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PROSTAGLANDIN E_1 HIGH AFFINITY BINDING SITES OF RAT THYMOCYTES

SPECIFICITY AND BLOCKADE BY NON-STEROIDAL ANTIINFLAMMATORY DRUGS AND LOCALIZATION IN A PLASMA MEMBRANE-ENRICHED FRACTION

INGER GRUNNET and EIGIL BOJESEN

Institute of Experimental Hormone Research, University of Copenhagen, 71, Nørre Alle, DK-2100 Copenhagen (Denmark)

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SUMMARY

Great specificity is demonstrated for the prostaglandin E_1 high affinity binding sites of rat thymocytes Whereas prostaglandin E_2 has the same affinity as prostaglandin E_1, 13 other prostaglandin derivatives and antagonists are bound with 2–1000 times smaller affinities

50 % inhibition of the high affinity binding of prostaglandin E_1 to rat thymocytes is demonstrated for three non-steroidal antiinflammatory drugs, indomethacin (3 6 10^{-5} M), salicylic acid (2 9 10^{-3} M) and acetylsalicylic acid (2 10^{-2}M) The low affinity binding of prostaglandin E_1 is enhanced by the same concentration of indomethacin, however, to a lesser degree and more variable than the inhibition of the high affinity binding of prostaglandin E_1

Like intact cells a 50-fold purified plasma membrane fraction, isolated from a homogenate of rat thymocytes, shows reversible high affinity binding of prostaglandin E_1 as well as irreversible binding of unidentified tritiated compounds The binding data are compatible with a localization in the plasma membrane of high affinity sites for reversible binding with a considerably higher dissociation constant than that found for whole cells Their identity remains to be demonstrated

INTRODUCTION

The presence of a limited number of high affinity binding sites for prostaglandin E_1 has been found in a number of tissues supposed to be targets for this substance, such as the thyroid gland [1], uterus [2], blood platelets [3, 4], corpus luteum [5], liver [6] and adipocytes [7, 8] In several of these investigations the data suggest that the binding sites are confined to the plasma membranes In accordance with these observations a correlation between the degree of saturation and the effect on the adenylate cyclase system or other cellular parameters has been found in few cases [9, 10] However, most other observations indicate that, in order to give half

maximal activation of the adenylate cyclase system, the prostaglandin must be present in a concentration 100- to 1000-fold the amount that saturates the binding sites half maximally, suggesting that the adenylate cyclase system is not involved These discrepancies show that the biological significance of these binding sites is not yet clear

The thymocytes are apparently an example of cells, where binding affinity and activation of the adenylate cyclase system are dissociated, since the half maximal effect of prostaglandin E_1 on the enzyme is obtained at 10^{-7}–10^{-6} M [11], whereas Schaumburg [12] has demonstrated half maximal saturation of the high affinity binding sites at 10^{-9} M at 37 °C The present studies were carried out as a continuation of these binding studies, assuming that more information may eventually point to the physiological significance of the binding It was considered doubtful that the small number of binding sites per cell found by Schaumburg [12] (300 per cell) was representative for thymocytes of different sizes Therefore, the binding capacity of a thymocyte suspension with a low fraction of large thymocytes was measured Furthermore, it was possible to produce a cell fraction with a large enrichment of plasma membrane constituents, and thus to see if prostaglandin E_1 binding sites are associated with the plasma membrane The ligand specificity of whole cells was studied by displacement of prostaglandin E_1 with different prostaglandins, prostaglandin derivatives and antagonists During this work prostaglandin synthetase inhibitors were applied and this led to the observation that indomethacin as well as other non-steroidal anti-inflammatory drugs were able to block the binding of prostaglandin E_1 to thymocytes

Part of this work has been reported in the proceedings from The International Conference on Prostaglandins, Florence, May 1975

MATERIALS AND METHODS

Chemicals

[5, 6-$^3H_2$]Prostaglandin E_1, 86 0, 59 1 and 59 0 Ci/mmol was supplied by Amersham, England Non-radioactive prostaglandin E_1, E_2, F_1 and A_1 were given by Dr Pike, Upjohn Co , Kalamazoo, Mich USA, nor-prostaglandin E_1 and homo-prostaglandin E_1 by Dr van Dorp, Unilever Research Laboratorium, Vlaardingen, Holland, 7-oxa-13-prostynoic acid by Dr Fried, University of Chicago, Chicago, USA and polyphloretinphosphate by Dr Hogsberg AB Leo, Halsingborg, Sweden The radioactive compounds were purified by thin-layer chromatography on silica gel (solvent system I[13]) before use, while the non-radioactive compounds were used without further purification 15-Keto-prostaglandin E_1 was produced by the method of Attenborrow et al [14], and the oxim of prostaglandin E_1 was made according to Pike et al [15] Both compounds were purified in the same way as the radioactive prostaglandin E_1 The methyl-, butyl- and isobutylesters and the butylamide of prostaglandin E_1 were prepared by the mixed anhydride method described by Bojesen and Bukhave [13] The methyl ester, analysed on thin-layer chromatography (solvent system I[13]), showed only one single compound with an R_F value of 0 54, whereas the methyl ester of prostaglandin A_1 was shown to have an R_F value of 0 75 Prostaglandin B_1 was synthesized by incubating prostaglandin E_1 in 0 05 M NaOH for 1 h at 37 °C

