Decrease of prostaglandin E_2 receptor binding is accompanied by reduced antilipolytic effects of prostaglandin E_2 in isolated rat adipocytes

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Abstract The effect of treatment of isolated rat adipocytes with prostaglandin E_2 (PGE_2) on subsequent $[^3H]PGE_2$ binding was studied. In addition, the antilipolytic effects of PGE_2, adenosine, and insulin were studied in control and PGE_2-treated adipocytes. Treatment of adipocytes with PGE_2 (1 μM) decreased the binding of $[^3H]PGE_2$ by 61% (from 11.0 to 4.6 fmol/10^6 cells, $P < 0.005$). Scatchard analysis of the binding data demonstrated that the decrease of PGE_2 receptor binding was due to a decrease in the apparent number of PGE_2 receptors while the apparent receptor affinity was unaltered. Reduction of the PGE_2 receptor binding was specifically regulated inasmuch as structurally related compounds such as $PGF_{2\alpha}$ and arachidonic acid had only minor effects on subsequent $[^3H]PGE_2$ receptor binding. Reduction of the available receptor number was associated with a significant decrease in the antilipolytic effect of PGE_2 on the isoproterenol-stimulated lipolysis ($P < 0.05$). The maximal antilipolytic effect of PGE_2 was decreased by 45%. Desensitization of the biological effect of PGE_2 (antilipolysis) was only partially specifically regulated inasmuch as the antilipolytic compound phenylisopropyladenosine also had reduced antilipolytic effect in PGE_2-treated cells. However, the antilipolytic effect of insulin was similar in control and PGE_2-treated cells. It was found that the PGE_2-induced decrease of $[^3H]PGE_2$ receptor binding may be due to a very tight coupling between the PGE_2 molecule and its specific receptor. This tight coupling may then represent an occupancy of the receptor rather than a true loss of receptors. In conclusion, it was demonstrated that PGE_2 was able to induce a longstanding occupancy of its own receptor. This decrease of available receptors for further PGE_2 binding was associated with a desensitization of PGE_2's antilipolytic effect. These findings further emphasize the tight coupling between the receptor binding of PGE_2 and the antilipolytic effect of PGE_2.—**Richelsen, B., and H. Beck-Nielsen.** Decrease of prostaglandin E_2 receptor binding is accompanied by reduced antilipolytic effects of prostaglandin E_2 in isolated rat adipocytes. *J. Lipid Res.* 1985. **26:** 127–134.

Supplementary key words antilipolysis · receptor occupancy

In the past few years much progress has been made in studying a variety of adenylate cyclase-linked hormone receptors by direct radioligand-binding techniques (1–3). Several studies have shown that, in intact cells exposed to a stimulatory hormone, the adenylate cyclase response becomes desensitized after repeated hormonal stimulations (4–8). This desensitization is often accompanied by a decrease of the hormone binding to the cells. These phenomena have been described for hormones that stimulate the adenylate cyclase complex such as beta-adrenergic agonists and prostaglandins of the E type (7, 9–11). However, prostaglandin E_2 (PGE_2) does not stimulate the adenylate cyclase in isolated adipocytes. On the contrary, this compound has an inhibitory effect on this enzyme which results in decrease of intracellular cyclic-AMP (12, 13). Reduction of PGE_2 binding sites and desensitization of the biological effect for hormones that interact with the adenylate cyclase system in an inhibitory manner have not yet been studied extensively.

It is generally accepted that hormone-sensitive adenylate cyclase systems consist of at least three cell membrane components: a specific receptor (R), a guanine nucleotide binding regulatory component (N), and a catalytic component of adenylate cyclase (C) (7, 14). Recently, it has been demonstrated that this N-component consists of a pair of homologous guanine nucleotide binding regulatory proteins, one of which mediates stimulation (N_s) of adenylate cyclase activity, while the other (N_i) is responsible for inhibition (15, 16). Thus, in adipocytes, PGE_2 may interact with the N_i subunit of the regulatory component.

We have recently demonstrated (17) the existence of specific PGE_2 binding sites on isolated human adipo-

Abbreviations: PGE_2, prostaglandin E_2; PIA, N^6-phenylisopropyladenosine.

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cytes. Furthermore, PGE_2 had pronounced antilipolytic effects both on basal and isoproterenol-stimulated lipolysis. This effect is probably mediated through inhibition of the adenylate cyclase system (13, 18). Since the equilibrium binding constant of PGE_2 for the high affinity binding sites was 2 nM and half-maximal inhibition of the isoproterenol-stimulated lipolysis was 3.8 nM, we suggested that the binding of PGE_2 to adipocytes was closely related to the antilipolytic effect of PGE_2. In order to better understand PGE_2 receptor regulation in adipocytes, we have studied whether these binding sites can be down-regulated in vitro and, if so, whether such changes in receptor concentration have functional consequences for the antilipolytic effect of PGE_2. Since we have found that PGE_2 binding characteristics of rat adipocytes and human adipocytes were similar, we used rat adipocytes for the present study.

