Factors regulating the production of prostaglandin E₂ and prostacyclin (prostaglandin I₂) in rat and human adipocytes

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The regulation of PGE₂ (prostaglandin E₂) and PGI₂ (prostaglandin I₂; prostacyclin) formation was investigated in isolated adipocytes. The formation of both PGs was stimulated by various lipolytic agents such as isoproterenol, adrenaline and dibutyryl cyclic AMP. During maximal stimulation the production of PGE₂ and PGI₂ (measured as 6-oxoPGF_{1α}) was 0.51 ± 0.04 and 1.21 ± 0.09 ng/2 h per 10⁶ cells respectively. Thus PGI₂ was produced in excess of PGE₂ in rat adipocytes. The production of the PGs was inhibited by indomethacin and acetylsalicylic acid in a concentration-dependent manner. The half-maximal effective concentration of indomethacin was 328 ± 38 nM and that of acetylsalicylic acid was 38.5 ± 5.3 μM. The PGs were maximally inhibited by 70–75 % after incubation for 2 h. In contrast with their effect on PG production, the two agents had a small potentiating effect on the stimulated lipolysis ($P < 0.05$). The phospholipase inhibitors mepacrine and chloroquine inhibited both PG production and triacylglycerol lipolysis and were therefore unable to indicate whether the PG precursor, arachidonic acid, originates from phospholipids or triacylglycerols in adipocytes. Angiotensin II significantly ($P < 0.05$) stimulated both PGE₂ and PGI₂ production in rat adipocytes without affecting triacylglycerol lipolysis. Finally, it was shown that PGE₂ and PGI₂ were also produced in human adipocytes, although in smaller quantities than in rat adipocytes. It is concluded that the production of PGs in isolated adipocytes is regulated by various hormones. Moreover, at least two separate mechanisms for PG production may exist in adipocytes: (1) a mechanism that is activated concomitantly with triacylglycerol lipolysis (and cyclic AMP) and (2) an angiotensin II-sensitive, but lipolysis (and cyclic AMP)-independent mechanism.

INTRODUCTION

Prostaglandins (PGs) are released from most tissues in response to diverse stimulations (hormonal, immunological and mechanical). Previously it was shown that PGs, especially those of the E series, are released from adipose tissue in response to nervous and hormonal stimulations (Shaw & Ramwell, 1968; Christ & Nugteren, 1970; Dalton & Hope, 1974). The substrate for PG synthesis is mainly arachidonic acid (Ramwell *et al.*, 1977; Irvine, 1982). In most cell types arachidonic acid is predominately present in phospholipids in cell membranes, almost exclusively in the 2-acyl position (Van den Bosch, 1980). Accordingly, arachidonic acid could be released for PG synthesis by the action of a specific phospholipase A₂ (or by other phospholipases or acylhydrolases) (Van den Bosch, 1980; Irvine, 1982). Since PGs are not stored within cells, biosynthesis may immediately precede their release. Thus, in most tissues, the synthesis of PGs seemed to be limited by the availability of their common precursor, free arachidonic acid, which possibly may be located in some specific intracellular compartments, since not all free arachidonic acid is converted into PGs (Christ & Nugteren, 1970; Dalton & Hope, 1974).

In adipocytes, however, arachidonic acid may also be stored in neutral (triacylglycerol) lipids (Christ & Nugteren, 1970; Dalton & Hope, 1974; Nègre & Ailhaud, 1981). Accordingly, this arachidonic acid may be available for PG synthesis in association with

triacylglycerol lipolysis. Whether arachidonic acid from the phospholipids or from neutral lipids is the most important precursor for PG production in adipocytes is at present unknown.

