

# Stimulatory effects of adenosine, adenosine analogs and insulin on adipose tissue lipoprotein lipase activity and their prevention by phosphodiesterase inhibitors

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Adenosine and its 'R<sub>i</sub>'- and 'P'-site analogs, *N*<sup>6</sup>-phenylisopropyladenosine and 2'-deoxyadenosine, stimulate, like insulin, lipoprotein lipase (LPL) activity in adipose tissue, an effect which is suppressed by cycloheximide. However, adenosine and its analogs do not potentiate the effects of submaximal insulin concentrations. As addition of cyclic AMP phosphodiesterase inhibitors abolishes the LPL stimulatory effects of insulin, adenosine and its analogs, this study suggests that these LPL effects are mediated through common mechanisms which are likely a decrease in cyclic AMP and an increase in LPL biosynthesis.

<i>Adipose tissue</i>	<i>Insulin</i>	<i>Adenosine</i>	<i>Adenosine analog</i>	<i>Lipoprotein lipase</i>	<i>Cyclic AMP</i>
					<i>Phosphodiesterase</i>

## 1. INTRODUCTION

Adenosine elicits some insulin-like effects on adipose tissue metabolism [1]. In fact, like insulin, adenosine reduces the responsiveness of rat adipocytes to different lipolytic hormones [2,3]. As in the case of insulin, these effects seem related to a decrease in intracellular cyclic AMP level since adenosine, like insulin [4,5], stimulates cyclic AMP phosphodiesterase [6] and inhibits adenylate cyclase [7]. It is well established that the adenosine-effect on adenylate cyclase involves the binding of adenosine to two distinct specific sites: one, R<sub>i</sub>-site, is located on the external side of the plasma membrane and has high affinity for adenosine, whereas the other, called the P-site, is located on the inner surface of the plasma membrane and binds adenosine with lower affinity [7-9].

Another important effect of insulin on adipose tissue metabolism is to stimulate lipoprotein lipase (LPL) activity [10,11] and it has been suggested that part of this effect could be related to a decrease in adipose tissue cyclic AMP level [12,13]. We have shown that adenosine and its catabolite

inosine also increase the LPL activity of isolated adipocytes [14]. In the same study, we also found that theophylline, a potent inhibitor of cyclic AMP phosphodiesterase [15] and of adenosine-binding to the R<sub>i</sub>-site [7], decreases LPL activity, an effect which appears unrelated to the adenosine antagonistic action of theophylline [14].

The aim of this study was to determine whether the LPL stimulation induced by adenosine in adipose tissue was a R<sub>i</sub>- or P-site-mediated effect. The effects of adenosine were therefore compared with those of the R<sub>i</sub>-site and P-site adenosine analogs, *N*<sup>6</sup>-phenylisopropyladenosine (PIA) and 2'-deoxyadenosine (DXA), respectively, using conditions under which adenosine deaminase is inhibited by deoxycoformycin [16]. Moreover, to determine whether a common mechanism may be involved in the adenosine and insulin effects on LPL, the role played by protein synthesis and cyclic AMP in these effects was also examined by testing the influence of cycloheximide and phosphodiesterase inhibitors on the abilities of adenosine and insulin to stimulate LPL activity.

## 2. MATERIALS AND METHODS

Porcine insulin (Actrapid, 40 IU/ml) and theophylline were purchased from Novo (France) and Merck (FRG) respectively.  $N^6$ -(L-Phenylisopropyl)adenosine (PIA) and adenosine deaminase were from Boehringer Mannheim (FRG). Adenosine, 2'-deoxyadenosine, cycloheximide and trioleylglycerol were obtained from Sigma (USA) and tri[1- $^{14}$ C]oleylglycerol (50 mCi/mmol) from the Radiochemical Centre (Amersham). Ro 20-1724 and 2'-deoxycoformycin were generous gifts of Hoffman La Roche (Basel) and Substantia Laboratories (France), respectively. All other chemicals were of analytical grade.

