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SPECIFIC PROSTAGLANDIN E_1 AND A_1 BINDING SITES IN RAT ADIPOCYTE PLASMA MEMBRANES

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SUMMARY

Rat adipocyte plasma membrane sacs have been shown to be a sensitive and specific system for studying prostaglandin binding. The binding of prostaglandin E_1 and prostaglandin A_1 increases linearly with increasing protein concentration, and is a temperature-sensitive process. Prostaglandin E_1 binding is not ion dependent, but is enhanced by GTP. Prostaglandin A_1 binding is stimulated by ions, but is not affected by GTP.

Discrete binding sites for prostaglandin E_1 and A_1 were found. Scatchard plot analysis showed that the binding of both prostaglandins was biphasic, indicating two types of binding sites. Prostaglandin E_1 had association constants of $4.9 \cdot 10^9$ l/mole and $4 \cdot 10^8$ l/mole, while the prostaglandin A_1 association constants and binding capacities varied according to the ionic composition of the buffer. In Tris–HCl buffer, the prostaglandin A_1 association constants were $8.3 \cdot 10^8$ l/mole and $5.7 \cdot 10^7$ l/mole, while in the Krebs–Ringer Tris buffer, the results were $1.2 \cdot 10^9$ l/mole and $8.6 \cdot 10^6$ l/mole.

Some cross-reactivity between prostaglandin E_1 and A_1 was found for their respective binding sites. Using Scatchard plot analysis, it was found that a 10-fold excess of prostaglandin E_1 inhibited prostaglandin A_1 binding by 1–20% depending upon the concentration of prostaglandin A_1 used. Prostaglandin E_1 competes primarily for the A prostaglandin high-affinity binding site. Similar Scatchard analysis using a 20-fold excess of prostaglandin A_1 inhibited prostaglandin E_1 binding by 10–40%. Prostaglandin A_1 was found to compete primarily for the E prostaglandin low-affinity receptor.

All of the bound [^{3}H]prostaglandin E_1, but only 64% of the bound [^{3}H]-prostaglandin A_1 can be recovered unmetabolized from the fat cell membrane. There is no non-specific binding of prostaglandin E_1, but 10–15% of prostaglandin A_1 binding to adipocyte membranes is non-specific. Using a parallel line assay to measure relative affinities for the E binding site, prostaglandin E_1 > prostaglandin A_2 > prostaglandin $F_{2\alpha}$. Prostaglandin E_2 and 16,16-dimethyl prostaglandin E_2 were equipotent with prostaglandin E_1, while other prostaglandins had lower relative affinities. 7-Oxa-13-prostynoic acid does not appear to antagonize prostaglandin activity in adipocytes at the level of the receptor.

Abbreviation: EGTA, ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*′-tetraacetic acid.

INTRODUCTION

Despite the varied pharmacological properties of the prostaglandins, little is known of their specific biological functions within the cell. Prostaglandin E_1 and E_2 inhibit hormone-stimulated lipolysis in adipocytes, presumably by an inhibition of adenylate cyclase[1,2]. Attempts to demonstrate direct prostaglandin inhibition of adenylate cyclase in broken cell preparations of fat cells have been unsuccessful[3,4] as was the case with insulin until recently[5,6]. Specific prostaglandin antagonists such as 7-oxa-13-prostynoic acid and SC-19220 (a dibenzoxazepine hydrazide) increase the rate of lipolysis induced by epinephrine[7]. Indomethacin, which blocks endogenous prostaglandin synthesis, increases epinephrine-stimulated and basal lipolysis in adipocytes[7]. Together these data strongly support the view that endogenously produced prostaglandin modulates lipolysis in fat cells.

This report deals with one of the initial events in the regulation of lipolysis by prostaglandins, their specific binding to rat adipocyte plasma membranes. Previous prostaglandin binding studies were reported in fat cell lipid cake homogenates[8] or in the rat forestomach[9], where binding studies were hampered by non-specific binding, and the rapid metabolism of the prostaglandins. We have found that adipocyte ghosts have essentially no non-specific binding, while they are known to retain many of the metabolic properties of an intact adipocyte[10].

MATERIALS AND METHODS

[5,6-$^3H_2$]Prostaglandin A_1, 110 Ci/mmole, and [5,6-$^3H_2$]prostaglandin E_1, 110 Ci/mmole, were purchased from New England Nuclear Co., and were chromatographed upon arrival against authentic prostaglandin standards. If the prostaglandins did not exhibit > 95% purity, they were not used in binding experiments. The [^{3}H]-methyl ester of prostaglandin E_1 was synthesized from [5,6-$^3H_2$]prostaglandin E_1, and purified using high-pressure liquid chromatography by Dr W. P. Schneider of The Upjohn Company. ATP, GTP and ethyleneglycol-bis-(β-aminoethylether)-*N,N'*-tetraacetic acid (EGTA) were obtained from the Sigma Chemical Company.