We have demonstrated that the anti-lipolytic effect of various PGs in adipocytes presumably was mediated by their interaction with the [³H]PGE₂ receptor (Richelsen *et al.*, 1984; Richelsen, 1987). The only PGs that have action in the nanomolar range were PGs of the E series, and these PGs had about 50 times higher sensitivity for inhibiting lipolysis than PGI₂ and PGF_{2α} (Richelsen, 1987). In addition, PGI₂ at low concentrations (10–80 nM) had a small potentiating effect on the stimulated lipolysis (Fredholm *et al.*, 1980; Richelsen, 1987). Thus PGI₂ and PGE₂ could possibly have antagonistic effects on lipolysis in the low-nanomolar concentration range that may have physiological relevance (Fredholm *et al.*, 1980). However, the possible role of endogenously released PGs in adipose tissue is still questionable (Dalton & Hope, 1973; Fain *et al.*, 1973).

The present study was performed in order to investigate some of the factors that regulate the production of PGs in adipocytes. Since only low levels of PGF_{2α} have been determined in adipocytes (Shaw & Ramwell, 1968; Dalton & Hope, 1974; Nègre & Ailhaud, 1981), only PGE₂ and PGI₂ (measured as the stable degradation product, 6-oxoPGF_{1α}) were measured by radioimmunoassay. In addition the important question as to whether or not non-steroid anti-inflammatory agents were able to inhibit PG synthesis in adipocytes was also examined.

concentrations of 1–100 µg/ml. The cells were harvested. Finally, preliminary data obtained with human adipocytes are presented.

MATERIALS AND METHODS

Fat-cell preparation

Rat adipocytes were obtained from epididymal fatpads of male Wistar rats (150–200 g). After fasting overnight, the rats were killed by cervical dislocation. Human subcutaneous adipose tissue was obtained from a biopsy from the gluteal region (Richelsen *et al.*, 1986). The volunteers (three females and three males) fasted overnight before tissue removal, and none of them had taken any anti-inflammatory agents in the last 4 days before the biopsy. The study was approved by the local Ethical Committee.

Rat and human adipocytes were isolated and cell concentration determined as previously described (Richelsen *et al.*, 1984; Richelsen & Pedersen, 1985). On optical-microscopic examination the adipocyte preparation was found to be homogeneous, without contamination with other cell types.

Adipocyte incubations

Adipocytes were incubated in 10 mM-Hepes buffer (Richelsen & Pedersen, 1985) with 2.5% (w/v) essentially fatty-acid-free human albumin and half the usual Ca^{2+} concentration (1.2 mM) (Van den Bosch, 1980; Axelrod & Levine, 1981). The concentration of adipocytes was usually about 2×10^5 cells/ml for rat adipocytes and $(0.5\text{--}1) \times 10^5$ cells/ml for human adipocytes. The incubations were performed in polypropylene tubes at 37 °C with gentle shaking, usually for 120 min. The incubations were terminated by removing the adipocytes by adding an oil layer at the top of the incubation mixture, followed by centrifugation at 3000 *g* for 1 min (4 °C) (Richelsen *et al.*, 1984). A small portion of the cell-free medium was taken for glycerol determination, the remainder being placed on an ice bath and extracted twice with methanol (1:3, v/v), followed by centrifugation at 4 °C. The methanol fraction was evaporated under a stream of N_2 .

Separation of PGs by Sep-pak

After extraction, the PGs were separated on Sep-pak C_{18} cartridges. The samples were eluted with 5 ml of ethyl acetate/methanol (9:1, v/v) (Gallon & Barcelli, 1986). This fraction was collected and evaporated to dryness under a stream of N_2 . The residue was dissolved in PGE_2 radioimmunoassay buffer and stored at –80 °C until assayed. PGs were routinely measured after this separation procedure.

Separation of PGs by t.l.c.

In some experiments, medium was further separated on t.l.c. plates (silica-gel 60; Merck). The PGs were separated on the plates with a mobile phase of chloroform/methanol/acetic acid/water (450:40:5:4) (Shaw & Ramwell, 1969; Grn *et al.*, 1978). The plates were air-dried and the lipid spots revealed with I_2 vapour.

