Studies on Mechanisms Involved in Hypoglycemia-Induced Platelet Activation

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SUMMARY

The aim of our study was to investigate the mechanisms involved in hypoglycemia-induced platelet activation. Sixteen healthy male subjects received a 60-min intravenous infusion of human regular insulin at the rate of 64 mU · m^{-2} · min^{-1}: throughout 150 min, we serially measured plasma concentrations of glucose, insulin, and counterregulatory hormones; platelet sensitivity to ADP, thrombin and platelet-activating factor; plasma concentrations of platelet markers for specific proteins of in vivo release reaction (β-thromboglobulin and platelet factor 4). Our study showed that insulin-induced hypoglycemia causes a significant increase in platelet sensitivity to aggregating agents in vitro and a platelet release reaction in vivo. Hypoglycemia-induced platelet activation was not correlated with plasma glucose concentrations at nadir and occurred before the increase of plasma growth hormone and cortisol.

To further elucidate the mechanisms of hypoglycemia-induced platelet activation, we incubated in vitro platelet-rich plasma (PRP) of seven fasting healthy subjects with the same concentrations of insulin, epinephrine, glucagon, growth hormone, and cortisol measured in vivo during insulin-induced hypoglycemia. Only epinephrine was able to increase platelet sensitivity to aggregating agents. To investigate the role of α-adrenergic receptors in this phenomenon, we also studied four healthy subjects on another occasion, repeating the above-described insulin infusion together with intravenous infusion of phentolamine (−15 to +150 min), 5 mg over 2 min followed by 500 μg/min. α-Blockade was able to suppress hypoglycemia-induced increase of platelet sensitivity to aggregating agents. A further study in vitro confirmed these results obtained in vivo, showing that incubation with phentolamine is able to inhibit the epinephrine-induced increase of platelet aggregation in response to ADP, thrombin, and platelet-activating factor.

In conclusion, insulin-induced hypoglycemia deeply influences platelet function, causing an increase of platelet sensitivity to aggregating agents in vitro and a release reaction in vivo. Through α-adrenoreceptors, epinephrine is responsible for the hypoglycemia-induced increase of platelet aggregation in response to ADP, thrombin, and platelet-activating factor. DIABETES 1986; 35:818–25.

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In recent years, many attempts have been made to attain near-normoglycemia in the treatment of insulin-dependent diabetes mellitus (type I) in the hope of preventing, delaying, and possibly reversing the vascular complications of the disease. Surprisingly, some reports have described a rapid deterioration of diabetic retinopathy shortly after the optimization of insulin therapy.[1–4] It may be postulated that increased frequency of hypoglycemic episodes may play a role in this phenomenon. Furthermore, some ophthalmologists observed a close temporal relationship between sudden microvascular accidents and the occurrence of hypoglycemia (F. L'Esperance and P. Brancato, personal communications).

These clinical observations prompted us to investigate the role of insulin-induced hypoglycemia on platelet function, a factor possibly involved in the pathogenesis of diabetic microangiopathy.[5,6]

Recent studies in this research area demonstrate that insulin-induced hypoglycemia increases platelet aggregation in response to ADP both in healthy controls and in type I diabetic patients[7,8] and stimulates, in type I diabetics, the release of β-thromboglobulin,[9] a marker of the platelet-release reaction in vivo.[10]

The aims of our study are to *1*) investigate whether the hypoglycemia-induced increase of platelet aggregation occurs also in response to aggregating agents, such as thrombin and platelet-activating factor, showing different mecha-

nisms of action to those of ADP; *2*) observe whether the hypoglycemia-induced in vivo platelet release reaction occurs not only in type I diabetic patients but also in healthy subjects, as a "normal" response of platelets to this particular metabolic condition; *3*) study the relationships between changes in platelet activation and changes in plasma glucose, insulin, and counterregulatory hormones during insulin-induced hypoglycemia; and *4*) elucidate the role of α-adrenergic receptors in hypoglycemia-induced platelet activation.

SUBJECTS AND PROTOCOL

Informed consent was obtained from 16 healthy male volunteers aged 26 ± 0.7 yr (mean ± SE), with body mass index 22 ± 0.5 (mean ± SE), who did not take medications during the 3 wk before the study and were on a weight-maintaining diet. We performed five different studies.

Study A. Sixteen subjects were studied between 0930 and 1000 h, after fasting overnight in the supine position. A 19-gauge butterfly needle was inserted into an antecubital vein for infusion of regular human insulin (Actrapid HM, Novo, Copenhagen, Denmark) at the rate of 64 mU · m^{-2} · min^{-1} for 60 min (0–60 min) with the infusion pump Perfusor (Braun, Melsungen, FRG). Another homolateral vein, distal to that used for insulin infusion, was catheterized with a 19-gauge butterfly needle kept open with a 0.9% NaCl infusion and used to take intermittent blood samples for 150 min at 5-min intervals for immediate plasma glucose determination, at 30-min intervals and as near as possible to the measured hypoglycemic nadir for measurement of plasma insulin, glucagon, growth hormone (GH), cortisol, and in 5 subjects, epinephrine. Four different venipunctures without stasis in contralateral veins were performed for measurement of platelet aggregation in vitro and for determination of plasma β-thromboglobulin and platelet factor 4 (markers of in vivo release reaction)[10] at the following times: 0 min, as near as possible to the hypoglycemic nadir, 90 min, and 150 min. β-Thromboglobulin and platelet factor 4 were determined in 11 subjects.

Study B. Four subjects were submitted to a control study, consisting of the infusion and sampling procedure described in study A, without including insulin in the infused saline solution.

Study C. Seven subjects were studied, after fasting overnight, on separate days. Venous blood samples were drawn without stasis to obtain PRP and platelet-poor plasma (PPP) as described in ANALYTICAL METHODS. Platelet sensitivity to ADP, thrombin, and platelet-activating factor was measured before and after incubation of PRP with concentrations of insulin, epinephrine, glucagon, GH, and cortisol similar to that measured in study A during insulin-induced hypoglycemia.

