Reversal of Abnormal Platelet Aggregability and Change in Exercise Tolerance in Patients with Angina Pectoris Following Oral Propranolol

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SUMMARY

Nineteen patients with severe but stable angina pectoris who had positive ECG response to exercise on a bicycle ergometer and 11 normal subjects were studied. Patients received placebo for six weeks and were then randomized into placebo (n = 9) and propranolol (n = 10) treatment groups. Threshold for platelet aggregation in response to adenosine diphosphate (ADP) was measured in fresh platelet rich plasma.

Mean concentration of ADP necessary for a biphasic threshold aggregation response was 1.56 \( \mu \text{M} \) (geometric mean) in patients and 3.85 \( \mu \text{M} \) in normals (\( P < 0.01 \)). Serial studies with placebo showed no significant change in ADP threshold. With propranolol, 80 mg/day, platelet aggregation in response to ADP was entirely normalized; 3.79 \( \mu \text{M} \) ADP produced maximal aggregation compared to 1.32 \( \mu \text{M} \) before therapy (\( P < 0.01 \)). No additional changes were noted with propranolol, 160 mg/day. In the propranolol-treated patients (80 mg/day) who demonstrated reduction in ADP-induced platelet aggregation, total work during exercise increased by 128\%, from 765 \( \pm \) 125 (standard error of mean) kpm during the control period to 1,792 \( \pm \) 285 kpm (\( P < 0.01 \)).

Thus, patients with angina and abnormal exercise tolerance demonstrate increased platelet aggregability in vitro which is restored toward normal with propranolol in dosage sufficient to improve exercise tolerance.

Additional Indexing Words:

Beta-adrenergic blockade  
Coronary artery disease  
Arteriosclerosis

SEVERAL OBSERVERS have suggested that platelet dysfunction may be a factor in the pathogenesis of coronary artery disease.\(^1\) Platelets initiate thrombosis by aggregating at the site of previous vascular injury.\(^3\) It is speculated that altered or accelerated platelet aggregation may play a significant role in the development and progression of arteriosclerotic lesions.

There are conflicting reports concerning the status of abnormal platelet function in patients with coronary artery disease.\(^7\)-\(^12\) Reliable and reproducible studies of human platelet aggregation are difficult because of the variety of factors which alter platelet responsiveness such as age,\(^13\) sex, diet,\(^14\) blood lipid and glucose levels,\(^15\),\(^16\) and smoking history.\(^17\) Many commonly used drugs significantly influence platelet aggregability in vitro.\(^18\)-\(^21\) Venupuncture techniques, rapidity of sample processing, and ambient changes in pH can all contribute to altering platelet responsiveness with any of the standard laboratory methods measuring aggregation threshold.\(^22\)

Platelet aggregates have been observed in the coronary microvasculature in a model following catecholamine infusion and after acute, severe stress.\(^23\),\(^24\) Infusions of adenosine diphosphate (ADP) induce coronary arterial platelet thrombi associated with myocardial necrosis in swine similar to that noted following catecholamine administration.\(^25\) It has been postulated that therapy directed at altering the platelet response to endogenous release of catecholamine or local release of ADP might alter the likelihood of acute thrombotic events in patients with coronary artery disease.

The present study was designed to test the hypothesis that patients with angina pectoris have altered platelet aggregability when compared to a normal group similar with regard to age and sex. In
addition, the effects of treatment with therapeutic doses of oral propranolol, a beta-adrenergic blocking agent with known anti-anginal properties but inadequately defined effects on platelet function, were also assessed.

Methods

Nineteen patients with presumed ischemic heart disease were studied. Criteria for inclusion were: 1) At least three attacks of angina pectoris per week, responsive to nitroglycerin, with no evidence of an accelerated course; 2) absence of valvular heart disease, hypertension, congestive heart failure, chronic obstructive pulmonary disease, diabetes, lipoprotein abnormalities, anemia, smoking history, or myocardial infarction within six months; 3) definite electrocardiographic (ECG) evidence of myocardial ischemia associated with chest pain during submaximal exercise stress testing. Pertinent clinical data of these 19 patients are presented in tables 1 and 2. There were eleven male and eight female patients with an average age of 54. Four patients had a history of previous myocardial infarction. Three patients without previous myocardial infarction had undergone coronary angiography. In each case, narrowing of at least 70% of one or more of the major coronary arteries was seen.

