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Relationship between binding and action of different prostaglandins in rat adipocytes with special reference to PGE_2 and PGI_2

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Prostaglandins (PGs) of the E-series (PGE_1 and PGE_2) have previously been shown to be potent inhibitors of stimulated cAMP accumulation and lipolysis both in rat [1–3] and human adipocytes [4–5]. These effects of PGEs are initiated by a binding reaction between PGE and a PG receptor located on the plasma membrane of the adipocytes [2–4, 6].

Recent studies by Axelrod *et al.* [7, 8] indicate that the only PGs that are produced in considerably sufficient amounts in rat adipocytes are PGE_2 and prostacyclin (PGI_2). In these studies and from our own study (to be published) it is shown that in adipocytes PGI_2 actually is produced in 2–4 times higher amounts than PGE_2. Although PGI_2 is produced in excess of PGE_2 the effect of PGI_2 on adipocyte metabolism has been much less studied. It has recently been shown that PGI_2 may have biphasic effects on the adenylate cyclase (AC) activity [9] and on lipolysis [10]. At low concentrations (nanomolar) PGI_2 enhanced the cAMP and the lipolytic responses. Whereas at higher concentrations (micromolar) PGI_2 had a similar effect to PGE_2 which is an inhibition of the AC complex and lipolysis [9, 10].

The present study was undertaken to determine if the effect of PGE_2 and PGI_2 were mediated by the same or by different receptors. In addition, the existence of other PG receptors in isolated rat adipocytes was also examined. The binding studies were related to the biological actions (antilipolysis) of these PGs.

Materials and methods

[^{3}H]PGE_2 (140–170 Ci/mmol), [^{3}H]PGI_2 (12.2 Ci/mmol), [^{3}H]$PGF_{2\alpha}$ (177 Ci/mmol) and [^{3}H]PGD_2 (131 Ci/mmol) were from New England Nuclear (Dreieich, F.R.G.). [^{3}H]PGA_2 (140 Ci/mmol) was from Amersham (U.K.). All other reagents were from Sigma (St Louis, MO).

Adipocytes from male Wistar rats were isolated as previously described [3, 4] with minor modifications since adenosine was added to the isolation buffer at a final concentration of 200 nmol/l in accordance to a recent report by Honnor *et al.* [11]. The adipocytes were then resuspended in a 10 mmol/l Hepes buffer (pH 7.4) [3, 4]. However, in studies with PGI_2, the pH of the incubation buffer was changed to 8.5 (at 37°) as described by Gaion *et al.* [11]. The alkanization of the incubation buffer was performed in an attempt to reduce the degradation of PGI_2, since PGI_2 is very labile under physiological conditions ($t^{1/2}$ = 3–5 min) [10, 12, 13].

Glycerol, cAMP and PG binding were determined under similar conditions. Adenosine deaminase (ADA) was present in a final concentration of 0.5 U/ml both in lipolysis and binding assays and these studies were performed for 60 min at 37° (in the PGI_2 studies the incubations were performed for 15 min). The contents of cAMP in adipocytes were determined after stimulation for 10 min with isoproterenol (500 nmol/l) by radioimmunoassay using an acetylation procedure [14].

PGE_2 binding sites in adipocytes were determined by [^{3}H]PGE_2 at a final concentration of 1–2 nmol/l. The binding affinity, expressed as the half-maximal inhibitory concentration (ED_{50}) of PGs, was determined from computer analysis of the individual competition curves. In some experiments the total binding capacity (B_{max}) and the binding affinity (K_d) were determined from Scatchard analysis [15] of the binding data.

