Dietary fat modifies thromboxane A₂-induced stimulation of rat platelets

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Diets containing high levels of monounsaturated, *n*–6 polyunsaturated and *n*–3 polyunsaturated fatty acids were fed to Wistar rats. This resulted in decreases in the arachidonate content in platelet phospholipids to 91%, 79% and 51% respectively of the level found after feeding a diet rich in saturated fatty acids. In the presence of CaCl₂, collagen- and thrombin-induced aggregation of washed platelets from the saturated-fat dietary group (with highest level of arachidonate) was low compared with that of platelets from the other dietary groups, despite a relatively high production of thromboxane B₂. On the other hand, *n*–3 polyunsaturated fatty acids in the diet resulted in platelets aggregating actively, but producing low levels of levels of thromboxane B₂. When indomethacin-treated rat platelets were activated with the thromboxane A₂ analogue U46619, the presence of a second agonist such as collagen, ADP or thrombin was necessary for aggregate formation. U46619-induced aggregation in combination with either co-activator was relatively low in arachidonate-rich platelets, and was higher in platelets with a low arachidonate content. Similarly, phospholipase C-catalysed formation of *L*-*myo*-inositol phosphates was higher in platelets with a low arachidonate content. We conclude that the ability of platelets to react with thromboxane A₂ is modified by diet in such a way that a decreased substrate-limited generation of thromboxane A₂ is compensated for by an increased response to thromboxane, and vice versa. No significant differences were detected in the binding of U46619 or SQ29548 to platelets from the various dietary groups. Therefore the changed response seems not to be caused by modified properties of the thromboxane A₂/prostaglandin H₂ receptors, but by altered transduction of the thromboxane signal.

INTRODUCTION

Human as well as rat platelets contain in their membrane phospholipids large amounts of arachidonic acid (AA), which is predominantly esterified at the *sn*-2 position of phosphatidylethanolamine, -inositol, -serine and -choline [1–4]. AA can be mobilized from these phospholipids in response to a number of platelet-stimulating agents, including collagen and thrombin. Generally, AA mobilization is considered to be a rate-limiting step in the biosynthesis of bioactive eicosanoids [4–7]. The eicosanoids produced, prostaglandin G₂ and H₂ and thromboxane A₂ (TxA₂), are potent platelet agonists, which amplify the activation process caused by collagen and thrombin by binding to a specific platelet receptor [8–10].

As AA is important in activation, it is expected that relatively low AA levels in platelets would result in a low tendency for platelet activation as a consequence of the limited production of prostaglandins and TxA₂. Evidence for such a relationship has been found in the decreased aggregation and the lower TxA₂ generation in platelets from Eskimos consuming a marine diet, where AA is substituted to a large extent by *n*–3 polyunsaturated fatty acids [11,12]. In a previous dietary study with rats, we examined the effect of the degree of saturation of dietary fat on the fatty acid composition in platelet phospholipids and on the tendency for platelet activation [13]. However, we found no evidence for a correlation between the AA content of rat platelets and platelet aggregability.

In order to clarify the importance of the content of endogenous AA for Tx-dependent platelet activation, we have again changed the fatty acid composition of rat platelets by dietary means, and studied the activation process with the TxA₂ analogue U46619. Evidence is presented that various types of high-fat diets not only

affect the amount of thromboxane that can be mobilized by the platelets, but also modulate the response of platelets to TxA₂. This changed response seems not to be caused by an altered binding of TxA₂ to the plasma membrane receptors, but by subtle changes in the plasma membrane structure.

