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INTERFERENCE OF EICOSAPENTAENOIC AND DOCOSAHEXAENOIC ACIDS WITH ARACHIDONATE- AND U46619-INDUCED PLATELET ACTIVATION AND DESENSITIZATION

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Abstract—In previous studies we showed that arachidonate (AA) and the cyclic endoperoxide/thromboxane (Tx) A_2 mimic U46619-induced auto- and cross-desensitization of human platelets to either agonist. The desensitizing effect of U46619 is direct, whereas that of AA is mediated by a cyclooxygenase-dependent metabolite. Desensitization by AA and U46619 is suppressed by antagonists of the endoperoxide/Tx receptor sites. In the present investigation we demonstrated that eicosapentaenoic (EPA) and docosahexaenoic acid (DCHA) the major polyunsaturated fatty acids of fish oil suppressed TxB_2 formation and prevented platelet activation by AA and U46619. This inhibition required the presence of EPA or DCHA, since platelets pre-treated with these fatty acids and washed before testing responded as controls to the stimulating agents. At 0.1 and 0.3 mM respectively, DCHA and EPA behaved as reversible inhibitors of cyclooxygenase or Tx synthetase (inhibition of the effects of AA) and as endoperoxides/TxA_2 receptor antagonist (inhibition of the effects of U46619). Co-exposure of DCHA (0.1 mM) with AA or U46619 prevents auto- and cross-desensitization to AA and U46619. Platelets exposed to 0.3 mM DCHA and washed became refractory to stimulation by AA, but responded as controls to U46619. EPA (0.3 mM) was fully removed from platelets, which responded to AA and to U46619.

EPA and DCHA antagonize endoperoxide/TxA_2 directly, and thus prevent the stimulation-dependent desensitization, and additionally, inhibit the cyclooxygenase activity required for desensitization.

Desensitization to a pharmacological agonist may be homologous (auto-desensitization) or heterologous (cross-desensitization). In the former case, a first exposure suppresses the response to a second exposure to the same agonist, whereas in the latter case the response to an unrelated agent is also reduced. We recently studied [1] the mechanisms of desensitization of human platelets by and to arachidonic acid (AA),† and demonstrated that those platelets are cross-desensitized to the PGH_2 analogue compound U46619 [2, 3]. Conversely, platelets exposed to the latter analogue were auto- and cross-desensitized to AA. In all instances, desensitized platelets maintained, at least in part, their responsiveness to thrombin. Since we also showed that inhibition of platelet Tx synthetase does not prevent desensitization by AA to itself or to U46619 even when activation is suppressed, and that auto- and cross-desensitization is reversed by different site antagonists of the cyclic endoperoxide/Tx, we suggested that the cyclooxygenase metabolite responsible for desensitization by AA interacts with a receptor-related target and is not TxA_2 itself [1].

Epidemiological and nutritional studies demonstrated the antithrombotic potential of ω3-polyunsaturated fatty acids of marine diets [4–7], such as EPA [8, 9] or DCHA [10, 11], which were converted respectively by cyclooxygenase to PGI_3 and TxA_3 [12, 13] and by lipoxygenase to 11- and 14-HDHE [14, 15]. Since EPA and DCHA inhibit platelet aggregation and arachidonate metabolism [16–18] we now correlated their platelet protective effects with their ability to interfere with desensitization under different conditions.

MATERIALS AND METHODS

Reagents. The purified fatty acids EPA (C20:5, ω-3) and DCHA (C22:6, ω-3) (respectively 99 and 90% purity, analysed by gas chromatography) were obtained by Jouvéinal Laboratories (France). These fatty acids were diluted in 0.9% NaCl (saline). The stable endoperoxide analogue 15(*S*)-hydroxy-11,9(epoxymethano) prosta-5Z,13E-dienoic acid (U46619) was a gift from Upjohn Co. (Kalamazoo, MI). Arachidonate (AA, C20:4, ω-6), stearic acid (SA, C18:0), bovine serum albumin (Fraction V) and bovine thrombin were from Sigma Chemical Co. (Dorset, U.K.). Stock solutions of U46619 and AA

† Abbreviations used: DCHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid; 12-HETE, 12-hydroxy eicosatetraenoic acid; 12-HPETE, 12-hydroperoxy eicosatetraenoic acid; 12-HEPE, 12-hydroxy eicosapentaenoic acid; 11- and 14-HDHE, 11- and 14-hydroxy docosahexaenoic acid; PGB_2, prostaglandin B_2; Tx, thromboxane.

in ethanol were stored at −20°, dilutions being freshly prepared before each experiment. Thrombin was solubilised in Michaelis Owren–Koller buffer (30 mM sodium veronal, 44 mM sodium acetate, 108 mM NaCl; pH was adjusted to 7.3 with HCl 0.1 N). Fibrinogen (Grade L) from Kabi (Stockholm, Sweden) was treated with diisopropyl fluorophosphate (DFP) by standard procedures to inactivate coagulant contaminants. Prostacyclin (PGI_2) and carbacyclin, dissolved in 0.05 M Tris buffer, pH 8.5 were gifts from Dr S. Moncada (The Wellcome Laboratories, Beckenham, U.K.). Compound L8027 (1′-isopropyl-2-indolyl)-3-pyrydyl-3-ketone from Sanofi (Toulouse, France) was solubilised in polyethylene glycol 300.