MATERIALS AND METHODS

Fat cell preparation

Adipose tissue was obtained from epididymal fat pads of male Wistar rats. Body weights of the animals ranged from 200 to 350 g. Adipocytes were isolated as previously described (17, 19) by collagenase digestion of fat tissue fragments in 10 mM HEPES buffer, pH 7.4, containing 2.5% bovine serum albumin (BSA) and 0.5 mg/ml of collagenase (37°C and light shaking). Isolated fat cells were obtained after 60 min of incubation. Then the adipocytes were filtered on a silk screen and washed three times with the incubation buffer and immediately used under the different assay conditions. The concentration of fat cells in adipocyte suspension was calculated as adipocyte volume fraction divided by mean adipocyte volume as previously described (17, 19).

Preincubations

Isolated adipocytes were resuspended in incubation buffer containing the following solute concentrations (mM): HEPES 10, NaCl 135, KCl 4.8, $MgSO_4$ 1.7, $CaCl_2$ 2.5, NaH_2PO_4 0.2, Na_2HPO_4 1.0, glucose 5.0, and BSA 5%. The pH was 7.4 (at 37°C). Adipocytes, in a volume fraction of 0.2, i.e., about 0.5×10^6 cells/ml, were preincubated in the presence or absence of PGE_2 at different concentrations, but routinely at a concentration of 1 μM. The incubation period was usually 50 min at 37°C. After this preincubation period adipocytes were washed four times in fresh buffer by centrifugation. The washing procedure usually took 25–30 min. In all the experiments control and PGE_2-treated cells were run in parallel.

Binding assay

Adipocytes were resuspended in incubation buffer at a volume fraction of 0.1–0.15, i.e., about $2–4 \times 10^5$ cells/ml. Binding studies were conducted in a final volume of 300 μl as previously reported (17). The incubation mixture consisted of 250 μl of adipocyte suspension, 25 μl of $[^3H]PGE_2$ in a final concentration of 0.5–3 nM, and 25 μl of buffer or unlabeled ligand for competition. The binding reaction was initiated by adding adipocytes to the incubation mixture. The binding study was carried out for 40 min at 22°C. Nonspecific binding was determined in the presence of 0.5 μM unlabeled PGE_2 and ranged from 10 to 20% of the total binding at a $[^3H]PGE_2$ concentration of 1 nM. Specific binding was taken as the total amount of radioactivity bound to the adipocytes minus nonspecific binding. The binding reaction was terminated as previously described by adding 9.75 ml of ice-cold saline to the incubation vessels followed by 1 ml of silicone oil (17, 19, 20). After centrifugation for 1 min at 2500 g, the fat cells were harvested from the oil layer. The fat cells were placed in 5 ml of scintillation fluid (Lipoluma, Lumac BV, Netherlands) and counted in a Rackbeta counter (LKB, Finland) with stored quench calibration curves and automatic dpm calculation.

Lipolysis and antilipolysis

Adipocytes were resuspended in incubation buffer at a volume fraction of 0.20, i.e., about 5×10^5 cells/ml. In the study of the lypolytic effect of isoproterenol, 200 μl of adipocyte suspension was incubated with isoproterenol in increasing concentrations for 90 min at 37°C. Concerning the antilipolytic effect of PGE_2, 200 μl of adipocyte suspension was incubated with PGE_2 in increasing concentration for 30 min at 37°C. Then isoproterenol was added in a final concentration of 5 μM and the incubation was continued for another 60 min. Glycerol release was taken as an index of lipolysis. All the experiments were performed in duplicate. Glycerol release was determined enzymatically (Peridochrom, Boehringer Mannheim) by a fluorometric method (21).

Materials

$[^3H]$Prostaglandin E_2 (sp act 140–170 Ci/mmol) was from Radiochemical Center, Amersham. Collagenase from *Clostridium histolyticum*, bovine serum albumin, prostaglandin E_2, prostaglandin $F_{2\alpha}$, arachidonic acid, adenosine deaminase, and phenylisopropyladenosine were from Sigma Chemical Co. (St. Louis, MO). Silicone oil 510/50, relative density 0.99, was from Dow Corning Cooperation (Midland, MI). Insulin was obtained from NOVO Research Institute (Copenhagen, Denmark).

