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DUAL-AGONIST ACTIVATED PLATELETS:

AN EMERGING COMPONENT OF THE PRIMARY HEMOSTATIC RESPONSE

Platelets activated at sites of vascular injury play two key roles in normal hemostasis. First, by adhering to exposed subendothelium, binding adhesive proteins and aggregating, they create a physical barrier that limits blood loss. Second, platelets accelerate coagulation by providing a surface which promotes two procoagulant reactions, generation of Xa and thrombin. A specific subset of dual-agonist activated platelets described in this thesis may affect both of these roles. Platelets activated simultaneously with collagen and thrombin reveal two distinct populations of activated cells. One population expresses very high levels of several  $\alpha$ -granule born procoagulant proteins including factor V, fibrinogen, von Willebrand factor, fibronectin,  $\alpha_2$ -antiplasmin, and thrombospondin while other does not. Those cells retaining high levels of procoagulant proteins are referred to as COAT-platelets, an acronym for collagen and thrombin activated platelets. These proteins are all transglutaminase substrates, and inhibitors of transglutaminase prevent the production of COAT-platelets. A synthetic transglutaminase substrate (CP<sub>15</sub>) also binds to COAT-platelets, and analysis by high performance liquid chromatography/mass spectrometry shows that a product is formed with a relative molecular mass (Mr) equal to CP<sub>15</sub> plus 176. Serotonin, an abundant component of platelet dense granules, has an Mr of 176, and fibrinogen isolated from COAT-platelets contains covalently linked serotonin. Synthetic bovine serum albumin-(serotonin)<sub>6</sub> binds selectively to COAT-platelets and also inhibits the retention of procoagulant proteins on COAT-platelets. These data indicate that COATplatelets use serotonin conjugation to augment the retention of procoagulant proteins on their cell surface through an as yet unidentified serotonin receptor. Similar subpopulation of platelets can also be generated by the combined stimulation of Fc $\gamma$ RIIA and thrombin receptors. Platelets activated in this manner are referred to as Fc receptor and thrombin-activated platelets, and they share many of the characteristics of the formerly observed COAT-platelets, including aminophospholipid exposure, adhesive and procoagulant protein enrichment, increased frequency among young platelets, and sensitivity to transglutaminase inhibitors