Male albino rats, 180–220 g, of The Upjohn colony, were used in all experiments. Animals were allowed free access to water, and fed *ad libitum*. Rats were killed by decapitation, exsanguinated, and the epididymal fat pads removed and placed in 3.0 ml of Krebs–Ringer Tris–albumin buffer at pH 7.4. Free adipocytes and adipocyte plasma membrane sacs were prepared from the fat pads according to Rodbell[11]. After preparation, the membranes were suspended in ice-cold Krebs–Ringer Tris buffer, pH 7.5, so that each 0.1 ml of suspension contained 30–50 μg of ghost membrane protein, determined by the method of Lowry *et al.*[12].

Prostaglandin-binding studies were conducted in a system that contained 0.5 ng of the appropriate [^{3}H]prostaglandin, 30–50 μg of ghost protein, and sufficient Tris–HCl or Krebs–Ringer Tris buffer to give a total volume of 1.0 ml. The system was incubated at 37 °C for 30 min, during which time complete equilibration occurred. At the end of the incubation period, the membranes were collected on HAWP 0.45-μm Millipore filters, and washed with 20 ml of cold buffer. The filters were then placed in 10 ml of Packard Insta-Gel scintillation fluid and counted in a Packard Model 3375 liquid scintillation spectrometer. In order to distinguish specific prosta-

glandin binding from non-specific binding, 1 μg of unlabeled prostaglandin E_1, or 10 μg of prostaglandin A_1 was added to the incubation system. The difference in binding with and without the unlabeled prostaglandin was interpreted as specific prostaglandin binding.

Bound [^{3}H]prostaglandin E_1 and [^{3}H]prostaglandin A_1 were eluted from the membranes by the following procedure. In six centrifuge tubes membranes and reaction components were incubated at 37 °C for 35 min. The tubes were centrifuged at $900 \times g$ for 15 min at 4 °C. The pellets were resuspended, and washed 3–5 times with buffer by centrifugation. After the final centrifugation, the pellets were extracted in ethyl acetate which was acidified with 2 M citric acid. Two aliquots from the first ethyl acetate extract were removed. One fraction was dried overnight, then reconstituted and the radioactivity determined. The second fraction was counted immediately. The remainder of the ethyl acetate extract was concentrated under a stream of N_2 and applied to an Analtech Silica Gel GF plate and developed in an A IX solvent system[13].

RESULTS

Fig. 1 shows the specificity and sensitivity of the adipocyte ghost system for studying prostaglandin binding. 0.5 ng of unlabeled prostaglandin E_1 competes to a significant level with 0.5 ng of [^{3}H]prostaglandin E_1. 10 ng of unlabeled prostaglandin E_1 almost completely eliminates binding. The same counts that are retained on the Millipore filter in the absence of ghost membranes are seen in the presence of membranes and 1 μg of unlabeled prostaglandin E_1, which indicates the complete absence of non-specific binding of prostaglandin E_1 to adipocyte membranes. The curve for prostaglandin A_1 binding showed that membranes even in the presence of 2 μg of unlabeled prostaglandin A_1, bind more than the Millipore alone, indicating some non-specific binding of prostaglandin A_1.

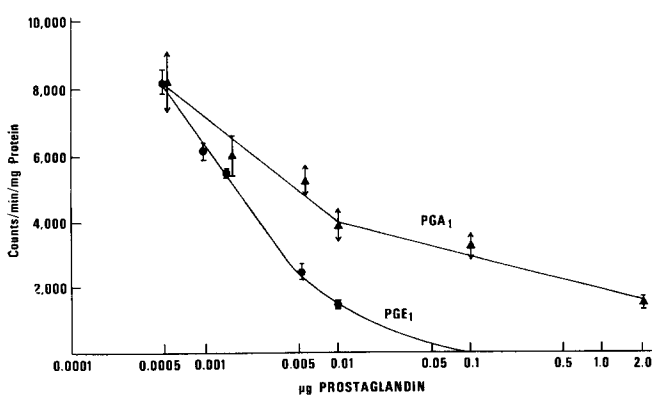

Fig. 1. Dose–response curve for prostaglandin E_1 (PGE_1) and prostaglandin A_1 (PGA_1) binding. Each incubation mixture contained 0.5 ng [5,6-$^3H_2$]prostaglandin E_1 or [5,6-$^3H_2$]prostaglandin A_1 and additional amounts of unlabeled prostaglandin E_1 or prostaglandin A_1 in a total volume of 1 ml 0.05 M Tris–HCl (pH 7.5) buffer. 30 min incubation at 37 °C. The Millipore background values were subtracted. The bars represent the S.D. of quadruplicate samples. ●—●, prostaglandin E_1; ▲—▲, prostaglandin A_1.