Eleven normal subjects with a negative stress test were studied along with the patient group. These subjects had no evidence of ischemic vascular disease, lipid abnormalities or diabetes, and each had a negative history for cigarette smoking and drug ingestion of any sort. Pertinent clinical data of the patients with angina are compared with those of the normal subjects in table 2.

Protocol

All normal subjects had platelet aggregation studies and exercise tests as described below.

The patients with angina pectoris were followed bimonthly by the same physician (W.F.). Informed consent was obtained, and all cardiovascular medications were discontinued except for nitroglycerin. An oral placebo* was administered for six weeks. Every patient received a resting electrocardiogram, chest roentgenogram, urinalysis, and blood analysis for cholesterol, triglycerides, glucose, blood urea nitrogen, creatine phosphokinase (CPK), lactic dehydrogenase (LDH), serum glutamic oxaloacetic transaminase (SGOT), hematoctit, hemoglobin, white blood cell count, and platelet count. Platelet aggregation studies were performed and exercise testing on a bicycle ergometer carried out as described below.

After the six week placebo period, patients were randomized into placebo and propranolol treatment groups. The placebo group (n = 9) remained on the same placebo regimen while the drug treatment group (n = 10) was started on 80 mg of oral propranolol daily in four divided doses. Neither patient nor physician knew whether therapy was placebo or drug. All patients continued taking nitroglycerin as needed. After two weeks, platelet aggregation studies were repeated. The propranolol dosage was then raised to 160 mg/day, again “double blind” as the pill was identical in appearance to the placebo. The drug was given in four divided doses and two weeks thereafter studies were repeated in both groups of patients.

Platelet Aggregation Studies

Blood was obtained after a 12-hour fast. After an initial resting period of 30 min, a 19 gauge needle was inserted, without use of a tourniquet, into an antecubital vein and a slow infusion of physiological saline begun. After 15 min of rest, with the patient relaxed, blood was sampled by free flow into plastic tubes containing 1/10 volume acid citrate dextrose (ACD). The blood specimens were then immediately processed for analysis. The specimens were coded so that those performing the aggregation studies had no knowledge of the patient’s identity, the diagnosis, or type of drug therapy.

Platelet aggregation studies were performed using the turbidometric method of Born,26 as modified by Mustard et al.27 The blood sample collected a few minutes earlier was placed in sterile polypropylene tubes and platelet rich plasma separated (PRP) by immediate centrifugation for 15 min at room temperature at 225 g. After removal of the upper 2/3 of the PRP, the remaining PRP was recentrifuged for 1,300 g for 10 min at 4°C to yield platelet poor plasma (PPP). Platelet counts were performed on a Coulter Model F counter, the count of the PRP was adjusted to the range 300–400,000/mm³ when necessary with the autologous PPP. Platelet aggregation studies were performed in duplicate within 90 min of venipuncture. The sample was kept tightly covered at room temperature until analyzed. Samples of 0.45 ml PRP were prewarmed for 1 min with stirring in siliconized cuvettes placed in a Payton dual channel aggregation module (Payton Associates, Buffalo, N.Y.) before aggregating agents were added. The light transmittance was recorded on a Riken Densi linear recorder. The blank for each study was a similarly treated sample of PPP. The aggregating agent tested was adenosine diphosphate (ADP) (Sigma, Inc., St. Louis, Mo.), maintained as a 10⁻⁴M frozen stock and diluted essentially twofold just before use over a range of 10⁻⁷M to 10⁻⁴M (0.1 to 100 μM). The ADP concentrations producing aggregation were 0.5, 1, 2, 5, 10, and 20 μM. The lowest concentration producing a full biphasic response was recorded as the threshold dose. The optimal ADP concentration was assessed without extrapolation between different concentrations. The change in light transmittance to increasing concentrations of ADP and the characteristic biphasic response at the threshold dose are illustrated in figure 1. In repeated determinations using coded samples, reproducibility was within 10% of mean.