Authentic PGE_2 and 6-oxoPGF $_{1\alpha}$ were added as standards. The zones which corresponded to these standards were scraped off and extracted twice with ethyl acetate/methanol (1:1, v/v). The R_F values for various

PGs were essentially as previously described (Shaw & Ramwell, 1969; Crutchley & Piper, 1976). The PGs were separated from their most predominant precursors, namely arachidonic acid and linoleic acid. However, the PGs of interest in the present study, PGE_2 and 6-oxoPGF $_{1\alpha}$, were not adequately separated (R_F 0.55 and 0.53 respectively). However, from the specificity of the two radioimmunoassay systems used (see below), no cross-reactivity between PGE_2 and 6-oxoPGF $_{1\alpha}$ would be expected to occur. The recoveries of the PGs by different extraction and separation procedures were determined from addition of [^3H]PGE $_2$ and [^3H]6-oxoPGF $_{1\alpha}$.

Radioimmunoassay of PGE_2 and 6-oxoPGF $_{1\alpha}$

PGE_2 and 6-oxoPGF $_{1\alpha}$ were measured with ^{125}I -PGE $_2$ and ^{125}I -6-oxoPGF $_{1\alpha}$ radioimmunoassay kits respectively. The lower limits of sensitivity of the PGE_2 assay were 0.25–0.5 pg of PGE_2 /100 µl. In the PGE_2 radioimmunoassay, the cross-reactivity with PGE $_1$ was 3.7% and with 6-oxoPGF $_{1\alpha}$, PGF $_{2\alpha}$ and PGB $_2$ it was less than 0.01%. 6-OxoPGF $_{1\alpha}$ was measured as the stable degradation product of PGI $_2$. The detection limit was about 2.5 pg of 6-oxoPGF $_{1\alpha}$ /100 µl. In the 6-oxoPGF $_{1\alpha}$ assay the cross-reactivity with PGF $_{2\alpha}$ was 2.6%, and with PGE_2 it was 1.1%.

Statistical analysis

Data are given as means $\pm$ S.E.M.. Incubations were performed in duplicate. In comparative studies significance was determined by using Student's paired *t* test.

Chemicals

PGE_2 and 6-oxoPGF $_{1\alpha}$ radioimmunoassay kits were from New England Nuclear Corp. (Boston, MA, U.S.A.). Collagenase from *Clostridium histolyticum* was obtained from Worthington Biochemical Corp. (Freehold, NJ, U.S.). Sep-pak (C_{18}) cartridges were from Waters Associates (Milford, MA, U.S.A.). All other reagents were from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Indomethacin was prepared daily in a phosphate buffer (0.066 M- Na_2HPO_4 /0.033 M- NaH_2PO_4).

RESULTS

Evaluation of the PGE_2 and 6-oxoPGF $_{1\alpha}$ radioimmunoassay systems

When the medium from adipocyte incubation was made alkaline with 1M-NaOH (Matsuda *et al.*, 1983), almost no immunoreactive PGE_2 (less than 5%) could be detected (results not shown). Presumably the alkali treatment converted all the PGE_2 into PGB $_2$ (Shaw & Ramwell, 1969). 6-OxoPGF $_{1\alpha}$ was affected only to a small extent by the alkali treatment (> 90% was recovered).

Multiple dilutions of samples from adipocyte incubations, obtained after Sep-pak separation, showed that the dilution curves were essentially parallel with the standard curves for PGE_2 and 6-oxoPGF $_{1\alpha}$ respectively (results not shown). In addition, when PGE_2 and 6-oxoPGF $_{1\alpha}$ were determined directly in the unextracted medium, it was found that these PGs could be measured in amounts similar to those measured after Sep-pak and t.l.c. separation. However, about 20% higher amounts of immunoreactive PGE_2 were measured in the unextracted medium than those measured after t.l.c. separation

Fig. 1. Time-dependency of the release of (a) glycerol, (b) 6-oxoPGF_{1α} and (c) PGE₂ from rat adipocytes

Adipocytes were incubated for different time periods at 37 °C with the indicated agents. PGE₂ and 6-oxoPGF_{1α} were measured by radioimmunoassay after Sep-pak separation. Results are means ± S.E.M. (*n* = 6–8). ●, Basal; ○, basal + indomethacin (10⁻⁵ M); ■, adenosine deaminase (0.5 unit/ml) + adrenaline (5 × 10⁻⁶ M); □, adenosine deaminase (0.5 unit/ml) + adrenaline (5 × 10⁻⁶ M) + indomethacin (10⁻⁵ M).