Study D. Study A was repeated in four subjects with the addition of an infusion of phentolamine (−15 to +150 min): a bolus of 5 mg i.v. over 2 min followed by continuous infusion at the rate of 500 μg/min i.v. Phentolamine was supplied by Ciba Geigy (Milan, Italy).

Study E. Platelet-rich plasma obtained from five fasting subjects was used to determine platelet sensitivity to ADP, thrombin, and platelet-activating factor both before and after incubation with epinephrine (as in study C) and epinephrine plus phentolamine. Phentolamine was added to reproduce in vitro conditions similar to those of study D.

ANALYTICAL METHODS

Plasma glucose. Blood for plasma glucose measurement was collected in 1.5-ml tubes containing 25 μl 10% EDTA-Na^+ and was immediately centrifuged at 8000 × *g* in Microfuge 11 (Beckman, Fullerton, CA). After plasma separation, plasma glucose was immediately determined, in duplicate, by means of the Beckman glucose analyzer.

Plasma hormones. Venous blood for determination of insulin, growth hormone, and cortisol was placed in chilled tubes containing 20 μl/ml of 10% EDTA-Na^+. Tubes for glucagon measurement also contained 1000 U aprotinin/ml blood. Tubes for epinephrine determination were heparinized and contained 100 μl of 10% sodium metabisulfite/10 ml blood. All tubes were immediately placed on ice and centrifuged at +4°C at 2500 × *g* in the J6M Beckman centrifuge. Plasma was separated and stored at −70°C until the time of assay, performed in duplicate. Insulin, glucagon, and cortisol were determined by RIA kit Biodata (Guidonia Montecelio, Rome, Italy); growth hormone was measured by RIA kit Lysophase Scalvo (Milan, Italy); epinephrine was determined by means of high-performance liquid chromatography (HPLC) with electrochemical detection. The method relies on a liquid/solid extraction of epinephrine onto alumina (AAO) followed by its elution with perchloric acid ($HClO_4$). Briefly, 2 ml of plasma were placed into reaction vials together with 25 μl of 100 ng/ml solution of the synthetic internal standard 3,4-dihydroxybenzylamine (DHBA) and 50 mg of AAO. For every set of samples, we prepared at least two standards as follows: 2 ml 0.1 mol/L phosphate buffer, pH 7; 25 μl DHBA standard; 50 μl epinephrine standard (25 ng/ml); and 50 mg AAO. After a suitable shaking period, AAO was washed and aspirated twice with water, followed by elution with 200 μl of 0.1 mol/L $HClO_4$ in a microfilter loaded with RC 58 membrane. One hundred microliters of acidic extract containing epinephrine were injected in HPLC (Beckman Model 110 A). The stationary phase was a Biophase ODS 5 μm column (250 × 4 mm) (Bioanalytical Systems, West Lafayette, IN); the mobile phase was 0.15 mol/L monochloroacetate buffer, pH 3, containing 2×10^{-3} mol/L EDTA-Na^+ and 25–30 mg/L sodium octylsulfate. Electrochemical detection was carried out at 650 mV by means of electrochemical transducer and LC-4B amperometric detector of Bioanalytical Systems. To determine epinephrine concentration in samples, peak height ratios relative to the internal standard DHBA for unknown plasmas were compared with those for this synthetic standard whose original concentrations were known according to the following formula:

$$|E|_{unknown} = \frac{|E/DHBA|_{unknown}}{|E/DHBA|_{known}} \times |E|_{known}$$

Platelet sensitivity to different aggregating agents. Eighteen milliliters of blood for measurement of platelet sensitivity to aggregating agents were drawn in 50-ml polypropylene tubes containing 2 ml of 3.8% sodium citrate and immediately centrifuged at 180 × *g* for 15 min to obtain PRP, which was put into 10-ml polypropylene tubes and used for the measurement of platelet sensitivity to different aggregating

agents in 250-μl aggregometer cuvettes. PPP was obtained by centrifugation of 1 ml of PRP at 2000 × *g* at room temperature for 10 min. In each sample of PRP, we determined the platelet count in an electronic Coulter counter (Model S-Plus, Hialeah, FL) to evaluate platelet recovery and, if necessary, to adjust the platelet count at 250,000/μl. Aggregation studies were carried out, always within 60 min from venous blood sample, by means of a double-channel aggregometer (Model 840, Elvi, Madrid, Spain), at 37°C, with a constant stirring at 900 rpm. The aggregometer was calibrated with PRP at the optical density of 0.95 and PPP at the optical density of 0.05. Aggregation profiles in response to aggregating agents were measured as the increase in light transmission after the addition of aggregating agents. To compare the different profiles, we used ED_{50} obtained from a dose-response curve at each experimental time point. ED_{50} is the concentration of each aggregating agent necessary to induce a maximal aggregation of 50%. Maximal aggregation is obtained according to the following equation: maximal aggregation (%) = OD at t_0 − minimum OD/OD at t_0 (where OD = optical density; t_0 = the time before the addition of aggregating agents).[11] We employed as aggregating agents both the classic agents ADP and thrombin and the recently discovered platelet-activating factor whose structure is: 1-*O*-alchyl-2-*O*-acetyl-*sn*-glycero-3-phosphorylcholine (AGEPc).[12] The agents were supplied by Sigma (St. Louis, MO). AGEPc was stored diluted in chloroform and recovered, after removing chloroform with a stream of N_2, in the Tris-buffered Tyrode-BSA, prepared as follows: KCl, 2.6×10^{-3} mol/L; $MgCl_2 \cdot 6H_2O$, 1.0×10^{-3} mol/L; NaCl, 1.37×10^{-1} mol/L; $CaCl_2 \cdot 6H_2O$, 1.3×10^{-3} mol/L; 0.1% glucose; tris(hydroxymethyl)aminomethane, 1×10^{-3} mol/L; 0.25% bovine serum albumin (BSA) (Pentex fraction V, Miles Laboratories, Kankakee, IL), the buffer was adjusted at pH 7.4 at room temperature.