Preparation of washed human platelets. Venous blood was obtained from healthy donors (Centre National de Transfusion Sanguine, Paris) who had received no medication which would influence platelet function for at least 10 days before venipuncture. Blood was anticoagulated with $\frac{1}{6}$ of its final volume of citric acid–citrate–dextrose (7 mM, 93 mM and 139 mM, respectively; pH 6.4) containing heparin Choay (20 U/ml). Platelet-rich plasma (PRP) was separated by centrifugation of blood (200 *g*; 20 min) at room temperature. The platelet pellet was obtained from PRP by centrifugation at 37° (1700 *g*, 15 min). Platelets were resuspended and washed in a modified Tyrode's buffer containing bovine serum albumin (0.35%) [19], in which apyrase was omitted and PGI_2 (1 nM) was added for the first two steps of washing. Platelets were resuspended in Tyrode's-albumin buffer adjusted to 5.10^8 cells/ml.

Procedure for studying the interference of eicosapentaenoic and docosahexaenoic acids with AA- and U46619-induced platelet desensitization (first phase experiments). Washed platelets were incubated with EPA (0.3 mM), DCHA (0.1–0.3 mM) or their solvent, and stirred for 2 min at 37° before the addition of AA (0.1 mM) or U46619 (1 μM). ATP release was determined 3 and 30 min after stimulation by the bioluminescence technique (luciferine–luciferase) [20] using a Pico-ATP device from Jobin Yvon (Paris, France). Platelet samples were collected in formaldehyde (2%) at the same time intervals and their number was estimated subsequently with a Coulter counter ZBI. At 4 min, 0.1 ml samples were collected for the radioimmunoassay of TxB_2. Five minutes later, 1 μM PGI_2 was added to disaggregate the platelets. Finally, 30 min after the first addition of AA or of U46619 the platelets were washed twice as indicated above and their final concentration was adjusted to 5.10^8 platelets/ml. At this stage, the final recovery of platelets for all the samples was 82–93%. These platelets were viable since they aggregated (see Results).

Platelet stimulation (second phase experiments). Platelet aggregation was measured in an Icare aggregometer (Marseille, France) set at 37° and 1100 rpm [21] on 0.4 ml samples of washed platelets. Platelets were challenged with AA (0.1–0.3 mM), U46619 (1–3 μM) or thrombin (0.25 U/ml). When AA or U46619 were studied, the content of fibrinogen was restored by the addition of DFP-fibrinogen at a final concentration of 0.28 mg/ml. ATP secretion and TxB_2 formation were evaluated 3 and 4 min respectively after adding the stimulating agent as indicated.

Extraction of the monohydroxy fatty acids and HPLC system. To 1 ml of each sample, acidified to pH 3.5 with acetic acid, were added 10 μg of PGB_2 as internal standard. The samples were extracted by shaking with 10 ml ethyl acetate, the extracts were concentrated under reduced pressure and dissolved in 0.2 ml acetonitrile.

The HPLC analysis were performed with an automatic gradient controller (M 80; Waters), two pump systems (M 6000; Waters), an automated injector (WISP 710B; Waters), a variable wavelength UV detector set a 234 nm (M481; Waters) and an integrator (SP4270; Spectra physics). The reverse phase column (Ultrasphere ODS, 4.6 × 150 mm; Becham) was packed with 5 μm particles. The solvent system was a non-linear gradient of acetonitrile (30–60%; 30 min) in H_2O/CF_3CO_2 (100/0.08%). pH adjusted to 6.1 with NH_4OH. The flow rate was 1 ml/min. In this system the retention time of PGB_2, 12-HEPE, 11- or 14-HDHE and 12-HETE were respectively at 18 ± 1, 32 ± 0.3, 35 ± 0.2 and 36 ± 0.3 min. These compounds were identified by co-chromatography of their ^{14}C derived compounds. The peaks of the 11- and 14-HDHE were not completely resolved but their relative intensity was 1:3 in accordance with Fischer *et al.* [15].

Radioimmunoassay of TxB_2. TxB_2 was determined according to Sors *et al.* [22] slightly modified [1]. Briefly, platelet samples were diluted in a phosphate-gelatin buffer, together with TxB_2 antiserum and ^{125}I–TxB_2. Bound fractions were precipitated with polyethylene glycol 6000 and after centrifugation the radioactivity was counted.

Statistical analysis. Statistical significance was analysed by Student's *t*-test for unpaired data.

