Restoration by Insulin of Impaired Prostaglandin E₁/I₂ Receptor Activity of Platelets in Acute Ischemic Heart Disease

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Treatment of normal platelet-rich plasma with a physiological amount of insulin (100 microunits/ml, optimum concentration) for 3 hours at 23°C stimulated the binding of prostaglandin E₁ by more than twofold (3,940±250 sites/10⁹ platelets) compared with the nontreated, control platelet-rich plasma (1,590±265 sites/10⁹ platelets). After platelet-rich plasma from patients with acute ischemic heart disease (n=43), whose platelets showed impaired prostaglandin E₁/I₂ receptor activity (850±100 sites/10⁹ platelets), was incubated with insulin (optimum amounts varied from 100 to 200 microunits/ml), the binding of the prostanoid was restored to normal levels (1,790±140 sites/10⁹ platelets) in 75% of the cases. Twenty-five percent of the patients did not respond to the stimulatory effect of insulin. The increased binding of the prostanoid to the insulin-treated platelets also resulted in increased cyclic AMP levels both in normal subjects (44.14±3.1 pmol/10⁹ [insulin-treated] platelets versus 16.35±2.91 pmol/10⁹ [control] platelets) and in patients with acute ischemic heart disease (23.87±4.1 pmol/10⁹ [insulin-treated] platelets versus 7.70±2.0 pmol/10⁹ [control] platelets) by the prostanoid (1.0 µM). The treatment of platelet-rich plasma with the hormone decreased the minimum inhibitory concentration of the prostanoid from 34±14 to 15±9 nM (p<0.001) in the case of normal volunteers and from 49±15 to 32±11 nM (p=0.002) in the case of “responder” patients. Insulin did not produce any effect on the inhibition of platelet aggregation by the prostanoid in “nonresponder” patients. In the follow-up study, although the stimulatory effects of insulin on platelets from responder patients were improved to normal levels, the platelets from nonresponder patients remained persistently unresponsive to the effect of the hormone. (Circulation Research 1991;68:245–254)

Aggregation of platelets induced by agonists like thrombin, ADP, l-epinephrine collagen, thromboxane A₂, serotonin, and, possibly, platelet-activating factor is believed to be critically important not only in normal blood coagulation but also in the pathogenesis of atherosclerosis and thrombosis (for a review see References 1 and 2). The aggregation of platelets is counterbalanced by several humoral factors, which include prostanoids such as prostacyclin (PGI₂) and prostaglandin E₁ (PGE₁) and proteinase such as blood coagulation factor Xa.3–5 The prostanoids inhibit platelet aggregation by increasing the intracellular cyclic AMP (cAMP) level through the activation of adenylate cyclase.3,6–8 The activation of adenylate cyclase itself, however, is initiated through the binding of these prostanoids to their specific receptors linked to adenylate cyclase in the membrane bilayer.7,8 Prostaglandins, through their inhibitory effect on platelet aggregation, are believed to play a key role in the prevention of thrombosis and atherosclerosis.9

Hyperactivity of platelets and their adhesion and aggregation at the site of endothelial injury have been shown to be crucially important in the pathogenesis of acute ischemic heart disease (AIHD). Previous studies have shown increased platelet-released products,10–12 aggregates of platelets in the circulation,13 and enhanced platelet aggregation14,15 in patients with acute myocardial infarction (AMI) and unstable angina (UA); recent studies have demonstrated that the conversion from chronic to acute ischemic syndromes is related to endothelial injury at the site of stenosis,16–19 probably by rupture or fissure of the atherosclerotic plaque.20–22 Platelet adhesion

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Received February 28, 1990; accepted September 4, 1990.
and aggregation at the site of endothelial injury, which release activating substances, further enhance platelet activity, platelet aggregation, and thrombus formation.12-14,16 Both clinical and autopsy studies have demonstrated obstructive thrombi and platelet aggregates in acutely occluded coronary vessels.15,20,23 The hyperactivity of platelets in the setting of AIHD is also reflected in their diminished response to the inhibitory effects of PGI₂, both in vivo and in vitro.24-27

We have recently demonstrated that the diminished response of platelets to the inhibitory effect of the prostanoïd in AIHD reflects decreased PGE₁/I₂ receptor numbers.28 Since prostaglandin receptor occupancy is related to the formation of cAMP,29 the impaired PGE₁/I₂ receptor numbers lead to a state of hyperactivation in these platelets due to the derangement of mechanisms counteracting the inhibition of platelet aggregation.30-32