In study C, we incubated PRP, at 37°C, with the following hormones before adding the aggregating agent: insulin (Actrapid HM, Novo) for 5 min at the final concentration of 140 μU/ml; epinephrine (Sigma) for 2 min at the final concentration of 500 pg/ml; glucagon (Novo) for 5 min at the final concentration of 160 pg/ml; GH (Serono, Rome, Italy) for 5 min at the final concentration of 20 ng/ml; and cortisol (Biodata) for 5 min at the final concentration of 250 ng/ml.

In study E, PRP was incubated with phentolamine (Ciba Geigy), at the final concentration of 20×10^{-6} mol/L + epinephrine (500 pg/ml) for 2 min.

Plasma β-thromboglobulin and platelet factor 4. Blood was placed in 9.4-ml chilled tubes Thrombotect (Abbott, Campoverde di Aprilia, Latina, Italy) and immediately centrifuged at +4°C in the J6M Beckman refrigerated centrifuge at 2500 × *g*. Plasma was stored at −70°C until the assay was carried out in duplicate by RIA (Amersham kit, Amersham, Bucks, UK, for β-thromboglobulin; Abbott kit, North Chicago, IL, for platelet factor 4).

STATISTICAL METHODS AND CALCULATIONS

All data in the text and figures are expressed as mean ± SE. Data were compared by means of paired Student's *t* test; correlation coefficients were calculated by the least-squares method.[13]

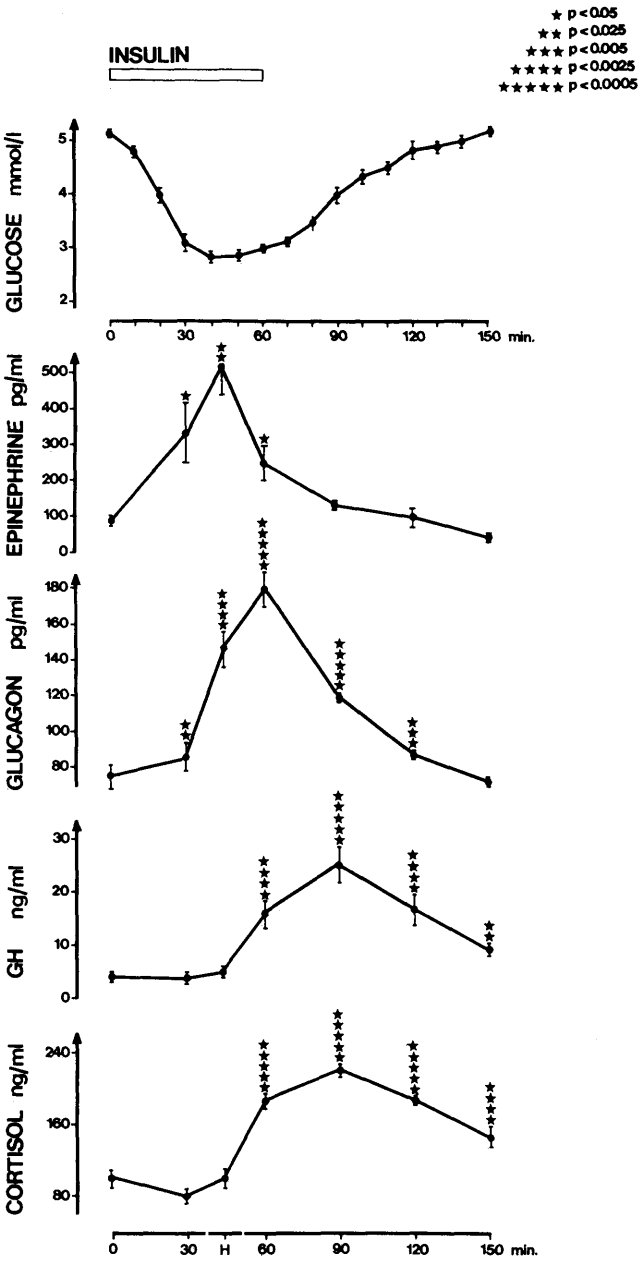

FIGURE 1. Plasma concentrations of glucose and counterregulatory hormones measured in healthy subjects receiving 60-min i.v. infusion of human regular insulin at rate of 64 mU · m^{-2} · min^{-1} after overnight fast. Values are expressed as means ± SE. Significance symbols refer to baseline concentrations. Significance concerning plasma glucose is not pictured. All values until 140 min are significantly lower than baseline values. Epinephrine has been measured in 5 out of 16 subjects studied. H, hypoglycemic nadir.

The rates of decrease in plasma glucose were calculated by dividing the differences between baseline and nadir plasma glucose concentrations by the time intervals. The rate of plasma glucose recovery was calculated by dividing the differences between plasma glucose values returned at baseline and values at nadir by the time intervals. When the postnadir plasma glucose concentrations did not reach baseline value within 150 min, the glucose value at 150 min was employed for calculations.

