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Research Article

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Aggregating Human Platelets Cause Direct Contraction and Endothelium-dependent Relaxation of Isolated Canine Coronary Arteries

Role of Serotonin, Thromboxane A₂, and Adenine Nucleotides

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Abstract

Aggregating human platelets contract isolated rings of canine coronary artery without endothelium, but relax rings with intact endothelium. We performed experiments to identify the substances released from platelets responsible for these effects. The contraction in rings without endothelium was reduced by treating the platelets with the thromboxane synthetase inhibitor, dazoxiben, or treating the vessels with the thromboxane-receptor antagonist, SQ 29548. The serotonergic antagonist, methiothepin, also reduced the platelet-induced contraction. The combination of methiothepin plus dazoxiben or SQ 29548 caused a further inhibition. The endothelium-dependent relaxation to platelets during contractions evoked by prostaglandin F_{2α} was nearly abolished by the ADP- and ATP-scavenger, apyrase. It was not inhibited by methiothepin, which antagonizes endothelium-dependent relaxations to serotonin. Thus, both serotonin and thromboxane A₂ contribute to the direct activation of coronary smooth muscle by aggregating human platelets, whereas adenine nucleotides are the principal mediators of the endothelium-dependent relaxation.

Introduction

Isolated canine coronary arteries denuded of endothelium contract to aggregating canine platelets. In contrast, platelets cause an endothelium-dependent relaxation of the same arteries (1–3). Both serotonin (e.g., 4) and thromboxane A₂ (e.g., 5) can contract coronary vessels and may contribute to the platelet-induced contraction. The relaxation caused by canine platelets is due principally to adenine nucleotides (ADP and ATP) (3), although serotonin released from the platelets might contribute (1, 2). Human platelets contain more than twice the total quantity of adenine nucleotides and less than half the serotonin compared with canine platelets (6). The present study was designed to determine whether aggregating human platelets also induce both endothelium-dependent relaxations and endothelium-independent contractions of coronary vascular smooth muscle and, if so, to identify the mediators of both responses.

Methods

Tissue preparation. Left circumflex coronary arteries were removed from mongrel dogs of either sex weighing 15–30 kg, which had been anesthe-

tized with sodium pentobarbital (30 mg/kg i.v.) and exsanguinated. The vessels were immediately placed in cold modified Krebs-Ringer bicarbonate solution (millimolar composition: NaCl, 118.3; KCl, 4.7; CaCl₂, 2.5; MgSO₄, 1.2; KH₂PO₄, 1.2; NaHCO₃, 25.0; calcium disodium edetate, 0.026; glucose, 11.1; control solution). Rings 4–6 mm long were cut, with up to eight rings from the same animal being studied in parallel as control and treatment groups. Some rings were denuded of endothelium by inserting a pair of fine forceps into the lumen and gently rolling the ring back and forth on a Krebs-Ringer-wetted paper. This procedure did not alter significantly the maximum tension the vessels could produce when contracted with 40 mM KCl solution (average increase in tension, 7.9±1.2 and 6.0±0.8 g in rings with and without endothelium, respectively; *n* = 10).

Organ chamber experiments. Rings were suspended in organ chambers made of nonsiliconized glass and filled with 15 ml of control solution maintained at 37°C and continuously bubbled with 95% O₂/5% CO₂. Isometric tension was measured by Gould UC2 strain gauges and recorded on a polygraph. Rings were gradually stretched in small increments up to a basal tension of 10–11 g (shown in previous experiments to be the optimum tension for isometric contraction under these conditions; 2, 3). They were then contracted repeatedly, by increasing the KCl concentration in the bath by 20 mM, until the response was stable.

After repeated rinses with control solution to restore basal tension, all vessels were contracted with prostaglandin F_{2α} (2×10^{-6} M), and tested for the presence of functional endothelium by the addition of acetylcholine (10^{-6} M) (7, 8). Rings from which the endothelium had been removed showed no change in tension upon addition of acetylcholine, whereas rings with endothelium relaxed to basal tension; rings not meeting these criteria were excluded.

The rings, after repeated rinses, were equilibrated for a further 30 min in the presence of antagonists. To exclude adrenergic effects of serotonin (9), all experiments were conducted in the presence of propranolol (10^{-6} M) and prazosin (5×10^{-7} M). Apyrase (0.67 U/ml) was added to the bath immediately before the addition of platelets; the concentration used here has little or no direct effect on the tension of the rings.

