Rapid Publications

Plasma β-Thromboglobulin Response to Insulin-induced Hypoglycemia in Type I Diabetic Patients

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SUMMARY

The effect of an insulin-induced hypoglycemia was examined in 14 type I diabetic patients. After an overnight blood glucose normalization, each patient received an additional intravenous bolus of 3 U regular insulin at 0900 h (time 0). Blood glucose was continuously recorded up to 180 min. Plasma samples were assayed for β-thromboglobulin (βTG, ng/ml), pancreatic glucagon (pg/ml), cortisol (μg/dl), and growth hormone (ng/ml) 30 min before the insulin stress, at time 0, at blood glucose nadir, and at 180 min. The blood glucose fell from a baseline level of 85.0 ± 3.2 mg/dl to a nadir value of 39.2 ± 1.9 mg/dl ($P < 0.001$) reached at an average time of 41.4 ± 4.9 min. Plasma βTG increased significantly ($P < 0.05$) during the insulin stress: 93.4 ± 23.7 ng/ml at nadir versus 42.5 ± 5.9 at time 0. Plasma cortisol and growth hormone were significantly increased ($P < 0.02$ and $P < 0.01$) at nadir compared with time 0 values. Plasma pancreatic glucagon was higher at nadir than at time 0, but the difference was not significant. The present results indicate that in vivo platelet activation can be triggered by hypoglycemic episodes in insulin-treated diabetic patients. DIABETES 1984; 33:907–909.

Near-normal circadian blood glucose, metabolic, and hormonal profiles can be achieved in insulin-dependent diabetic patients by either continuous subcutaneous insulin infusion or intensified conventional insulin therapy,[1–3] but such treatments do not result in a total eradication of the hypoglycemic risk.[4,5] Although it has been reported that severe hypoglycemia can occur during continuous subcutaneous insulin infusion,[4] it seems that the hypoglycemic episodes usually remain moderate or asymptomatic[5] provided that the patients have no defect in glucose counterregulatory system[6] and adhere to careful self-monitoring of blood glucose. However, each hypoglycemic episode, even asymptomatic, can stimulate the secretion of hormones such as glucagon and catecholamines,[7] the latter being recognized as potent stimulators of platelet aggregation.[8,9] It is possible, therefore, that the persistence of hypoglycemic episodes, even moderate, in pump-treated patients can contribute to maintain a state of increased platelet aggregation, even when the other metabolic parameters have been markedly improved. For that reason we were led to study the effects of an insulin-induced hypoglycemia on plasma levels of β-thromboglobulin (βTG), a platelet-specific protein that is released during the aggregation process.[10,11]

MATERIAL AND METHODS

SUBJECTS

Fourteen insulin-requiring diabetic patients, 16–52 yr old (mean, 27 yr), were studied after their informed consent had been obtained. The mean percentage ideal body weight of the patients was 102% (range, 81–130%). Mean ideal body weight was estimated from the tables of the Metropolitan Life Insurance Co. In order to avoid any interference from treatment on platelet function, the patients selected for the study received no medication other than insulin. All subjects included had been free of both any intercurrent disease and any treatment with anti-inflammatory, hypolipidemic, or oral contraceptive drugs for at least 1 mo before the investigation. Glycosylated hemoglobin percentages at the time of the investigation ranged from 8% to 12.5% (mean, 10.2%). In the 14 patients, plasma βTG levels at time 0 were between 15 and 80 ng/ml. All patients except one (βTG = 80 ng/ml) had βTG levels within the normal range (9–68 ng/ml) determined in a group of 17 age-matched control subjects. Twelve patients had no diabetic complications while the other two suffered from peripheral neuropathy and/or retinal complications.