RESULTS

Effects of eicosapentaenoic and docosahexaenoic acids on platelet activation (first phase experiments)

In presence of DCHA (0.1 mM) or EPA (0.3 mM) AA-induced platelet activation and TxB_2 formation were suppressed (Figs 1, 2 and Table 1). At these concentrations, DCHA and EPA also inhibited aggregation and secretion of ATP induced by low concentration of thrombin (0.05 U/ml) and by U46619 (1 μM) but did not inhibit aggregation by a high concentration of thrombin (0.25 U/ml). Platelet pre-treatment with aspirin did not suppress the protective effects of DCHA or of EPA over thrombin and U46619 (Table 2). In contrast to DCHA and EPA, stearic acid (0.3 mM) was inactive against platelet activation by AA or U46619 (data not shown).

Effect of docosahexaenoic acid on platelet desensitization (second phase experiments)

The effects of DCHA varied according to the concentrations used. Thus, platelets pre-treated with DCHA (0.1 mM) and then washed, aggregated and secreted as much as control platelets in response to AA and U46619 (Fig. 3). Platelets exposed to AA or to U46619 in the presence of DCHA (0.1 mM) were not desensitized to either, as compared to plate-

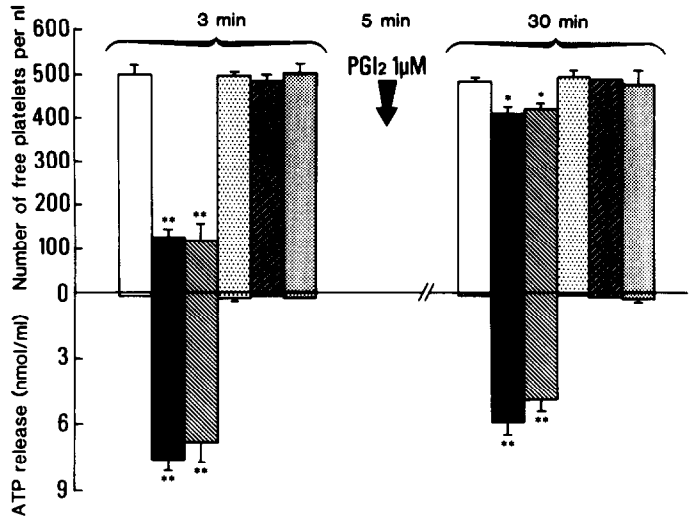

Fig. 1. Inhibition by docosahexaenoic acid (DCHA) of arachidonate (AA)- and U46619-induced platelet activation. Stirred platelets (37°) were exposed to saline (control, □), to AA 0.1 mM (■), to U46619 1 μM (▧), and to DCHA 0.1 mM alone (▩) or in combination with agonists (AA 0.1 mM, ▨; U46619 1 μM, ▩). The number of free platelets and the amounts of ATP released were determinated after 3 and 30 min. Five min after the addition of the agonists, PGI_2 1 μM was added to each sample. The number of free platelets is indicated above the horizontal zero line and ATP secretion below. The results are the mean ± SEM of four separate experiments. Significantly different (*$P < 0.05$, **$P < 0.001$) when compared to control platelets.

lets exposed to AA or U46619 alone. Aspirin did not prevent the protective effects of DCHA (0.1 mM) towards desensitization by and to U46619 (Fig. 4). Contrasting with the lack of direct effects of 0.1 mM of DCHA, platelets exposed to 0.3 mM and then recovered, were desensitized to AA, but not to U46619 or thrombin. Thus, the DCHA (0.3 mM)-treated platelets aggregated and secreted ATP as much as control platelets when stimulated by U46619 or thrombin, under conditions where responsiveness to AA was suppressed (Fig. 5). TxB_2 formation from AA was also markedly reduced by pre-exposure to 0.3 mM of DCHA (Table 3). Selective DCHA (0.3 mM)-induced desensitization to AA was also not prevented by co-exposing the platelets with the cyclooxygenase inhibitor compound L8027 (30 μM) or with carbacyclin (0.3 μM), which prevent against AA and U46619-induced homologous and heterologous desensitization [1].

Effect of eicosapentaenoic acid on platelet desensitization (second phase experiments)

Platelets exposed to up to 0.3 mM of EPA and then recovered, maintained their responsiveness to

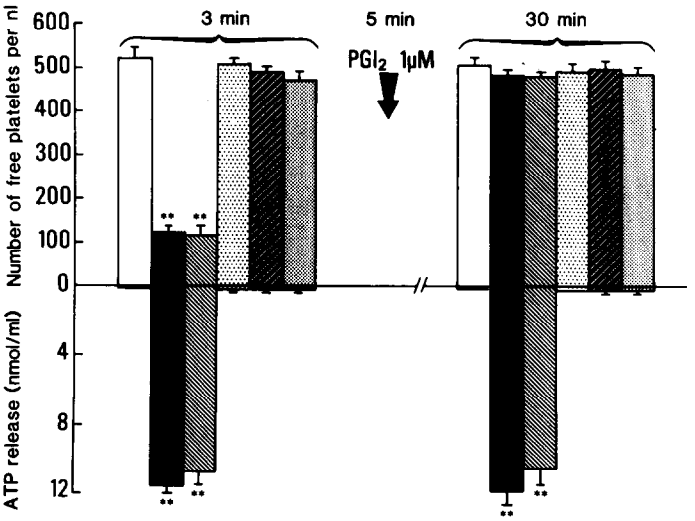

Fig. 2. Inhibition by eicosapentaenoic acid (EPA) of arachidonate (AA)- and U46619-induced platelet activation. Legend as in Fig. 1; EPA replacing DCHA.

