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# Stimulation and inhibition of cyclic AMP formation in isolated rat fat cell by prostacyclin ($PGI_2$)

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Since prostaglandins may be formed by adipose tissue and prostaglandins of the E-series are known to inhibit cyclic AMP formation and lipolysis in fat cells, a feed-back-regulatory role for these compounds has been proposed (for ref. see [1,2]). However, for several reasons it has been concluded that a significant physiological role for E-prostaglandins is unlikely [2–5]. After the isolation of prostaglandin endoperoxides, it was suggested that these compounds, rather than their metabolites (PGE's), played a regulatory role [6]. However, using intact fat cells we found that $PGH_2$ was considerably less potent than $PGE_2$ as an inhibitor of cyclic AMP formation and concluded that neither the endoperoxides nor the thromboxanes were likely to play a role as antilipolytic regulators [7]. Recently, still another biologically active metabolite of the prostaglandin endoperoxides, (5*Z*)-9-deoxy-6,9a-epoxy-$\Delta^5$-$PGF_{1\alpha}$ (prostacyclin or $PGI_2$), was isolated and characterized by Vane and co-workers [8,9]. Since $PGI_2$ has similar effects as the PGE's in several systems, but is more potent [10,11], the effect of the $PGI_2$ on cyclic AMP formation in rat fat cells was tested.

Fat cells were isolated from male Sprague–Dawley rats (180–220 g) and incubated at a concentration of 50–150,000 cells/ml as described earlier [12]. Cyclic AMP accumulation was stimulated by noradrenaline (as the hydrochloride, Sigma, NA) in the presence or absence of theophylline (as the ethylenediamine salt, Oxyphylline, Astra). The incubation was terminated by trichloroacetic acid (final concentration 10%). After removing the TCA by extracting four times with ether, cyclic AMP content was determined directly on an aliquot of the deproteinized extract by the method of Brown *et al.* [13]. $PGI_2$ was dissolved in ethanol: 0.05 M Tris buffer, pH 9 (9:1), in which it is stable at −70°. Immediately prior to use it was diluted in 0.5 M Tris buffer, pH 9. Aliquots (10 $\mu$l) of this solvent with or without $PGI_2$ were added per ml of the incubate. The solvent *per se* had no effect on the fat cells. $N^6$-Phenylisopropyl adenosin (PIA), a kind gift of Dr. H. Storck of Boehringer, Mannheim, was dissolved in water.

NA ($10^{-6}$M) caused a rapid increase of fat cell cyclic AMP levels both in the absence and in the presence of theophylline ($10^{-3}$ M). In both cases a maximal or close to maximal cyclic AMP level was found after 10 min incubation (Fig. 1). The accumulation was inhibited by a high ($10^{-6}$ M) but not by a low ($10^{-8}$ M) concentration of $PGI_2$. The inhibitory effect was also essentially maximal after 10 min incubation and decreased thereafter. Thus, for all subsequent studies this time of incubation was used.

In Fig. 2 the effect of preincubating the cells with $PGI_2$ before the addition of NA is shown. The inhibitory effect of $PGI_2$ ($10^{-7}$ and $10^{-6}$ M) decreased as a consequence of preincubation. Thus the percentage inhibition caused by $10^{-7}$ M $PGI_2$ decreased from 34 ± 4 to 7 ± 4 per cent and that caused by $10^{-6}$ M from 68 ± 2 to 47 ± 5 per cent. This fall in inhibitory potency by incubation tallies with the known instability of $PGI_2$ in aqueous solution [9]. Two