The inhibition of binding of [^{3}H]prostaglandin E_1 is specific for prostaglandins, and non-related compounds such as insulin, serotonin, morphine, or cholesterol even at concentrations up to 10 μg/ml, have no effect on the binding of prostaglandin E_1 (Gorman, R. R. and Miller, O. V., unpublished).

At equilibrium, prostaglandin E_1 and A_1 binding has a linear relationship to increasing protein concentration. Prostaglandin E_1 gave a slope of 8 fmoles/mg protein, and prostaglandin A_1 a slope of 25 fmoles/mg protein.

Binding of prostaglandin E_1 reaches equilibrium under our assay conditions in less than 30 min at 37 °C (Fig. 2). The prostaglandin A_1 time course was found to be the same as the prostaglandin E_1 time course.

Prostaglandin binding is very temperature sensitive. No prostaglandin E_1 and very little prostaglandin A_1 is bound at 0 °C, when compared to the amount of prostaglandin that binds at 37 °C (Table I).

We have found no change in prostaglandin E_1 binding regardless of the ionic composition of the buffer (Table II). The binding is the same in Krebs–Ringer Tris, Tris–HCl, or Krebs–Ringer Tris without Ca^{2+} with 1 mM EGTA. Prostaglandin A_1 binding is stimulated by ions. Over twice as much prostaglandin A_1 is bound when Krebs–Ringer Tris buffer is used, than when the incubation occurs in Tris–HCl

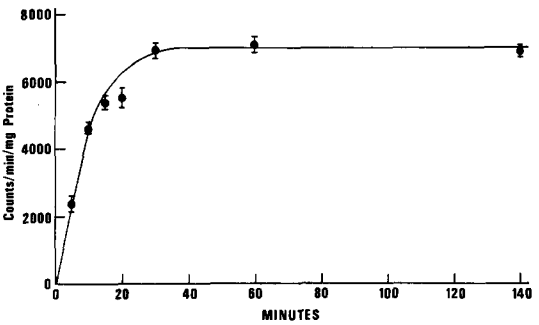

Fig. 2. Time course of prostaglandin E_1 binding at 37 °C. Incubation was in Krebs–Ringer Tris buffer with 0.5 ng [5,6-$^3H_2$]prostaglandin E_1. The Millipore background values were subtracted. The bars represent the S.D. of quadruplicate samples.

TABLE I

EFFECT OF TEMPERATURE ON PROSTAGLANDIN BINDING

Incubation conditions as described in Methods. Data presented as Mean ± S.D. of quadruplicate samples.

Time (min)	*cpm/mg protein*			
	Prostaglandin E_1		*Prostaglandin A_1*	
	0 °C	*37 °C*	*0 °C*	*37 °C*
5	0 ± 28	3117 ± 146	259 ± 19	4321 ± 147
15	476 ± 37	6198 ± 330	240 ± 21	5841 ± 239
30	0 ± 20	8664 ± 330	798 ± 32	7374 ± 383

buffer. EGTA did not significantly reduce prostaglandin A_1 binding, indicating Ca^{2+} is not an essential ion for binding.

An enhancement of prostaglandin E_1 binding by guanine and possibly adenine nucleotides, is shown in Table III. GTP at $1 \cdot 10^{-5}$ M significantly stimulates binding. The stimulation with ATP at a much higher ($1 \cdot 10^{-3}$ M) concentration was not statistically significant. Prostaglandin A_1 binding was not influenced by GTP, but in the presence of $1 \cdot 10^{-3}$ M ATP, binding was significantly stimulated (Table III).

All of the [^{3}H]prostaglandin E_1 bound by adipocyte membranes can be extracted, chromatographed, and identified as authentic prostaglandin E_1 (Table IV). When [^{3}H]prostaglandin A_1 was extracted from adipocyte membranes, only 64% was prostaglandin A_1 and approximately 29% of the bound radioactivity proved to

TABLE II

EFFECT OF IONS ON [^{3}H]PROSTAGLANDIN E_1 BINDING

Assay conditions as described in Fig. 1 except for the change in buffer. Data presented as mean ± S.E. of quadruplicate measurements. N.S., not significant.

