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PROSTACYCLIN AND LIPOLYSIS IN RAT FAT CELLS*

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Abstract—In rat fat cells incubated for 15 min at 37° and pH 8.5, glycerol release was highly stimulated both by norepinephrine and by theophylline. Prostacyclin (PGI_2) (10^{-8}–10^{-7} M) did not alter the basal rate of glycerol release but potentiated the lipolytic effect of 2×10^{-6} M norepinephrine. The rate of norepinephrine-induced glycerol release was increased by PGI_2 during 10 min of incubation and then maintained for the next 5 min. Lipolysis induced by concentrations of norepinephrine which produced maximal effects was not altered by PGI_2. PGI_2 (10^{-7}–10^{-6} M) also potentiated the effect of 5×10^{-4} M theophylline on glycerol release, but antagonized the stimulation induced by a maximally effective concentration of the methylxanthine (2×10^{-3} M). Incubation of the cells with norepinephrine in the presence of 2×10^{-4} or 5×10^{-4} M theophylline caused a loss of the potentiating effect of PGI_2 on norepinephrine-induced lipolysis. In the presence of 10^{-3} M theophylline, the lipolytic action of norepinephrine was inhibited by PGI_2. In fat cells incubated with adenosine deaminase (0.5 U/ml), 2.5×10^{-7} M PGI_2 did not alter the response to 5×10^{-4} M theophylline and inhibited the effect of norepinephrine both in the absence and in the presence of theophylline. The present results show that, under appropriate experimental conditions, PGI_2 may act as a lipolytic agent in isolated fat cells and that some kind of interaction exists between stimulation of methylxanthine-sensitive adenosine receptors and stimulation of PGI_2 receptors.

Prostacyclin (PGI_2) synthesis has been recently shown to occur in fat cells [1] as well as in endothelial cells of adipose tissue artery [2].

Very little is known about the effects of PGI_2 on lipolysis in adipose tissue and isolated adipocytes. According to Lambert and Jacquemin [3] PGI_2 inhibits catecholamine-induced lipolysis both in rat fat cells and in adipose tissue incubated in the presence of a methylxanthine. This is in agreement with the marked antilipolytic activity of PGI_2 previously observed in intact rat adipose tissue [4], while no effect of PGI_2 was detected by other authors in isolated fat cells, under a variety of incubation times and temperatures [1].

As PGI_2 is very unstable in aqueous solutions and its degradation rate is markedly influenced by temperature and pH [5–8], the various experimental conditions chosen for the above mentioned studies may account, at least in part, for the discrepancies among the previously reported results.

Moreover a possible interference of endogenous adenosine with the effects of prostaglandins on lipolysis is suggested by the following observations: prostaglandin production inhibitors potentiate lipolysis stimulated by the adenosine receptor antagonist, theophylline, but not by isoproterenol [9]; the antilipolytic action of PGE_1 is increased in fat cells incubated with adenosine deaminase [10]; PGE_1 is much more effective against theophylline than against catecholamine-induced lipolysis [11, 12]. Thus also the possibility that the action of PGI_2 on lipolysis is affected by adenosine released by fat cells during the incubation should be taken into consideration.

The aim of the present study is to reexamine the possible effects of PGI_2 on lipolysis in rat adipocytes by incubating the cells in a condition which would delay the inactivation of this prostaglandin without hindering a normal response to lipolytic agents. For this purpose the influence of buffer pH and temperature as well as of the incubation time on the lipolytic response was examined. Furthermore the possible interference of adenosine with PGI_2 action was evaluated by incubating the cells in the presence of theophylline and/or adenosine deaminase.