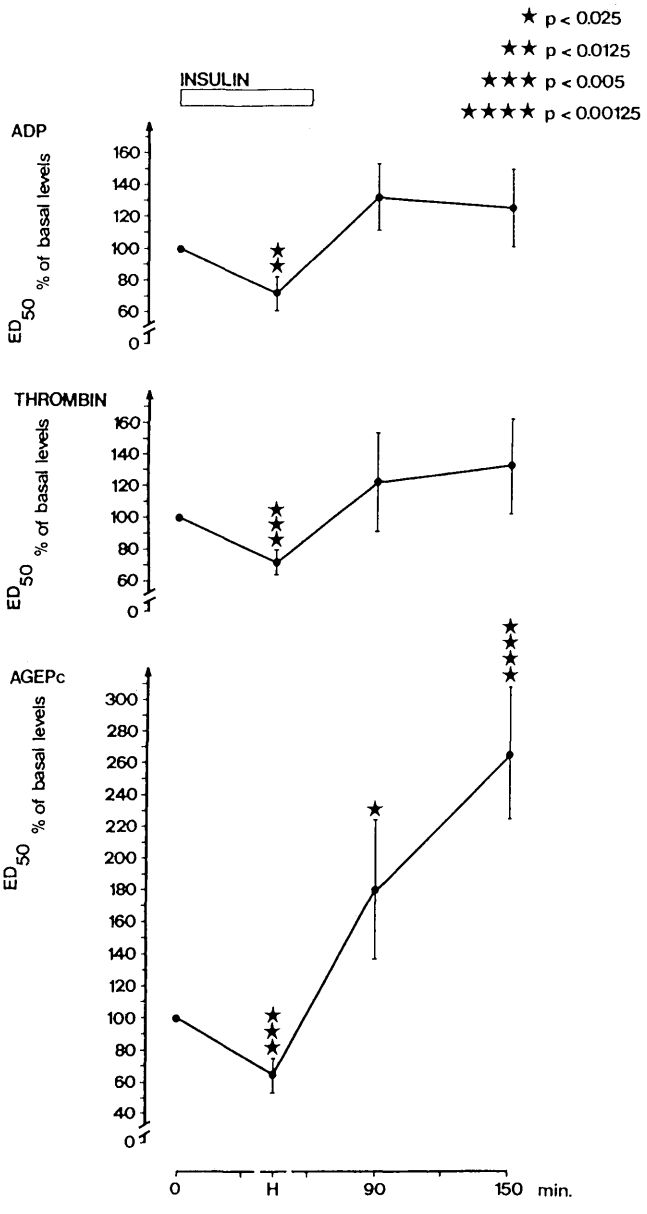

FIGURE 2. Platelet sensitivity to ADP, thrombin, and platelet-activating factor (AGEPc) measured in healthy subjects receiving 60-min i.v. infusion of human regular insulin at rate of 64 mU · m^{-2} · min^{-1} after overnight fast. Data (means ± SE) are expressed as percent of baseline ED_{50} of each aggregating agent. Significance symbols refer to baseline values. H, hypoglycemic nadir.

RESULTS

STUDY A

Plasma insulin. The mean baseline plasma insulin concentration was 11.9 ± 1.3 μU/ml. Insulin infusion induced an increase of plasma insulin concentrations, reaching values of 118 ± 9 μU/ml at 30 min, 136 ± 11 μU/ml at the hypoglycemic nadir, and 132 ± 10 μU/ml at 60 min. After the end of insulin infusion, the insulin concentrations returned toward the basal values: 9.7 ± 1.5 μU/ml at 90 min, 9.3 ± 1.5 μU/ml at 120 min, and 9.4 ± 1.4 μU/ml at 150 min.

Plasma glucose. The mean basal plasma glucose concentration was 5.12 ± 0.059 mmol/L. During the insulin infusion, the mean plasma glucose reached a nadir of 2.72 ± 0.098 mmol/L at 45 min. However, the mean of the values of plasma glucose at each subject's individual nadir (occurring in the range 30–55 min) was 2.36 ± 0.077 mmol/L (range 1.69–2.88 mmol/L). The rate of plasma glucose decrease was 0.069 ± 0.003 mmol · L^{-1} · min^{-1}. The rate of plasma glucose recovery was 0.029 ± 0.0019 mmol · L^{-1} · min^{-1} (Figure 1).

Plasma epinephrine, glucagon, growth hormone, and cortisol. All these counterregulatory hormones increased throughout the study. At the hypoglycemic nadir, only plasma concentrations of epinephrine and glucagon were significantly greater than baseline values (Figure 1).

Platelet sensitivity to ADP, thrombin, and AGEPc. Platelet sensitivity to aggregating agents showed a significant increase at the hypoglycemic nadir, as demonstrated by the signficant decrease of ED_{50}. Platelet sensitivity to AGEPc was significantly lower in the recovery phase than at baseline (Figure 2).

Plasma β-thromboglobulin and platelet factor 4. Plasma concentrations of these platelet-specific proteins were significantly higher than their basal values at the hypoglycemic nadir and at 90 min (Figure 3).