Platelet preparation. The blood of nine healthy nonsmoking male volunteers taking no medications was drawn from the antecubital vein into citrate anticoagulant to yield final concentrations of 9.3 mM sodium citrate/0.7 mM citric acid/14 mM dextrose (10). The blood was centrifuged for 40 min at 100 *g* at room temperature and the platelet-rich plasma was pipetted off. An equal volume of cold citrate anticoagulant solution (93 mM sodium citrate, 7 mM citric acid, 105 mM dextrose, and 5 mM KCl, pH 6.5) was added to the platelet-rich plasma, and the mixture was centrifuged for 20 min at 500 *g*. The supernatant was discarded, and the remaining platelet pellet was resuspended in a small volume of the second citrate anticoagulant mixture and kept on ice until use. A platelet count of this suspension was then obtained (Coulter Electronics, Inc., Hialeah, FL), and the volume of the suspension was adjusted so that when added to the organ chamber (in a dilution of 1:40 or higher) the resulting platelet concentration in the bath was 70,000/μl. When added to the organ chambers, the platelets aggregated upon exposure to glass, the collagen of the cut vessel surfaces, and the calcium in the Krebs-Ringer solution, while being stirred with bubbling by the gas mixture. Completion of aggregation was evidenced in all cases by clearing of the initially turbid solution and formation of visible platelet clumps and strands. In some cases, the platelets were incubated in the presence of the selective inhibitor of thromboxane synthetase, dazoxiben (3.7×10^{-3} M; 11), for 40 min before addition to the organ bath. The resultant

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bath concentration of dazoxiben ($\sim 10^{-4}$ M) when added by itself caused no changes in tension of quiescent rings ($n = 6$).

Drugs. The following drugs were used: acetylcholine chloride (Sigma Chemical Co., St. Louis, MO), apyrase (ATPase and ADPase; Grade V from potato, Sigma Chemical Co.), dazoxiben HCl (Pfizer, Inc., Groton, CT), methiothepin maleate (Hoffmann-La Roche, Inc., Nutley, NJ), prazosin HCl (Pfizer, Inc.), DL-propranolol (Sigma Chemical Co.), prostaglandin $F_{2\alpha}$ (Sigma Chemical Co. or Upjohn Co., Kalamazoo, MI), serotonin creatinine sulfate (Sigma Chemical Co.), sodium pentobarbital (Fort Dodge Laboratories, Fort Dodge, IA) and [1S-[1 α ,2 β (5Z),3 β ,4 α]]-7-[3-[[2-[(phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo [2.2.1] hept-2-yl]-5-heptenoic acid (SQ 29548; E. R. Squibb and Sons, Inc., Princeton, NJ). Prazosin was dissolved in dimethylsulfoxide (final bath concentration, 3.5×10^{-5} M) and then diluted with distilled water. SQ 29548 was dissolved in ethanol (final bath concentration, 6.6×10^{-4} M) and diluted in 2 mM Na_2CO_3 (final concentration, 7.6×10^{-7} M) and then in distilled water. Apyrase was suspended in control solution and added to organ baths at a concentration of 10 U ADPase and 7.5 U ATPase activity per 15 ml (as defined by supplier, 1 U activity liberates $1 \mu\text{mol PO}_4/\text{min}$). Other drugs were dissolved in distilled water. Drug concentrations are reported as the final molar concentration in the bath (and in the case of dazoxiben, in the platelet suspension); apyrase concentration is reported as the ADPase activity.

Statistical analysis. Data are expressed as means \pm SEM. In each series, n refers to the number of dogs from which vessels were taken; in most cases a different platelet-donor was used with each dog. When vessels were contracted with prostaglandin $F_{2\alpha}$, changes in tension induced by platelets are expressed as a percentage of the prostaglandin-induced tension. When platelets were added under basal conditions, changes in tension were expressed as a percentage of the contraction produced in the same ring by raising the concentration of KCl in the bath solution by 40 mM. Relaxations can go below basal tension because the canine coronary artery exhibits some intrinsic tone. Time-course analysis was performed by measuring the tension in each ring every 30 s over the 5 min after addition of platelets; the mean response at each interval was then calculated, and time-courses generated under different experimental conditions were compared by paired t test.

