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PLATELET-ACTIVATING FACTOR (PAF)-INDUCED INTRACELLULAR Ca^{2+} MOBILIZATION IN HUMAN PLATELETS. K.Matsuno(1), F.Katabami(1), M.Koyama(2), K.Abe(2), K.Sakurada(2), T.Miyazaki(2), S.Ozasa(2), H.Saitoh(2), I.Maekawa(2), H.Matsumiya(1). Department of Laboratory Medicine(1), and The Third Department of Internal Medicine, Hokkaido University School of Medicine, Sapporo, Japan.(2)

PAF-induced intracellular Ca^{2+} mobilization and platelet aggregation were investigated in human platelets. Cytosolic free Ca^{2+} concentration ($[Ca^{2+}]$cyt) was measured by using fluorescent probe quin2 and fura-2, and photoprotein aequorin. $^{45}Ca^{2+}$ uptake was measured after stimulation by PAF. Platelet aggregation was studied by recording the change in light transmission with platelet rich plasma (PRP) or washed platelet suspension (WPS).

These three Ca^{2+}-indicators could determine the elevation of $[Ca^{2+}]$cyt that was stimulated by PAF in the presence of extracellular Ca^{2+} (quin2 method: 98.2nM to 289.7nM; fura-2 method: 102.0nM to 351.4nM; aequorin method: 4.1μM to 8.2μM). In the absence of extracellular Ca^{2+}, however, little elevation of $[Ca^{2+}]$cyt was detected after stimulation by PAF. PAF could evoke the transient Ca^{2+} uptake.

New PAF specific antagonist, ONO-6240 inhibited PAF-induced platelet aggregation at a concentration from 1μM dose-dependently, whereas it didn't inhibit collagen- and thrombin-induced platelet aggregation at a concentration of 100μM. ONO-6240 inhibited PAF-induced increase in $[Ca^{2+}]$cyt in a dose-dependent manner as determined by these Ca^{2+}-indicators, as well as platelet aggregation.

These results suggest the increase in $[Ca^{2+}]$cyt is responsible for platelet aggregation induced by PAF, and the increased Ca^{2+} is derived from external Ca^{2+} influx chiefly.

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IS IT POSSIBLE TO IDENTIFY HUMAN PLATELETS IN VITRO AS BEING DESENSITIZED TO PLATELET ACTIVATING FACTOR (PAF-ACETHER,PAF) IN VIVO? Cs. Perger, A. von Felten. Laboratory of Blood Coagulation, Dept. of Int. Medicine, University Hospital, Zürich, Switzerland.

PAF is suggested to be of pathophysiological importance in a variety of diseases. Since platelets exhibit a reduced sensitivity to PAF after a contact with this agent, this behavior may be used as indicator of PAF released into the circulation. In extrinsic asthma, platelets show a diminished reaction to PAF after exposition of the patients to the antigen compared to their own platelets before exposition (Beer and von Felten, Adv. Inflamm. Res. 10:323,1986). We were therefore looking for a test system indicating directly whether platelets had been in contact with PAF.

- Preparation of PAF-desensitized platelets: Citrated PRP was placed in a cuvette of an aggregometer, and PAF was added in 10 portions at intervals of 10 sec (37°C, constant stirring) to a final concentration of 10 to 100 nM, depending on the individual sensitivity of each platelet preparation. Therby, only a minimal, completely reversible aggregation was registered without any release of serotonin (ST) or β-thromboglobulin (BTG). Control platelets were pretreated with buffer instead of PAF. Both platelets preparations were kept at 37°C for 45 min. Whereas control platelets showed a secondary aggregation to PAF (5x conc. used for desensitization), PAF-pretreated platelets were only reversibly aggregated.
- Sensitivity of PAF-desensitized and control platelets to other platelet agonists: No difference in aggregation, ST-or BTG-release was observed after stimulation with several concentrations of ADP, collagen and arachidonate ($p > 0.05$, n= 41).
- Binding of ^{3}H-PAF to platelets: PAF-desensitized and control platelets were separated from plasma by filtration through sepharose CL-2B (Pharmacia) in hepes-buffered Tyrode's solution. After incubation with 3H-PAF, platelets were washed on Whatman 934-AH filters (vacuum filtration). On desensitized and control platelets, we found 175±48 (mean±sd) and 231±70 ^{3}H-PAF molecules / platelet respectively after incubation with 5 nM ^{3}H-PAF, 399±36 and 504±66 ^{3}H-PAF molecules / platelet after incubation with 20 nM. In spite of a statistically significant reduction of PAF-binding after desensitization ($p < 0.01$), the variability of PAF-binding between platelets of different individuals is too high to allow a discrimination of normal from PAF-desensitized platelets.