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Statistics

Data in the text are given as mean ± 1 SD; data in figures and tables are given as mean ± 1 SEM. In comparative studies Student's paired *t*-test was used.

RESULTS

Binding studies

Binding of $[^3H]PGE_2$ to rat adipocytes was very similar to the binding data obtained in isolated human adipocytes (17). Steady state binding was achieved after incubation for 20 min at 22°C and was then constant for more than 1 hr (data not shown). In the present study control and PGE_2-treated rat adipocytes were run in parallel incubations. After preincubation in the presence and absence of unlabeled PGE_2 for 50 min at 37°C, adipocytes were washed vigorously four times by centrifugation. The subsequent ability of adipocytes to bind $[^3H]PGE_2$ was then determined. When adipocytes were preincubated with PGE_2 at a concentration of 1 μM, the specific binding of $[^3H]PGE_2$ was decreased by 62 ± 12%, from 11.0 ± 2 fmol/10^6 cells in controls to 4.6 ± 1.6 fmol/10^6 cells in PGE_2-treated cells ($P < 0.005$) (**Fig. 1A**). Nonspecific binding was similar in control and treated adipocytes. The PGE_2-induced decrease of $[^3H]PGE_2$ binding could occur through a decrease in receptor number and/or in receptor affinity. To evaluate these possibilities, competition binding curves for controls and PGE_2-treated cells were constructed (Fig. 1A). Scatchard analysis of the data revealed two straight lines that had different x-axis intercepts on the abcissa (B_{max}) but equal slopes (Fig. 1B), indicating the same affinities but different concentrations of receptors. In these experiments the apparent receptor concentration was decreased from 29 fmol/10^6 cells to 14 fmol/10^6 cells and the apparent affinities were similar in control and PGE-treated cells (K_D = 1.2 nM and 1.7 nM, respectively). This decrease of $[^3H]PGE_2$ binding to adipocytes was already observed after incubation with unlabeled PGE_2 for 10 min. The maximal decrease of binding was obtained after incubation for 40–60 min (data not shown).

The effect of PGE_2, in increasing concentrations, on subsequent binding of $[^3H]PGE_2$ is shown in **Fig. 2.** A dose-dependent decline in receptor binding was observed and half-maximal decrease in receptor binding was reached at a PGE_2 concentration of 3.5 nM.

Reduction of available PGE_2 receptors seemed to be a specific effect of preincubation with PGE_2, inasmuch as structurally related compounds such as $PGF_{2\alpha}$ and arachidonic acid had only minimal effects on $[^3H]PGE_2$ binding (**Fig. 3**). Preincubation with $PGF_{2\alpha}$ and arachidonic acid at a concentration of 1 μM decreased the subsequent PGE_2 binding by 10–15%. Before the adipocytes were washed, $PGF_{2\alpha}$ and arachidonic acid at these concentrations (1 μM) inhibited the