MATERIALS AND METHODS

Epididymal fat pads were removed from male Wistar rats (180–240 g b.w.) under light ether anaesthesia, and immediately washed in Krebs–Ringer bicarbonate (K–R b) buffer, pH 7.4, containing 3% bovine serum albumin fraction V. The composition of the buffer was the following (mM): NaCl 119; KCl 4.5; $CaCl_2$ 2.7; KH_2PO_4 1.2; $MgSO_4$ 1.2; $NaHCO_3$ 24.2. The tissue was minced and the fragments were suspended in the same buffer in the presence of crude collagenase (1 mg/ml). Fat cells were isolated according to the method of Rodbell and Krishna [13]. After the last cell wash, the final cell suspension was made with K–R b albumin buffer having a pH of either 7.4 or 8.5. The latter was obtained by the addition of an appropriate volume of glycine buffer (pH 11) to the K–R b albumin buffer pH 7.4. Glycine buffer was used instead of Tris or NaOH, because it caused no salt precipitation in the solution and

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Table 1. Influence of incubation temperature and pH on the response of rat fat cells to norepinephrine and theophylline

Temperature (°C)	pH	Glycerol (μmoles/10^6 cells/15 min) Control	Norepinephrine (2×10^{-6} M)	Theophylline (3×10^{-4} M)
37	7.4	0.074 ± 0.015	0.181 ± 0.017*	0.148 ± 0.017†
37	8.5	0.063 ± 0.013	0.236 ± 0.016*	0.172 ± 0.015*
30	7.4	0.043 ± 0.016	0.104 ± 0.027	0.089 ± 0.022
30	8.5	0.055 ± 0.019	0.111 ± 0.018‡	0.102 ± 0.013

Fat cells were incubated in Krebs–Ringer bicarbonate albumin buffer at a pH of 7.4 or 8.5, as indicated. The incubation temperature was 30° or 37°. After 15 min preincubation norepinephrine or theophylline were added and the samples were further incubated for 15 min.

Each value is the mean (±S.E.) of data obtained from 9 incubations in 3 experiments. The significance of the differences between glycerol levels measured in the presence of norepinephrine (second column) or theophylline (third column) and the respective controls (first column) was calculated by Student's *t*-test. Where the P value is not indicated, the difference is not significant ($P > 0.05$).

* $P < 0.001$; † $P < 0.005$; ‡ $P < 0.05$.

allowed the highest reproducibility in the results. Furthermore at pH 8.5 the buffer remained stable throughout the incubation period.

The number of fat cells in the final suspension (25,000–50,000 cells/ml) was estimated by the dilution method and counting under the microscope as described by Rodbell and Krishna [13]. Aliquots of 2 ml of the cell suspension were preincubated for 15 min in a metabolic shaker, in the absence or presence of adenosine deaminase (0.5 U/ml). PGI_2, norepinephrine and/or theophylline were then added in 10 μl volumes and the samples further incubated for 15 min unless otherwise stated.

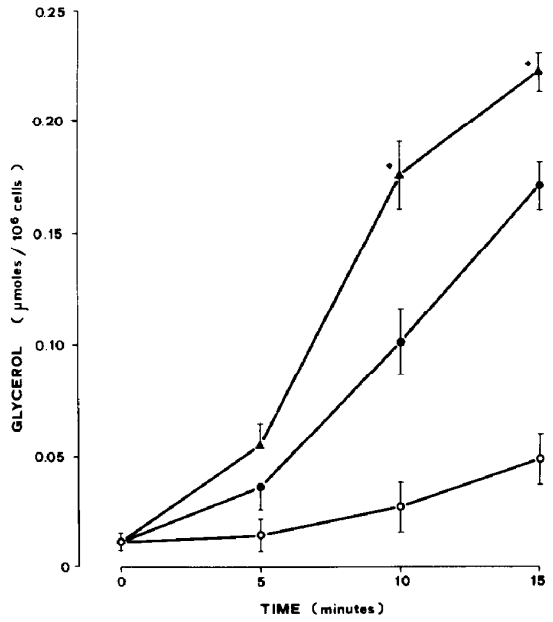

Fig. 1. Time course of lipolysis in the absence and presence of norepinephrine and norepinephrine plus PGI_2. Fat cells were preincubated for 15 min in Krebs–Ringer bicarbonate albumin buffer (pH 8.5) at 37° before the addition of norepinephrine and PGI_2. The reaction was then stopped at different times as indicated. Each value is the mean (±S.E.) of the data obtained from 9 incubations in 3 experiments. The statistical significance of changes induced by PGI_2 was calculated by Student's *t*-test: ○—○, basal; ●—●, 2×10^{-6} M norepinephrine; ▲—▲, 2×10^{-6} M norepinephrine + 10^{-7} M PGI_2. * $P < 0.005$.

