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Exposure of latent prostaglandin-binding sites in the rat epididymal adipocyte membrane

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The addition of albumin to a suspension of plasma membranes isolated from rat epididymal fat cells increases both the initial binding rate and the maximal specific binding of prostaglandin E_2 to these membranes. The presence of albumin affects neither the dissociation rate of bound prostaglandin E_2 nor its dissociation constant. The results indicate that the interaction of albumin with the isolated membranes exposes latent prostaglandin-binding sites. Albumin fails to alter the specific binding of prostaglandin E_2 to membranes isolated from frog erythrocytes or from rat peritoneal fat cells. These results indicate that the effect of albumin on the specific binding of prostaglandin E_2 characterizes a specific molecular property of the prostaglandin receptors in rat epididymal adipocytes.

Introduction

Prostaglandins E_1 and E_2 possess antilipolytic activity in rat [1–3] and human [4] adipocytes. The effect is mediated by occupation of specific prostaglandin receptors which are embedded in the adipocyte membrane [5–7]. Measurements of adenylate cyclase activity in isolated adipocyte membranes show that prostaglandins E_1 and E_2 inhibit the rate of cyclic AMP production [8,9]. Study of the cyclic AMP levels and the lipolysis in intact adipocytes demonstrates that the increase in the prostaglandin binding is concomitant both with a reduction in the cyclic AMP levels within the cell and with an inhibition of lipolytic activity [10,11]. As was shown for other inhibitory receptors of the adenylate cyclase system, e.g., adenosine receptor in fat cells [12] or noradrenaline receptor in platelets [13], the prostaglandin receptors are coupled to the adenylate cyclase catalytic unit via the inhibitory regulatory component of the adenylate cyclase system (Gi).

In the present work, it is demonstrated for the first time that albumin, at concentrations which are usually used in experiments with isolated adipocytes [9,14,15], increases the specific binding of prostaglandin E_2 to isolated epididymal adipocyte plasma membranes. Since albumin itself has a low affinity to prostaglandins [16,17], it is expected that it will decrease rather than increase the specific binding of prostaglandin E_2 to the isolated membranes. Further inspection of the phenomenon shows that albumin increases the binding capacity of the isolated epididymal adipocyte membranes by exposure of latent prostaglandin-binding sites. Possible implications of this finding, with reference to the regulation of the prostaglandin receptor, are discussed.

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Materials and Methods

Materials. Bovine serum albumin was either ultrapure globulin-free, or 96–99% fraction V purchased from Sigma; both gave the same results. [^{3}H]Prostaglandin E_2 (spec. act. 160 Ci/mmol), 99% pure, was obtained from New England Nuclear. Synthetic prostaglandin E_2 was a gift from Dr. J. Pike, Upjohn, Kalamazoo. Percoll was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). All other chemicals were of the highest purity available.

Source of tissue. Fat pads were obtained from male albino Charles River rats (body wt. 200–250 g) with free access to water and stock laboratory diet.

Preparation of epididymal adipocyte membranes. Plasma membranes were prepared according to Belsham et al. [18]. Pads from the epididymis of two rats were immediately homogenized with Polytron PT20 homogenizer at position 3 for 4 s in 8 ml extraction medium at 0°C. The extraction medium comprised 0.25 M sucrose/10 mM Tris-HCl/2 mM EGTA/20 mM NaF (pH 7.4). The crude extract was centrifuged at $1000 \times g$ for 30 s. The infranatant was removed by syringe from between the fat plug and the cell debris and centrifuged 30 min at $30000 \times g$. The pellet was suspended in 6.9 ml of extraction medium and mixed with 1.4 ml of Percoll and 0.2 ml of solution which comprised 2 M sucrose/80 mM Tris-HCl/8 mM EGTA (pH 7.4). The mixture was centrifuged in a fixed-angle rotor at $10000 \times g$ for 15 min. After centrifugation, the plasma membrane band below the surface was taken by automatic pipette and washed from the Percoll mixture by further centrifugation of 30 min at $30000 \times g$ in Krebs-Ringer. The pellet was suspended in Krebs buffer to a final concentration of about 1–2 mg membrane protein/ml. All the operations were carried out at 0–4°C.

Preparation of peritoneal fat cell membranes. Peritoneal fat tissue was collected from around the kidneys. The subsequent steps of membrane isolation were performed exactly as described above.