Buffer	*[^{3}H]prostaglandin E_1 binding (cpm/mg protein)*	*P value compared to Tris–HCl*	*[^{3}H]prostaglandin A_1 binding (cpm/mg protein)*	*P value compare Tris–HC*
Tris–HCl	7356 ± 257	—	7 896 ± 184	—
Krebs–Ringer Tris	7004 ± 392	N.S.	16 872 ± 622	< 0.05
Krebs–Ringer Tris +1 mM EGTA without Ca^{2+}	6589 ± 357	N.S.	14 760 ± 294	< 0.05

TABLE III

ADENINE AND GUANINE NUCLEOTIDES AND PROSTAGLANDIN BINDING

Incubation conditions as described in Methods except either ATP or GTP was added. Data expressed as Mean ± S.E. of six separate observations for prostaglandin E_1 and four separate observations for prostaglandin A_1.

Additions (M)		*Prostaglandin E_1 (cpm/mg protein)*	*Prostaglandin A_1 (cpm/mg protein)*
None		7 816 ± 453	13 212 ± 560
GTP	10^{-6}	9 124 ± 516	15 577 ± 992
	10^{-5}	12 449 ± 467*	15 884 ± 1173
	10^{-4}	9 720 ± 266	13 718 ± 824
	10^{-3}	8 748 ± 232	12 833 ± 162
ATP	10^{-6}	8 380 ± 182	11 902 ± 956
	10^{-5}	8 848 ± 531	14 530 ± 199
	10^{-4}	8 848 ± 744	13 501 ± 758
	10^{-3}	9 890 ± 208	21 317 ± 779*

* $P < 0.05$. Compared to control (no additions) response.

be polar metabolites which did not leave the origin on the thin-layer chromatogram (Table IV). At present, we do not know if these polar compounds represent metabolism of specifically, or non-specifically bound prostaglandin A_1.

TABLE IV

ELUTION OF PROSTAGLANDIN E_1 AND PROSTAGLANDIN A_1 FROM "GHOSTS"

Experimental procedures as described in Methods.

Elution procedure	*Total cpm*	
	Prostaglandin E_1	*Prostaglandin A_1*
Substrate	$3.3 \cdot 10^6$	$3.2 \cdot 10^6$
Supernatant of reaction mixture after 30 min incubation	$3.2 \cdot 10^6$	$3.3 \cdot 10^6$
Wash 1	69 876	133 488
2	3 438	16 704
3	1 665	5 597
4	—	3 080
5	—	1 958
Ethylacetate extract of ghosts No. 1	13 856	7 322
Ethylacetate extract of ghosts No. 1 dried and reconstituted	14 338	6 965
Ethylacetate extract of ghosts No. 2	1 155	88
Prostaglandin bound (cpm/mg protein)	8 714	5 881
Thin-layer chromatography of extracts	97.6% as prostaglandin E_1	66.1% as prostaglandin A_1

Because of the small quantity of bound prostaglandin, we could not yet demonstrate the biological activity of the eluted materials. There was no ^3H_2O found in the eluate from either the prostaglandin E_1 or A_1 extraction.

The relative affinities as competitors of [^{3}H]prostaglandin E_1 for the E prostaglandin-binding site were determined for a few naturally occurring prostaglandins, prostaglandin metabolites, and a prostaglandin antagonist by a parallel line assay[14]. Fig. 3 illustrates the relative affinities of prostaglandin E_1, A_2, $F_{2\alpha}$, and 15-epiprostaglandin A_2. The relative affinities were prostaglandin E_1 > prostaglandin A_2 > prostaglandin $F_{2\alpha}$, and the 15-epiprostaglandin A_2 was the least effective. The 15-epiprostaglandin A_2 did not give a parallel response, indicating a different type of competition may be involved. Table V summarizes the data for all the analogs tested. Prostaglandin E_2 and 16,16-dimethylprostaglandin E_2 are equipotent with prostaglandin E_1. 15[S]-15-methyl prostaglandin $F_{2\alpha}$ although of low relative potency, was approximately twice as active as prostaglandin $F_{2\alpha}$. The 13,14-dihydroprostaglandin E_1 and the methyl ester of prostaglandin E_1 were relatively potent antagonists, but not equal to prostaglandin E_1. We have completed some preliminary binding studies with the [^{3}H]methyl ester of prostaglandin E_1, and the data indicate a large degree of nonspecific binding by the ester. This could tend to decrease the effective concentration

of the ester at the E prostaglandin-binding site, making it appear to have a lower affinity than prostaglandin E_1. The ester also binds to the glassware, therefore all equipment was siliconized. Elution and chromatography of the bound ester showed that about 40% of the radiolabeled material was prostaglandin E_1 free acid, and 60% prostaglandin E_1 methyl ester. Work is in progress to determine if hydrolysis of the ester linkage is a prerequisite for specific binding, or if the hydrolysis occurs on the non-specific sites.