Exercise Testing

Bicycle ergometry testing was performed without knowledge of the patients’ drug status during the study. A modification of the ear-ensiform system of Abarquez et al.28 was used with three apical leads recorded in the supine position and on the bicycle ergometer prior to exercise. The placement of electrodes was as follows: right arm in the left V₅ position, left arm on the forehead, left leg over the ensiform (xiphoid) process and right leg over the right V₆ position. Simultaneous recordings were taken of aV₅, aV₁, and aV₉, yielding modified orthogonal X, Y, and Z leads. The precordial leads were placed in the V₅ and V₆ position and the third placed either in the V₃ or V₄ positions so as to record the transitional QRS over the precordium. The modified X, Y, and Z leads were monitored continuously on

*Carrnitol, 225 mg. (Ayerst Laboratories).
Table 1

Clinical Data in Nineteen Patients with Angina Pectoris

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<tr>
<th></th>
<th>Age</th>
<th>Sex</th>
<th>Angina pectoris</th>
<th>Attacks per week</th>
<th>History M.I.*</th>
<th>Positive exercise tolerance test</th>
<th>Hematocrit (%)</th>
<th>Blood urea nitrogen (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Fasting blood sugar (mg/dl)</th>
<th>Glutamic oxaloacetic transaminase (units)</th>
<th>Lactic dehydrogenase (units)</th>
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<td>16-20</td>
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<td>3-10</td>
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<td>240</td>
<td>120</td>
<td>82</td>
<td>20</td>
<td>190</td>
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</table>

Normal values: Hematocrit, 38-53%; blood urea nitrogen, <22 mg/dl; cholesterol, <300 mg/dl; triglycerides, 0-160 mg/dl; fasting blood sugar, 62-108 mg/dl; glutamic oxaloacetic transaminase, 4-40 units; lactic dehydrogenase, 90-225 units.

*Myocardial infarction.*
Table 2

Comparison of Untreated Patients with Angina Pectoris and Normal Subjects

<table>
<thead>
<tr>
<th>No.</th>
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<th>Age</th>
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<th>Angina patients</th>
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<td>M</td>
<td>32</td>
<td>None</td>
<td>365 ± 7</td>
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<td>3</td>
<td>M</td>
<td>8</td>
<td>None</td>
<td>146 ± 7</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>8</td>
<td>None</td>
<td>229 ± 8</td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>11</td>
<td>All</td>
<td>238 ± 8</td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>54</td>
<td>All</td>
<td>225 ± 6</td>
</tr>
</tbody>
</table>

Plaquelet counts: See Table 1. Normal values: See Table 1. Normal count, 150,000/450,000/um m.

Figure 1

Aggregation response of platelets from a normal subject induced by different concentrations of adenosine diphosphate (ADP). Degree of aggregation is related to the increase in light transmission since clumping reduces light absorption. Arrows indicate the addition of different concentrations of ADP to stirred platelet-rich plasma. 5 μM ADP induces the characteristic biphasic aggregation threshold response of an initial plateau followed by maximal aggregation. 1 μM and 2 μM ADP are inadequate to achieve threshold or maximal aggregation. 10 μM ADP induces maximal aggregation but at this higher concentration the biphasic threshold cannot be detected.

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a three-channel oscilloscope throughout the study. Tracings were recorded of both lead groups every minute during the procedure.

Multistage graded exercise was performed utilizing a bicycle ergometer (Schwinn ergometric exerciser) as the patient pedaled against a predetermined load. After blood for platelet studies had been obtained, patients were started at a work load of 150 kpm/min. The work load was increased by 150 (kilo-pond meters) kpm/min every three minutes until either chest pain, fatigue, or a heart rate of 150 beats/min occurred. An abnormal electrocardiographic response was defined as a flat or downsloping ST-segment depression, 1 mm, measured 0.08 seconds after the termination of the QRS complex with the P-Ta segment as the baseline of reference.

