ISOLATION OF PLATELET MEMBRANE COLLAGEN RECEPTOR. A. Silver, R.L. Swanson, H. Schwenzerstahl, and D. Michaeli. University of California, San Francisco, California, U.S.A.

A fraction which competes with intact platelets for interaction with collagen was isolated from human platelets by Sepharose A-collagen (SC) affinity columns, followed by lysis with a nonionic detergent and extensive wash with water (treated SC columns). Reduced the columns' capacity to subsequently bind platelets and induce serotonin release. This reduction was proportional to the number of platelets that had been applied to SC columns. Treated SC columns could be partially regenerated with solutions of high ionic strength (1 M NaCl or Tris- HCl) and most effectively with 0.3M sodium dodecylsulfate (SDS), but not with 5 M urea. 15% ethanol or lipopolysaccharide X-100, indicating an ionic interaction. A fraction eluted with SDS from treated SC columns manifested receptor activity: when rebound to collagen it caused a dose-dependent decline in interaction of the collagen with intact platelets, as measured by binding and serotonin release. The receptor activity was sensitive to heat and was absorbed by an anionic exchange resin. When the membrane of intact platelets were labeled with 125I and iodotyrosine, the derived receptor fraction contained a small proportion (about 1%) of the label.

PLATELET ALPHA-ADRENERGIC RECEPTORS: DIRECT IDENTIFICATION BY [3H] DITHYDROSCORPTYLINE.
L.T. Williams, K.H. Neuman and R.J. Leikowitz. Duke University, Durham, North Carolina. Platelet aggregation induced by epinephrine is an α-adrenergic response which is blocked by α-adrenergic antagonists. We now report the successful identification of human platelet α-adrenergic receptors (AR) by direct binding studies with the potent α-adrenergic antagonist [3H] dithydroscorptyline (DHS). Specific DHS binding to platelet AR was assayed by incubating DHS with platelet lysates for 17 at 25°C. Binding of DHS had the specificity expected of binding to AR. The α-adrenergic agonists (-)epinephrine and (-)norepinephrine (-)-isoepinephrine) displaced the radioligand in a high affinity binding site, causing half-maximal inhibition of DHS binding at a concentration (EC50) of 0.8 μM. A series of α-adrenergic agonists competed for the binding sites in an order of potency (-)epinephrine (EC50 = 0.09 μM) > (-)norepinephrine (EC50 = 0.14 μM) > (-)-isoepinephrine (EC50 = 0.93 μM). Competition for binding sites by α-adrenergic agonists was stereospecific. The (-) stereoisomers of epinephrine and norepinephrine being 7-10 fold more potent than the corresponding (+) stereoisomers. The α-adrenergic antagonist phentolamine inhibited binding with an EC50 of 0.04 μM while α-adrenergic antagonists propranolol, propranolol and dichloroisoproterenol competed only at very high concentrations (10 μM). Other α-adrenergic agents such as phenylephrine (EC50 = 0.02 μM) and clonidine (EC50 = 0.05 μM) also competed for the binding sites. Dopamine (EC50 = 9.4 μM) and serotonin (EC50 = 90 μM) competed for binding at high concentrations. Catecholamine metabolites and structural analogues devoid of α-adrenergic activity did not compete for the binding sites. The results indicate that human platelet AR can be directly labeled and studied with DHS.


Serotonin and dopamine are transported by platelets, and serotonin also stimulates platelets to change shape. En values for transport are around 1 μM for serotonin and 40 μM for dopamine. Both amines also enter platelets by diffusion. Antidepressant drugs (e.g. chlorimipramine; Lilly 110440) inhibit serotonin and dopamine uptake with equal potency and also block the stimulatory action of serotonin, but serotonin antagonists (e.g. 2-10 μM) reduce the formation of 3-4-phenylserpine acid diethylamylide) which are potent inhibitors of the serotonin-induced shape-change do not affect the transport of serotonin or dopamine. Binding studies with [3H]-serotonin show heterogeneity of receptors: one site has characteristics consistent with the uptake carrier, and another is selectively blocked by serotonin antagonists. We conclude that dopamine is transported into platelets by the serotonin carrier (although with a much lower affinity) and that a separate site mediates platelet stimulation by serotonin. The concept of platelets as models for amineergic neurons in the central nervous system may be valid for serotonin but does not hold for dopamine.