(results not shown). This latter finding could possibly be due to albumin interfering with the PGE₂ radioimmunoassay. Actually, 2.5 and 5% (v/v) albumin inhibited the antigen-antibody reaction in the PGE₂ assay by about 30 and 43% respectively (results not shown). Taking the different recoveries into account, it was found that Sep-pak and t.l.c. separations gave comparable results. In the studies described below the PGs were measured after Sep-pak separation.

Fig. 2. Effects of different PG inhibitors on release of PGE₂ (a) and (b) glycerol

Rat adipocytes were incubated for 120 min in the presence or absence of the indicated agents. PGE₂ production and lipolysis were stimulated by adenosine deaminase (0.5 unit/ml) in combination with adrenaline (5 × 10⁻⁶ M). The action of the inhibitors is expressed in relation to values in the absence of these inhibitors. Results are means ± S.E.M. for eight experiments (indomethacin, ●) or four experiments [acetylsalicylic acid (○), mepacrine (□) and chloroquine (■)] respectively.

Time-dependence of PG production in adipocytes

In these studies the adipocytes were stimulated by adenosine deaminase (0.5 units/ml) in combination with 5 μM-adrenaline. The release of 6-oxoPGF_{1α} was linear for the first 2 h of incubation, whereas the release of PGE₂ was linear for 3 h (Figs. 1b and 1c). On the other hand the glycerol release was linear with time for up to 4 h of incubation (Fig. 1a). After 4 h of incubation with continuous shaking, the PG production increased considerably, and the difference between the stimulated and the non-stimulated PG release diminished (Fig. 1). Since the release of both PGE₂ and 6-oxoPGF_{1α} was still linear after 2 h of incubation, this incubation period was chosen for the following studies.

In the presence of indomethacin (10 μM), both PGE₂ and 6-oxoPGF_{1α} were greatly decreased. After incubation for 4 h, the stimulated 6-oxoPGF_{1α} production was inhibited by 89% and the PGE₂ production was inhibited by 85%. By contrast, indomethacin had a small potentiating effect on the stimulated glycerol release, but had no effect on the 'basal' glycerol release (Fig. 1a).

Table 1. Action of different lipolytic compounds on PG formation

Adipocytes were incubated for 2 h in the presence or absence of the indicated compounds. Results are means $\pm$ S.E.M. for the number (*n*) of experiments given. Abbreviations: ADA, adenosine deaminase; ISOP, isoproterenol; PRO, propranolol; d-cAMP, dibutyryl cyclic AMP. Significance: **P* < 0.01 compared with no additions.

Addition	<i>n</i>	PG formation (pg/2 h per 10 ⁶ cells)	
		PGE ₂	6-oxoPGF _{1α}
None	8	232 $\pm$ 26	512 $\pm$ 61
ADA (0.5 unit/ml)	8	252 $\pm$ 35	808 $\pm$ 69*
ISOP (1 μ M)	8	562 $\pm$ 58*	1234 $\pm$ 82*
ISOP (1 μ M)	6	235 $\pm$ 29	531 $\pm$ 67
+PRO (100 μ M)			
d-cAMP (2 mM)	5	477 $\pm$ 67*	1167 $\pm$ 91*

Action of different PG inhibitors

Both indomethacin and acetylsalicylic acid inhibited PGE₂ production in isolated rat adipocytes (Fig. 2a). The production of 6-oxoPGF_{1 α} was inhibited in a very similar manner (results not shown). After 2 h the PGE₂ production was maximally inhibited (77 $\pm$ 4%) by an indomethacin concentration of 10 μ M (Fig. 2a). The IC₅₀ of indomethacin was 328 $\pm$ 38 nM. The IC₅₀ value of acetylsalicylic acid was 38.5 $\pm$ 5.3 μ M. That indomethacin inhibited PG production by 70–80% was also confirmed by radioimmunoassay measurements after t.l.c. separation of the samples (results not shown).