Table 1. Inhibition by eicosapentaenoic (EPA) and docosahexaenoic (DCHA) acids of thromboxane B_2 (TxB_2) generation by arachidonate (AA)-stimulated platelets

Treatment	Thromboxane B_2 (ng/ml)	
	Without AA (Controls)	With AA
Saline (no treatment)	3.7 ± 0.6†	543 ± 134.8
EPA 0.3 mM	82.9 ± 63.2*	130 ± 51.2*
DCHA 0.1 mM	5.9 ± 3†	114 ± 31.5*
DCHA 0.3 mM	46.1 ± 23.4*	NT

Platelets were exposed to EPA, DCHA or to AA alone or associated at the indicated final concentrations. The amounts of TxB_2 (ng/ml) formed after 4 min are indicated. The results are means ± SEM of four separate experiments; NT not tested because of toxic effects of the association. Significantly different (*$P < 0.05$, †$P < 0.01$) when compared to the untreated platelets stimulated with AA.

AA and U46619 (Fig. 6); EPA thus failed to desensitize platelets. At 0.3 mM, EPA prevented U46619-induced platelet desensitization to the stimulating effects of AA and U46619. In contrast, when platelets were co-exposed to AA and to EPA, only heterologous desensitization (to U46619) was prevented, whereas homologous desensitization (to AA) was maintained (Fig. 6). Under those conditions, the production of TxB_2 from AA was also reduced (Table 3).

Contrary to EPA and DCHA, stearic acid (0.3 mM) did not prevent AA and U46619-induced platelet desensitization (Fig. 7).

Identification of the metabolites of DCHA and EPA in presence or absence of arachidonic acid or U46619

Washed human platelets did not convert EPA to 12-hydroxyeicosapentaenoic acid (12-HEPE) (Fig. 8B) and to TxB_3, as previously reported [23]. The addition of U46619 to washed platelets did not induce EPA conversion, whereas the addition of AA stimulated EPA metabolism (Figs 8C and 8D).

Under the same conditions we examined the conversion of DCHA; as shown in Fig. 8(F), the washed human platelets converted DCHA alone into 11- and 14-HDHE.

DISCUSSION

Pharmacological studies demonstrate that DCHA and EPA, contrary to saturated fatty acids, such as stearic acid which was used as a control, inhibit AA-induced platelet activation and TxB_2 formation [16–19]. Inhibition requires the presence of DCHA or EPA or of their hypothesized metabolites. In our experiments with DCHA, two different effects were observed, depending upon the concentrations used. Platelets pre-exposed to 0.1 mM of DCHA, alone or together with AA or U46619, and then washed, aggregated and secreted ATP as much as control platelets (Fig. 3), suggesting that at this concentration DCHA prevents the site-directed desensitization at the level of the endoperoxide/Tx platelet "receptor". This competition resembles that obtained with reversible cyclooxygenase inhibitors such as methyl salicylate and compound L8027. When platelets were pretreated with aspirin and co-exposed to DCHA 0.1 mM (or 0.3 mM, see below) and U46619, they were recovered as fully responsive to a second challenge with U46619, i.e. the protective effect of DCHA persisted (Fig. 4). This excludes the possibility that DCHA protects against platelet desensitization by a cyclooxygenase-dependent metabolite. Nevertheless, as the formation of TxB_2 from AA is inhibited by DCHA (Table 1) we cannot conclude that DCHA acts exclusively as a site-protective agent.

In contrast to 0.1 mM, platelet exposure to 0.3 mM of DCHA alone became unresponsive to AA, but maintained fully their sensitivity to U46619 and partially to thrombin. Absence of significant synthesis of TxB_2 when DCHA (0.3 mM)-pretreated platelets were exposed to AA (0.1–0.3 mM) (Table 3), shows a dissociation between an effect of DCHA involving

Table 2. Inhibition by eicosapentaenoic (EPA) and docosahexaenoic (DCHA) acids of thrombin (0.05 U/ml)-induced platelet aggregation

Pretreatment (incubation for 2 min)	Percent light transmission						
	Non aspirin-treated platelets				Aspirin-treated platelets		
	AA 0.1 mM	U46619 1 μM	Thrombin 0.25 U/ml	Thrombin 0.05 U/ml	AA 0.1 mM	Thrombin 0.25 U/ml	Thrombin 0.05 U/ml
Saline (no pretreatment)	84 ± 1	83 ± 1	75 ± 2	73 ± 6	0 ± 0	79 ± 3	65 ± 6
DCHA 0.1 mM	9 ± 6‡	10 ± 6‡	77 ± 1	44 ± 16	NT	82 ± 1	43 ± 17
DCHA 0.3 mM	0 ± 0‡	0 ± 0‡	76 ± 1	15 ± 6†	NT	70 ± 9	5 ± 5†
EPA 0.3 mM	0 ± 0‡	0 ± 0‡	78 ± 3	20 ± 13*	NT	80 ± 4	23 ± 12*

