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RAT ADIPOCYTE LIPOPROTEIN LIPASE ACTIVITY IS INHIBITED BY THEOPHYLLINE AND STIMULATED BY INOSINE THROUGH ADENOSINE-INDEPENDENT MECHANISMS.

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SUMMARY : Incubation of rat adipose tissue or isolated rat adipocytes with high (50 mM) but not with low concentrations (0.5 mM) of theophylline results in a decrease of lipoprotein lipase (LPL) activity. This effect is not altered by the addition of adenosine deaminase, indicating that the decrease of adipose LPL activity by theophylline is not due to the competition of theophylline with adenosine. On the contrary, incubation of isolated fat cells with adenosine (0.1 - 100 µM) results in an increase of the intracellular form of LPL activity. As this effect is also observed in cells incubated with adenosine deaminase (40 mU/ml) or with inosine (0.1 - 100 µM) but not in cells incubated with the adenosine analog N^6-phenylisopropyladenosine, it is concluded that the increase in the intracellular form of LPL found after incubation with adenosine is not due to adenosine per se but to inosine generated from the breakdown of endogenous adenosine by adenosine deaminase.

INTRODUCTION :

Different studies have provided indirect evidence that cyclic AMP may play a role in the regulation of lipoprotein lipase (LPL) in adipose tissue. As a matter of fact, it was reported that dibutyryl cyclic AMP, catecholamines and glucagon decreased LPL activity in adipose tissue in vitro (1 - 3), while insulin promoted this activity (4). Theophylline, a potent inhibitor of cyclic nucleotide phosphodiesterase (5), has also been shown to decrease adipose tissue LPL activity (6 - 7). However, as theophylline also potently inhibits the R-site mediated adenosine effect (8), i.e. inhibition of adenylate cyclase in adipose tissue (9 - 10), it is not known whether the inhibitory effect of theophylline on adipose tissue LPL is the consequence of the interaction of theophylline with adenosine or with phosphodiesterase.

To answer this question, we have investigated the effects of different concentrations of theophylline, adenosine and its analog N^6-phenylisopropyladenosine

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(PIA) (8), adenosine deaminase and inosine on LPL activity in adipose tissue and/or in isolated adipocytes from the rat. The results presented here indicate that theophylline decreases LPL activity through phosphodiesterase inhibition and that inosine increases LPL activity in rat adipocytes. Finally, the failure to find in this study any significant effect of PIA on this enzyme activity *in vitro* also suggests that the recently reported stimulation of adipose tissue and post-heparin plasma LPL activities by PIA *in vivo* (11) is not due to a direct effect of this compound on these activities.

MATERIAL AND METHODS :

Sources of materials were as follows : tri(1-^{14}C) oleylglycerol (50 mCi/mmol), Amersham, Radiochemical Centre (England), trioleylglycerol, adenosine and inosine, Sigma Chemical Co (USA) ; theophylline, Merck (Western Germany) ; adenosine deaminase and N^6-phenylisopropyladenosine (PIA),Boehringer Mannheim GmbH (Western Germany). All other chemicals were of analytical grade. Ion exchange resin (Amberlite IRA-400) was prepared according to Kelley (12).

Male Wistar rats (120-150 g) were fasted overnight before sacrifice. After decapitation, epididymal fat pads were excised and isolated fat cells were prepared as previously described (13). Adipose tissue (200 mg/ml) or isolated fat cells (500 µl packed cells/ml) were incubated under O_2/CO_2 (95/5 ; v/v) in the absence or presence of the compounds to be tested in Krebs - Ringer bicarbonate buffer (pH 7,4) containing 1.25 mM calcium, 5 mM glucose, 20 mg/ml dialysed bovine albumin (fraction V) and an amino-acid mixture (final concentration 390 mM) the composition of which corresponded to the amino-acid concentration of rat plasma (14).

Incubation of isolated fat cells were performed in the same buffer containing 3.8 % fresh rat serum. After a two hour incubation at 37°C (adipose tissue) or 25°C (fat cells), adipose tissue fragments or fat cells were removed from the incubation buffer, homogenized at 4°C in 50 mM NH_4OH-NH_4Cl buffer (pH 8.6) and centrifuged at 4°C. The clear supernatant and the fat cell incubation medium were used for the assay of LPL.

