

ISOLATION OF PLATELET MEMBRANE COLLAGEN RECEPTOR. A. Livne, A.L. Swanson, H. Scheuenstuhl, 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 2B-collagen (SC) affinity columns. Binding of platelets to SC 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 0.3% sodium dodecylsulfate (SDS), but not with 8 M urea, 15% ethanol or 1% Triton 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 ^{125}I and lactoperoxidase, the derived receptor fraction contained a small proportion (about 1%) of the label.

PLATELET ALPHA-ADRENERGIC RECEPTORS: DIRECT IDENTIFICATION BY [^3H] DIHYDROERGOCRYPTINE.

L.T. Williams, K.D. Newman and R.J. Lefkowitz. 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] dihydroergocryptine (DHE). Specific DHE binding to platelet AR was assayed by incubating DHE with platelet lysates for 17' at 25°C. Binding of DHE had the specificity expected of binding to AR. The α -adrenergic agonist (-)epinephrine, had a high affinity for the binding site, causing half-maximal inhibition of DHE binding at a concentration (EC_{50}) of $0.8 \mu\text{M}$. A series of α -adrenergic agonists competed for the binding sites in an order of potency ((-) epinephrine > (-)norepinephrine > (-)isoproterenol) identical to their order of potency in stimulating α -adrenergic mediated platelet aggregation. Competition for DHE 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 EC_{50} of $0.04 \mu\text{M}$ while β -adrenergic antagonists practolol, propranolol and dichlorisoproterenol competed only at very high concentrations ($10 \mu\text{M}$). Other α -adrenergic agents such as phenylephrine ($\text{EC}_{50}=4 \mu\text{M}$) and clonidine ($\text{EC}_{50}=0.05 \mu\text{M}$) also competed for the binding sites. Dopamine ($\text{EC}_{50}=9 \mu\text{M}$) and serotonin ($\text{EC}_{50}=90 \mu\text{M}$) 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 DHE.

INTERACTION OF PLATELETS WITH SEROTONIN AND DOPAMINE A.H. Drummond, H.J. Olverman
D.E. MacIntyre and J.L. Gordon University Department of Pathology and A.R.C. Institute of Animal Physiology, Cambridge, U.K.

Serotonin and dopamine are transported by platelets, and serotonin also stimulates platelets to change shape. Km values for transport are around $1 \mu\text{M}$ for serotonin and $40 \mu\text{M}$ for dopamine. Both amines also enter platelets by diffusion. Antidepressant drugs (e.g. chlorimipramine; Lilly 110140) inhibit serotonin and dopamine uptake with equal potency and also block the stimulatory action of serotonin, but serotonin antagonists (e.g. methysergide; d-lysergic acid diethylamide) 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 aminergic neurons in the central nervous system may be valid for serotonin but does not hold for dopamine.