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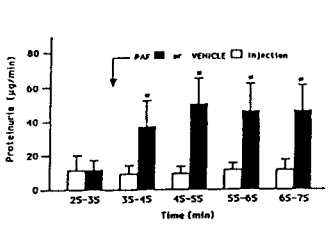
PLATELET ACTIVATING FACTOR (PAF) AS A MEDIATOR OF PROTEINURIA IN ISOLATED PERFUSED KIDNEY (IPK). F. Delaini, M. Tagliaferri, D. Macconi, C. Lupini, N. Perico and G. Remuzzi. Mario Negri Institute for Pharmacological Research, Bergamo, Italy.

PAF amplifies tissue damage in glomerulonephritis and can promote proteinuria stimulating platelet and neutrophil cationic protein release. We used IPK to establish whether PAF directly causes proteinuria. Kidneys were isolated from male Sprague-Dawley rats and perfused at constant pressure (100 mmHg) in a closed circuit with a Krebs-Henseleit buffer containing glucose urea creatinine BSA (1%), Ficoll 70 (3.5%) and amino acids. After 25 min stabilization period, a basal 10 min clearance period was followed by PAF (1.8 nM f.c. n = 6) or vehicle (n = 5) injection into the renal artery. As seen in the figure PAF but not vehicle significantly ($p<0.01$) increased urine protein excretion. No significant changes in GFR (as creatinine clearance) were observed after PAF or vehicle injection (Basal: 0.786 ± 0.075 PAF: 0.658 ± 0.070. Basal 0.653 ± 0.081 vehicle 0.639 ± 0.074 ml/min/g kidney). The data indicate that PAF may directly increase glomerular permeability to proteins.



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SYNTHESIS OF PLATELET ACTIVATING FACTOR BY HUMAN BLOOD PLATELETS. A. Sturk (1), A. Prins (1), M.C.L. Schaap (1), J.W. ten Cate (1) and H. van den Bosch (2). Div. of Hemostasis and Thrombosis, Academic Medical Centre, Amsterdam (1) and Dept. of Biochemistry, Univ. of Utrecht (2), The Netherlands.

Synthesis of platelet activating factor (PAF) by human blood platelets is a controversial issue. Whereas some groups have reported the synthesis, induced by thrombin, collagen or Ca^{2+}-ionophore A_{23187}, others were unable to obtain for instance the thrombin-induced PAF synthesis. Also, synthesis of only up to 6 pMoles PAF/10^{8} platelets has been reported, but leucocytes may synthesize up to 6000 pMoles PAF/10^{8} cells. Only an 0.1% leucocyte contamination would thereby explain the "platelet PAF synthesis". We therefore optimized the PAF synthesis by human blood platelets and leucocytes, induced by thrombin and A_{23187}. As platelets have been reported to show an increased PAF synthesis upon treatment with phenylmethylsulfonylfluoride (PMSF), this was also investigated.

Leucocytes were optimally stimulated with 10 uM A_{23187} (mean ± SD 4678 ± 2033, range 1698-7058 pMoles PAF/10^{8} cells, n=6), but could not be stimulated by thrombin. PMSF treatment itself induced PAF synthesis by these cells, but this was not influenced by thrombin or A_{23187}.

Platelet suspensions, with <0.005% leucocyte contamination as determined by light microscopy after Jenner-Giemsa staining, could synthesize PAF when treated with PMSF and stimulated with 2.5 IU/ml thrombin (mean ± SD 0.6 ± 0.3, range 0.3-1.0 pMoles PAF/10^{8}platelets, n=6), but in these suspensions A_{23187}-induced synthesis could not be demonstrated.

The results indicate that synthesis of PAF by human blood platelets is not due to contaminating leucocytes, if thrombin is used as the stimulus. These results were confirmed by ^{3}H-acetate uptake experiments.