The incubation temperature was 37°, except for the preliminary experiments in which the influence of a lower temperature (30°) on lipolysis was tested (see Table 1).

The incubation was terminated by the addition of 0.2 ml of 50% trichloroacetic acid (TCA). After mixing and centrifugation of the samples, 0.5 ml aliquots of the supernatant fluid were used for the estimation of the glycerol content, which was taken as an index of lipolysis. Glycerol was determined according to the method of Lambert and Neish [14] by using the acetyl acetone reagent as proposed by Nash [15]. Samples containing known amounts of glycerol dissolved in K–R b albumin buffer were added in each experiment as a standard.

Stock solutions of PGI_2 (2 mM) in absolute ethanol were stored at −35° and diluted with ethanol immediately before use. The indicated amounts of PGI_2 were added to the samples in 10 μl volumes. The same volume of ethanol was added where PGI_2 was not present.

Theophylline, (−)norepinephrine bitartrate, bovine serum albumin fraction V and crude collagenase (from Clostridium venom, type II) were purchased from Sigma Chemical Co. (St. Louis, MO). Prostacyclin sodium salt was a generous gift by Carlo Erba (Milano, Italy). All other chemicals and reagents were of analytical grade.

RESULTS

Choice of the experimental conditions: temperature, pH and incubation time

As the rate of PGI_2 inactivation can be reduced by increasing the pH of the medium [8] or lowering the incubation temperature [6, 7], the influence of such changes on the lipolytic response of fat cells to norepinephrine and theophylline was tested. For this purpose the cells were incubated in K–R b albumin

Table 2. Influence of different PGI_2 concentrations on basal and stimulated lipolysis

PGI_2 (M)	Glycerol (μmoles/10^6 cells/15 min) Control	Norepinephrine (2×10^{-6} M)	Theophylline (5×10^{-4} M)
0	0.078 ± 0.008	0.235 ± 0.019	0.153 ± 0.006
10^{-9}	0.071 ± 0.019	0.297 ± 0.025	0.160 ± 0.005
10^{-8}	0.065 ± 0.008	0.380 ± 0.017*	0.160 ± 0.007
10^{-7}	0.068 ± 0.012	0.395 ± 0.034†	0.211 ± 0.019‡
10^{-6}	0.063 ± 0.012	0.204 ± 0.019	0.207 ± 0.014†

Fat cells were preincubated in Krebs–Ringer albumin buffer (pH 8.5) at 37° for 15 min. Norepinephrine, theophylline and PGI_2 were then added where indicated and the reaction was stopped 15 min later by the addition of 0.2 ml of 50% TCA.

Each value is the mean (±S.E.) of data obtained from 10 to 12 incubations in 4 experiments. The statistical significance of the changes induced by PGI_2 was calculated according to Student's *t*-test. Where the P value is not indicated, the difference from the respective control (first value of each column) is not significant ($P > 0.05$).

* $P < 0.001$; † $P < 0.005$; ‡ $P < 0.01$.

buffer at pH 7.4 as well as 8.5 and the incubation temperature was maintained at 30° or 37°.

At 37° (Table 1) incubation of fat cells for 15 min with 2×10^{-6} M norepinephrine or 3×10^{-4} M theophylline caused a marked and highly significant stimulation of glycerol release. Under these conditions the highest rates of stimulated lipolysis were measured in the samples incubated at pH 8.5.

At 30° (Table 1) the increases of glycerol release induced by norepinephrine or theophylline were not statistically significant from control except for the change caused by norepinephrine at pH 8.5.