Indomethacin from Dumex, Copenhagen, salicylic acid from British Drug House and acetylsalicylic acid from Mecobenzon, Copenhagen were used as supplied without purification

Experimental procedures and analyses

The binding experiments with thymocytes were performed in the following way as previously described by Schaumburg [12] Young female rats (Sprague-Dawley, 120–130 g) were decapitated, and the thymi removed and cooled at 4 °C in a modified Krebs-Ringer phosphate buffer [12] The suspension of thymocytes was obtained by mincing the thymi at 4 °C, whereupon the cells were gently washed three times During the incubation period the suspension of thymocytes in Krebs-Ringer phosphate buffer was gently stirred After the incubation the cells were isolated by centrifugation in polypropylene microtest tubes through a layer of silicone oil By this technique a cell pellet containing as little as 10 % (v/v) incubation medium was obtained [16] The radioactivity of the pellet was determined by cutting the test tube and dropping the tube section into the scintillation vials containing scintillation liquid [12] The incubated number of cells was determined as described [12]

Removal of large thymocytes from a thymocyte suspension The large thymocytes were removed from the thymocyte suspension by filtration through a column of glass beads (diameter = 90 μm), as described by Shortman [17], at 4 °C and with a flow rate of 10 ml/h, using a modified Krebs-Ringer phosphate buffer [18] (pH 7 4) containing 0 45 % bovine serum albumin as medium

The number of cells was counted in a Fuchs-Rosenthal chamber (depth 0 200 mm) Differential counting of small and large thymocytes was done with a phase contrast microscope on unfixed cells The numbers counted corresponded to a standard error of less than 4 % The viability of the cells was estimated by the fraction coloured after 5 min by 1 % eosin (w/v) in Krebs-Ringer phosphate containing 0 45 % bovine serum albumin

Preparation of a plasma membrane-enriched fraction The washed cells were resuspended in 0 20 M sucrose plus 3 mM $CaCl_2$ and homogenized with ten strokes in a Potter-Elvehjem homogenizer 2 5 ml of the homogenate, adjusted to isotonicity (0 25 M—3 mM $CaCl_2$), was layered on top of the two-layer sucrose gradient, generally used to separate smooth and rough membranes [19] (1 0 ml 2 0 M sucrose+ 3 mM $CaCl_2$ and 1 5 ml 1 3 M sucrose+3 mM $CaCl_2$) After centrifugation for 90 min at 90 000 $\times g$, the band at the interface between 0 25 M sucrose and 1 3 M sucrose was collected, diluted 5-fold with buffer (according to volume) and centrifuged at 105 000 $\times g$ for 60 min The pellet containing the membranes was suspended in Krebs-Ringer phosphate buffer The protein in the pellet amounted to 0 75 $\pm$ 0 02 % of the protein content of the starting material The enrichment of the plasma membrane marker, 5'-nucleotidase (EC 3 1 3 5), in the pellet was determined by Sigma Kit (No 675-PB) The ratio of the specific activities of the cytoplasmatic enzyme, lactate dehydrogenase (EC 1 1 1 27), in the pellet and the homogenate was determined using a Biochemica Test Combination for ultraviolet determination of this enzyme (Cat No L5977)

Binding experiments with the membrane fraction The plasma membrane-enriched fraction (350–450 μg protein/ml) suspended in Krebs-Ringer phosphate buffer was incubated with radioactive prostaglandin E_1 in a thermostated water bath

at 22 °C for various times The membranes of 100 μl incubate were separated from the medium on 0 2 μm Metricel membrane filters (Gelman) and washed with 2 ml buffer The filters absorbed 0 3 % non-protein-bound [^{3}H]-prostaglandin E_1 The filters were placed in scintillation vials containing the same scintillation liquid as used in the experiments with whole cells [12]

The protein content of the incubate was estimated by the method of Lowry et al [20]

