Impaired Prostaglandin E₁/I₂ Receptor Activity of Human Blood Platelets in Acute Ischemic Heart Disease

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The platelets from 74 patients with acute myocardial infarction or with unstable angina showed decreased prostaglandin E₁/I₂ receptor activity when compared with that of 56 normal volunteers by using [³H]prostaglandin E₁ as a probe. In normals, Scatchard analyses showed the presence of one high-affinity–low-capacity (Kₒ=9.0±1.2 nM [mean±SD]; nₒ=120±30 sites/cell) and one low-affinity–high-capacity (Kₒ=1.1±0.5 μM; nₒ=1,460±250 sites/cell) prostaglandin E₁/I₂ receptor population in platelets. In contrast (p<0.01), platelets from patients showed decreased ligand binding (nₒ=40±20 sites/cell; nₒ=800±210 sites/cell) with little change in the affinity of the receptors (Kₒ=7.50±1.6 nM; Kₒ=0.68±0.24 μM). On the other hand, the platelets from the patients with dilated cardiomyopathy (n=7) who were hospitalized for acute chest pain but had normal coronary arteries did not show any impairment of the receptor activity. The plasma prostacyclin level of the patients with acute ischemic heart disease was similar to that of normal volunteers; this finding indicated that the defective receptor function was not related to the prostaglandin receptors occupancy in vivo. The impaired receptor activity was temporary in nature. The follow-up studies showed that the prostaglandin receptor activity of the patients’ platelets improved to “normal” levels within 2–8 weeks. The decreased prostaglandin E₁ binding to its receptors in the platelets from acute ischemic heart disease also resulted in the similar reduction of the formation of cyclic AMP by 1.0 μM prostaglandin E₁ (7.5±2.0 pmol/10⁸ cells [mean±SD]) when compared with the control platelets (16.35±0.91 pmol/10⁸ cells). These results show that the defective prostaglandin E₁/I₂ receptors of platelets are probably pathophysiologically more important than the altered synthesis of prostacyclin in acute ischemic heart disease. (Circulation Research 1990;66:932–940)

Aggregation of human blood platelets is critically important in the events ranging from normal blood coagulation to the extremes of thrombosis and atherosclerosis (see References 1 and 2 for review). The aggregation of platelets is induced by several agonists, including ADP, l-epinephrine, collagen, or thrombin, and the process is believed to be mediated, at least in part, through intracellular formation of prostaglandin G₂ and thromboxane A₂.³ Homeostasis is achieved by the countervailing effect of several humoral factors, most notably by prostacyclin (PGI₁), through the inhibition of platelet aggregation.⁴ PGI₁ and other prostanooids, which include prostaglandin E₁ (PGE₁) and prostaglandin D₂, inhibit platelet aggregation⁵–⁶ by increasing cyclic AMP levels through the activation of adenylate cyclase. The activation of the enzyme and, consequently, the inhibition of platelet aggregation are initiated through the interaction of these autacoids with their respective receptors on the cell membrane.⁷–⁹ Although PGD₂ has its own receptors on platelet membrane, PGI₁ and PGE₁ bind to the same receptors.⁷–⁹

Platelet hyperactivity plays an important role in the pathogenesis of acute ischemic heart disease. Previous studies have demonstrated increased circulating platelet release products,¹⁰–¹² platelet aggregates,¹³ and enhanced platelet aggregability¹⁴,¹⁵ in patients with acute myocardial infarction. Clinical and autopsy studies have shown obstructive thrombi and platelet aggregates in acutely occluded coronary vessels,¹⁶,¹⁷ which have been confirmed by coronary angiography.¹⁸ Originally, suggested by the work of Hirsh et al¹⁹ and Willerson et al²⁰ and subsequently extended by several investigators,²¹–²³ the concept of the “dynamic” coronary lesion has arisen. This lesion
develops by plaque rupture, hemorrhage, and platelet aggregates from a “stable” obstructive coronary lesion. Although PGI₂, through its potent antiplatelet activity, is believed to play an important role in the prevention of thrombosis, it remains controversial whether the altered synthesis of the eicosanoid itself plays any significant role in acute ischemic heart disease. On the other hand, platelets from patients with acute ischemic heart disease have been shown to be less responsive to the inhibitory effect of PGI₂, both in vitro and in vivo. Since the antiplatelet activity of prostaglandins depends, among other factors, on the occupancy of the membrane receptors by the agonist for the formation of cyclic AMP, we examined the receptor function of the prostanoid and the formation of cyclic AMP in the platelets from the patients with acute myocardial infarction (AMI) or unstable angina (UA) by using [3H]PGE₁ as a probe. For comparison, we also studied the PGE₁ incubated receptor activities of the platelets from hospitalized patients with noncardiac chest pain as well as normal volunteers.