In a previous study,33 we have shown that the receptors for PGE₁/I₂ and insulin are functionally related in that the incubation of human erythrocyte membranes with either of these prostanoïds increased the insulin receptor numbers by exposing "spare" binding sites in the membrane bilayer. On the other hand, incubation of platelets from normal volunteers with physiological amounts of insulin increased the binding of PGE₁ and thereby stimulated the activation of adenylate cyclase by the prostanoïd.34 In an effort to restore the impaired prostanoïd receptor activity of platelets in AIHD, we studied the effect of physiological concentrations of insulin on the PGE₁/I₂ receptor number in platelets from patients with AMI or UA and compared it with platelets from normal volunteers.

Materials and Methods

Ethical Clearance

The protocol was approved by the Institutional Review Board for Clinical Research, Montefiore Medical Center.

Patient Selection Criteria

Two categories of AIHD patients were studied: 1) patients with UA and 2) patients with AMI.

Inclusion criteria. All patients were admitted to the Coronary Care Unit or Stepdown Unit of Montefiore Medical Center. Patients were included in the study if they met the following criteria for UA: 1) severe angina at rest of more than 10-15 minutes but less than 30 minutes, 2) transient ST segment and/or T wave changes in at least two contiguous leads in the electrocardiogram (ECG) or, if ECG changes are not present, documentation of coronary artery disease (previous myocardial infarction or abnormal coronary arteriogram), and 3) no elevation of plasma creatine kinase (CK) and no appearance of CK-MB isoenzyme in plasma.

Patients were included in the study if they met the following criteria for AMI: 1) chest pain characteristic of myocardial ischemia for 30 minutes or more, 2) ST segment elevation of at least two leads of the ECG, reflecting a single myocardial region, and 3) confirmation of the diagnosis of AMI by elevated CK level and by CK-MB isoenzyme in plasma.

Anginal attack or clinical onset of infarction must be less than 12 hours before blood sampling for inclusion in the study, and the patient must be less than 70 years old.

Exclusion criteria. Patients were excluded from the study if one of the following applied: 1) diabetes mellitus, 2) bundle branch block or left ventricular hypertrophy in ECG, not allowing the diagnosis of ischemia/infarction, 3) infusion of a thrombolytic agent within 4 weeks of the study, or 4) use of inotropic agents including epinephrine, norepinephrine, dopamine, or dobutamine.

Patients using common cardiac medications (see below) were not excluded.

Patient Population

Group 1. Seventy patients, 56 men and 14 women between the ages of 38 and 68 (median, 54) years, who were studied within 5.2±3.2 (mean±SD) hours after the onset of ischemic chest pain were included. Forty-six patients had AMI (61% with anterior/lateral infarction, 39% with inferior/posterior infarction), and 24 patients had UA. Serum CK and CK-MB levels were elevated in all AMI patients and in no UA patients.

Group 2. Sixty-two normal volunteers, 42 men and 20 women between the ages of 28 and 60 (median, 52) years, were studied. None of the volunteers were smokers, and none had taken any medication at least for 2 weeks before the study.

Concomitant Medical Therapy

Forty-two AMI patients received an intravenous infusion of lidocaine (2 mg/hr), and two other patients received the drug at a rate of 3 mg/hr. Five AMI and 19 UA patients received an intravenous infusion of heparin to keep the partial thromboplastin time 1.5 to two times above the control level. Eight AMI and three UA patients had taken aspirin (80-325 mg) before the study. The majority of patients were maintained on antiangiatal therapy, including propranolol, nitropreparations, diltiazem, nifedipine, or verapamil. Ten AMI and two UA patients had received morphine sulfate (2-8 mg i.v.).

