Physiological Concentrations of Epinephrine Potentiate Thromboxane A₂ Release from Platelets in the Isolated Rat Heart

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SUMMARY. The isolated rat heart perfused with washed platelets was used as a model to examine platelet-vessel wall interactions. Release of prostacyclin and thromboxane A₂ was measured, using a cascade of smooth muscle bioassay tissues or radioimmunoassays of the stable hydration products. In hearts perfused with rabbit or human platelets, injection of sodium arachidonate caused release of both prostacyclin and thromboxane A₂. In hearts perfused with aspirin-pretreated platelets, arachidonate released only prostacyclin indicating that thromboxane A₂ originates largely in the platelets. Infusion of epinephrine (0.6–6 nmol/liter) through the heart potentiated arachidonate-induced release of thromboxane A₂. Similar potentiation of thromboxane A₂ release was observed in rat hearts perfused with either rabbit or human platelets, and in rabbit hearts perfused with rabbit platelets. In contrast, when rabbit platelets were infused through an incubation coil of tubing in place of the heart, epinephrine did not alter thromboxane A₂ release. There was no significant loss of rabbit platelets on perfusion through rat hearts, and no aggregates were observed in the effluent either before or immediately after arachidonate injections, even in the presence of epinephrine. Thus, potentiation of thromboxane A₂ production could not be explained by aggregation. However, it is clear from these studies that physiological concentrations of epinephrine can potentiate thromboxane A₂ release from platelets when they are stimulated by arachidonic acid within the heart. This could result from a redirection of arachidonate metabolism to a local potentiating factor in the vessel wall. Potentiation of thromboxane A₂ release might contribute to myocardial ischemia associated with platelet activation. (Circ Res 58: 172–176, 1986)

EPINEPHRINE induces direct aggregation of human platelets at concentrations in the micromolar range (O’Brien, 1963) and potentiates platelet aggregation to other agonists such as adenosine diphosphate (Ardlie et al., 1966) at somewhat lower concentrations (threshold 50–100 nmol/liter). However, the lowest concentrations at which epinephrine interacts positively with other agonists in platelet-rich plasma are at least two orders of magnitude higher than the normal blood concentrations, and at least one order of magnitude higher than concentrations achieved during pathological stress (Grant and Scrutton, 1980). It has also been reported that platelet aggregation in vivo may be enhanced under conditions that result in elevated circulating levels of catecholamines, including exercise-induced stress (Siess et al., 1982), tobacco smoking (Grignani et al., 1977), and hypertension (Vlachakis and Aledort, 1980). We have studied the effect of very low concentrations of epinephrine on thromboxane A₂ (TxA₂) release from platelets when they have the opportunity to interact with blood vessel walls. The model we chose was the rat isolated heart, perfused by the Langendorff technique with washed platelets.

Methods

Preparation of Platelets

Platelets obtained from anesthetized rabbits, or 3-day-old human platelet concentrates, were isolated and washed by the method of Vargas et al. (1982). The platelet concentrations of these preparations ranged between 3.5 and 5.0 x 10⁹ platelets/µl. They were stored in Tyrode’s solution containing bovine serum albumin (BSA) (3 mg/ml) at 4°C for 24 hours before use. In some experiments, rabbit platelets were incubated with aspirin (1 mmol/liter) for 60 minutes at 37°C, centrifuged at 600 g and resuspended so that the preparation was aspirin-free when used to perfuse hearts.

Perfused Hearts and Bioassay Tissue

Female Sprague-Dawley rats (200–250 g) were anesthetized with diethylether/oxygen mixture and given heparin (2.0 U/g), intravenously. Hearts were removed, placed in ice-cold Krebs-Henseleit solution, and mounted rapidly on a Langendorff perfusion apparatus. The Krebs-Henseleit solution contained, in mmol/liter: NaCl, 118; KCl, 4.6; CaCl₂, 2.5; MgSO₄, 7H₂O, 1.2; NaHCO₃, 25.0; KH₂PO₄, 1.2; and glucose, 11.0, and was gassed with 5% carbon dioxide in oxygen. The hearts were perfused at a
constant flow rate of 10 ml/min with Krebs’ buffer at 37°C and maintained in a water-filled, temperature-controlled jacket. Peak systolic tension and heart rate were recorded from a strain gauge transducer attached to the apex of the ventricle (Nayler et al., 1984). In four experiments, hearts obtained from male rabbits (2–3 kg) were used, and they were perfused similarly at a flow rate of 40 ml/min.

