Regulation of prostaglandin E_2 receptors in vivo by dietary fatty acids in peritoneal macrophages from rats

F. A. Opmeer,[1] M. J. P. Adolfs, and I. L. Bonta

Department of Pharmacology, Medical Faculty, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands

Abstract Groups of rats were pretreated with 4-week diets containing 12.5% corn oil or linseed oil. At the end of this period peritoneal macrophages were elicited and isolated. These cells were used for binding experiments with ^{3}H-PGE_2 and for estimation of prostaglandin-stimulated cAMP production. Specific binding of ^{3}H-PGE_2 was saturable, reversible, protein-dependent, and correlated with stimulation of cAMP production, indicating that specific binding referred to receptor binding. PGE_1 and PGI_2 were far less effective than PGE_2 in competition of binding with ^{3}H-PGE_2, indicating receptor selectivity for PGE_2. Scatchard analysis of the secific binding data revealed a high affinity component (K_d 17 nM) and a low affinity component. The total number of high- and low-affinity binding sites, respective K_d values, and PG stimulation of cAMP production of cells from rats fed the linseed oil diet were comparable to controls. The corn oil diet, however, resulted in a twofold increase in total number of high- and low-affinity binding sites, while respective K_d values were unchanged. This enhancement of binding capacity could be explained by an increased density of binding sites on the cells, and may itself be responsible for the increased sensitivity of the macrophages in this diet group for PG-stimulated cAMP production. The data suggest a regulatory mechanism at the receptor level and are discussed in terms of possible altered bioavailability of arachidonic acid-derived PGE_2.—**Opmeer, F. A., M. J. P. Adolfs, and I. L. Bonta.** Regulation of prostaglandin E_2 receptors in vivo by dietary fatty acids in peritoneal macrophages from rats. *J. Lipid Res.* 1984. **25:** 262–268.

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It is now well established that certain essential fatty acids (EFAs) are the precursors for prostaglandins (PGs) and other oxygenated products that modulate various pathophysiological processes in specific and often opposing ways. In part, the type and amounts of PGs produced depend on what unsaturated fatty acids are available as substrates. The type of dietary fat determines which acids are potentially available and modifies activities of specific enzymes involved in the generation of arachidonic acid (AA) and other PG precursors (1). Consequently, dietary variation in EFA content affects PG synthesis and release, and may influence immuno-inflammatory processes or other pathophysiological conditions (2). An integrated control mechanism of dietary fat on PG biosynthesis can be demonstrated (3). Since PGs act in a manner consistent with current models of hormone receptor interactions, it is conceivable that long-term shifts in PG concentrations may influence certain PG receptor populations, resulting in an adaptation to the altered environmental conditions. This type of interaction has been reported for PGE_1 receptors in liver membranes and erythrocytes (4, 5), and for $PGF_{2\alpha}$ receptors in skin membranes (6). Moreover, tissues of animals maintained on EFA-deficient diets, showed markedly reduced PG synthesis (7, 8) and an increased specific binding capacity for $PGF_{2\alpha}$, which could be restored to normal values after refeeding the animals with an EFA-rich diet (6).

A basal dietary intake of EFA equivalent to 1–3% of the calories is generally accepted now to prevent symptoms of EFA deficiency and to provide sufficient amounts of precursor for the PG production. A moderate increase in the basal requirement of EFA may be indicated in various pathophysiological conditions; at least such an increase in basal EFA intake enhances PG production (9, 10). However, dietary administration of very high amounts of EFAs to infants (11) and rats (12) or in vitro incubation of skin with [^{14}C]arachidonic acid (AA) in the presence of high concentrations of various EFAs (1), resulted in reduced PG synthesis. It is not known whether such treatment simultaneously affects PG binding sites, as has been shown for the EFA-deficient state on $PGF_{2\alpha}$ binding (6).

Immuno-inflammatory stimuli attract and activate macrophages. These major effector cells in most inflammatory events discharge products such as PGE_2, which have stimulating or suppressive effects, depending on the inflammatory conditions (10). The lipids of peritoneal

Abbreviations: EFA, essential fatty acid; AA, arachidonic acid; PG, prostaglandin.