Collection of Blood and the Preparation of Platelet-Rich Plasma

Blood samples (40-50 ml) were collected in sodium citrate (0.013 M final concentration) from the patients by using 19-gauge siliconized needles in plastic tubes. Platelet-rich plasma (PRP) was then prepared by centrifuging the blood samples at 200g for 15 minutes at 23°C.35
Incubation of PRP With Insulin and the Assay of Platelet PGE₁/₁ Receptors

Unless otherwise stated, PRP was incubated with 100 microunits/ml insulin (HumulinR, Eli Lilly, Indianapolis, Ind.) for 3 hours at 23°C. After incubation, platelets were washed with an equal volume of Tyrode’s buffer (without Ca²⁺), pH 7.5, containing 100 microunits/ml insulin and resuspended in a small volume (one tenth of the original) of the same buffer containing 5 mM MgCl₂. Typically, 10⁸ platelets were incubated with 3 nM [³H]PGE₁ (specific activity, 50.3 Ci/mmol; New England Nuclear, Boston) in a total volume of 200 μl at 23°C for 15 minutes to attain equilibrium. After incubation, the reaction mixture was diluted to 1.2 ml with the buffer and filtered on glass microfiber filters (GF/C, Whatman Inc., Clifton, N.J.) presoaked with the same buffer under mild vacuum. The filters were then washed with 5 ml Tyrode’s buffer for a second time. Under these conditions, the platelets remained adsorbed to the membrane filter, and the free ligand was removed by washing. The filters were subsequently dried, and the radioactivity was determined by suspending them in ACS II solvent (Amersham Corp., Arlington Heights, III.) in a liquid scintillation counter (Isocap/300, Searle Pharmaceuticals, Chicago) with 60% efficiency for tritium. Nonspecific binding was determined by adding excess (15 μM) unlabeled prostaglandin to the assay mixture. Specific binding was calculated by subtracting the nonspecific binding from the total binding. The total PGE₁/₁, receptor number was calculated by determining the specific binding of the ligand at saturated concentration (1.0 μM) as previously described.

In certain phases of this work, it was necessary to test the effect of epinephrine and glucagon on the binding of PGE₁ to platelets. These experiments were done by incubating PRP with epinephrine (4 μM) or glucagon (100 μM) for 3 hours, and the binding of [³H]PGE₁, to the platelets was determined as described above.

Scatchard Analysis of [³H]PGE₁ Binding to Platelets

The dissociation constants (Kd) and the capacities (receptor numbers) of the PGE₁/₁ receptors of platelets were determined by Scatchard analysis. Platelets were incubated with 3 nM [³H]PGE₁ plus 0–3 μM unlabeled PGE₁ for 15 minutes at 23°C. The binding of [³H]PGE₁ to the platelets was determined for each concentration of the autacoid by calculating the specific activity of the ligand obtained by diluting [³H]PGE₁ with a known concentration of the unlabeled prostaglandin. The dissociation constants and the capacities were obtained from a nonlinear regression analysis of equilibrium binding by a nonweighted, iterative, least-squares algorithmic analysis by a microcomputer (Elsevier-Biosoft, Cambridge, UK).

cAMP Assay

cAMP formation in platelets was determined by the protein kinase binding method. Typically, washed platelets (~2×10⁸) in Tyrode’s buffer, pH 7.5, containing 5.0 mM MgCl₂ were incubated with 10 mM theophylline in a total volume of 200 μl for 2 minutes at 23°C. PGE₁ (1.0 μM) was then added to the cell suspension, and the mixture was incubated for 1 minute more. After the second incubation, ice-cold trichloroacetic acid (5% final concentration) was added to the mixture, and the concentration of cAMP in the extract was determined. Briefly, assay mixture containing 0.97 pmol [³H]cAMP (60,000 disintegrations per minute; Dupont–New England Nuclear, Boston) with 50 μg cAMP-dependent protein kinase, 200 μg protein kinase inhibitor, 50 mM sodium acetate buffer, pH 4.0, and the platelet extract was incubated for 1 hour at 0°C. The mixture was then filtered over a filter (HA, Millipore Corp., Bedford, Mass.) and washed two times with 5 ml of 20 mM sodium phosphate buffer, pH 6.0. The dried filter was dissolved in 1 ml acetonitrile, and the radioactivity was determined as described above. The cAMP contents of the extracts were determined by adding known quantities of unlabeled cAMP to the assay mixture.

Incubation of Normal Platelets With Cardiovascular Medications

To determine the effect of commonly used cardiovascular drugs on the PGE₁/₁ receptor activity of platelets, PRP from normal volunteers was incubated with various medications for 3 hours in the presence of insulin at 23°C. After incubation, the binding of [³H]PGE₁ to platelets was determined as described above.

Aggregation of Platelets

Aggregation of platelets by ADP and the inhibition by PGE₁ was studied in an aggregometer (Chronolog, Broomall, Pa.) by stirring PRP at 1,200 rpm at 37°C as previously described.