DCHA (0.1–0.3 mM) was pre-incubated with platelets and after 2 min AA (0.1 mM) or U46619 (1 μM) was added to initiate aggregation. The table shows that DCHA 0.1 mM markedly inhibits aggregation by AA or U46619 and partially inhibits the response to a low concentration of thrombin (0.05 U/ml). High concentrations of DCHA or EPA (0.3 mM) suppressed aggregation by AA or by U46619 and further reduced that induced by low concentration of thrombin (0.05 U/ml), but not by a high concentration (0.25 U/ml). The responses are unaffected when platelets are pre-treated with aspirin. The results are the mean ± SEM of percentage light transmission (aggregation) from three separate experiments. NT not tested. Significantly different (*$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$) when compared to the non-treated platelets stimulated with each agonist.

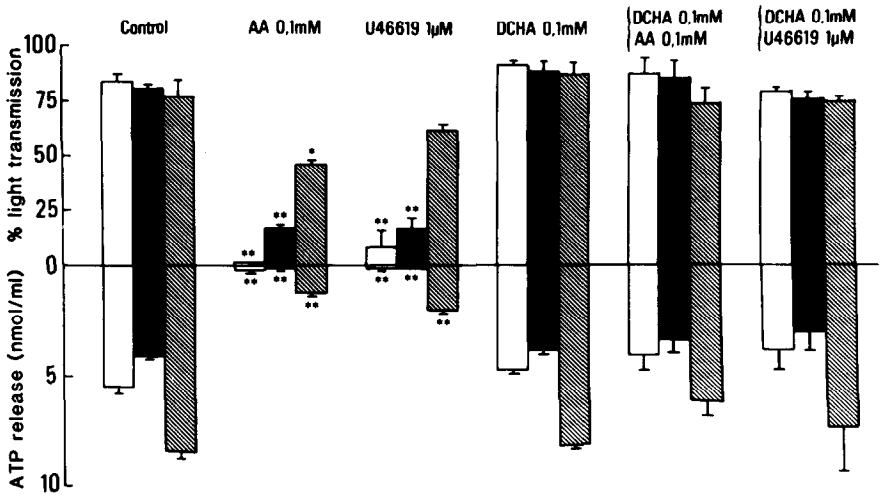

Fig. 3. Reversal by docosahexaenoic acid (DCHA) of arachidonate (AA)- and U46619-induced platelet desensitization. Platelets pre-treated as in Fig. 1 were washed and adjusted to 5.10^8 cells/ml. Platelets were stimulated with AA (0.1 mM; □), U46619 (1 μM; ■) or thrombin (0.25 U/ml; ▨). % light transmission is shown above the horizontal zero line and ATP secretion below it. The results are the mean ± SEM from four separate experiments. Significantly different (*$P < 0.05$, **$P < 0.001$) when compared to control platelets.

the cyclooxygenase–Tx synthetase system, which is possibly modified, and the membrane "receptor" target for U46619, which is not affected by DCHA. It is apparent that at 0.3 mM DCHA exerts an antagonistic effect similar to that noted at 0.1 mM (Fig. 3), plus an effect involving the interference with the metabolism of AA (Fig. 5). Whether this is accounted for by DCHA itself, or by one or more metabolites [14, 15], is still an open question. Since the effects of 0.3 mM of DCHA persisted when platelets were incubated with the reversible cyclooxygenase inhibitor L8027 [25], it is unlikely that the metabolites responsible for the desensitizing effects of DCHA are cyclooxygenase-dependent. The hypothesized substance may in fact be a lipoxygenase metabolite, such as 11- and/or 14-HDHE (Fig. 8F), the major DCHA platelet metabolites [14, 15]. The absence of cross-desensitization between AA and U46619 (Fig. 5) on one side, and the abrogation of the capacity to synthesize Tx (Table