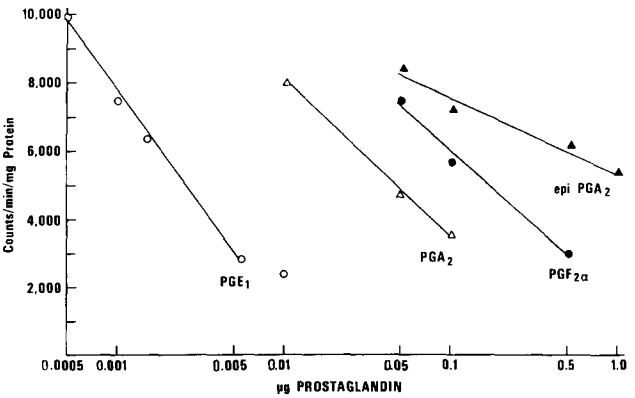

Fig. 3. Parallel line assay of prostaglandins as competitors of prostaglandin E_1 binding. Each tube contained 0.5 ng [^{3}H]prostaglandin E_1 (PGE_1) and the appropriate amounts of prostaglandins as inhibitors. Incubation was for 15 min at 37 °C in Krebs–Ringer Tris buffer. PGA_2, prostaglandin A_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$.

TABLE V

RELATIVE AFFINITIES OF PROSTAGLANDINS AND PROSTAGLANDIN ANALOGS AS COMPETITORS OF THE BINDING OF PROSTAGLANDIN E_1

Assay conditions as described for Figs 2 and 4.

Compounds	*Concentration range of prostaglandins (μg/ml)*	*Relative affinities*	*95% confidence limits*		*λ* index of precision*
			Upper	*Lower*	
Prostaglandin E_1 (standard)	0.0005–0.005	100			
Prostaglandin E_2	0.0005–0.005	93.0	122.5	70.4	0.116
Prostaglandin A_2	0.01 –0.1	4.1	6.0	2.8	0.162
Prostaglandin $F_{2\alpha}$	0.05 –0.5	1.1	1.7	0.7	0.184
15-epiprostaglandin A_2	0.05 –1.0	0.3	0.5	0.2	0.212
15[S]-15-methyl prostaglandin $F_{2\alpha}$	0.01 –0.5	2.3	3.8	1.5	0.189
13,14-dihydro prostaglandin E_1	0.005 –0.05	25.5	51.5	13.7	0.250
16,16-dimethyl prostaglandin E_2	0.0005–0.001	82.4	124.0	54.5	0.198
Prostaglandin E_1 methyl ester	0.001 –0.01	41.4	54.8	30.8	0.129
7-Oxa-13-prostynoic acid	1.0 –10.0	<0.04	—	—	0.107

* λ=S.D./slope.

Figs 4 and 5 show the concentration dependence of prostaglandin E_1 and A_1 binding. Prostaglandin A_1 binding is shown with and without ions. It is apparent, that when binding occurs in the presence of ions, the binding of prostaglandin A_1

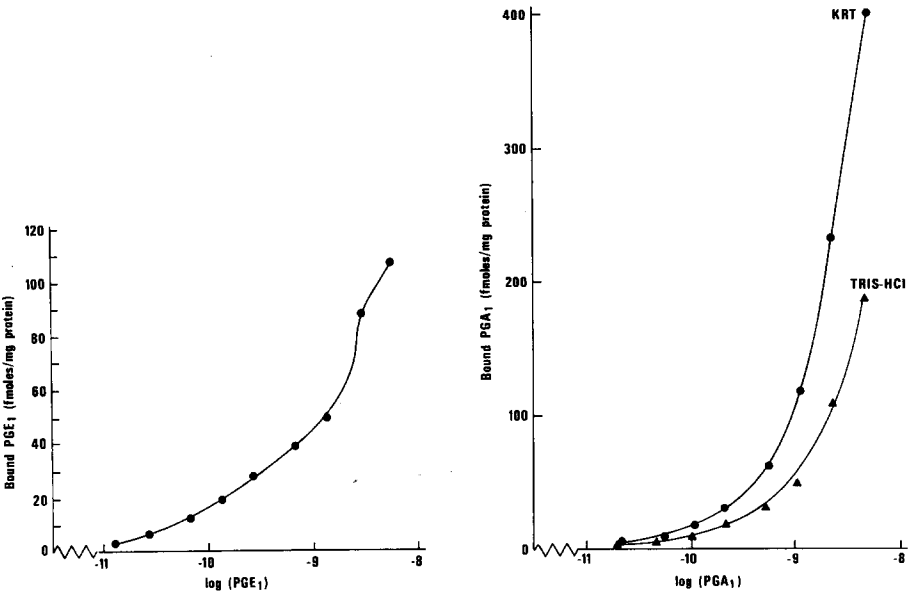

Fig. 4. Concentration dependence of prostaglandin E_1 (PGE_1) binding. Increasing concentrations of [^{3}H]prostaglandin E_1 over a 3 log range were added to 100 μg of membrane protein, and the binding measured. Incubations were for 40 min in Tris–HCl buffer at 37 °C.

