Modification of thrombin-induced platelet aggregation by estrogen. H. Maggs, H. Steiner, and J. Maclachlan. The Memorial Hospital and Bronx, N.Y.A.

The effect of estrogens on platelet function has remained a subject of considerable controversy. Neither in vivo nor in vitro studies have yet established a basis for a possible mode of action of these hormones on platelets. Our studies were prompted by one of us suggesting a direct interaction of estrogens with antithrombin III (AT III). Platelets were isolated by conventional methods from freshly drawn blood of volunteers, washed twice with Ca2+-free Tyrode buffer and finally suspended in medium at a concentration of 4.5 x 10^7 platelets/mL. Aggregation was induced by addition of 0.01 units of purified bovine thrombin (390 NIH units/mg protein). Aggregation was immediate reaching a maximum within 1-2 min. AT III purified from human plasma (2 U/mg protein) inhibited thrombin-induced aggregation in a predictable, concentration-dependent manner. Addition of 0.06 U AT III produced almost complete inhibition. The inhibiting effect of AT III was found to be related to the platelet concentration. Increasing the latter diminished progressively the effect of AT III on thrombin-induced aggregation. Beta-estradiol also inhibited the AT III effect on thrombin-induced aggregation abolishing it at a concentration of 5 x 10^-7M. The minimal concentration of I-estradiol which produced a recognizable effect in this system was 5 x 10^-8M. These results indicate a direct effect of estrogen on AT III, modifying the protein in such a way that subsequent interaction with thrombin either becomes impossible or does not lead to the inactivation of the enzyme. In addition a possible neutralization of AT III by intact platelets is suggested from our data.

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Aspirin, collagen and the collagen-platelet interaction. John C. Whitin and Elizabeth B. Simms. Boston University School of Medicine, Boston, Massachusetts, U.S.A.

While the effect of aspirin (ASA) on platelets has been investigated by many laboratories, the effect of ASA on collagen in collagen-initiated platelet aggregation has not. We report here on the rate of collagen fibril formation in the presence of ASA from 0.1 to 15 mg, and on the subsequent perturbation of platelet aggregation by such agents as, collagen, adenosine diphosphate, and fibrinopeptide A. The kinetics of fibril formation of rat tail tendon collagen are linear at 50°C in the absence of perturbants and as well in the presence of low (0.5mM) concentrations of ASA. Higher (9.7mM) concentrations of ASA lead to an induction period whose length progressively decreases as the concentration of fibrils decreases. This induction period increases gradually, eventually attaining the control level. Fibrils formed in the presence of 10mM ASA contain one 'C acetyl group per collagen x chain. Preliminary experiments with sodium salicylate (Nasa) and acetylsalicylic acid (AI) were also performed. Nasa and Nac did not affect collagen fibril formation. AI, on the other hand, closely resembled ASA in perturbation of fibril formation and of platelet aggregation. The effect on platelets of aliquots of semi-soluble fibrils (as determined by high speed scattering) can be compared. Collagen fibrils formed in the presence of ASA or AI do initiate a platelet response even though collagen NA groups have been acetylated. At high ASA, AI, or Nasa concentrations these measurements were perturbed by the large amount of residual nonreacted reagent in the fibril aliquots. These in vitro results indicate that aspirin treatment slows but does not prevent the formation of collagen fibrils capable of interacting with platelets. The effects may be significant, particularly for collagen newly synthesized in vivo in the process of repair.


Serotonin, PG-endoperoxides and thromboxane A2 released from platelets by collagen for example - contract vascular smooth muscle in vivo. Platelets adhering to subendothelium in vivo undergo the release reaction. Platelet adhesion to subendothelium and concomitant vasoconstriction were therefore measured morphometrically in cross sections of rabbit iliac arteries which had been fixed by perfusion of glutaraldehyde at 110°C for 1 (1) 1.25, 2.5, 5, 10 and 20 min after complete demucosalization of endotethium by balloon catheter injury; (2) 10 min after partial denudation; (3) 10 min after complete demucosalization in rabbits made thromboembolic by injection of a heterologous antibody against platelets. The non-ballooned iliac artery served as control. In vivo operated rabbits the vessel diameter (D) as derived from the length of the internal elastic lamina in cross sections was similar for both iliac arteries. (4) 10 min after demuconisation - when surface coverage with platelets approached 100% and aggregation was maximal - D was reduced by 28±4% (mean±SE, n=20, p<0.001). At 1.25 or 20 min - when few platelets or only degenerated platelets, respectively, adhered - D of balloon and control arteries was again similar.

(2) Localized platelet adhesion caused localized vasoconstriction. The extent of platelet adhesion and vasoconstriction correlated (r=0.46, n=44, p<0.001). (3) In thromboembolic rabbit surface coverage with 25±7% platelets was associated with reduction of D by only 10±3%.

Thus platelets freshly adhering to subendothelium in vivo appear to induce a transient contraction of medial smooth muscle cells during a few minutes.