MODIFICATION OF THROMBIN-INDUCED PLATELET AGGREGATION BY ESTROGEN. H. Naganawa, N. Steiger and M. Ando. The Memorial Hospital and Brown Univ.

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 this hormone 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 method from freshly drawn blood of volunteers, washed twice with Ca²⁺/Mg²⁺-free Tyrode buffer and finally suspended in medium at a concentration of 4.5 x 10⁹ platelets/ml. Aggregation was induced by addition of 0.01 units of purified bovine thrombin (300 NIH units/mg protein). Aggregation was immediate reaching a maximum within 1-2 min. AT III purified from human plasma (2 U/mgprot) 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 10⁻⁹M. The minimal concentration of 1-estradiol which produced a recognizable effect in this system was 5 x 10⁻¹⁰M. 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. Simmons. 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 µM, and on the subsequent aggregation of platelets. Aggregation by aminonaphthylsulfonyl fluoride (SNAF) of fibrillar collagen is inhibited by ASA in a dose dependent manner. The kinetics of fibril formation of rat tail tendon collagen are linear at 30°C in the absence of perturbants as well as in the presence of low (0.5-5.0 µM) concentrations of ASA. Higher (87-250 µM) concentrations of ASA lead to a premature period whose length increases with increasing concentration of ASA. The rate of fibril formation increases gradually, eventually attaining the control level. Fibrils formed in the presence of 1000 U/ml ASA contain one 1-6 acetyl group per collagen w chain. No acetyl groups were detected in fibrils formed in the absence of ASA. Both sodium salicylate (SAL) and aspirin (AI) were also performed. SAL and AI did not perturb 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 plasma at the same concentration as aspirin (AI) interferes with light scattering (able to inhibited by light scattering) can be compared. Collagen fibrils formed in the presence of ASA or AI do not initiate a platelet response even though collagen NAG groups have been acetylated. At high ASA, AI, or NAG concentrations these measurements were perturbed by the large amount of residual unacetylated 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.

PLATELET-MEDIATED VASOCONSTRICTION: EVIDENCE IN VIVO. H.R. Baugartner and Th.B. Tschopp. P. Hoffmann-La Roche & Co., Ltd., Basel, Switzerland

Serotonin, DOP-endo peroxides and thromboxane A₂ - released from platelets by collagen for example - contract vascular smooth muscles in vitro. Platelets adhering to subendothelium in vivo undergo the release reaction. Platelet adhesion to subendothelium and concomitant vasoconstriction were therefore measured morphoanatomically in cross sections of rabbit iliac arteries which had been fixed by perfusion of glutaraldehyde at 110 mm Hg (1) At 1.25, 2.5, 5, 10 and 20 min after complete denudation of endothelium by balloon catheter injury; (2)10 min after partial denudation; (3)10 min after complete denudation in rabbits made thrombocytopenic by injection of a heterologous antibody against platelets. The non-ballooned iliac artery served as control. In shaven operated rabbits the vessel diameter (2) as derived from the length of the internal elastic lamina in cross sections was similar for both iliac arteries. (110)10 min after denudation - when surface coverage with platelets approached 100 % and aggregation was maximal - D was reduced by 20±5 % (mean ± SD, n = 20, zp = 0.001). At 1.25 or 20 min - when few platelets or only degranulated platelets, respectively, adhered - D of balloon and control arteries was again similar. Localized platelet adhesion caused localized vasoconstriction. The extent of platelet adhesion and vasoconstriction correlated (R = 0.64, n = 44, zp = 0.001). In thrombocypoeenic rabbits surface coverage with 20±5 % platelets was associated with reduction of D by only 15±5 %.

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