Definitions and calculations

The amount of radioactivity taken up by the cells divided by the amount of radioactivity in the same volume of incubation medium was named Q [18] The Q value of the high affinity bound prostaglandin E_1 is the difference between the Q value found at low medium concentration of prostaglandin E_1 and that of high medium concentration [18]

The K_d and K_i values were calculated either by non-linear regression analysis of the plots, using the general method described by Marquardt [21], or by linear regression analysis of the corresponding Hanes [22] plots

RESULTS

Specificity of the prostaglandin E_1 binding sites

The maximal Q value of the saturable binding sites is obtained as the difference between the total Q value at 10^{-10} M prostaglandin E_1 and the Q value found at 10^{-6} M prostaglandin E_1 This formalism is based upon the assumption that the Q value of the unsaturable accumulation is independent of the concentration Along

TABLE I

ANALYSIS OF THE SPECIFICITY OF THE PROSTAGLANDIN E_1 HIGH AFFINITY BINDING SITES OF RAT THYMOCYTES AT 37 °C

The apparent K_i values for the different analogues and antagonists shown below were measured after 30 min incubation of the thymocyte suspension with 10^{-10} M radioactive prostaglandin E_1 at various concentrations of the tested compound The affinity relative to prostaglandin E_1 is given as K_d/K_i ($K_d = 2\ 10^{-9}$ M)

Compound	K_d/K_i	Assayed concentration range (M)
Prostaglandin E_2	1	$4\,8\ 10^{-12}$–$9\,5\ 10^{-6}$
Methyl ester of prostaglandin E_1	0 08	$1\,5\ 10^{-11}$–$9\,0\ 10^{-7}$
Butyl ester of prostaglandin E_1	0 1	$5\,9\ 10^{-12}$–$1\,1\ 10^{-6}$
Isobutyl ester of prostaglandin E_1	0 01	$1\,5\ 10^{-11}$–$1\,0\ 10^{-6}$
Butylamide of prostaglandin E_1	0 002	$4\,8\ 10^{-12}$–$8\,0\ 10^{-7}$
15-keto-prostaglandin E_1	0 09	$6\,6\ 10^{-12}$–$1\,2\ 10^{-6}$
Nor-prostaglandin E_1	0 5	$5\,0\ 10^{-12}$–$8\,8\ 10^{-7}$
Homo-prostaglandin E_1	0 4	$5\,4\ 10^{-12}$–$9\,5\ 10^{-7}$
Prostaglandin B_1	0 004	$3\,5\ 10^{-9}$ –$5\,0\ 10^{-4}$
Prostaglandin A_1	0 01	$1\,6\ 10^{-8}$ –$1\,2\ 10^{-5}$
Prostaglandin $F_{1\alpha}$	0 02	$1\,0\ 10^{-10}$–$1\,0\ 10^{-7}$
Prostaglandin E_1-oxim	0 1	$6\,2\ 10^{-12}$–$1\,1\ 10^{-6}$
7-oxa-13-prostynoic acid	0 0002	$1\,8\ 10^{-11}$–$9\,6\ 10^{-6}$
Polyphloretin phosphate	0 002	$1\,5\ 10^{-12}$–$7\,9\ 10^{-7}$

with a common estimation of the Q value for the unsaturable binding (10^{-6} M prostaglandin E_1 in the medium) the total maximal Q value was measured after 30 min incubation at 37 °C with 10^{-10} M [^{3}H]prostaglandin E_1 in the medium together with different concentrations of analogues and antagonists as shown in Table I

The apparent K_i values of the analogues and antogonists, calculated on the basis of the assumption that the inhibition is competitive, are shown together with the inhibitor concentrations in Table I Apparently the substituents in both the ring and the side chains are of great importance for the affinity A double bond in one of the side chains (as in prostaglandin E_2) and the length of the alkyl side chain (as in nor- and homo-prostaglandin E_1) are on the other hand less important The two antagonists, 7-oxa-13-prostynoic acid and polyphloretin phosphate are not bound to the high affinity binding sites of the thymocytes in the assayed concentration ranges It can be concluded that the binding sites for prostaglandin E_1 in rat thymocytes possess a high degree of specificity

Binding experiments with a thymocyte suspension with a lowered amount of large thymocytes

The filtration of cells through the column of glass beads does not damage the recovered cells, since an unaltered fraction of the cells was coloured by eosin (4 8 $\pm$0 5 % versus 4 5$\pm$0 6 %) Before and after filtration the small thymocytes constituted 86 4$\pm$0 9 % and 92 6$\pm$0 9 %, respectively, of the cell suspension From this it is evident that two times more of the large thymocytes than of the whole cell population are lost through passage through the column, the yield of which was about 10 % The maximal Q value of the saturable binding sites remains, however, unchanged (Table II) Thus it is not only the large thymocytes that has the saturable prostaglandin E_1 binding sites The possibility that large thymocytes are unable to bind prostaglandin E_1 with high affinity is not excluded by the data, because of the low fraction of these cells