Blood pressure in the brachial artery was recorded by sphygmomanometer prior to and immediately after exercise. The heart rate-blood pressure product, an approximation of myocardial oxygen consumption, was calculated from measurements obtained immediately after cessation of exercise.

Propranolol Blood Levels

The concentration of propranolol in blood was measured from coded samples without knowledge of previous therapy. The fluorometric method of Black and associates, as modified by Shand et al., was used. Four milliliters of plasma, obtained approximately two hours after the most recent dose of propranolol or placebo, were alkalinized with 1 ml of 1 N NaOH and extracted into 12 ml of heptane containing 1.5% isomyl alcohol. After centrifugation, 10 ml of the organic phase was extracted into 1.5 ml of 0.1 N HCl and the fluorescence of the acid phase measured in an
PROPRANOLOL AND PLATELET ACTIVITY

Aminco-Bowman spectrophotometer (Maximum excitation, 295 μ; maximum emission, 360 μ; uncorrected). For each patient duplicate plasma blanks and a standard of 100 ng per ml of known propranolol added to the plasma blank were determined.

Statistical Analysis

For all of the variables except ADP, arithmetic group means with the standard error of the mean are presented. For ADP data, geometric means are presented. Geometric means are preferred since the concentrations of ADP used were essentially geometric dilutions. Tests of significance for results obtained in normals and untreated patients, as well as between the two groups of untreated patients, were performed utilizing the Wilcoxon Rank Sum Test. Comparison of data from the same group with various experimental protocols were performed using the Wilcoxon Signed Rank Test or by the Sign Test.

Results

Platelet Aggregation Studies

Patients with angina pectoris demonstrated increased platelet sensitivity to aggregating concentrations of ADP when compared to the control subjects exposed to the same concentration of ADP. Mean concentration of ADP necessary for a biphasic aggregation response was 1.56* μM in the group with angina pectoris, a value significantly less than the 3.85* μM observed in the normal controls (P < 0.01) (table 3, figure 2).

Serial studies performed on the patients with angina pectoris receiving placebo showed no change in the increased platelet sensitivity to ADP (table 3).

Administration of propranolol in a dose of 80 mg/day had a dramatic effect in reducing platelet sensitivity to ADP in patients with angina pectoris. After propranolol a mean of 3.79* μM ADP was required to produce a biphasic aggregation response compared to 1.32* μM before therapy (P < 0.01). No additional changes in ADP concentration to effect aggregation threshold were noted after the propranolol dosage was increased to 160 mg/day, (fig. 3, table 3). The mean concentration of ADP required to produce the threshold aggregation response after 80 mg/day of propranolol was not significantly different from the value observed in the normal controls, but was significantly different from placebo-treated patients (P < 0.01) (table 3). An example of the effect of propranolol in increasing the dose of ADP required for platelet aggregation in a patient with angina pectoris is shown in figure 4.

Mean serum concentration of propranolol was 21 ± 4.0 ng/ml (range: 11–49 ng/ml) at a dose of 80 mg/day and 49 ± 9 ng/ml (range: 25–110 ng/ml) at a dose of 160 mg/day.

There were no significant changes from control in the mean level of blood glucose, urea nitrogen, SGOT, CPK, LDH, cholesterol, triglycerides, hematocrit, or platelet count (table 4) during the serial sampling periods in either the control, placebo or drug treatment group.

Exercise Tests

During the control period, all 19 patients with angina had a positive electrocardiographic response to

Table 3

<table>
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<th>Normal subjects</th>
<th>Age</th>
<th>ADP μM*</th>
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<td>11. W.P.</td>
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Table 3

Individual and Group Mean Concentrations of ADP Inducing Platelet Aggregation in Patients with Angina Pectoris (Treated and Untreated) and Normal Subjects