Statistical Analyses

Results are shown as mean±SD. Data were analyzed by paired Student’s t test, and values of p<0.01 were considered significant.

Results

Effects of Incubation of PRP With Insulin on the Binding of [³H]PGE₁ to Platelets in Normal Subjects and AIHD Patients

Incubation of PRP from normal volunteers (n=35) with 100 microunits/ml insulin at 23°C for various periods showed that the binding of [³H]PGE₁ to the hormone-treated platelets increased with the increase in incubation time (Figure 1). The maximum binding of the prostaglandin to the platelets was achieved between 2 and 3 hours of incubation. Further incubation of PRP with the hormone, up to 6 hours, did not increase or decrease the binding of [³H]PGE₁ to these platelets. The plasma components of PRP did not influence the effect of the hormone, since washed platelets, when incubated with insulin,
also showed similar increases in $[^3]$H$PGE_1$ binding (not shown). The nonspecific binding of $[^3]$H$PGE_1$, which was 10–15% of the total, remained the same when platelets were incubated with insulin.

In a different experiment, PRP preparations from normal and AIHD patients were incubated with increasing concentrations of insulin (0–300 microunits/ml) for 3 hours to obtain maximum $[^3]$H$PGE_1$ binding. It was found that the incubation of PRP from AMI ($n=30$) or UA ($n=13$) patients with insulin resulted in increased specific binding of $[^3]$H$PGE_1$ in 75% of the patients ("responders") (Figure 2). About 25% of these patients ("nonresponders") did not show any enhanced binding of the prostaglandin to the platelets when these cells were similarly treated with the hormone. The nonspecific binding of $[^3]$H$PGE_1$ to the platelets from responder or nonresponder AIHD patients was similar (10–15% of the total) and was comparable with that of the normal platelets. Although the increase of $[^3]$H$PGE_1$ binding to platelets from AIHD patients in the presence of insulin was more than 2.5 times greater than the nontreated platelets ($p<0.001$), the actual amount of the radioligand bound to these cells was almost 50% lower when compared with normal platelets under identical conditions. However, the quantity of PGE bound to the insulin-treated platelets from AIHD patients ($2.3±0.20$ fmol/10$^8$ platelets) was increased to a level similar to that bound to the normal control platelets ($2.15±0.15$ fmol/10$^8$ cells). When washed platelets, instead of PRP, from the responder AIHD patients were incubated with insulin, the binding of the radioligand did not improve further ($2.2±0.13$ fmol/10$^8$ platelets; data not shown).

Since hyperglycemia frequently occurs in the absence of any history of glucose intolerance in patients during cardiac ischemia,$^{38–41}$ the effect of glucose on the binding of $[^3]$H$PGE_1$ to platelets was also determined. Incubation of normal PRP with glucose (150 mg/dl, an amount comparable with the mean plasma glucose level in AIHD) up to 3 hours at 37°C did not change the binding of $[^3]$H$PGE_1$ to these platelets ($2.35±0.20$ fmol/10$^8$ cells) compared with control platelets ($2.20±0.16$ fmol/10$^8$ cells, $n=3$).

Unlike insulin, neither epinephrine nor glucagon, which might be responsible for the decrease of plasma insulin level in AIHD$^{42}$ and consequently downregulate the expression of PGE$_1$ receptors in platelets, produced no effect on the prostaglandin binding. Incubation of normal PRP with epinephrine (4 μM) or a physiological amount of glucagon (100 pM) for 3 hours did not change the binding of $[^3]$H$PGE_1$ to these platelets ($2.10±0.16$ fmol/10$^8$ platelets in the case of epinephrine; $2.45±0.17$ fmol/10$^8$ platelets in the case of glucagon; $n=3$) compared with control platelets ($2.20±0.16$ fmol/10$^8$ platelets; $n=3$).