[1] To whom reprint requests should be addressed.

macrophages contain high levels (25%) of esterified AA. Inflammatory stimuli can promote the release of 40–50% of this AA store and the major part of it is recovered as PGs (13). Consequently, elicited peritoneal macrophages were the prime targets for our studies on the effects of dietary fatty acids on PGE_2 receptor characteristics. Since regulatory changes at the receptor level may have consequences on the adenyl cyclase-cAMP system, the dietary influence on stimulated cAMP production was investigated as well.

MATERIALS AND METHODS

Diets and isolation of peritoneal macrophages

Two groups of male Wistar rats (4 weeks old) were pretreated during a 4-week period with diets enriched with corn oil or linseed oil (12.5%). The diets consisted of caseine (62 g), vitamin mix (1 g), salt mix (5.4 g $CaCo_3$ + Ca $(PO_4)_2$ + KCl + NaCl + MgO), cellulose (15 g), cerelose (149 g), and 27 g of linseed oil or corn oil, respectively. Animals were housed separately in metal cages (23°C ± 1°C, relative humidity 60% ± 10%). Weights were controlled weekly. At day 27 the rats were given an intraperitoneal injection of 5 ml of a starch solution in order to elicit a population of peritoneal macrophages. After 24 hr rats were killed and the peritoneal cavity was flushed with 2 × 25-ml portions of a cold solution (pH 7.8) containing NaCl (0.9%), Tris–HCl (15 mM), $CaCl_2$ (0.05 mM), KCl (5 mM), $MgSO_4$ (1 mM), glucose (0.1%), and indomethacin (10^{-5} M). The harvested cells were spun down (10 min at 4°C; 800 *g*) and layered over Ficoll-Isopaque. Centrifugation (30 min at 4°C, 400 *g*) resulted in two cell layers, the upper one consisting of at least 90% viable macrophages as examined by Trypan Blue exclusion. These cells were aspirated and washed after which appropriate dilutions were made for the incubations. Samples (0.1 ml) were taken for protein assay (14). cAMP levels were determined by the protein binding method (15) as described earlier (16).

^{3}H-PGE_2 binding studies

Aliquots of the cell suspension (0.3 ml containing 1–3 mg of protein/ml) were added to 5 nM ^{3}H-PGE_2 dissolved in buffer, in the presence or absence of various concentrations of unlabeled PGs. Incubations (assay volume: 0.36 ml; 4°C; 90 min) were carried out in a metabolic shaker. The incubation was terminated by diluting triplicate samples (0.1 ml) to 4 ml with ice-cold buffer in siliconized glass tubes, followed by rapid filtration under reduced pressure through Whatman GF/A glass filters. The glass filters and tubes were then quickly washed with four portions of 5 ml of ice-cold buffer. The residue on the dry filters was subsequently extracted with 0.2 ml of Soluene 350 for 16 hr. Radioactivity (dpm) was determined by liquid scintillation spectrometry (counting efficiency 46%).

Analysis of data

Scatchard plots were analyzed using the equations as described by Rosenthal (17). The lines drawn on the Scatchard plots are the theoretical lines generated by employing the constants so obtained from the binding data. Hill plots were obtained by transferring the binding data in the appropriate formula. The lines were fitted to sets of data points using least squares regression analysis, allowing estimates to be made of slopes and intercepts.

Materials

^{3}H-PGE_2 (sp act 160 Ci/mmol) was obtained from Amersham BV Utrecht, The Netherlands, with a radiochemical purity greater than 98% as judged by thin-layer chromatography. The prostaglandins PGE_1 and PGE_2 were gifts from Dr. A. Vergroesen, Unilever Research Lab., Vlaardingen, The Netherlands. PGI_2-sodium salt was obtained through the courtesy of Dr. E. Schillinger, Schering AG, Berlin, F.R.G. (±)-5E-13,14-Didehydro-carbo-prostacyclin (DDH-carbo-PGI_2) was from Prof. C. A. Gandolfi (Farmitalia, Carlo Erba, Milan, Italy). Rats were kept on diets at Unilever, Vlaardingen, The Netherlands. Linseed oil consists of the following fatty acids: 19.0% oleic acid, 24.1% linoleic acid, 47.4% α-linolenic acid, and 9.5% saturated fatty acids. Corn oil consists of 1.5% palmitoleic acid, 49.6% oleic acid, 34.3% linoleic acid, and 14.6% saturated fatty acids.