TABLE II

PROSTAGLANDIN E_1 HIGH AFFINITY BINDING OF THE THYMOCYTE SUSPENSION AT 4 °C BEFORE AND AFTER REMOVAL OF A FRACTION OF THE LARGE THYMOCYTES

The maximal Q value of the saturable binding ($\pm$S E) was estimated as the difference between the total Q value found at 10^{-10} M radioactive prostaglandin E_1 and the Q value found with 10^{-6} M unlabelled and 10^{-10} M labelled prostaglandin E_1 in the medium after incubation for 30 min The values are corrected for differences in cell number in the two populations

Experiment No	Maximal Q value		Concentration of prostaglandin E_1 (M)
	Generally used thymocyte suspension	Thymocyte suspension with reduced number of large cells	
1	0 382$\pm$0 110	0 338$\pm$0 090	4 5 10^{-10}
2	0 353$\pm$0 043	0 473$\pm$0 040	2 3 10^{-10}
3	0 284$\pm$0 024	0 299$\pm$0 026	3 8 10^{-10}
4	0 373$\pm$0 159	0 408$\pm$0 031	3 0 10^{-10}

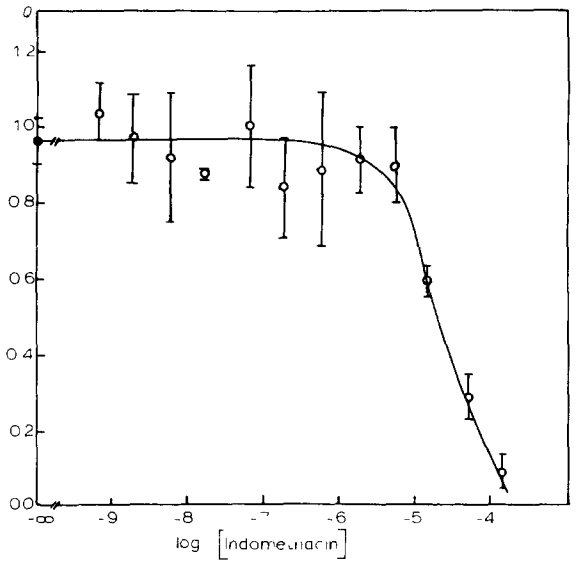

Fig 1 Inhibition of the equilibrium uptake of [^{3}H]prostaglandin E_1 by indomethacin after incubation for 130 min at 4 °C in the absence and presence of 3.7×10^{-6} M prostaglandin E_1. The curve shown in the figure appears as the difference between these two experiments (± S.E.). The maximal Q value of the [^{3}H]prostaglandin E_1 equilibrium uptake in the absence of indomethacin was also estimated (●).

The effect of non-steroidal antiinflammatory drugs on the binding of prostaglandin E_1 to thymocytes

When thymocytes were incubated for 30 min at 4 °C in Krebs-Ringer phosphate buffer with a low concentration of [^{3}H]prostaglandin E_1 (10^{-10} M), the cellular accumulation of this compound could be inhibited by the addition of indomethacin in different concentrations, as shown in Fig. 1. In three experiments the mean 50% inhibition was obtained with an indomethacin concentration of $3.6 \times 10^{-5} \pm 1.2 \times 10^{-5}$ M. Higher concentrations of the related drugs acetylsalicylate (2×10^{-2} M) and salicylate (2.9×10^{-3} M) also gave a 50% inhibition in similar experiments.

In Fig. 2 are shown the results from two equilibrium experiments with various concentrations of prostaglandin E_1 in the presence or absence of 6.7×10^{-5} M indomethacin. In the presence of indomethacin, the dissociation constant for the high affinity binding sites was increased from 9.5×10^{-10} to 4.5×10^{-9} M, and the calculated number of binding sites per cell also increased from 1.0×10^{-9} to 2.1×10^{-9} M, showing that the effect of indomethacin is quite complex. This is further emphasized by the observation that the unsaturable, low affinity binding of [^{3}H]prostaglandin E_1 is increased about 10% in the presence of indomethacin.

Binding of prostaglandin E_1 to a plasma membrane-enriched fraction

The specific and total activity of 5′-nucleotidase and lactate dehydrogenase in the membrane fraction and the homogenate are shown in Table III. It appears that the specific activity of 5′-nucleotidase is 40–50 times greater in the membrane fraction than in the homogenate. The membrane fraction is only slightly contaminated with the cytoplasmic enzyme (0.2–0.3% of the total activity).

