# REVERSAL OF PLATELET AGGREGATION BY AORTIC MICROSOMES

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SUMMARY

The microsomal fraction of dog aortas inhibited human platelet aggregation induced by arachidonic acid, ADP, or thrombin. When aortic microsomes were added to a preparation of irreversibly aggregated platelets, the aggregates dispersed after 4-6 minutes. The fact that aortic microsomes inhibit platelet aggregation induced by ADP suggests that its effect is probably on the cellular function of platelets and not in direct competition against thromboxane $A_2$.

In human platelets, arachidonic acid is converted by the microsomal cyclo-oxygenase and thromboxane synthetase enzyme complex to thromboxane $A_2$ which causes platelet aggregation (1-3). Recently, Moncada *et al.* (4) reported the presence of an enzyme in arteries that transformed prostaglandin endoperoxides ($PGG_2$ or $PGH_2$)[1] to an unstable substance, designated as PGX that inhibited human platelet aggregation.

Generation of PGX by vessel walls is suggested to be the biochemical mechanism underlying their unique ability to resist platelet adhesion. It is unclear, however, whether the aortic microsomes contain the cyclo-oxygenase enzyme and whether PGX inhibits platelet aggregation induced only by arachidonic acid.

We have discovered that aortic microsomes inhibit platelet aggregation induced by various agents, including arachidonic acid, ADP, and thrombin. We also found that

1 Abbreviations: $PGG_2$, prostaglandin $G_2$; $PGH_2$, prostaglandin $H_2$; PGX, unstable substance synthesized by aortic microsomes; thromboxane $A_2$, unstable platelet-aggregating factor derived from prostaglandin endoperoxides.

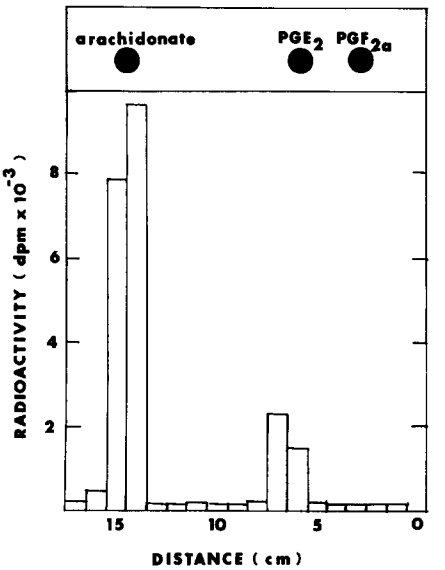


Fig. 1. Thin-layer radiochromatogram of the product obtained after incubation of $^{14}$C-arachidonic acid as described in the text. Solvent system = chloroform:methanol:acetic acid (90:5:5 v/v/v).

aortic microsomes redispersed platelets after complete aggregation had occurred.

## MATERIALS AND METHODS

Dog aortas were stripped of adventitia, snap-frozen in liquid nitrogen, crushed into a fine powder, resuspended (1:10 w/v) in 0.05 M Tris buffer (pH 7.5), and homogenized at high speed in a Virtis 45 homogenizer with Turbo shear blades. The lyophilized microsomal powder was obtained according to the method of Moncada et al. (4). The yield averaged 30 mg powder [55.5% protein (5)] per 4 g of aorta tissue. Cyclo-oxygenase and thromboxane synthetase activities were assayed as previously described (3), and the products were separated by thin-layer chromatography. Platelet aggregation was monitored with a Chromolog aggregometer as previously described (2).

## RESULTS

Aortic microsomes (500 µg) were incubated with 5 µM $^{14}$C-arachidonic acid (sp. act. = 50 µc/µmole, New England Nuclear), 50 mM Tris·HCl buffer, pH 8.0, 5 mM L-tryptophan, and 2 µM methemoglobin in a total volume of 0.2 ml. The reaction was performed for five minutes at 37°. The radio-

