ENHANCED ASSOCIATION OF FIBRINOGEN WITH ITS PLATELET RECEPTOR DUE TO SODIUM CITRATE INDICATION FOR ONE FIBRINOGEN RECEPTOR ONLY. S.K. Bowry, Clinical Research Unit for Blood Coagulation and Thrombosis of the Max-Planck-Gesellschaft, Gaffkystr. 11, D-6300 Giessen, West-Germany.

Washed platelet suspensions are almost always prepared from blood anticoagulated with sodium citrate. As citrate has been implicated in platelet function and as estimates of the number of fibrinogen (Fbg) binding sites on platelets range from 4,100 to 82,500, we examined the involvement of citrate on the fibrinogen-platelet interaction. The binding of 125I-fibrinogen to washed platelets from citrated blood and those from gel-filtered non-anticoagulated blood (PNB) from the same donor showed linear Scatchard plots for PNB and curvilinear plots for PCB. Assuming the presence of two classes of binding sites, a high affinity site (Kd = 7.5 x 10-10, 6,520 molecules/platelet; Kd = 1.02 x 10-9 M) were determined for PCB. However, PNB indicated a single class of binding sites with 16,430 ± 2,000 Fbg molecules/platelet. When blood from one donor was collected into 10 mM and 20 mM citrate, increased binding of Fbg was observed on platelets exposed to 20 mM citrate. The effects of citrate appear to be on the platelet Fbg receptor since non-specific binding was not affected by the citrate. As an 14C-citric acid binding to platelets was observed, citrate may affect the receptors without binding to the platelets. The dependence of binding on the pH of the citrate suggests ionic interactions between platelets and fibrinogen. Different amounts of Fbg were bound when four different preparations of sodium citrate varying in concentration and pH were used. Our data suggest that a combination of the direct effects of citrate on the Fbg binding of platelets, together with the variable concentrations and pH of the different citrate preparations routinely used to anticoagulate blood, may explain why some investigators obtain upwardly different preparations of sodium citrate. The results indicate that the major reason for the disparities in the Fbg binding data is due to the effects of citrate on platelets.

ABOLITION OF IN VIVO PLATELET THERUSM FORMATION WITH MONOCOFAALANTIGENS TO THE PLATELET GP IIb/IIIa RECEPTOR CORRELATES WITH PLATELET AGERGATION AND BLOODSTING TIME. W. Koller, J. Mikolaj, R. Tippel, S. B. Smith and L.R. Schodder, SUNY-Stony Brook, NY, and C. Neumann, Madison, WI, USA.

We previously reported that 6.8 mg/kg of the Fab'2 fragment of anti-GP IIb/IIIa was capable of abolishing thrombus formation on partially stenosed arterial arteries in monkeys (Mnks). The present study was designed to test another antibody to GPIIb/IIIa, PNB, (2) find the minimum effective dose, and (3) correlate this effect with changes in the platelet bleeding time (PT) and platelet aggregation (PA). Periodic platelet and PA were examined (11,020 ± 6,520 molecules/platelet; Kd = 1.02 x 10-9 M) were determined for PCB. However, PNB indicated a single class of binding sites with 16,430 ± 2,000 Fbg molecules/platelet. When blood from one donor was collected into 10 mM and 20 mM citrate, increased binding of Fbg was observed on platelets exposed to 20 mM citrate. The effects of citrate appear to be on the platelet Fbg receptor since non-specific binding was not affected by the citrate. As an 14C-citric acid binding to platelets was observed, citrate may affect the receptors without binding to the platelets. The dependence of binding on the pH of the citrate suggests ionic interactions between platelets and fibrinogen. Different amounts of Fbg were bound when four different preparations of sodium citrate varying in concentration and pH were used. Our data suggest that a combination of the direct effects of citrate on the Fbg binding of platelets, together with the variable concentrations and pH of the different citrate preparations routinely used to anticoagulate blood, may explain why some investigators obtain upwardly different preparations of sodium citrate. The results indicate that the major reason for the disparities in the Fbg binding data is due to the effects of citrate on platelets.