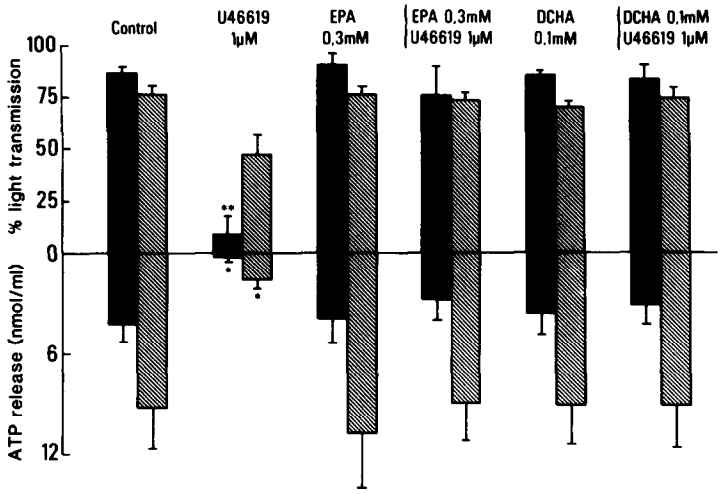

Fig. 4. U46619-induced desensitization of aspirin-treated human platelets: reversal by eicosapentaenoic acid (EPA) and by docosahexaenoic acid (DCHA). Platelets pretreated with aspirin 30 μM for more than 10 min were incubated with saline in control, with U46619, with EPA, with DCHA and with U46619 in presence of EPA or of DCHA. Platelets were washed and recovered as indicated in Methods and challenged with U46619 (1 μM; ■) or thrombin (0.25 U/ml; ▨). The results are the mean ± SEM of three separate experiments. Significantly different (*$P < 0.05$, **$P < 0.001$) when compared to control platelets.

Table 3. Interference of eicosapentaenoic acid (EPA) and docosahexenoic acid (DCHA) with the generation of thromboxane B_2 (TxB_2) from arachidonate (AA)

	Control (no pretreatment)	Thromboxane B_2 (ng/ml)					
		AA 0.1 mM	DCHA 0.1 mM	DCHA 0.3 mM	EPA 0.3 mM	AA 0.1 mM EPA 0.3 mM	AA 0.1 mM DCHA 0.3 mM
AA 0.1 mM	293.3 ± 32	121.6 ± 27.2*	262.1 ± 67.2	34.2 ± 28†	202.7 ± 42.3	74.5 ± 38.3*	239.3 ± 83.9
AA 0.3 mM	NT	615.4 ± 64.5	NT	203.1 ± 62.8‡	NT	304.2 ± 61‡	NT

Platelets were pre-treated with saline (control), with AA, with EPA or DCHA alone or in combination with AA. Platelets were then recovered as described in Methods and restimulated with AA (0.1–0.3 mM). TxB_2 (ng/ml) was determinated 4 min after stimulation. The results are the mean ± SEM of three to four separate experiments. NT not tested. Significantly different when compared to the control platelets stimulated with AA 0.1 mM (*$P < 0.05$, †$P < 0.01$) and to AA 0.1 mM pretreated platelets stimulated with AA 0.3 mM (‡$P < 0.05$).

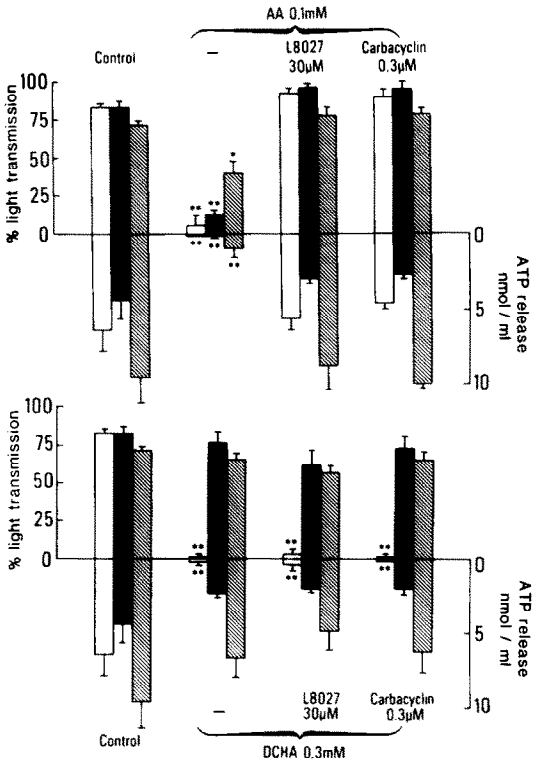

Fig. 5. Docosahexaenoic acid (DCHA; 0.3 mM)-treated platelets are desensitized to AA but not to U46619. The columns above the horizontal zero lines show the % light transmission across platelet suspensions and below the zero line show the amounts of ATP secreted. The results are the mean ± SEM of four separate experiments. Significantly different (*$P < 0.01$, **$P < 0.001$) when compared to untreated control platelets. Upper panel: Platelets pre-exposed to saline in control, to AA 0.1 mM alone, to AA 0.1 mM in presence of L8027 30 μM or carbacyclin 0.3 μM as indicated above the columns were recovered and challenged with AA (0.1 mM; □), U46619 (1 μM; ■) or thrombin (0.25 U/ml; ▨). Lower panel: Legends as above. As indicated in the figure, platelets exposed to DCHA 0.3 mM alone or in the presence of L8027 or carbacyclin became refractory to AA, whereas responsiveness to U46619 and to thrombin was maintained.