Preparation of frog erythrocyte membranes. The membranes were obtained from Jumbo grass frogs according to Mukherjee et al. [19]. The intact erythrocytes were washed three times with a cold solution of 110 mM NaCl/10 mM Tris-HCl (pH 7.4) lysed in 5 mM Tris-HCl buffer (pH 8.1) and centrifuged at $30000 \times g$ for 15 min. The lysis was repeated three times. The membranes were suspended by homogenization in 50 mM Tris-HCl/15 mM $MgCl_2$ (pH 7.4) with or without 1% albumin.

Binding assay. The isolated membranes were incubated with 10^{-8} M of [^{3}H]prostaglandin E_2 for 45 min at 37°C in Krebs-Ringer buffer at a final volume of 120 µl in the presence or the absence of 1% albumin. At the end of the incubation, the membranes were separated from the suspension by filtering the samples through Whatman GF/C filters [20] immediately after the addition of 4 ml cold buffer. After two additional washes of the filters with 4 ml cold buffer, the filters were transferred to 10 ml Packard Insta-gel scintillation fluid. Radioactivity was counted in a Packard Model 3330 liquid-scintillation counter. In order to eliminate any possible effects of the albumin presence in the incubation medium on the filtration process, 4 ml cold buffer containing an equivalent amount of albumin was added before the filtration step to the albumin-free incubation medium of the control samples. Specific binding was defined as the difference in the amount of [^{3}H]prostaglandin E_2 bound in the presence and in the absence of 1000-fold excess of unlabelled prostaglandin E_2. When specific binding of prostaglandin E_2 to frog erythrocyte membranes was measured, 50 mM Tris-HCl (pH 7.4) [20] was substituted for the Krebs buffer. All the remaining details of the binding were as described above.

Dissociation measurements. In order to test the effect of albumin on the dissociation rate of [^{3}H]prostaglandin E_2 which was bound to the adipocyte membranes in the presence or absence of albumin, the experiments were conducted with two groups of membrane samples. Membrane samples of the first group were incubated with [^{3}H]prostaglandin E_2 in the presence of albumin. Samples of the second group were incubated with [^{3}H]prostaglandin E_2 in the absence of albumin. At the end of the incubation, all the samples were washed by further centrifugation ($30000 \times g$) with cold Krebs buffer. Half of the samples of each group were resuspended in Krebs buffer containing albumin and the other half were resuspended in Krebs buffer without albumin. Dissoci-

ation of bound prostaglandin E_2 was initiated [21] by the addition of prostaglandin E_2 to each test-tube, which yielded a final concentration of 10^{-5} M of unlabelled prostaglandin E_2. The dissociation process was terminated at successive times by the addition of 4 ml ice-cold buffer and filtration, as described above. All the experiments were carried out with at least four membrane preparations and each assay was run in triplicate.

Results

The effect of albumin on prostaglandin E_2 binding to isolated epididymal adipocyte membranes

Inspection of the time dependence of prostaglandin E_2 binding to adipocyte plasma membranes in a saturating concentration of prostaglandin E_2 reveals that the presence of albumin in the medium increases both the initial binding rate and the maximal binding level by approx. 2-fold (Fig. 1). Adipocyte plasma membranes, the specific binding of which was eliminated by preheating (15 min at 80°C) or by long storage (30 days at −20°C), restored neither the prostaglandin E_2 specific binding nor the effect of albumin. Measurements of the time-dependent dissociation of bound [^{3}H]prostaglandin E_2 (Fig. 2), initiated by the addition of 1000-fold excess of unlabelled prostaglandin (10^{-5} M), clearly show that the presence of albumin in the medium has no effect on the release of the bound prostaglandin from its binding site.

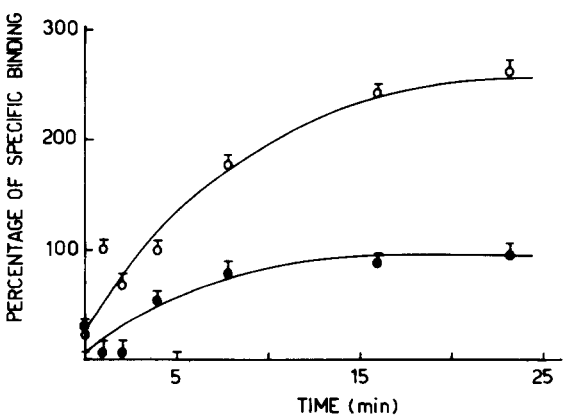

Fig. 1. Time dependence of the specific binding of prostaglandin E_2 at saturating concentration (10^{-8} M) to isolated rat epididymal adipocyte membranes in the presence (○) or absence (●) of 1% albumin in the incubation medium. Maximal binding in the absence of albumin is expressed as 100%. The bars represent the S.E. of triplicate determinations.

