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Biochemical and Biophysical Research Communications 310 (2003) 28–31
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Dipeptidyl peptidase-IV expression and activity in human glomerular endothelial cells

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Received 29 July 2003

Abstract

Glucagon-like peptide-1 (GLP-1), a meal-stimulated gastrointestinal insulinotropic hormone inactivated by dipeptidyl peptidase-IV (DPP-IV), is reduced in type 2 diabetic patients. The present study shows that 2-week exposure of human glomerular endothelial cells to high glucose (22 mM) determines a highly significant increase in DPP-IV activity and mRNA expression, which cannot be entirely accounted for by hyperosmolarity. On the other hand, incubation of purified DPP-IV in a buffer solution added with high glucose does not affect enzyme activity. These results suggest that high glucose increases expression and activity of DPP-IV, possibly contributing to GLP-1 reduction in type 2 diabetic patients.

Keywords: Glucose; Peptidase; GLP-1; Endothelial cells; Diabetes mellitus

Dipeptidyl peptidase-IV (DPP-IV) is an enzyme, which is produced by endothelial cells in different districts and circulates in plasma [1]. DPP-IV, which has been proposed as a target for pharmacological intervention in patients with type 2 diabetes [2], catalyses the inactivation of several hormones and neuropeptides. It has been reported that GLP-1 levels after a mixed meal [3,4] and after an oral glucose load [5,6] are reduced in patients with type 2 diabetes; this could be due either to impaired secretion, increased degradation, or both. GLP-1 and GIP gene expression and peptide synthesis have been reported to be unmodified with respect to controls in rodent models of type 1 and type 2 diabetes [7], but no data on humans are available. GLP-1 kinetics was reported to be unmodified with respect to control subjects in a small sample of type 2 diabetic patients [8]; however, GLP-1 doses used in that study were such as to obtain a largely circulating hormone level more than 10-fold higher than those usually observed in the post-prandial state [3], so that the possibility of increased GLP-1 degradation of type 2 diabetic patients in more physiological conditions cannot be excluded. Circulating DPP-IV levels have been reported to be reduced in a small sample of elderly patients with type 2 diabetes [9]; in a study on another small sample of subjects, no significant difference in circulating enzyme activity was detected between type 2 diabetic patients and matched healthy controls [3]. However, endothelial DPP-IV activity, which appears to be more relevant than circulating enzyme activity [10], has not been studied in type 2 diabetic patients so far.

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0006-291X/$ - see front matter © 2003 Published by Elsevier Inc.
doi:10.1016/j.bbrc.2003.08.111

In order to explore the effects of chronic hyperglycemia, DPP-IV activity, and mRNA expression were measured in cultured human glomerular endothelial cells exposed to high glucose. The effect of prolonged incubation with high glucose on the activity of purified DPP-IV in vitro was also assessed.

Materials and methods

Cell cultures. Human glomerular endothelial cells were obtained as described elsewhere [11]. The cells were cultured in different glucose concentrations (5.5, 11, or 22 mmol/L), or in glucose 5.5 mM and mannitol 16.5 mM, for a week before the experiments.

Buffer studies. Purified DPP-IV (Sigma, Milan, Italy) 0.065 U/ml was incubated in a buffer (Hepes, pH 8) with different glucose concentrations (glucose 5.5 mM, glucose 22 mM, or glucose 5.5 mM plus mannitol 16.5 mM); DPP-IV activity was measured at 4, 12, 24, 48, and 72 h and after a week.

Immunohistochemical studies. Immunohistochemical studies were performed, in order to verify positivity for CD26/DPP-IV, as described elsewhere [10]. Cells grown on sterile slides were fixed in 3.7% paraformaldehyde in PBS for 15 min at room temperature, followed by permeabilisation in 3.7% paraformaldehyde in PBS containing 0.1% Triton X-100 for 15 min, and overnight incubation ay 4 °C with the primary antibody (mouse monoclonal) against CD26 diluted 1:50 (Technogenetics, SpA Italiana Laboratori Bouty, Milan, Italy). The slides were rinsed in PBS and incubated at room temperature for 45 min with fluoresceinated secondary antibody (goat anti-mouse, 1:100). Controls were performed by processing slides lacking the primary antibodies or stained with the corresponding non-immune serum. The slides were examined with a phase contrast microscope equipped with epifluorescence (Nikon Microphot-FX microscope; Nikon, Tokyo, Japan).