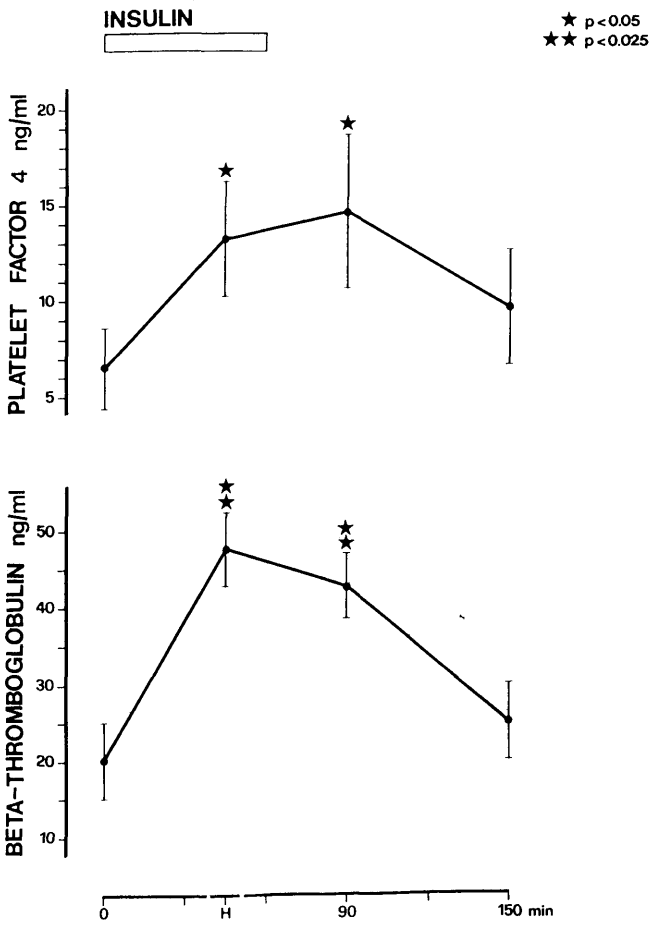

FIGURE 3. Plasma concentrations of β-thromboglobulin and platelet factor 4 measured in healthy subjects receiving 60-min i.v. infusion of human regular insulin at rate of 64 mU · m^{-2} · min^{-1} after overnight fast. Values are expressed as means ± SE. Significance symbols refer to baseline concentrations. H, hypoglycemic nadir.

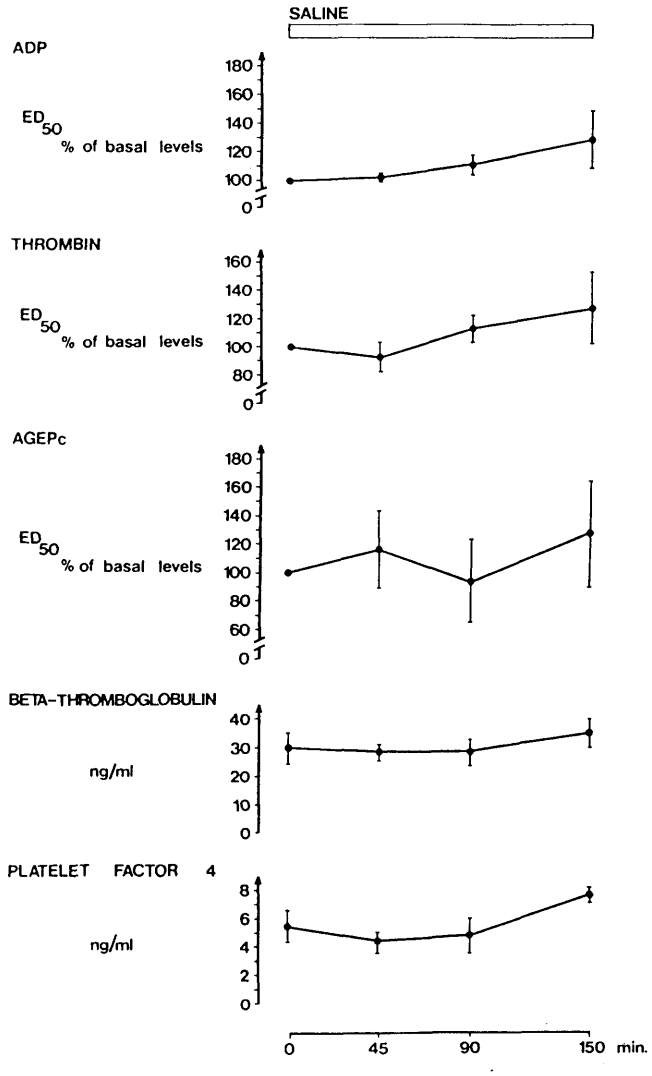

FIGURE 4. Platelet sensitivity to ADP, thrombin, and platelet-activating factor (AGEPc) and plasma levels of β-thromboglobulin and platelet factor 4 measured in healthy subjects in control study with saline infusion. Data concerning platelet aggregation are expressed as percent of baseline ED_{50} of each aggregating agent. Values are expressed as means ± SE.

Correlation between plasma glucose response to insulin infusion and platelet activation. No correlation was found between the degree of in vitro or in vivo platelet activation and the plasma glucose concentrations at nadir or the plasma glucose decrease rate.

STUDY B

No significant changes in platelet sensitivity to aggregating agents or in plasma concentrations of β-thromboglobulin and platelet factor 4 were observed throughout the control study with saline infusion (Figure 4).

STUDY C

Figure 5 shows platelet sensitivity to aggregating agents both with and without the incubation of PRP with the same concentrations of insulin and counterregulatory hormones observed in vivo during study A. Insulin showed an inhibitory influence on platelet aggregation. At the employed concentrations, epinephrine did not induce platelet aggregation per se; however, it significantly increased platelet sensitivity to ADP, thrombin, and AGEPc. Glucagon, GH, and cortisol did not significantly modify platelet response to aggregating agents.

STUDY D

Figure 6 shows plasma glucose and hormone concentrations in the same subjects receiving insulin and insulin plus phentolamine infusion on two different occasions.

Plasma glucose. Plasma glucose concentrations showed a smaller decrease when insulin was infused with phentolamine (study D) than when insulin was infused alone (study A). In particular, when the single hypoglycemic nadirs are considered, whenever they occurred, the mean value was 2.51 ± 0.10 mmol/L in study A and 2.80 ± 0.16 mmol/L in study B ($P < .05$). The rate of plasma glucose decrease was 0.064 ± 0.007 mmol · L^{-1} · min^{-1} in study A and 0.050 ± 0.007 mmol · L^{-1} · min^{-1} in study B (NS). The rate of plasma glucose recovery was 0.031 ± 0.003 mmol · L^{-1} · min^{-1} in study A and 0.036 ± 0.008 mmol · L^{-1} · min^{-1} in study B (NS). Plasma glucose concentrations were higher in study B than in study A at the following time points: 20, 25, 30, 35, 70, 75, 80, 85, 90, 95, 100, 105, 110, and 120 min (range of significancies, $P < .05$ to $P < .005$).