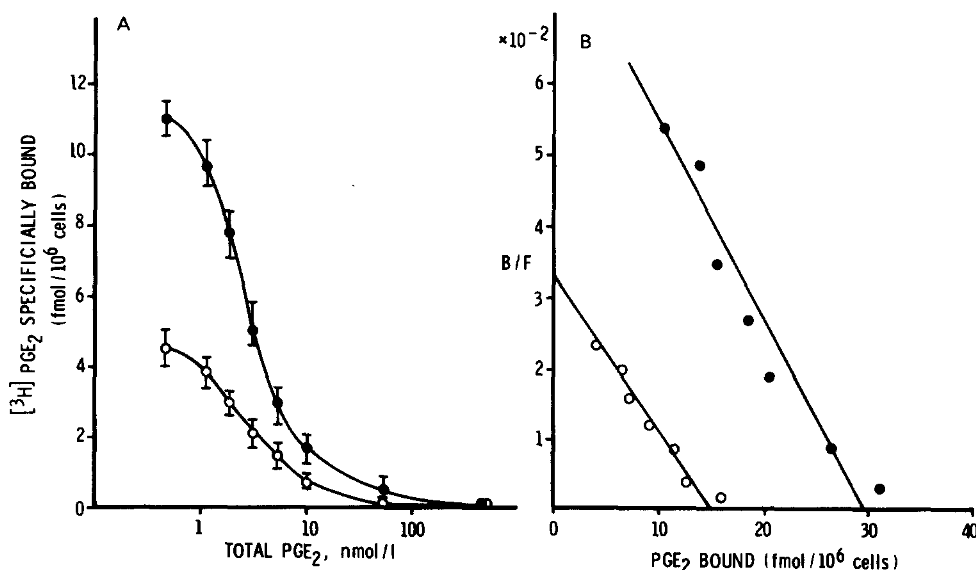

Fig. 1 Specific binding of $[^3H]PGE_2$ to control (●) and PGE_2-treated (○) rat adipocytes. Panel A: Competition curves of unlabeled PGE_2 with $[^3H]PGE_2$ in rat adipocytes. Rat adipocytes were incubated in the presence and absence of PGE_2 (1 μM) for 50 min at 37°C. After the washing procedure the binding study was performed with $[^3H]PGE_2$ (0.6 nM) and unlabeled PGE_2 for 40 min at 22°C. Data are expressed as specifically bound $[^3H]PGE_2$ to adipocytes in fmol per 10^6 cells and are given as mean ± SEM for five paired experiments. Panel B: Using the Scatchard plot (obtained from the competition data in panel A), the following equilibrium binding constants (K_D) and total binding capacities (B_{max}) for control and PGE_2-treated cells were: K_D, 1.2 nM and 1.7 nM; B_{max}, 29 fmol/10^6 cells and 14 fmol/10^6 cells, respectively.

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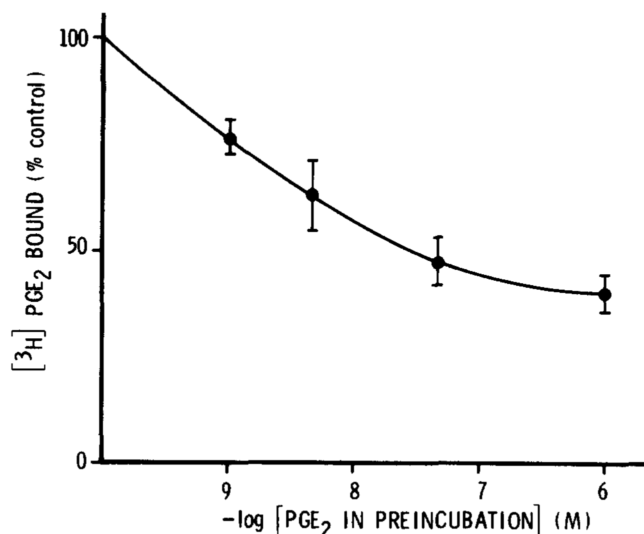

Fig. 2 Concentration dependence of PGE_2-induced decrease in [^{3}H]PGE_2 binding in rat adipocytes. Adipocytes were incubated with unlabeled PGE_2 in different concentrations for 50 min at 37°C followed by four washes. The subsequent [^{3}H]PGE_2 binding to fat cells was measured as described in Materials and Methods. The concentration of [^{3}H]PGE_2 was 1 nM. Data are expressed as mean ± SEM for three experiments performed in duplicate.

PGE_2 receptor binding by 60% and 30%, respectively (data not shown).

To characterize further this process of interaction between PGE_2 and its receptor, adipocytes were preincubated with [^{3}H]PGE_2 for 50 min at 24°C. Adipocytes were then washed as previously described both in the presence and absence of unlabeled PGE_2 (1 μM) in the washing medium. Unlabeled PGE_2 was included in the medium to accelerate the dissociation of [^{3}H]PGE_2. [^{3}H]PGE_2 binding to adipocytes before and after washing is shown in **Table 1**. It was found that vigorous washing for 30 min only removed about 50% of adipocyte-bound radioactivity when a concentration of 3 nM [^{3}H]PGE_2 was used. Unlabeled PGE_2 in the washing medium did not significantly reduce the residual amount of bound radioactivity.

Lipolysis studies

Studies were also performed to determine whether this PGE_2-induced decrease in available PGE_2 receptors was related to parallel changes in the biological effect of PGE_2. Thus, the lipolytic effect of isoproterenol and the antilipolytic effect of PGE_2 and other compounds were examined in control and PGE_2-treated adipocytes. Isoproterenol stimulated glycerol release in a dose-dependent manner both in control and PGE_2-treated cells (**Fig. 4**). Half-maximal stimulatory effect of isoproterenol was reached at a concentration of 100 nM in control cells and at 320 nM in PGE_2-treated cells, indicating a reduced sensitivity of isoproterenol in PGE_2-treated cells. However, the maximal lipolytic response of isoproterenol was similar in both groups [125% (control) and 118% (treated) above basal glycerol release]. To examine the antilipolytic effect of PGE_2, isoproterenol at a concentration of 5 μM was used to stimulate lipolysis. This concentration of isoproterenol was chosen because it resulted in maximal lipolytic response in both control and PGE_2-treated cells (Fig. 4).