RT-PCR. Total RNA was extracted with RNeasy Mini Kit (Qiagen, Valencia, CA). Two micrograms of each sample was used for cDNA synthesis with MoMLV-Reverse Transcriptase (Gibco-BRL, Life Technologies, Gaithersburg, MD). Semiquantitative analysis of resulting cDNA was performed by co-amplification of the gene together with the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using corresponding gene-specific primer sets: GAPDH FW: 5′-CCATGGAGAAGGCTGGGG-3′; GAPDH RV: 5′-CAAAGTTGTCATGGATGACC-3′, which gave rise to 440 bp product. To determine specifically the presence of CD26, we used the following set of primers: CD26 FW: 5′-GAATGCCAGGAGGAA GGAATCT-3′, CD26 RV:5′TATTCCACACTTGAACACGCCA-3′, which gave rise to a 767-bp product; 1 μl cDNA was added to 30 ml of final volume PCR containing: reaction buffer (10 mM Tris–Cl, 50 mM KCl, and 0.1% gelatin), 1.5 mM $MgCl_2$, 200 mM of each dNTP, 20 pmol of each primer, and 1.5 U Taq polymerase (Promega, Madison, WI). PCR amplification was carried out as follows: 95 °C for 5 min, then 35 cycles of 94 °C for 1 min, 54–63 °C for 1.5 min, 72 °C for 1.5 min, and finally 72 °C for 5 min. The PCR products were loaded on a 1.8% agarose gel and visualised by ethidium bromide. The data were compared after being normalised by the intensity of GAPDH. The semiquantitative analysis was performed using the GS-670 Imaging Densitometer (Bio-Rad, CA).

DPP-IV activity assay. DPP-IV activity was measured on homogenate of cells grown in different glucose concentrations for a week and using purified DPP-IV (Sigma, Milan, Italy) in buffer with different glucose concentrations.

DPP-IV activity was measured by a colorimetric assay. Gly-Pro-4-*p*-nitroanilide, a chromogenic substrate of DPP-IV, is hydrolysed into the dipeptide Gly-Pro and the product 4-nitroaniline, whose rate of appearance can be measured spectrophotometrically [10,12]. To evaluate within-run, and between-run precision of the DPP-IV assay, the activities of a low (15 U/L), middle (30 U/L), and high (70 U/L) activity medium sample were assessed 10 times in five days. The coefficients of variation were 2.6%, 3.4%, and 2.3%, respectively, for within-run precision, and 1.5%, 4.8%, and 4.7%, respectively, for between-run precision.

Results

Immunostaining

A positivity for CD-26 (DPP-IV) at immunofluorescent immunostaining in human Glomerular Endothelial Cells (GENC) was observed, showing that these cells could be used as an in vitro model for the assessment of the effects of high glucose on the expression of DPP-IV gene and activity in microvascular endothelial cells.

Effects of high glucose on DPP-IV gene expression and activity in endothelial cells

Exposure of GENC to high glucose (22 mmol/L) or high osmolarity (glucose 5.5 mmol/L and mannitol 16.5 mmol/L) for one week determined an increased expression of mRNA for DPP-IV/CD-26, assessed with semiquantitative PCR (Fig. 1). When performing a densitometric analysis, assuming density for glucose 5.5 mmol/L (normoglycemia) as 1, after normalisation for the levels of GADPH in each lane, the expression of mRNA for DPP-IV/CD-26 was 0.13 at glucose 11 mmol/L, 3.52 at glucose 22 mmol/L, and 3.71 at glucose 5.5 mmol/L plus mannitol 16.5 mmol/L. When DPP-IV activity was measured in GENC grown for one week in culture media with different glucose concentrations, a dose-dependent increase of enzymatic activity determined by exposure to high glucose ($p < 0.0001$ at ANOVA) was observed. Hyperosmolarity, obtained by adding mannitol 16.5 mM to the culture medium with glucose 5.5 mM, induced a significant increase of DPP-IV activity, which was inferior to that observed with an equimolar concentration of glucose (Fig. 2).

