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Fatty acid binding protein (FABP) modulates prostaglandin E binding to rat epididymal adipocyte membrane similarly to albumin

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Albumin enhances prostaglandin E_2 (PGE_2) binding to isolated epididymal adipocyte membrane and also binds PGE_2 with low affinity. On the other hand, S-100, ovalbumin and albumin-stearate failed to bind PGE_2, as shown by ultrafiltration, and also failed to enhance PGE_2 binding to the isolated adipocyte membranes. These results suggested that albumin enhances PGE_2 binding possibly by serving as a carrier for the prostaglandin molecules. 3 mM warfarin or 1 mM phenylbutazone inhibited PGE_2 binding to albumin by 70% and 95%, respectively, but both drugs failed to affect the enhancement of PGE_2 binding to the isolated adipocyte membrane in the presence of albumin. These results exclude the possibility that PGE_2 bound to albumin is more accessible to the prostaglandin receptor than free PGE_2 in solution. Finally it is shown that fatty acid binding protein (FABP), a cytosolic protein which binds specifically PGE_1 but not PGE_2, enhances PGE_1 and PGE_2 binding to isolated adipocyte membranes similarly to albumin. The physiological implications of these findings are discussed.

Introduction

Incubation of PGE_2 with isolated rat epididymal adipocyte membranes in the presence of albumin resulted in a substantial increase in the specific binding of PGE_2 to the membranes [1,2]. Since preincubation of albumin with the isolated membranes failed to enhance PGE_2 binding, it is unlikely that albumin increases PGE_2 binding by elution of membranal components such as proteins or fatty acids [1,3]. The failure of albumin to enhance PGE_2 binding to intact cells [2] suggests that albumin mimics the activity of a cytoplasmic protein, which modulates the binding to the prostaglandin receptor at the internal side of the adipocyte plasma membrane. Albumin also increased PGE_2 binding to a solubilized complex of the prostaglandin receptor and G binding protein [3]. The latter finding supports the possibility that albumin interacts directly with an internal domain of the prostaglandin receptor or with the G binding protein, which increases the prostaglandin binding affinity in the presence of GTP [3]. Analysis of the binding data showed that albumin, similarly to GTP, increased the affinity of the low-affinity, high-capacity PGE_2 binding sites. Treatment of the isolated adipocyte membranes with low concentration of *N*-ethylmaleimide completely abolished the stimulatory effect of GTP on PGE_2 binding, but only partially inhibited the enhancement of PGE_2 binding by albumin. These results and the finding that the enhancement of PGE_2 binding by albumin, unlike the effect of GTP, is independent of the presence of Mg^{2+}, did not support the possibility that albumin interfered with GTP at the same G binding protein [3]. It was also possible that prostaglandin molecules bound to albumin are more accessible to the membranal prostaglandin receptors than free prostaglandin molecules in solution. If this possibility is correct, it is expected that a correlation could be found between the capacity of albumin to bind free prostaglandin molecules and the degree of the enhancement of PGE_2 specific binding to the membranes by albumin. The aim of the present study was to test this possibility.

Materials and Methods

Materials. Warfarin, phenylbutazone, diazepam, bovine serum albumin (fraction V), fatty acid-free albumin and ovalbumin were purchased from Sigma. [^{3}H]PGE_2 (specific activity 160–170 Ci/mmol, 99% pure) and [^{3}H]PGE_1 (60 Ci/mmol) were obtained from New England Nuclear. Unlabeled PGE_2 was a gift from

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Dr. John Pike, Upjohn, Kalamazoo. Percoll was obtained from Pharmacia Fine Chemicals. Albumin stearate was a gift from Dr. S. Braun, the Hebrew University of Jerusalem. Protein S-100, from cardiac muscle, was a gift from Dr. K. Kato, Institute for Developmental Research Aichi Prefectural Colony, Kasugai, Aichi, Japan. Fatty acid binding protein (FABP), from rat liver, was a gift from Dr. A.K. Dutta-Roy, Wright State University, Dayton, Ohio, U.S.A.

Source of tissue. Epididymal fat pads were obtained from male albino Charles-River derived rats, which had free access to water and stock laboratory chow.