On the basis of these results in all the following experiments fat cell samples were incubated at 37° and pH 8.5. Furthermore incubation times longer than 15 min could not be used, as the potentiating

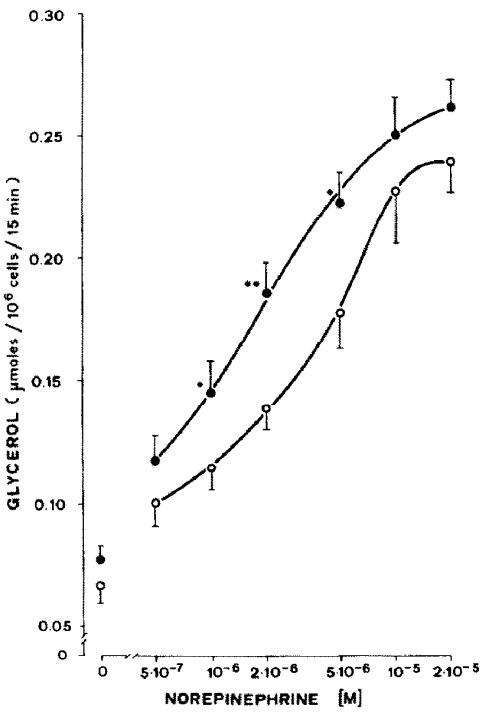

Fig. 2. Concentration–response curve for norepinephrine in the absence and presence of 10^{-7} M PGI_2. After 15 min preincubation of fat cells in Krebs–Ringer bicarbonate albumin buffer (pH 8.5) at 37°, various concentrations of norepinephrine with or without 10^{-7} M PGI_2 were added to the samples. The reaction was stopped 15 min later. Each value is the mean (±S.E.) of the data obtained from 12 incubations in 4 experiments. The statistical significance of the changes induced by PGI_2 was calculated by Student's *t*-test: ○—○, norepinephrine; ●—●, norepinephrine + 10^{-7} M PGI_2. * $P < 0.05$; ** $P < 0.001$.

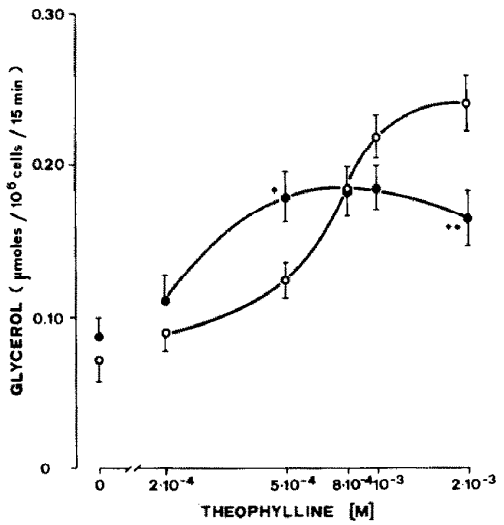

Fig. 3. Concentration–response curve for theophylline in the absence and presence of 10^{-7} M PGI_2. Fat cells were preincubated for 15 min in Krebs–Ringer bicarbonate albumin buffer (pH 8.5) at 37°. Various concentrations of theophylline with or without 10^{-7} M PGI_2 were then added to the samples, which were further incubated for 15 min. Each value is the mean (±S.E.) of the data obtained from 12 incubations in 4 experiments. The statistical significance of the changes induced by PGI_2 was calculated by Student's *t*-test: ○—○, theophylline; ●—●, theophylline + 10^{-7} M PGI_2. * $P < 0.05$; ** $P < 0.01$.