Bioassay Tissues and Radioimmunoassay

Effluent from the heart was directed over a cascade of smooth muscle bioassay tissues including pig and bovine coronary artery and rat stomach strip. The tissues were superfused with a mixture of phenoxybenzamine (0.3 μmol/liter), propranolol (6 μmol/liter), and ketanserin (10 μmol/liter) to abolish any direct effects of catecholamines or serotonin (Dusting and Nolan, 1981). Indomethacin (10 μmol/liter) was also continuously superfused over the tissues.

In another series of experiments, the effluent from eight rat hearts was collected into tubes containing indomethacin (28 μmol/liter), for subsequent radioimmunoassay of thromboxane B₂ and 6-keto-prostaglandin F₁₋ (6-keto-PGF₁₋) (Dusting and Nolan, 1981). Full details of the radioimmunoassay have been published elsewhere (Nolan et al., 1981).

Experimental Procedure

Rabbit or human platelets were infused into the aortic cannula at a flow rate of 0.1 ml/min, to give a final platelet concentration in the perfusate of between 3.5 and 5.0 × 10⁵ platelets/ml for the rat hearts, and 0.9 to 1.3 × 10⁶ platelets/ml for rabbit hearts. Sodium arachidonate was injected into the aortic cannula below the platelet infusion line allowing approximately 5 seconds contact time with the platelets before they reached the heart. Epinephrine could also be infused into the aortic cannula between the platelet infusion line and the site of arachidonate injection, allowing approximately 8 seconds contact with the platelets before reaching the heart.

Responses of the bioassay tissues to prostanoid release were recorded with a Grass model 7D polygraph. The effluent from six rat hearts was collected during infusion of rabbit platelets, for 30-second periods before and immediately after arachidonic acid administration, in both the absence and presence of epinephrine infusion. The number of platelets in each sample was counted 5 times in a hemacytometer chamber immediately after collection, and examined microscopically for any platelet aggregates.

Drugs

Suppliers: aspirin (Fawns and McAllan); arachidonic acid (Sigma); epinephrine hydrochloride (David Bull Laboratories); prostacyclin and 15s-hydroxy-11a-9a-epoxy-methano-prostaglandin A₂ (U46619, Upjohn). All reagents were used of analytical grade, and drug solutions were freshly prepared. Arachidonic acid was dissolved in Na₂CO₃ (0.1 mol/liter) to make the sodium salt. Prostacyclin was dissolved in Na₂CO₃ (0.01 mol/liter, pH 10) and diluted with Tris buffer (0.05 mol/liter, pH 7.5) immediately before use. Epinephrine was dissolved in normal saline (0.15 mol/liter) containing sodium ascorbate (0.023 mol/liter) to retard oxidation. Other substances were dissolved in normal saline.

Results

Rat Hearts

Injection of sodium arachidonate (5–20 μg) into Krebs-perfused rat hearts, caused the release of prostacyclin, as indicated by relaxation of the superfused bovine coronary artery (Fig. 1) and the 5-fold increase of 6-keto-PGF₁₋ in the effluent (from 0.5 ± 0.2 to 2.4 ± 0.4 ng/ml). In Krebs-perfused hearts, there was no bioassay evidence for TXA₂ release. TXB₂ in the effluent was barely detectable, and it increased only 2-fold with arachidonate injection (from 0.08 ± 0.02 to 0.16 ± 0.03 ng/ml). In contrast, in platelet-perfused hearts, arachidionate (5–20 μg) caused a biphasic effect on bovine coronary artery (contraction followed by relaxation), and contraction of pig coronary artery, indicating release of both TXA₂ and prostacyclin (Fig. 1). After arachidonate injection, 6-keto-PGF₁₋ in the effluent achieved levels in platelet-perfused hearts (from 0.8 ± 0.1 to 2.6 ± 0.3 ng/ml) that were similar to those in Krebs-perfused hearts, whereas TXB₂ increased 7-fold in the platelet-perfused heart (from 0.10 ± 0.01 to 0.69 ± 0.09 ng/ml). These data indicate that, in this system, prostacyclin is released only from the heart, whereas TXA₂ is produced mainly by the perfusing platelets. Furthermore, in four hearts that were perfused with platelets that had been preincubated with aspirin (1 mmol/liter for 60 minutes), arachidonate-induced TXA₂ release was abolished, and prostacyclin release was unchanged.