Materials and Methods

Ethical Clearance

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

Patient Selection Criteria

Two categories of patients were studied:

1) patients with UA and 2) patients with AMI.

Inclusion criteria. Patients with UA severe enough to require admission to the Coronary Care Unit or Stepdown Unit of Montefiore Medical Center were included in the study. UA is defined as 1) severe angina at rest of more than 10-minute but less than 30-minute duration, 2) transient ST segment and/or T wave changes in at least two contiguous leads in the electrocardiogram (ECG) or documentation of coronary artery disease if ECG changes are not present (previous myocardial infarction or abnormal coronary arteriogram), and 3) no elevation of plasma creatine kinase (CK) and no appearance of CK-MB in plasma.

Patients with AMI were also included in the study. AMI is defined as 1) chest pain characteristic of myocardial ischemia for 30 minutes or more, 2) ST segment elevation of at least 0.1 mV in at least two leads of the ECG, reflecting a single myocardial region, and 3) confirmation of the diagnosis of acute myocardial infarction 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. The patient must also 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 on ECG, not allowing for the diagnosis of ischemia/infarction, 3) injection of a thrombolytic agent within 4 weeks of the study, or 4) use of ionotropic agents including epinephrine, norepinephrine, dopamine, or dobutamine.

Commonly used cardiac medications (see below) represented no exclusion.

Patient Population

Group 1. Seventy-four patients, 58 males and 16 females, aged 32–62 years (median, 57 years), were studied an average of 5.2±3.2 hours (mean±SD) after the onset of ischemic chest pain. Forty-six patients had AMI (61% with anterior/lateral infarction, 39% with inferior/posterior infarction), and 28 patients had UA. Serum CK and CK-MB were elevated in all AMI patients and in none of those with UA.

Group 2. Fifty-six normal volunteers, 39 males and 17 females, aged 28–64 years (median, 52 years), were studied. None of the volunteers were smokers, and none had taken any medication 2 weeks before the study.

Group 3. Seven patients, 5 males and 2 females, aged 22–62 years (median, 47 years), were studied. These patients were admitted to the Coronary Care Unit because of chest pain. The subsequent workup, including right and left heart catheterization, revealed dilated cardiomyopathy in all seven patients. The coronary arteries were normal or had only minor disease.

Concomitant Medical Therapy

Forty AMI patients received an intravenous infusion of lidocaine at a rate of 2 mg/hr, and two other patients received the drug at a rate of 3 mg/hr. Twenty-nine AMI and 17 UA patients received an intravenous infusion of heparin to keep the partial thromboplastin time one and one half to two times above the control level. One UA and four AMI patients had received aspirin (80–325 mg p.o.) before the study. The majority of the patients were maintained on antianginal therapy, consisting of propranolol, nitro preparations, diltiazem, nifedipine, or verapamil. One UA and eight AMI patients had received morphine sulfate (2–8 mg i.v.).

Collection of Blood and the Preparation of Washed Platelets

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 was then prepared by centrifuging the blood samples at 200g for 15 minutes at 23°C. Next, the platelets were washed two times with 2 vol Tyrode’s buffer (without Ca²⁺) at pH 7.5 containing 1.0 mM EDTA as described before. Since the presence of Mg²⁺ is essential for the binding of prostaglandins to the receptors, platelets were washed with the chelating agent to release any bound prostaglandin from the receptor sites that might have been occupied by the agonist in vivo. The
platelets were then washed with 2 vol of the same buffer (without EDTA) containing 5 mM MgCl₂. The platelet number was adjusted to 6.6×10⁹ cells/ml by use of a Coulter counter.