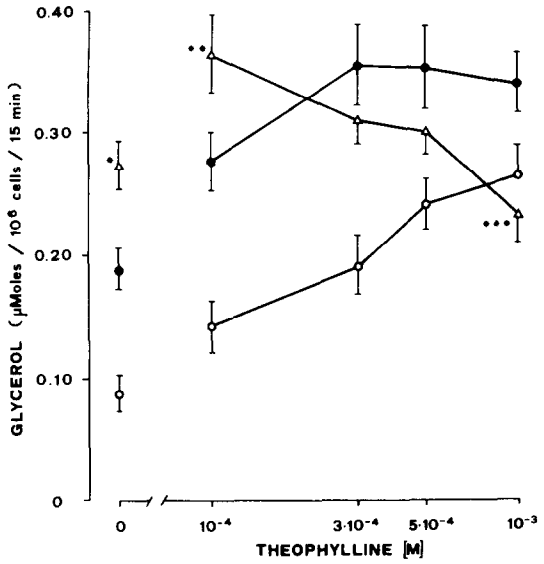

Fig. 4. Influence of PGI_2 on norepinephrine-induced glycerol release in the presence of different theophylline concentrations. Fat cells were preincubated for 15 min in Krebs–Ringer bicarbonate buffer (pH 8.5) at 37° before the addition of the drugs. Theophylline, at the indicated concentrations, was added to all the samples, except for the control ones (values indicated on the left side of the figure). Norepinephrine (2×10^{-6} M) and PGI_2 (5×10^{-8} M) were also added where indicated. The reaction was stopped 15 min later: ○—○, theophylline; ●—●, theophylline plus norepinephrine; △—△, theophylline plus norepinephrine plus PGI_2. Each value is the mean (±S.E.) of the data obtained from 9 incubations in 3 experiments. The statistical significance of the changes induced by PGI_2 on norepinephrine-induced glycerol release was calculated by Student's *t*-test. * $P < 0.005$; ** $P < 0.05$; *** $P < 0.02$.

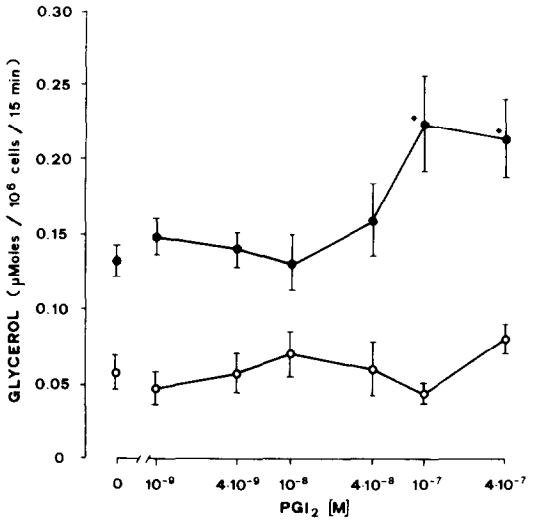

Fig. 5. Influence of PGI_2 on lipolysis in the absence and presence of adenosine deaminase. Adenosine deaminase (0.5 U/ml) was added to the samples at the beginning of the 15 min preincubation period. Incubation was then started by the addition of various PGI_2 concentrations, as indicated and stopped 15 min later: ○—○, no adenosine deaminase; ●—●, 0.5 U/ml adenosine deaminase. Each value is the mean (±S.E.) of the data obtained from 8 incubations in 2 experiments. The statistical significance of the changes induced by PGI_2 is indicated. * $P < 0.025$.

effect of PGI_2 on norepinephrine-induced lipolysis, which was measurable at this time (Fig. 1), did not last up to 30 min (not shown).

Influence of PGI_2 on norepinephrine-induced lipolysis

In all the experiments PGI_2 did not alter the basal rate of lipolysis, at any concentration tested (10^{-9}–10^{-6} M) (Table 2). When the release of glycerol was submaximally stimulated by norepinephrine (2×10^{-6} M), 10^{-8} and 10^{-7} M PGI_2 significantly potentiated the effect of this catecholamine (Table 2).

Figure 1 shows the time course of lipolysis in the absence and presence of norepinephrine (2×10^{-6} M) and of PGI_2 (10^{-7} M). The rate of norepinephrine-induced lipolysis was increased by PGI_2 only during the first 10 min of incubation, while from 10 to 15 min no further stimulation was observed. The basal rate of lipolysis was not altered by PGI_2 (not shown).

Concentration–response curves for norepinephrine were obtained in the absence and presence of PGI_2 (10^{-7} M). As shown in Fig. 2, PGI_2 significantly potentiated the effect of submaximal (10^{-6}–5×10^{-6} M), but not maximal (10^{-5}–2×10^{-5} M) norepinephrine concentrations, thus shifting the concentration–response curve to the left without significant alterations in the maximal effect.

Influence of PGI_2 on theophylline-induced lipolysis

The stimulation of glycerol release induced by 5×10^{-4} M theophylline was potentiated by PGI_2 (Table 2). The concentration range in which PGI_2 elicited this effect (10^{-7}–10^{-6} M) was higher as compared to the one required for increasing the rate of norepinephrine-induced lipolysis. Furthermore, at concentrations of theophylline higher than the EC_{50}, PGI_2 was ineffective and it inhibited the release of glycerol maximally activated by 2×10^{-3} M theophylline (Fig. 3).