In order to demonstrate whether phospholipases are also involved in PG production in adipocytes, the effect

of three phospholipase A₂ inhibitors (mepacrine, chloroquine and *p*-bromophenacyl bromide) were examined (Vargaftig, 1977; Van den Bosch, 1980). Mepacrine and chloroquine inhibited PGE₂ (and 6-oxoPGF_{1 α}) production with IC₅₀ values of about 71 μ M and 420 μ M respectively (Fig. 2a). However, in contrast with indomethacin and acetylsalicylic acid, these agents also had pronounced inhibitory effects on glycerol release over the same concentration range at which they inhibited PG production (Fig. 2b).

In the presence of indomethacin and acetylsalicylic acid, the stimulated lipolysis was actually further enhanced (Fig. 2b). At indomethacin concentrations of 1, 5 and 10 μ M the enhancement was significant (*P* < 0.05, *n* = 8). However, the action of indomethacin on lipolysis was relatively small (maximal enhancement of about 15–18%).

Action of lipolytic agents on PG production

Basal release of PGE₂ was 232 $\pm$ 26 pg/2 h per 10⁶ cells in rat adipocytes. Adenosine deaminase had very little effect on PGE₂ release, in contrast with its significant stimulatory effect on the formation of 6-oxoPGF_{1 α} (Table 1). In the presence of the lipolytic agents isoproterenol (1 μ M) and dibutyryl cyclic AMP (2 mM), the PGE₂ production was more than doubled (Table 1). Similarly, these compounds also more than doubled the release of 6-oxoPGF_{1 α} (Table 1). Addition of propranolol (100 μ M) totally abolished the action of isoproterenol on the formation of both PGE₂ and PGI₂ (Table 1). Propranolol also prevented adrenaline-stimulated PG formation, but had no effect on the action of dibutyryl cyclic AMP (results not shown).

Effects of angiotensin II on PG and glycerol release

In these studies the incubations were performed for only 60 min. This incubation time was used because the action of angiotensin II was most clearly demonstrated at short incubation times (results not shown).

Fig. 3. Effects of angiotensin II (ATII) on PG production (a, b) and glycerol release (c) in isolated rat adipocytes

Adipocytes were incubated for 60 min in the absence or presence of angiotensin II in the indicated concentrations. Results are means $\pm$ S.E.M. (*n* = 6). Significance: **P* < 0.05.

Table 2. PGE₂ and 6-oxoPGF_{1α} production in human subcutaneous adipocytes

Human adipocytes were incubated with the indicated agents in duplicate for 2 h at 37 °C. Results are means ± S.E.M. (*n* = 6). Abbreviations and concentrations: ADA, adenosine deaminase (0.5 units/ml); ISOP, isoproterenol (1 μM); INDO, indomethacin (10 μM).

Addition	PG production (pg/10 ⁶ cells)			
	PGE ₂		6-oxoPGF _{1α}	
	–INDO	+INDO	–INDO	+INDO
None (basal)	117 ± 13	39 ± 3	584 ± 68	271 ± 24
+ADA	127 ± 17	–	877 ± 75	–
+ADA + ISOP	174 ± 24	43 ± 3	1029 ± 79	309 ± 32

Angiotensin II (8–400 nM) significantly stimulated both PGE₂ and 6-oxoPGF_{1α} production to 35–45 % above basal levels (*P* < 0.05, Fig. 3). By contrast, angiotensin II had no effect on the glycerol release (Fig. 3). Addition of propranolol had no effect on the angiotensin-stimulated PG formation (results not shown).