MATERIAL AND METHODS

Fish oil (*ω*-3 oil 30) was supplied by Hoffmann–La Roche (Basel, Switzerland), hydrogenated coconut oil and sunflower seed oil were from Unilever (Vlaardingen, The Netherlands), and palm oil was from Porim (Kuala-Lumpur, Malaysia). The TxA₂ mimetic U46619, (5*Z*,9*a*,13*E*,15*S*)-9,11-(epoxymethano)prosta-5,13-dien-1-oic acid (C₂₁H₃₄O₄), was a gift from Upjohn (Kalamazoo, MI, U.S.A.), and the antagonist SQ29548, [1*S*]-[1*a*,2*a*-(5*Z*)-3*a*,4*a*]-7-[3-[[2-[(phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo-(2.2.1)hept-2-yl] 5-heptenoic acid (C₂₁H₂₉N₃O₄), was from Squibb (Princeton, NJ, U.S.A.). Radioactive [15-³H(n)]U46619 and [5,6-³H]SQ29548 were bought from Du Pont/New England Nuclear (Bad Homburg, Germany), and *myo*-[2-³H]inositol (TRK 911) was from Amersham International (Amersham, Bucks., U.K.). Other chemicals were from Sigma (St. Louis, MO, U.S.A.).

Animals and diets

Male Wistar rats aged 5 weeks were provided *ad libitum* with high-fat diets containing: (i) 45% of digestible energy (en%) as hydrogenated coconut oil plus 5 en% sunflower seed oil, to prevent linoleic acid deficiency of the animals; (ii) 50 en% palm oil; (iii) 50 en% sunflower seed oil; or (iv) 45 en% fish oil plus 5 en% sunflower seed oil, to prevent linoleate deficiency. Diets

Abbreviations used: AA, arachidonic acid; TxA₂ and TxB₂, thromboxanes A₂ and B₂.

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did not contain cholesterol and had equal energy contents of 19.5 kJ/g; the remaining energy was provided by casein and corn starch. The fatty acid composition of the diets is described in [13]. The type of diet did not influence the weight of the animals or the concentration of platelets in whole blood.

Platelet isolation and activation

After a period of the various diets of 9–12 weeks, the rats were bled, blood was collected into anticoagulant buffer, and platelets were isolated and washed twice [13]. Washed platelets from two animals were pooled and finally suspended in 136 mM-NaCl, 11.9 mM-NaHCO₃, 5.6 mM-glucose, 5 mM-Hepes, 2.7 mM-KCl, 2 mM-MgCl₂ and 0.42 mM-NaH₂PO₄ (pH 7.4 with NaOH). Platelet concentrations were determined with a thrombocounter [14]. The cells were kept at 37 °C and were used within 3 h of preparation.

Aggregation of platelets (1.25×10^8 platelets, 0.5 ml) was recorded with a Chrono-log (Havertown, PA, U.S.A.) aggregometer. Platelet activations were started by the addition of thrombin (0.5–2 nM), collagen (10 µg/ml) or ADP (10 µM) (final concentrations). Where indicated, indomethacin (55 µM) and CaCl₂ (300 µM) were given 2 min before the start of activation, whereas U46619 (10–500 nM) and SQ29548 (0.5–5 nM) were supplied 10 s before the start.

Inositol metabolism

Washed platelets (1.3×10^{10} , 2 ml) were loaded with *myo*-[2-³H]inositol (1.5 MBq) in the presence of 5 µM-prostaglandin E₁ and 0.1 mM-EGTA for 3 h at 37 °C. The inositol-labelled platelets were washed and then equilibrated in the presence of 20 mM-LiCl for 30 min. Platelets (2.0×10^9 , 2 ml) were subsequently activated at 37 °C, with stirring at 900 rev./min, and incubations (all in duplicate) were stopped by the addition of chloroform/methanol/HCl (50:100:1, by vol.). Phase separation was achieved by the addition of chloroform and water, and the aqueous phase was dried under N₂ and re-dissolved in 50 mM-Tris/HCl (pH 8.0) containing 5 mM unlabelled *L*-*myo*-inositol. The labelled *myo*-inositol phosphates were then separated on Bio-Rad (Richmond, CA, U.S.A.) AG 1-X8 anion-exchange resin (formate form), as described [15,16]. Identification of the eluted products was by comparison with labelled reference compounds, the recovery being 97% or higher. In control experiments, the chloroform phase was also analysed by t.l.c. for labelled phosphoinositides [15]. Detection of lipids was as before [17].