The antilipolytic effect of PGE_2 on isoproterenol-stimulated lipolysis was significantly reduced in PGE_2-treated cells (**Fig. 5**). Basal lipolysis was decreased in PGE_2-treated cells compared to controls, from 312 ± 45 to 290 ± 44 nM glycerol per 10^6 cells per 90 min ($P < 0.05$). Maximal inhibition of PGE_2 on isoproterenol-stimulated lipolysis was reduced from an inhibition of 61 ± 16% in controls to an inhibition of 34 ± 12% in treated cells ($P < 0.01$). Hence, preincubation of adipocytes with PGE_2 (1 μM) resulted in a decrease of the antilipolytic effect of PGE_2 by about 45%. Contrary to the decreased maximal responsiveness of PGE_2 in treated adipocytes, the sensitivity of PGE_2, expressed as half-maximal inhibition (IC_{50}), was similar in the two groups [IC_{50} (control) = 0.7 nM vs. IC_{50} (treated) = 1.2 nM, $P > 0.05$].

Furthermore, to examine whether this reduced antilipolysis was a specific effect of PGE_2, we studied the antilipolytic effect of such unrelated compounds as insulin and N^6-phenylisopropyladenosine (PIA, a non-metabolizable analogue of adenosine) in control and PGE_2-treated cells. PIA, like PGE_2, inhibits lipolysis through a decrease of intracellular cyclic-AMP (22) and this effect is mediated via binding to specific adenosine

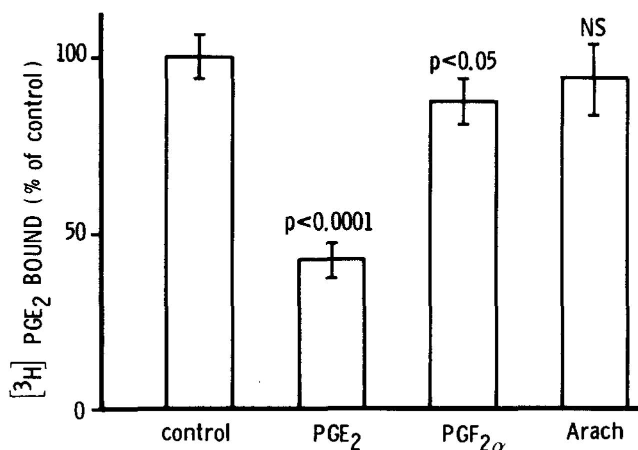

Fig. 3 Specificity of prostaglandin-induced reduction of PGE_2 binding sites in rat adipocytes. Adipocytes were incubated with PGE_2 (1 μM), $PGF_{2\alpha}$ (1 μM), and arachidonic acid (1 μM) for 50 min at 37°C. Control cells were incubated without added prostaglandins. After the washing procedure the binding of [^{3}H]PGE_2 was measured as described in Materials and Methods. Data are expressed as mean ± SEM for five experiments made in parallel.

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TABLE 1. Binding of [^{3}H]PGE$_2$ to rat adipocytes before and after wash

	[^{3}H]PGE$_2$ Bound before Wash	[^{3}H]PGE$_2$ Bound after Wash	[^{3}H]PGE$_2$ Bound after Wash with PGE$_2$ (1 μM)
		fmol/10^6 cells	
Additions			
[^{3}H]PGE$_2$ (0.5 nM)	10.3 ± 2.1 (100%)	3.4 ± 1.0 (33%)	3.0 ± 0.8 (29%)
[^{3}H]PGE$_2$ (3.0 nM)	23.3 ± 1.9 (100%)	11.5 ± 2.0 (49%)	9.3 ± 2.2 (40%)

Adipocytes were incubated with [^{3}H]PGE$_2$ in the indicated concentrations for 50 min at 24°C. After the adipocyte-bound [^{3}H]PGE$_2$ was determined, the remaining adipocytes were washed in the presence or absence of unlabeled PGE$_2$ (1 μM). Then residual [^{3}H]PGE$_2$ binding to the adipocytes was measured. Data are expressed as mean ± SEM for three experiments.