Cumulative concentration-response curves to serotonin in the presence or absence of antagonists were compared by calculating the EC_{50} s for each ring (i.e., the concentration of serotonin producing half its maximal contraction) and taking their geometric mean. Maximal contractions were also compared.

Statistical comparisons were performed by paired two-tailed Student's t test (or by two-way random-block-design analysis of variance if more than two groups were compared), since in all cases rings obtained from the same dogs were studied in parallel. If parametric testing was precluded by significant variance inhomogeneity (as indicated by Bartlett's test), a signed-rank test was used. Significance was accepted at the 0.05 level.

Serotonin determination. Samples of fluid were withdrawn from the organ baths 5 min after addition of platelets. 0.5 ml of the fluid was added to 120 μl of cysteine (1% by weight in distilled water) and proteins precipitated by adding ZnSO_4 and NaOH and centrifuging at 3,000 g for 30 min at 4°C . The resulting supernatant was frozen until analysis. On the day of analysis, the supernatant was filtered through centrifugal microfilters (Bioanalytical Systems, Inc., West Lafayette, IN) with regenerated cellulose membranes (0.2- μm pore size). The amine in the resulting supernatant was quantitated by reverse-phase high pressure liquid chromatography with electrochemical detection (12).

Thromboxane B_2 determination. 1-ml aliquots of the fluid collected 5 min after addition of platelets were centrifuged (3,000 g , 10 min, 4°C) and frozen until analysis. They were then brought to pH 3.5 with 1 N hydrochloric acid. Thromboxane B_2 was extracted using octadecylsilyl columns (Bond Elut C-18; Analyti-Chem International, Harbor City, CA) by the method of Powell (13). Further purification was accomplished by eluting the samples with 2 ml ethyl acetate onto silica columns (Bond Elut Si; Analyti-Chem International). After washing with 2 ml 80:20 benzene/ethyl acetate, thromboxane B_2 was eluted with 4 ml 60:40:40 benzene/ethyl acetate/methanol and evaporated to dryness in a 37°C

water bath under nitrogen. 100- μl aliquots of standards and diluted samples were assayed by displacement of [^3H]thromboxane B_2 (New England Nuclear, Boston, MA) from thromboxane B_2 -antiserum (Seragen, Boston, MA) in a total incubation volume of 300 μl at 4°C . After addition of 1% dextran/1% charcoal (600 μl) and centrifugation (3,000 g , 5 min, 4°C), the supernatant containing the antibody-bound [^3H]thromboxane B_2 fraction was counted in a scintillation counter, and the concentration of thromboxane B_2 was estimated by comparison with a standard curve (14).

Results

Basal tension. Unstimulated rings of coronary artery without endothelium contracted on exposure to aggregating platelets, reaching a peak tension which averaged $26.8 \pm 9.4\%$ of the tension induced by KCl (40 mM) (Figs. 1, 2). Peak tension was