RESULTS

Effects of dietary fatty acid composition on the elicited population of peritoneal macrophages

Aliquots of the cell population isolated from the upper layer after Ficoll-Isopaque density centrifugation were used to determine total number of harvested cells and cell viability by Trypan Blue exclusion. It appeared that viability of the cells was at least 90%. Total number of harvested macrophages per rat after eliciting this population by a starch injection was 26×10^6 (n = 180) and 28×10^6 (n = 40) in control and linseed oil groups, respectively. A 25% higher amount of macrophages was harvested from the corn oil diet rats (36×10^6 macrophages per rat (n = 40)). The number of harvested resident (non-elicited) peritoneal macrophages is normally 2 to 4×10^6 cells/rat. For differential cell counts, smears were stained with May Grünwald-Gemsa solution. Microscopic examination revealed that the percentage of harvested macrophages was unaffected by the diets (± 92% monocytes of total cell count). Additionally, the shape and size of the macrophages seemed to be unaf-

fected by the dietary pretreatments of the rats. Moreover, protein content of the cells averaged 77 ± 8 $\mu g/10^6$ cells.

Effects of dietary fatty acid composition on PG-stimulated cAMP production in elicited peritoneal macrophages

Basal levels of cAMP of elicited peritoneal macrophages were between 20 and 30 pmol/mg protein and were not altered by dietary pretreatments of the animals. Stimulation of cAMP production with various PGE_2 concentrations (**Fig. 1A**) showed that the dose-response curve obtained with cells from the linseed oil diet rats was similar to that with cells harvested from control rats. However, compared to this curve, the PGE_2 dose-response curve obtained with cells from the corn oil diet rats showed a parallel shift to the left, while maximal stimulation was not changed. A comparable situation was observed with the stimulation of cAMP production with a stable analogue of PGI_2 (DDH-carbo-PGI_2). The dose-response curve with cells from the corn oil diet rats was shifted to the left. Although approximately the same maximal cAMP production could be attained as with PGE_2, 20- to 30-fold higher concentrations of DDH-carbo-PGI_2 were required to produce half-maximal stimulation of cAMP production and the slopes of the curves were less steep than that obtained with PGE_2 (Fig. 1B).

Effects of dietary fatty acid composition on PGE_2 receptor binding of elicited peritoneal macrophages

Linearity with protein. Suspensions of peritoneal macrophages from rats that were kept on the 4-week diets were diluted to various protein concentratons and incubated with a fixed concentration of ^{3}H-PGE_2 (5 nM). These incubations were performed in the absence and in the presence of excess unlabeled PGE_2 (5 μM). Total binding and the difference of binding in the absence minus binding in the presence of unlabeled PGE_2 (specific binding) revealed that, over the concentration range that was tested, the binding was linear to the protein concentration. Most experiments were performed with 1.5 to 2.5 mg protein/ml. Under these conditions, less than 1% of the total ^{3}H-PGE_2 added was bound, so that the free ligand concentration was essentially constant throughout an experiment.

Concentration dependence of binding. **Fig. 2** shows the results of experiments in which the ligand concentration was increased by addition of unlabeled PGE_2 to 5 nM ^{3}H-PGE_2. Specific binding of PGE_2 to isolated peritoneal macrophages from rats kept for 4 weeks on diets enriched with corn oil or linseed oil, respectively, is plotted as a function of the diluting ligand. Specific binding seemed to be saturable at ligand concentrations between 10^{-5} and 10^{-4} M. Control data from specific binding of ^{3}H-PGE_2 to elicited macrophages from control rats are inserted for comparison. It appeared that the pretreatment of rats with the corn oil diet had markedly increased specific binding of PGE_2 to macrophages at all ligand concentrations tested.