Preparation of epididymal adipocyte membranes and binding assay. Plasma membranes were separated from mitochondria and other cell components by centrifugation in the presence of a self-forming gradient of Percoll as described by Belsham et al. [4]. Binding assay was performed on freshly prepared membranes according to Cohen-Luria and Rimon [3]. Briefly, the isolated membranes were incubated with 3 nM [^{3}H]PGE_2 or [^{3}H]PGE_1, for 30 min at 37°C, in 25 mM Tris buffer (pH 7.4) and 2 mM $MgCl_2$. At the end of the incubation the membranes were separated from the suspension by filtering through Whatman GF/c filters. In order to eliminate any possible effects of albumin or other protein present in the incubation medium on the filtration process, an equivalent amount of albumin was added before the filtration step to the protein-free samples. Non specific binding, defined as the binding in the presence of 1000-fold excess of unlabeled PGE_2 or PGE_1, did not exceed 25–30% of the total binding. Competition on [^{3}H]PGE_2 binding by other prostaglandins was in the following order of potency: PGE_1 = PGE_2 = sulprostone (a PGE_2 analogue) ≫ PGF_{2a}.

Measurement of PGE_2 binding to protein. 1% albumin or another protein was dissolved in 25 mM Tris buffer (pH 7.4), and incubated with 3 nM [^{3}H]PGE_2 for 30 min at 37°C. At the end of this incubation albumin was separated from the incubation medium by ultrafiltration on Amicon YMT membranes, using Amicon MPS-1 micropartition system. Binding of prostaglandin to albumin was calculated by measurement of the reduction of the free prostaglandin concentration in the filtered solution due to the binding to albumin. The non specific binding of PGE_2 to the filtration apparatus contributed less than 5% to the reduction of the free prostaglandin concentration in the solution. The effects of warfarin, diazepam or phenylbutazone on prostaglandin binding to albumin were monitored by direct addition of these drugs to the incubation medium.

Results and Discussion

Binding of PGE_2 to albumin

In order to test the possibility that albumin may function as a carrier for the PGE_2 molecules, direct measurements of PGE_2 binding to albumin were performed. Using ultrafiltration, it was found that albumin binds PGE_2 with low affinity (dissociation constant of $3 \cdot 10^{-4}$ M; a similar binding constant was reported by Raz et al. [5]). Since in the nM range of PGE_2 only a minor fraction of albumin (0.0018%) is occupied by PGE_2, a linear correlation exists between the total and the bound PGE_2 (Fig. 1). Therefore it is expected that at each concentration of PGE_2, a constant fraction of the total PGE_2 is bound to albumin. This assumption was corroborated by the results (Fig. 1), implying that the presence of albumin does not distort the concentration dependence of PGE_2 binding to the adipocyte isolated membranes.

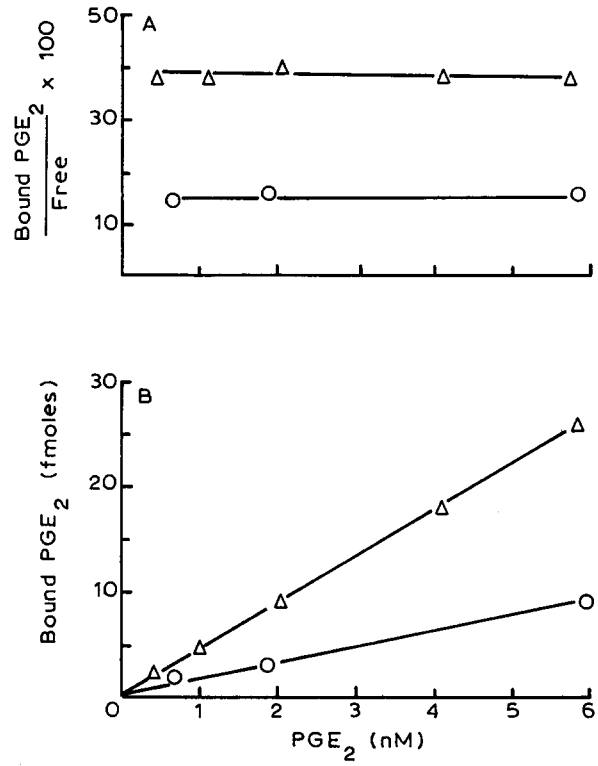

Fig. 1. Binding of PGE_2 to albumin. Binding experiments were performed at (○) 23 or (△) 37°C. (A) The ratio of bound PGE_2 over free PGE_2, as a function of PGE_2 concentration. (B) Binding of PGE_2 to 1% albumin as a function of PGE_2 concentration. The results are means of four experiments.