3), on the other side, distinguishes clearly desensitization to AA by DCHA at 0.3 mM, from that which is due to AA itself. Furthermore, DCHA 0.3 mM is probably not toxic to the platelets, since responsiveness to U46619-induced activation after the elimination of DCHA was maintained. Finally, the increase of the cyclic AMP platelet content with carbacyclin [26], which prevents AA- and U46619-induced desensitization [27] did not prevent the desensitizing effect of DCHA (0.3 mM), which once again distinguishes from that due to AA.

Platelets treated with EPA (0.3 mM) alone, contrary to those treated with DCHA 0.3 mM, were not desensitized and behaved as controls. When co-exposed to AA and EPA, and then washed, platelets maintained their sensitivity to thrombin and U46619, but became refractory to AA (Fig. 6). These AA-desensitized platelets also failed to form Tx when re-exposed to it (Table 3). We reported [1] that AA

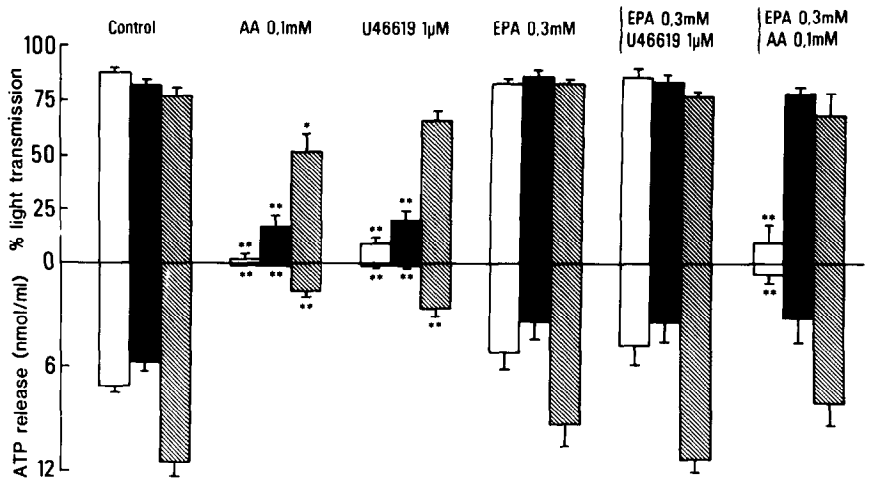

Fig. 6. Arachidonate (AA)-treated platelets are desensitized to AA but not to U46619 in the presence of eicosapentaenoic acid (EPA). Platelets treated as indicated above the columns were recovered according to Methods and challenged with AA (0.1 mM; □), U46619 (1 μM; ■), or thrombin (0.25 U/ml; ▨). The results are the mean ± SEM of four separate experiments. Significantly different (*$P < 0.05$, **$P < 0.001$) when compared to untreated control platelets.

and U46619 cross-desensitize; we now show that in presence of EPA (0.3 mM) only desensitization to AA persists. This dissociation is difficult to explain, unless it results from cooperation between AA and EPA, as supported by the results with HPLC (Fig. 8) and by the work of Morita *et al.* [16, 23], showing that AA stimulates the metabolism of EPA by platelets, probably via 12-HPETE. It is likely that specific desensitization to AA, which does not cross with U46619, is due to a metabolite of EPA, which does not require the presence of AA for its synthesis. Our results confirm the concept that the intact human platelets do not metabolize EPA unless AA is present [16, 23]. Accordingly, U46619, which does not interfere with the metabolism of EPA (Fig. 8), stimulated the EPA-pretreated platelets refractory to AA.

In conclusion, our results show that EPA and DCHA inhibit AA- and U46619-induced activation and desensitization of platelets by two similar mechanisms: a direct effect, as antagonists at the level of the endoperoxide/Tx receptor, allowing inhibition of platelet activation and desensitization by U46619

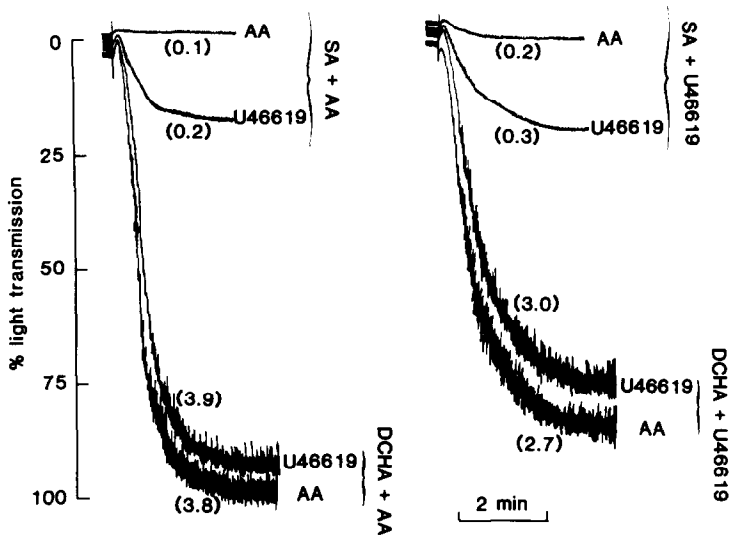

Fig. 7. Reversal by docosahexaenoic acid (DCHA) but not by stearic acid (SA) of arachidonate (AA)- and U46619-induced platelet desensitization. Representative tracings of platelet aggregation by AA or by U46619. Platelets were pre-exposed during the first phase experiments to AA (1 mM) or to U46619 (1 μM) in the presence of SA (0.3 mM) or DCHA (0.1 mM) as indicated next to each tracing. Platelets were then recovered and stimulated during the second phase experiments with AA (0.1 mM) or with U46619 (1 μM). The values in brackets indicate the amounts of ATP (nmol/ml) released by each sample during this phase.

