Effect of Propranolol on Platelet Function

By Babette B. Weksler, Muriel Gillick, and Jane Pink

Excessive reactivity of blood platelets may contribute to atherosclerotic vascular disease. Hence drugs which alter platelet function may be protective. Prompted by findings that propranolol therapy normalized hyperactive platelet aggregation in patients with coronary artery disease, we studied propranolol in vitro to assess its action on platelets. At concentrations similar to those achieved in vivo (0.1-1 μM), propranolol raised the thresholds for aggregation of some normal platelets by adenosine diphosphate (ADP). At higher concentrations (10-50 μM), propranolol abolished the second wave of platelet aggregation induced by ADP and epinephrine, and inhibited aggregation induced by collagen, thrombin, and the ionophore A23187. Propranolol blocked the release of ¹⁴C-serotonin from platelets, inhibited platelet adhesion to collagen, and interfered with clot retraction. Propranolol blocked ionophore-induced uptake of ⁴⁵Ca by platelets. Inhibition appeared unrelated to beta-adrenergic blockade, as d(+) propranolol (which lacks beta-blocking activity) was equipotent with l(−) propranolol. Moreover, practolol, a beta-blocking drug which is nonlipophilic, did not inhibit platelet function. These studies suggested that propranolol, like local anesthetics, decreased platelet responsiveness by a direct action on the platelet membrane, possibly by interfering with calcium availability. Modulation of platelet function by propranolol may occur at concentrations achieved at usual clinical doses of the drug.

PLATELETS contribute to the development of occlusive vascular disease both by mechanical obstruction of the microcirculation and by release of vasoactive mediators that affect the blood vessel wall. The vasoactive materials in turn alter permeability and lead to proliferative changes1 or to deposition of materials in the vascular wall.2 The platelets of patients with thrombotic vascular disease show increased turnover rates3,5 and augmented responses to a variety of aggregating agents.6,8 These findings suggest a pathophysiologic process characterized by increased platelet consumption. Clinical interest has therefore developed in the pharmacologic control of increased platelet reactivity and in the mechanisms by which drugs might normalize platelet behavior. We have observed that the platelets of patients with angina pectoris have exaggerated aggregation responses which become normal during propranolol therapy.8 The studies presented here have been undertaken to examine the effect of propranolol upon platelets in vitro and to elucidate the mechanism by which the drug alters platelet responsiveness.

MATERIALS AND METHODS

Blood was drawn by a two-syringe technique from healthy, fasting, normal subjects who had not ingested aspirin-containing compounds for at least 1 wk. The blood was mixed with 0.1 volume...
of 3.2% sodium citrate in polypropylene tubes and centrifuged at 240 g for 15 min at room temperature to yield platelet-rich plasma (PRP). PRP was used at platelet counts of 300,000-400,000/μl. Platelet counts were performed with a Coulter Counter Model ZBI (Coulter Electronics, Hialeah, Fla.). Washed platelet suspensions were prepared from PRP by the method of Mustard et al.9 and were used at a concentration of 300,000/μl.

**Aggregation studies.** Platelet aggregation was carried out by a modification of the method of Born10 using a dual-channel Payton aggregation module and Riken-Denshi recorder (Payton Associates, Buffalo, N.Y.). Light transmission was set at 0% with PRP and 100% with platelet-poor plasma. All tests were performed in duplicate. Aggregating agents (adenosine diphosphate [ADP], epinephrine, collagen, and thrombin) were diluted in Tris-buffered saline, pH 7.35. The ionophore A23187 was dissolved in 25% dimethylsulfoxide (DMSO)-75% absolute ethanol and diluted in Tris saline. The DMSO-ethanol solution at similar dilutions (final concentration less than 0.1%) did not affect platelet function.

The effect of propranolol on the aggregation of platelets was measured by adding propranolol in vitro to a suspension of platelets prior to addition of the threshold concentration of aggregating agent.