Influence of theophylline on PGI_2-induced potentiation of lipolysis stimulated by norepinephrine

The potentiating effect of PGI_2 (5×10^{-8} M) on lipolysis stimulated by 2×10^{-6} M norepinephrine was reversed by theophylline (10^{-4}–10^{-3} M) in a concentration-dependent manner (Fig. 4). At 10^{-4} M theophylline the potentiation by PGI_2 was still significant; at 3×10^{-4} and 5×10^{-4} M theophylline, norepinephrine-induced glycerol release was not altered by PGI_2 while in the presence of 10^{-3} M theophylline it was inhibited (Fig. 4). Similarly PGI_2 (10^{-7} M) was unable to alter the effect of a wide range of norepinephrine concentrations (10^{-7}–

Table 3. Influence of adenosine deaminase and theophylline on PGI_2-induced changes of basal and norepinephrine-stimulated lipolysis

Drugs (M)	Glycerol (μmoles/10^6 cells/15 min)			
	Control		ADA (0.5 U/ml)	
	—	+PGI_2 (2.5×10^{-7} M)	—	+PGI_2 (2.5×10^{-7} M)
—	0.071 ± 0.010	0.092 ± 0.017	0.146 ± 0.016	0.212 ± 0.018*
Theophylline 5×10^{-4}	0.146 ± 0.017	0.198 ± 0.016†	0.194 ± 0.016	0.182 ± 0.009
Norepinephrine 2×10^{-6}	0.172 ± 0.012	0.235 ± 0.017*	0.337 ± 0.026	0.239 ± 0.026‡
			—	+PGI_2 (10^{-7} M)
Theophylline 5×10^{-4}			0.189 ± 0.018	0.163 ± 0.010
Norepinephrine 10^{-6}			0.235 ± 0.007	0.187 ± 0.016*
Norepinephrine 10^{-6} + theophylline 5×10^{-4}			0.239 ± 0.015	0.189 ± 0.018†
Norepinephrine 5×10^{-6}			0.276 ± 0.007	0.228 ± 0.012†
Norepinephrine 5×10^{-6} + theophylline 5×10^{-4}			0.284 ± 0.018	0.218 ± 0.016*

Fat cells were preincubated for 15 min in the absence or presence of adenosine deaminase (ADA). Theophylline (5×10^{-4} M), PGI_2 (2.5×10^{-7} M) and the indicated concentrations of norepinephrine were then added and the reaction was stopped 15 min later. The results shown in the table were obtained in two sets of 3 experiments. Each value is the mean (±S.E.) of the data obtained from 8 to 10 incubations. The statistical significance of the changes induced by PGI_2 is indicated.

* $P < 0.025$; † $P < 0.05$; ‡ $P < 0.02$.

5×10^{-6} M) when 5×10^{-4} M theophylline was present in the incubation medium (not shown).

Influence of adenosine deaminase on the effects of PGI_2

Addition of adenosine deaminase (0.5 U/ml) to the incubation medium markedly increased the rate of glycerol release from fat cells (Fig. 5). Under these conditions PGI_2 (10^{-7} and 4×10^{-7} M) exerted a significant stimulatory effect on lipolysis, which was not observed in the absence of adenosine deaminase (Fig. 5).

The potentiation of theophylline (5×10^{-4} M) effect by 2.5×10^{-7} M PGI_2 was abolished by adenosine deaminase treatment (Table 3). In these experiments glycerol levels measured after addition of PGI_2 in cells treated with theophylline, adenosine deaminase or adenosine deaminase plus theophylline were not statistically different (Table 3).

Adenosine deaminase also converted the stimulatory effect of PGI_2 on norepinephrine-induced lipolysis into an inhibitory one (Table 3). Under these conditions PGI_2 (10^{-7}, 2.5×10^{-7} M) reduced the effect of any norepinephrine concentration tested (10^{-6}–5×10^{-6} M) and this antagonism was not affected by the addition of 5×10^{-4} M theophylline (Table 3).