PG production in human adipocytes

Human adipocytes produce considerably smaller amounts of PGs than do rat adipocytes (Table 2). If PG release is expressed in relation to the adipocyte surface instead of to the adipocyte number, this difference would be even greater (results not shown). Compared with that of 6-oxoPGF_{1α} the production of PGE₂ was considerably lower in human adipocytes than in rat adipocytes. Thus the formation of 6-oxoPGF_{1α} was about 2 times higher than the formation of PGE₂ in rat adipocytes, whereas in human adipocytes about 5 times more 6-oxoPGF_{1α} than PGE₂ was produced. Finally, indomethacin (10 μM) also inhibited PG production in human adipocytes, by 55–75 % after incubation for 2 h (Table 2).

DISCUSSION

Measurements by radioimmunoassay of PGs in adipocyte incubations have been associated with certain difficulties, mainly attributable to cross-reactivity of the antibodies with various non-esterified fatty acids (Gold & Edgar, 1978; Samuelsson *et al.*, 1978; Metz *et al.*, 1979). However, within the last few years, more specific antibodies have been developed which bind PGs with higher specificity. Recently, a 6-oxoPGF_{1α} radioimmunoassay has been used measuring 6-oxoPGF_{1α} in unextracted medium from adipocyte incubations (Axelrod & Levine, 1981; Axelrod *et al.*, 1985), and the direct measurements were confirmed by h.p.l.c. (Axelrod *et al.*, 1985).

We found that using the Sep-pak separation procedure described above, both PGE₂ and 6-oxoPGF_{1α} could be measured in adipocyte incubations with great precision as compared with the t.l.c. separation. The specificity of the radioimmunoassay systems was determined from the fact that: (1) the inhibition curves of ¹²⁵I-PGE₂ antibody and ¹²⁵I-6-oxoPGF_{1α} antibody binding by immunoreactive compounds in the samples parallel that of

authentic PGE₂ and 6-oxoPGF_{1α} respectively and (2) alkali treatment of samples almost completely degrades immunoreactive PGE₂. The degradation referred to is caused by a specific dehydration of the β-ketol system in the five-membered ring of PGE₂ to form PGB₂ (Shaw & Ramwell, 1969). Accordingly, compounds that possess no such β-ketol system, i.e. PGFs and non-PG compounds, would remain unaltered by this treatment, as exemplified by 6-oxoPGF_{1α}. Finally, the agreement between radioimmunoassay results obtained after Sep-pak separation compared with t.l.c. separation confirmed the specificity of the assay systems.

PG production was greatly enhanced after incubation for 4 h; however, this increased PG release was not associated with accelerated lipolysis (Fig. 1). These findings may indicate that the PG-generating system may be nearly maximally activated after 4 h of incubation, possibly attributable to its sensitivity to continuous mechanical vibration (Flower, 1978). However, triacylglycerol breakdown was still linear and fully activatable by diverse lipolytic stimulation, indicating that the lipolytic system is less sensitive to mechanical stimulation and, furthermore, that the enhanced PG production is not due to lysis of the adipocytes. After incubation for 4 h, indomethacin still blocked the PG synthesis and, then, in relative terms, has a more pronounced inhibitory effect during long-term than during short-term incubations.

PG production was stimulated by various lipolytic agents as previously demonstrated (Shaw & Ramwell, 1968; Christ & Nugteren, 1970; Dalton & Hope, 1974). The action of adrenaline and isoproterenol on the formation of PGE₂ and PGI₂ seemed to be mediated by the β-adrenergic receptor, since their actions were blocked by propranolol (Table 1). However, β-receptor activation is not necessary, since the cyclic AMP analogue dibutyryl cyclic AMP also had a stimulatory effect on PG production. The association between lipolytic agents and PG production seems to be related either to triacylglycerol lipolysis itself or to a cyclic AMP-dependent mechanism that is activated concomitantly with lipolysis [e.g. phospholipase A₂ activation (Chiappe De Cingolani *et al.*, 1972)].