The threshold for ADP-induced aggregation was defined as the minimal concentration of ADP producing a biphasic aggregation response which resulted in a final optical density within 10% of that of platelet-poor plasma (complete aggregation). The threshold for epinephrine was the minimum concentration which produced a complete biphasic aggregation, even if delayed. The threshold for collagen or thrombin was the minimum concentration producing complete aggregation. All platelet preparations used produced biphasic responses with ADP and epinephrine.

Platelet shape change was assessed visually and by changes in oscillation pattern on the aggregation module recorder.

**Serotonin labeling and release.** 14C-serotonin (as 5-hydroxy-2-14C-tryptamine creatinine sulfate, specific activity 57 mCi/mM) was incubated with PRP or washed platelet suspensions at a final concentration of 0.45 μM at 37°C for 15 min with gentle shaking. Samples of labeled platelets were stirred with aggregating agents in the aggregation module; aggregation responses were recorded and the cuvettes were rapidly placed in a melting ice bath. The platelets were sedimented by centrifugation at 4°C at 4340 g for 10 min. Then 50 μl of supernatant was added to 0.5 ml of 2 N NaOH and 10 ml PCS. Radioactivity was measured in a liquid scintillation counter. Release was expressed as percentage of total radioactivity in the platelet suspension (radioactivity of supernatant of experimental samples minus radioactivity of control supernatants divided by total radioactivity).

In some experiments platelets were also labeled with 3H-adenine (specific activity 19 Ci/mM, final concentration 1.06 nM). The total 3H count in the supernatant of experimental samples was measured and corrected for residual counts in control supernatants.

**Uptake of propranolol.** PRP or washed platelets were incubated with 14C-propranolol (5.42 mCi/mM) at concentrations of 0.1-10 × 10⁻⁶ M for up to 15 min at 0°, 25°, or 37°C. In PRP studies, 0.8 mg/ml 3H-inulin (175 μCi/mg), which is not taken up by platelets, was included as a plasma marker. The platelets were sedimented by centrifugation through silicone oil for 2 min at 8000 g. Supernatant radioactivity was measured as described. The platelet pellets were dissolved in NCS and counted as described above.

**Uptake of 45CaCl₂.** Washed platelets were incubated for 5-15 min at 37°C with a final concentration of 3.9 μM 45CaCl₂ (29.1 mCi/mg Ca) in 2mM unlabeled CaCl₂ in the presence or absence of 0.5 × 10⁻⁶ M A23187 and/or 2.5-5 × 10⁻⁷ M propranolol. The tubes were then placed in ice and the platelets separated by centrifugation. Platelet pellets were dissolved in NCS and counted as described above.

**Platelet adhesion to collagen.** The capacity of platelets to adhere to collagen was measured by the method of Cazenave et al.12 using new glass tubes coated with collagen. Washed platelets were labeled with 14C-serotonin and were suspended in Tyrode’s buffer containing apyrase (0.05 mg/ml) to inhibit aggregation. The platelet suspensions were rotated in the tubes at 25°C for 10 min at 17 rpm, poured into fresh tubes, centrifuged, and the supernatants tested for serotonin release.
The collagen-coated tubes were then rinsed four times in saline, drained, and treated with 0.5 ml NCS/tube in order to solubilize the residual radioactivity, representing platelets adherent to collagen. Aliquots of NCS were then counted in Toluene-Liquifluor.

Clot retraction. Citrated PRP was clotted in glass tubes by addition of 0.1 ml Reptilase diluted 1:5 as described by de Gaetano et al.\textsuperscript{13} Duplicate samples were incubated for 1 hr at 37°C. The clot was gently removed from each tube on a curved glass rod left in the tube during incubation and the expressed serum was measured with a Hamilton syringe. Clot retraction was evaluated in the presence of platelet aggregating agents, saline, and/or propranolol.

Platelet factor 3 availability. The method of Spaet and Cintron\textsuperscript{14} was used, employing the Stypven clotting time. Platelets were activated by Celite, ADP, or collagen in the presence or absence or propranolol.

