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RESEARCH LETTER

GLP-1 reduces metalloproteinase-14 and soluble endoglin induced by both hyperglycemia and hypoglycemia in type 1 diabetes

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Endoglin (also known as CD105), is weakly expressed in resting endothelial cells, but increases during angiogenesis [1]. Soluble endoglin (sEng) enhances atherogenesis via down regulation of eNOS expression and inhibition of TGF-β signaling [2]. The sEng has recently been associated with cardiovascular damage, also in diabetes [3, 4]. The sEng is generated by the cleavage of the extracellular domain of the protein by the metalloproteinase-14 (MMP-14) [2]. The activity of the metalloproteinases is enhanced by hyperglycemia through the generation of an oxidative stress [5], and, recently, we have reported that in human endothelial cells, high glucose also induces sEng over-secretion through the oxidative stress [6].

Recently, much attention is paid to the possibility that glucagon like peptide-1 (GLP-1) and GLP-1 receptor agonists (GLP-1RA) can be used in combination with insulin in the management of type 1 diabetes [7]. The possible usefulness of this combination seems to be not only related to the possibility of decreasing the insulin dose, body weight gain, and the risk of hypoglycemia [7], but also to a direct protective effect of GLP-1 [8].

In particular, we have recently shown that in type 1 diabetes, GLP-1 protects endothelial function and reduces inflammation during both acute hyperglycemia and hypoglycemia [8]. This protective action seems partly related to the ability of GLP-1 of increasing intracellular antioxidant defenses and decreasing the oxidative stress [8–10]. Therefore, the aim of this study has been to evaluate the impact of both acute hyperglycemia and hypoglycemia on sEng and MMP-14 plasma levels and the possible protective effect of GLP-1.

Research design and methods

Plasma samples regarding the experiments performed in type 1 diabetes were from the study previously published [8]. A total of 30 type 1 diabetic patients (15 males and 15 females) participated in such study (8). For this new study, 30 healthy (17 males and 13 females), age (24.0 ± 2.3 vs. 24.4 ± 2.2 years, mean ± SE), and BMI (23.5 ± 2.0 vs. 23.7 ± 2.2 kg/m^2) matched controls were recruited. All subjects were nonsmokers and had a normal blood count, plasma lipids, plasma electrolytes, liver, and renal function, and were normotensive. Studies were approved by the Ethical Committee, and all participants gave written informed consent.

The diabetic patients were divided in two groups [8]. In the group 1, two different experiments were planned for each subject in a randomized order: a period of 2 h of hypoglycemia (2.9 ± 0 mmol/l), with or without GLP-1 (0.4 pmol/kg/min) infusion [8].

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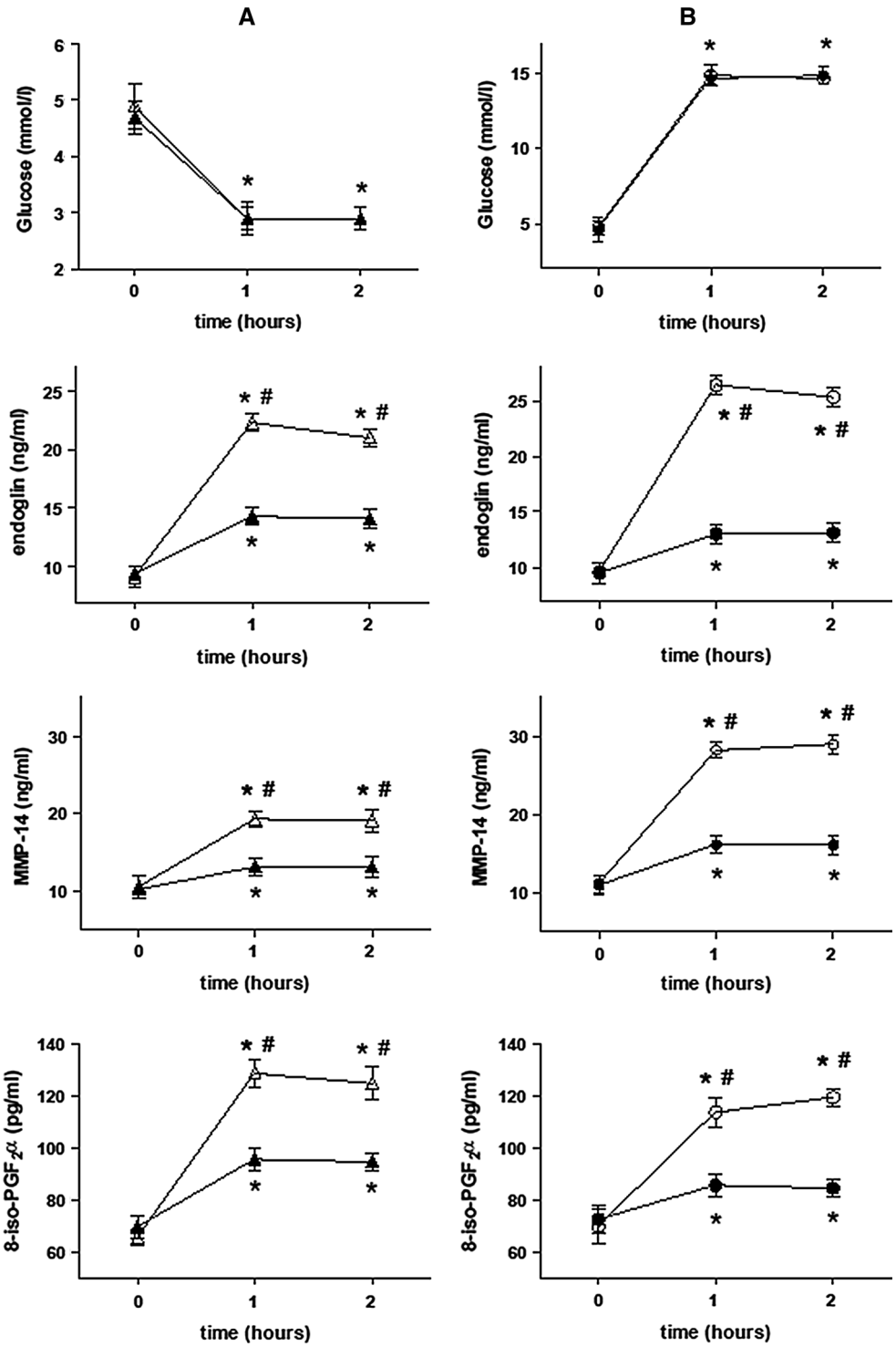