**Determination of Plasma PGI₂ Levels**

PGI₂ is an unstable compound that is readily converted into 6-ketoprostaglandin F₁₇₆ (6-keto-PGF₁₇₆) by diluted HCl. The plasma PGI₂ was determined in its 6-keto-PGF₁₇₆ form by a radioimmunoassay kit bought from New England Nuclear, Boston, Massachusetts. Typically, plasma samples in 1 mM EDTA were treated with 1.0N HCl (final), extracted, and quantitated by the methods described before. The recovery was determined by adding 0.2 µCi of tritiated 6-keto-PGF₁₇₆ to the extract. Approximately 80% of the added [³H]6-keto-PGF₁₇₆ was recovered. Appropriate correction was then made to determine the amount of 6-keto-PGF₁₇₆ in the plasma. The cross-reactivity of other prostanooids including thromboxane B₂, prostaglandin E₂, prostaglandin F₂α, prostaglandin D₂, and prostaglandin A₂ were <0.3%, <1.5%, <0.05%, <0.01%, and <0.3%, respectively, when compared with 6-keto-PGF₁₇₆ (100%). The assay method could detect as little as 1 pg of 6-keto-PGF₁₇₆ in the assay mixture (0.1 ml). Since PGI₁ synthesis is inhibited by aspirin, patients who had taken the medication were excluded from this analysis.

**Platelet PGI₁ Receptor Assay**

Since PGI₂ and PGE₁ bind to the same receptors on platelet membrane and radiolabelled PGI₂ as free-acid form is not yet commercially available, [³H]PGE₁ (5,6α-[³H]PGE₁, specific activity 50-3 Ci:mmol, New England Nuclear, Boston, Massachusetts) was used as a probe to assess the PGI₁ receptor activity in these cells. Typically, approximately 10⁶ platelets in Tyrode’s buffer, pH 7.5, containing 5 mM MgCl₂ were incubated with 3 nM [³H]PGE₁ (60,000 disintegrations per minute) 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 microfiltration filters (GF/C, Whatman, Clifton, New Jersey) 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 bound to the membrane filter, and the free ligand that did not bind to the filter was removed by washing. The filters were subsequently dried, and the radioactivity was determined by suspending them in ACS II solvent (Amersham, Arlington Heights, Illinois) in a liquid scintillation counter (Isocap/300, Searle Pharmaceuticals, Chicago, Illinois) with 60% efficiency for tritium. The nonspecific binding was determined by adding excess (15 µM) unlabeled prostanooid to the assay mixture. The specific binding was calculated by subtracting the nonspecific binding from the total binding.

In some phases of the work, it was necessary to establish that the preoccupation of the PGE₁ binding sites in platelets by circulating prostaglandins in vivo would not interfere with the PGE₁/1₂ receptor assay. This was done by incubating normal platelet-rich plasma with 1.0 µM unlabeled PGE₁ for 30 minutes at 23°C. After incubation, the platelets were washed with 5 mM MgCl₂. The [³H]PGE₁ binding activity of the washed platelets was then determined and compared with those platelets that had not been exposed to the prostaglandin as described above.

**Incubation of Normal Platelets With Cardiovascular Medications or With Plasma From UA or AMI Patients**

To determine the effect of commonly used cardiovascular drugs on the PGE₁/1₂ receptor activity of platelets, platelet-rich plasma from normal volunteers was incubated with various medications for 3 hours at 37°C. After incubation, gel-filtered platelets (GFPs) were prepared, and the binding of [³H]PGE₁ to GFPs was determined by the method described above.

In a separate experiment, platelet-free plasma from the patients’ blood samples was prepared by centrifuging platelet-rich plasma from UA or AMI patients at 10,000g for 20 minutes at 8°C. GFPs prepared from normal platelet-rich plasma were then suspended in the patients’ plasma (=10⁸ cells/ml) and incubated at 23°C for 3 hours. The platelets were subsequently isolated by gel filtration, and the binding of [³H]PGE₁ to these cells was determined. Control experiments were carried out by incubating the GFPs with normal platelet-free plasma under identical conditions.