**Dose–Response Relation of Normal and AIHD Platelets to the Stimulatory Effect of Insulin**

The concentration of insulin that resulted in the maximal binding of $[^3]$H$PGE_1$ was approximately 100 microunits/ml in normal PRP (Figure 3). On the other hand, the concentration of the hormone needed to maximize the binding of the radioligand to the platelets from responder AIHD patients varied considerably when compared with that of the normal subjects (Figure 3). Although almost 50% of PRP samples from these patients showed maximal $[^3]$H$PGE_1$ binding at 100 microunits/ml insulin, approximately 30% and 20% of these PRP samples needed 150 and 200 microunits/ml insulin, respectively, for the maximum binding of the radioligand. However, it should be
Heart disease was incubated with increasing concentrations of insulin to maximize the binding of isolated, and the binding of was similar in AMI and UA patients. Twenty-three Platelets were isolated, and the binding of was similar in AMI and UA patients. Twenty-three Platelets showed no interaction is comparable with three other experiments. Triangles represent normal platelet-rich plasma in the presence (●) or absence (○) of insulin. Circles represent platelets from patients with acute ischemic heart disease with (●) or without (○) the hormone.

FIGURE 3. Graph showing variation of optimum concentrations of insulin to maximize the binding of [3H]prostaglandin E, (3H-PGE,) to platelets from normal subjects and patients with acute ischemic heart disease. Normal platelet-rich plasma --- or platelet-rich plasma from patients with acute ischemic heart disease --- was incubated with increasing concentrations of insulin as shown. After incubation, platelets were isolated, and the binding of 3H-PGE, was determined.

Effect of CommonlyUsed Cardiac Medications on the Insulin-Stimulated [3H]PGE, Binding to Normal Platelets

The possibility of interference with the stimulatory effect of insulin by commonly used cardiac medications was tested by incubating normal PRP with these drugs in the presence of insulin and measuring the binding of [3H]PGE, to these platelets (Table 1). Incubation of normal PRP with these medications for up to 3 hours did not interfere significantly with the insulin-induced stimulation of the binding of [3H]PGE,.

Binding Characteristics of [3H]PGE, to the Platelets From Normal Volunteers and AIHD Patients in the Presence of Insulin

The stimulation by insulin of the binding of [3H]PGE, to the platelets from six normal volunteers and six responder patients was next analyzed by Scatchard plot. For comparison, the PRP samples were obtained from only those patients whose platelets showed the maximal binding of [3H]PGE, at 100 microunits/ml insulin. Scatchard plot of the equilibrium binding of [3H]PGE, to platelets in the presence or absence of insulin was curvilinear in samples from normal volunteers and from AIHD patients (Figure 4). The curvilinear nature of the plots indicated heterogeneity of the binding sites. However, whether the curvilinearity of the Scatchard plots was due to a negative cooperativity within the single class of receptor molecules created by increased ligand receptor interaction is not known. Computer analy-
ses of the binding of $[^3]H$PGE$_1$ to the platelets are summarized in Table 2. These data indicate that the treatment of PRP from either normal volunteers or AIHD patients with insulin increased both the high and low affinity receptor numbers (capacity) without affecting their affinity (dissociation constant). Although the total number of receptors in both cases was increased by approximately 2.5-fold in the presence of insulin, the actual number of receptors in platelets from AIHD patients remained 50% lower than in normal platelets. However, treatment of PRP from AIHD patients increased the PGE$_1$ receptor numbers, of both the high and low affinity binding sites, to normal levels.

**Effect of Increased PGE$_1$ Binding to Platelets From Normal and AIHD Patients by Insulin on Intracellular cAMP Synthesis**

Since the occupancy of both the high and low affinity PGE$_1$ receptors on the platelet surface is directly related to the intracellular synthesis of cAMP,$^{29}$ the effect of the stimulated prostanoiid binding to the platelets by insulin (100 microunits/ml) on the cyclic nucleotide level was determined. To compare the effect of insulin on PGE$_1$-induced cAMP synthesis in normal and AIHD platelets, only those patients whose platelets showed maximal binding of $[^3]H$PGE$_1$ at 100 microunits/ml insulin concentration were included in these experiments. It was found that in platelets from normal volunteers and in platelets from responder AIHD patients the increased PGE$_1$ binding to these platelets by insulin resulted in the increased formation of cellular cAMP (Figure 5). The basal cAMP level in normal platelets, which was $1.95 \pm 0.42$ (mean $\pm$SD) pmol/10$^8$ cells, increased to $16.35 \pm 2.91$ pmol/10$^8$ platelets in the presence of 1.0 $\mu$M PGE$_1$ ($n=16$). When these normal platelets were treated with insulin and then challenged with a similar concentration of PGE$_1$, the cyclic nucleotide level was increased to $44.14 \pm 3.10$ pmol/10$^8$ cells. Insulin itself had no effect on the cellular cAMP level (2.5$\pm$0.51 pmol/10$^8$ cells). In contrast to normal platelets, the treatment of platelets from AIHD patients with 1.0 $\mu$M PGE$_1$ increased the cAMP level to only $7.70 \pm 2.0$ pmol/10$^8$ cells from a basal level of 2.0$\pm$0.30 pmol/10$^8$ cells. The treatment of these platelets with insulin increased the cAMP level to $23.87 \pm 4.1$ pmol/10$^8$ cells ($n=12$) in the presence of 1.0 $\mu$M PGE$_1$. As in the case of normal platelets, the hormone itself did not affect the cyclic nucleotide level (2.1$\pm$1.1 pmol/10$^8$ cells) of AIHD platelets (2.15$\pm$1.1 pmol/10$^8$ cells).