Assay of LPL was performed according to a modification of the procedure of Greten and Walter (15) as previously described (13). LPL activity was calculated as µmol free fatty acids (FFA) released per gram fat pad or gram cell lipid per hour. Each value is the mean ± S.E. of three to four experiments performed in triplicate. Statistically significant differences were determined by using Student's "t" test. P values equal to or less than 0.02 were considered significant.

RESULTS :

1- Adipose tissue

Fig. 1,A shows the time course of the effect of a high concentration of theophylline (50 mM) on adipose tissue LPL activity. As can be seen, in the absence of theophylline, LPL activity declined with the time of incubation (the activity found after 2 hours being only 30-35 % of the activity at zero time), a result which is in agreement with the observations of Cunningham and Robinson (16). In the presence of theophylline this decline was more pronounced, the activity found at each time being 32 - 35 % lower than in the controls ($P < 0.001$). When

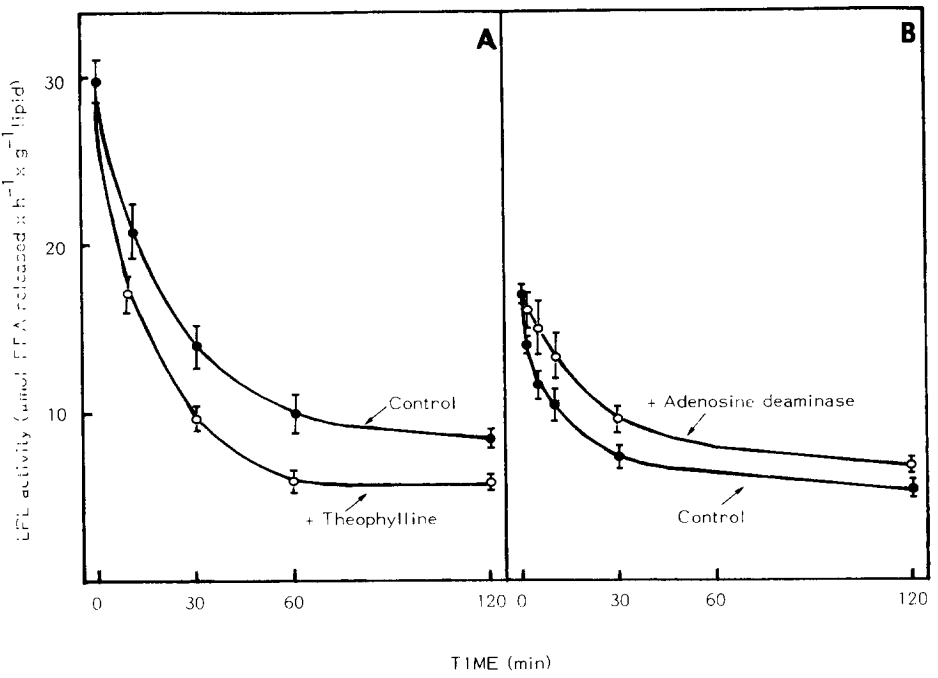

Fig. 1 : Influence of theophylline (50 mM) and adenosine deaminase (40 mU/ml) on the loss of adipose tissue lipoprotein lipase (LPL) activity during incubation at 37°C. Adipose tissue fragments were incubated for different periods, at 37°C, in the absence or presence of 50 mM theophylline (A) or 40 mU/ml adenosine deaminase (B), after which adipose tissue LPL activity was determined as described in the text. Each point represents the mean of three separate experiments performed in triplicate and the vertical lines represent 2 S.E.

lower concentrations of theophylline (1 - 100 µM) were used, no significant effect was observed (data not shown), suggesting that the inhibitory effect of high concentrations of theophylline on adipose tissue LPL activity could be due to intratissular cyclic AMP accumulation secondary to the inhibition of phosphodiesterase induced by theophylline under these conditions (5).

To further improve the validity of this hypothesis, the influence of adenosine removal induced by addition of adenosine deaminase (40 mU/ml) was studied under the same experimental conditions as above. As shown in Fig. 1,B a time-dependent decrease in LPL activity was also found, but, in contrast with the data found with theophylline, this decrease was less pronounced in the presence than in the absence of adenosine deaminase (the activity at each time being 20-28 % higher than in controls, $0.001 < P < 0.01$). When incubations were now performed for 2 hours in the presence of both theophylline (50 mM) and adenosine deaminase (40 mU/ml), the potency of theophylline to inhibit the LPL activity stimulated by adenosine deaminase was the

same (48 % inhibition, data not shown) as that found on LPL activity studied in the absence of adenosine deaminase (47 % inhibition).