Materials. ADP, bovine tendon collagen, and apyrase were obtained from Sigma Chemical Co. (St. Louis, Mo.); \textsuperscript{3}H-serotonin, \textsuperscript{3}H-adenine, \textsuperscript{45}CaCl\textsubscript{2}, PCS, and NCS from Amersham/Searle (Arlington Heights, Ill.); bovine thrombin and epinephrine from Parke, Davis (Ann Arbor, Mich.); Stypven from Burroughs Wellcome (Research Triangle Park, N. C.); and Reptilase from Abbott Laboratories (Chicago, Ill.). Propranolol, practolol and \textsuperscript{14}C-propranolol were the gifts of Ayerst Laboratories (New York, N.Y.). A23187 was the gift of Dr. Robert Hamill of Eli Lilly & Co. (Indianapolis, Ind.). Liquifluor was from New England Nuclear (Boston, Mass.).

RESULTS

Effect of Propranolol on Platelet Aggregation

Propranolol, \(0.1-1 \mu M\), raised the ADP threshold for second-phase platelet aggregation in PRP (Fig. 1) when the threshold was low (less than \(2 \times 10^{-6} M\)). Propranolol altered both the rate and extent of the second phase and promoted disaggregation without affecting primary aggregation. The inhibition produced by a given concentration of propranolol depended upon the ADP threshold of the platelet suspension (Table 1), platelets with lower thresholds being more easily inhibited. The primary phase of aggregation induced by threshold ADP was abolished only by propranolol concentrations greater than \(2 \times 10^{-4} M\).

Platelet aggregation by epinephrine, collagen, and thrombin was also altered by propranolol (Table 1). Second-phase aggregation induced by threshold epinephrine was delayed and inhibited by \(2 \times 10^{-5} M\) propranolol, whereas the primary phase was not altered (represented by \(99\%\) aggregation in the presence of \(10^{-4} M\) propranolol). The rate and extent of collagen-induced ag-
Table 1. Effect of Propranolol on Platelet Aggregation by Threshold Concentrations of Aggregating Agents

<table>
<thead>
<tr>
<th>Threshold Aggregating Agent*</th>
<th>Propranolol Concentrations (%)</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP (1.5 x 10^-6 M)</td>
<td>100</td>
<td>60</td>
<td>50</td>
<td>33</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>ADP (5.0 x 10^-6 M)</td>
<td>100</td>
<td>98</td>
<td>—</td>
<td>61</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Epinephrine (0.5 x 10^-6 M)</td>
<td>100</td>
<td>98</td>
<td>37</td>
<td>18</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Collagen (50 μg/ml)</td>
<td>100</td>
<td>63</td>
<td>—</td>
<td>30</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Thrombin (0.025 units/ml)</td>
<td>100</td>
<td>91</td>
<td>68</td>
<td>43</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

Values are percentages of control aggregation. Each figure represents the mean value from 3–5 experiments using platelets from different normal donors. In each experiment, the extent of aggregation in the presence of propranolol, as maximal change in light transmission after addition of aggregant, was expressed as per cent of that achieved in absence of propranolol.

*Minimum concentration which produced complete aggregation, as defined in Materials and Methods, in PRP, except for experiments with thrombin, which were performed with washed platelets.

Aggregation were inhibited by propranolol and the lag phase was markedly prolonged. Similarly, thrombin-induced aggregation (of washed platelets) was both slowed and diminished by the drug at concentrations of 10^-5 M or more.

Propranolol inhibited platelet aggregation immediately and preincubation of platelets with drug did not increase the inhibitory effect. When added to platelets after an aggregating agent but before the onset of the second wave, propranolol blocked further aggregation by epinephrine (Fig. 2A, B) or ADP (not shown). Adding the drug after the second wave had begun halted or reversed further aggregation (Fig. 2C), depending upon drug concentration. Shape change induced by aggregating agents was not inhibited by propranolol.