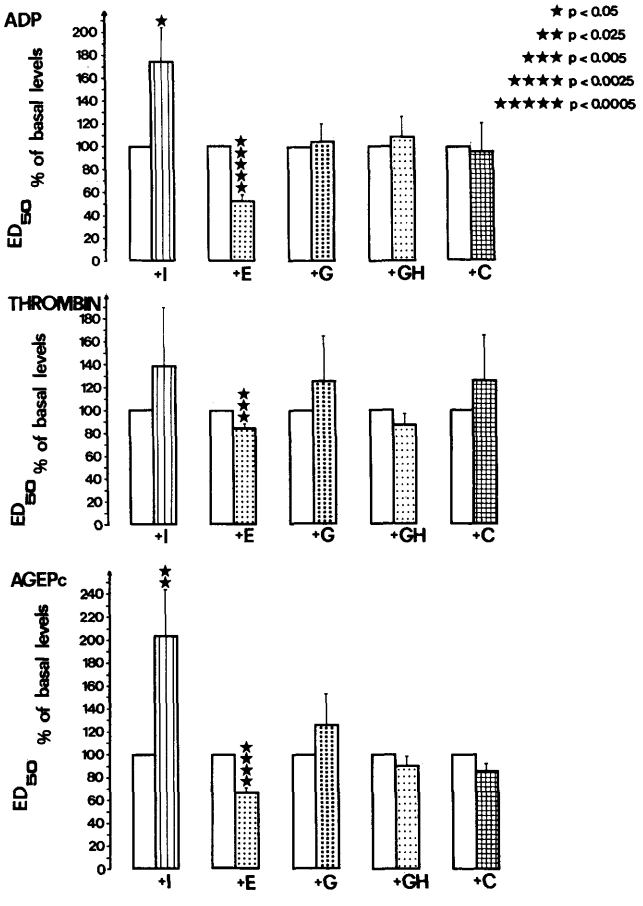

FIGURE 5. Platelet sensitivity to ADP, thrombin, and platelet-activating factor (AGEPc) measured on PRP of healthy fasting subjects both with and without its incubation with insulin (I), 140 μU/ml for 5 min; epinephrine (E), 500 pg/ml for 2 min; glucagon (G), 160 pg/ml for 5 min; growth hormone (GH), 20 ng/ml for 5 min; cortisol (C), 250 ng/ml for 5 min. Data (means ± SE) are expressed as percent of ED_{50} of each aggregating agent in experiments without incubation with hormones.

Plasma glucagon, GH, and cortisol. Similar concentrations of plasma glucagon and plasma cortisol were reached in studies A and D, whereas the plasma GH increase was blunted in study D. At the hypoglycemic nadir, only plasma glucagon concentrations were significantly higher than at baseline ($P < .05$).

Platelet sensitivity to aggregating agents. Hypoglycemia-induced increase of platelet sensitivity to aggregating agents was suppressed by phentolamine infusion (Figure 7).

STUDY E

Figure 8 shows platelet sensitivity to aggregating agents with and without incubation of PRP with epinephrine and epinephrine plus phentolamine. The increase of platelet aggregation in response to ADP, thrombin, and AGEPc observed after incubation with epinephrine was suppressed when PRP was incubated with epinephrine plus phentolamine.

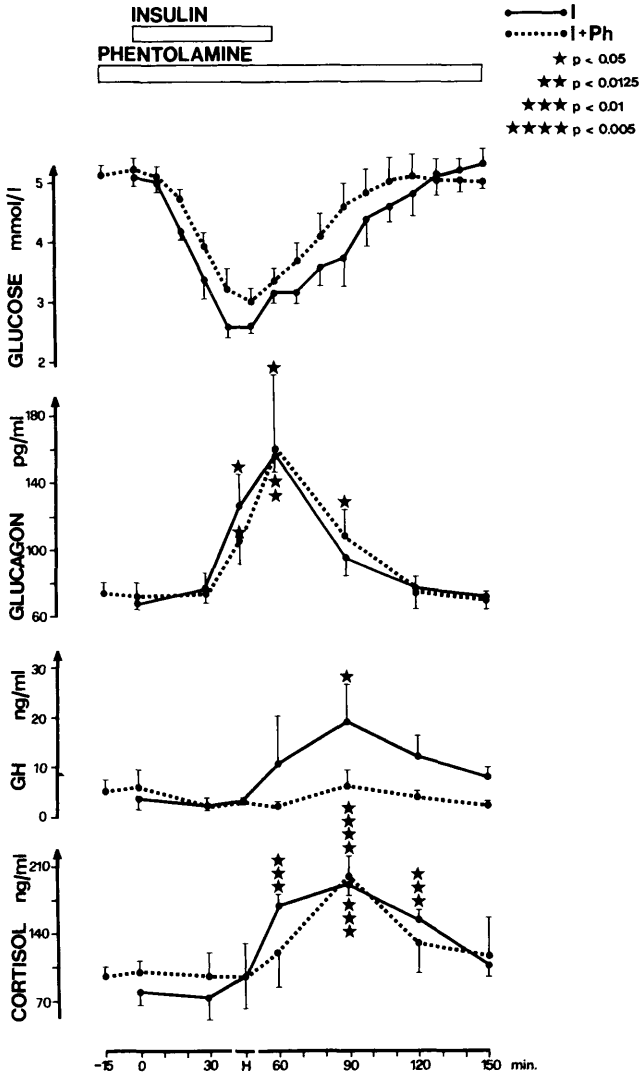

FIGURE 6. Plasma concentrations of glucose and counterregulatory hormones in healthy subjects receiving, after overnight fast, 60-min i.v. infusion of human regular insulin at rate of 64 mU · m^{-2} · min^{-1}, with (*dotted line*) or without (*solid line*) i.v. infusion of phentolamine (−15 to +150 min), 5 mg over 2 min followed by 500 μg/min. Values are expressed as means ± SE. Significance symbols refer to values at 0 min. H, hypoglycemic nadir.