**Role of Insulin on the PGE$_1$-Induced Inhibition of Platelet Aggregation**

The effect of insulin on the inhibition of platelet aggregation by PGE$_1$ was studied by determining the minimum inhibitory concentration of the pros-

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**TABLE 2. Summary of Scatchard Analyses of the Binding of $[^3]H$Prostaglandin E$_1$ to Platelets From Normal Subjects and From Responder Patients With Acute Ischemic Heart Disease in the Presence and Absence of Insulin**

<table>
<thead>
<tr>
<th>Platelets</th>
<th>Addition</th>
<th>Affinity High ($K_a$) (nM)</th>
<th>Affinity Low ($K_a$) (µM)</th>
<th>Capacity High affinity receptor (n$_i$) (sites/cell)</th>
<th>Capacity Low affinity receptor (n$_i$) (sites/cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>None</td>
<td>9.1$\pm$1.2</td>
<td>1.1$\pm$0.5</td>
<td>120$\pm$30</td>
<td>1,460$\pm$250</td>
</tr>
<tr>
<td>Normal</td>
<td>Insulin</td>
<td>8.2$\pm$1.5</td>
<td>1.5$\pm$0.7</td>
<td>320$\pm$40</td>
<td>3,620$\pm$210</td>
</tr>
<tr>
<td>AIHD</td>
<td>None</td>
<td>8.5$\pm$2.0</td>
<td>0.9$\pm$0.6</td>
<td>40$\pm$20</td>
<td>810$\pm$80</td>
</tr>
<tr>
<td>AIHD</td>
<td>Insulin</td>
<td>9.8$\pm$2.5</td>
<td>1.2$\pm$0.4</td>
<td>110$\pm$30</td>
<td>1,680$\pm$110</td>
</tr>
</tbody>
</table>

Values are mean$\pm$SD of six different patients in each group. AIHD, acute ischemic heart disease.

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**FIGURE 5. Plot showing cyclic AMP formation in platelets incubated in the presence of insulin by prostaglandin E$_1$ (PGE$_1$) in normal subjects and in responder patients with acute ischemic heart disease. Platelet-rich plasma samples were incubated with insulin as described. After incubation, the platelets were isolated and treated with 1.0 $\mu$M PGE$_1$ in the presence of 10 mM theophylline. Cyclic AMP formation was then determined. A, normal platelets; B, normal platelets treated with PGE$_1$; C, normal platelets incubated with insulin (100 microunits/ml) and treated with PGE$_1$; D, platelets from patients with acute ischemic heart disease; E, platelets from patients with acute ischemic heart disease treated with PGE$_1$; F, platelets from patients with acute ischemic heart disease incubated with insulin and treated with PGE$_1$.**
tanoid before and after the incubation of the same PRP from either normal volunteers or AIHD patients with insulin (Figure 6). Treatment of normal PRP with 100 microunits/ml insulin decreased the minimum inhibitory concentration of PGE₁ from 34±14 to 15±9 nM (p<0.001). Similarly, when PRP preparations from responder AIHD patients, whose platelets showed maximal [³H]PGE₁ binding at 100 microunits/ml insulin, were incubated with the same concentration of the hormone, the minimum inhibitory concentration of PGE₁ decreased from 49±15 to 32±11 nM (p=0.002). In contrast, incubation of PRP from the nonresponder AIHD patients (n=6) with insulin, which failed to show increased [³H]PGE₁ binding to these platelets (Figure 2), also failed to decrease the amount of PGE₁ needed to inhibit platelet aggregation (52±14 versus 50±12 nM; p=NS).