![Fig. 2](image_url)
Effect of Propranolol on the Platelet Release Reaction

The release of $^{14}$C-serotonin from platelets was inhibited by propranolol pretreatment when platelets were subsequently stimulated by any of the aggregating agents used above. The parallel inhibition of both aggregation and $^{14}$C-serotonin release from propranolol-treated platelets after collagen stimulation is shown in Fig. 3. Both processes were inhibited by $5 \times 10^{-6}$ to $10^{-4}$ M propranolol, whereas higher concentrations produced a nonspecific loss of both serotonin and $^3$H-adenine nucleotides in the absence of aggregation, suggesting platelet membrane damage. The release of $^{14}$C-serotonin from platelets during ADP- or epinephrine-mediated aggregation was inhibited by even lower concentrations of propranolol. Inhibition of serotonin release from thrombin-stimulated platelets, however, required $5 \times 10^{-5}$ M drug or more.

Platelets stimulated with small amounts of epinephrine and ADP, each at a concentration too low to produce a threshold aggregation response, undergo full aggregation accompanied by an augmented release of serotonin. Propranolol inhibited such potentiation of platelet aggregation and $^{14}$C-serotonin release (Table 2). The inhibition in the potentiated system required less propranolol than was needed to inhibit platelet aggregation by the threshold ADP alone.

Table 2. Effect of Propranolol on Platelet Aggregation Produced by a Combination of Subthreshold Epinephrine and ADP

<table>
<thead>
<tr>
<th>Propranolol Concentration ($\times 10^{-6}$ M)</th>
<th>Platelet Aggregation (%)</th>
<th>Serotonin Release (% of Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>68.6</td>
<td>52</td>
</tr>
<tr>
<td>20</td>
<td>40.5</td>
<td>11.3</td>
</tr>
<tr>
<td>40</td>
<td>38</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Mean of four experiments, done in duplicate, using platelets from different donors. Platelet aggregation was induced by a combination of epinephrine (1/10 threshold concentration) and ADP (1/4 threshold concentration). Threshold values were determined for each donor. All experiments performed in PRP.
Effect of Propranolol on Platelet Responses to the Ionophore A23187

The divalent cation ionophore A23187 induces platelet aggregation and the release reaction,\textsuperscript{15} as depicted in Fig. 4. Propranolol, added prior to ionophore, inhibited the aggregation and release of \textsuperscript{14}C-serotonin (latter not shown). Added after ionophore, propranolol did not alter the platelet response to A23187 and did not reverse aggregation.

Low concentrations of ionophore which caused neither aggregation nor serotonin release (0.5–2 × 10\textsuperscript{-6} M in PRP, less than 10\textsuperscript{-4} M in washed platelets) potentiated the effects of subthreshold ADP and collagen (Table 3) and epinephrine (not shown). The potentiation of aggregation and serotonin release was also blocked by propranolol (Table 3).

The ionophore A23187 has previously been shown to promote uptake of \textsuperscript{45}CaCl\textsubscript{2} by platelets.\textsuperscript{15} The uptake of \textsuperscript{45}CaCl\textsubscript{2} by washed platelets was enhanced 2.5-fold by 0.5 × 10\textsuperscript{-6} M ionophore, a concentration which did not induce platelet aggregation or serotonin release. Since the volume of trapped medium in the platelet pellet, measured with \textsuperscript{3}H-inulin, did not differ between ionophore-
Table 3. Inhibition by Propranolol of Ionophore-potentiated Platelet Aggregation and Release

<table>
<thead>
<tr>
<th>Agents Added to Platelets</th>
<th>Platelet Aggregation (%)</th>
<th>Release of $^{14}$C-Serotonin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionophore A23187</td>
<td>5.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Collagen</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>Ionophore + collagen</td>
<td>93.7</td>
<td>39.2</td>
</tr>
<tr>
<td>Propranolol (10$^{-6}$ M)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ ionophore + collagen</td>
<td>14.9</td>
<td>3.4</td>
</tr>
<tr>
<td>ADP</td>
<td>9.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Ionophore + ADP</td>
<td>86.5</td>
<td>27.8</td>
</tr>
<tr>
<td>Propranolol (10$^{-6}$ M)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ ionophore + ADP</td>
<td>11.0</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Representative experiment in PRP. Collagen 50 µg/ml, ionophore 3 x 10$^{-6}$ M (this concentration alone did not aggregate the platelets), ADP 0.6 x 10$^{-6}$ M.

containing and control samples, the uptake of $^{45}$CaCl$_2$ represented increased cell-bound calcium. Propranolol (2.5–5 x 10$^{-5}$ M) inhibited the ionophore-induced enhancement of calcium uptake by platelets.