LIPROTEIN BINDING TO HUMAN PLATELETS IS LOCATED AT GPP/IIb/IIIa COMPLEX. E.Koller (1) and F.Koller (2).

Institut für Medizinische Physiologie (1) and Institut für Allergische Biochimie (2), University of Vienna, Vienna, Austria.

Human platelets possess specific binding sites for low density lipoproteins (LDL) and high density lipoproteins (HDL). Binding of both classes of plasma lipoproteins, though competitive, has been shown by several groups to facilitate platelet activation. Isolated washed platelets specifically aggregate upon addition of high concentrations of LDL even in the absence of known platelet activators. The proteins responsible for these binding have been visualized by ligand blotting (2). Both types of ligand specifically bind to two glycoproteins with molecular weights of 135 and 115 kD. Conditions of binding to these two proteins, however, markedly differ from those known for other lipoprotein receptors. Following extensive purification, these two species are still present at concentrations relative to each other that depend markedly on the conditions of purification. The purified, solubilized receptor was tested under various conditions, including in the absence and presence of calcium, after disulfide-reduction, and following chymotryptic digestion. In parallel experiments, the proteins were treated with respect to binding of fibrinogen, different lectins, and the alloantibody anti-P1. The results strongly support the assumption that the two protein bands associated with lipoprotein binding are constituents of the GP-IIb/IIIa complex.

Our results may have great implications for our understanding of the mechanism by which lipoproteins facilitate platelet stimulation.


UNMQUIRED DEFICIENCIES CAN AFFECT SEPARATELY THE PLATELET MEMBRANE GLYCOPROTEINS IIB-IIa COMPLEX AND THE LEUKOCYTE LFA-1, MAC-1 AND P50,95 COMPLEXES. D. Piderit (1), A. Fischer (2), C. Fouillet (3), F. Ledeist (2) and A.T. Rutherford (2)

(1) INSERM, U 334 CNRS, Hospital Lariboisière (1) and U 135 INSERM, Hôpital Necker (2), Paris, France.

The human platelet membrane glycoprotein (GP) Iib-IIa complex and a family of functional leukocyte cell membrane antigens, LFA-1 (L), MAC-1 (M) and P50,95 (K), possess known structural analogy with the structure of the GP-IIb/IIIa complex. The proteins responsible for these binding have been visualized by ligand blotting (2). Both types of ligand specifically bind to two glycoproteins with molecular weights of 135 and 115 kD. Conditions of binding to these two proteins, however, markedly differ from those known for other lipoprotein receptors. Following extensive purification, these two species are still present at concentrations relative to each other that depend markedly on the conditions of purification. The purified, solubilized receptor was tested under various conditions, including in the absence and presence of calcium, after disulfide-reduction, and following chymotryptic digestion. In parallel experiments, the proteins were treated with respect to binding of fibrinogen, different lectins, and the alloantibody anti-P1. The results strongly support the assumption that the two protein bands associated with lipoprotein binding are constituents of the GP-IIb/IIIa complex.

Our results may have great implications for our understanding of the mechanism by which lipoproteins facilitate platelet stimulation.


The human platelet membrane glycoprotein (GP) Iib-IIa complex and a family of functional leukocyte cell membrane antigens, LFA-1 (L), MAC-1 (M) and P50,95 (K), possess known structural analogy with the structure of the GP-IIb/IIIa complex. The proteins responsible for these binding have been visualized by ligand blotting (2). Both types of ligand specifically bind to two glycoproteins with molecular weights of 135 and 115 kD. Conditions of binding to these two proteins, however, markedly differ from those known for other lipoprotein receptors. Following extensive purification, these two species are still present at concentrations relative to each other that depend markedly on the conditions of purification. The purified, solubilized receptor was tested under various conditions, including in the absence and presence of calcium, after disulfide-reduction, and following chymotryptic digestion. In parallel experiments, the proteins were treated with respect to binding of fibrinogen, different lectins, and the alloantibody anti-P1. The results strongly support the assumption that the two protein bands associated with lipoprotein binding are constituents of the GP-IIb/IIIa complex.

Our results may have great implications for our understanding of the mechanism by which lipoproteins facilitate platelet stimulation.