Fig. 5. Concentration dependence of prostaglandin A_1 (PGA_1) binding. Increasing concentrations of [^{3}H]prostaglandin A_1 over a 3 log range were added to 100 μg of membrane protein and the binding measured. Incubations were for 30 min in either Krebs–Ringer Tris (KRT) or Tris–HCl buffer at 37 °C. ●—●, prostaglandin A_1 in KRT buffer; ▲—▲, prostaglandin A_1 in Tris–HCl buffer.

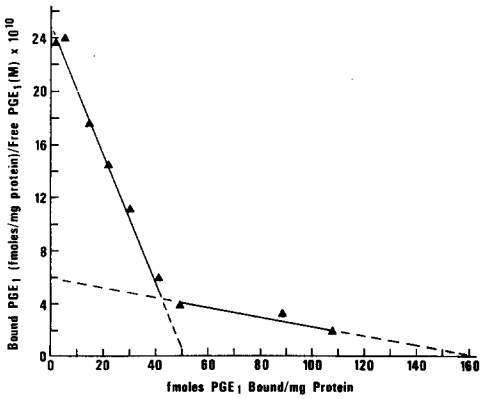

Fig. 6. Scatchard plot of prostaglandin E_1 (PGE_1) binding. The data shown in Fig. 5 were plotted according to Scatchard, and the extrapolated intercepts of the plots were used to calculate the association constants and the number of binding sites per mg membrane protein. $K_1 = 4.9 \cdot 10^9$ l/mole; $N_1 = 51$ fmoles/mg protein. $K_2 = 4 \cdot 10^8$ l/mole; $N_2 = 157$ fmoles/mg protein.

is stimulated. The binding data shown in Figs 4 and 5 were evaluated by Scatchard plot analysis[15], and presented in Figs 6 and 7. The binding is biphasic for both prostaglandin E_1 and A_1, indicating that there are two classes of binding sites. From the extrapolated intercepts of the linear segments of the plots, the high-affinity E prostaglandin sites had an association constant of $4.9 \cdot 10^9$ l/mole and a total binding capacity of 51 fmoles of prostaglandin E_1/mg membrane protein. The low affinity E prostaglandin sites had an association constant of $4 \cdot 10^8$ l/mole and a binding capacity of 157 fmoles/mg membrane protein. Two Scatchard plots were made for prostaglandin A_1, one with, and one without ions (Fig. 7). Without ions, the high affinity prostaglandin A_1 binding sites had an association constant of $8.3 \cdot 10^8$ l/mole and bound a total of 100 fmoles/mg protein. The lower affinity sites in the same system had an association constant $5.7 \cdot 10^7$ l/mole with a binding capacity of 780 fmoles/mg protein. The addition of ions to the buffer changed the high affinity constant to

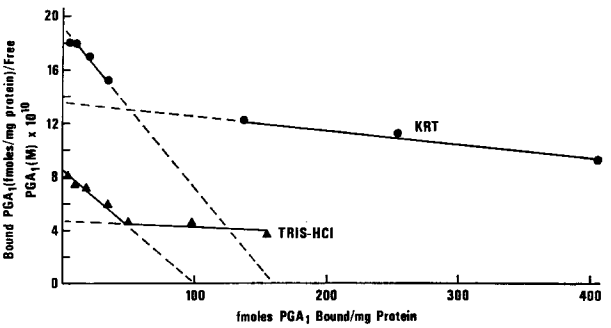

Fig. 7. Scatchard plot of prostaglandin A_1 (PGA_1) binding. The data shown in Fig. 6 were plotted according to Scatchard, and the association constants and number of binding sites/mg protein determined. Tris buffer: $K_1 = 8.3 \cdot 10^8$ l/mole; $N_1 = 100$ fmoles/mg protein. $K_2 = 5.7 \cdot 10^7$ l/mole; $N_2 = 780$ fmoles/mg protein. Krebs–Ringer Tris buffer: $K_1 = 1.2 \cdot 10^9$ l/mole; $N_1 = 160$ fmoles/mg protein. $K_2 = 8.6 \cdot 10^6$ l/mole; $N_2 = 1552$ fmoles/mg protein. ●—●, prostaglandin A_1 in Krebs–Ringer Tris (KRT) buffer; ▲—▲, prostaglandin A_1 in Tris–HCl buffer.