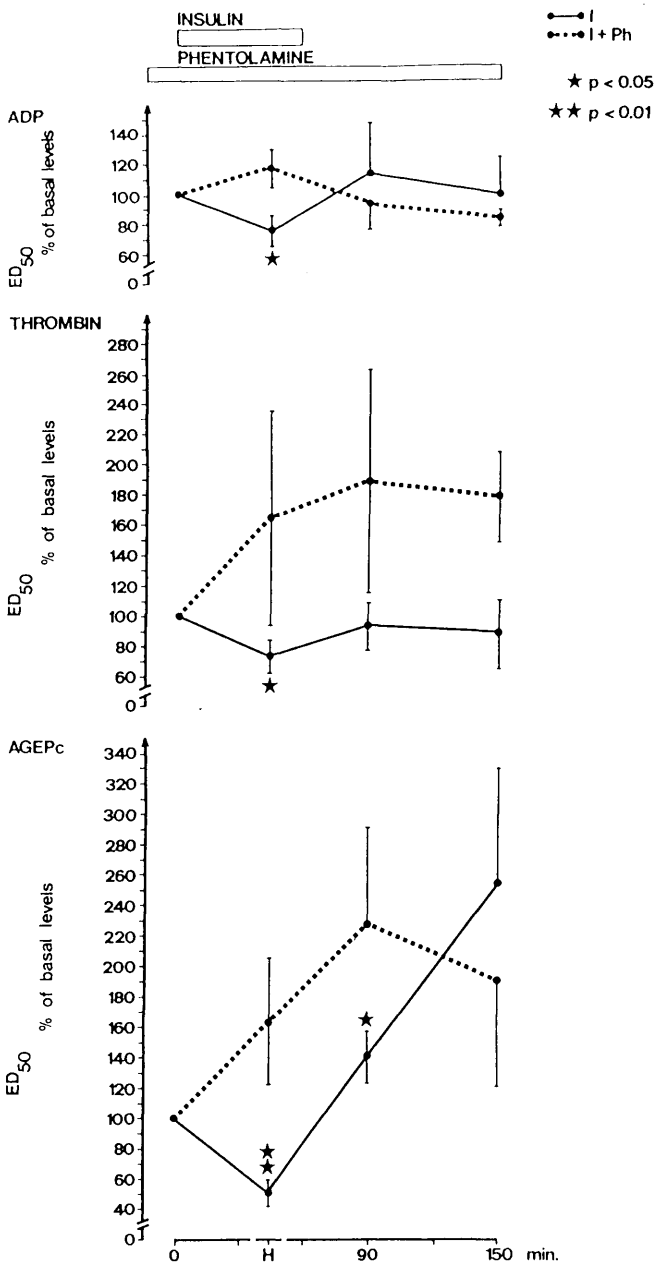

FIGURE 7. Platelet sensitivity to ADP, thrombin, and platelet-activating factor (AGEPc) measured in healthy subjects receiving, after overnight fast, 60-min i.v. infusion of human regular insulin at rate of 64 mU · m^{-2} · min^{-1} with (*dotted line*) or without (*solid line*) i.v. infusion of phentolamine (−15 to +150 min), 5 mg over 2 min followed by 500 μg/min. Data (means ± SE) are expressed as percent of ED_{50} of each aggregating agent at 0 min. Significance symbols refer to values at 0 min.

DISCUSSION

Our study demonstrates that insulin-induced hypoglycemia deeply influences platelet function; it actually increases platelet sensitivity to aggregating agents in vitro and induces the release reaction in vivo.

Note that at hypoglycemic nadir, platelets showed a similar increase of the aggregating response to substances with different mechanisms of action, e.g., ADP, thrombin, and AGEPc. In the recovery phase after the hypoglycemic nadir, platelet sensitivity to AGEPc was deeply and significantly