In rat hearts perfused with rabbit washed platelets, infusion of epinephrine (0.6–6 nmol/liter) greatly potentiated the contractions of pig and bovine coronary arteries produced by arachidonate injection (Fig. 2A). Arachidonate-induced TXB₂ release was also significantly potentiated (P < 0.01) by epinephrine (6 nmol/liter). Similar results were obtained in four hearts with two separate batches of washed human platelets. In contrast, when rabbit platelets were perfused through an incubation coil of inert tubing, arachidonate-induced TXB₂ formation was not altered by epinephrine (Fig. 2B). 6-Keto-PGF₁₋ levels were not altered by epinephrine in platelet-perfused hearts, and remained below the detection limit of the assay in the tubing experiments. In addition, epinephrine (6 nmol/liter) had no significant effect on TXB₂ produced by incubating washed rabbit platelets (5 × 10⁷/μl) with arachidionate (30–100 μmol/liter) for 4 minutes at 37°C in vitro.

The lower concentration of epinephrine (0.6
nmol/liter) did not alter significantly the peak systolic tension developed by the hearts, but at 6 nmol/liter, there was a slight increase in developed tension (13.5 ± 1.5 to 15.7 ± 1.6 g). The heart rate declined from 249 ± 10.7 to 219 ± 8.2 beats/min with 0.6 nmol/liter epinephrine, but remained at the resting level with 6 nmol/liter.

During infusion with washed rabbit platelets, the effluent collected from six rat hearts immediately before and after arachidonic acid administration was examined microscopically. Platelet aggregates were not observed in any of the samples, and the platelet counts before and after arachidonate did not differ significantly from those of the platelet concentrate examined before infusion through the hearts (one-way analysis of variance between a sample of platelet concentrate and nine samples of effluent with five counts on each, $F_{9,90} = 0.4, P > 0.05$). No 2

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Prostanoids released by sodium arachidonate (AA) from a buffer-perfused heart (left-hand panels) and from the same heart during perfusion with rabbit platelets (right-hand panels). Bioassay records are from pig coronary artery (PCA), bovine coronary artery (BCA), and rat stomach strip (RSS) treated with antagonists (see text) and superfused, in cascade with the cardiac effluent. AA was injected through the heart (TH), and the thromboxane mimetic (U46619) and prostacyclin (PGI₂) were given directly over the tissues. AA causes release of PGI₂ in the buffer-perfused heart, and both TxA₂ and PGI₂ in the platelet-perfused heart.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Thromboxane release from platelet-perfused hearts in the presence of epinephrine (ADR, 0.6 nmol/liter). Panel A: typical record of a pig coronary artery and TxB₂ release (expressed as percent of control response to arachidonate) from eight perfused hearts. U46619 was given directly over the bioassay tissue, and arachidonate (AA) was injected through the heart (TH). Panel B: similar experiments in which an incubation coil of tubing replaced the rat heart. Control release of TxB₂ 30 seconds after AA injection in the perfused hearts was 1.6 ± 0.7 ng/ml and 2.3 ± 0.5 ng/ml in the perfused tubing. The vertical bars represent SEM.
significant loss of platelets was observed during, and 15 minutes after, infusion of epinephrine (0.6 nmol/liter) through these hearts (Fig. 3).

Rabbit Hearts

Pig coronary artery became very insensitive to U46619 and arachidonate-induced TxA2 release when it was superfused with the effluent from rabbit hearts. However, as in the rat hearts, bovine coronary artery relaxed after arachidonate injection in buffer-perfused rabbit hearts (due to prostacyclin release) and contracted in platelet-perfused hearts (Fig. 4). Arachidonate-induced contractions of bovine coronary artery in these four hearts were potentiated during epinephrine infusion (0.6 nmol/liter), again indicating augmented TxA2 release from the platelets (Fig. 4).