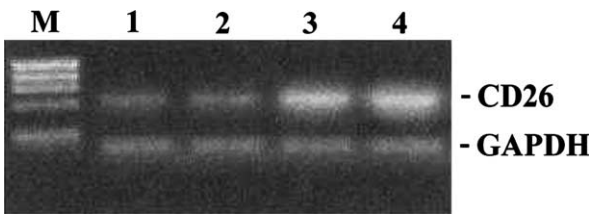

Fig. 1. mRNA for DPP-IV in human glomerular endothelial cells in different culture conditions. Lane 1, glucose 5.5 mM; lane 2, glucose 11 mM; lane 3, glucose 22 mM; and lane 4, glucose 5.5 mM plus mannitol 16.5 mM.

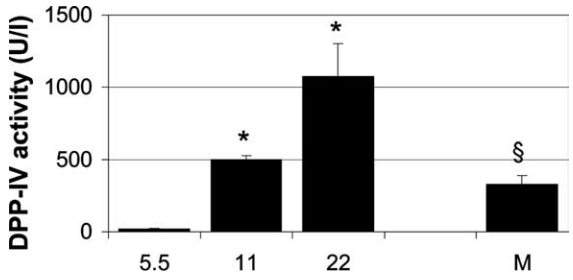

Fig. 2. DPP-IV activity (mean) in glomerular endothelial cells in different culture conditions. Mean ± SD with glucose 5.5, 11, or 22 mM, or glucose 5.5 mM plus mannitol 16.5 mM (M). $^{*}P < 0.0001$ vs. 5.5 mM; $^{\S}P < 0.0001$ vs. 5.5 mM and vs. 22 mM.

DPP-IV activity in buffer solution

The activity of purified DPP-IV in a buffer solution was not modified by glucose 22 mM (Fig. 3). Furthermore, when adding mannitol 16.5 mM to buffer solution with 5.5 mM glucose, DPP-IV activity after a week of incubation was not significantly different from that observed with glucose 5.5 mM only (data not shown).

Discussion

DPP-IV, which is a circulating enzyme, is also expressed by endothelial cells [1]; in particular, microvascular endothelial cells of some districts, including kidneys, appear to be the main source of endogenous DPP-IV [10]. For this reason, we have chosen human glomerular endothelial cells as a model for the study of the effects of high glucose on DPP-IV expression and activity.

In this model, high glucose determined a dose-dependent increase of endothelial DPP-IV activity, which was evident at 11 mmol/L. Hyperosmolarity also determined an increase in enzyme activity, which could not account entirely for the effects of high glucose. The effect of hyperglycaemia on DPP-IV activity appears to be due, at least partly, by modulation of enzyme mRNA expression. In fact, glucose 22 mmol/L induced a relevant up-regulation of DPP-IV gene expression. It should be observed, however, that a similar increase of specific mRNA was observed with glucose 5.5 mmol/L plus mannitol 16.5 mmol/L, suggesting that the effect of high glucose on DPP-IV gene expression could be explained by hyperosmolarity; furthermore, glucose 11 mmol/L, which significantly increased enzyme activity, did not modify gene expression. These data suggest that other mechanisms beside modulation of DPP-IV gene expression could be involved in the increase of enzyme activity induced by high glucose, although results obtained with prolonged incubation of purified DPP-IV with high glucose exclude a direct effect of glucose on DPP-IV activity.

The increase of endothelial DPP-IV activity determined by high glucose could induce an increase in GLP-1 degradation and therefore provide an explanation for the reduction of active hormone concentrations observed in diabetic patients. Lower GLP-1 level reported in type 2 diabetes [7] could be due either to increased degradation or to decreased degradation. Although some previous studies did not show any

