1663

PLASMINOGEN ACTIVATOR INHIBITORS OF TYPE 1 (PAI-1) AND TYPE 2 (PAI-2) ARE PRESENT IN ASCITIC FLUID FROM PATIENTS WITH OVARIAN CARCINOMA. I. Lecander (1), G. Martinsson (1), L.S. Nielsen (2), P.A. Andreasen (2), K. Danø (2) and B. Astedt (1). Research Laboratory of the Department of Obstetrics and Gynecology, University of Lund, Lund, Sweden (1) and Finsen Laboratory, Rigshospitalet, Copenhagen, Denmark (2).

The fast acting specific plasminogen activator inhibitor PAI-1 produced in endothelial cells and present in plasma and thrombocytes is known also to be released from certain tumour cell lines. The specific plasminogen activator inhibitor PAI-2 of placental type and present in pregnancy plasma is also released from a histocytoma cell line.

We examined ascitic fluid for the presence of PAI-1 and PAI-2 in five patients with advanced ovarian carcinoma. The antigen levels of PAI-1 and PAI-2 were assayed with sandwich ELISAs using a combination of monoclonal and polyclonal antibodies and the activity of PAI-1 was in addition measured by an activity assay (BioPool). The antigen level of PAI-1 ranged between 110 and 800 µg/l (mean 315 µg/l) and the activity between 5 and 16 U/ml (mean 12 U/ml). Antigen levels of PAI-2 ranged from 10 to 62 µg/l (mean 30 µg/ml), i.e. approximately 35 (12-72) % of the concentration of PAI-2 in term plasma. In blood samples obtained from three of the patients during surgery from veins draining the tumors, the concentration of PAI-2 was 0, 7 and 12 µg/l, respectively. PAI-2 could not be detected in the peripheral blood.

PLATELET ACTIVATION

1664

ADP-BINDING SITES IN PLATELETS: CHARACTERIZATION BY PHOTOAFFINITY LABELING AND BINDING STUDIES WITH FIXED PLATELETS. J.R. Jefferson, J.T. Harmon and G.A. Jamieson. American Red Cross Laboratories, Rockville, MD 20855, U.S.A.

Attempts to photoaffinity label platelet ADP receptors with 2-azidoADP have not been successful possibly due to the absence of a spacer arm between the nucleotide and the photolabile group. We have synthesized a probe having a long spacer arm by coupling 2-(3-aminopropylthio)-ADP to succinimidyl 4-^{3}H-azidobenzoate. Labeling competable by ADP could not demonstrated with intact platelets. With isolated platelet membranes, three bands (Mr 140,000, 110,000 and 46,000) were labeled that were not competed by ADP while three other bands (Mr 188,000, 92,000 and 51,000) were competable by 100 uM ADP.

Another problem in characterizing ADP receptors has been complications due to ADP metabolism and secretion from the dense granules. To avoid this problem we have measured the binding of ADP and analogues to formalin-fixed platelets. ADP bound to two sites (K1, 0.35 ± 0.04 uM; R1, 160,000 ± 20,000 sites/platelet; K2 7.9 ± 2.0 uM; R2, 400,000 ± 40,000 sites/platelet) with low non-specific binding: these values are in agreement with ADP concentrations required for activation. Affinity at the high affinity site was in the sequence ADP(0.35 uM)=ATP(0.4 uM)> 2-MeS.ADP(6.8 uM)> GDP(49 uM) > AMP(360 uM); adenosine did not compete. Binding at the high affinity site was blocked by pMBS (EC_{50} 250 uM) and 5-fluorosulfonylbenzoyladenosine (EC_{50} 1 mM). This is the first report of photoaffinity labeling of putative ADP receptors. Our experiments with fixed platelets suggest that they may be useful in testing agonists, antagonists and inhibitors in the absence of complications due to secretion and metabolism.

1665

PLATELET AGGREGATION DYNAMICS TO ADENOSINE DIPHOSPHATE IN NON-STIRRED SUSPENSIONS: LONG-RANGE INTERACTIONS FOR HUMAN, BUT NOT RABBIT, PLATELETS. K. Longmire and M.M. Frojmovic. Physiology Department, McGill University, Montreal, Canada.

The simplest experimental approach for a theoretical description of platelet aggregation is based on kinetics of early multiplet formation (< 4 platelets per aggregate) occurring with diffusion-dependent particle collisions (no flow). The Smoluchowski theory was used to calculate collision efficiencies, α_B, from a linear plot of platelet particle count $(N_t)^{-1}$ vs time (t) following addition of adenosine diphosphate (ADP) to citrated platelet-rich-plasma (PRP) for 7 human (H) and 2 rabbit (R) donors. A 0.1 ml sample of PRP was stirred with ADP for 0.5s, then immediately transferred to a 37°C bath for no-stir (diffusion) studies or further stirred with ADP for stir-induced aggregation studies. Samples were fixed with 0.5 ml 0.8% glutaraldehyde with particle count (N_t) determined with a resistive counter and % aggregation (PA) computed (reproducibility/sensitivity < 5%). For stir conditions, R platelets were as sensitive and as rapidly aggregated by ADP (2-10 µM) as H platelets, with ~ 1 s time lag for onset of PA. However, for no-stir conditions, linear regression analysis of data for ADP (5-10 µM) induced PA for H platelets for 0-30 s gave α_B = 7.5±4.6 ($\bar{r}$ = 0.9±0.05). Analysis at longer "diffusion" times showed a second phase (60-300 s) in some H donors with α_B = 0.5±0.4 (4/9 donors), while R platelets showed only 1 phase with α_B = 0.65±0.15 (0-60 to 0-900 s) ($\bar{r}$ = 0.8±0.1). The ADP sensitivity ($[ADP]_{\frac{1}{2}}$ corresponding to 50% of maximal changes) for the abnormally rapid PA in no stir H PRP for early times, measured over 0.4-100 µM range, was found to be ~9 µM (5-17 µM range) and 3.5 µM (3-10 µM) for measurements respectively at 5-10 and 20-30s; these values were ~ 3-8 x greater than $[ADP]_{\frac{1}{2}}$ measured for stirred suspensions for rate/extent of PA or rate of turbidometrically-measured macroaggregation (TA), while > [ADP] threshold for secondary aggregation in TA (10 H donors). These abnormally large α_B values and their ADP sensitivity observed for human platelets are consistent with long-range interactions mediated by "chemotactic" agents released from the cells but distinct from normal dense granule release requiring macroaggregation, or by as yet uncharacterized membrane or polymetric bridges.