The rate of uptake of [^{3}H]prostaglandin E_1 in thymocyte membrane fragments was followed by removing aliquots from shaken homogeneous incubate (22 °C) and measuring the radioactivity of the fragments collected and washed on a filter as

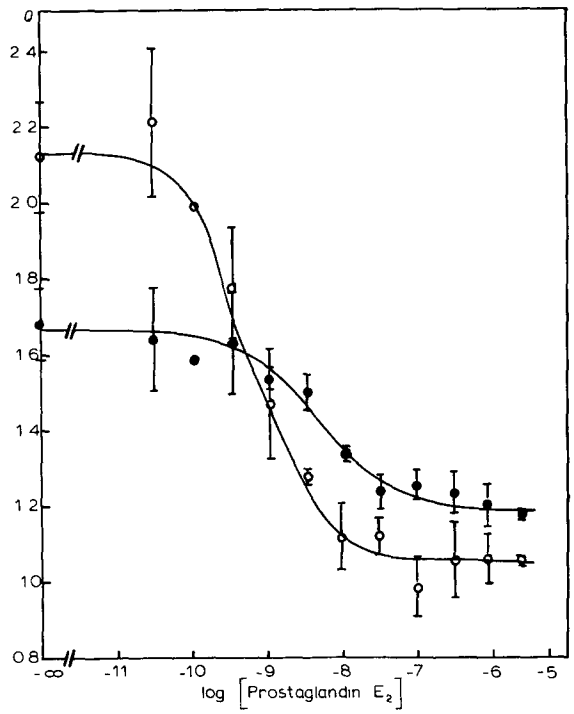

Fig 2 The equilibrium uptake of 8 3 10^{-11} M [^{3}H]prostaglandin E_1 was measured as a function of the concentration of non-radioactive prostaglandin E_1 in absence (○) and presence (●) of 6 7 10^{-5} M indomethacin after 130 min incubation at 4 °C It appears from the curve (±S E) that the non-saturable low affinity binding of prostaglandin E_1 is higher in the experiment with indomethacin than in the one without this drug

TABLE III

SPECIFIC AND TOTAL ACTIVITY OF 5′-NUCLEOTIDASE AND LACTATE DEHYDROGENASE IN THE HOMOGENATE AND THE MEMBRANE FRACTION OF RAT THYMOCYTES

The specific activity of 5′-nucleotidase is given as μmol P_i formed/h per mg protein, and the specific activity of lactate dehydrogenase is expressed as munits per mg protein The values in the table are from two different experiments

	5′-Nucleotidase		Lactate dehydrogenase	
	Homogenate	Membranes	Homogenate	Membranes
Specific activity	0 30	12 1	159 5	66 0
	0 14	7 3	241 5	82 0
Total activity (%)	100	30	100	0 3
	100	40	100	0 2

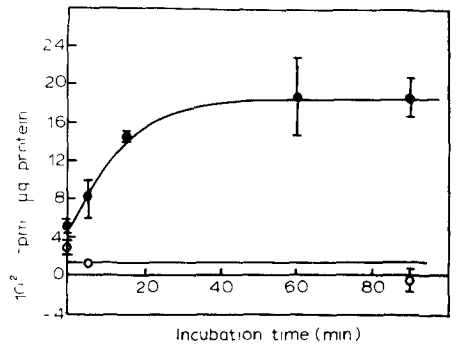

Fig 3 The uptake (±S E) of 1 1 10^{-10} M [^{3}H]prostaglandin E_1 by the plasma membrane fraction of rat thymocytes was measured in the absence (●) and presence (○) of 3 1 10^{-6} M unlabelled prostaglandin E_1 at 22 C

described For each incubation period, duplicates could be processed within 45 s It was expected that this technique, in contrast to that used for whole cells, would eliminate the contribution of any low affinity unsaturable binding, since it included washing of the fragments However, the membranes incubated with high concentrations of prostaglandin E_1 showed a rapid uptake of tritium, which was not washed out on the filters At low concentrations (10^{-10} M) a similar instantaneous uptake of irreversibly bound radioactivity took place, resulting in a 2–3 times higher uptake

