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CHARACTERIZATION OF FIBRINOGEN RECEPTORS ASSOCIATED WITH GLYCOPROTEIN IIb/IIIa (GPIIb/GPIIIa) COMPLEX BY TRIGRAMIN, A UNIQUE LOW MOLECULAR WEIGHT PEPTIDE PROBE. Tur-Fu Huang, H. Lukasiewicz, J.C. Holt, S. Niewiarowski, Thromb. Ctre., Dept. Physiol., Temple University School of Medicine, Phila., PA

Trigramin (Mr weight 10 kDa), an acidic, cysteine rich peptide purified into homogeneity from Trimeresurus gramineus snake venom contained a single protein chain with EAGE at the NH2 terminal end. It inhibited platelet aggregation induced by various agents without affecting release reaction. It blocked competitively the binding of ^{125}I-fibrinogen to ADP stimulated and chymotrypsin treated platelets (Ki= 2 X 10^{-8}M). ^{125}-I trigramin bound to intact and to ADP stimulated platelets in a saturable manner (approx. 16,000 sites per platelet). However, ADP increased 5 fold, the binding affinity of trigramin to platelets (to Kd = 4 X 10^{-8}M) suggesting that ADP is changing the conformation of receptors associated with GPIIb/GPIIIa complex. The binding of trigramin to thrombasthenic platelets was markedly reduced. The binding to normal platelets was significantly inhibited by EDTA and by monoclonal antibodies directed against GPIIb/GPIIIa complex but not by the antibodies directed against GPIIb or GPIIIa molecules. The binding of ^{125}I-trigramin to ADP-stimulated platelets was inhibited by RGDS (IC_{50} = 125 μM) and by YHHLGGAKQAGDV (C-terminal fragment of fibrinogen gamma chain, IC_{50} = 250 μM) suggesting that these or similar peptide sequences are required for interactions of various ligands with GPIIb/GPIIIa complex.

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EFFECT OF FIBRINOGEN RECEPTOR BLOCKERS ON HUMAN PLATELET AGGREGATION, ATP SECRETION AND THROMBOXANE B_2 PRODUCTION. R. Kerry, V. Findlay, J. Ambler and R.B. Wallis. Ciba-Geigy Pharmaceuticals, Wimblehurst Road, Horsham, West Sussex, U.K.

The sequences of those parts of fibrinogen involved in the binding of fibrinogen to activated platelets are known and mimetics of these sequences, namely $Fib(\gamma)_{402\_411}$ and $Fib(A\alpha)_{572\_575}$ are selective fibrinogen receptor blockers. By competing with fibrinogen, these agents prevent the platelet: fibrinogen interaction and hence represent agents capable of selectively preventing platelet:platelet interaction (i.e. aggregation). In the experiments described here these peptides are used as tools to elucidate the interdependence of platelet aggregation, secretion and thromboxane (TxB_2) production using a range of aggregating agents.

Both peptides gave quantitatively similar results but $Fib(A\alpha)_{572\_575}$ was ten times more potent than $Fib(\gamma)_{402\_411}$. The results are summarised in the table:

		RESPONSE		
		Aggregation	Secretion (ATP)	Thromboxane (B_2)
AGGREGATING AGENT	PAF	-	-	-
	Collagen	-	-	+
	Thrombin	-	+	+

(-) = inhibition (+) = no inhibition

Experimentally aggregation and secretion were measured simultaneously in a lumi-aggregometer. Thromboxane B_2 was subsequently determined by RIA in the same samples. The concentration of $Fib(\gamma)_{402\_411}$ (1mM) and $Fib(A\alpha)_{572\_575}$ (100 μM) employed were those that gave half maximal inhibition of an aggregatory response.

The results show that a) PAF induced thromboxane production and ATP secretion are dependent on platelet aggregation, b) collagen induced ATP secretion is dependent on platelet aggregation while thromboxane production is independent of aggregation and c) thrombin induced ATP secretion and thromboxane production can both occur independently of platelet aggregation.

These observations throw further light on the interdependence of platelet responses and show fibrinogen receptor blockers to be true anti-aggregatory agents.

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FIBRINOGEN RECEPTORS IN BLOOD PLATELETS OF MIGRAINE PATIENTS. B. Walkowiak (1), W. Kozubski (2), Z. Pawlowska (1) and C.S. Cierniewski (1). Department of Biophysics (1), Department of Neurology (2), Medical School of Lodz, Poland.

The augmentation of platelet aggregability in migraine patients was found in many studies, by the use of both *in vivo* and *in vitro* techniques. In order to evaluate the platelet characteristics responsible for the increased aggregability of migraine platelets we determined their binding capacities and the apparent dissociation constant ($K_{D^{APP}}$) of fibrinogen receptors. Twelve non-pregnant women in age ranged from 20 to 44 years with unequivocal history of common migraine and 10 control healthy age matched women were used in these studies. The patients were assessed in headache-free intervals. Blood was drawn into acid citrate dextrose containing apyrase (0.1 mg/ml) and platelets were isolated by differential centrifugation. The mean number of platelet fibrinogen receptors exposed by ADP in migraine patients (65197 ± 6276) was statistically ($p<0.05$) higher than that obtained in healthy controls (40217 ± 7678). The apparent K_D for fibrinogen receptors in migraine platelets (6.01 ± 1.11 x 10^{-7} M) was lower than that in control women about threefold (2.17 ± 0.56 x 10^{-6} M). The difference was statistically significant ($p<0.02$). With these results we conclude that the increased capacity and binding affinity of fibrinogen receptors may be responsible for the elevated number of circulating platelet aggregates in migraine patients and for the prevalance of various kinds of strokes during migraine attack.

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DIRECT BINDING OF FIBRINOGEN-GOLD PROBES DOES NOT DISCLOSE THE ENTIRE POPULATION OF BOUND FIBRINOGEN ON ADHERENT PLATELETS. D.W. Estry (1), J.C. Mattson (2), Medical Technology Program, Michigan State University, East Lansing, MI, U.S.A. (1) and Department of Clinical Pathology, William Beaumont Hospital, Royal Oak, MI, U.S.A. (2).

The binding characteristics of fibrinogen to adherent platelets were determined using both direct protein-gold labeling and an immunogold procedure. The binding of colloidal gold-fibrinogen was studied in whole mounts of contact activated, gel filtered platelets by transmission electron microscopy. There was a transition from minimal binding of fibrinogen in dendritic platelets to marked zonal binding in fully spread platelets. The pattern of direct fibrinogen binding in fully spread platelets appeared to orient itself with the underlying filamentous cytoskeleton, a pattern that is consistant with that previously reported by us and others. In contrast, when bound fibrinogen was assayed using a rabbit anti-fibrinogen antibody, the pattern of bound fibrinogen was diffuse and strong labeling was present in both early dendritic forms as well as late fully spread platelets. To further confirm these observations the direct labeling technique was combined with the immunogold labeling procedure using two different sized gold probes. Platelets previously incubated with fibrinogen-gold (20 nm) were fixed, incubated with rabbit anti-human fibrinogen and then with gold conjugated goat anti-rabbit IgG (10 nm). The morphologic organization of the direct label was unchanged. However, the immunogold technique demonstrated a diffuse binding pattern over the entire cytoplasmic veil including areas previously unlabeled by the direct technique. This suggested that in addition to fibrinogen receptors identified by direct labeling, there are other receptors that either already contain bound fibrinogen, compete for released fibrinogen more affectively, or represent membrane bound granule fibrinogen that is exposed rather than released and rebound and is therefore identified only by the immunogold labeling procedure.