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<td>Propranolol 160 mg/day</td>
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<td>1.32†</td>
<td>3.79†</td>
<td>3.46†</td>
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*Adenosine diphosphate in concentration needed to reach aggregation threshold.
†Geometric mean.
exercise. Administration of propranolol was followed by a significant increase in exercise tolerance in the patients with angina pectoris. In the ten patients who received propranolol (80 mg/day), total work during exercise increased by 128%, from 765 ± 125 kpm during the control period to 1,790 ± 285 after propranolol (P < 0.01) (fig. 5). This beneficial effect on work performance was associated with a significant drop in the heart rate-blood pressure product from 16,800 ± 1,500 before to 12,000 ± 890 after propranolol (P < 0.01) (fig. 6). In each case typical anginal pain was the end-point. In contrast, there was no significant change in exertional tolerance in patients with angina pectoris who received a placebo. When the dose of propranolol was increased to 160 mg/day, work performed during maximal tolerated exercise decreased slightly with either pain or fatigue the end-point, but the mean for the group was not significantly different from that observed with 80 mg/day.

Table 4

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<th>No.</th>
<th>Age</th>
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<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Platelet count (x 10^9)</th>
<th>Exercise improved (x 10^9)</th>
<th>Propranolol Blood pressure (mmHg)</th>
<th>Heart rate (beats/min)</th>
<th>Blood urea nitrogen (mg/dl)</th>
<th>Fasting blood sugar (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Creatinine clearance (ml/min)</th>
<th>Hematocrit (vol%)</th>
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Clinical Data in Patients Treated with Placebo and Propranolol

![Figure 3](image)

Effect of propranolol on platelet aggregability in patients with angina pectoris. After propranolol, 80 mg/day, platelets of treated patients are less sensitive to ADP (geometric mean) compared to before treatment and not significantly different from normal. Platelet responsiveness was not further changed when the dose of propranolol was doubled to 160 mg/day.

![Figure 2](image)

Comparison of ADP-induced platelet aggregability in normal subjects and patients with angina pectoris. Platelets of normal subjects require significantly higher concentrations of ADP (geometric mean) to attain aggregation threshold than do platelets of patients with angina pectoris.
Discussion

A number of abnormalities in platelet function have been reported in patients with ischemic heart disease.7-11,32-34 Platelet hyperresponsiveness has been described in myocardial infarction7-9,32 and cerebrovascular disease.35 However, other investigators have failed to detect differences in platelet function in patients with arteriosclerotic heart disease when compared to normal subjects.9,10,12,36 Wide differences in methodology and patient selection may account in part for these divergent findings.22,36

Some factors that influence the reliability and reproducibility of platelet aggregation analyses have been identified. Repeated measurements of human
Platelet aggregation induced by ADP vary from day to day depending on the subject’s emotional state, smoking history, or with acute changes in blood lipids, glucose, free fatty acids, catecholamines, hematocrit, and platelet count. Differences in age and sex of the subjects studied may also affect the comparability of previously reported studies. The technique of blood sampling and processing and the rapidity with which studies are performed is also important. Since many drugs influence platelet function, it is imperative that this factor be rigidly controlled. In our study an attempt was made to minimize the effects of the variable factors influencing platelet function by carefully selecting and closely following a population of reliable patients with documented ischemic heart disease and comparing them to a group of normal controls, similar in age and sex.

The present study demonstrates a highly significant increase in ADP-induced platelet aggregability in patients with angina pectoris when compared to normal subjects. There are several possible explanations for this observation. Platelets are known to react with components of injured vascular endothelium such as exposed basement membrane and collagen by adhesion and aggregation, an effect mediated by ADP. Platelets passing through narrow and sclerotic vessels may thus be rendered hyperaggregable. Platelet aggregation induced by these or other mechanisms might cause intravascular thrombi further narrowing the lumen. Thrombi produced by platelet aggregation on ruptured atherosclerotic plaques may induce acute myocardial infarction or ischemia by obstruction or distal embolization of the aggregates. Our results may be reflecting the secondary effect of vascular disease on platelet aggregation. We demonstrated coronary artery disease in three of our patients by coronary arteriography. The other patients had typical angina pectoris with abnormal exercise electrocardiograms and it seems probable that most of them had significant coronary vascular disease as well.