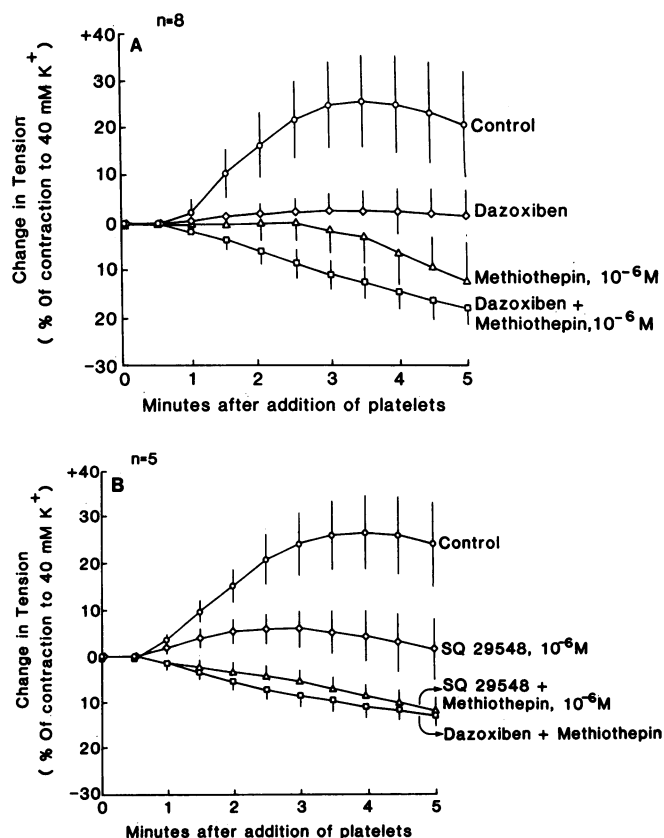


Figure 1. Time course of the tension response of rings of canine coronary artery without endothelium to the addition of human platelets (70,000/ μl) in control solution and in the presence of inhibitors of serotonin and of thromboxane A_2 . Changes in tension are expressed as a percentage of the contraction of the rings to 40 mM K^+ . (A) Mean contraction to 40 mM KCl for control group, 5.3 ± 0.9 g ($n = 8$); other groups did not differ significantly. The difference between the responses of control rings and rings in the presence of methiothepin (10^{-6} M) was significant at 2.5 and 3 min; between control rings and rings exposed to dazoxiben-treated (3.7×10^{-3} M) platelets, at 2–4.5 min; between rings in the presence of methiothepin and rings exposed to the combination of methiothepin plus dazoxiben-treated platelets, at 2.5 min; and between rings exposed to dazoxiben-treated platelets and rings exposed to methiothepin plus dazoxiben-treated platelets, at all points after 1 min. (B) Mean contraction to 40 mM KCl for control group, 6.8 ± 1.3 g ($n = 5$); other groups did not differ significantly. The difference between the responses of control rings and rings in the presence of SQ 29548 (10^{-6} M) was significant at 3–5 min.

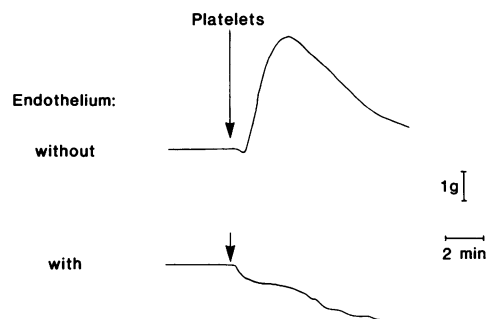


Figure 2. Traces of isometric tension recordings of two rings of the same canine coronary artery. In the ring from which the endothelium had been removed (*top*), aggregating human platelets ($70,000/\mu\text{l}$) induce a marked contraction. In the ring with endothelium (*bottom*), a relaxation of the vessel occurs.

reached an average of 3.5 min after addition of the platelets, which was followed by a gradual decline in tension.

The 5HT_1 - and 5HT_2 -serotonergic antagonist (15), methiothepin (10^{-6} M), present in the bath solution significantly depressed the contraction in rings without endothelium. Treatment of the platelets with the selective thromboxane synthetase inhibitor (16), dazoxiben, also significantly inhibited the platelet-induced contraction. In combination, methiothepin plus dazoxiben caused a significantly greater inhibition of the platelet-induced contraction than either methiothepin or dazoxiben alone. Methiothepin or the combination of methiothepin plus dazoxiben converted the response of endothelium-denuded rings from a contraction into a gradual relaxation (Fig. 1 A). Likewise, the thromboxane receptor antagonist (17) SQ 29548 (10^{-6} M) in the organ bath significantly inhibited platelet-induced contractions, and in combination with methiothepin (10^{-6} M) caused a gradual decline in tension upon addition of platelets which was nearly identical to that produced by dazoxiben and methiothepin (Fig. 1 B).

Addition of the platelet suspension to the organ bath is accompanied by foam formation. If the active tone of denuded coronary rings was eliminated by relaxation with papaverine (3×10^{-4} M), generation of foam by the addition of dazoxiben-treated platelets in the presence of methiothepin caused gradual decreases in measured tension qualitatively similar to those seen in rings at basal tension not treated with papaverine (data not shown).

