conclusion: Cardio-respiratory control imbalance is a common finding in early type 1 diabetes. A reduced sensitivity to hypoxia seems a primary factor leading to reflex sympathetic activation (enhanced hypercapnic chemoreflex and baroreflex depression), hence suggesting a functional origin of cardio-respiratory control imbalance in initial diabetes.

1. Introduction

In diabetes, the impairment in autonomic function is associated with increased morbidity and mortality [1]. This abnormality could be observed at an early stage by a depressed baroreflex sensitivity [2,3], which is also a well-established marker of cardiovascular mortality for cardiovascular causes [4,5].

Recent data from our laboratory suggests that the baroreflex abnormality could be to some extent functional, and evidences point to similarities to the more general dysregulation occurring in congestive heart failure [6,7]. In chronic heart failure, a reduced baroreflex and a sympathetic overactivity, are linked to an overactivity of the reflexes that regulate ventilation (chemoreflexes), in a clear inverse relationship [8]. These functional abnormalities are implicated in the reduced exercise performance, and interventions aimed at restoring the cardio-respiratory control (e.g. physical exercise) improved both cardiovascular regulation and exercise tolerance, hence resulting in important clinical improvement in patients with heart failure [9].

On the other hand, we previously found that severe autonomic damage (as it occurs in familial dysautonomia) is associated with a reduction in both chemo- and baroreflexes [10].

In diabetes the cardio-respiratory integrated control remains to a large extent to be investigated. Although several studies have tested the hypothesis of altered respiratory or cardiovascular control, the respiratory and cardiovascular reflexes were tested only in small number of subjects and only separately on chemoreflex (hypercapnic only [11], hypoxic only [12], both chemoreflexes [13]) or baroreflex

Abbreviations: BRS, baroreflex sensitivity; $CO2_{et}$, end-tidal carbon dioxide pressure; DLCO, diffusing capacity of the lung for carbon monoxide; FEV_1, forced expiratory volume (at the end of the first second of forced expiration); FVC, forced vital capacity; HIF-1, hypoxia-inducible factor-1; HVR, hypoxic chemoreflex (ventilatory response); HCVR, hypercapnic chemoreflex (ventilatory response); HRV, heart rate variability; N+ subgroup, presence of incipient autonomic dysfunction; N- subgroup, absence of autonomic dysfunction; SDNN, standard deviation of all RR intervals; T1D, type-1 diabetic subjects; VE, minute ventilation; Vt, tidal volume; VT-CO_2, ventilatory threshold for carbon dioxide; CXCR4, C-X-C chemokine receptor type 4; eNOS, endothelial nitric oxide synthase; EPC, endothelial progenitor cells; GLO-1, glyoxalase-1; GSH, glutathione; MGO, methylglyoxal; ROS, reactive oxygen species; SDF-1, CXCR4-ligand stromal cell-derived factor-1; VEGF, vascular endothelial growth factor.

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[2,3]. Results were interpreted simply as yet another additional evidence of neuropathy and thus of little novelty.

Therefore, a comprehensive evaluation of cardiovascular, hypoxic and hypercapnic reflexes is missing so far, thus preventing us to understand how these reflexes might interact in diabetes.

This is particularly relevant in view of the previous hypothesis of an initially functional (hence reversible) autonomic abnormality in type 1 diabetes. In that case the different reflexes should be linked in a mutual reciprocal interaction, like in heart failure (e.g. ventilatory reflexes enhanced and cardiovascular reflexes depressed). Conversely, a generalized reduction in reflexes could be the result of an established neuropathy, as shown in familial dysautonomia [10].

In the present study we then evaluated the baroreflex sensitivity and the central and peripheral chemoreflex sensitivities in subjects with absent or minimal autonomic dysfunction (as evaluated by a standard battery of cardiovascular reflex tests). The type of the link should give us an indication of whether the extent of the possible abnormality should be interpreted. We reasoned that a generalized depression in both cardiovascular and respiratory reflexes could be an indication of autonomic neural damage (e.g. similar to familial dysautonomia), whereas an inverse relationship between cardiovascular and respiratory reflexes (depressed baroreflex and enhanced chemoreflex gain) would be an indication of functional disorder, eg similar to heart failure.

2. Methods

We studied 46 type-1 diabetic subjects (T1D) (33 ± 1 year, 22 female) and 103 age-matched controls. Clinical characteristics of study participants are shown in Table 1.

Type 1 diabetes was defined as C-peptide deficiency (<0.03 nmol/l) and initiation of permanent insulin treatment within one year after the diagnosis of diabetes. None of the patients showed clinical signs of cardiovascular disease. Only two patients had antihypertensive medication (ACE-inhibitors). The healthy control subjects were recruited by email advertisements among university students and staff. Only individuals with normal fasting glucose and without 1st degree relatives with diabetes mellitus were included. Before participation, all subjects gave their written informed consent. The study protocols were approved by the Ethics Committee of University of Pavia.

2.1. Experimental set-up

All subjects were investigated in a quiet room, at a temperature between 19 and 23 °C, between 8 a.m. and 2 p.m. Before the examination, the subjects received instructions to refrain from alcohol for 36 h, caffeinated beverages and cigarettes for 12 h. A light meal was permitted 2 h before testing. Electrocardiogram was recorded using a bipolar precordial lead. Continuous blood pressure was monitored with validated [14] applanation tonometry (Colin CBM 7000, San Antonio, USA) from the wrist of the left arm held at heart level. A respiratory signal (by inductive respiratory belts positioned around the chest), pulse oximetry (Ohmeda Pulse Oxymeter, Englewood, Colorado, USA) and carbon dioxide were also recorded (COSMOplus, Novametrix Wallingford, Connecticut, USA). All signals were acquired continuously on a personal computer (Apple Macintosh), at 300 samples/s/signal.