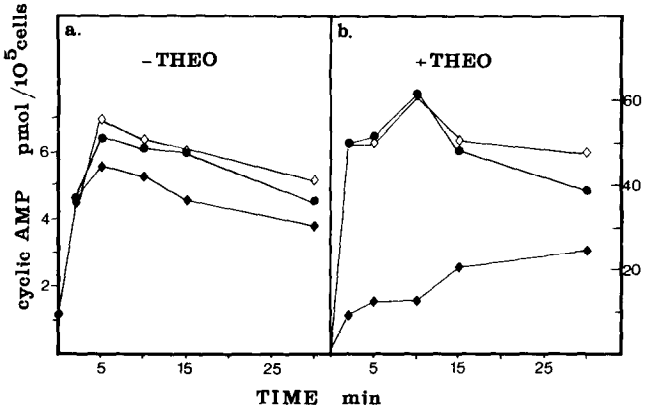


Fig. 1. Time-course of cyclic AMP accumulation in fat cells (85,000 cells/ml) following the administration of noradrenaline (1 $\mu$M) in the absence (●——●) and presence of $PGI_2$ ($10^{-8}$ (◇——◇) and $10^{-6}$ M (◆——◆)). Panel a: no theophylline present. Panel b: theophylline (1 mM) added together with the other drugs. Mean of triplicate determinations.

Table 1. The effect of increasing doses of $PGI_2$ on cyclic AMP accumulation induced by noradrenaline (1 $\mu$M) and theophylline (1 mM)*

| | | Cyclic AMP (% of control) | | |
|---|---|---|---|---|
| $PGI_2$ | $10^{-9}$ M | 103 ± 7 | (6)† | N.S.‡ |
| | $10^{-8}$ M | 121 ± 6 | (22) | P<0.01‡ |
| | $10^{-7}$ M | 72 ± 5 | (10) | P<0.01‡ |
| | $10^{-6}$ M | 35 ± 2 | (16) | P<0.01‡ |
| 6-keto-$PGF_{1\alpha}$ | $10^{-6}$ M | 103 ± 6 | (5) | N.S.‡ |
| $PGE_2$ | $2.10^{-8}$ M | 11 ± 4 | (4) | P<0.01§ |

* The prostaglandins were added simultaneously with noradrenaline. Results are expressed in per cent of corresponding controls without prostaglandins. Mean ± s.e.m. from 2–5 separate experiments.

† Number of determinations within parentheses.

‡ Student's *t*-test.

§ Data from [7].

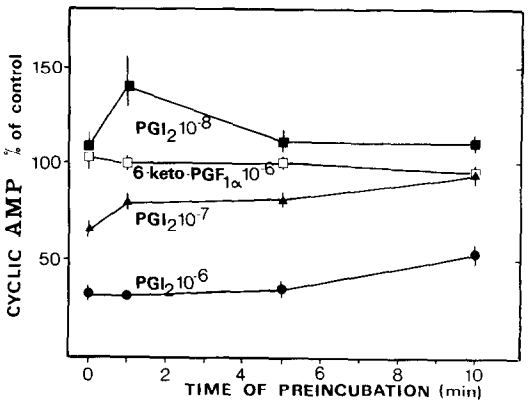


Fig. 2. The effect of preincubation on the actions of $PGI_2$ and 6-keto-$PGF_{1\alpha}$. The prostaglandins were added 10, 5 and 1 min before or at the same time as noradrenaline (1 M) to the fat cell suspension (100,000 cells/ml). Theophylline was present during a 5 min preincubation. After 10 min incubation with the lipolytic drugs, the incubation was terminated as described previously and the cyclic AMP content determined. The results are expressed as per cent of the control value (in the absence of prostaglandin) at the corresponding time. Mean ± s.e.m. of 5 determinations from two separate experiments. In the absence of lipolytic drug the cyclic AMP content was 2.1 ± 0.2 pmole/$10^5$ cells. In the presence of noradrenaline and theophylline it was 53 ±-4 pmole/$10^5$ cells.

other interesting features are present in Fig. 2. First, a low dose of $PGI_2$ ($10^{-8}$ M) stimulated rather than inhibited cyclic AMP accumulation. Second, the product of $PGI_2$ rearrangement in aqueous solution, 6-keto-$PGF_{1\alpha}$ [9], had no discernible effect on cyclic AMP accumulation.

