SUMMARY  To study the possible role of catecholamines in platelet activation, platelet aggregation stimulated by ADP, collagen, arachidonic acid and l-epinephrine, thromboxane B₂ (TXB₂) formation and plasma levels of catecholamines and renin were studied in healthy men both before and after 6 days of propranolol treatment (40 mg three times daily) under control conditions and during sympathoadrenergic stimulation by physical exercise (200 W) or smoking. Exercise markedly increased plasma norepinephrine from 128 ± 28 to 998 ± 418 pg/ml (± SD), and plasma renin activity from 1.0 ± 0.5 to 4.2 ± 1.8 ng AI/ml-hour. Smoking predominantly increased plasma epinephrine, from 47 ± 25 to 154 ± 76 pg/ml. Propranolol did not consistently influence these variables, but blunted the circulatory response to exercise and smoking. Despite the marked increases in plasma catecholamines after both stimuli with and without β blockade, platelet aggregation stimulated by ADP, l-epinephrine, collagen and arachidonic acid and associated TXB₂ formation were not enhanced. Moreover, as already suggested by a trend toward reduced aggregability in these settings, plasma norepinephrine levels in the same range (745 ± 368 pg/ml) due to infusion (5 μg/min) significantly reduced platelet aggregation with low-dose collagen (0.25–0.75 μg/ml), l-epinephrine (0.2–1.0 μM) and ADP (0.5–1.5 μM). These data do not support a role of endogenous catecholamines in initiating platelet activation and TXB₂ formation.

SMOKING, a well-documented cardiovascular risk factor, and physical effort, which may precipitate myocardial infarction in patients with preexisting coronary artery disease, stimulate catecholamine release.1–6 Abnormally reactive platelets may play a role in the development of coronary heart disease and acute myocardial infarction.7,8 Several studies have suggested an association between physical stress and platelet activation in coronary artery disease.9–14 After physical exercise11,12 and pacing-induced tachycardia in patients with coronary artery disease,9–14 as well as after acute15–16 or chronic smoking,17 alterations of platelet function have been reported. Epinephrine and, to a lesser extent, norepinephrine aggregate platelets in vitro and potentiate the response to other aggregating compounds18–22 that can be antagonized by α blockers and membrane-stabilizing β blockers.18,23,24 Propranolol pretreatment blunts the increase of platelet aggregation during tachycardia in coronary patients,13 and antiplatelet drugs or thrombocytopenia can protect against epinephrine-induced experimental myocardial necrosis.25 We therefore studied platelet aggregation and thromboxane B₂ (TXB₂) formation during exercise- or smoking-induced sympathoadrenergic activation both before and after propranolol pretreatment in healthy men.

Methods and Materials

Study Protocol

Six healthy male volunteers, mean age 30 ± 4 years (± SD), who had not taken any medication for 3 weeks were studied after an overnight fast both before and after 1 week of treatment with propranolol, 40 mg three times daily. Data were assessed after 45 minutes of supine rest at 8 a.m. (control), after treadmill exercise (4 minutes at 100 W and 4 minutes at 200 W), 2 hours later after smoking of two cigarettes (1.6 mg of nicotine each) and, in a second experimental setting, before and after a 15-minute norepinephrine infusion (5 μg/min). Blood pressure was recorded by sphygmomanometry, heart rate by ECG, and blood for laboratory studies was drawn from the antecubital vein in a carefully standardized manner.

Platelet Aggregation and Thromboxane Formation

TXB₂ formation and platelet aggregation were estimated as described earlier.26 Briefly, blood was drawn into sodium citrate (3.8%, 1/10 vol/vol) and centrifuged (150–200 g for 5 minutes) at room temperature to prepare platelet-rich plasma (PRP). PRP was adjusted to a platelet count of 250,000/μl with autologous platelet-poor plasma (PPP). Tubes were capped to prevent loss of carbon dioxide. Platelet aggregation was studied by the method of Born27 using a “Labor-Aggrometer” (Fresenius). The percent change of light transmission after addition of aggregating agents was recorded; 100% corresponded to a change of 0.14 optical density. The following aggregating agents were tested at fixed times after blood sampling to eliminate time drifts of platelet sensitivity:26 Collagen (Horm Chemie), 0.25–10 μg/ml PRP (50–60 minutes after
blood sampling); arachidonic acid, 1.8 mM (at 90 minutes); ADP, 0.5–2.5 μM (at 80–90 minutes); l-epinephrine, 0.2–5.0 μM (at 70–80 minutes) (all from Serva). Aggregation-associated thromboxane formation was measured by radioimmunoassay of TXB₂, the stable hydrolytic product of TXA₂, after acidification and organic solvent extraction of the samples at 5 minutes. A specific TXB₂ antiserum (provided by L. Levine, Brandeis University), standard TXB₂ (provided by J. Pike, Upjohn Co.), and (³H)-TXB₂ (NEN) with a specific activity of 150 Ci/mM, were used.