**Scatchard Analysis of [³H]PGE₁ Binding to Platelets**

The dissociation constants (Kᵦₛ), and the capacities (nₛ, receptor numbers) of the PGE₁/1₂ receptors of platelets from patients with acute ischemic heart disease were determined by Scatchard analyses. The GFPs were incubated with 3 nM [³H]PGE₁ plus 0–30 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 Kᵦₛ and the capacities were obtained from a nonlinear regression analysis of equilibrium binding by a nonweighted, iterative, least-squares algorithmic analysis by a microcomputer (model 4, Radio Shack TRS 80). In parallel experiments, normal platelets obtained from sex- and age-matched volunteers were used to determine the Kᵦₛ and the receptor numbers.

**Cyclic AMP Assay**

The basal cyclic AMP level and the formation of cyclic AMP in platelets by PGE₁ were determined by the protein kinase binding method. Typically,
TABLE 1. Effect of Medications on Platelet Prostaglandin E\textsubscript{1}/I\textsubscript{2} Receptor Activity In Vitro

<table>
<thead>
<tr>
<th>Addition</th>
<th>(n_1) (Sites/cell)</th>
<th>(n_2) (Sites/cell)</th>
<th>(K_{d1}) (nM)</th>
<th>(K_{d2}) ((\mu)M)</th>
<th>Number of studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>120±30</td>
<td>1,460±250</td>
<td>9.0±1.2</td>
<td>1.1±0.5</td>
<td>20</td>
</tr>
<tr>
<td>Lidocaine (25 (\mu)M)</td>
<td>145±20</td>
<td>1,480±150</td>
<td>9.2±1.2</td>
<td>1.2±0.6</td>
<td>3</td>
</tr>
<tr>
<td>Aspirin (1.0 (\mu)M)</td>
<td>150±40</td>
<td>1,500±200</td>
<td>8.8±1.6</td>
<td>1.2±0.8</td>
<td>3</td>
</tr>
<tr>
<td>Morphine sulfate (3.0 (\mu)M)</td>
<td>135±50</td>
<td>1,440±150</td>
<td>9.5±2.0</td>
<td>1.0±0.6</td>
<td>3</td>
</tr>
<tr>
<td>Nitrogllycerin (25 (\mu)M)</td>
<td>140±30</td>
<td>1,390±260</td>
<td>9.2±2.0</td>
<td>1.2±0.5</td>
<td>3</td>
</tr>
<tr>
<td>Heparin (0.1 units/ml)</td>
<td>110±60</td>
<td>1,350±200</td>
<td>9.1±1.6</td>
<td>0.9±0.5</td>
<td>3</td>
</tr>
<tr>
<td>Propranolol (4.0 (\mu)M)</td>
<td>120±60</td>
<td>1,380±250</td>
<td>9.2±1.0</td>
<td>0.9±0.4</td>
<td>3</td>
</tr>
<tr>
<td>Diltiazem (1.5 (\mu)M)</td>
<td>135±70</td>
<td>1,450±210</td>
<td>8.5±2.1</td>
<td>1.1±0.6</td>
<td>3</td>
</tr>
<tr>
<td>Verapamil (4.0 (\mu)M)</td>
<td>145±60</td>
<td>1,480±240</td>
<td>8.0±2.0</td>
<td>1.2±0.5</td>
<td>3</td>
</tr>
<tr>
<td>Nifedipine (6.0 (\mu)M)</td>
<td>125±50</td>
<td>1,380±220</td>
<td>9.1±2.5</td>
<td>0.9±0.5</td>
<td>3</td>
</tr>
</tbody>
</table>

Values are mean±SD. Platelet-rich plasma from normal volunteers was incubated with different compounds, as indicated above, for 3 hours at 37°C. After incubation, platelets were separated by gel filtration. The capacities (\(n_1\) and \(n_2\)) and affinities (\(K_{d1}\) and \(K_{d2}\)) for the binding of [\(^3\)H]prostaglandin E\textsubscript{1} to these cells were determined by Scatchard analyses.\textsuperscript{27}

**Results**

**Effects of Commonly Used Cardiovascular Medications and Plasma Factors of UA or AMI Patients on the PGE\textsubscript{1}/I\textsubscript{2} Receptor Activity of Normal Platelets**

Since the patient population in our study would be expected to be under various cardiovascular medications, the effects of these compounds on the platelet PGE\textsubscript{1}/I\textsubscript{2} receptors was determined by using platelets from normal volunteers. It was found that the incubation of normal platelet-rich plasma with different cardiovascular medications, in amounts similar to the reported concentrations needed for their pharmacological effects, did not influence the prostanoid receptor activity of GFPs (Table 1).