Specific binding data, corrected for the dilution of specific activity, can be treated by Scatchard analysis. Two straight lines were obtained for each dietary group, demonstrating high and low affinity binding activities (**Fig. 3**). It appeared that binding data from the linseed oil diet rats could easily be plotted together with such data obtained from experiments performed with macrophages from control rats. Hill plots of the same data (Fig. 3, insert) show straight parallel lines. The interaction coefficients (slopes of the lines) were found to be 0.90 and

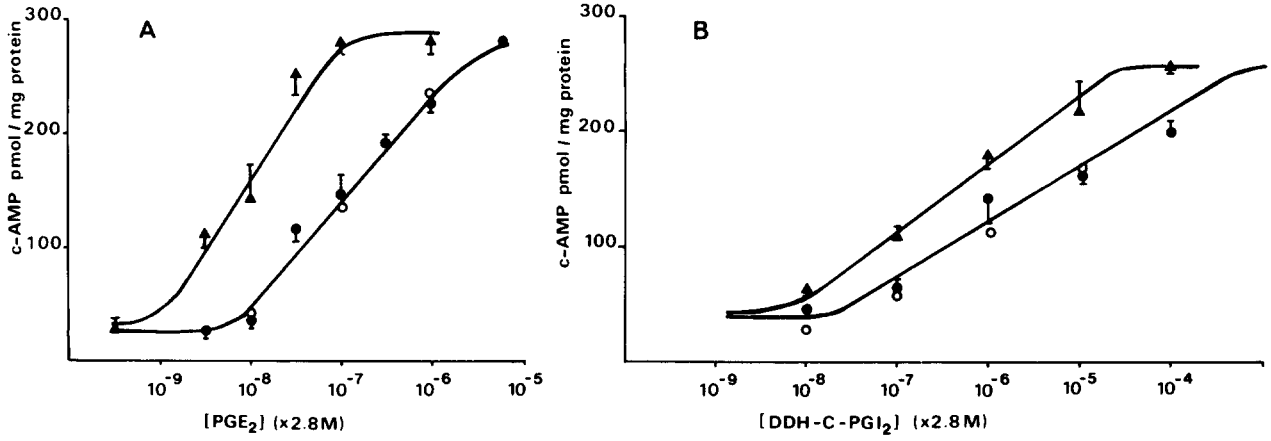

Fig. 1. Stimulation of cAMP production by prostaglandins in elicited peritoneal macrophages from control rats (○) and from rats maintained for four weeks on diets enriched with linseed oil (●) or corn oil (▲). Macrophages were incubated (10 min, 37°C) in the presence of isobutylmethyl xanthine (IBMX) and various concentrations of PGE_2 (A) or DDH-c-PGI_2 (B). cAMP production, expressed as pmol/mg protein is shown as the mean (±SEM) of triplicate determinations from four experiments. Linear parts of the dose-response curves were calculated by the method of least squares. Slopes of the lines in A were not significantly different but they differed in slope from the parallel lines in B. (A: r = 0.89, 0.98, and 1.00 for the corn oil, linseed oil, and control groups, respectively, slopes: NS), (B: r = 0.96, 0.83, and 0.99 for the corn oil, linseed oil, and control groups respectively, slopes: NS).

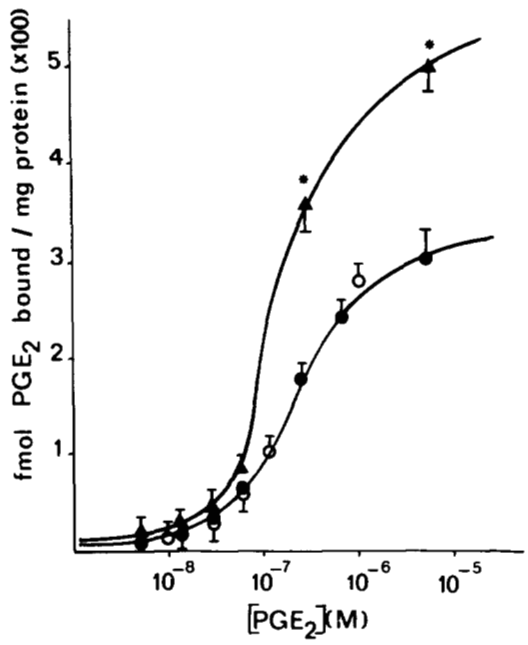

Fig. 2. Concentration dependence of ^{3}H-PGE_2 binding to peritoneal macrophages derived from control rats (○) and rats kept on diets enriched with linseed oil (●) or corn oil (▲). Specific bound PGE_2 per mg protein (corrected for specific activity) is plotted as a function of diluting concentrations of PGE_2. For methods see text. Each data point shows the result of the mean ± SEM of triplicate determinations from two different experiments. *, Means significantly different from respective data of the linsead oil curve ($P < 0.05$; Student's *t*-test).