Comparison of Propranolol Isomers

The propranolol used to inhibit platelet aggregation and serotonin release in the above studies was a racemic mixture of d(+) and l(−) isomers (the clinical form of the drug). The separate d(+) and l(−) isomers of propranolol were then compared: d(+) propranolol and l(−) propranolol were equipotent as inhibitors of platelet aggregation by ADP, and their effects did not significantly differ from the effect of racemic propranolol. Both isomers also produced equal inhibition of platelet aggregation and release induced by the other aggregating agents discussed above.

In contrast, another beta-adrenergic blocking drug, practolol, did not affect platelet aggregation even at ten times the highest concentration of propranolol tested (1 mM practolol). Furthermore, practolol did not alter the inhibitory effect of propranolol on platelet responses.

Uptake of Propranolol by Platelets

At plasma concentrations of 10$^{-7}$–10$^{-5}$ M propranolol, the cells accumulated 10–30 times the plasma levels of drug, the binding increasing with the drug concentration (Table 4). Uptake occurred equally well at 0° as at 25° or 37°C.

Table 4. Accumulation of $^{14}$C-Propranolol by Platelets in PRP

<table>
<thead>
<tr>
<th>Concentration of Propranolol in Plasma ($\times$ 10$^{-6}$ M)</th>
<th>Ratio of Propranolol Concentration in Platelets/Plasma*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>11.1 ± 2.6†</td>
</tr>
<tr>
<td>1.0</td>
<td>13.6 ± 2.2</td>
</tr>
<tr>
<td>5.0</td>
<td>22.1 ± 2.7</td>
</tr>
<tr>
<td>10.0</td>
<td>25.6 ± 5.7</td>
</tr>
</tbody>
</table>

*PRP (300,000 platelets/µl) incubated 10 min at 37°C with 0.1–10 × 10$^{-6}$ M $^{14}$C-propranolol and $^{3}H$-inulin, platelets separated by centrifugation through silicone oil for 2 min at 8000 g.

†Mean ± SD.
and was rapid, reaching the maximum in 1 min. The bound drug exchanged easily with unlabeled propranolol. When platelets labeled with $^{14}C$-propranolol were fractionated, the membrane fraction showed highest drug specific activity, although granules and cytosol were also labeled. The ease of exchange of labeled drug may account for its presence in all subcellular fractions.

**Effect of Propranolol on Platelet Adhesion to Collagen**

The effect of propranolol on the adhesion of $^{14}C$-serotonin-labeled platelets to collagen-coated tubes was measured and was compared to the effect of aspirin. Platelet adhesion to collagen was inhibited by concentrations of propranolol from $10^{-7}$ to $10^{-5} M$, as shown in Fig. 5. The inhibitory effect of racemic d,l-propranolol was similar in magnitude to that produced by either d(+) or l(−) isomer alone, the two isomers having roughly equal inhibitory effects.

Propranolol was a more potent inhibitor of platelet adhesion to collagen than was aspirin in this test system (Table 5). The two drugs used together decreased platelet adhesion to collagen only slightly more than propranolol alone. The lack of additive effect was observed at all concentrations of drugs tested.

**Table 5. Effects of Aspirin and Propranolol on Platelet Adherence to Collagen-coated Tubes**

<table>
<thead>
<tr>
<th>Agent Tested</th>
<th>Adherence in Presence of Drug (% of Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$1 \times 10^{-8} M$</td>
</tr>
<tr>
<td>Aspirin</td>
<td>91.8</td>
</tr>
<tr>
<td>Propranolol</td>
<td>72.1</td>
</tr>
<tr>
<td>Aspirin + propranolol*</td>
<td>70.3</td>
</tr>
</tbody>
</table>

Values are means of quadruplicate determinations in three experiments using washed platelets.

