MODIFICATION OF THROMBIN-INDUCED PLATELET AGGREGATION BY ESTROGEN. H. Nagasawa, M. Steiner and M. Baldini. The Memorial Hospital and Brown University, Providence, Rhode Island, U.S.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 this hormone on platelets. Our studies were predicated on previous results obtained 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 Ca^{2+} -free Tyrode buffer and finally suspended in this medium at a concentration of 4.5×10^5 platelets/ mm^3 . 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 1.5-2 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 $5 \times 10^{-9}\text{M}$. The minimal concentration of β -estradiol which produced a recognizable effect in this system was $5 \times 10^{-9}\text{M}$. 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 R. Simons. 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 mM, and on the resultant perturbation of platelet aggregation by such ASA-treated collagen. 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 ($<7.5\text{mM}$) concentrations of ASA. Higher ($>7.5\text{mM}$) concentrations of ASA lead to an inductive period whose length is a function of ASA concentration. The rate of fibril formation increases gradually, eventually attaining the control level. Fibrils formed in the presence of 10mM ASA contain one ^{14}C acetyl group per collagen α chain. Parallel experiments with sodium salicylate (NaSA), sodium acetate (NaAc) and acetyl imidazole (AI) were also performed. NaSA and NaAc 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 equal size fibrils (as monitored by light scattering) can be compared. Collagen fibrils formed in the presence of ASA or AI do initiate a platelet response even though collagen NH_2 groups have been acetylated. At high ASA, AI, or NaSA concentrations these measurements were perturbed by the large amount of residual unreacted 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. Baumgartner and Th.B. Tschopp, F. Hoffmann-La Roche & Co., Ltd., Basel, Switzerland

Serotonin, PG-endoperoxides and thromboxane A_2 - released from platelets by collagen for example - contract vascular smooth muscle in vitro. 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 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 sham 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. (1) 10 min after denudation - when surface coverage with platelets approached 100% and aggregation was maximal - D was reduced by $28 \pm 2\%$ (mean $\pm$ SE, $n = 20$, $2p < 0.001$). At 1.25 or 20 min - when few platelets or only degranulated platelets, respectively, adhered - D of ballooned and control arteries were again similar. (2) Localized platelet adhesion caused localized vasoconstriction. The extent of platelet adhesion and vasoconstriction correlated ($r = 0.64$, $n = 44$, $2p < 0.001$). (3) In thrombocytopenic rabbits surface coverage with $22 \pm 7\%$ platelets was associated with reduction of D by only $10 \pm 3\%$.

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