0.89 for the two dietary groups. Scatchard analysis yielded dissociation constants (K_d) and binding capacities (B_{max}) as follows: high affinity K_d values were 1.7×10^{-8} M and 1.9×10^{-8} M for the linseed oil and corn oil groups, respectively. Low affinity K_d values for these groups were 11.7×10^{-8} M and 10.9×10^{-8} M, respectively. In contrast to the comparable high or low affinity K_d values for the two diet groups, marked differences were found for the binding capacities. It appeared that the corn oil diet resulted in increased B_{max} values for both the high and low affinity components of PGE_2 binding to macrophages. The linseed oil diet, which can be compared to standard rat food in this respect, resulted in high and low affinity B_{max} values of 30 and 600 fmol/mg, respectively, while the data resulting from rats fed the corn oil diet were 50 and 1280 fmol/mg, respectively.

Dietary fatty acid influence on specificity of PG binding. The specificity of PG binding to elicited peritoneal macrophages was determined by competition between ^{3}H-PGE_2 (5 nM) and unlabeled PGE_2, PGE_1, and PGI_2. **Fig. 4** summarizes the interactions of these PGs with macrophages from rats of the two diet groups. The data are expressed as percent of the specific binding of ^{3}H-PGE_2, obtained with cells from rats fed standard diets, as a function of the concentration of the competing ligand. It appeared that there was no influence of diet on the slopes of the curves for PGE_2, PGE_1 and PGI_2. The highest competing potency was found with PGE_2 (concentration at which half-maximal binding occurs: 10×10^{-8} M, for cells derived from linseed oil diet rats). The order of potency was $PGE_2 > PGE_1 > PGI_2$. For all competing ligands tested, the corn oil diet resulted in absolute shifts to the right of the competition curves (e.g., PGE_2 concentration at which half-maximal binding occurs: 20×10^{-8} M for cells derived from corn oil diet rats). A marked difference in slope was noted for the parallel curves of PGE_1 and PGI_2 competition, compared to that of PGE_2.

DISCUSSION

Experiments were performed to assess the feasibility of measuring PGE_2 specific binding to intact macrophages from control rats, which was coupled to the adenyl cyclase-cAMP system. Optimal binding conditions were determined and a detailed characterization of PGE_2 receptors (saturation and specificity of binding, stability of the ligands during incubations, K_d and B_{max} values, etc.) using viable macrophages and membrane fractions from these cells has recently been described[2] (18). These studies (not shown) revealed that specific binding of ^{3}H-PGE_2 was enhanced in plasma membrane fractions of peritoneal macrophages and could be subdivided in high affinity (K_D ± 30 nM) and low affinity (K_D ± 220 nM) sites. Optimal conditions resulted in 50–60% specific binding. For competition studies it was found that pGI_2 was stable during the incubation period at a temperature of 4°C. Inasmuch as a higher incubation temperature (37°C) is used in the cAMP assay, the stable analogue DDH-carbo-PGI_2 was used here instead of PGI_2, which had comparable activity as measured by inhibition of ADP-induced platelet aggregation.

The marked increase in specific binding of PGE_2 to macrophages due to the corn oil diet as presented in Fig. 2 could indicate an increase in affinity and/or receptor capacity or a shift of receptor populations, e.g., towards more high affinity sites, or changes in specificity for other PGs like PGE_1 or PGI_2. Further analysis of the data (Hill and Scatchard plots, Fig. 3) and additional experiments in which the competition for the binding sites is examined should give more information about the nature of the observed effects of the corn oil diet on PGE_2 binding and on stimulated cAMP production. The observed interaction coefficients in the Hill plots were found to be less than 1.0. This may be an indication of the heterogeneity of the receptor population or could be due to negative cooperativity between binding sites. Based on present steady state binding studies, however, it is not possible

[2] Opmeer, F. A., M. J. P. Adolfs, and I. L. Bonta. Unpublished results.