These results led to conclude that the decrease in LPL activity observed in the presence of high concentrations of theophylline is not due to a competition of theophylline with adenosine but is more likely linked to phosphodiesterase inhibition. In addition, these observations also suggest that adenosine may exert an inhibitory action on adipose tissue LPL activity or that a metabolite of adenosine, possibly inosine, may stimulate this enzyme.

2- Isolated fat cells.

Nilsson-Ehle et al. (17) have shown that during incubation of isolated adipocytes, two different forms of LPL activity could be identified : one form (fraction "a") is released into the incubation medium, whereas the other form (fraction "b") is retained inside the cell. To get more information on the site of action of theophylline and adenosine deaminase on adipose tissue LPL activity, the following experiments were performed using isolated fat cells and the LPL activities were determined both in the cells and in the incubation medium. Fig.2 shows the variations of both forms of LPL activity as a function of the time of incubation as well as the influence of theophylline (50 mM) on these activities. In control cells (no theophylline) and in agreement with Cryer et al. (18), the intracellular LPL activity increased only during the first hour of incubation whereas the LPL release from the cell was linear at least during the first two hours of incubation. In the presence of theophylline, the intracellular activity also increased with time, but the magnitude of this effect was markedly reduced compared to the control (50 % less after 1 hour incubation, $P < 0.001$). Theophylline also induced an almost complete abolition of the LPL release from the cell (Fig. 2).

As shown in Table I, when cells were incubated with adenosine deaminase (40 mU/ml) for two hours, a 50 % increase ($P < 0.001$) of the intracellular form of LPL activity was observed. However, the release of LPL activity from the cells was unaffected under these conditions. Data in Table I also show that, as already observed above in intact adipose tissue, the addition of adenosine deaminase failed to influence the effects of theophylline on both the intra- and extra-cellular LPL forms.

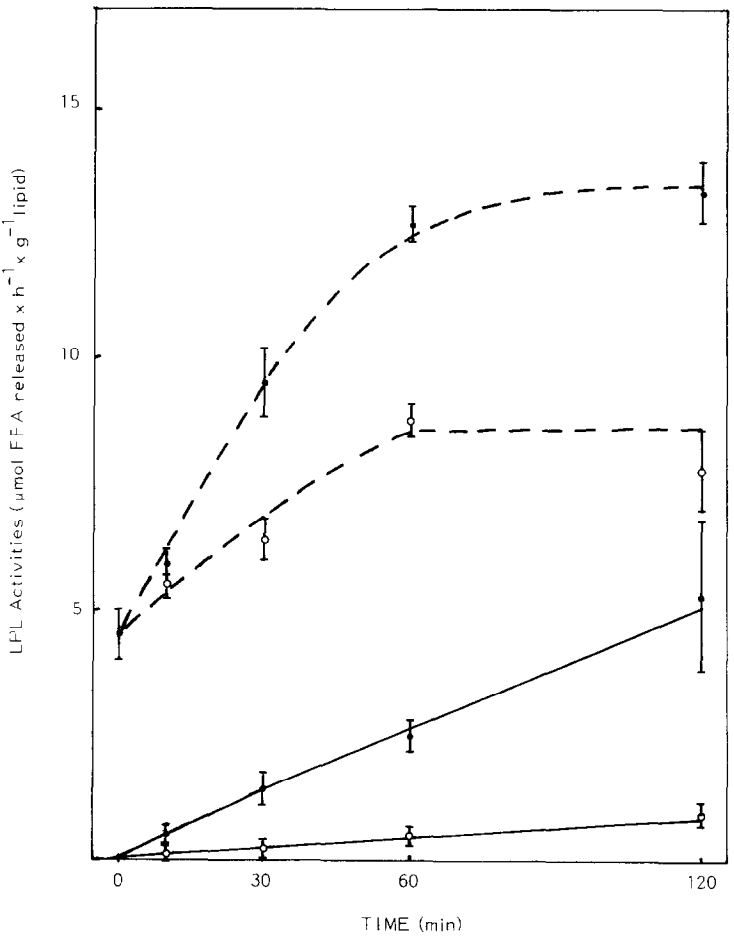

Fig. 2 : Influence of theophylline (50 mM) on the time dependent increase of the intra- (broken lines) and extra-cellular (solid lines) forms of lipoprotein lipase (LPL) activities of rat fat cells. Isolated fat cells were incubated for different periods at 25°C in the absence (•) or presence (o) of 50 mM theophylline, after which the intra- and extra-cellular LPL activities were determined as described in the text. Each point represents the mean of three separate experiments performed in triplicate and the vertical lines represent 2 S.E.