Binding of PGE_2 to soluble proteins and albumin derivatives

S-100 is a cytoplasmic acidic protein which was identified in certain cells including adipocytes and the biological function of which is still unclear [7]. The data presented in Table I show that S-100 and ovalbumin, which is another hydrophilic protein [8], failed to enhance PGE_2 binding to the membranes. The failure of these hydrophilic proteins to enhance PGE_2 binding might suggest that hydrophobic interactions are necessary for the interaction of albumin with the membranes.

TABLE I

Percent of PGE_2 binding to soluble proteins and to isolated adipocyte membranes in the presence of these proteins

Binding of PGE_2 to the proteins or to the membranes was performed as described in Materials and Methods. Protein concentration was 1%. PGE_2 binding to the membrane in the absence of proteins is defined as 100%. PGE_2 binding to protein is given as the percentage of the bound PGE_2 over the total concentration of PGE_2. (*) PGE_2 binding to protein was not measured. The results are means ± S.E. of four experiments.

Protein	PGE_2 binding to protein	PGE_2 binding to isolated membranes
Albumin (fraction V)	40.50 ± 1.44	200 ± 9
Ovalbumin	0.20 ± 0.25	87 ± 14
S-100	−0.10 ± 0.15	92 ± 11
Succinyl albumin	*	175 ± 9
Albumin-stearate	1.40 ± 0.02	89 ± 13

But the findings that succinyl albumin, a hydrophilic albumin derivate [9], enhanced PGE_2 binding, while albumin stearate, a hydrophobic albumin derivate, failed to enhance PGE_2 binding to the membranes, do not support a simple relationship between the hydrophobicity of the protein and its ability to potentiate PGE_2 binding to the isolated membranes. Inspection of the data presented in Table I shows that only albumin bound PGE_2 and enhanced PGE_2 binding to the membranes. Fatty acid-free albumin yielded similar results (not shown). The other three proteins failed to bind PGE_2 and to enhance PGE_2 binding to the membranes. These results may suggest a correlation between the capability of albumin to bind PGE_2 and its ability to enhance PGE_2 binding to the membranes. Furthermore, one may be lead to conclude that bound PGE_2 could be more accessible than free PGE_2 to the prostaglandin receptor.

Binding of PGE_2 to albumin and to isolated membranes in the presence of warfarin and phenylbutazone

Spectroscopic studies have shown that the presence of albumin catalysed a very slow dehydration process of PGE_2, with a half-time of 11–18 hours. In these studies warfarin and phenylbutazone were effective inhibitors of the dehydration process, while diazepam, fatty acids and steroids were ineffective [10]. Since the enhancement of PGE_2 binding by albumin in our studies was completed within 20 minutes [1], it is unlikely that the dehydration process of PGE_2 by albumin could account for the effects reported here. In order to further investigate the relationship between the binding capacity of albumin and its potential to enhance PGE_2 binding to the adipocyte membranes, we measured the effects of warfarin, phenylbutazone and diazepam on PGE_2 binding to albumin on the one hand, and the effects of these drugs on the enhancement of PGE_2 binding to the adipocyte membranes in the presence of 1% albumin, on the other hand. Albumin in these particular experiments affected a 4-fold increase in PGE_2 specific binding to the membranes. Diazepam failed to affect significantly PGE_2 binding to albumin, but warfarin and phenylbutazone inhibited PGE_2 binding to albumin. 3 mM warfarin inhibited PGE_2 binding to albumin by 70%, and 1 mM phenylbutazone inhibited PGE_2 binding almost completely (Fig. 2A). If the enhancement of PGE_2 binding depends on the concentration of the PGE_2 that is bound to albumin, it is expected that the presence of warfarin and phenylbutazone should reduce the albumin enhancement of PGE_2 binding to the adipocyte membrane by the same proportion. As is demonstrated in Fig. 2B, these drugs had no effect on PGE_2 binding to the isolated membrane in the absence of albumin, and similarly to diazepam, did not affect the enhancement of PGE_2 binding to the isolated membrane in the presence of albumin. The failure of these drugs to inhibit the stimulatory effect of albumin on PGE_2 binding to the isolated membranes suggested that