*Both drugs present at concentration noted in column headings.
Table 6. Propranolol-induced Inhibition of Clot Retraction in PRP Clotted by Reptilase in the Presence of ADP or Epinephrine

<table>
<thead>
<tr>
<th>Agent</th>
<th>Propranolol Concentrations ($\times 10^{-6} M$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>ADP</td>
<td></td>
</tr>
<tr>
<td>1.6*</td>
<td>100</td>
</tr>
<tr>
<td>0.4</td>
<td>80</td>
</tr>
<tr>
<td>Epinephrine</td>
<td></td>
</tr>
<tr>
<td>2.5*</td>
<td>100</td>
</tr>
<tr>
<td>0.8</td>
<td>92</td>
</tr>
</tbody>
</table>

Values indicate clot retraction as per cent of control, calculated from mean of duplicate determinations in a single representative experiment. Five experiments were performed.

*Threshold concentration for platelet aggregation.

Effects of Propranolol on Clot Retraction

PRP clotted by Reptilase (Botrops atrox venom) undergoes only minimal clot retraction unless a platelet-activating agent is added to the plasma. We confirmed the findings of de Gaetano et al.\textsuperscript{13} that ADP, epinephrine, and collagen promote clot retraction in Reptilase-clotted PRP, as does the ionophore A23187. These agents supported maximal retraction when used at the threshold concentrations for platelet aggregation, but even subthreshold amounts of ADP and epinephrine produced retraction. Propranolol inhibited clot retraction of PRP incubated with ADP or epinephrine (Table 6). The extent of inhibition depended on the amount of aggregating agent present, and in general required $10^{-5} M$ propranolol for ADP-mediated retraction and $5 \times 10^{-5} M$ or greater for retraction in response to other agents.

Effects of Propranolol on Platelet Factor 3 Availability

The Stypven time in PRP incubated with Celite to induce maximal availability of platelet factor 3 was not altered by propranolol ($5-500 \times 10^{-6} M$). Shortening of the Stypven time of PRP activated by ADP or collagen, which are weaker activators of factor 3, was decreased 43\% and 71\%, respectively, by $7.5 \times 10^{-5} M$ propranolol, but not by lower drug concentrations.

DISCUSSION

Propranolol, added to platelets in vitro, inhibited platelet aggregation induced by ADP, epinephrine, collagen, thrombin, and the ionophore A23187. Propranolol inhibited mainly the secondary phase of platelet aggregation and serotonin release, while it had little effect upon primary aggregation. Reptilase-induced clot retraction, platelet adhesion to collagen, and availability of platelet factor 3 were also inhibited by the drug.

Because of the increasingly widespread use of propranolol in clinical settings ranging from angina pectoris to hypertension, it was of interest to ascertain whether this drug altered platelet functions at concentrations achieved in clinical practice. We have previously shown\textsuperscript{8} that the low thresholds for platelet aggregation by ADP and epinephrine present in patients with severe angina pectoris became normal (i.e., the platelets were less responsive) during therapy with 80-160 mg/day propranolol for angina. After propranolol ther-
apy was stopped, the initial low aggregation thresholds were again observed. The question arose, could this change in platelet function be a direct effect of propranolol on platelets?

Peak plasma levels of propranolol following usual clinical doses range from 20 to 200 ng/ml ($10^{-7}$-$10^{-6} M$). In the present study we found that propranolol added in vitro altered platelet aggregation, serotonin release, and adhesion to collagen at similar concentrations. Inhibition of aggregation by $10^{-7}$-$10^{-6} M$ propranolol was only observed in platelets with low thresholds for aggregation by ADP. Platelets from normal donors with less sensitive thresholds required more than $10^{-5} M$ drug for inhibition in vitro. While the ADP threshold phenomenon, i.e., ADP-induced second-phase aggregation and serotonin release, results from the low concentration of ionized calcium in citrated plasma, it correlates well with other measurements of platelet reactivity. Propranolol may reset the threshold of platelet responsiveness, but in a clinical context the effect is limited to platelets which have shown increased sensitivity to aggregating stimuli. Such “sensitive” platelets have been observed in patients with hypercholesterolemia, diabetes mellitus, recent stroke or myocardial infarction, and chronic angina pectoris.