Results and discussion

The potency order of several PGs for inhibition of PGE_2 binding was comparable with their antilipolytic potencies, with $PGE_2 \geq PGE_1 \gg PGI_2 \geq PGF_{2\alpha} \gg PGA_1 > PGD_2 >$ 6-keto-$PGF_{1\alpha}$ > arachidonic acid (Table 1). The K_d of the [^{3}H]PGE_2 receptor binding was 1.3 nmol/l (estimated from Scatchard plot). Thus, these PGE_2 binding sites had about 50 times higher affinity for PGs of the E-series than for the next group of PGs which includes PGI_2 and $PGF_{2\alpha}$ (Table 1). Except from PGI_2 no other prostaglandins

Table 1. Affinities (ED_{50}) and sensitivities (IC_{50}) of different PGs on [^{3}H]PGE_2 binding and lipolysis in rat adipocytes

Drugs	N	Binding (ED_{50}) (nmol/l)	Lipolysis (IC_{50}) (nmol/l)
PGE_2	8	2.5 ± 0.3	1.6 ± 0.3
PGE_1	4	3.4 ± 0.5	2.7 ± 0.6
PGI_2*	8	98.2 ± 8	183 ± 24
$PGF_{2\alpha}$	4	122 ± 12	153 ± 32
PGA_1	5	1352 ± 141	977 ± 136
PGD_2	4	4357 ± 520	3025 ± 551
6-Keto-$PGF_{1\alpha}$	4	13,000 ± 3200	15,000 ± 2700
Arachidonic acid	4	48,000 ± 3900	63,000 ± 4000

Each value is the mean ± SEM of the indicated number of experiments (N).

ED_{50} is the half-maximal inhibitory concentration of the PGs determined from competition curves with [^{3}H]PGE_2 (1.0 nmol/l).

IC_{50} is the half-maximal inhibitory concentration of the PGs which inhibit the stimulated lipolysis with 50%. Lipolysis and binding were performed in the presence of ADA (0.5 U/ml) for 60 min at 37°. 6–10 different ligand concentrations were used for each PG.

* Binding and lipolysis were performed for 15 min at 37° (pH 8.5).

($PGF_{2\alpha}$, PGA_1, PGD_2) were found to have biphasic effect on lipolysis (data not shown).

In the studies comparing PGE_2 and PGI_2 both binding and lipolysis were performed for 15 min (pH 8.5) (Fig. 1). The [^{3}H]PGE_2 binding was inhibited by PGE_2, PGI_2, and 6-keto-$PGF_{1\alpha}$ (stable degradation product of PGI_2) with ED_{50}-values of about 2.9 nmol/l, 98 nmol/l, and >10 μmol/l, respectively. The antilipolytic action of these PGs was demonstrated in a similar concentration range (Fig. 1). Thus, the antilipolytic effects of these PGs were presumably mediated by their interaction with the PGE_2 receptor. However, at low concentrations (<80 nmol/l), PGI_2 actually enhanced the stimulated glycerol release. At a concentration of 40 nmol/l, PGI_2 enhanced lipolysis with 21 ± 2% ($P < 0.05$, Fig. 1). PGI_2 at very high concentration (20 μmol/l) only inhibited the stimulated lipolysis by 75% whereas PGE_2 at a concentration of 100 nmol/l was able completely to antagonize the ADA-stimulated lipolysis (Fig. 1). The stimulatory effects of low concentrations of PGI_2 (40 nmol/l) was only observed for the first 20–25 min of the incubation. After this period the stimulatory effect of PGI_2 disappeared and a small inhibitory effect was discovered. Only the inhibitory action was observed at higher PGI_2 concentrations (>200 nmol/l, data not shown). These findings indicate that the stimulatory effect of low concentrations of PGI_2 may only be due to genuine, intact PGI_2 and not to any degradation products of PGI_2.

The alkali buffer does not seem to be responsible for the stimulatory action of PGI_2 since binding and action of PGE_2 were similar at pH 7.4 and 8.5, respectively (Table 1 and Fig. 1). However, selective effects of the alkali buffer on, for example, a possible PGI_2 receptor could, of course, not completely be excluded.