Concentration-response curves to serotonin. Cumulative addition of serotonin to the organ bath (10^{-9} to 10^{-4} M) caused contraction of coronary rings without endothelium, with a maximal tension $\sim 20\%$ of that produced by KCl (40 mM). This contraction was not significantly affected by the presence of dazoxiben (10^{-4} M) in the organ bath but was slightly reduced by SQ 29548 (10^{-6} M) though this was not significant below 10^{-5} M serotonin (Fig. 3). The concentration-response curve to serotonin was shifted approximately 230-fold to the right by methiothepin (10^{-6} M) (Fig. 3).

Contracted rings. Quiescent rings of coronary artery with endothelium showed an immediate relaxation below basal tension on exposure to aggregating platelets (Fig. 2). To better study these relaxations, rings were contracted with prostaglandin $\text{F}_{2\alpha}$ (2×10^{-6} M).

Rings without endothelium consistently showed a transient further increase in tension after exposure to aggregating platelets;

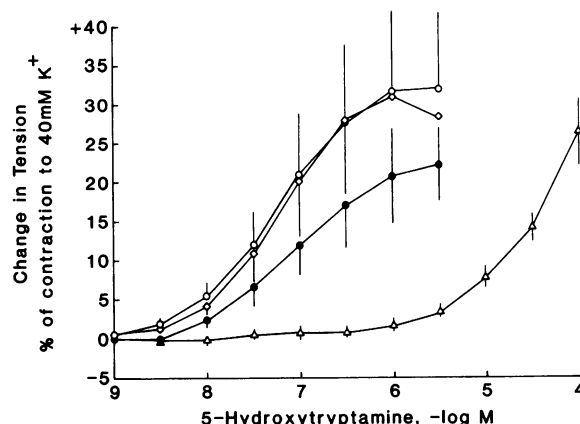


Figure 3. Cumulative concentration-response curves for rings of canine coronary artery without endothelium to serotonin (5-hydroxytryptamine). Tension is expressed as a percentage of the contractile response of each ring to KCl (40 mM). The control curve (\circ ; $n = 13$) is pooled from controls for experiments with dazoxiben (\bullet ; 10^{-4} M) and methiothepin (\triangle ; 10^{-6} M) ($n = 8$) and from experiments with SQ 29548 (\diamond ; 10^{-6} M) ($n = 5$). Statistical comparisons are between paired responses. The EC_{50} for serotonin was highly significantly increased by treatment of rings with methiothepin (10^{-6} M) but neither the EC_{50} nor the maximal response was significantly affected by treatment of the rings with dazoxiben (10^{-4} M) or with SQ 29548 (10^{-6} M).

no acute relaxations to platelets were observed in endothelium-denuded rings (Figs. 4, 5). Rings of coronary artery with endothelium exhibited a marked acute relaxation on exposure to aggregating platelets, which was typically maximal within the first 2 min after addition. Neither the contraction to prostaglan-

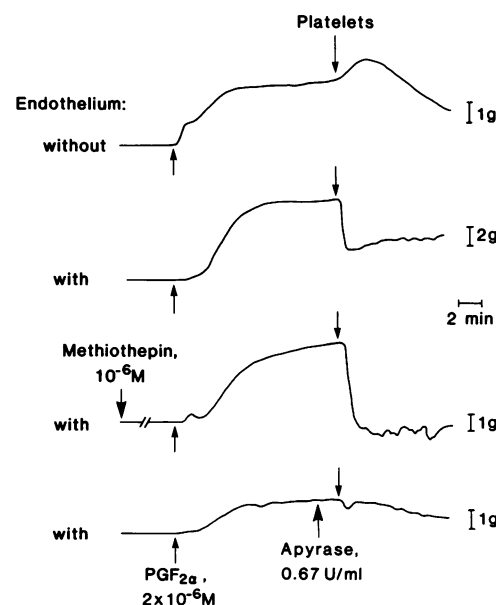


Figure 4. Traces of isometric tension recordings of rings of canine coronary artery taken from the same vessel. Human platelets ($70,000/\mu\text{l}$) are added to the organ bath after stabilization of contraction to prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$, 2×10^{-6} M). From top to bottom: ring denuded of endothelium; control ring with intact endothelium; ring with intact endothelium, in the presence of methiothepin (10^{-6} M); ring with intact endothelium, in the presence of apyrase (0.67 U/ml).