Discussion

The infusion of washed platelets into the Langendorff-perfused rat heart allowed the generation of both prostacyclin and TxA2 in this experimental model. Although resting levels of TxA2 were low, these were markedly elevated after injection of sodium arachidonate. The release of prostacyclin was much higher under resting conditions and was elevated further by the injection of arachidonate. That aspirin pretreatment of the platelets abolished TxA2 release from the system indicates that platelets are the major source of TxA2. Moreover, prostacyclin release was not altered by aspirin treatment of the platelets alone, suggesting that, under these conditions with low platelet number, the platelets do not supply substrate endoperoxide for prostacyclin biosynthesis by the vessel wall.

The major finding in this study was that low concentrations of epinephrine, well within the physiological range of circulating blood levels, can act to potentiate the release of TxA2 from the platelets. By examining microscopically the effluent from platelet-perfused rat hearts, we were unable to detect the loss of platelets during passage through the heart, during arachidonic acid administration, during infusion of epinephrine, or both together. Thus, it is highly unlikely that aggregation of platelets could account for the potentiation of TxA2 release, although we cannot exclude the possibility that some aggregation, although undetectable, may have occurred with these treatments. Certainly, others have found that very high concentrations of epinephrine are required to aggregate washed rabbit platelets directly (Barthel and Markwardt, 1974). Furthermore, the potentiation cannot be explained by interaction of platelets with the vasculature of heterologous species, for it also occurred in rabbit hearts perfused with rabbit washed platelets.

Clearly, the potentiation of TxA2 release by epinephrine is not a direct effect on the platelet, because it did not occur when an incubation coil of tubing replaced the rat heart; neither did it occur in platelets

![Figure 3](image-url)  
**Figure 3.** Histograms showing the number of platelets recovered following infusion of rabbit platelets through 6 rat hearts expressed as percentages of the number infused. The number of platelets counted before and immediately after arachidonate administration (10 μg) did not differ significantly from 100%. Counts are shown before, during, and after infusion of epinephrine (ADR, 0.6 nmol/liter).

![Figure 4](image-url)  
**Figure 4.** Prostanoid release by sodium arachidonate (AA) from a buffer-perfused rabbit heart (lefthand panel) and a rabbit heart perfused with rabbit platelets (righthand panel). Arachidonate causes release of PGI2 in the buffer-perfused heart, and of TxA2 in the platelet-perfused heart. TxA2 release was potentiated during infusion of epinephrine (0.6 nmol/liter) through the heart.
isolated and incubated in vitro. These data suggest that epinephrine can potentiate TxA2 release from the platelet-perfused rat heart only when the platelets are able to interact with the coronary vessels in the presence of arachidonic acid. One explanation of this interesting phenomenon is that potentiation of TxA2 release could require the production of an intermediate potentiating factor by the coronary vessel wall. Indeed, we have recently found that the effluent from perfused hearts contains a stable, nonprostanooid factor that also potentiates thromboxane release when incubated with platelets (Purchase et al., 1985). However, the identity, or cell origin, of this factor, is yet to be determined. It is unlikely that the potentiating action of epinephrine is secondary to inotropic or chronotropic effects on the heart, for these were minimal at the concentrations used. As a working hypothesis, we suggest that the TxA2-potentiating effect of epinephrine is due to enhanced synthesis of a factor within the coronary vessel wall, that, in turn, activates platelet cyclooxygenase. Because epinephrine does not appear to affect vascular cyclooxygenase and prostacyclin production, the possibility that this potentiating factor acts on platelet thromboxane synthetase should also be considered.

Whatever the mechanism, these findings raise the possibility that epinephrine release could be partly responsible for the elevation of coronary sinus TxB2 measured during and after episodes of myocardial ischemia in experimental animals and in man (Coker et al., 1981; Robertson et al., 1981; Hirsh et al., 1981). Elevated TxB2 has also been measured in the coronary artery distal to a site of lumen obstruction in dogs, and here sampling was through a short catheter unlikely to have caused artifactual platelet activation (Bush et al., 1984). It is also known that arachidonate is mobilized in the ischemic myocardium (Chien et al., 1984), and this may be a substrate source for further synthesis of TxA2 by the platelets, and perhaps for a potentiating factor within the vascular tissue. Clearly, our finding of thromboxane potentiation suggests a new interpretation of the deleterious effects of circulating epinephrine released following myocardial ischemia, tobacco smoking, and psychological stress, and the platelet hyperactivity that is observed after myocardial infarction.

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