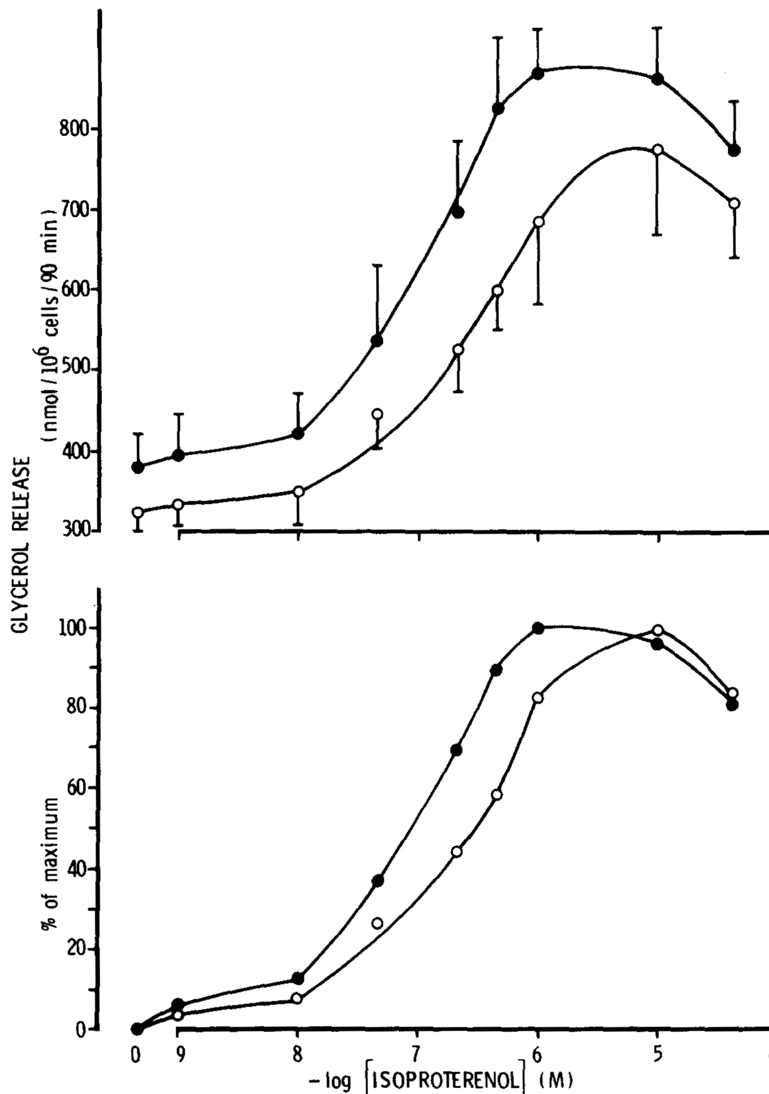

Fig. 4 Upper panel: Concentration dependence of isoproterenol-induced glycerol release in control (●) and PGE$_2$-treated (○) rat adipocytes. Incubations with adipocytes were carried out in the presence and absence of PGE$_2$ for 50 min at 37°C. After the washing procedure adipocytes were incubated with isoproterenol in increasing concentrations for 90 min at 37°C. Data are expressed as mean ± SEM for three paired experiments performed in duplicate. Lower panel: Data are expressed as percentage of the maximal response of isoproterenol.

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receptors (23). Which mediators are responsible for the antilipolytic effect of insulin is still controversial but cyclic-AMP does not seem to be directly involved (24–26). As shown in **Table 2** we found decreased antilipolytic effects of PIA in PGE_2-treated fat cells ($P < 0.05$). However, the antilipolytic effect of insulin was similar in the two groups. The highest concentration of insulin and PIA used in these experiments was expected to result in maximal antilipolytic effect of these compounds.

DISCUSSION

The present report demonstrates that preincubation with PGE_2 was able to induce a decline of the receptor binding in rat adipocytes assayed by $[^3H]PGE_2$ binding techniques. Scatchard analysis of the specific binding data revealed a decrease in the number of PGE_2 receptors by about 60% in PGE_2-treated cells. This decrease of binding sites was accompanied by a decrease of the maximal antilipolytic of PGE_2 by about 45%; however, the antilipolytic effect of PIA was also reduced (Table 2). Nonspecific binding was approximately the same in both groups. We have recently demonstrated (17) that PGE_2 binds to specific receptors on isolated human adipocytes with an equilibrium binding constant of 2 nM and that PGE_2 also has strong antilipolytic effects in these cells with an IC_{50} value of 3.8 nM on isoproterenol-stimulated lipolysis. These data, together with our present results, even though they have been obtained in rat adipocytes, further indicate that the PGE_2 receptor binding to adipocytes is closely related to the antilipolytic effect of PGE_2. Compared with our data obtained in human adipocytes, there was a reduced number (B_{max}) of PGE_2 binding sites on rat adipocytes (58 fmol/10^6 cells in humans vs. 29 fmol/10^6 cells in rats). However, if the binding was expressed per cm^2 cell surface, the total binding capacity was similar (19.3 fmol/100 cm^2 vs. 20.7 fmol/100 cm^2, mean surface of rat and human adipocytes was $15 \times 10^{-9}m^2$ and $28 \times 10^{-9}m^2$, respectively). The equilibrium binding constants were also similar in rat and human adipocytes with K_D = 1.2 nM and K_D = 2 nM, respectively. However, maximal antilipolytic effect of PGE_2 in human adipocytes was about 90% of the isoproterenol-stimulated lipolysis and in rat adipocytes maximal antilipolytic effect was only about 50% under the same experimental conditions. From these comparisons it is obvious that PGE_2 has a more pronounced antilipolytic effect in human adipocytes than in rat adipocytes, even though the binding data are similar.