Since catecholamines are known to alter platelet function, an elevation of plasma catecholamine levels in the patient population could have influenced our findings. Experimentally, catecholamine infusion or acute stress induce platelet aggregation in the coronary microcirculation with subsequent myocardial necrosis. Antiaggregation substances protect against this effect. Catecholamine levels were not measured in either the normal or patient group, and it is possible that anticipation of exercise, particularly in those patients prone to pain, might have acutely elevated plasma catecholamines and affected platelet aggregability. However, in the patient group no evidence of excessive catecholamine release could be detected by gross measurements such as heart rate, and all studies were done with the patients relaxed and reassured. Furthermore, platelet aggregability studies were performed in other clinical settings, when establishing reproducibility of assay, and the results were consistent with pre-exercise values.

Free fatty acids can also alter platelet aggregability but increased levels are generally secondary to stress-induced rise in catecholamines. An alpha-globulin that inhibits platelet aggregation has recently been described in normal plasma. The role of this factor in platelet aggregability of patients with angina pectoris is conjectural.

Alternatively, our findings may be a reflection of a primary platelet abnormality in patients with angina pectoris. This was suggested by Hampton and Gorlin who showed abnormalities in platelet function not only in patients with symptomatic coronary vascular disease, but also in their healthy relatives, raising the possibility of a familial defect.

Although the exact mechanism for the increased platelet aggregability in patients with angina pectoris remains unknown, the clinical implications of this observation are of interest. Experimental myocardial infarction has been observed secondary to platelet aggregation although the aggregates persisted for only a few minutes. Haerem reported that platelet aggregates in the epicardial coronary arteries of patients who died suddenly of cardiac causes were more numerous and larger than those found in patients without cardiac disease. Extension or reinfarction is common in patients dying with cardiogenic shock and may be due to formation of microaggregates mediated by a local release of ADP from ischemic myocardial cells, circulating platelets, and red blood cells. Agents which could alter platelet aggregability may have a role in the treatment of coronary artery disease, possibly in the prevention of acute ischemic complications. Further clinical data will be required, however, to evaluate the significance of platelet dysfunction in the infarction syndrome.

In this study, ten patients who received propranolol in dosage of 80 mg/day showed a dramatic and significant increase in performance during standard exercise. Propranolol improved the exercise response and restored the increased platelet aggregability induced by ADP toward normal in most patients with angina pectoris. The platelet effect was noted with serum levels of propranolol as low as 11 ng/ml, was not further influenced by increasing the dose, and persisted over a long period of time with chronic administration of the drug. It is also of interest that the improvement in exercise tolerance was also maximal at the lower dose of propranolol and was not enhanced by increasing dosage.

The improved exercise tolerance in patients with...
angina pectoris receiving propranolol was associated, in all cases, with a decrease in the heart rate-blood pressure product, an effect which has been demonstrated by several investigators. While the improved exercise performance in patients with angina pectoris receiving propranolol can be correlated with reduction of myocardial oxygen consumption, the possibility that the decrease in platelet aggregability influenced cardiac performance, perhaps by improving capillary blood flow, merits consideration.

The effect of propranolol itself on platelet function is controversial. It has been suggested that catecholamine-induced aggregation is an alpha-adrenergic response since phentolamine prevents epinephrine-induced platelet aggregation. Others have speculated that with beta-adrenergic blockade, the alpha receptor on the platelet could be unmasked, and aggregation responsiveness heightened. Thomas, however, showed that propranolol, in vitro, using a concentration of 10 μM, reduced platelet aggregability. Haft and his associates have recently demonstrated that propranolol prevents isoproterenol-induced intravascular platelet in the rat.

Propranolol has multiple physiologic actions and may be of potential value in patients with ischemic heart disease through several mechanisms. In addition to reducing cardiac work during exercise and thus reducing myocardial oxygen demand, the drug shifts the oxyhemoglobin dissociation curve to the right and also has an antitremor effect. In addition, the present study documents that propranolol abolishes platelet hyperresponsiveness to ADP in patients with angina pectoris.

If hyperaggregable platelets play a role in the pathogenesis of myocardial ischemia, an agent which reverses this abnormality may be an important adjunct in chronic treatment. The mechanism of action of propranolol in reducing platelet hyperaggregability and the long-term effectiveness of oral propranolol in preventing thrombotic events in patients with coronary artery disease remains to be determined.

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