

IS PHOSPHOLIPASE A<sub>2</sub> (PLA<sub>2</sub>) INVOLVED IN PAF-ACETHER FORMATION BY PLATELETS? M. Chignard, B.B. Vargaftig, J.P. Le Couedic and J. Benveniste. Institut Pasteur Paris and INSERM U 200 Clamart-Paris, France.

PAF-acether (platelet-activating factor) has been recently identified as 1-O-alkyl-2-acetyl-sn-glyceryl-phosphorylcholine, and later chemically synthesized. Platelets form PAF-acether upon stimulation with the calcium ionophore A 23187 or with more physiological stimuli such as thrombin or collagen. By contrast, arachidonic acid (AA) and adenosine diphosphate (ADP) do not trigger formation of PAF-acether. Since 1) PAF-acether is a phospholipid derivative and 2) aggregating agents which trigger PAF-acether formation are potent platelet PLA<sub>2</sub> stimulators, we speculated that PLA<sub>2</sub> could be implicated in its formation.

Rabbit washed platelets were incubated at 37°C in the presence of thrombin (2.5 U/ml) or of ionophore A 23187 (2.5 µM) for 7 min and ethanol (80 % final) was added. After centrifugation, the supernatant was evaporated and concentrated. The extract was tested for its aggregating property on rabbit washed platelets preincubated with a cyclo-oxygenase inhibitor (aspirin) and an ADP scavenging system (creatine phosphate and creatine phosphokinase).

In the presence of calcium chelating agents such as EDTA (5 mM) and EGTA (5 mM) most of the synthesis of PAF-acether was suppressed (93 % and 100 % of inhibition respectively). Dibutyl cyclic AMP (5 mM) also suppressed PAF-acether formation from platelets challenged by thrombin or by the ionophore A 23187 (100 % and 62 % inhibition respectively). Bromophenacyl bromide (0.1 mM) and compound CB 874 (0.1 mM) proved also to be very potent inhibitors of PAF-acether synthesis (100 % inhibition both). All these drugs are well-known platelet PLA<sub>2</sub> inhibitors. Upon stimulation platelets also form a deacetylated PAF-acether (lyso-PAF-acether) which could be the direct precursor of PAF-acether. The release of lyso-PAF-acether and the blockade of PAF-acether formation by various molecules having in common a PLA<sub>2</sub>-inhibitory activity lead us to conclude that a PLA<sub>2</sub> may be implicated in PAF-acether formation from platelets. Alternative explanations include the possibility that the various inhibitors act on other membrane-related sites.

SPECIFIC DESENSITIZATION OF WASHED RABBIT PLATELETS BY PAF-ACETHER AND DERIVATIVES. C. Lalau Keraly, M. Tencé, F. Heymans and J. Benveniste. INSERM U 200 Clamart-Paris, and Université Paris VII, France.

PAF-acether-aggregated rabbit platelets did not respond to a second challenge with the same agonist after their spontaneous disaggregation, but still aggregated in the presence of arachidonic acid (AA). When using 1 nM of PAF-acether in the first stimulation, aggregation in response to a second challenge with the same dose was nil. However, when 0.34 nM was used in the first step, aggregation in response to 0.64 nM PAF-acether was 59 %, as compared to control platelets. By contrast, aggregation of platelets pretreated with 1 nM PAF-acether was 72 % in the presence of 2.8 µM AA. Adding fibrinogen (0.34 mg/ml) before the second stimulation did not modify the desensitization phenomenon. Supernatants from platelets desensitized with 1 nM PAF-acether exhibited neither aggregating nor inhibitory activity. PAF-acether-induced desensitization could always be overcome, since aspirin-pretreated platelets first stimulated with 10 nM PAF-acether still aggregated with 100 nM of the same agonist, i.e. when using 100 times the amount which induced maximal aggregation. All our experiments were performed in the presence of ADP scavengers and, except for AA-induced aggregation, aspirin. We tested PAF-acether from 4 different cell origins and the semi-synthetic and synthetic compounds. Platelets were cross-desensitized towards PAF-acether from any source. Totally synthetic PAF-acether bearing a C<sub>16</sub> alkyl chain at position 1 of the glycerol moiety desensitized platelets as well as the C<sub>18</sub> synthetic analog. Lyso-PAF-acether (i.e. a compound lacking the acetyl group at the position 2 of the glycerol) (0.5-10.0 nM) and PAF-acether enantiomer (0.5-5.0 nM) neither aggregated nor desensitized platelets to PAF-acether. These results indicate that 1) PAF-acether from any source exhibit at least an identical active molecular site; 2) the presence and the stereospecific position of the 2-acetyl group are critical for the interaction of PAF-acether with platelets, a result which could indicate the existence of a platelet acceptor (receptor) for PAF-acether. However, these postulated platelet membrane acceptors were never saturated even using very high amounts of the agonist.