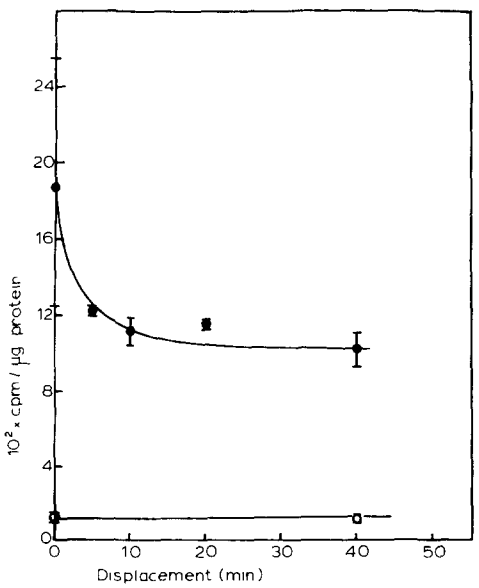

Fig 4 Time course of the displacement of plasma membrane-bound [^{3}H]prostaglandin E_1 The membranes were preincubated for 60 min with 1 1 10^{-10} M radioactive prostaglandin E_1 in presence (○) and absence (●) of 3 1 10^{-6} M unlabelled prostaglandin E_1 at 22 °C, and the uptake of radioactivity was determined Thereupon, unlabelled prostaglandin E_1 was added to the incubation mixtures, so that the final concentration of non-radioactive prostaglandin E_1 became 3 1 10^{-6} M The time course of the displacement (±S E) was followed by taking aliquots of the membrane suspensions at different times

Only at low concentrations this rapid irreversible binding was succeeded by a slow accumulation, which reached a constant level after 60 min, thus showing a time constant similar to that of the high affinity binding sites of whole cells By application of linear regression analysis, an initial rate of uptake could be calculated $v_{(1\,1\ 10^{-10}\ \text{M prostaglandin } E_1)} = 1\,6\ 10^{-19}$ mol/μg protein per min That a large part of this slowly accumulated radioactivity was reversibly bound prostaglandin E_1 was revealed by the displacement data shown in Fig 4

The rate of displacement was determined by the same technique after addition of a high concentration of prostaglandin E_1 (10^{-6} M) to the medium containing membrane fragments preincubated for 60 min with 10^{-10} M [^{3}H]prostaglandin E_1 Fig 4 shows the results of this experiment A relatively large fraction (50 %) of the radioactivity bound to membranes incubated with 10^{-10} M [^{3}H]prostaglandin E_1 was irreversibly absorbed or metabolised prostaglandin E_1, since it was not displaced by high concentrations of prostaglandin E_1 in the medium This phenomenon is qualitatively in agreement with the observation in the association experiment, showing that some rapidly bound tritium-labelled compounds could not be washed out of the membranes The rate constant of displacement, calculated on basis of this experiment, is 0 18 min^{-1} and $t_{\frac{1}{2}} = 4$ min

Since the irreversibly bound tritiated compounds must also have been produced and accumulated in the membrane fraction in the association experiment with 10^{-10} M prostaglandin E_1, the initial rate of uptake of reversibly bound prostaglandin E_1 has been somewhat slower than indicated in Fig 3

An attempt was made to determine a dissociation constant (K_d) by equilibrating the membranes for 60 min at 22 °C with various concentrations of prosta-

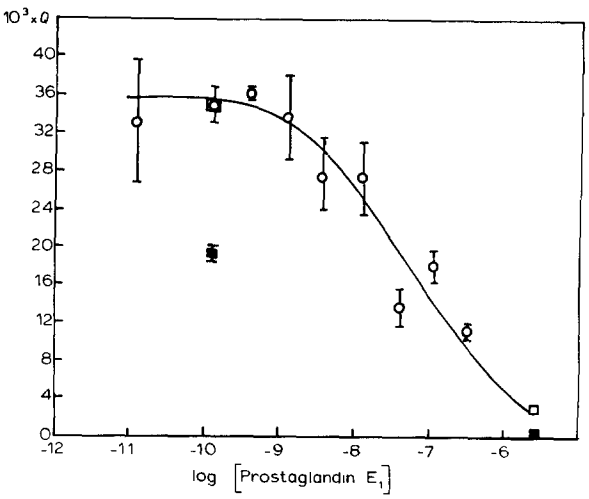

Fig 5 Equilibrium uptake of 9 6 10^{-11} M [^{3}H]prostaglandin E_1 by rat thymocyte, plasma membranes at various concentrations of unlabelled prostaglandin E_1 at 22 °C The uptake is expressed as the concentration ratio Q (cpm per μg protein/cpm per μl medium) $\pm$S E (○) The Q values at 3 10^{-6} and 1 10^{-10} M prostaglandin E_1 calculated from the displacement data (□) and the Q values at 3 10^{-6} and 1 10^{-10} M prostaglandin E_1 corrected for the irreversible binding on basis of the displacement experiment (■) are also indicated in the figure