[^{3}H]*prostaglandin binding to adipocytes.* Although various experimental conditions were performed no specific PGI_2 binding could be detected in adipocytes by the use of [^{3}H]PGI_2. However, [^{3}H]PGI_2 is in comparison with [^{3}H]PGE_2 a bad tracer since [^{3}H]PGI_2 could only be

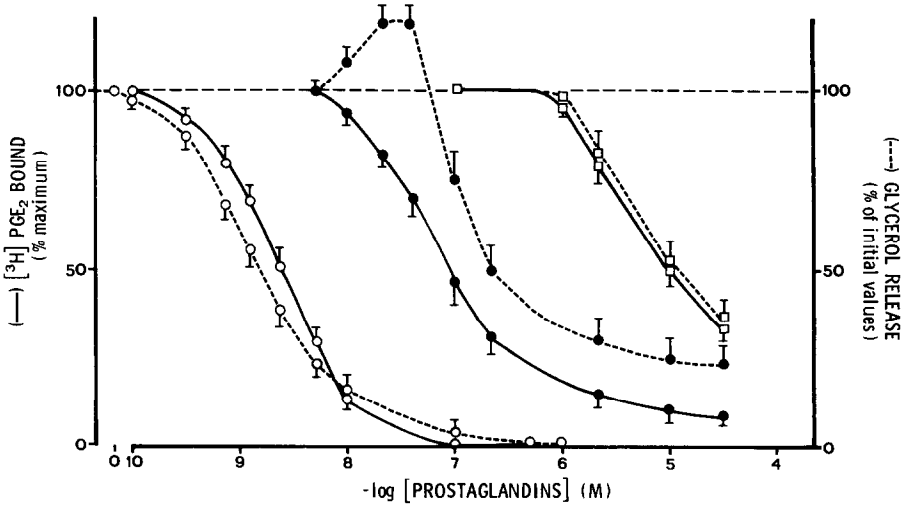

Fig. 1. Relationships between [^{3}H]PGE_2 binding and antilipolysis. The binding study was performed with [^{3}H]PGE_2 (1.0 nmol/l) and different concentrations of PGE_2 (○), PGI_2 (●), and 6-keto-$PGF_{1\alpha}$ (□). The antilipolytic effects (dotted lines) of the different PGs were determined on lipolysis stimulated by ADA (0.5 U/ml). The incubations were performed for 15 min at 37° (pH 8.5). Mean ± SEM of 8 experiments for PGE_2 and PGI_2 and 4 experiments for 6-keto-$PGF_{1\alpha}$.

obtained with very low specific activity (12.2 Ci/mmol). At least some binding should have been found since PGI_2 actually interacted with the PGE_2 receptor (Fig. 1). Thus, until a better PGI_2 tracer is available the presence of specific high affinity PGI_2 binding sites to adipocytes could not absolutely be ruled out.

The binding of $[^3H]PGF_{2\alpha}$ and $[^3H]PGD_2$ was considerably smaller than the binding of $[^3H]PGE_2$ (Table 2). Competition with unlabelled $PGF_{2\alpha}$ and PGD_2, respectively, revealed no high affinity binding sites (Table 2). From Scatchard analysis of the binding data (indicating a homogeneous receptor population) it was found that the B_{max} of $PGF_{2\alpha}$ was similar to the total binding capacity of PGE_2 (49 vs 44 fmol/10^6 cells). The apparent K_d value for the $PGF_{2\alpha}$ binding was, however, much higher (43.4 nmol/l vs 1.4 nmol/l, Table 2). These findings may indicate that PGE_2 and $PGF_{2\alpha}$ bind to the same population of receptors with different affinities. The binding of $[^3H]PGD_2$ was so small that the ED_{50}-value could only be determined with uncertainty (Table 2). Thus, no high affinity binding sites for $PGF_{2\alpha}$ or PGD_2 were found in rat adipocytes, and the binding data are in accordance with the assumption that these tracers are interacting with the PGE_2 receptor.

In contrast to these PGs $[^3H]PGA_2$ seemed, in agreement with a previous study [16], to have its own specific binding sites in rat adipocytes (Table 2). However, the $[^3H]PGA_2$ binding was only displaced by PGA_1 and PGA_2 (equipotent) but was not displaced by any other PGs even at extreme high concentrations (>10 μM, data not shown). In addition the specific binding of $[^3H]PGA_2$ gradually diminished when the binding reaction was performed for more than 120 min at 37° mainly because of enhanced non-specific binding (data not shown). The mechanism(s) for this latter phenomenon is unknown. However, since the K_d, determined from Scatchard plot, was about 54 nM but the antilipolytic action of PGA_2 (and PGA_1) was in the concentration range about 1 μM (Table 1) these findings may indicate that the antilipolytic action of PGAs are mediated by their interaction with the PGE_2 receptor (Table 1) and, thus, the physiological relevance of the PGA binding sites is at present completely obscure.