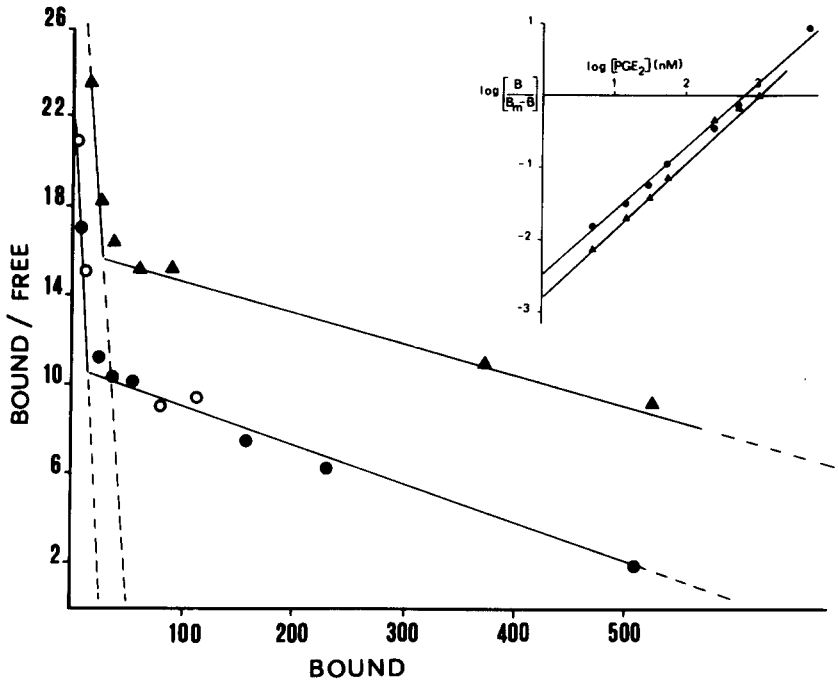

Fig. 3. Scatchard plot of specific binding of PGE_2 to peritoneal macrophages from experimental rats (●, linseed oil diet, ▲, corn oil diet) and from control rats (○). The units are: ordinate, femtomoles of PGE_2 bound per mg of protein/$[PGE_2] \times 10^{-8}$ M; abscissa, femtomoles of PGE_2 bound per mg of protein. Peritoneal macrophages (protein content between 1 and 2 mg/ml) were incubated (90 min, 4°C) with ^{3}H-PGE_2 (concentration range 3 to 7 nM) in the absence and presence of excess (5 μM) unlabeled PGE_2. The theoretical lines were obtained by substitution of the estimated constants into the binding equations and into appropriate expressions for intercepts of the tangent lines (see text and ref. 17). Equations of the lines were as follows: high affinity lines $y = -0.73x + 22.0$ and $y = -0.70x + 35.0$ for linseed oil and corn oil diets, respectively; low affinity lines $y = -0.018x + 10.8$ and $y = -0.013x + 16.0$ for linseed oil and corn oil diets, respectively. Insert: Hill plots of the data. B (specific binding at any particular PGE_2 concentration) and B_{max} (binding at a saturation ligand concentration) were determined from the Scatchard analysis. Lines are fitted to sets of data points using least squares regression analysis. Triplicate binding data from four different experiments were used.