glandin E_1 (10^{-11}–10^{-6} M) The data are presented in Fig 5 and an apparent K_d value of 3 10^{-8} M fits the data fairly well However, the data must be corrected for the irreversibly bound compounds revealed by the displacement experiment Such a correction can be performed on the basis of the displacement experiment, provided the ratio between reversible and irreversible binding is dependent only on the concentration of prostaglandin E_1 in the medium and the incubation time The ratio was, however, only measured at 10^{-10} M prostaglandin E_1, and it is therefore not possible to calculate a K_d value of the reversible high affinity binding The calculated Q value of reversibly bound prostaglandin E_1 at 10^{-10} M is shown in Fig 5 The tritium activity remaining in the membrane fraction after incubation with a high concentration of prostaglandin E_1 (10^{-6} M) in the displacement experiment (Fig 4) is probably irreversibly bound, since the membranes were washed on the filter The Q values at 3 10^{-6} and 1 10^{-10} M prostaglandin E_1 measured in the displacement experiment are in agreement with the Q value observed in the equilibrium experiment at 1 10^{-10} M and the Q value obtained by extrapolation of the equilibrium data to 3 10^{-6} M This indicates that the concentration-dependent irreversible accumulation of labelled compounds, apparent from the data of the displacement experiment, have been about the same in the two types of experiments

DISCUSSION

The low dissociation constant, as well as the specificity of the high affinity binding sites of rat thymocytes for prostaglandin E_1 (and E_2), suggests that these sites have a receptor function In both respects they have similar properties to the prostaglandin binding sites on presumptive targets cells [2, 5–8], only with the difference that in thymocytes the methyl ester has an at least 10-fold lower affinity than prostaglandin E_1 The two compounds have been reported to have nearly the same affinities to presumptive targets such as liver cell membranes [6], uteri [2] and membranes of adipocytes [7] (Table IV) The information available does not, however, exclude the possibility that the difference is due to the presence of esterases in such membrane preparations

Compared with well defined hormone receptors [18, 25] the number of prostaglandin E_1 high affinity binding sites per thymocyte is low (about 300) [12] The possibility that only the small fraction of large thymocytes is responsible for the whole binding capacity was ruled out by the observation that preparations of thymocytes considerably depleted of large thymocytes and the original preparations had nearly identical binding capacities per cell

Two recent reports strongly suggest that the high affinity binding sites of thymocytes are indeed functioning as receptors Ishizuka et al [26] have found that very low concentrations of prostaglandins E_1 (3 10^{-12} M) enhance the antibody formation in spleen cells in vitro More conclusive, however, is the report of Bach and Bach [27] Under conditions where the T-cell characteristics of the rosette-forming spleen cells are at a low level, this can be normalized by prostaglandin E_1 in vitro in a dose-dependent fashion, as would be expected from the dissociation constant of the binding sites Moreover, the authors claim that prostaglandin E_1 (and E_2) mimics the effect of a thymic polypeptide in generating T-cell characteristics of spleen cells from the thymus-deficient mice strain Nu-Nu That the high affinity binding sites

TABLE IV

COMPARISON OF THE SPECIFICITY OF THE PROSTAGLANDIN E_1 HIGH AFFINITY BINDING SITES OF DIFFERENT TISSUES

The affinity of the analogues and antagonists relative to prostaglandin E_1 is given as the ratio K_d/K_1, where K_d is the dissociation constant for prostaglandin E_1 in the tissue concerned

	Prostaglandin E_2	Prostaglandin $F_1\alpha$	Prostaglandin $A_{1\,2}$	Prostaglandin B_1	15-keto-prostaglandin $E_{1\,2}$	Prostaglandin E_1-methyl ester	7-oxa-13-prostynoic acid	Polyphloretin phosphate
Liver membranes [6]	0 80	$9\ 10^{-4}$	0 75	$5\ 10^{-4}$	–	0 92	–	–
Corpus luteum membranes [5, 23]	–	$3\ 10^{-3}$	–	–	–	–	$1\ 10^{-6}$	–
Uterine pieces [24]	0 87	–	$8\ 10^{-3}$	$1\ 10^{-2}$	–	–	–	–
Uterus slices [2]	0 85	–	0 47	–	$3\ 10^{-2}$	0 50	–	–
Adipocytes [8]	0 6	$8\ 10^{-3}$	$1\ 3\ 10^{-2}$	–	$5\ 10^{-3}$	–	–	–
Adipocyte-membranes [7]	0 93	–	$4\ 10^{-2}$	–	–	0 41	$4\ 10^{-4}$	–
Thymocytes	1 0	$2\ 10^{-2}$	$1\ 10^{-2}$	$4\ 10^{-3}$	$9\ 10^{-2}$	$8\ 10^{-2}$	$\sim 2\ 10^{-4}$	$\sim 2\ 10^{-3}$

of thymocytes are closely related to such effects of prostaglandins on thymus-derived and splenic precursor cells is further supported by the observation that indomethacin inhibits the effects of prostaglandin E_1, since this drug, according to the present work inhibits the binding of the prostaglandin to thymocytes