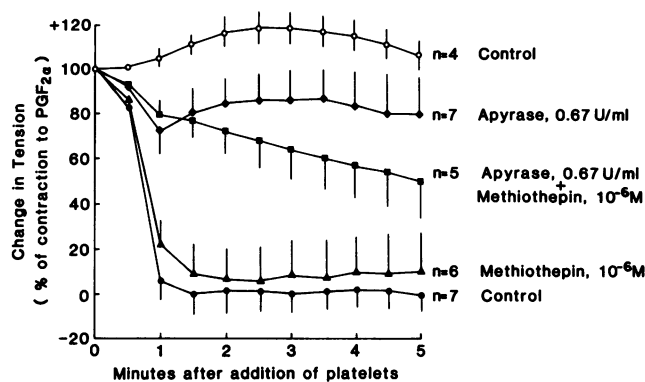


Figure 5. Time course of the tension response of rings of canine coronary artery with (♦, ■, ▲, ●) and without (○) endothelium to the addition of human platelets (70,000/ μ l). Changes in tension are expressed as a percentage of the contraction of the rings to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 2×10^{-6} M). Mean contraction to $PGF_{2\alpha}$ of control group = $42 \pm 5\%$ of response to 40 mM KCl; other groups did not differ significantly. All rings were taken from seven dogs; in each case, significance was determined by paired *t* test between appropriate subgroups of paired rings taken from the same animals. The difference between the responses of control rings with and without endothelium was significant at all points; between control rings with endothelium and rings treated with apyrase, at all points except 0.5 min; between control rings with endothelium and rings treated with methiothepin, at no points; and between rings treated with apyrase and rings treated with apyrase plus methiothepin, at no points.

din $F_{2\alpha}$ ($n = 10$) nor the relaxation to platelets ($n = 6$) was affected significantly by the serotonergic antagonist, methiothepin (10^{-6} M), in the bath solution (Figs. 4, 5). However, the platelet-induced relaxation was significantly inhibited by apyrase (0.67 U/ml), an ADPase and ATPase. The effect of the combination of apyrase plus methiothepin was not significantly different from that of apyrase alone (Figs. 4, 5).

The platelet-induced relaxation was significantly augmented by treatment of the platelets with dazoxiben (3.7×10^{-3} M) (Fig. 6).

Assay of serotonin. Aggregating platelets released serotonin into the bath fluid, at a concentration of 35.2 ± 2.5 ng/ml ($\sim 2 \times 10^{-7}$ M), measured 5 min after addition of platelets. The release of serotonin was not significantly inhibited by either the presence of methiothepin (10^{-6} M) in the bath fluid, the incubation of the platelets with dazoxiben (3.7×10^{-3} M), or both (Fig. 7, top).

Assay of thromboxane. Thromboxane B_2 levels measured in the bath fluid were 2.2 ± 0.5 ng/ml (5.9×10^{-9} M) 5 min after addition of platelets. The release of thromboxane was not affected by the presence of methiothepin (10^{-6} M) in the bath, but was significantly reduced by incubation of the platelets with dazoxiben (3.7×10^{-3} M) (Fig. 7, bottom).

Discussion

This study shows that human platelets trigger contraction of canine coronary arterial rings from which the endothelium has been removed. Two substances are released by aggregating platelets that are known contractile agents in certain vascular smooth muscle preparations: serotonin and thromboxane A_2 . In particular, serotonin can contract isolated canine coronary arteries (e.g., 2, 4, 18, 19). Thromboxane A_2 released from plate-

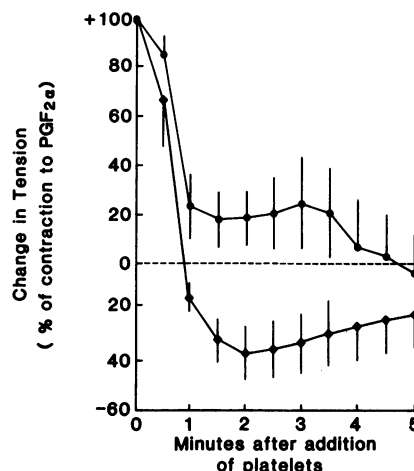


Figure 6. Time course of the tension response of paired rings with endothelium to the addition of human platelets (70,000/ μ l) to the organ bath. Rings are first contracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 2×10^{-6} M) and subsequent changes in tension are expressed as a percentage of that contraction. Mean contraction to $PGF_{2\alpha}$ of control group = $59 \pm 9\%$ of response to 40 mM KCl; response of dazoxiben-treated rings did not differ significantly. The difference between responses to dazoxiben-treated (—♦—) (3.7×10^{-3} M) and control platelets (—○—) is significant at 1–2 min ($n = 5$).