PROTOCOL

During the 12 h before the beginning of the investigation (0800 h), each subject underwent an overnight fast and an

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overnight intravenous insulin infusion. The insulin doses were carefully adjusted to stabilize blood glucose levels between 70 and 140 mg/dl and to avoid hypoglycemic episodes. From 0800 to 0900 h (time 0) each diabetic patient was connected to an artificial pancreas in order to obtain an optimal insulin delivery and, therefore, the best possible fasting blood glucose concentrations. Individual blood glucose values at time 0 were between 65 and 102 mg/dl (mean, 85 mg/dl). In all patients, the insulin-induced hypoglycemia was initiated at 0900 h (i.e., time 0) by an intravenous injection of an additional insulin bolus consisting of 3 U of regular insulin (Novo Actrapid Monocomponent, Novo Industri, Copenhagen, Denmark). Blood glucose was continuously monitored for a 4-h period (from 0800 to 1200 h) over the entire duration of the investigation. The blood was withdrawn from a cubital vein through an indwelling catheter connected to a glucose Auto-Analyzer apparatus (Technicon). The blood was assayed for glucose by the glucose-oxidase method. This continuous blood glucose monitoring allowed accurate determinations of both blood glucose nadir and basal blood glucose value at the initiation of the insulin-induced hypoglycemia. Furthermore, the connection to the artificial pancreas permitted a progressive and safe recovery from the insulin-induced hypoglycemia.

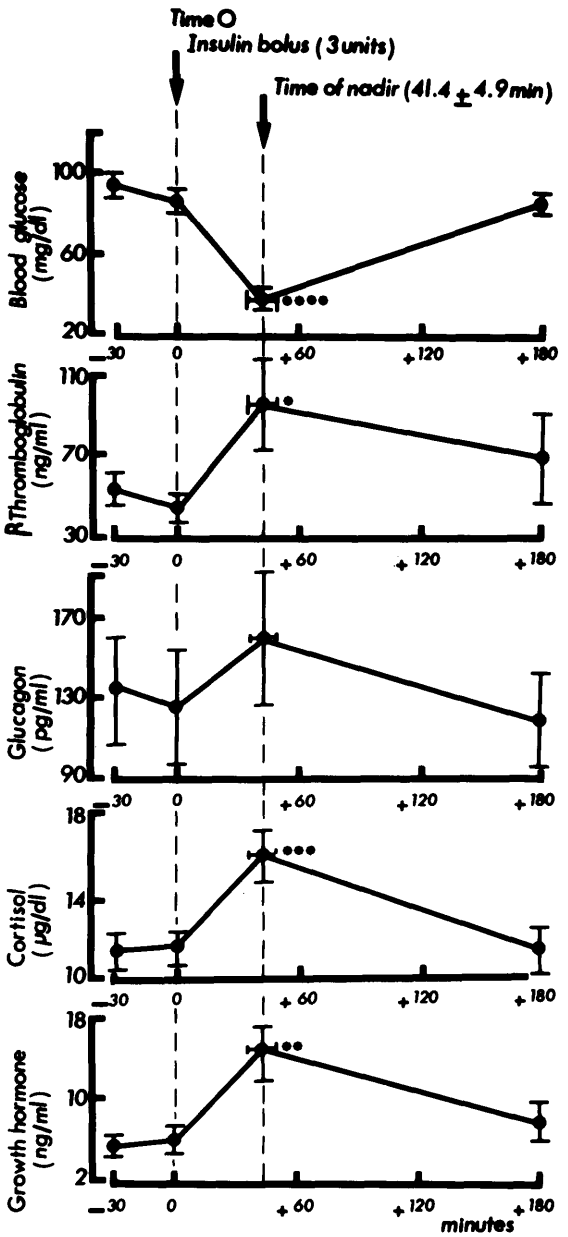

FIGURE 1. Responses of blood glucose, plasma βTG, pancreatic glucagon, cortisol, and growth hormone during the insulin-induced hypoglycemia that was initiated at time 0 in 14 insulin-dependent diabetic patients. All data were given as mean ± SEM. Mean comparisons between the data obtained at the different times of the investigation and the 0 values were made using Student's *t*-test for paired data. Statistical differences were only indicated when significant. *P < 0.05; **P < 0.02; ***P < 0.01; ****P < 0.001.

Blood samples were drawn 30 min before the insulin stress, at time 0 at the blood glucose nadir and at 180 min after the insulin bolus in a contralateral antecubital vein by using repeated venipunctures with fine needles.

Plasma thromboglobulin, pancreatic glucagon, cortisol, and growth hormone were determined on all collected samples.

