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/10⁸ platelets) compared with the nontreated, control platelet-rich plasma (1,590±265 sites/10⁸ 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/10⁸ platelets), was incubated with insulin (optimum amounts varied from 100 to 200 microunits/ml), the binding of the prostanoïd was restored to normal levels (1,790±140 sites/10⁸ 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 prostanoïd to the insulin-treated platelets also resulted in increased cyclic AMP levels both in normal subjects (44.14±3.1 pmol/10⁸ [insulin-treated] platelets versus 16.35±2.91 pmol/10⁸ [control] platelets) and in patients with acute ischemic heart disease (23.87±4.1 pmol/10⁸ [insulin-treated] platelets versus 7.70±2.0 pmol/10⁸ [control] platelets) by the prostanoïd (1.0 μM). The treatment of platelet-rich plasma with the hormone decreased the minimum inhibitory concentration of the prostanoïd from 34±14 to 15±5 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 prostanoïd 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 the 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, α-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 hemural factors, which include prostanoïds such as prostacyclin (PGI₂) and prostaglandin E₁ (PGE₁) and proteinase such as blood coagulation factor Xa.3–5 The prostanoïds 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 prostanoïds 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
and aggregation at the site of endothelial injury, which release activating substances, further enhance platelet activity, platelet aggregation, and thrombus formation. Both clinical and autopsy studies have demonstrated obstructive thrombi and platelet aggregates in acutely occluded coronary vessels. 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.

We have recently demonstrated that the diminished response of platelets to the inhibitory effect of the prostanoid in AIHD reflects decreased PGE₁/PGI₂ receptor numbers. Since prostaglandin receptor occupancy is related to the formation of cAMP, the impaired PGE₁/PGI₂ receptor numbers lead to a state of hyperactivation in these platelets due to the derangement of mechanisms counteracting the inhibition of platelet aggregation.

In a previous study, we have shown that the receptors for PGE₁/PGI₂ and insulin are functionally related in that the incubation of human erythrocyte membranes with either of these prostanoids 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 prostanoid. In an effort to restore the impaired prostanoid receptor activity of platelets in AIHD, we studied the effect of physiological concentrations of insulin on the PGE₁/PGI₂ 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 characteris-
Incubation of PRP With Insulin and the Assay of Platelet PGE₁/I₂ 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, 10⁶ 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, Ill.) 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/I₂ 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 (Kᵣ) and the capacities (receptor numbers) of the PGE₁/I₂ 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 (~2X10⁶) 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, 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/I₂ 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 [3H]PGE1 binding (not shown). The nonspecific binding of [3H]PGE1, 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 [3H]PGE1 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 [3H]PGE1 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 [3H]PGE1 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 [3H]PGE1 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 PGE1 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 [3H]PGE1 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 [3H]PGE1 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 AIHD42 and consequently downregulate the expression of PGE1/1 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 [3H]PGE1 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 [3H]PGE1 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 [3H]PGE1 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
Platelets showed the maximal binding of [3H]PGE1 at 100 micromoles/ml insulin. Scatchard plot of the equilibrium binding of [3H]PGE1 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.8 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 \[^{[3]}\text{H}\text{PGE}_1\] 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\textsubscript{1} receptor numbers, of both the high and low affinity binding sites, to normal levels.

**Effect of Increased PGE\textsubscript{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\textsubscript{1} receptors on the platelet surface is directly related to the intracellular synthesis of cAMP,\textsuperscript{29} the effect of the stimulated prostanoid binding to the platelets by insulin (100 microunits/ml) on the cyclic nucleotide level was determined. To compare the effect of insulin on PGE\textsubscript{1}-induced cAMP synthesis in normal and AIHD platelets, only those patients whose platelets showed maximal binding of \[^{[3]}\text{H}\text{PGE}_1\] 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\textsubscript{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±0.42 (mean±SD) pmol/10\textsuperscript{8} cells, increased to 16.35±2.91 pmol/10\textsuperscript{8} platelets in the presence of 1.0 \(\mu\text{M}\) PGE\textsubscript{1} (\(n=16\)). When these normal platelets were treated with insulin and then challenged with a similar concentration of PGE\textsubscript{1}, the cyclic nucleotide level was increased to 44.14±3.10 pmol/10\textsuperscript{8} cells. Insulin itself had no effect on the cellular cAMP level (2.5±0.51 pmol/10\textsuperscript{8} cells). In contrast to normal platelets, the treatment of platelets from AIHD patients with 1.0 \(\mu\text{M}\) PGE\textsubscript{1} increased the cAMP level to only 7.70±2.0 pmol/10\textsuperscript{8} cells from a basal level of 2.0±0.30 pmol/10\textsuperscript{8} cells. The treatment of these platelets with insulin increased the cAMP level to 23.87±4.1 pmol/10\textsuperscript{8} cells (\(n=12\)) in the presence of 1.0 \(\mu\text{M}\) PGE\textsubscript{1}. As in the case of normal platelets, the hormone itself did not affect the cyclic nucleotide level (2.1±1.1 pmol/10\textsuperscript{8} cells) of AIHD platelets (2.15±1.1 pmol/10\textsuperscript{8} cells).

**Role of Insulin on the PGE\textsubscript{1}-Induced Inhibition of Platelet Aggregation**

The effect of insulin on the inhibition of platelet aggregation by PGE\textsubscript{1} was studied by determining the minimum inhibitory concentration of the pros-
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/10⁶ platelets) in the acute phase of the attack returned to normal levels (2.34±0.20 fmol/10⁶ 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/10⁶ 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/10⁶ platelets in control versus 1.03±0.17 fmol/10⁶ platelets in the presence of insulin; p=NS; n=3), remained nonresponsive to insulin when these patients were restudied (2.05±0.18 fmol/10⁶ 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/10⁶ 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 prostanoiad 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 PGE$_1$ and the minimum inhibitory concentration of the prostanoid 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 PGE$_1$ 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.$^{24-28}$ We$^{28}$ and other investigators$^{43,44}$ have demonstrated that during ischemia the reduced response of platelets to the inhibitory effect of PGE$_1$ relates to the impaired PGE$_1$/I$_2$ 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 PGE$_1$ 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 PGE$_1$ 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 PGE$_1$ 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 PGE$_1$/I$_2$ 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 [H]PGE$_1$ 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 PGE$_1$/I$_2$ 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 [H]PGE$_1$ binding of platelets in the responder patients returned to near normal level, the platelets of nonresponder 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 PGE$_1$ or PGF$_2$ 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 PGE$_1$/I$_2$ receptor numbers. However, the control of platelet

<table>
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<th>Cyclic AMP (fmol/10$^6$ cells)</th>
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<td>Responder</td>
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<td>33±9</td>
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<tr>
<td>Nonresponder</td>
<td>Insulin</td>
<td>31±6</td>
<td>13.2±4.5</td>
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</table>

Values are mean±SD of six responders and three nonresponders with acute ischemic heart disease. PGE$_1$, minimum inhibitory concentration of prostaglandin E$_1$. Cyclic AMP formation was determined by treating platelet-rich plasma with 1.0 μM prostaglandin E$_1$. 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.²²,²³,²⁴ 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 prostanoid 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.

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References


**KEY WORDS** • prostaglandin E1 • prostacyclin • prostaglandin E2/I2 receptors • platelets • acute ischemic heart disease
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