In order to determine whether the increase in the intracellular form of LPL activity which is induced by adenosine deaminase is related to an inhibitory effect of adenosine or to a stimulatory effect of its metabolite inosine, the influence of these nucleosides were also tested. As shown in Fig. 3, none of these nucleosides affected the release of LPL activity. However, both adenosine and inosine increased dose-dependently the intracellular activity, inosine being more potent than adenosine. In fact, although the concentrations of these nucleosides required for half-maximal stimulation of the intracellular LPL activity were similar(2,5µM), a significantly higher stimulation was observed with inosine than with adenosine (115 ± 5 % stimula-

TABLE I : Effects of theophylline and/or adenosine deaminase on the intra- and extra-cellular forms of lipoprotein lipase (LPL) activities of rat fat cells.

	LPL activities (μmol FFA released $\times h^{-1} \times g^{-1}$ lipid)	
	Intra-cellular	Extra-cellular
Control	9.3 ± 0.24	4.3 ± 0.21
Theophylline 5×10^{-2}M	6.5 ± 0.20 P< 0.001 (a)	0.8 ± 0.13 P < 0.001 (a)
Adenosine deaminase 40 mU/ml	14.1 ± 0.35 P < 0.001 (a)	4.1 ± 0.20 P >0.1 (a)
Adenosine deaminase and Theophylline	8.4 ± 1.00 P < 0.001 (b)	1.0 ± 0.12 P < 0.001 (b)

Isolated fat cells were incubated for two hours at 25°C in the absence or presence of theophylline and/or adenosine deaminase, after which the intra- and extra-cellular LPL activities were determined as described in the text. Each value is the mean ± S.E. of three to four separate experiments performed in triplicate. Statistical significance : (a) compared to control ; (b) compared to adenosine deaminase.

tion at 100 μM inosine vs. 90 ± 16 % stimulation at 100 μM adenosine). Contrasting with these data, PIA had no significant influence on the intra- and extra-cellular forms of LPL activity (data not shown).

DISCUSSION :

The present study has shown that the inhibitory effect of theophylline on LPL activity in adipose tissue and in adipocytes is not due to competition of theophylline with adenosine but is likely related to phosphodiesterase inhibition. This further underlines the possible existence of a cyclic AMP-dependent regulation of LPL activity in adipose tissue as was already suggested by the works of Robinson et al. (1,2,6).

Adenosine deaminase, a lipolytic agent (19-21), induced an unexpected increase in LPL activity in both adipose tissue and isolated fat cells. To explain this effect, the influence of adenosine and inosine on LPL activity in adipocytes was also investigated. A significant stimulation of the intracellular form of LPL activity was observed with both nucleosides, inosine being, however, more potent than adenosine. Under the same conditions, however, the adenosine analog,PIA, acting at the R-site (8),