In another experiment, when the GFPs from normal volunteers were suspended in fresh plasma from different patients with UA or AMI and the [\(^3\)H]PGE\textsubscript{1} binding activities of these cells were assayed, it was found that neither high-affinity nor low-affinity PGE\textsubscript{1}/I\textsubscript{2} receptor activity of normal platelets was affected (Figure 1).

These results showed that neither the commonly used cardiovascular drugs nor the plasma factor(s) of the UA or AMI patients, when removed by gel filtration, interfered with the binding of [\(^3\)H]PGE\textsubscript{1} to the platelets of normal volunteers.

**Plasma PGI\textsubscript{2} Levels in AMI or UA Patients and in Normal Volunteers**

The plasma PGI\textsubscript{2} level, as determined in its 6-keto-PGF\textsubscript{1\alpha} form, in AMI patients (\(n=10\)) varied between 7.56 and 12.43 \(\text{pM}\). In UA patients (\(n=6\)), the plasma level of the prostanoid was also in the same range (between 7.50 and 12.97 \(\text{pM}\)). There

![Figure 1](http://circres.ahajournals.org/)

**FIGURE 1.** Plot showing effect of plasma from the patients with unstable angina or acute myocardial infarction on the binding of [\(^3\)H]prostaglandin E\textsubscript{1} to normal gel-filtered platelets. Each open triangle (\(\triangle\)) and circle (\(\bigcirc\)) represents high- and low-affinity binding capacity of prostaglandin E\textsubscript{1} receptor, respectively, of platelets from a single individual (A). Each solid triangle (\(\blacktriangle\)) and circle (\(\bullet\)) shows high- and low-affinity binding capacity, respectively, of the same platelets after they were exposed to the plasma of a particular patient (B). Each point is mean of three experiments.
was no statistical difference between the prostaglandin levels in AMI or UA patients. The plasma PGI₂ level in normal volunteers (n=16) varied in the range between 8.10 and 16.25 pM (Table 2). These values were not significantly different from those obtained from the patients with acute ischemic heart disease (p>0.1).

**Binding of [³H]PGE₁ to Platelets From Normal, UA or AMI, or Cardiomyopathic Patients**

To circumvent the possibility of the occupancy of the binding sites of PGE₁/I₂ in platelets by the prostaglandins in the circulation, normal platelets were incubated with 1.0 µM unlabeled PGE₁. After incubation, the platelets were washed by the EDTA/Tyrode’s buffer, and the binding of [³H]PGE₁ to these cells was compared with those platelets that had not been treated with the prostaglandin. It was found that the PGE₁-treated platelets bound 2.33±0.21% (mean±SD) of the total radioactivity; the untreated cells bound 2.30±0.20% (mean±SD) of the total radioligand. These results indicated that the washing of the platelets by EDTA/Tyrode’s buffer effectively made available the prostaglandin binding sites by removing the bound ligand.

The specific binding of [³H]PGE₁ to platelets was 0.8% in patients with UA or AMI. This value was significantly (p<0.01) lower compared with 2.3% binding to platelets of normal volunteers (Figure 2). Scatchard analyses of the prostanoid binding showed that the decreased binding of the ligand was due to the decreased capacities of the receptors rather than to their decreased affinities (Figure 3). The same analyses of [³H]PGE₁ binding of normal platelets showed the presence of one high-affinity–low-capacity (Kₐ₁=9.0±1.2 nM [mean±SD]; n₁=120±30 sites/cell) and one

**TABLE 2. Plasma 6-Ketoprostaglandin F₁α Levels in Patients With Acute Myocardial Infarction and Unstable Angina and in Normal Volunteers**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Mean±SD</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMI (n=10)</td>
<td>9.59±1.43</td>
<td>9.18</td>
<td>7.56–12.43</td>
</tr>
<tr>
<td>UA (n=6)</td>
<td>9.72±2.08</td>
<td>9.45</td>
<td>7.50–12.97</td>
</tr>
<tr>
<td>Normal (n=16)</td>
<td>11.10±3.2*</td>
<td>10.13*</td>
<td>8.10–16.25*</td>
</tr>
</tbody>
</table>

6-Ketoprostaglandin F₁α was measured by radioimmunoassay. AMI, acute myocardial infarction; UA, unstable angina.