TABLE VI

INHIBITION OF PROSTAGLANDIN E_1 BINDING BY PROSTAGLANDIN A_1

Increasing concentrations of [^{3}H]prostaglandin E_1 were incubated with a 20-fold excess of prostaglandin A_1 as a competitive inhibitor of prostaglandin E_1 binding. Binding determined as described in Methods. Data expressed as per cent inhibition at each concentration.

[^3H]*prostaglandin* E_1 *concn* (*M*)	*Prostaglandin* A_1 *concn* (*M*)	*% inhibition*
$5.2 \cdot 10^{-9}$	$1.04 \cdot 10^{-7}$	44
$2.63 \cdot 10^{-9}$	$5.01 \cdot 10^{-8}$	40
$1.37 \cdot 10^{-9}$	$2.51 \cdot 10^{-8}$	33
$6.32 \cdot 10^{-10}$	$1.26 \cdot 10^{-8}$	31
$2.69 \cdot 10^{-10}$	$5.02 \cdot 10^{-9}$	29
$1.39 \cdot 10^{-10}$	$2.51 \cdot 10^{-9}$	27
$6.96 \cdot 10^{-11}$	$1.26 \cdot 10^{-9}$	10
$2.78 \cdot 10^{-11}$	$5.02 \cdot 10^{-10}$	20

$1.2 \cdot 10^9$ l/mole with a binding capacity of 160 fmoles/mg, and the low affinity constant to $8.6 \cdot 10^6$ l/mole and a total capacity of 1552 fmoles/mg protein.

Scatchard plots were also used to investigate the cross-reactivity we had previously found between prostaglandin E_1 and A_1 for their respective receptors. By incubating [^{3}H]prostaglandin E_1 with a 20-fold excess of prostaglandin A_1, prostaglandin A_1 was found to compete poorly with prostaglandin E_1 for the high affinity E prostaglandin-binding sites. The data from the Scatchard plots are presented as per cent inhibition at the respective prostaglandin concentrations (Table VI). 10–40% of the prostaglandin E_1-binding sites could be blocked with the 20-fold excess of prostaglandin A_1. These data indicate that prostaglandin A_1 cross-reactivity occurs primarily on the low-affinity E prostaglandin-binding sites.

When [^{3}H]prostaglandin A_1 binding was analyzed in the presence of a 10-fold excess of prostaglandin E_1, and the data presented as per cent inhibition, prostaglandin E_1 was found to inhibit 1–20% of the prostaglandin A_1 binding, and to compete primarily for the high-affinity A prostaglandin-binding sites. At the highest concentration of [^{3}H]prostaglandin A_1, prostaglandin E_1 had only a 1% inhibition (Table VII).

TABLE VII

INHIBITION OF PROSTAGLANDIN A_1 BINDING BY PROSTAGLANDIN E_1

Increasing concentrations of [^{3}H]prostaglandin A_1 were incubated with a 10-fold excess of prostaglandin E_1 as a competitive inhibitor of prostaglandin A_1 binding. Binding determined as described in Methods. Data expressed as per cent inhibition at each concentration.

[^{3}H]prostaglandin A_1 concn (M)	*Prostaglandin E_1 concn (M)*	*% inhibition*
$4.46 \cdot 10^{-9}$	$4.46 \cdot 10^{-8}$	1.11
$2.23 \cdot 10^{-9}$	$2.23 \cdot 10^{-8}$	8.6
$1.12 \cdot 10^{-9}$	$1.12 \cdot 10^{-8}$	13.8
$5.58 \cdot 10^{-10}$	$5.58 \cdot 10^{-9}$	24.2
$2.23 \cdot 10^{-10}$	$2.23 \cdot 10^{-9}$	18.2
$1.12 \cdot 10^{-10}$	$1.12 \cdot 10^{-9}$	16.1
$5.58 \cdot 10^{-11}$	$5.58 \cdot 10^{-10}$	15.8
$2.23 \cdot 10^{-11}$	$2.23 \cdot 10^{-10}$	17.4

DISCUSSION

Although the existence of discrete receptors for prostaglandins had been inferred, it has been only during the last year that published accounts of prostaglandin-binding activity have appeared[8,9]. Both of these studies reported the existence of an E-type receptor with little affinity for prostaglandins of the A or F series.

This report extends the findings of E-binding activity in adipocytes[8], and for the first time suggests that there may be distinct binding sites for A prostaglandins.