INTERFERENCE OF SULPHINPYRAZONE WITH THE PLATELET AND BRONCHIAL EFFECTS OF "PLATELET-ACTIVATING FACTOR". B.B. Vargaftig, J. Lefort, F. Wal and M. Chignard. Institut Pasteur Paris, France.

Platelet-activating factor (PAF-acether, 1-O-alkyl-2-acetyl-sn-glyceryl-phosphorylcholine) stimulates platelets independently from ADP release and formation of arachidonate (A) metabolites, is released from platelets by aggregating amounts of thrombin or collagen, and may account for physiopathologically relevant aggregations. PAF-acether induces reversible aspirin-resistant thrombocytopenia and bronchoconstriction (BC) when given iv at 60-120 ng/kg to propranolol-treated guinea-pigs. BC is platelet-dependent, and is suppressed by prostacyclin or by platelet depletion. BC was reduced for 30-60 min by iv sulphinpyrazone (S, 100 mg/kg). Inhibition by S of A-induced BC and thrombocytopenia lasted 6 hrs. This agrees with the fact that S is metabolized to a more effective cyclooxygenase inhibitor. Surprisingly, S was inactive against thrombocytopenia by PAF-acether, suggesting that BC was not inhibited because of a primary platelet site of action of S. Nevertheless, when platelets were collected from S-treated animals, aggregation and the release reaction by PAF-acether were inhibited. Inhibition of aggregation was surmounted by increasing the amounts of PAF-acether, whereas the release reaction was still inhibited by 50 % when 35 times more PAF-acether were added. Aggregation by ADP was unaffected by S, whereas the time course of inhibition of A was as against BC. *In vitro*, S (0.1-1 mM) inhibited the release reaction by AA and by PAF-acether; this was surmounted with high amounts of the latter, but not of the former: the mechanism of inhibition by S of PAF-acether and of A thus differs *in vivo* and *in vitro* and this may be relevant to its therapeutic effectiveness.

THE APPEARANCE OF PF1 AND PF3 IN ACTIVATED HUMAN PLATELETS. H. Sandberg<sup>†</sup>, A.P. Bode<sup>†</sup>, F.A. Dombros<sup>†</sup>, L.-O. Andersson<sup>‡</sup> and B.R. Lentz<sup>§</sup>. Depts. of Pathology<sup>†</sup> and Biochemistry<sup>§</sup>, Center for Thrombosis and Hemostasis, University of North Carolina, Chapel Hill, NC 27514, USA and Dept. of Biochemistry, Kabi AB, Stockholm, Sweden

Collagen and thrombin induced platelet activation were examined, *in vitro*, with regard to the appearance of surface-associated Factor V-like activity (PF1) and catalytic phospholipid-like surface activity (PF3). Two test systems were used: a clotting assay (a modified KAPTT) and a chromogenic substrate assay (maximum hydrolysis of S-2238). Following stimulation of normal platelets, both PF1 and PF3 appeared simultaneously in the supernatant and platelet pellet. When normal platelets were collected and carefully washed in a buffer containing adenosine, PGE<sub>1</sub>, and theophylline, the appearance of both PF1 and PF3 was blocked, as was the release of ATP from dense granules, the release of β-TG and PF4 from α-granules, and the occurrence of aggregation. When platelets were collected in this same inhibitor-containing buffer, and then gel filtered/centrifuge-washed in an inhibitor-free buffer, the appearance of PF1 and PF3 was still blocked. This occurred even though release of ATP, β-TG and PF4 as well as aggregation followed a pattern equivalent to platelets never exposed to these inhibitors. When the release supernatant from normal platelets isolated in the absence of inhibitors was gel filtered on Sepharose CL-4B in the presence of EDTA, the carbohydrate-free, lipid-protein particles (70-170 nm diam.) that provide PF3 appeared in the void volume. When the release supernatant from normal platelets was gel filtered in the presence of Ca<sup>2+</sup>, both PF1 and PF3 eluted in the void volume. With platelets isolated from severe F.V.-deficient donors, only PF3 was found in the void volume, in the presence or absence of Ca<sup>2+</sup>. It seems that the appearance of PF1 and PF3 as coagulant activities is completely separate from both the release of dense granule and α-granule contents as well as platelet aggregation and that the appearance of PF1 requires the presence of Ca<sup>2+</sup>.