Binding experiments

Washed platelets (2.5×10^8 , 0.5 ml) were incubated with [15-³H(n)]U46619 (1.67 kBq, diluted with 0–500 nM unlabelled U46619) or with [5,6-³H]SQ29548 (55.5 Bq, diluted with 0–50 nM unlabelled SQ29548) in the presence of 1 µM-prostacyclin for 20–30 min at 37 °C. The platelets were then transferred to 6 ml of ice-cold 0.9% NaCl, rapidly filtered through a Whatman GF/C filter and rinsed three times with 6 ml of ice-cold 0.9% NaCl. The filtration procedure was completed within 10–15 s, after which the filters were counted for radioactivity [10,17]. Control incubations to determine the aspecific binding contained additionally a 10 µM excess of unlabelled ligands. All incubations were carried out in duplicate.

Binding parameters for U46619 and SQ29548 were calculated by non-linear regression least-squares curve fitting, using the non-transformed data (B.M.D.P. Software, Los Angeles, CA, U.S.A.). Assuming a single high-affinity binding site for each compound [9,18–20], the amount of (measured) total d.p.m. bound was fitted as a function of the ligand concentration, analogous to the method described in [21]. Three parameters

were fitted simultaneously: $B_{\text{aspec.}}$ (aspecific binding), K_d and $B_{\text{max.}}$. The generated curves were used to calculate the specific binding of U46619 and SQ29548 at fixed ligand concentrations.

Analyses

The enzymic activity of thrombin was measured as before [13]. TxB₂ was quantified using the radioimmunoassay kit from Du Pont (Boston, MA, U.S.A.). Fatty acid analysis and statistical evaluation of the data other than binding data were as described elsewhere [22].

RESULTS

Effect of dietary fatty acids on platelet arachidonate content

In order to modify endogenous AA content in platelet phospholipids [13], Wistar rats were provided with one of four high-fat diets containing fatty acids with different degrees of saturation. The hydrogenated coconut oil diet contained large amounts of saturated fatty acids, and the palm oil diet contained equal amounts of monounsaturated oleic acid and saturated palmitic acid. The sunflower seed oil diet was strongly enriched in the *n*–6 polyunsaturated linoleic acid, and the fish oil diet was enriched in *n*–3 polyunsaturated fatty acids. The exact fatty acid composition of the diets is given in [13]. The AA level in all platelet phospholipids was modified considerably by the type of diet. The highest level of AA was found in platelets from rats fed on the saturated fat diet (hydrogenated coconut oil). Feeding the rats on diets rich in monounsaturated or *n*–6 or *n*–3 polyunsaturated fatty acids resulted in decreases in the AA in total phospholipids to $91 \pm 3.1\%$, $79 \pm 1.4\%$ and $51 \pm 1.1\%$ respectively (mean $\pm$ S.E.M., *n* = 6–8) compared with results with the saturated diet (see also [13]). Similar dietary effects on platelet phospholipid composition have been reported in studies from other laboratories [23,24].

Effect of dietary fatty acids on platelet activation

Rat platelets require extracellular Ca²⁺ in order to form aggregates [25]. Adjustment of the external Ca²⁺ concentration to 300 µM was sufficient to induce high platelet aggregation with collagen (Fig. 1a). Collagen-induced aggregation was significantly lower with platelets from rats fed on the saturated fat diet than on the other diets (Fig. 2). Thrombin-induced aggregation was affected by the type of diet in a similar way, but the differences between diet groups were smaller.

We activated platelets from all dietary groups with collagen in the presence of various CaCl₂ concentrations, and then measured aggregation and production of TxB₂ (Fig. 3). Higher CaCl₂ concentrations led to increased aggregate formation and increased TxB₂ production. The saturated fat diet resulted in platelets with a relatively low aggregation tendency, but with a high TxB₂ production, whereas the platelets from fish-oil-fed animals combined high aggregatability with a low TxB₂ generation (Fig. 3). Upon activation with thrombin, only small amounts of TxB₂ were produced. Nevertheless, platelets from rats fed on the saturated fat and *n*–3 polyunsaturated fat diets differed in their relationship between aggregate formation and TxB₂ production in a similar way to that discussed for collagen (see Fig. 2). We conclude that the content of endogenous AA in platelet phospholipids is not the primary factor that determines aggregation with either collagen or thrombin as agonist.