We have also shown that platelets accumulate $^{14}C$-propranolol in vitro 10-30-fold over plasma concentrations. Preferential accumulation of the drug by platelets in vivo may thus account for altered platelet responsiveness at the plasma propranolol concentrations measured in vivo.

Several lines of evidence suggest that propranolol alters platelet behavior by mechanisms other than beta-adrenergic blockade. Propranolol is known to have a “local anesthetic” or membrane-stabilizing effect. This effect has been shown in red blood cells, nerve, and skeletal and cardiac muscle, and has previously been suggested as the mechanism for inhibition of epinephrine-induced platelet aggregation. Concentrations of propranolol needed for a membrane effect are higher ($10^{-6}$-$10^{-4} M$) than for beta-adrenergic blockade ($10^{-9}$-$10^{-8} M$). Also, the membrane-stabilizing effect is not stereospecific, unlike beta blockade. Thus, $d(+)$ and $l(-)$ propranolol have similar membrane effects, whereas the $d(+) isomer has only 1% of the beta-blockading capacity of the $l(-)$ isomer. The concentration of propranolol required to inhibit platelet function in vitro ($10^{-7}$-$10^{-4} M$) is high, suggesting a membrane effect. This conclusion is supported by the finding that $d(+)$ and $l(-)$ forms are equipotent inhibitors of platelet aggregation, serotonin release, and platelet adhesion to collagen. Furthermore, practolol, a potent beta-adrenergic blocking drug which lacks membrane stabilizing activity, has no effect upon platelet function. Thus, the membrane effect and not beta-adrenergic blockade appears crucial for the action of propranolol on platelets.

Like other membrane-stabilizing substances, the main effect of propranolol was to inhibit the second phase of platelet aggregation and serotonin release. Shape change was not inhibited by propranolol, suggesting that the drug did not interfere with the initial contact or binding of aggregants with the platelet surface. All platelet functions affected by propranolol are calcium dependent, whereas platelet shape change, which is independent of divalent cations, was not affected.

Propranolol may alter platelet function by affecting the availability of
calcium within platelets. The drug displaces calcium from membrane sites\(^3\) and inhibits lipid-facilitated transport of calcium.\(^3\) Both platelet aggregation and the release reaction involve the transport of calcium ions into the platelet cytoplasm from other sites—either the dense tubular system in the platelet\(^1\) or the external medium.\(^3\) Platelet dense tubular membranes, like the sarcoplasmic reticulum of muscle, sequester calcium.\(^3\) Ionophores such as A23187 can transport extracellular calcium ions into cells and can shift intracellular calcium from physiologically sequestered locations into the cytoplasm, as has been shown for muscle.\(^3\)

Propranolol inhibited ionophore-mediated potentiation of platelet aggregation by subthreshold amounts of ADP and collagen and inhibited uptake of \(^{45}\)CaCl\(_2\) induced by A23187. The effects were observed only when the propranolol was introduced prior to the ionophore, suggesting that propranolol may act to inhibit the access of ionophore to calcium at critical intracellular sites. While inhibition of ADP-induced platelet aggregation in citrated PRP might reflect the low plasma concentration of ionized calcium, we also found that propranolol was inhibitory to washed platelets suspended at physiologic calcium ion concentrations. Therefore low external calcium concentration was not crucial to demonstration of a propranolol effect.

We conclude that propranolol is bound by platelets and renders the platelet membrane less sensitive to the action of aggregant substances. At clinically achieved levels of drug, propranolol affects platelet function much less than does aspirin. The modulation of platelet membrane function by propranolol does not involve beta-adrenergic blockade; the drug may alter platelet reactivity by interfering with internal shifts of calcium ions at intracellular sites.

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Effect of propranolol on platelet function

BB Weksler, M Gillick and J Pink