Stimulation of [³H]PGE₁ Binding to Platelets From Responder and Nonresponder AIHD Patients by Insulin in Follow-up Study

In a follow-up study, the effect of treatment of PRP with insulin on the binding of [³H]PGE₁ to the platelets from nine responder and three nonresponder AIHD patients was determined after 4–8 weeks of recovery (Figure 7). It was found that, in the case of the responders, not only was the impaired binding of PGE₁ to platelets (1.16±0.21 fmol/10⁸ platelets) in the acute phase of the attack returned to normal levels (2.34±0.20 fmol/10⁸ platelets; p<0.001; n=6) during recovery but the diminished response of these platelets to insulin was also normalized (4.35±0.41 fmol/10⁸ platelets; p<0.001; n=6) in the follow-up study. In contrast, the platelets from nonresponder patients, who failed to respond to the stimulatory effect of insulin during ischemia (1.10±0.15 fmol/10⁸ platelets in control versus 1.03±0.17 fmol/10⁸ platelets in the presence of insulin; p=NS; n=3), remained nonresponsive to insulin when these patients were restudied (2.05±0.18 fmol/10⁸ platelets). However, the impaired binding of [³H]PGE₁ to their platelets during the ischemic attack improved to normal levels after 4–8 weeks of recovery (2.41±0.17 fmol/10⁸ platelets).

cAMP Formation and the Inhibition of Platelet Aggregation by PGE₁ in Responder and Nonresponder AIHD Patients in Follow-up Study

The formation of cAMP by PGE₁ and the minimum inhibitory concentration of the prostanoid needed to inhibit platelet aggregation improved to normal levels in both insulin-treated and untreated PRP from responder patients when compared with controls (Table 3) at 4–8 weeks of follow-up. In the
case of nonresponder patients, although the cAMP formation in platelets by PGE₁, and the minimum inhibitory concentration of the prostaglandin were improved in the recovery period, the stimulatory effects of insulin on the platelets could not be demonstrated.

Discussion

These results demonstrate that insulin at physiological concentrations (postprandial) regulates platelet function through increased formation of cAMP and by stimulating the binding of PGE₁ to these cells. The hormone itself has no effect on the cellular cAMP level. The hyperactivity of platelets and the reduced response to the inhibitory effect of prostaglandin in AIHD have been reported before.²⁴⁻²⁸ We²⁸ and other investigators⁴³,⁴⁴ have demonstrated that during ischemia the reduced response of platelets to the inhibitory effect of PGE₁ relates to the impaired PGE₁/1₂ receptor activity of these cells and that the receptor activity returns to normal level during the recovery period. The present data demonstrate that the impaired PGE₁ binding of platelets in AIHD could be restored to “normal” levels in 75% of the patients by treating these platelets with physiological amounts of insulin (Figure 2).

The platelets from AIHD patients were found to be considerably resistant to the inhibitory effect of PGE₁ when compared with normal platelets (Figure 6). Although the stimulatory effect of insulin in the case of platelets from AIHD patients was 50% less than that in the case of normal platelets, the hormone nevertheless decreased the minimum inhibitory concentration of PGE₁ in platelets to normal levels. To our knowledge, such normalization of the sensitivity of platelets to the inhibitory effect of prostaglandin in AIHD, albeit in vitro, by a physiological agent has not been achieved before.

Although the mechanism of the increase of PGE₁/1₂ receptor numbers in platelets by insulin is not known, the time course of the stimulatory effect of insulin (Figure 1), which followed the time course of insulin binding to these cells,³⁴,⁴⁵ suggests a dependence of the process on the insulin receptor interaction. It has been shown above that, unlike the normal platelets, the platelets from the AIHD patients showed considerable variation in the optimum dose of insulin for maximizing the binding of [³H]PGE₁ to these platelets (Figure 3). Since this variation of the optimum concentration of the hormone could not be corrected by washing these platelets nor did it appear to arise from interference caused by the presence of cardiac medications in the samples of PRP (Table 1), it is possible that the above variation was related to the insulin receptor status of the platelets.