* p>0.1 compared with AMI or UA platelets by paired t test.

**FIGURE 2. Graph showing specific binding of prostaglandin E₁ to platelets of unstable angina (UA) or acute myocardial infarction (AMI) patients and control subjects. Results shown here are mean±SEM percentage of [³H]prostaglandin E₁ bound to 10⁶ platelets from 58 patients and 40 normal volunteers.**
Interestingly, this decrease in the specific binding of $[^3H]PGE_1$ was not observed in the platelets from dilated cardiomyopathy patients with normal coronary arteries ($n_1=110\pm40$ sites/cell, $n_2=1,460\pm150$ sites/cell; $K_d=8.5\pm1.5$ nM, $K_d=0.91\pm0.3$ μM). These patients had been admitted to the Coronary Care Unit because of chest pains similar to those of the patients admitted because of UA or AMI.

**Reversibility of Impaired PGE$_1$/I$_2$ Receptor Activity of Platelets From UA and AMI Patients**

To determine whether the impaired prostaglandin receptor function of the platelets from the two patient populations with acute ischemic heart disease was restored to "normal" levels over time, the $[^3H]PGE_1$ binding activity of these platelets was assayed during the acute phase of the disease and during the recovery periods (2 weeks to 2 months) in four AMI and five UA patients. The prostaglandin binding, which was impaired during the acute ischemic episode (Figure 4, A and C) was found to return to the normal level at the follow-up study (Figure 4, B and D). Both high- and low-affinity receptors of $[^3H]PGE_1$ binding sites of the platelets were improved.

**Comparison of PGE$_1$/I$_2$ Receptor Activity of Platelets From UA and AMI Patients**

Although AMI is frequently considered a progression from UA with similar coronary pathophysiology (both entities represent acute myocardial ischemia),
we compared the prostaglandin receptor activities of platelets of the two groups to ensure comparable responses. Platelets from 26 UA and 32 AMI patients showed similar impairment of $[^{3}H]PGE_{1}$ binding. There was no difference in the autacoid receptor activity including affinities ($K_{d}$) or binding sites (n), $K_{d_{1}}$ (7.5±1.0 nM), $K_{d_{2}}$ (0.7±0.2 µM), n$_{1}$ (50±20 sites/cell), and n$_{2}$ (820±210 sites/cell) in the cases of UA were very similar to $K_{d_{1}}$ (7.0±1.5 nM), $K_{d_{2}}$ (0.75±0.4 µM), n$_{1}$ (40±20 sites/cell), and n$_{2}$ (800±220 sites/cell) in the cases of AMI.

**Formation of Cyclic AMP in Platelets From AMI and UA Patients by PGE$_{1}$**

Since the occupancy of PGE$_{1}$ receptors in platelets by the agonist is directly related to the formation of cyclic AMP through the activation of adenylate cyclase, the increase of PGE$_{1}$ challenge was also determined. In normal platelets, the basal cyclic AMP level, which was 1.95±0.42 pmol/10$^8$ cells (mean±SD), increased to 16.35±0.91 pmol/10$^8$ cells (n=16) when these cells were treated with 1.0 µM PGE$_{1}$ (Figure 5). In contrast, the basal cyclic AMP level in platelets from UA and AMI patients, which was 2.0±0.3 pmol/10$^8$ cells, increased to only 7.70±2.0 pmol/10$^8$ cells in response to the same concentration of PGE$_{1}$. The basal level of cyclic AMP (2.08±0.29 pmol/10$^8$ cells) and the stimulation of cyclic AMP formation (7.5±2.1 pmol/10$^8$ cells) by PGE$_{1}$ in platelets from UA patients (n=6) were similar to those in the case of AMI (n=10; basal level, 2.21±0.39 pmol/10$^8$ cells; cyclic AMP formed in the presence of PGE$_{1}$, 7.89±2.41 pmol/10$^8$ cells).