Effect of PGI_2 *on cAMP accumulation.* Indomethacin (10 μmol) was used in these studies in an attempt to suppress the formation of endogenous PGs in adipocytes [7, 17, 18]. The cAMP accumulation was stimulated by isoproterenol (500 nmol/l). In the absence of indomethacin low concentrations of PGI_2 (20–100 nmol/l) had small stimulatory effects (about 10% increment) on the cAMP accumulation ($0.1 > P > 0.05$, $N = 6$, Fig. 2). At higher concentrations PGI_2 (> 100 nmol/l) had pronounced inhibitory effects on the stimulated cAMP. In the presence of indomethacin (10 μmol/l) the stimulated cAMP response

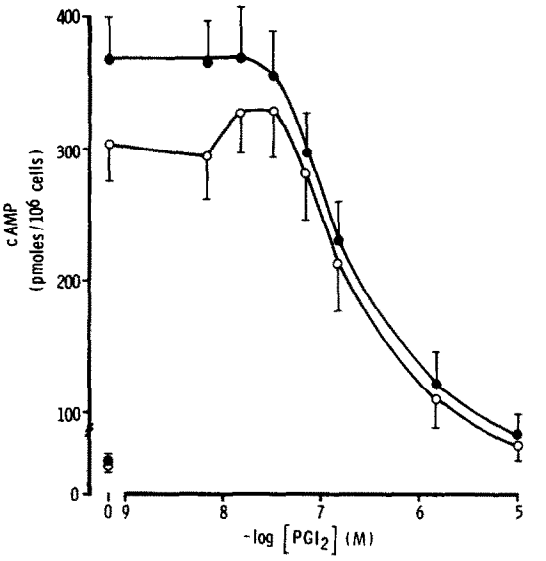

Fig. 2. Effect of PGI_2 on cAMP accumulation. Adipocytes were preincubated in the presence (●) and absence (○) of indomethacin (10 μmol/l) for 30 min at 37° (pH 8.5). Then increasing concentrations of PGI_2 were added together with theophylline (2 mmol/l) and isoproterenol (500 nmol/l) and the incubation was continued for a further 10 min. cAMP was measured by RIA. Mean ± SEM of six parallel experiments.

was significantly enhanced ($P < 0.01$) which possibly was due to the fact that indomethacin through its inhibition of the PG production [7, 17] may prevent the inhibitory effects of endogenous PGs. In addition, the stimulatory action of low concentrations of PGI_2 disappeared in the presence of indomethacin, and, thus, PGI_2 had inhibitory actions at all concentrations tested (Fig. 2). Preincubation with indomethacin also reduced the stimulatory effect of low concentrations of PGI_2 on lipolysis by about 50% and, thus, did not completely abolish the PGI_2-stimulated lipolysis.

Low concentrations of PGI_2 (20–100 nmol/l) had no stimulatory actions on either the non-stimulated AC activity or on the non-stimulated lipolysis. Thus, since PGI_2 has no stimulatory effect alone and its potentiating effect at least partially can be blocked by indomethacin (presumably by reducing the endogenous level of PGs), these data may indicate that the stimulatory action of PGI_2 is

Table 2. Comparison of the binding of different PGs to rat adipocytes

	Bound* (fmol/10^6 cells)	B_{max}	ED_{50} (nmol/l)	K_d
Additions:				
$[^3H]PGE_2$	27.6 ± 1.7	44 ± 5	3.6 ± 0.4	1.4 ± 0.2
$[^3H]PGF_{2\alpha}$	2.5 ± 0.3	49 ± 7	47.6 ± 4.2	43.4 ± 3.2
$[^3H]PGD_2$	0.4 ± 0.1	—	>500	—
$[^3H]PGA_2$	26.4 ± 3.6	731 ± 63	59.3 ± 7.1	54.4 ± 5.7