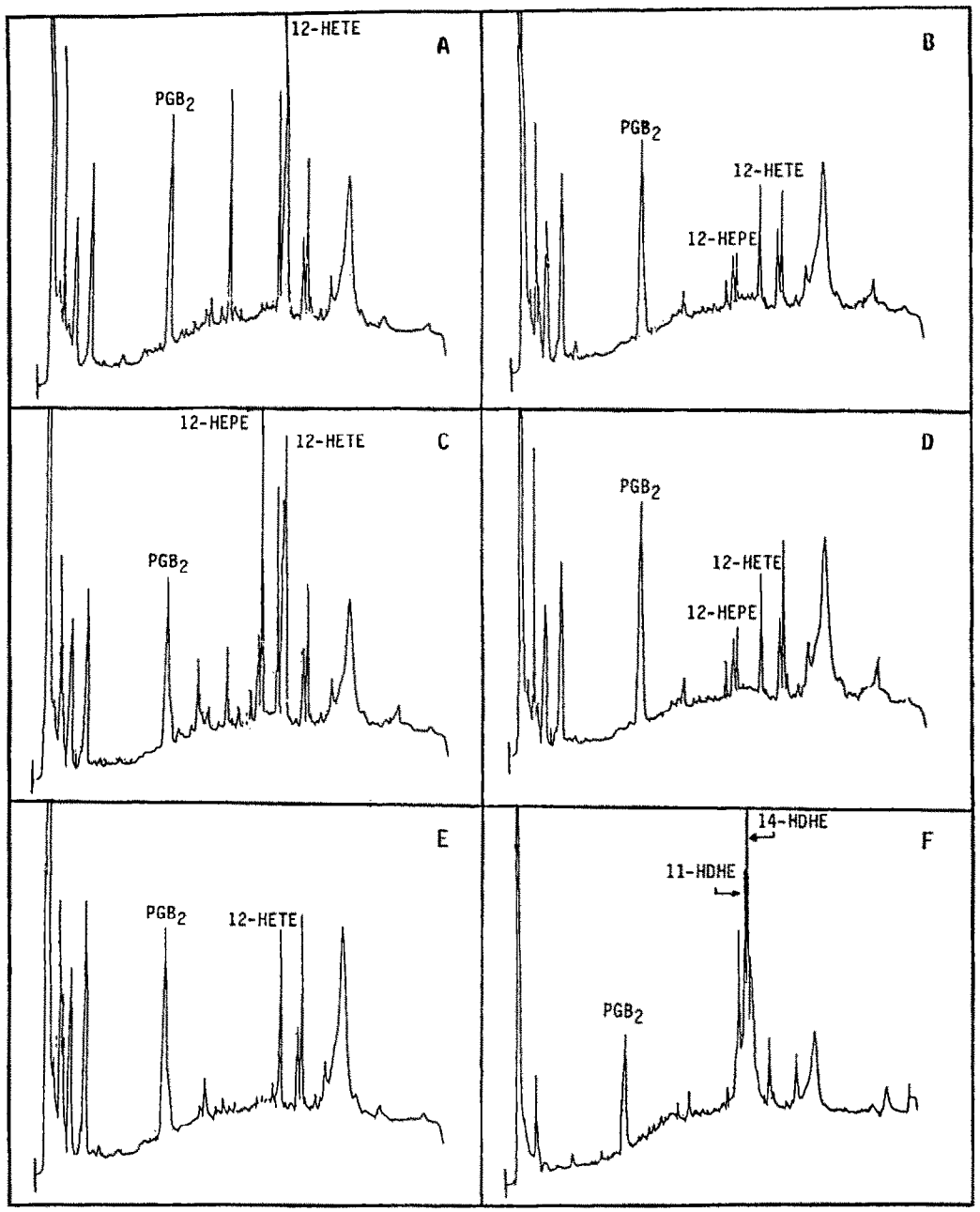

Fig. 8. RP-HPLC chromatograms of extracts obtained from incubation of human platelets with: AA 100 μM, A; EPA 300 μM, B; EPA 300 μM plus AA 100 μM, C; EPA 300 μM plus U46619 1 μM, D; U46619 1 μM, E and DCHA 300 μM, F (see Methods).

and prevention of its desensitizing effect, and an indirect effect, observed at high concentrations of EPA and DCHA, which is probably mediated by one of their metabolites.

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