DISCUSSION

The results of the present study show that, under appropriate experimental conditions, PGI_2 elicits stimulatory effects on fat cell lipolysis.

The first objective was to choose the incubation temperature, pH and time most suitable for delaying PGI_2 inactivation and evaluating its effect in a system in which a clear lipolytic response to stimulatory agents was maintained.

Gryglewski *et al.* showed that, at pH 7.5, inactivation of PGI_2 occurs within 10 min of incubation at 37° [6] and within 20 min at 22° [7]. It is also known that low temperature reduces basal as well as stimulated lipolysis in adipose tissue [16]. In the present experiments lowering the incubation temperature from 37° to 30° caused a decrease in the amount of glycerol released from fat cells in response to norepinephrine and to theophylline and these effects were highly variable. This made the lowering of incubation temperature as a means for preserving PGI_2 not a viable method. Moncada *et al.* demonstrated that, at 22°, the antiaggregatory activity of PGI_2 is undiminished after 20 min incubation at pH 8.5 or 9.5 [7] and Mosinger reported that a pH related peak in the free fatty acid release from adipose tissue in response to epinephrine occurs at pH 8.4 [16]. According to the present results glycerol release from fat cells incubated at 37° was markedly increased by lipolytic agents at pH 8.5. Thus this pH was used throughout the study. Furthermore a 15 min incubation time was chosen, as it allowed the detection of significant changes of glyercol level in response to norepinephrine and theophylline as well as to PGI_2, while a loss of PGI_2 effect occurred at longer times.

PGI_2 stimulated the release of glycerol induced by submaximally effective norepinephrine concentrations. This stimulation was short lasting, which may be due to the rapid inactivation PGI_2 undergoes in aqueous solution. The lack of PGI_2 effect on norepinephrine-induced lipolysis observed by other authors [1] in fat cells incubated for 10 or 45 min at 37° or 25° might be explained by an even faster inactivation of PGI_2 at physiological pH. Similarly some degradation product of PGI_2 might be responsible for the reported inhibition of catecholamine-stimulated lipolysis by PGI_2 in fat cells incubated for 60 min at neutral pH [3]. PGI_2 did not significantly alter the rate of lipolysis in the presence of maximally effective catecholamine concentrations, but shifted to the left the concentration–response curve for

norepinephrine, indicating that this potentiation might be of competitive nature. A stimulatory effect of PGI_2 on catecholamine-induced accumulation of cAMP in fat cells was previously observed by Fredholm *et al.* [20], but changes in lipolysis were not evaluated in parallel in that study. Thus the possible involvement of cAMP in the lipomobilizing effect of PGI_2 remains to be elucidated.

The interactions between PGI_2 and theophylline appear to be related, at least in part, to the blockade of adenosine R-receptors operated by this methylxanthine [17–19]. This is suggested by the similarity between the effects induced by PGI_2 in fat cells incubated with theophylline and in adenosine deaminase-treated cells. In fact PGI_2 increased the rate of lipolysis induced by low theophylline concentrations. The concentrations of PGI_2 exerting this effect were higher than those required to potentiate norepinephrine action, but were comparable to those eliciting a lipolytic effect in adenosine deaminase-treated cells. Moreover, both theophylline and adenosine deaminase caused a reversal of PGI_2 effect on norepinephrine-activated lipolysis. The changes of PGI_2 action on basal as well as catecholamine-induced glycerol release, which occurred upon adenosine deaminase treatment, were not modified by further addition of theophylline to the incubation system. These results indicate that the influence of theophylline on PGI_2 effects is secondary to the interference of this methylxanthine with the adenosine system, which is also the site of adenosine deaminase action.

The inhibitory action of PGI_2 on norepinephrine-induced lipolysis, which was observed in fat cells incubated with theophylline and/or adenosine deaminase, is in accordance with the previously reported inhibition of catecholamine-activated cAMP accumulation [20] and adenylate cyclase activity [21] by PGI_2 in rat fat cells.

Two major conclusions emerge from the present results, i.e. that PGI_2, under certain conditions, may act as a lipolytic agent and that an interaction exists between stimulation of adenosine receptors and stimulation of putative prostacyclin receptors.

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