Adipocytes were incubated with different tritium labelled PGs (2 nmol/l) for 60 min at 37°. Competition was performed with unlabelled PGE_2, $PGF_{2\alpha}$, PGD_2 and PGA_2, respectively. Non-specific binding was determined with unlabelled PGE_2 (0.5 μmol/l), $PGF_{2\alpha}$ (25 μmol/l), PGD_2 (50 μmol/l) and PGA_2 (50 μM), respectively. The binding data are given directly from the competition binding curves (bound and ED_{50}) and from analysing the data by the method of Scatchard (B_{max} and K_d). Values are mean ± SEM for 4–5 experiments for each PG.

* Specific adipocyte binding at tracer concentrations (2 nmol/l).

mediated by some indirect mechanisms and presumably not through a specific PGI_2 receptor.

Preliminarily it is hypothesised that the minor stimulatory action of low concentrations of PGI_2 might be due to some kind of PGE_2 receptor antagonism since PGI_2 is interacting with the PGE_2 receptor in the relevant concentration range (Fig. 1). Maybe low concentrations of exogenous PGI_2, through this receptor antagonism, to some extent could prevent the antilipolytic effect mediated by endogenous PGE_2. This latter suggestion is in agreement with the observations that when the level of endogenous PGs (including PGE_2) was reduced by indomethacin the small stimulatory effect of PGI_2 was partially abolished. The concentrations of PGE_2 and PGI_2 which may be of most "physiological" relevance are probably in the nanomolar range [9]. Accordingly, PGE_2 and PGI_2 could very well have antagonistic actions on the AC complex (and lipolysis) in isolated adipocytes as well as in the adipose tissue *in vivo*. In light of the pronounced production of PGI_2 in adipocytes [7], these latter considerations might possibly explain many of the previous difficulties in determining any action of endogenous PGs in adipocytes by using indomethacin [18, 19] since indomethacin inhibits all PGs including PGE_2 and PGI_2 [7, 17]. Thus, to differentiate between the action of these two PGs highlights the need of specific PGI_2 and PGE_2 receptor antagonists in further studies.

In summary, it is demonstrated that the antilipolytic action of all PGs in adipocytes seems to be mediated by their interaction with the PGE_2 receptor. However, whether the action of low concentrations of PGI_2 also is due to interaction with the PGE_2 receptor is at present an open question.

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Na^+-independent, pyridine nucleotide-linked efflux of Ca^{2+} from preloaded rat heart mitochondria: induction by chlortetracycline

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Chlortetracycline is a polycyclic antibiotic of the naphthacenecarboxamide group which, in a non-aqueous environment, forms highly fluorescent complexes with divalent cations [1]. The initial report [1] describing the properties of CTC* indicated that, at concentrations below 20 μM, CTC did not alter mitochondrial respiration or oxidative phosphorylation. As a result the drug has been widely used as a probe of intracellular Ca^{2+} disposition [2] and mitochondrial Ca^{2+} fluxes [3–6].

Numerous observations suggest, however, that CTC concentrations as low as 5 μM are not entirely without effect on mitochondrial function. Mitochondrial Ca^{2+} uptake measured in the presence of CTC is, under some conditions, transient [5, 6]. In mitochondria from systems as diverse as mammalian liver [3], Jerusalem artichoke tubers [7], and *Tetrahymena pyraformis* [6], CTC uncouples respiration and collapses membrane potential in a Ca^{2+}-dependent fashion. It has therefore been suggested that CTC, like ionophore A23187, mediates the electroneutral release of Ca^{2+} from mitochondria [6].

Agents, including palmitoyl-CoA [8], substrates such as oxaloacetate [9] and *t*-butyl hydroperoxide [10], *N*-ethylmaleimide [11], high concentrations of inorganic phosphate [11, 12], alloxan [13], menadione [14], and divicine [15], induce Ca^{2+} release from preloaded mammalian mitochondria. Release occurs via a process that is Na^+-inde-

* Abbreviations: CTC, chlortetracycline; F-CCP, carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone; and MOPS, 3-(*N*-morpholino) propanesulfonic acid.