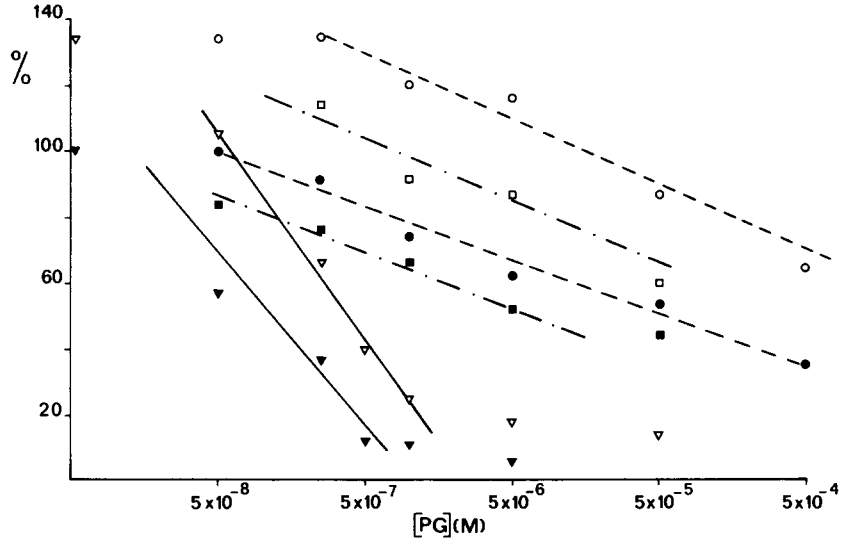

Fig. 4. Competition between ^{3}H-PGE_2 and PGE_2 (▽), PGE_1 (□), and PGI_2 (○) for specific binding to peritoneal macrophages elicited in rats pretreated with 4-week diets enriched with linseed oil (filled symbols) or corn oil (open symbols). ^{3}H-PGE_2 (5 nM) was added to cell suspensions simultaneously with various concentrations of the unlabeled ligands and incubated at described in Methods. The results (mean values of triplicate determinations of four experiments) are expressed as percentage of binding of ^{3}H-PGE_2 (5 nM) to macrophages from control rats as a function of the competing ligand concentration ([PG](M)). Lines were drawn according to the description in Methods.

to distinguish between these alternatives (19). Assuming that multiple receptor sites for PGE_2 are present on peritoneal macrophages, it is possible to resolve the Scatchard plot into two straight lines (17), representing two noninteracting forms, one of high affinity and low capacity and the other representing the majority of the receptor population with a low affinity. The dissociation constants for both high and low affinity calculated from the slopes of the Scatchard lines are within the concentration range at which PGE_2 shows elevated cAMP levels (Fig. 1A) between approximately 20 to 95% of the maximal response. This may indicate that occupation of both high and low affinity sites is apparently necessary to give a maximal cAMP production in vitro. Whether such high levels of cAMP are reached in vivo or whether predominantly high affinity sites are involved, is still an open question.

Although the corn oil diet shifted the competition curves (Fig. 4) to the right, the curves shifted in a parallel manner, indicating that more competing ligand was needed to displace 50% of the labeled PGE_2 (the IC_{50} had increased). This may indicate that the specificity of the sites was not altered by the diets. The potency series for competition ($PGE_2 > PGE_1 > PGI_2$) was not affected. Accordingly, Scatchard analysis revealed that both high and low affinity constants were not altered by the diets but the corn oil diet resulted in a marked increase in receptor capacity: both total numbers of high and low affinity sites for PGE_2 were increased.

Comparing the microscopic results and protein contents of the experimental groups, it appeared that the diets did not influence viability, population (92% of cells was identified as monocytes), macrophage size, and protein content ($77 \pm 8\ \mu g/10^6$ cells), However, more cells were harvested from the rats fed the corn oil diet. This 25% increase paralleled the 25% increase in total protein recovery of the harvested cells, indicating that the corn oil diet may have enhanced the chemotactic response to the starch injection, resulting in an increased amount of elicited peritoneal macrophages. Independent of this increased response, specific binding in the corn oil diet group showed a marked increase in receptor capacity as stated above. Since specific binding data are expressed per unit of protein, the finding of an increase in receptor capacity (expressed as fmol/mg protein) suggests an enhanced receptor density on the cells.

The results of the displacement studies (Fig. 4) provide additional evidence for the observation that the enhanced specific binding in the corn oil diet rats was independent of the increase in total harvested cells per rat. All displacement curves with macrophages from the corn oil diet rats were shifted in a parallel manner to the right, and binding of ^3H-PGE_2 in the absence of displacing prostaglandins was $\pm 135\%$ from control binding. Stimulation of cAMP production with PGE_2 or DDH-carbo-PGI_2 resulted in comparable maximal cAMP levels in control macrophages, indicating roughly an equal intrinsic activity for these PGs. However, the 20- to 30-fold higher concentration of DDH-carbo-PGI_2 required to produce half-maximal cAMP production indicates that the "apparent" affinity of PGE_2 is higher than that of DDH-carbo-PGI_2 as measured by this assay. Apart from the differences in "apparent" affinity for the PGs, the linseed oil diet did not affect cAMP production but the corn oil diet affected "apparent" affinity for both PGs similarly, since the dose-response curves were shifted to the left in a parallel manner (Fig. 1 A and B) while basal and maximal cAMP levels were not altered as compared to control and the linseed oil group.