When rat adipocytes were preincubated with radioactive PGE_2 and then washed, the residual radioactivity bound to the adipocytes was present in an amount of about 50% of the originally bound radioactivity (Table 2). This residual radioactivity bound to the adipocytes agreed very well with the reduction of available binding sites induced by preincubation with unlabeled PGE_2 (about 60%). Thus, the loss of $[^3H]PGE_2$ binding sites after pretreatment with PGE_2 probably represents an occupancy of the binding sites with PGE_2 rather than a true loss of binding sites.

From these observations, together with our previous study in human adipocytes (17), it seems reasonable to assume that about 50% of the PGE_2 binding is readily reversible and the rest of the binding (50%) is very slowly reversible. That the "down-regulated" receptors are occupied by very tightly bound PGE_2 molecules is

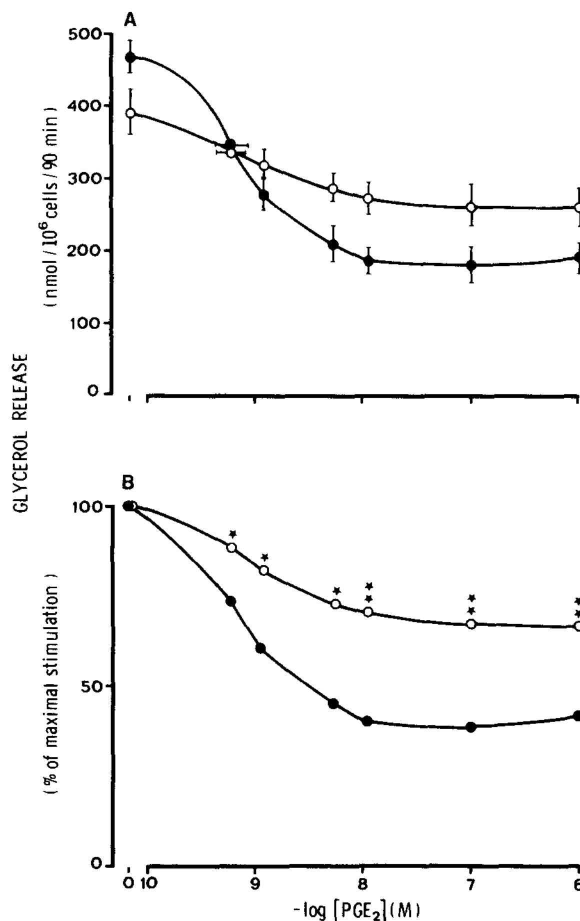

Fig. 5 Antilipolytic effect of PGE_2 in control (●) and PGE_2-treated (○) rat adipocytes. Adipocytes were preincubated with and without PGE_2 and washed as previously described. The adipocytes were then incubated with PGE_2 in increasing concentration for 90 min at 37°C. Isoproterenol (5 μM) was added for the last 60 min of the incubation to stimulate lipolysis. Basal lipolysis in control and PGE_2-treated cells was 312 ± 45 and 290 ± 44 nM glycerol per 10^6 cells per 90 min, respectively. Lipolysis is expressed as isoproterenol-stimulated glycerol release minus basal glycerol release. Data are given as mean ± SEM for five paired experiments (A). In panel B, data for the antilipolytic effect of PGE_2 are given as percent inhibition of maximal stimulated lipolysis for each group. *$P < 0.05$; **$P < 0.01$.

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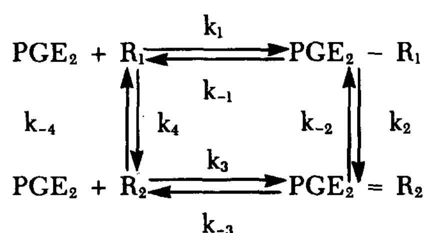
TABLE 2. Antilipolytic effect of insulin and phenylisopropyladenosine (PIA) in control and PGE_2-treated rat adipocytes