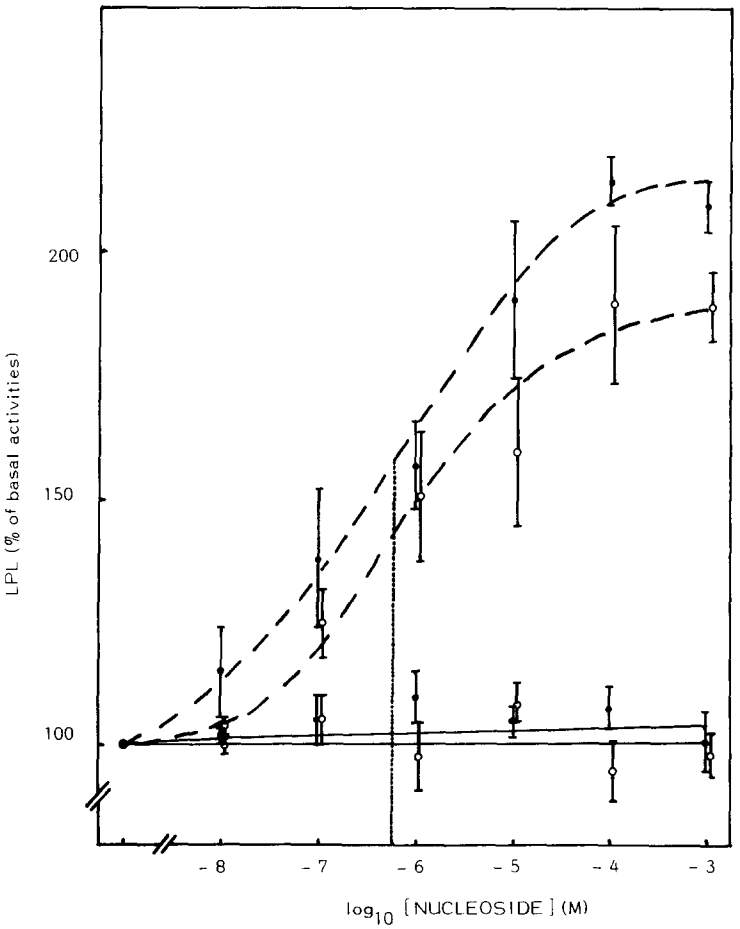

Fig. 3 : Influence of various concentrations of adenosine and inosine on the intra- (broken lines) and extra-cellular (solid lines) forms of lipoprotein lipase (LPL) activities of rat fat cells. Isolated fat cells were incubated for two hours at 25°C in the presence of various concentrations of adenosine (o) or inosine (•) after which the intra- and extra-cellular LPL activities were determined as described in the text. Results were expressed as a percentage of control values, each point representing the mean of three to five separate determinations and the vertical lines represent 2 S.D. The concentration of adenosine or inosine giving half maximal stimulation is indicated by the vertical dotted line.

had no significant effect on the intra- and extra-cellular forms of LPL activity. As the metabolic effects of adenosine are now considered to be initiated by the binding of adenosine to its receptors at both the P- and the R-sites (22), these data suggest that the stimulatory effect of adenosine on LPL activity is likely due to inosine issued from deamination of adenosine rather than to adenosine per se. Giving further support to this are the unexpected opposite effects of adenosine deaminase and theophylline on LPL activity. In fact, because adenosine is an inhibitor of adenylate cyclase in the fat cells (9,10), removal of adenosine by adenosine deaminase should increase

the cyclic AMP level in the cell as does the phosphodiesterase inhibitor,theophylline, and should also inhibit LPL activity as does theophylline. Nevertheless, an increase in LPL activity was observed with adenosine deaminase, indicating thus that this increase was not due to a cyclic AMP-dependent mechanism and therefore not to adenosine but likely to inosine generated from adenosine.

The lack of any significant effect of PIA on adipocyte LPL activity shown in this study is in direct contrast with the recent report of Ohisalo et al. (11). In fact, these authors found that 90 min after the intraperitoneal administration of PIA, both the post-heparin plasma LPL activity and the heparin-releasable adipose tissue LPL activity were increased. Although the influence of PIA on the heparin-stimulated release of LPL from isolated adipocytes was not investigated presently, it can be, however, reasonably assumed from our present results that PIA has no direct effect on the two forms of LPL in the adipocyte. One possible explanation to the in vivo findings of Ohisalo et al. (11) is the fact that PIA and to a lesser extent adenosine increase the blood flow in adipose tissue (23), an effect which leads to an increased tissular supply of circulating substrates. Among these substrates the case of VLDL is of particular interest since an increased supply of VLDL results in an increased supply of the LPL activator factor, the apo C II fraction (24-25), and consequently in an increased LPL activity in adipose tissue.

To conclude, this study has shown : 1) that theophylline inhibits adipose tissue LPL activity through a mechanism which does not involve competition with adenosine, 2) that inosine increases the intra- but not the extra-cellular form of adipose tissue LPL activity. The latter finding underlines the existence of different regulatory processes for these two enzymic forms, a phenomenon which suggests that the effects of inosine described herein could be physiologically relevant. Further investigations are necessary, however, to test this hypothesis and to elucidate the mechanism through which inosine stimulates in vitro the intracellular LPL activity of the adipocyte.

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