Effect of diets on TxA₂-induced platelet activation

In a second study, the effect of dietary fatty acids was examined on Tx-dependent platelet activation and on binding of Tx to the platelets. For this study the dietary regimens were chosen that resulted in the most prominent changes in platelet AA content,

Fig. 1. Synergistic effects of Ca²⁺ and U46619 on aggregation of platelets from rats fed on a sunflower seed oil diet
(a) Platelets were activated with collagen (10 µg/ml) in the presence of various concentrations of Ca²⁺_{free}. (b) and (c) Indomethacin-treated platelets were activated with collagen (10 µg/ml), thrombin (1 nM) and/or U46619 (100 nM) in the presence of 300 µM-CaCl₂, as indicated. Tracings of light transmission are given.

Fig. 2. Effect of high-fat diets on collagen- and thrombin-induced platelet aggregation
Washed platelets were activated in the presence of CaCl₂ (300 µM) with collagen (10 µg/ml) or with thrombin (2 nM). The platelets were pre-incubated with indomethacin (indo; 55 µM) where indicated. Presented are percentage changes in light transmission (%T; mean values ± s.e.m., *n* = 6–8) after 5 min of activation. The number above each bar indicates the amount of TxB₂ (in ng/ml) produced during activation. Diets: □, hydrogenated coconut oil; ▨, palm oil; ■, sunflower seed oil; ▩, fish oil. Significance of differences is indicated by **P* < 0.05, ***P* < 0.01 compared with platelets from rats fed on the hydrogenated coconut oil diet.

Fig. 3. Correlation between platelet aggregation and TxB₂ production in platelets
Washed platelets were activated with collagen (10 µg/ml) in the presence of 0–500 µM-CaCl₂, as indicated. Shown are changes in light transmission (%T) as a function of the TxB₂ formed during 5 min of activation. Mean values ± s.e.m. (*n* = 6–10) are given for platelets from rats fed on diets of hydrogenated coconut oil (○), palm oil (△), sunflower seed oil (●) and fish oil (▲).

i.e. the hydrogenated coconut oil, sunflower seed oil and fish oil diets. Platelet AA levels were determined and found to be changed by diet in the expected way (results not shown).
In indomethacin-treated washed rat platelets, the TxA₂ mimetic U46619 by itself induced only shape change, but no aggregation (Fig. 1b). On the other hand, U46619 strongly potentiated platelet aggregation in combination with other agonists such as collagen (Fig. 1b), thrombin (Fig. 1c) or ADP (Fig. 4) when supplied in doses that did not induce high aggregation by themselves.

U46619-induced shape change was influenced by dietary fatty acids at low U46619 concentrations (Table 1). Platelets from rats fed on the *n*–3 polyunsaturated fat diet responded better than did platelets from the other groups to 10 nM-U46619. At 50 nM, almost the maximal shape change was reached in each diet group, and effects of diet type were no longer significant. When co-activated with collagen (in the presence of indomethacin), ‘fish-oil platelets’ aggregated already at low doses of U46619

Fig. 4. Effect of U46619 on ADP- and thrombin-induced aggregation of platelets

Washed platelets were activated in the presence of indomethacin ($55 \mu\text{M}$) and CaCl_2 ($300 \mu\text{M}$) with either (a) ADP ($10 \mu\text{M}$) plus fibrinogen ($20 \mu\text{g/ml}$) or (b) thrombin (2 nM). Where indicated, U46619 (50 nM) was present. Changes in transmission (%T; mean values $\pm$ S.E.M., $n = 8-12$) are given, recorded during 5 min of activation. Diets: □, hydrogenated coconut oil; ■, sunflower seed oil; ▨, fish oil. Significance of differences is indicated by * $P < 0.05$, ** $P < 0.01$ compared with platelets from hydrogenated coconut oil group.

Table 1. Effect of diets on platelet shape change induced by various concentrations of U46619

Platelets were preincubated with indomethacin. Shown is the percentage decrease in light transmission (mean $\pm$ S.E.M., $n = 7-12$). Abbreviations: FO and HCO, fish oil and hydrogenated coconut oil diet (each including 5 en % of sunflower seed oil), SO, sunflower seed oil diet (see the Materials and methods section for details). *Significantly different from platelets from HCO and SO groups; $P < 0.001$.