Table 1
Clinical characteristics of study participants (mean ± standard error).

	T1D	Controls	p value
N	46	103	
M/F	24/22	52/51	ns
Age (years)	33.2 ± 1.1	36.4 ± 1.4	ns
Weight (Kg)	63.1 ± 1.5	67.2 ± 1.1	0.025
Height (cm)	167.1 ± 1.4	171.3 ± 0.9	0.008
Body mass index (Kg/m^2)	22.5 ± 0.3	22.9 ± 0.3	ns
Years from diagnosis	7.0 ± 0.9	–	
HbA1c (%) (mmol/mol)	8.19 ± 0.24 66	–	
Fasting glucose (mg/dL)	184.4 ± 12.7	–	
Diabetes complications	Diabetic retinopathy: Proliferative: 2 Non-proliferative: 6 Microalbuminuria: 2	–	
Other pathologies	Hypertension: 2 Depressive sd.: 1 H. Pylori gastritis: 1 Hepatitis B: 2 Hepatitis C: 1 Hiatal hernia: 1	–	
Medications	ACE-inhibitors: 2 pz	–	
Autonomic score	0.8 ± 0.1	0.0 ± 0.0	–

2.2. Autonomic testing

The subjects underwent five cardiovascular autonomic function tests using previously described methods [15]: 1) the difference between expiratory and inspiratory heart rate during slow deep breathing (6 cycles per minute), 2) the maximum/minimum 30/15 ratio of RR interval during active standing, 3) the systolic blood pressure response to standing, and 4) the maximum/minimum ratio of RR interval during a Valsalva manoeuver, 5) the diastolic blood pressure increase during handgrip, obtained by compressing a sphygmomanometer cuff partially inflated and keeping a pressure of 30% of maximal strength for 2 min.

2.3. Baroreflex testing

Electrocardiogram and continuous blood pressure were recorded in the supine position at rest during 5 min of spontaneous breathing, to measure baroreflex sensitivity (BRS).

2.4. Chemoreflex testing

During chemoreflex testing, the participants were seated, and connected to a rebreathing circuit through a mouthpiece, in a fashion similar to that previously described and validated [16,17]. Rebreathing into a closed circuit causes a progressive reduction in inspired oxygen and increase in carbon dioxide concentration, both of which stimulate ventilation. When the response to varying oxygen was to be assessed (hypoxic chemoreflex), end-tidal carbon dioxide pressure (CO_2et) was kept constant at 40 ± 1 mm Hg by passing a portion of the expired air into a scrubbing circuit (containing soda lime) before returning it to the rebreathing bag.

Conversely, when the response to carbon dioxide was to be tested (hypercapnic chemoreflex), oxygen was continuously supplied at a very low flow (1 l/min) to the rebreathing circuit in order to maintain the percentage of arterial oxygen saturation (SaO_2) at baseline values (>95%). The amount of air in the rebreathing circuit was set at 5 l, in order to maintain the duration of each test (and hence the individual's compliance) at about 7 min. Before each rebreathing test, the subjects breathed room air through the same mouthpiece as during the rebreathing, in order to collect baseline data. The rebreathing tests terminated when SaO_2 reached 80% (response to hypoxia) and when CO_2et reached 55 mm Hg (response to hypercapnia) (Fig. 1). In each condition, we continuously measured the carbon dioxide via the COSMOplus connected to the mouthpiece and SaO_2. The airway flow was measured continuously by a heated Fleish pneumotachograph (Metabo, Epalinges, Switzerland) connected to a differential pressure transducer (RS part N395-257, Corby, UK) connected in series in the expiratory arm of the rebreathing circuit.

2.5. Assessment of baroreflex sensitivity and heart rate variability

Previous studies have shown a poor correlation between different indices of BRS, while, on the other hand, no method has shown clear superior performance over the other [18]. Accordingly, we have used all the most common methods, and analysed the BRS as their average, as previously validated [19]. BRS was determined from the time series of RR interval and systolic blood pressure using the sequence method for 1) positive and 2) negative sequences, or spectral analysis for the 3) low frequency, 4) high frequency, and 5) for the average of the low- and high-frequency components, 6) the transfer function technique and 7) by the standard deviation method (BRS-SD), following the technical details previously explained [19]. Additionally, the standard deviation of all RR intervals (SDNN) was considered an index of global RR interval variability.

2.6. Analysis of respiration/chemoreflex sensitivity

The ventilatory flow signal was integrated by software and each breath was identified. Breathing rate, tidal volume (Vt) and minute ventilation (VE) relative to each breath were recognized, with their corresponding values of SaO_2 and CO_2et.

The chemoreflex sensitivity to hypoxia (hypoxic ventilatory response) or hypercapnia (hypercapnic ventilatory response) was obtained from the slope of the linear regression line of minute ventilation plotted against SaO_2 or CO_2et, respectively (Fig. 1). The change in ventilation due to hypercapnia is interpreted as the central chemoreflex sensitivity, whereas the change in ventilation due to hypoxia is interpreted as peripheral chemoreflex sensitivity. The point at which the ventilation started to increase during the progressive HCVR (called ventilatory threshold (VT-CO_2, Fig. 1) was identified by interpolating the ventilation/CO_2et plot using a fourth-order polynomial function.

2.7. Statistical analysis

Data are presented as mean ± standard error. Differences were analysed by one-way ANOVA factorial design. If overall significant changes were observed ($p < 0.05$), then significance was tested by Sheffe's test. Correlation between different variables was evaluated by linear regression analysis.

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