Fig. 1 **a** Glycemia, 8-iso-PGF2a, sEng, and MMP-14 in type 1 diabetes during hypoglycemia. *White triangle* hypoglycemia. *Black triangle* hypoglycemia + GLP-1. $^{*}p < 0.01$ versus basal. $^{\#}p < 0.01$ versus hypoglycemia + GLP-1. **b** Glycemia, 8-iso-PGF2a, sEng, and MMP-14 in type 1 diabetes during hyperglycemia. *White circle* hyperglycemia. *Black circle* hyperglycemia + GLP-1. $^{*}p < 0.01$ versus basal. $^{\#}p < 0.01$ versus hyperglycemia + GLP-1. The graphics of glucose and 8-iso-PGF2a in the figure **a** and **b** have already been published in Ceriello et al. [8]

Two different experiments were planned for each subject of the group 2 in a randomized order: a period of 2 h of hyperglycemic clamp (15 mmol/l), with or without GLP-1 (0.4 pmol/kg/min) [8] infusion. For more details see Ref. 8.

At baseline and after 1 and 2 h, glycemia, plasma 8-iso prostaglandin F2alpha (8-iso-PGF2a, Cayman Chemical, Ann Arbor, MI, USA), sEng (R&D Systems, Inc., Minneapolis, MI, USA), and MMP-14 (Uscn Life Science, Inc., Houston, TX, USA) plasma levels were measured.

Statistical analysis

Data are expressed as mean ± SE. The Kolmogorov–Smirnov algorithm was used to determine whether each variable had a normal distribution. Comparisons of baseline data among the groups were performed using unpaired Student's *t* test or Mann–Whitney *U* test, where indicated. The changes in variables during the tests were assessed by two-way ANOVA with repeated measures or Kolgorov–Smirnof test, where indicated. If differences reached statistical significance, post hoc analyses with two-tailed paired t test or Wilcoxon signed-rank test for paired comparisons were used to assess differences at individual time periods in the study. Statistical significance was defined as $p < 0.05$.

Results

Baseline 8-iso-PGF2a (67.5 ± 4.8 vs. 34.5 ± 4.5 pg/ml, $p < 0.01$), sEng (9.6 ± 0.4 vs. 4.2 ± 0.3 ng/ml, $p < 0.01$), and MMP-14 (11.3 ± 1.0 vs. 5.0 ± 0.8 ng/ml, $p < 0.01$) were increased in diabetic patients compared to controls.

After 2 h of hypoglycemia, 8-iso-PGF2a, sEng, and MMP-14 significantly increased, compared to basal values (Fig. 1). When hypoglycemia was accompanied by the simultaneous infusion of GLP-1, all these phenomena were significantly attenuated (Fig. 1). Similar results were obtained in the hyperglycemic clamp experiments. After 2 h of hyperglycemia, 8-iso-PGF2a, sEng, and MMP-14 increased, compared to basal values (Fig. 1). When hyperglycemia was accompanied by the simultaneous infusion of GLP-1, all these phenomena were significantly attenuated (Fig. 1).

Discussion

In this study, we show an increase of sEng and MMP-14 plasma levels in type 1 diabetes. Moreover, for the first time, we report that both acute hypoglycemia and hyperglycemia induce an increase of these molecules and that GLP-1 can counterbalance this effect. It is worthy of interest that the increase of sEng and MMP-14 is simultaneous: the hypothesis is that the increase of MMP-14 favors the cleavage of sEng from the endothelium [2]. Previous evidences suggest that both sEng and MMP-14 can be induced by hyperglycemia through an oxidative stress [6, 11]. We have demonstrated that also hypoglycemia produces an oxidative stress [8]. Therefore, our data suggest that sEng and MMP-14 are activated by acute hyperglycemia and hypoglycemia because they generate an oxidative stress. The data of 8-iso-PGF2a support this hypothesis.

GLP-1 counteracts the effects of both hyperglycemia and hypoglycemia, probably because of its antioxidant activity [8–10]. Consistently, it has been already reported that antioxidants can reduce the expression of MMP-14 [11, 12].

Acute hyperglycemia, hypoglycemia, and sEng have been all involved in the development of cardiovascular disease in diabetes [3, 13, 14]. On the other hand, MMP-14 promotes vulnerable plaque morphology [15]. Our data suggest oxidative stress as a possible link between them. Currently, there is a raising interest about the possibility of using the GLP-1RA for the therapy of type 1 diabetes [7]. Recent evidence show that also GLP-1 RA, widely used in clinical practice are able to reduce oxidative stress in diabetes [16, 17], therefore our data further support the possible usefulness of these compounds in type 1 diabetes.

Conflict of interest The authors do not have any conflict of interest to disclose.

Ethical statement All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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