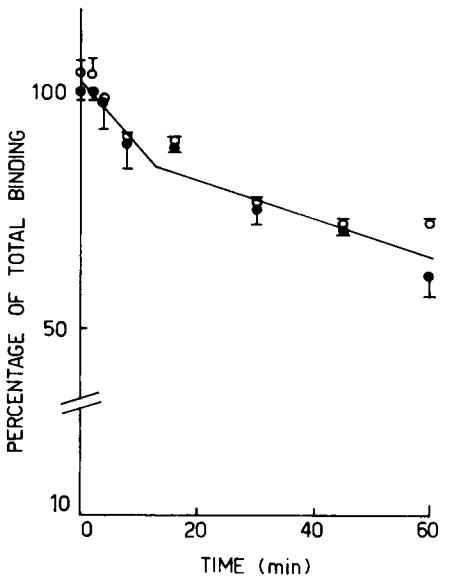

Fig. 2. The dissociation time dependence of [^{3}H]prostaglandin E_2 bound to isolated epididymal adipocyte membranes in the presence (○) or absence (●) of 1% albumin in the incubation medium. The dissociation of [^{3}H]prostaglandin E_2 was initiated by the addition of prostaglandin E_2 (10^{-5} M) as described in Materials and Methods. The bars represent the S.E. of triplicate determinations.

No effect of albumin on the dissociation process was observed when the [^{3}H]prostaglandin E_2 binding was performed in the presence of albumin. The dissociation half-times of [^{3}H]prostaglandin E_2 which was bound in the absence or presence of albumin were found to be identical (70 ± 5 min, not shown). The above results indicate that albumin interferes only with the binding process of prostaglandin but does not affect the dissociation process of the bound prostaglandin. Scatchard analysis of the specific binding of [^{3}H]prostaglandin E_2 as a function of its concentration (Fig. 3) demonstrates that in the presence of 1% albumin the number of prostaglandin-binding sites increases by a factor of 1.7, while the dissociation constant is insignificantly reduced from $2 \cdot 10^{-8}$ to $1.36 \cdot 10^{-8}$ M^{-1}.

Both the initial binding rate and the final level of specific binding are linearily proportional to the number of binding sites, whereas the dissocia-

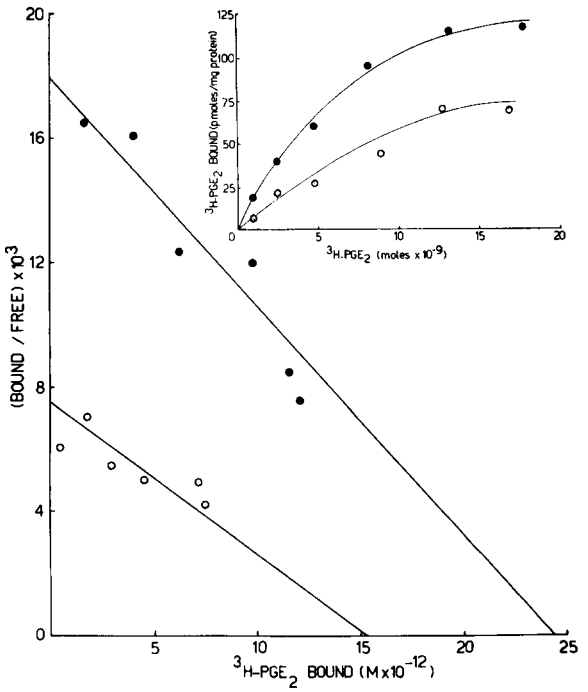

Fig. 3. Scatchard analysis of [^{3}H]prostaglandin E_2 specific binding to isolated rat epididymal adipocyte membranes in the presence (●) or absence (○) of 1% albumin in the medium. Inset: concentration dependence of [^{3}H]prostaglandin E_2 binding in the presence (●) or absence (○) of 1% albumin in the incubation medium. Each point is the mean of triplicate determinations.

tion rate is independent of the number of binding sites and is correlated only to the dissociation constant [22]. It is thus expected that albumin, which increases the number of binding sites without a significant alteration in the dissociation constant, will enhance the initial binding rate without affecting the release of the bound prostaglandin E_2.

Dependence of the prostaglandin E_2 binding capacity on the albumin concentration

An increase in the albumin concentration of up to 2% in the presence of a saturating concentration of [^{3}H]prostaglandin E_2 (Fig. 4) results in a gradual approach of the specific binding towards a plateau level, as would be expected when only a limited number of prostaglandin binding sites are exposed.