The E- and A-binding sites in adipocyte membranes share several characteristics. Their binding is linear with increasing protein concentration, the rate of binding is temperature sensitive, and binding equilibrium is reached in less than

30 min at 37 °C. Although there are similarities, there are also distinct differences. Prostaglandin E_1 is not sensitive to ions, while prostaglandin A_1 binding is stimulated in Krebs–Ringer Tris buffer. The addition of ions increased the number of high- and low-affinity A prostaglandin-binding sites, and increases the high-affinity association constant while lowering the low-affinity association constant. The finding that bound prostaglandin E_1 is not metabolized, while some metabolism of prostaglandin A_1 was detected, suggests prostaglandin E_1 and A_1 may be metabolized by different routes in adipose tissue. Nucleotides also alter prostaglandin E_1 and A_1 binding differently. Prostaglandin E_1 but not prostaglandin A_1 binding is stimulated by GTP. Krishna *et al.*[16] reported that the stimulation of platelet adenylate cyclase by prostaglandin E_1 was enhanced by GTP, and an enhancement of the prostaglandin E_1 activation of thyroid adenylate cyclase by GTP has also recently been reported[17]. Our data suggest these reports may represent a facilitation of prostaglandin E_1 binding in these tissues by GTP.

The relative activities of prostaglandin E_1, E_2, A_1, and $F_{2\alpha}$ as competitors of prostaglandin E_1 binding generally were found to parallel their respective biological potency in adipose tissue[18–20]. Although the methyl ester of prostaglandin E_1 demonstrated only a 41% relative activity in our system, it has potent biological activity in smooth muscle (Weeks, J. R., personal communication). It is probable that the non-specific binding of the methyl ester of prostaglandin E_1 reduces its apparent relative potency. The possible necessity of hydrolysis of the ester before binding cannot be ruled out at this time. The 16,16-dimethylprostaglandin E_2 which has been reported to have 50 times the antisecretory activity of prostaglandin E_2 (ref. 21), was found to have a high affinity for the E binding site. The activity of 7-oxa-13-prostynoic acid, a prostaglandin antagonist, was found to be so low relative to prostaglandin E_1, that this antagonist must exert its effect at a place other than the prostaglandin binding sites.

There is very close agreement between our data and the data of Kuehl[8] and Miller[9] on the relative affinities of prostaglandin E_1, $F_{2\alpha}$, and 13,14-dihydroprostaglandin E_1. There are also dissimilarities. Kuehl found prostaglandin E_2 to have 65% of the affinity of prostaglandin E_1, while our laboratory found it to be equipotent to prostaglandin E_1 in rat stomach and rat fat cell ghosts. Our calculated high affinity constant also agrees closely with Kuehl's constant, but Kuehl using Lineweaver–Burke plots, reported only one class of E prostaglandin binding sites. Our work employing Scatchard plot analysis of prostaglandin E_1 and A_1 binding demonstrates the existence of two classes of binding sites for each prostaglandin. Both prostaglandins have high and low affinity binding sites, but prostaglandin A_1 has many more low affinity sites than prostaglandin E_1. It is apparent that the low-affinity A prostaglandin binding sites may have a non-specific component.

Considering that the concentration range studied by Scatchard plot did not exceed $1 \cdot 10^{-8}$ M for either prostaglandin E_1 or A_1, it is conceivable that both the high and the low affinity sites may be available for binding of prostaglandins *in vivo*.

If prostaglandin binding is an initial event in the modulation of lipolysis, the prostaglandins must first migrate from the endoplasmic reticulum, where they are probably formed[22], to the plasma membrane before regulation is initiated. The regulation of lipolysis by prostaglandins at the level of the plasma membrane would certainly involve adenylate cyclase, but there are systems controlled by prostaglandins

that do not involve adenylate cyclase. Direct prostaglandin effects on enzymes[23,24] and mitochondrial Ca^{2+} binding[25] have been reported. The local transport of prostaglandins from the endoplasmic reticulum to other points within the cell may be important to the overall cellular processes that are controlled by prostaglandins, but not mediated by cyclic AMP. In view of these prostaglandin actions that appear to be unrelated to cyclic AMP, the localization of prostaglandin binding sites in the plasma membrane should not exclude the existence of other prostaglandin binding sites, or imply that all prostaglandin regulation is initiated by a plasma membrane–prostaglandin interaction.

It will be of particular importance to the understanding of prostaglandin control mechanisms to expand prostaglandin-binding studies to other tissues. Binding studies with epinephrine[26,27] and insulin[28,29] have indicated that the hormone receptors in adipose tissue are similar, if not identical to those found in the liver.

A preliminary report[30] of prostaglandin binding sites in the liver indicate this may be true for prostaglandins as well.

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