lets induces contraction of porcine coronary arteries (5); the thromboxane mimetics, U44069 and U46619, activate the smooth muscle of the canine coronary artery (19, 20). The pres-

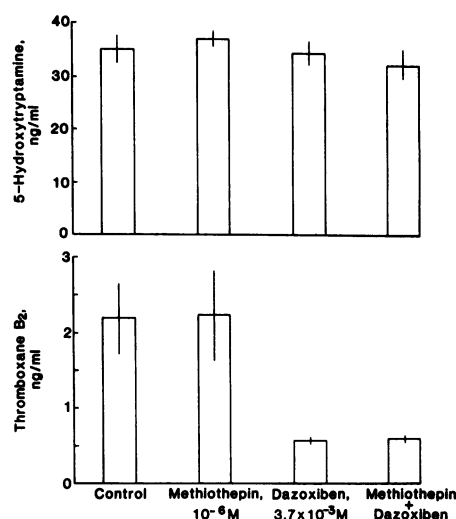


Figure 7. Top, Release of serotonin from human platelets, as determined by high performance liquid chromatography assay of samples withdrawn from the organ bath 5 min after addition of platelets (70,000/ μ l); $n = 6$. Concentrations of serotonin (5-hydroxytryptamine) were not significantly different in any of the four groups. "Methiothepin" indicates the presence of methiothepin (10^{-6} M) in the organ bath at the time of platelet addition; "dazoxiben" indicates incubation of the platelets with dazoxiben (3.7×10^{-3} M) before addition to the bath. Bottom, production of thromboxane B_2 by aggregating human platelets, as determined by radioimmunoassay of the same samples withdrawn from the organ bath 5 min after addition of platelets (70,000/ μ l); $n = 6$. Concentrations of thromboxane were not significantly affected by methiothepin (10^{-6} M) in the bath but were significantly and markedly suppressed by treatment of the platelets with dazoxiben (3.7×10^{-3} M).

ent study shows that both serotonin and thromboxane A₂ are released during aggregation of human platelets under the experimental conditions imposed, and that both contribute to the contractions they evoke in coronary rings; Moulds et al. (21) reached similar conclusions with human digital arteries. The concentration of serotonin measured in the bath in our experiments is above the EC₅₀ determined for the contraction to the monoamine. Further evidence that serotonin contributes to the contraction to human platelets is provided by the observation that the serotonergic antagonist, methiothepin (at a concentration that prevented the contractile response of coronary rings to a concentration of serotonin equivalent to that found in the bath, but did not inhibit the release of thromboxane from the platelets), inhibited the contractile response to platelets.

Evidence pointing to a contribution by thromboxane A₂ to the platelet-induced contraction is that treatment of the platelets with the selective thromboxane synthetase inhibitor, dazoxiben (at a concentration that markedly reduced the generation of thromboxane B₂), also decreased the contractile response of endothelium-denuded rings to aggregating platelets. This could not be attributed to a nonspecific effect on the release of serotonin or on the responsiveness of the vessels to the monoamine, since neither of these was affected by dazoxiben. Dazoxiben did not inhibit platelet aggregation in this system, to judge from the fact that dazoxiben-treated aggregating platelets induced a greater (rather than lesser) endothelium-dependent relaxation than control platelets; this observation suggests that the direct constrictor effect of thromboxane A₂ weakly opposes the endothelially mediated relaxation.

Confirmation of the role of thromboxane A₂ in the platelet-induced contraction is provided by SQ 29548. This compound selectively antagonizes thromboxane receptors, but does not inhibit ADP-induced aggregation of human platelets or the formation of thromboxane A₂ by human platelet membrane preparations (17). At a concentration which did not significantly depress contractile responses to serotonin, SQ 29548 inhibited the platelet-induced contraction of endothelium-denuded coronary rings. The degree of inhibition was similar to that caused by dazoxiben-treatment of the platelets. Likewise, the degree of inhibition caused by the combination of SQ 29548 and methiothepin was almost identical to that caused by dazoxiben plus methiothepin.