U46619 (nM)	Type of diet...	Decrease in light transmission (%)		
		HCO	SO	FO
10		4.8 ± 0.4	5.9 ± 0.5	$7.9 \pm 0.2^*$
50		14.6 ± 0.8	15.9 ± 0.8	16.4 ± 0.5
100		15.4 ± 0.6	17.3 ± 0.6	17.1 ± 0.5

(10 nM), and aggregated rapidly with higher doses (Fig. 5a). Platelets from animals fed on saturated fatty acids had a relatively small response to U46619, whereas platelets from the sunflower seed oil group were intermediate in reactivity. When ADP or thrombin was used as second agonist, the same differences in platelet responses to U46619 were found (Fig. 4) as observed in combination with collagen. Thus, irrespective of the type of co-activator used (collagen, ADP or thrombin), a saturated fat diet decreased and an $n-3$ polyunsaturated fat diet increased the platelet aggregation reaction with U46619, when compared with the $n-6$ polyunsaturated fat diet.

We studied the properties of the TxA_2 /prostaglandin H_2 receptors by measuring the binding to platelets of radioactive [^3H]U46619 and [^3H]SQ29548, the latter being a high-affinity TxA_2 antagonist [19]. At none of the concentrations of U46619 or SQ29548 tested was a significant effect of diet measured on the amount of label bound to the platelets (results not shown). Subsequently, binding curves were calculated using non-linear regression analysis and assuming the presence of one high-affinity binding site for each compound [9,18-20]. This resulted in a set of binding parameters for U46619 and SQ29548, which again

Fig. 5. Effect of diets on U46619-dependent platelet aggregation and on binding of U46619

(a) Indomethacin-treated platelets were activated with collagen ($10 \mu\text{g/ml}$) and U46619 ($0-500 \text{ nM}$) in the presence of $300 \mu\text{M}$ CaCl_2 . (b) Platelets were incubated with [^3H]U46619 ($12-500 \text{ nM}$) and the specific binding at various concentrations of U46619 was calculated from a fitted binding curve, as described in the Materials and methods section. Data are mean values $\pm$ S.E.M., ($n = 6-8$). Diets: □, hydrogenated coconut oil; ■, sunflower seed oil; ▨, fish oil. Significant differences from platelets from hydrogenated coconut oil group: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Significant differences from platelets from sunflower seed oil group: † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$.

Table 2. Characteristics of binding of TxA_2 analogues to diet-modified platelets

The binding data for U46619 and SQ29548 were calculated from a fitted curve, as described in the Materials and methods section. Data are mean values $\pm$ S.E.M. ($n = 6$). Platelets from the same animals were also used for aggregation studies (see Figs. 4 and 5). Abbreviations for diets are as in Table 1.

Type of diet...	HCO	SO	FO
U46619			
K_d (nM)	43.4 ± 7.1	50.7 ± 16.1	56.7 ± 9.6
B_{max} (fmol/ 10^8 platelets)	84 ± 18	92 ± 15	97 ± 15
SQ29548			
K_d (nM)	1.95 ± 0.16	1.62 ± 0.10	1.85 ± 0.08
B_{max} (fmol/ 10^8 platelets)	206 ± 27	194 ± 11	183 ± 11

were similar for platelets from animals on all types of diet (Table 2).

For a further evaluation of the binding data, the fitted curves were used to calculate specific binding at fixed concentrations of U46619. The results were compared with the effect of the same concentrations of U46619 on platelet aggregation (Fig. 5). Clearly, the type of diet influenced greatly U46619-dependent platelet aggregation, but not the amount of U46619 bound to the platelets. Moreover, when the activation data obtained with each pool of platelets (i.e. platelets from each pair of animals) were plotted as a function of the binding data, a correlation coefficient

Fig. 6. Phospholipase C-catalysed formation of *myo*-inositol phosphates in platelets