Effect of albumin on the inactivation of [^{3}H]prostaglandin E_2 binding

Preincubation of the isolated epididymal adipocyte membranes at 37°C before the initiation of the binding experiment results in a slow time-dependent decrease of [^{3}H]prostaglandin E_2 specific binding. Comparison of the time-dependent inactivation curves (Fig. 5) of adipocyte membranes that were preincubated for 10, 20 and 35 min in Krebs buffer at 37°C in the absence or the presence of albumin shows that the presence of albumin during the preincubation period does not affect the decay rate of the prostaglandin E_2 specific binding. The identity of the inactivation rates in the absence or presence of albumin suggests that the exposed prostaglandin binding sites are similar to the preexisting binding sites, not only in their affinity to prostaglandin E_2 but also in their denaturation properties.

The effect of albumin on the specific binding of [^{3}H]prostaglandin E_2 to membranes isolated from frog erythrocytes and rat peritoneal adipocytes

In order to find out whether the increase in prostaglandin E_2 binding capacity is specific to the epididymal adipocyte membrane or whether it is a more general one, the effect of albumin on prostaglandin E_2 binding to membranes isolated

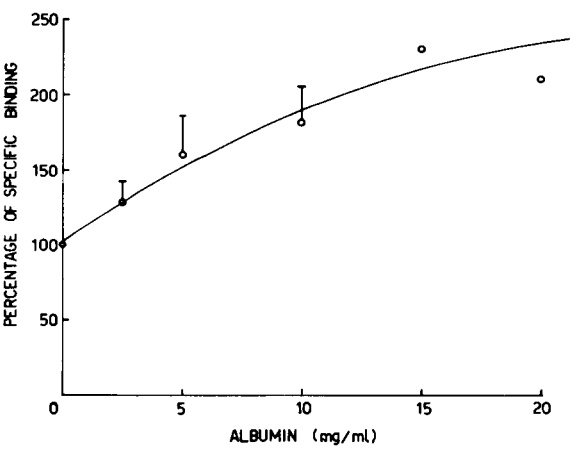

Fig. 4. Specific binding of [^{3}H]prostaglandin E_2 at saturating concentration (10^{-8} M) to isolated rat adipocyte membranes as a function of albumin concentration in the incubation medium. Maximal binding of [^{3}H]prostaglandin E_2 in the absence of albumin is represented as 100% of binding. The bars represent the S.E. of triplicate determinations.

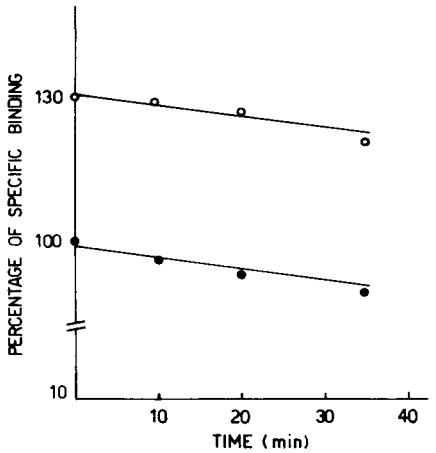

Fig. 5. The inactivation time dependence of [^{3}H]prostaglandin E_2 binding to rat adipocyte membranes. Membranes were preincubated in the absence (●) or presence (○) of 1% albumin at 37°C in Krebs buffer for 0, 10, 20 and 35 min before the initiation of [^{3}H]prostaglandin E_2 binding. Binding was performed as described in Materials and Methods. Each point is the mean of triplicate determinations.

from frog erythrocytes or rat peritoneal fat cells was tested. Inspection of the results given in the first row of Table I reveals that the presence of 1% albumin in the incubation medium decreases the total binding of prostaglandin E_2 to membranes isolated from frog erythrocytes by about 10%, but does not alter the specific binding of prostaglandin E_2. When the effect of albumin on the binding of prostaglandin E_2 to membranes isolated from rat peritoneal fat cells (second row, Table I) is tested, a reduction in the total binding without alteration in specific binding is also achieved. Since the total binding is the sum of specific and nonspecific binding, these results indicate that albumin decreases nonspecific binding in frog erythrocytes and peritoneal fat cell membrane without effect on specific binding. The results presented in the first and second rows of Table I are in accordance with those obtained by Moore and Wolf [23], who showed that bovine serum albumin had no effect on the specific binding of prostaglandin E_2 to isolated bovine thyroid membranes.

The third row in Table I demonstrates a typical increase in the specific binding of prostaglandin E_2 to epididymal adipocyte membranes due to the presence of albumin. It can be concluded that the exposure of latent prostaglandin E_2-binding sites by albumin is a specific characteristic of the prostaglandin E_2-binding sites in the epididymal rat fat cell membrane.