The increase in PGE_2 binding site density of macrophages derived from the corn oil diet rats itself offers an explanation for physiological consequences, such as stimulation of cAMP production, by PGs. Thus, first the dose response curve of PG-induced stimulation of adenyl cyclase, from a population of macrophages containing an increased density of PG receptors, will consequently shift to the left in a parallel manner. Second, the resemblance of PG binding characteristics of macrophages from linseed oil and control diet rats paralleled the findings on PG-stimulated cAMP elevation in these macrophages. Furthermore, the data represent strong evidence that PGE_2 bindings sites are coupled to adenyl cyclase, because an increase in receptor density is followed by parallel changes in PGE_2-induced cAMP production. Since intrinsic activity was not changed by the diets, it might be speculated that the adenyl cyclase-cAMP system itself was unaffected by the corn oil diet. The experiments provide the novel demonstration that regulatory changes in PGE_2 receptor site density on macrophages can be induced in vivo by modulation of the dietary fatty acid composition. The unsaturated fatty acid compositions of linseed oil and corn oil (see Materials) do not easily permit a simple explanation for the observed effects. Unsaturated fatty acids are desaturated and elongated in the endoplasmatic reticulum and the sequences of reactions that take place in the linoleic, α-linolenic, and oleic acid series are similar. Common enzymes in these series are $\Delta 6$- and $\Delta 5$-desaturase; their activities are differentially modulated by various factors like hormonal and dietary changes (see ref. 1 for review). Moreover, it was shown that the substrates compete for the rate-limiting enzyme $\Delta 6$-desaturase and that linoleic and linolenic acids inhibit desaturation of oleic acid (1). Local substrate turnover and product concentrations thus finally control the availability of PG precursors such as AA. It might well be that the various amounts of oleic, linoleic, and α-linolenic acid in linseed oil present a dietary composition which affects the PG-receptor interactions of elicited peritoneal macrophages of rats in a similar way as standard rat food. High dietary amounts of linoleic acids have been shown to reduce tissue

levels of AA in rats (20) and infants (11). The release of polyunsaturated fatty acids other than AA was increased, AA concentration in plasma and platelet phospholipids decreased, and the PG synthesis seemed to be inhibited (11). The conversion of AA to PGE_2 could also be inhibited in vitro by high concentrations of linoleic acid (3). Recently, a competition between linoleic acid and AA for esterification and storage in tissue lipids was suggested (11). Thus, PG synthesis may be influenced ultimately at various levels by a high linoleic acid intake. Corn oil consists of large amounts (34.3%) of linoleic acid and contains no α-linolenic acid, in contrast to linseed oil (47.4% α-linolenic acid), and therefore the above mentioned effects of high dietary linoleic acid on PG generation might be present during the dietary corn oil period. Assuming that a deficient synthesis (and release) of PGE_2 had been induced by the corn oil diet, altered ligand-site interactions could consequently develop. The observed changes may be interpreted then as a protective mechanism, whereby the PGE_2 receptor density of the macrophages increased to overcome insufficient stimulation. Interestingly, $PGF_{2\alpha}$ synthesis could be depressed in rats receiving low dietary fat, saturated fat, and high amounts of unsaturated fat (20% corn oil) (21). It might well be that the diet with the high corn oil level induced comparable changes in PGE_2 synthesis in the present experiments. EFA-deficient animals are being used to study pathophysiological roles of PGs. Altered platelet function, dermal changes, and the roles of PGs in models of acute and chronic inflammation may be related to changes in PG production in these deficient animals. There are limitations to the usefulness of this deficiency disease. Complete absence of linoleic acid in the diet may result in undesirable and irreversible degeneration of membranes (7). It should, therefore, be determined whether certain diets containing high levels of EFA, which may induce a state resembling a "functional EFA-deficiency," are suitable for the study of PG-modulated pathophysiological conditions.

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