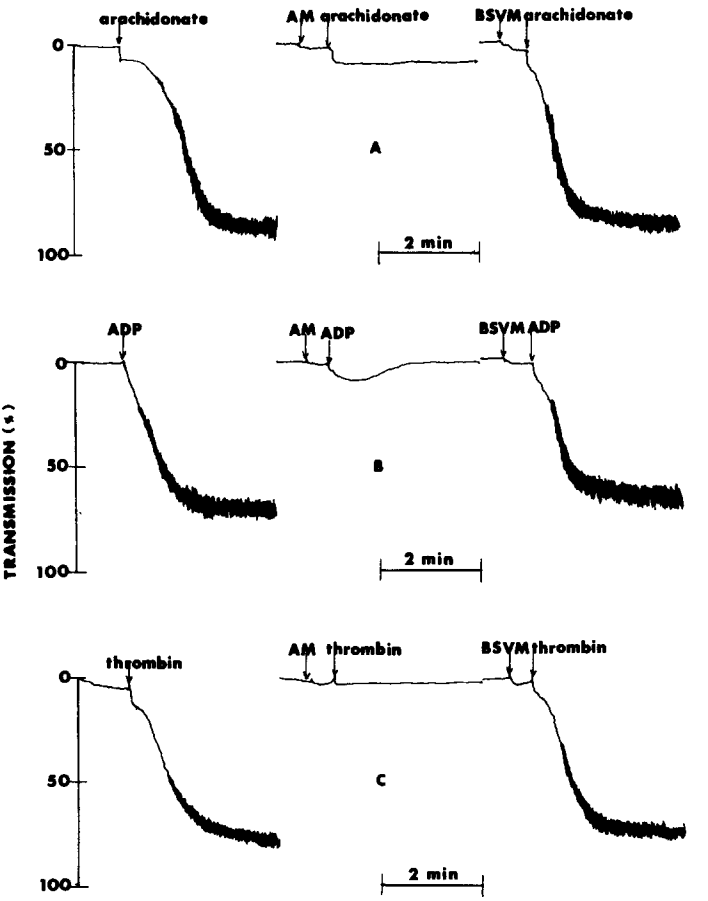


Fig. 2. Platelet aggregation induced by (A) 0.5 mM arachidonic acid, (B) 50 µM ADP, and (C) 0.5 unit/ml of thrombin. The aggregator tube contained 0.5 ml of platelet suspension. At the indicated arrows, 50 µl of microsome suspension (10 mg/ml) containing 5 mM tryptophan and 2 µM methemoglobin were added and followed by the aggregating agent. AM = dog aortic microsomes, BSVM = bovine seminal vesicular gland microsomes.

chromatogram of the products is shown in Fig. 1. An unknown substance that migrated slightly faster than $PGE_2$ was obtained. The chemical characterization of this product is now in progress.

The effect of aortic microsomes on platelet aggregation was examined. Although an initial experiment was performed

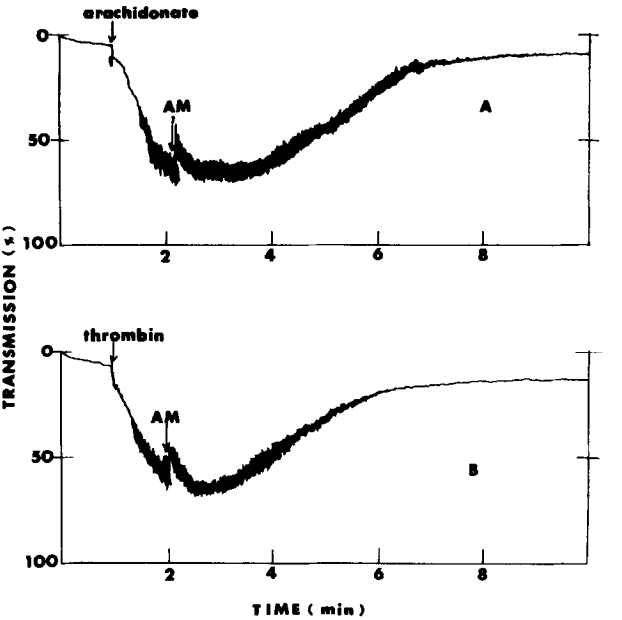


Fig. 3. Dispersion of platelet aggregates by aortic microsome. Aggregations were induced by (A) 0.5 mM arachidonic acid, and (B) 0.5 unit/ml of thrombin. When large aggregates began to form, 50 µl of aortic microsome (AM) suspensions (10 mg/ml) containing 5 mM tryptophan and 2 µM methemoglobin were added.

with a preincubated reaction mixture containing 12.5 µM arachidonic acid, it was subsequently discovered that aortic microsomes alone at 1 mg/ml completely inhibited platelet aggregation induced by 0.5 mM arachidonic acid (Fig. 2). Furthermore, such inhibition was observed with various aggregating agents, which included ADP (50 µM) and thrombin (0.5 unit/ml). Since the aortic microsomes were not preincubated with arachidonic acid to generate PGX, one can speculate that the rate of PGX synthesis from the released arachidonic acid and prostaglandin endoperoxide (6) must be extremely fast. To further demonstrate that such an inhibitory property is unique to aortic microsomes, bull

seminal vesicular microsomes at the same concentration were shown to be of no effect.

The well-documented irreversible aggregation patterns produced by arachidonic acid and thrombin are shown in Fig. 2. When aortic microsomes were added to the preparation of irreversibly aggregated platelets, the aggregates dispersed after 4-6 minutes (Fig. 3). Since the suspension returns to its original turbidity, the dispersion phenomenon is probably not due to a lysis of the aggregated platelets. However, the integrity of the re-dispersed platelets remains to be proven only after electron-microscopic examination.

## DISCUSSION

Although the platelet-aggregating activity of arachidonic acid has been attributed to the enzymic conversion of arachidonic acid by platelet microsomes to thromboxane $A_2$, the mechanism of aggregation induced by ADP has not been associated with either prostaglandin endoperoxides or thromboxane $A_2$ (6). The fact that aortic microsomes [or PGX, which is synthesized from the released arachidonic acid and prostaglandin endoperoxides, as suggested by Moncada *et al.* (4)] can inhibit ADP-induced aggregation and disperse platelet aggregates suggests that its effect is probably on the cellular function of the platelets and not in direct competition against thromboxane $A_2$. Cyclic AMP levels in platelets have been suggested to mediate the inhibition of aggregation (6). Whether aortic microsomes or PGX will elevate cyclic AMP levels remains a subject for further investigation.

Our present data show that not only the endothelial lining of blood vessels possess its own mechanism to prevent platelets from adhering to its surface or to each other but it also is capable of dispersing platelet aggregates.

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