The results of several experiments on the effect of $PGI_2$ are summarized in Table 1. It is seen that at concentrations between $10^{-9}$ and $10^{-7}$ M there was a significant potentiation of the cyclic AMP response. At higher concentrations, $PGI_2$ was inhibitory and fifty per cent inhibition was obtained between $10^{-7}$ and $10^{-6}$ M $PGI_2$. The metabolite of $PGI_2$, 6-keto $PGF_{1\alpha}$, was ineffective, while $PGE_2$ was several times more potent than $PGI_2$ (see Table 1).

The results presented in Table 2 demonstrate that the low dose of $PGI_2$ ($10^{-8}$ M) potentiated cyclic AMP accumulation induced by several concentrations of NA. Conversely, $10^{-6}$ M $PGI_2$ inhibited cyclic AMP accumulation induced by NA $10^{-8}$to $10^{-6}$ M, except when the effect of NA was antagonized by PIA, in which case $PGI_2$ ($10^{-6}$ M) had no further inhibitory effect. This could indicate that the two compounds have a similar or identical point of attack.

The present results thus show that $PGI_2$ in a low concentration range may enhance rather than inhibit noradrenaline induced cyclic AMP accumulation, although the effect is small. A similar behaviour has been reported earlier for $PGH_2$ and $PGD_2$ [7]. Since these low concentrations are the ones likely to be encountered *in vivo*, the effect of these prostaglandins might be, if anything, stimulatory, in obvious contradiction to a proposed feed-back inhibitory role.

The present findings further show that even though $PGI_2$ is more potent than $PGE_2$ on e.g. vascular relaxation, on blood platelets and on human lymphocytes [10–11, 14], this order or potency is not universal. This suggests that there are differences in ligand affinity of prostaglandin receptors in different tissues.

Inspection of the data concerning the conversion of $PGH_2$ in fat cells and fat cell homogenates published earlier [7] reveals that there is no significant formation of 6-keto-$PGF_{1\alpha}$, the stable metabolite of the unstable $PGI_2$ [9]. This implies that there is little formation of $PGI_2$ by these cells. On the other hand, $PGI_2$ may be formed by the vascular cells in adipose tissue, but then $PGI_2$ would have to diffuse from this site of formation to the fat cells in order to exert

Table 2. Effect of $PGI_2$ on cyclic AMP accumulation induced by noradrenaline (NA) alone or in combination with theophylline (Theo) and phenylisopropyladenosine (PIA)*

| Treatment* | NA or PIA concn. ($\mu$M) | Cyclic AMP (pmoles/$10^5$ cells) $PGI_2$ None | $10^{-8}$M | $10^{-6}$M |
|---|---|---|---|---|
| None | | 2.8 ± 0.8 | 2.0 ± 0.8 | 1.9 ± 0.3 |
| NA | 1 | 7.8 ± 1.4 | 6.6 ± 1.2 | 3.6 ± 1.2 |
| Theo | | 3.3 ± 1.3 | 3.3 ± 0.7 | 3.6 ± 1.2 |
| Theo + NA | 0.01 | 4.1 ± 1.0 | 7.9 ± 3.0 | 3.0 ± 1.2 |
| Theo + NA | 0.1 | 22.8 ± 5.0 | 37.6 ± 6.9 | 6.6 ± 1.4 |
| Theo + NA | 1 | 45.0 ± 5.0 | 68.4 ± 11.4 | 25.2 ± 5.4 |
| Theo + NA + PIA‡ | 1 | 16.4 ± 1.8 | 28.6 ± 5.2 | 14.8 ± 5.2 |

* Mean ± s.e.m. of triplicate determination (100,000 cells/ml). The drugs were added simultaneously and the incubation was allowed to proceed for 10 min.

† Theophylline (Theo) concentration was always 1 mM.

‡ Phenylisopropyladenosine (PIA) concentration was always 0.1 mM.

its action on adipocytes. It has been proposed that $PGI_2$ is a circulating hormone [15]. Therefore circulating $PGI_2$ could possibly modulate adipocyte function. In either case the $PGI_2$ levels are such as to have no effect or even cause a stimulation rather than inhibition of cyclic AMP formation. Thus, in spite of the recent developments, there seems to be little reason to change the previous negative conclusions [1–5] concerning the physiological importance of prostaglandins as negative feed-back regulators in adipose tissue.

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