The modest, gradual loss of tension of rings without endothelium in the presence of combined thromboxane and serotonergic inhibition could reflect the release of an as-yet unidentified vasodilator substance by the platelets. However, it may as well reflect an artifact of the experimental system. Traction exerted by rising foam on the thread connecting the vessels to the force transducer would unload the transducer, as suggested by similar platelet-suspension-induced declines of measured tension in rings treated with papaverine to eliminate active tone.

This study shows that human, like canine platelets (1–3, 22), can induce an endothelium-dependent relaxation of isolated coronary arteries. Earlier studies (1, 2, 22) suggested that serotonin, which can induce endothelium-dependent relaxations in the canine coronary artery (19), is released from canine platelets and might contribute to the endothelially mediated relaxation. Since human platelets contain less than half the secretable serotonin that canine platelets do (6), it seems unlikely that the monoamine would be a major contributor to the endothelium-dependent relaxation to human platelets. In confirmation of this, methiothepin, a serotonergic antagonist that at the concentration

used abolishes the endothelium-dependent relaxation of coronary arterial rings to serotonin (3, 23), failed to affect the relaxation induced by human platelets.

Another substance released from aggregating platelets that can induce endothelium-dependent relaxations is platelet-activating factor; however, such relaxations occur at concentrations of the substance that are unlikely to occur in vivo (24). More probable candidates as the mediators of this relaxation are the adenine nucleotides, ATP and ADP. These nucleotides can induce potent, endothelium-dependent relaxations in canine femoral and coronary arteries (3, 8). They appear to be the principal mediators of the relaxation of coronary arteries induced by canine platelets (3). Human platelets contain more than twice the total content of adenine nucleotides (ATP plus ADP) that canine platelets do (6). Apyrase is an enzyme that hydrolyses ATP and ADP to AMP and adenosine (the latter two products being much less potent relaxing agents of large coronary arteries) and consequently inhibits the endothelium-dependent relaxation of rings of coronary artery to exogenous ADP without affecting responses to serotonin (3). Apyrase present in the organ chamber at the time of platelet aggregation nearly abolished the human platelet-induced relaxation. The modest, transient relaxation persisting in the presence of apyrase is most readily explained by ADP and ATP temporarily escaping degradation by the enzyme, as this residual relaxation is not prevented by methiothepin. These findings strongly suggest that adenine nucleotides are the principal mediators of the endothelium-dependent relaxation of coronary arterial rings induced by human platelets.

The nature of the signal for relaxation transferred from the endothelium to the smooth muscle in response to stimulation by ADP or ATP (or to stimulation by a variety of other pharmacological agents) remains unknown. Bioassay experiments have suggested that it is a diffusible factor (25). It is apparently not prostacyclin or another cyclo-oxygenase or lipoxygenase product since, at least in the canine femoral artery, ATP-induced relaxations are not inhibited by indomethacin, 5-*S*-11-14-eicosatetraenoic acid (ETYA), or quinacrine (26). Likewise, in the coronary artery, relaxations to adenine nucleotides released by aggregating canine platelets are not inhibited by meclofenamate (3).

Thus, a variety of vasoactive substances released from aggregating human platelets can be identified: thromboxane A₂ and serotonin, which favor contraction, and the adenine nucleotides (ATP and ADP) which favor relaxation in the presence of endothelial cells. In the coronary artery, the balance of their effects depends on the presence or absence of endothelium. In its presence, the net effect is relaxation, whereas if it is removed or damaged, contraction ensues. We therefore speculate that coronary vasospasm may be, in at least some instances, due to endothelial dysfunction. In one animal model, intact endothelium reduced vasoconstriction to serotonin in blood-perfused canine coronary arteries (27); in another, intact endothelium prevented the development of platelet-induced coronary spasm in fluorocarbon-perfused isolated rabbit hearts (28). Our results suggest that blockade of the synthesis by platelets of thromboxane A₂, or of the appropriate vascular serotonergic or thromboxane receptors, may be of therapeutic benefit in coronary vasospastic disease; both may be required for full effect.

Acknowledgments

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