Discussion

The experimental results show that albumin enhances the initial binding rate of prostaglandin E_2 (Fig. 1) and increases the prostaglandin maximal binding to isolated epididymal adipocyte plasma membrane without changing the release kinetics of the bound prostaglandin (Fig. 2). These results are in accordance with the Scatchard analysis of prostaglandin E_2 binding (Fig. 3) which clearly shows that in the presence of albumin, there is an increase in the binding capacity of the isolated membranes without significant alteration in the dissociation constant. The fact that the

TABLE I

EFFECT OF ALBUMIN ON [^{3}H]PROSTAGLANDIN E_2 BINDING TO MEMBRANES ISOLATED FROM FROG ERYTHROCYTES, PERITONEAL ADIPOCYTES AND EPIDIDYMAL ADIPOCYTES

The results are given as counts ± S.E. per min/mg protein. Membrane preparation and binding measurements were conducted as described in Materials and Methods.

Source of membrane	Total binding		Specific binding	
	− albumin	+ albumin	− albumin	+ albumin
Frog erythrocytes	5330 ± 220	4600 ± 230	3750 ± 330	3400 ± 620
Peritoneal rat adipocytes	8695 ± 140	8353 ± 60	6395 ± 430	6663 ± 170
Epididymal rat adipcytes	9150 ± 180	15460 ± 90	7050 ± 230	13580 ± 22

exposure of the prostaglandin E_2-binding sites did not significantly alter the dissociation rate of the bound prostaglandin E_2, the dissociation constant or the time-dependent inactivation of prostaglandin E_2 specific binding (Fig. 5), suggests that the exposed prostaglandin E_2-binding sites are similar in their kinetic and denaturation properties to the preexisting sites. A close resemblance was also found between the equilibrium binding properties of latent membranal insulin receptors in 3T3-L1 adipoctyes and the cell surface insulin receptors [24].

Albumin failed to influence the specific binding of prostaglandin E_2 to membranes isolated from frog erythrocytes (Table I), from bovine thyroid [15] or from rat peritoneal fat cells (Table I). These findings strongly suggest that the phenomenon of the exposure of latent prostaglandin-binding sites by albumin represents a specific molecular property of the prostaglandin E_2-binding sites embedded in the rat epididymal adipocyte membrane. There are several possible mechanisms by which albumin might expose latent binding sites: one possibility is that the new exposed binding sites are not susceptible to binding by prostaglandin E_2 molecules in the solution but rather are available for interaction with prostaglandin E_2 molecules which are already bound to albumin [16,17]. Another possibility is that albumin itself exposes hidden binding sites by direct interaction with the cell membrane. Exposure of a concanavalin A-binding site as a result of a direct interaction between albumin and the concanavalin A receptors was demonstrated by Ketis and Grant [25] both in liposomes bearing reconstituted concanavalin A receptors and in intact human erythrocytes.

Although albumin probably interacts with the cell membrane via nonspecific hydrogen-bonding [26], the mechanism by which it modifies a certain membranal system might be a very specific one. An interesting example is the interaction of albumin with rod disk membranes. In these membranes, albumin inhibits the light activation of cyclic GMP phosphodiesterase by a selective elution of GTP-binding protein which mediates the interaction between the light-activated rhodopsin and the phosphodiesterase [27].

The physiological relevance of the exposure phenomenon is not clear, but if a similar process takes place in the intact cell, it is reasonable to assume that the exposure process occurs in the cytoplasmatic side of the cell membrane, where a large amount of cytoplasmatic proteins might interact with the cell membrane. Since albumin is not present in the cytoplasm, it is expected that other types of water-soluble proteins also have the ability to expose latent prostaglandin-binding sites.

The existence of a large fraction of latent binding sites in the adipocyte membrane indicates that in the intact cell a constant process of down-regulation of the prostaglandin-binding sites takes place, and as a result, a substantial potential for up-regulation exists in these cells. The phenomenon of up- and down-regulation of the prostaglandin-binding site have already been shown in rat fat cells [28] and in plasma membranes isolated from rat liver cells [29,30].

Intensive studies revealed that regulation of insulin binding to fat cells involves a transition of a precursor form [32] of the insulin receptor to a regular surface form and internalization of the surface insulin receptors [14,31]. According to these findings, the latent prostaglandin E_2-binding sites may represent a precursor form of the prostaglandin E_2 receptors or an early membranal stage of internalized prostaglandin E_2 receptors.

Acknowledgements

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