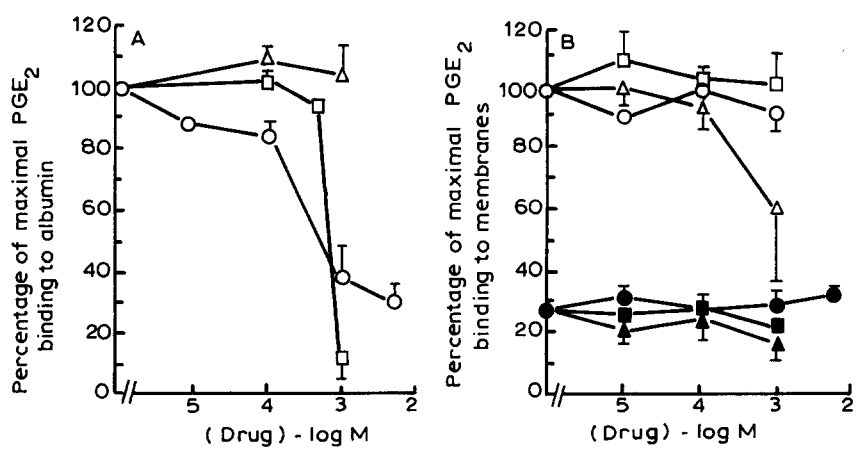

Fig. 2. The effects of diazepam (△, ▲), phenylbutazone (□, ■) and warfarin (○, ●) on PGE_2 binding to albumin (A) and to isolated adipocyte membranes (B) in the presence (open symbols) or absence (closed symbols) of 1% albumin. The data are means ± S.E. of six experiments.

the enhancement of PGE_2 binding was not related to the ability of albumin to bind PGE_2. It may also be suggested that only a small fraction of albumin, free of bound warfarin or phenylbutazone, is needed in order to obtain maximal enhancement of PGE_2 binding to the isolated membranes. Knowledge of the free and albumin-bound PGE_2 concentrations and the dissociation constant allows one to calculate that in the presence of 3 nM PGE_2 and 1 mM phenylbutazone, 0.118% albumin is needed to yield maximal enhancement of PGE_2 binding to the adipocyte membranes. Our previous studies showed that the half-maximal effect of albumin is obtained at a concentration of about 1% albumin [1], while at concentration of 0.1%, albumin had no significant effect on the enhancement of PGE_2 binding to the adipocyte membranes. The discrepancy between the calculated albumin concentration and the experimental observation excludes the suggestion that PGE_2 bound to albumin could be more accessible to the prostaglandin receptor than free PGE_2 molecules in solution, and is incompatible with the suggestion that albumin may serve as a carrier for the prostaglandin molecule.

Effect of FABP on the enhancement of PGE_2 binding to isolated epididymal adipocyte membranes

Since albumin is not a cytosolic protein, its effect on PGE_2 binding as shown in our previous reports [1–3] is physiologically meaningless, unless it can be shown that a cytosolic protein has a similar effect on the enhancement of PGE_2 binding. FABP, also known as protein Z, is a soluble protein which is abundant in the cytosol of many cells types [11], including adipocytes [12]. The cellular concentration of FABP is about 5% of the total cytosolic proteins [13]. At certain locations in the cell the concentration of FABP approaches 7.2 mg/ml [14]. A previous study showed that FABP binds PGE_1, but not PGE_2, specifically and reversibly [15]. Also, Raza et al. [16] reported recently specific high-affinity binding of lipoxygenase metabolites by liver FABP. Presently, incubation of PGE_2 or PGE_1 with the isolated membranes in the presence of FABP, resulted in an increase in the binding of both prostaglandin E_2 and E_1 to the isolated membranes (Fig. 3). The enhancement of PGE_2 binding to the membranes by FABP, in spite of the fact that it does not bind PGE_2, is in accordance with our present finding that the increased prostaglandin binding to the isolated membranes by albumin is not involved with binding of prostaglandin molecule to the protein. The finding that FABP enhanced binding of prostaglandins E_1 and E_2 to isolated membranes further support our suggestion [1] that albumin mimics the function of a cytosolic protein. The FABP used in this investigation is from rat liver [7]. Since there are several distinct FABP proteins [15], the possibility that the FABP from adipose tissue does not carry out the stimulatory effect on PGE_2 binding cannot be ruled out. The observation that FABP and albumin have a comparable effect on PGE_2 binding is not unexpected, as both proteins have other common activities, besides binding of fatty acids. Both accelerate long chain acyl-CoA: glycerol-3-phosphate acetyltransferase, stimulate mitochondrial and microsomal acyl-CoA synthetase activities [17] and stimulate sodium-dependent synaptosomal uptake [18]. Vis-à-vis our previous observation that albumin increases PGE_2 binding also to a solubilized complex of PGE_2 receptor and G binding protein [3], we suggest that FABP or albumin increase PGE_2 binding by a direct interaction with an internal domain of the receptor or its close vicinity, perhaps a G binding protein. It is also probable that there exists another cytosolic protein that enhances PGE_2 binding to the membrane at much lower concentrations than FABP. It was demonstrated recently that GAP (GTPase activating protein), a cytosolic protein, regulates the GTPase activity of P21 [19], a membranal rats protein [20] which shares structural and biochemical properties with the signal-transducing G proteins [21].