In the studies of Schaumburg [12] indomethacin was added in low concentrations to the incubation medium in order to inhibit any potential prostaglandin production By increasing the concentration to 10^{-5} M a pronounced inhibition of the high affinity binding of prostaglandin E_1 was noticed Although ligand competition plays a part, attempts to clarify the mechanism in detail were unsuccessful since the drug also increased the low affinity accumulation of tritiated compounds in the cells Unpublished data suggest that this increased "noise level" is not due to metabolites, but to the prostaglandin itself Thus the drug may enhance the permeability of some cellular compartments to which the prostaglandins normally have no access

Thymocytes and splenic T-cells are not unique with regard to the binding and effects of prostaglandin E_1 being inhibited by indomethacin Thus Rao [5] has reported that $1\,4\ \ 10^{-4}$ M indomethacin blocks 43 % of the prostaglandin E_1 binding capacity of cell membranes from corpus luteum Others [28] have found that low concentrations of indomethacin inhibit the prostaglandin effects on rat uterus ($5\ \ 10^{-5}$ M) and on guinea pig ileum ($7\ \ 10^{-5}$ M) Thus the phenomenon may be quite general These effects of indomethacin are reproduced by higher concentrations of other members of the family of aspirin-like drugs, in an analogous manner to the effect of indomethacin on the synthetase [29–31] and the 15-hydroxyprostaglandin dehydrogenase from lung tissue [32] Since the synthetase is only slightly more sensitive to this family of drugs than the two other systems it is attractive to propose a common mechanism for all three drug effects According to Gryglewski [33] the effects of synthetase inhibitors, including the weak inhibitors lauryl sulphate and myristate, may be understood to some extent on the basis of ligand competition, provided that the enzyme-bound substrate has a conformation closely resembling the "hair-pin" model of arachidonic acid This assumption is not invalidated by the recent demonstration that this conformation is not the generally favoured one [34] Since the prostaglandins can be regarded as long chain fatty acids in a ' locked hair-pin" conformation, the hypothesis of Gryglewski implies that the same drugs which block the synthetase also inhibit the reactions in which the prostaglandins are the ligands, although perhaps at somewhat higher concentrations This seems actually to be the case The high affinities and specificities of receptors could then be due to cooperative effects between such features of the binding sites which they share with the synthetase and group as well as stereospecific loci

Our attempts to localize the high affinity binding sites to the plasma membrane have not resulted in an unambiguous conclusion although the membrane preparation was probably rather pure A high degree of purity is inferred from the enrichment factor of 5'-nucleotidase (about 40) and the good recovery (30–40 %) from the homogenate since others [35, 36] have shown that this enzyme is mainly if not entirely present in this organelle of lymphocytes They showed that the enrichment factors of the enzyme and surface antigens were nearly identical and a little lower than in our preparation, in agreement with the higher specific activity of the enzyme More recently, another group [37] has obtained a plasma membrane preparation similar to ours with regard to the specific activity of the enzyme Although it was possible

to show that the membrane preparation contained reversible binding sites, a rather large degree of irreversible binding prevented direct calculation of the dissociation constant From the Q value of the reversibly bound prostaglandin E_1 at a low medium concentration (10^{-10} M) the number of reversible binding sites per mg protein can be calculated by assuming that the dissociation constant is the same for the membranes and for cells at the same temperature (1 1 10^{-9} M) [12] Computed in this way the membrane preparation was not enriched at all with regard to the binding sites However, the concentration dependence of the total Q values including the irreversibly bound radioactivity (Fig 5) is more compatible with a much higher dissociation constant, provided the concentration dependencies of reversible and irreversible bindings are not very different A K_d value of 3 10^{-8} M will then be in harmony with both the binding curve (Fig 5) and the enrichment factor for the enzyme marker Thus the data are compatible with a localization in the plasma membrane provided the same binding sites have a much higher dissociation constant for the same ligand in the isolated membrane fragments, than in situ We have concluded that the question of whether or not the high affinity binding sites of the thymocytes are confined to the plasma membrane cannot be answered unambiguously by the approach we have chosen and is still an open one

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