	Control Cells	PGE_2-Treated Cells	Statistics
	% of maximal lipolysis		
Additions			
None	100	100	
Insulin (80 pmol/l)	80.8 ± 4.1	80 ± 4.4	NS
Insulin (640 pmol/l)	56.6 ± 7.5	62.5 ± 5.3	NS
PIA (0.1 μmol/l)	55.8 ± 8.2	66.7 ± 6.4	$P < 0.05$
PIA (50 μmol/l)	45.8 ± 8.5	60.2 ± 5.8	$P < 0.05$

Adipocytes were incubated with and without PGE_2 (1 μM). After washing, adipocytes were then incubated with adenosine deaminase (1 μg/ml) and insulin or PIA in the indicated concentrations for 90 min. Isoproterenol (5 μM) was added for the last 60 min of this incubation to stimulate lipolysis. Basal lipolysis values in control and PGE_2-treated cells were 310 ± 44 and 292 ± 44 nM glycerol per 10^6 cells per 90 min, respectively. Isoproterenol-stimulated lipolysis values were 645 ± 130 and 538 ± 80 nM glycerol per 10^6 cells per 90 min, respectively. Data are expressed as percentage inhibition of the isoproterenol-stimulated lipolysis, mean ± SEM for five paired experiments. NS, not significant.

consistent with previous observations in frog erythrocytes (9), in cultured cell membranes (2), and in human adipocytes (17). The molecular basis for this densensitization or "down-regulation" phenomenon has been proposed by Brunton et al. (2) and Lefkowitz et al. (9) by the following scheme:



PGE_2 binds to the receptor (R_1) to form the ligand-receptor complex ($PGE_2 - R_1$) which is readily reversible. This complex is then more slowly changed into a stable ligand-receptor complex ($PGE_2 = R_2$) which has a very slow dissociation constant (k_{-2} or k_{-3}). This complex presumably represents the desensitized or "down-regulated" receptor. However, some extent of internalization of the ligand-receptor complex could not be excluded from the present study.

Robertson et al. (11) have also studied down-regulation and desensitization of PGE receptors. They induced down-regulation of the receptor in vivo in rat hepatocytes by a 16,16-dimethyl analogue of PGE_2. The PGE receptor binding was decreased by 37% without any change of the affinity. This down-regulation was followed by a significant decrease of the PGE-stimulated adenylate cyclase activity. Taking into consideration the observation that PGE stimulates cyclic-AMP in hepatocytes and inhibits cyclic-AMP in adipocytes, these observations are in reasonable agreement with our findings. However, the down-regulation induced in hepatocyte membranes in vivo was found to be caused by a true loss of PGE receptors, since the amount of extracted PGE from control and treated membranes was similar in the two groups. This is in contrast to our findings in vitro in intact rat adipocytes. We found that the decrease of available PGE_2 receptors presumably was due to a very tight binding between the receptor and the PGE_2 molecule. Since the experimental conditions were so different in the two studies, it is difficult to explain the discrepancy.

The reduction of the PGE_2 binding seemed to be a specific effect of PGE_2 inasmuch as structurally related compounds such as $PGF_{2\alpha}$ and arachidonic acid had only minor effects on the subsequent PGE_2 binding (Fig. 3). PGE_2-induced desensitization of the antilipolysis was only partially specifically regulated. Insulin had similar antilipolytic effects in control and PGE_2-treated cells but the antilipolytic effect of PIA was significantly reduced in PGE_2-treated cells (Table 2). Furthermore, basal lipolysis was reduced and the sensitivity of isoproterenol-stimulated glycerol release was decreased in PGE_2-treated cells (Fig. 4). These findings indicate that pretreatment with PGE_2 in some way may desensitize the adenylate cyclase system in a more heterogenous manner both for stimulatory (isoproterenol) and inhibitory (PIA, PGE_2) compounds. Clark and Butcher (4) and Kassis and Fishman (8) have reported similar findings concerning the effect of exposure to PGE on the subsequent stimulation of cyclic-AMP in fibroblasts. They proposed that the heterogenous desensitization of the adenylate cyclase system induced by PGE involved an alteration of the nucleotide-binding protein (N) which resulted in less efficient coupling between the receptor (stimulatory or inhibitory) and the catalytic component (adenylate cyclase).

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In conclusion, the present study demonstrated that a decrease in the number of functional PGE_2 receptors in rat adipocytes resulted in a concomitant decrease of the maximal antilipolytic effect of PGE_2. However, the antilipolytic effect of PIA was also reduced. The tight coupling between the molecule and the PGE_2 receptor seemed to be a specific effect of PGE_2, whereas desensitization of the antilipolysis was heterogenously regulated. Thus, PGE_2 may play a role in the modulation of adenylate cyclase-mediated pathways (e.g., lipolysis) by regulation of available cell surface PGE_2 receptors and, presumably, by regulation of nucleotide-binding protein (N). ■

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