**Discussion**

The interaction between the prostaglandins and their receptors has been shown to be a prerequisite for the activation of adenylate cyclase for the inhibition of platelet aggregation. The impairment of PGE$_{1}$/I$_{2}$ receptor function of platelets from AMI or UA patients would explain, at least partly, the mechanism of resistance of these cells to the inhibitory effect of PGI$_{2}$ reported by several investigators. This impairment of PGE$_{1}$/I$_{2}$ receptor activity of platelets from patients could not be due to the blockage of PGE$_{1}$/I$_{2}$ receptor sites by the presence of excess amounts of PGI$_{2}$ in the circulation since the plasma 6-keto-PGF$_{1a}$ concentrations in the cases of both normal volunteers and the patients were similar (Table 2). Further, the washing procedure described in the text effectively removed all the bound prostanoids from the binding sites. Similarly, the presence of inhibitor(s) or autoantibody that might interfere with the binding of $[^{3}H]PGE_{1}$ to AMI or UA platelets was also ruled out (Figure 1).

The impairment of $[^{3}H]PGE_{1}$ binding appeared to be rather selective. The platelets from patients with dilated cardiomyopathy, who were hospitalized with acute chest pain and later ruled out for coronary heart disease, did not show any derangement of PGE$_{1}$ binding activity. The defective prostanoid bind-
We have also previously demonstrated that the increased binding of PGE_{1} to its receptors does not induce a negative cooperativity within the receptor molecules to "down-regulate" its own interaction with the binding sites but that the binding of the prostanooid to its receptors in platelets is regulated by cyclic AMP. It should be mentioned that the solubilization of PGE_{1}/I_{2} receptors by Triton X-100 from the patients' platelets, which releases over 90% of the prostaglandin receptor from platelet membrane, also showed that these cells contained less amounts of the macromolecule (2.8±0.42 μg/10^9 cells; n=3) when compared with the control platelets (5.0±0.52 μg/10^9 cells) (authors' unpublished observation). All these results indicate that the observed decrease of the PGE_{1}/I_{2} receptor number in platelets of patients with acute ischemic hearts was not related to the occupancy of the receptors by the prostanooid in vivo or to the "down-regulation" of the receptors but probably represented a real decrease in the numbers of the receptor sites.

We have reported earlier that the PGE_{1}-induced activation of adenylyl cyclase, which increases cellular cyclic AMP level in platelets, is directly related to the occupancy of PGE_{1} receptors by the agonist. The binding of PGE_{1} to its receptors in platelets from patients with acute ischemic heart disease was approximately 50% less than that of normal controls (Figure 3). The decreased PGE_{1} binding resulted in a similar reduction of cyclic AMP formation in these cells by PGE_{1} when compared with normal platelets (Figure 5). These results indicated that the decreased formation of the cyclic nucleotide in platelets from AMI and UA patients was a direct consequence of decreased PGE_{1} binding to its receptors in the adenylyl cyclase-receptor complex and was not due to impaired cyclase-receptor coupling. On an individual basis, however, impairment of cyclase-receptor coupling leading to decreased formation of cyclic AMP cannot be ruled out by these data.

PGI_{2}, through its antiplatelet activity and vasodilating effect, has been shown to be an important factor in the prevention of acute ischemia and thrombosis. We have demonstrated herein that this important mechanism is defective in AMI and UA patients due to impaired PGE_{1}/I_{2} receptor function of platelets. It is interesting to note that the reduction of PGE_{1}/I_{2} receptor numbers in the platelets from UA or AMI patients was not seen in the cases of dilated cardiomyopathic patients. Other investigators also have reported that platelets from stable angina patients have shown normal prostaglandin binding. Although our results suggest that the reduction of PGE_{1}/I_{2} receptor numbers on platelets could be pathophysiologically important in acute ischemic heart disease, it remains to be addressed whether the ischemic event was indeed a consequence of a reduction of the prostaglandin receptor numbers in platelets. However, if the impairment of PGE_{1} binding in platelets does precede clinical onset of acute ischemic heart disease, then reduced PGE_{1} receptor numbers of these cells could be a risk factor associated with impending UA or AMI.

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