To estimate platelet sensitivity to prostacyclin, PGI₁, (provided by J. Pike) was dissolved in a 1:10 solution of 0.1 M TRIS HCl buffer (pH 9.0) and ethanol. From this stock, the test solutions were prepared each day in 0.1 M TRIS HCl buffer (pH 9.0), added to PRP to give final concentrations of 50–2000 pg/ml PRP and preincubated at 37°C for 1 minute. For these studies, aggregation was challenged either with ADP (5 μM) or collagen (1 μg/ml PRP).

Plasma catecholamines were determined by a sensitive and specific radioenzymatic assay, and plasma renin activity by radioimmunoassay according to Haber et al.

The data were analyzed using the t test for paired and unpaired values.

**Results**

Physical exercise markedly increased plasma norepinephrine, whereas cigarette smoking predominantly stimulated plasma epinephrine levels. Systolic blood pressure, heart rate and plasma renin activity were markedly elevated by physical exercise. Smoking increased heart rate only (fig. 1). Over the whole range, a close relation (y = 53.5 ± 0.01x; r = 0.66) between plasma norepinephrine and heart rate was found. Propranolol pretreatment lowered basal systolic and diastolic blood pressure as well as heart rate and attenuated the circulatory response to both exercise and smoking and the increase of plasma renin activity after exercise. Basal and stimulated plasma catecholamine levels were not significantly influenced by propranolol (fig. 1).

Despite markedly increased endogenous catecholamine levels, platelet aggregation and associated TXB₂ formation induced by arachidonic acid, collagen, l-epinephrine and ADP were not enhanced after either physical exercise or smoking. Platelet sensitivity to prostacyclin was not affected. Both exercise and smoking tended to decrease aggregation with low-dose collagen, ADP and especially l-epinephrine. In addition, propranolol pretreatment, which did not detectably influence platelet aggregation, TXB₂ formation or platelet sensitivity to prostacyclin under baseline conditions, seemed to shift back to control levels the reduced platelet aggregability after exercise and smoking (fig. 2).

To investigate this weak but consistent trend in more detail, we studied platelet aggregation before and after infusion of norepinephrine, so as to exclude mechanisms other than sympathoadrenergic arousal as mediators of this tendency toward reduced platelet aggregability after exercise or smoking.

Norepinephrine infusion (5 μg/min) increased plasma norepinephrine levels from 173 ± 17 to 745 ± 368 pg/ml (± SD) (p < 0.01) and blood pressure from 119 ±

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**Figure 1.** Blood pressure, heart rate, plasma norepinephrine, plasma epinephrine and plasma renin activity during rest (left), exercise (center) and smoking (right) without (black) and with (white) propranolol pretreatment (40 mg three times daily). Values are mean ± SEM (n = 6). *p < 0.05, **p < 0.01 vs rest; $p < 0.05, §§p < 0.01$ vs without propranolol pretreatment.
8/80 ± 3 to 138 ± 11/91 ± 5 mm Hg (p < 0.01); these changes were similar to those after treadmill exercise. Norepinephrine infusion decreased heart rate from 63 ± 9 to 55 ± 7 beats/min (p < 0.05), but did not affect plasma epinephrine levels (38 ± 12 vs 44 ± 15 pg/ml).

To test the stability of catecholamines during the platelet studies, aliquots of PRP were split off at several points during aggregation studies in some subjects and at the end (90 minutes after blood sampling) in all, centrifuged to yield PPP, and analyzed for catecholamines. Norepinephrine, as well as epinephrine, slowly but continuously decreased during processing, reaching −28 ± 5% in control studies and −39 ± 6% after norepinephrine infusion at the end of aggregation studies compared with levels measured immediately after blood sampling.

Platelet aggregability was significantly reduced after norepinephrine infusion, especially when induced by l-epinephrine (at all concentrations studied), or by low concentrations of collagen and ADP, whereas aggregation with higher doses of collagen, ADP and arachidonic acid was unchanged (fig. 3). The mean minimal dose of ADP required for irreversible aggregation was significantly increased, from 1.3 ± 0.24 to 1.71 ± 0.4 μM (p < 0.01).