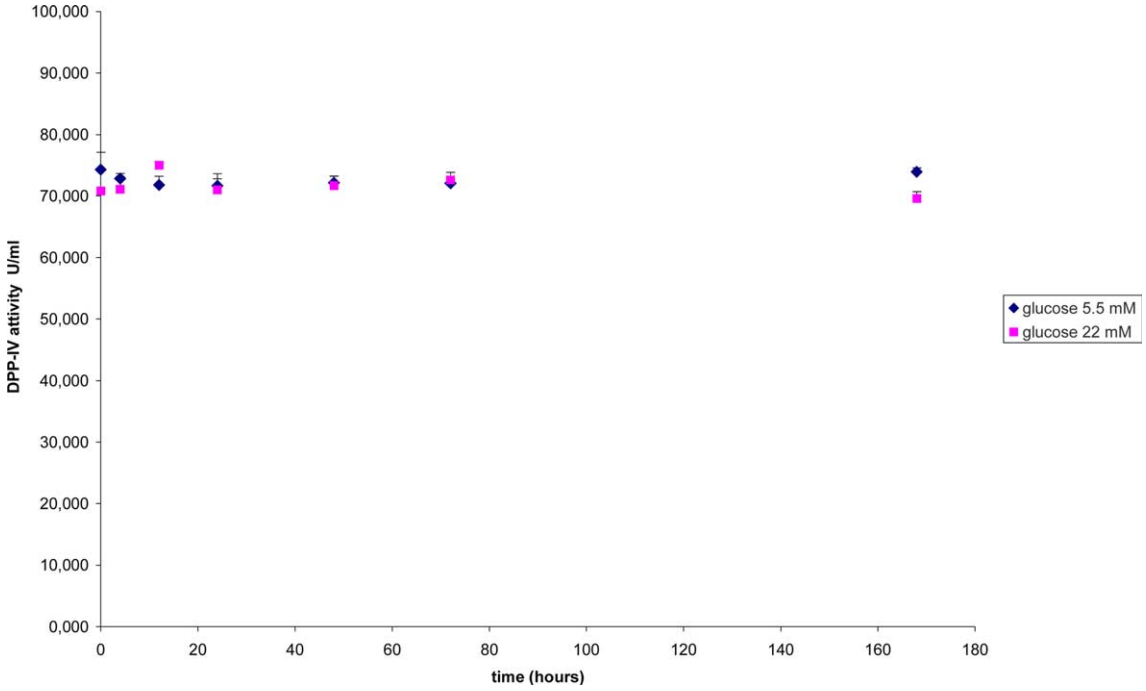

Fig. 3. Purify DPP-IV activity (mean) in buffer with glucose 5.5 and 22 mM. No significant different there is between two concentrations.

increase of circulating DPP-IV activity in type 2 diabetic patients when compared to healthy control subjects [3,9], this does not exclude the possibility of an increased endothelial DPP-IV activity. A study on a very small sample of type 2 diabetic patients showed a kinetic of GLP-1 after intravenous injection similar to that observed in healthy subjects [8]. The limited size of the sample could have prevented the detection of small kinetic differences between diabetic and non-diabetic subjects. In addition, the doses of GLP-1 administered in that study were such as to obtain plasma concentrations of the active hormone more than 10-fold than peak postprandial levels previously reported by the same group [3]; it should be considered that differences in GLP-1 kinetics could theoretically be present at lower hormone concentrations. Furthermore, the majority of diabetic patients enrolled in the previous cited study were currently taking medication possibly interfering with DPP-IV activity [13], making interpretation of results problematic.

A glucose-induced increase of DPP-IV activity could theoretically determine a reduction of GLP-1 levels in type 1, as well in type 2, diabetic patients. A recent study did not show any significant difference in post-prandial GLP-1 levels between type 1 diabetic patients and healthy subjects [14]; however, considering the relevant interindividual variability of the parameters studied, the size of the sample (only eight patients) was not sufficient to exclude differences between groups in GLP-1 response to meals. Glucose-dependent Insulinotropic Peptide (GIP) is another known substrate of DPP-IV; therefore, a hyperglycaemia-induced increase of endothelial DPP-IV activity is consistent with the previously reported reduction of GIP half-life in type 2 diabetic patients [15].

In conclusion, the present data show that hyperglycaemia is capable of increasing in a dose-dependent manner DPP-IV activity in microvascular endothelial cells and that this effect is at least partly due to modulation of gene expression. Chronic hyperglycaemia could establish a vicious cycle, determining an increase of DPP-IV activity, which induces a reduction of active GLP-1 levels, leading to the impairment of insulin secretion, which worsens hyperglycaemia. Other glucose-dependent or independent mechanisms, including impairment of hormone secretion, probably contribute to the reduction of GLP-1 levels in type 2 diabetes; their role needs to be assessed through further, specifically designed studies.

Acknowledgment

This study was supported by grants from the Ministry of Scientific Research (40% and 60% funds MIUR).

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