On the basis of the response of their platelets to the stimulatory effect of insulin, AIHD patients were subgrouped as responders or nonresponders (Figure 2). The effect of insulin on the platelets from the nonresponder AIHD patients was tested by using up to 200 microunits/ml of the hormone. It is not known whether these nonresponder platelets will respond in the presence of higher amounts of insulin. However, although the impairment of PGE₁/1₂ receptor activity in these platelets was transient, the lack of response of platelets from the nonresponder AIHD patients was longer lasting. Even after 4–8 weeks of recovery, when the [³H]PGE₁ binding of platelets in the responder patients returned to near normal level, the platelets of nonresponders remained persistently unresponsive to the effect of insulin (Figure 7). Although it is possible that the nonresponder platelets might eventually respond after a longer period of recovery, similar lack of response to the stimulatory effect of insulin hormone is also known to occur in the case of platelets from diabetes mellitus (type II) patients with impaired insulin binding to these cells.⁴⁶ It remains to be determined whether the lack of response of platelets from these AIHD patients was due to disorders in the insulin receptor binding or was the consequence of derangement in post-receptor interaction phenomena. The lack of the hormonal stimulation might, however, predispose the nonresponder AIHD patients to a higher risk of developing recurrent cardiac ischemic conditions, particularly in the case of reduced availability of PGE₁ or PG₁₂ in the circulation. The hyperactivity of platelets in AIHD patients has been shown to be a significant factor in the pathogenesis of recurrent cardiac ischemia. We have found the platelets from AIHD patients (n=54) who had not taken aspirin required significantly lower amounts of ADP for aggregation (2.01±1.7 μM) when compared with control platelets (n=62) (3.62±1.80 μM; p<0.001). It is not known whether the platelet hyperactivity in AIHD is a consequence of the loss of PGE₁/1₂ receptor numbers. However, the control of platelet

Table 3. Inhibition of Platelet Aggregation and the Cyclic AMP Formation in Platelets From Responder and Nonresponder Patients With Acute Ischemic Heart Disease in the Follow-up Study

<table>
<thead>
<tr>
<th>Platelets</th>
<th>Addition</th>
<th>PGE₁ (nM)</th>
<th>Cyclic AMP (fmol/10⁸ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responder</td>
<td>None</td>
<td>33±9</td>
<td>15.2±3.5</td>
</tr>
<tr>
<td>Responder</td>
<td>Insulin</td>
<td>17±7</td>
<td>37.5±5.2</td>
</tr>
<tr>
<td>Nonresponder</td>
<td>None</td>
<td>35±5</td>
<td>14.5±3.1</td>
</tr>
<tr>
<td>Nonresponder</td>
<td>Insulin</td>
<td>31±6</td>
<td>13.2±4.5</td>
</tr>
</tbody>
</table>

Values are mean±SD of six responders and three nonresponders with acute ischemic heart disease. PGE₁, minimum inhibitory concentration of prostaglandin E₁. Cyclic AMP formation was determined by treating platelet-rich plasma with 1.0 μM prostaglandin E₁. Platelets were obtained from these patients after 6–8 weeks of recovery.
function by infusion of PGI₂ in this condition has been proved to be disappointing,²⁴⁻²⁷ partly due to the loss of sensitivity of platelets to the inhibitory effect of the prostaglandins.²²,³³,³⁴ Although the impaired PGE₁/I₂ receptors of platelets in AIHD patients would play a critical role in the development of coronary artery thrombosis, endothelial injury and dynamic vasoconstriction would also be equally important in the process.¹⁶⁻²² Restoration of the prostanoiad receptor activity of these platelets by insulin to a normal level might inhibit, at least partly, thrombus formation; this suggests a new approach to the reduction of hyperactivity in platelets in AIHD. However, the extension of the in vitro stimulatory effect of insulin on platelet PGE₁/I₂ receptors to in vivo conditions must be made with caution.

Acknowledgments

We thank Dr. Peter Buttrick for his critical review of the manuscript. We are indebted to Mr. David Wilson, BS; Mrs. Jackie Assante, BS; Ms. Kathy Hemingway, RN; and Ms. Barbara Ventura, RN, for their expert technical assistance and to Mrs. Janice Brewton for the preparation of this manuscript.

References


KEY WORDS • prostaglandin E1 • prostacyclin • prostaglandin E1/I2 receptors • platelets • acute ischemic heart disease
Restoration by insulin of impaired prostaglandin E1/I2 receptor activity of platelets in acute ischemic heart disease.

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doi: 10.1161/01.RES.68.1.245

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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