In two additional experiments, blood samples from resting subjects were split; to one half, exogenous norepinephrine (10 ng/ml) was added, PRP was prepared as usual and aggregation studies were performed in parallel in native and norepinephrine-enriched PRP. Again, aggregability with all stimuli was consistently reduced after norepinephrine addition. This high pharmacologic dose of norepinephrine was virtually 100% carried through the procedure.

Although the platelet count in blood was not significantly altered, the platelet yield in PRP preparations was significantly increased after exercise both before and after propranolol; for aggregation studies, the platelet count in PRP was effectively adjusted (table 1).

**Discussion**

Several facts suggest that catecholamines may affect platelet function: l-epinephrine is a potent aggregating agent in vitro that is antagonized by α-receptor blockers, and platelets are endowed with a catecholamine-uptake mechanism and atypical α receptors, but obviously lack β receptors. Some antiplatelet activity has been attributed to propranolol, however, which has been linked to its non-receptor-mediated membrane-stabilizing properties.

Although in the present study, physical exercise or smoking markedly increased plasma levels of norepinephrine and epinephrine in healthy men, these catecholamine changes did not detectably enhance platelet aggregation and associated TXB₂ formation or blunt platelet sensitivity to prostacyclin. On the contrary, platelets aggregability tended to be reduced after exercise or smoking. In addition, propranolol pretreatment alone in a dosage sufficient to blunt the circulatory responses was without effect on platelet function, but seemed to abolish the reduced sensitivity of platelets. This tendency toward reduced platelet aggregability preexposed to high catecholamine levels could be statistically confirmed in a more detailed experiment in which infusion was used to increase plasma levels of norepinephrine. Thus, catecholamines could be more reliably singled out as the mediator of this reduced aggregability with several stimuli. Based on our data, the mechanisms of reduced platelet response after norepinephrine preexposure cannot be elucidated: Reduced response to l-epinephrine could easily be ex-

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**Figure 2.** Platelet aggregation (columns) and associated TXB₂ formation (brackets) at rest (left), after exercise (center) and smoking (right) without (black) and with (white) propranolol pretreatment (40 mg three times daily) using collagen (1 and 10 μg/ml PRP), arachidonic acid (C20:4, 500 μg/ml), l-epinephrine (5 or 10 μM) and ADP (2.5 μM) as stimulating agents. ID₅₀ for PGI₂ = concentration of prostacyclin (pg/ml PRP) needed for 50% inhibition of aggregation induced by collagen (1 μg/ml PRP). Values are mean ± SEM (n = 6). PRP = platelet-rich plasma.
explained by down regulation, as apparent decrease in receptor number or affinity after agonist exposure has been reported for several hormonal systems. However, as the platelet response to several other aggregating compounds, which act probably independent of or distal to adrenergic receptors, was reduced in a similar fashion, a mode of action not restricted to sympathetic-adrenergic signal transmission in the platelet seems more likely.

These findings do not substantiate a role of catecholamines in initiating platelet-related mechanisms that precipitate events of coronary heart disease, at least at a range of concentrations of endogenous catecholamines achievable in our experimental setting in healthy subjects. In fact, the concentrations in our in vivo experiments were 10–100 times lower than minimal epinephrine concentrations needed to challenge isolated platelet aggregation in vitro. In addition, platelet aggregability as tested in our in vitro setting seemed reduced after preexposure to catecholamine levels achievable in vivo.

Platelet aggregation studies in vitro are easily invalidated by the instability of PRP, and both platelet concentration in PRP and test time after sampling influence aggregation and TXB₂ formation. By standardizing test time after blood sampling and adjusting platelet count in PRP, platelet aggregation studies are reproducible. The marked increase of platelet count in PRP after physical exercise and the lesser increase after smoking can mimic an increase of platelet aggregation and TXB₂ formation if platelet counts in PRP are not adjusted. Therefore, platelet counts in PRP must be adjusted before studying aggregation and TXB₂ formation.

In several studies in patients with coronary heart disease, signs of platelet activation have been reported after physical exercise or pacing-induced tachycardia. For healthy subjects, only few data on the effect of physical exercise on platelet function are available. Our data in healthy subjects do not exclude the possibility that in patients with ischemic myocardial disease, in whom plasma levels of catecholamines and thromboxane are reported to be increased and platelets abnormally reactive, exercise- and smoking-induced sympathetic-adrenergic stimulation may enhance untoward platelet dysfunction at plasma catecholamine levels that have no proaggregatory influence in healthy subjects without endothelial damage.

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