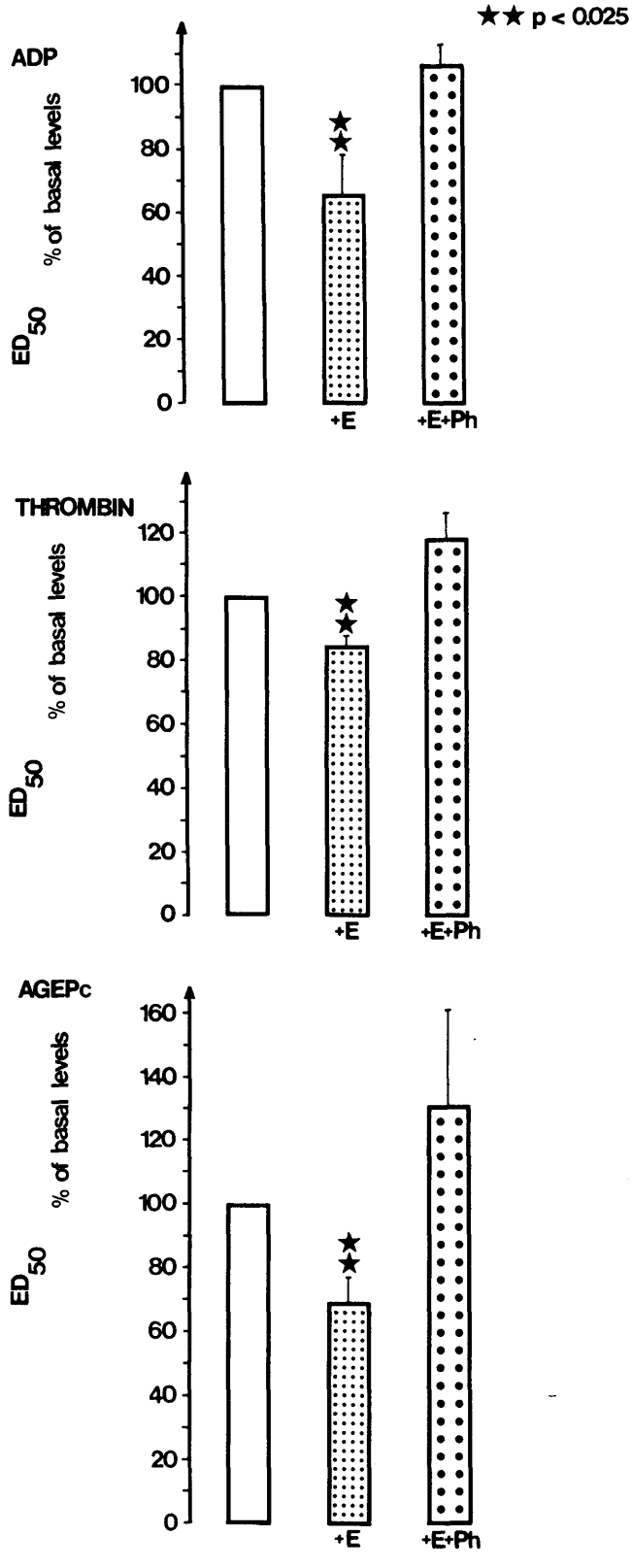

FIGURE 8. Platelet sensitivity to ADP, thrombin, and platelet-activating factor (AGEPc) measured on PRP of fasting healthy subjects both with and without its incubation with epinephrine (E): 500 pg/ml for 2 min; and epinephrine plus phentolamine (Ph): 20 × 10^{-6} mol/L for 2 min. Data (means ± SE) are expressed as percent of ED_{50} of each aggregating agent in experiments without incubation with E or E + Ph.

depressed; this intriguing phenomenon needs further investigation.

We examined the pathophysiologic mechanisms of hypoglycemia-induced platelet activation. First, our results show that the value of plasma glucose at nadir is not correlated with the changes in platelet function. This fact suggests that hypoglycemia, per se, is not the direct cause of platelet hyperactivation. Second, we can suppose an influence of the increased plasma concentrations of insulin or of counterregulatory hormones.

The decrease of platelet response to aggregating agents observed when PRP is incubated with insulin is a very interesting phenomenon, showing that the direct effect of insulin on platelets is not the cause of the increased platelet sensitivity to aggregating agents observed during insulin-induced hypoglycemia.

In regard to the role of counterregulatory hormones, GH and cortisol are not involved in hypoglycemia-induced platelet activation, because they are not significantly increased in vivo at the hypoglycemic nadir when platelet activation occurs and because they do not influence platelet response to aggregating agents when added in vitro. We could suppose a role of glucagon that is significantly increased immediately after the hypoglycemic nadir. However, despite the temporal relationships, data in vitro show that, when PRP is incubated with glucagon, platelet sensitivity to aggregating agents is not modified. On the other hand, the role of epinephrine in hypoglycemia-induced platelet activation is supported by much evidence. First, plasma epinephrine concentrations peak at hypoglycemic nadir, when platelet changes take place. Furthermore, epinephrine added in vitro at the same concentrations reached in vivo is able to reproduce platelet aggregation changes similar to those observed in vivo at hypoglycemic nadir. The influence of epinephrine, at concentrations too low to cause aggregation per se, on platelet response to agents that act by ways independent of adrenergic receptors has also been described by other investigators and ascribed to membrane modulation mechanisms involving the α-adrenergic receptors.[14–16] The phenomenon is further confirmed by the blunted increase of platelet aggregation observed when insulin-induced hypoglycemia was carried out in the presence of α-blockade. Under this condition we observed a smaller plasma glucose decrease and a blunted plasma GH increase with respect to the experiments performed with insulin infusion alone.

The inhibition of GH response to hypoglycemia during α-blockade is in keeping with previous reports.[17] The smaller plasma glucose decrease is an intriguing phenomenon. We can suppose that the striking increase of plasma norepinephrine caused by α-blockade[18] can produce an extrastimulation of β-adrenergic receptors that are deeply involved in the hyperglycemic action of catecholamines in humans.[19,20] However, because our study shows that plasma glucose concentrations in the hypoglycemic range are not correlated per se with hypoglycemia-induced platelet activation, it is unlikely that differences in plasma glucose values can account for the blunted increase of platelet sensitivity to aggregating agents observed during α-blockade. This finding could rather be interpreted in the light of the modulation exerted by phentolamine on platelet response to aggregating agents. In fact,

phentolamine added in vitro completely abolished the epinephrine-induced increase of platelet aggregation in response to ADP, AGEPc, and thrombin. Our study therefore supports the conclusion that epinephrine, through α-adrenergic receptors, is directly responsible for the hypoglycemia-induced increase of platelet sensitivity to aggregating agents. A role of norepinephrine can not be completely ruled out; however, personal unpublished data agree with the observation that norepinephrine is far less powerful than epinephrine on platelets.[15]

From the clinical point of view, the results of our study demonstrate that insulin-induced hypoglycemia in the range between 1.7 and 2.9 mmol/L causes platelet activation in nondiabetic subjects. Activation therefore is the "normal" response of normal platelets to hypoglycemia. Consequently, it might occur in diabetic patients also before the appearance of angiopathy, of abnormalities of platelet function, or of alterations of the counterregulatory response to hypoglycemia. An "aggressive" insulin therapy to achieve near normoglycemia is often pursued from the onset of the disease in type I diabetic patients in the hope of preventing vascular complications. The results of this study induce us to consider the possible hazards of the intensified insulin therapy if it is the cause of increased frequency of hypoglycemic episodes. Actually, hypoglycemia must be carefully prevented not only to avoid neuroglycopenia, as generally accepted, but also to avoid possible platelet-mediated vascular damage.

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