Non-steroidal anti-inflammatory agents are well-known PG-synthesis inhibitors (Flower & Vane, 1974). However, some controversy exists about their action in adipocyte incubations (Dalton & Hope, 1973; Fain *et al.*, 1973). In the present study both indomethacin and acetylsalicylic acid were able to inhibit PGE₂ and 6-oxoPGF_{1α} production (Fig. 2). PG production was maximally inhibited by 70–75 % after incubation for 2 h. In addition, the sensitivity of indomethacin and acetylsalicylic acid in adipocytes was comparable with their sensitivities in other tissues (Flower & Vane, 1974). In an attempt to disclose the relative importance of the phospholipid as against the triacylglycerol pathway for PG production, different phospholipase inhibitors were examined. Mepacrine and chloroquine significantly inhibited PG production (Fig. 2). Unfortunately, all these agents also inhibited triacylglycerol lipolysis (Fig. 2). These findings may be related to the fact that these agents are not specific phospholipase inhibitors but, in fact, have more general anti-enzyme activities (Hofmann *et al.*, 1982; Best *et al.*, 1984). Thus these agents do not further elucidate the more specific pathway for PG production in adipocytes.

In accordance with the results of a study by Axelrod *et al.* (1985), we also found, in the present study, that angiotensin II was able to stimulate PGI_2 production without affecting lipolysis (Fig. 3). However, in contrast with the study of Axelrod *et al.* (1985), we found that PGE_2 was also stimulated by angiotensin II in a similar manner to PGI_2 . The fact that Axelrod *et al.* (1985) were unable to find any effect of angiotensin II on the PGE_2 release may possibly be related to their less-specific PGE_2 assay. This assumption is supported by the fact that they were unable to inhibit the PGE_2 production by indomethacin, indicating major interference with the PGE_2 assay, possibly involving non-esterified fatty acids and albumin (Axelrod & Levine, 1981).

Angiotensin II stimulated the formation of PGE_2 and 6-oxo $\text{PGF}_{1\alpha}$ without affecting lipolysis (Fig. 3) and without affecting the concentration of cyclic AMP (Axelrod *et al.*, 1986). Thus it is possible that angiotensin II may stimulate PG production by a direct activation of phospholipase A_2 (or C), as has been demonstrated in other tissues (Van den Bosch, 1980; Wuthrich & Vallotton, 1986).

Since PGE_2 has pronounced anti-lipolytic effects and PGs are produced in adipocytes in response to lipolytic stimulation, it is possible that endogenous PGE_2 is able to attenuate the lipolytic response in adipocytes. However, in the present study, indomethacin, which inhibits both PGE_2 and PGI_2 formation, enhanced lipolysis to only a very small extent (Fig. 2). PGI_2 has considerably smaller anti-lipolytic effect than PGE_2 (Richelsen, 1987), but is a very potent vasodilator; thus it could be an important factor in the vasodilatation in adipose tissue that occurs in response to lipolytic stimulation (Lewis & Matthews, 1970; Bowery & Lewis, 1973). Finally, the possible implications of the antagonistic effects of nanomolar concentrations of endogenous PGI_2 and PGE_2 on the regulation of lipolysis are at present unknown (Fredholm *et al.*, 1980; Richelsen, 1987).

In conclusion, both PGE_2 and 6-oxo $\text{PGF}_{1\alpha}$ are produced in rat and human adipocytes. The formation of both PGE_2 and PGI_2 seems to be stimulated by at least two pathways: by a lipolysis (and cyclic AMP)-dependent mechanism and by an angiotensin II-sensitive but lipolysis (and cyclic AMP)-independent mechanism. In addition, the stimulation and inhibition of these two PGs roughly parallel each other under all the experimental conditions used in the present study. Finally, it is demonstrated that, under appropriate incubation conditions, non-steroidal anti-inflammatory agents have considerable inhibitory effects on PG formation (both PGE_2 and PGI_2) in isolated adipocytes.

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