ANALYTIC PROCEDURES

Plasma βTG concentration. Venous blood samples were drawn without stasis and 2.75 ml immediately transferred into precooled plastic tubes containing EDTA and theophylline, but not PGE 1. The tubes were inverted three times and centrifuged at 2300 × *g* for 30 min at 4°C in order to obtain a platelet-poor plasma. According to the data of Paulsen et al.[12] only the 0.5 ml plasma upper layer was removed, stored at −20°C, and assayed for βTG concentrations within 4 wk using the Amersham radioimmunoassay kit (Radio Chemical Centre, England). Results are expressed as nanograms per milliliters of platelet-poor plasma.

Plasma pancreatic glucagon was assayed by radioimmunoassay using the K30 antiserum of Unger.[13]

Plasma cortisol was determined by using a competitive binding assay kit (Biolab, Limal, Belgium).

Plasma growth hormone concentrations were measured by using a radioimmunoassay kit (CEA-SORIN, Gif sur Yvette, France).

STATISTICAL ANALYSIS

All data were given as mean ± SEM. Mean comparisons between the data obtained at the different times of the investigation and the 0 values were made using Student's *t*-test for paired data.

RESULTS

The results at the different times of the investigation are shown in Figure 1. The blood glucose nadir was reached at an average time of 41.4 ± 4.9 min (range, 10–85 min). The mean blood glucose was significantly lower ($P < 0.001$) at nadir than at time 0 (39.2 ± 1.9 versus 85.0 ± 3.2 mg/dl.) Plasma βTG levels increased significantly ($P < 0.05$) during the insulin stress from a basal value of 42.5 ± 5.9 ng/ml to a value of 93.4 ± 23.7 ng/ml at the time of blood glucose nadir. At 180 min plasma βTG levels remained slightly elevated (67.1 ± 20.8 ng/ml) compared with baseline, but the difference was not significant.

Plasma glucagon increased slightly from 122.5 ± 27.2 (baseline value) to 159.6 ± 33.4 pg/ml at blood glucose

nadir, but the difference was not statistically significant. On the contrary, significant increases in plasma cortisol and growth hormone were observed. Plasma cortisol rose from 11.6 ± 0.7 (0-value) to 15.1 ± 1.3 μg/dl at blood glucose nadir ($P < 0.01$). Plasma growth hormone increased from a baseline value of 5.8 ± 1.3 to 14.1 ± 2.6 ng/ml at blood glucose nadir ($P < 0.02$).

DISCUSSION

The present study indicates that plasma βTG levels rise during insulin-induced hypoglycemia. These results are consistent with those previously found by other authors[14,15] who have clearly established that in both diabetic and nondiabetic subjects, insulin-induced hypoglycemia can result in enhanced platelet aggregation. For instance, Hilsted et al. have recently observed that hypoglycemia produces an enhancement in ADP-induced platelet aggregation and a reduction in platelet counts.[15] These authors have suggested that these findings can be explained by an intravascular platelet aggregation induced by the blood glucose fall. The significant increase in plasma βTG that we observed during hypoglycemia provides strong support for the latter hypothesis since plasma βTG is usually considered a reflection of in vivo platelet activation and release reaction.[10,11]

All these data, which indicate that intravascular platelet aggregation is activated during hypoglycemia, are not surprising since it is well known that hypoglycemia stimulates the secretion of epinephrine,[16,17] which is a powerful stimulator of platelet aggregation.[8,9] Furthermore, insulin-induced hypoglycemia is accompanied by a transient increase in free insulin levels that can result in an inhibition of PGI_2 synthesis by the endothelial cell[18,19] and contributes in turn to the enhanced platelet aggregability. The persistence of slightly elevated βTG levels in plasma at 180 min may be simply explained by the plasma half-life of βTG, which is equal to approximately 100 min.[20]

Although the precise mechanisms cannot be discussed further from the present results, it appears that in vivo platelet activation is triggered by insulin-induced hypoglycemia. One should therefore recommend that hypoglycemic episodes be avoided or at least maintained within reasonable limits in all insulin-treated diabetic patients, even in those who are submitted to intensified insulin therapies with pumps.

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