Washed platelets were preloaded with *myo*-[³H]inositol as described in the Materials and methods section. The degree of labelling was similar in platelets from all diet groups. (a) Indomethacin-treated platelets were activated with 200 nM-U46619 and 20 µg of collagen/ml. (b) Platelets were activated with 2 nM-thrombin. All activations were carried out with 1×10^9 platelets/ml (2 ml) in the presence of CaCl₂ (300 µM) for 20 s. These incubation conditions induced the expected diet-induced differences in the platelet aggregation (compare with Figs. 2 and 5). Changes are given in the labelling of *myo*-inositol phosphates relative to that in unstimulated platelets (mean values $\pm$ S.E.M., $n = 8$). Diets: □, hydrogenated coconut oil; ■, sunflower seed oil; ▨, fish oil. Significant differences from platelets from hydrogenated coconut oil group: * $P < 0.05$, ** $P < 0.01$.

was obtained of -0.08 ± 0.54 (Spearman rank coefficient with $n = 18$; 50 nM-U46619), again suggesting a lack of correlation between activation and binding.

In human as well as in rat platelets, the strong agonists thrombin and U46619 induce rapid phospholipase C-catalysed formation of *L*-*myo*-inositol phosphates [26–29]. In order to measure initial responses of diet platelets, we monitored *myo*-inositol metabolism in the early stages of activation. Platelets preloaded with *L*-*myo*-[³H]inositol were activated during 20 s with either U46619 (in the presence of indomethacin and collagen) or thrombin, and labelled *myo*-inositol phosphates were analysed. Fig. 6(a) shows that the U46619/collagen-induced formation of *myo*-inositol phosphates was modified by diet in a similar way as was platelet aggregability (see Fig. 5a). Control experiments in which the individual contributions of U46619 and collagen were determined indicated that U46619 accounted for 70–75 % of the phosphoinositide hydrolysis (results not shown). Thrombin-induced inositol metabolism was not significantly changed by diet, although differences between the different dietary groups showed the same trend as seen with U46619/collagen (Fig. 6b).

DISCUSSION

Modulation by high-fat diets of platelet reactivity with TxA₂

Our results demonstrate that high-fat diets, which modify the level of endogenous AA in platelet phospholipids as well as the amount of Tx produced upon platelet stimulation, change the tendency of platelets to be activated in a way that is not proportional to the effect on AA metabolism. Three lines of evidence indicate that diets modify platelet activation by means

of an altered response to TxA₂. Firstly, diets change the quantitative relationship between the formation of aggregates and the amount of TxB₂ produced upon aggregation (Fig. 3). Further, diets change the synergistic effect of the TxA₂ analogue U46619 on collagen-, ADP- or thrombin-induced platelet aggregation (Figs. 4 and 5a). Finally, diets affect the amount of *myo*-inositol phosphates formed by phospholipase C in response to U46619/collagen (Fig. 6a).

The rat platelets reacted very slowly or not at all with platelet-activating factor, 5-hydroxytryptamine or adrenaline (results not shown). Therefore these compounds are unlikely to interfere with the synergistic effect of U46619 in collagen-, ADP- and thrombin-induced aggregation, or with the modulation of this effect by diet. It is thought that TxA₂/prostaglandin H₂ receptors are coupled to phospholipase C by means of GTP-binding proteins [28–30]. The number of platelets available limited the study of *myo*-inositol metabolism to a single activation with U46619 in combination with collagen. Since U46619 was a stronger stimulator of *myo*-inositol phosphate formation than was collagen, our results suggest that the Tx-induced phospholipase C activity is altered by diet, although an effect of diet on its potentiation by collagen cannot be excluded. In any case, we believe that the primary effect of diet on TxA₂ aggregation is early in the signal transduction pathway, although secondary effects (such as altered secretion of ADP or fibrinogen, or modified exposure of fibrinogen receptors) may potentiate the initial effect. In rabbit platelets activated with thrombin, indeed, it has been shown that polyunsaturated fatty acids in the diet can modulate the phospholipase C-dependent production of inositol phosphates independently of their effect on TxB₂ production [16].

Taken together, the present results indicate that platelets from rats fed on saturated fatty acids are rather insensitive to TxA₂, that $n-6$ polyunsaturated fatty acids in the diet make platelets more reactive towards TxA₂, and that the diet containing $n-3$ polyunsaturated fatty acids results in platelets with greatest sensitivity to Tx. Consequently, the ability of platelets to react with TxA₂ seems to be modified by diet in such a way that a decreased substrate-limited generation of TxA₂ is compensated for by an increased response to TxA₂, and vice versa.