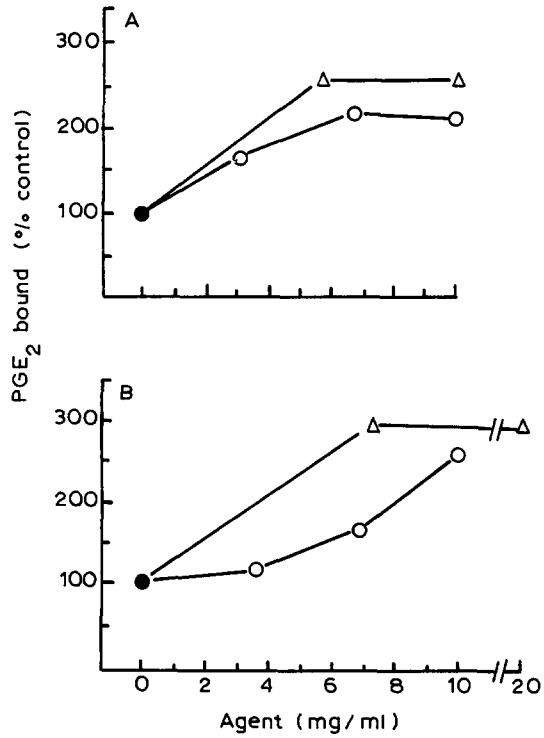

Fig. 3. Binding of PGE_1 (A) and PGE_2 (B) to isolated adipocyte membranes in the presence of increasing concentrations of albumin (△) or FABP (○). 100% represents PGE_2 binding in the absence of albumin or FABP. The results are means of three experiments.

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References

1 Luria, R. and Rimon, G. (1986) Biochim. Biophys. Acta 857, 68–74.
2 Luria, R. and Rimon, G. (1986) Biochim. Biophys. Acta 887, 323–326.
3 Cohen-Luria, R. and Rimon, G. (1989) Second Messengers and Phosphoproteins, in press.
4 Belsham, G.J., Denton, R.M. and Tanner, J.S. (1980) Biochem. J. 192, 457–467.
5 Raz, A. (1972) Biochem. J. 130, 631–636.
6 Suzuki, F. and Kato, K. (1986) Biochim. Biophys. Acta 889, 84–90.
7 Suzuki, F., Nakajima, T. and Kato, K. (1982) J. Biochem. (Tokyo) 92, 835–838.
8 McClure, W.O. and Edelman, G.M. (1966) Biochemistry 5, 1908–1919.
9 Qasim, M.A. (1986) J. Biochem. 100, 1125–1136.
10 Fitzpatrick, F.A., Ligget, W.F. and Wynalda, M.A. (1984) J. Biol. Chem. 259, 2722–2727.
11 Bass, N.M. and Manning, J.A. (1986) Biochem. Biophys. Res. Commun. 137, 929–935.
12 Haq, R.U., Christodoulides, L., Ketterer, B. and Shrago, E. (1982) Biochim. Biophys. Acta 713, 193–198.
13 Bass, N.M., Manning, J.A. and Ockner, R.K. (1985) J. Biol. Chem. 260, 9603–9607.
14 Fournier, N.C. and Rahim, M. (1985) Biochemistry 24, 2387–2396.
15 Dutta-Roy, A.K., Gopalswamy, N. and Trulzsch, D.V. (1987) Eur. J. Biochem. 162, 615–619.
16 Raza, H., Pongubala, J.R. and Sorof, S. (1989) Biochem. Biophys. Res. Commun. 161, 448–455.
17 Burnett, D.A., Lysenko, N., Manning, J.A. and Ockner, R.K. (1979) Gastroenterology 77, 241–249.
18 Rhoads, D.E., Ockner, R.K., Peterson, N.A. and Raghupathy, E. (1983) Biochemistry 22, 1965–1970.
19 Gibbs, J.B., Schaber, M.D., Allard, W.J., Sigal, I.S. and Scolnick, E.M. (1988) Biochemistry 85, 5026–5030.
20 Trahey, M. and McCormick, F. (1987) Science 238, 542–545.
21 Sigal, I.S. (1988) Nature 332, 485–486.