Effect of high-fat diets on platelet TxA₂ receptor properties

We could not obtain evidence for an altered binding of TxA₂/prostaglandin H₂ analogues to the receptors on platelets from different dietary groups. In binding studies with U46619 or SQ29548, neither the K_d nor the B_{max} were changed significantly by the type of diet (Table 2). The K_d and B_{max} values obtained are in agreement with those reported previously for rat [18] and human [19,31] platelets. The apparent lack of effect of diet on TxA₂ binding is also compatible with a lack of effect on the IC₅₀ value (concn. causing 50 % inhibition) for SQ29548 in collagen-induced platelet aggregation (results not shown).

Recently the development of new high-affinity TxA₂ analogues has led to the identification of two subtypes of TxA₂/prostaglandin H₂ receptor sites in human platelets, one mediating platelet shape change and the other (linked to phospholipase C) resulting in platelet aggregation [29,32]. At present, it is not clear whether these subtypes also exist in rat platelets. The inability of U46619 to induce aggregation of rat platelets in the absence of a co-activator (the present paper; [27]) may suggest that platelets from man and rat differ in the population of their receptor sites, but could also point to differences in regulatory function of the TxA₂ receptors. On the other hand, Arita and coworkers [27,28] have distinguished two phases in the response to U46619 of platelets from both man and rat, which seems compatible with the existence of two receptor subtypes in both species. We

measured an influence of high-fat diets on U46619-induced platelet aggregation in the presence of co-activators (Figs. 4 and 5a), and also on U46619-induced shape change (Table 1), and conclude that both types of signal transduction may be modified; notably, the type mediated by receptors linked to phospholipase C seems to be affected.

The question remains of how (almost) unchanged occupation of the TxA_2 /prostaglandin H_2 receptors can lead to differences in the platelet activation tendency and, particularly, in generation of *myo*-inositol phosphates. One possibility is that slightly changed receptor properties (being too small to be detected in the assay) lead to magnified changes in activation. However, the dose-response relationship of U46619 (with an affinity supposed to be similar to that of TxA_2) does not predict that minor changes in binding would result in major differences in aggregation (Fig. 5). Alternatively, the diet-induced change in the fatty acid composition of phosphatidylinositol and, consequently, in its phosphorylated derivatives may alter the phospholipase C-catalysed degradation of phosphoinositides. Finally, diets may alter the physical properties of the plasma membrane, resulting in an altered coupling of TxA_2 receptors with effector proteins such as phospholipase C. The last explanation is supported by the previously observed low cholesterol/phospholipid ratio and the high degree of unsaturation of phospholipids in platelets from rats fed on hydrogenated coconut oil, compared with the sunflower seed oil and fish oil groups. These changes were expected to influence platelet membrane fluidity, although the changes were too small to detect by fluorescence polarization [13].

Implications

Many studies on the biological effects of dietary polyunsaturated fatty acids concentrate on the altered cellular lipid and eicosanoid metabolism. However, the present data suggest that the cellular responses to eicosanoids can also be influenced by dietary means. This can explain, at least partially, the rather complicated relationship between platelet phospholipid composition and the tendency of platelets to be activated [1,23,24].

An unexpected observation was the consistently high reactivity of platelets from rats fed on *n*-3 polyunsaturated fatty acids. Extensive examination of the literature revealed that the effect of a fish oil diet on platelet aggregation is rather equivocal [12]. We emphasize that our measurements were carried out with washed platelets in the presence of high Ca^{2+} concentrations (300 μM) and that platelets from fish-oil-fed animals activated at lower Ca^{2+} concentrations (20–50 μM) were rather inactive (J. W. M. Heemskerk & M. A. H. Feijge, unpublished work). Therefore the Ca^{2+} concentration as well as the responsiveness to endogenously generated TxA_2 should be taken into account in studies in which platelet activation is manipulated by dietary means. Data obtained with citrated platelet-rich plasma [11,23] may thus differ from the present ones with high Ca^{2+} concentrations.

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