Male Wistar rats ( $140 \pm 20$  g) were fasted overnight before killing. Epididymal fat pads were quickly removed, washed, cut into small pieces and incubated (200 mg/ml) under  $O_2/CO_2$  (95/5; v/v)

in Krebs-Ringer bicarbonate buffer (pH 7.4) added or not with the compounds to be tested and containing 1.25 mM calcium, 5 mM glucose, 20 mg/ml dialysed bovine albumin (fraction V), 400 nM dexamethasone, 2 U/ml heparin [17] and an amino acid mixture (final concentrations 390 mM) corresponding to the amino acid composition of rat plasma [18].

After a 2 h incubation at 37°C (corresponding to the optimal conditions for insulin action) adipose tissue fragments were separated 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 was used for LPL assay which was performed according to a modification of the procedure in [19] as previously described [11]. LPL activity was expressed as  $\mu$ mol  $^{14}C$  free fatty acids (FFA) released/g fat pad per h. Each value is the mean  $\pm$  SE of 3-4 experiments

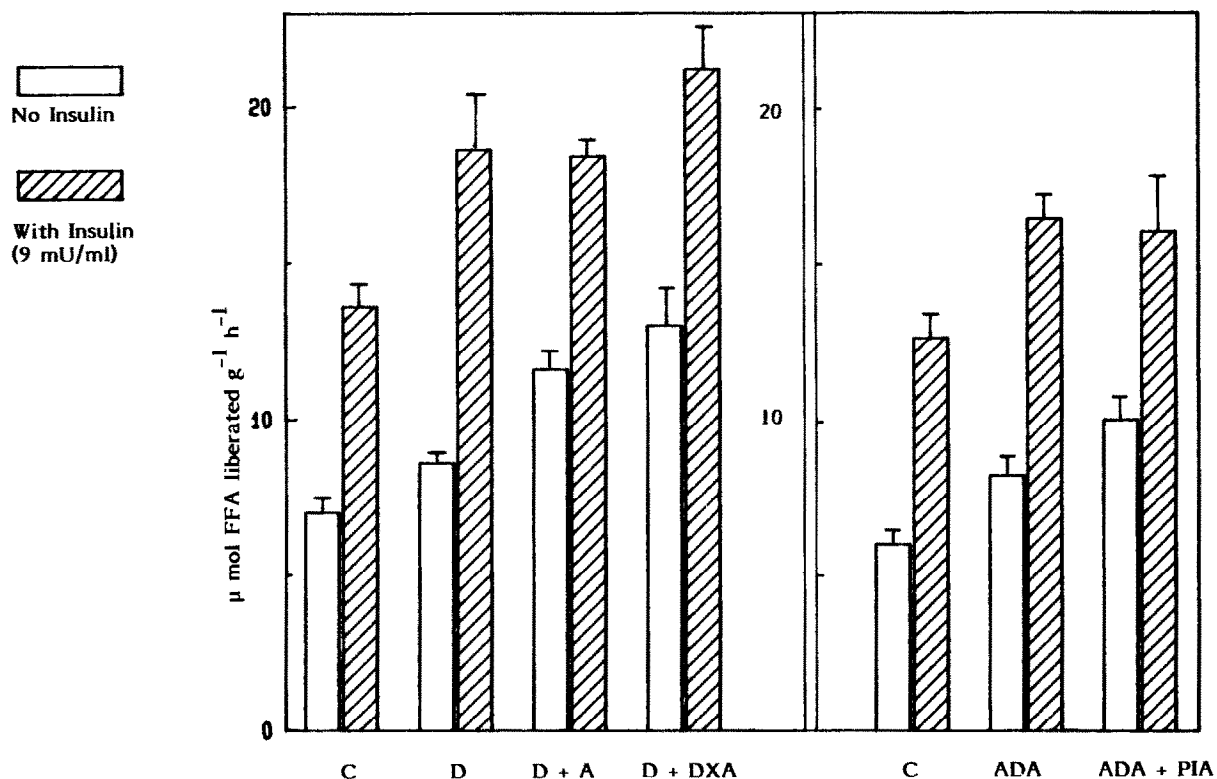


Fig.1. Stimulation of adenosine, deoxyadenosine and PIA-stimulated adipose tissue LPL activity by insulin. The values are means  $\pm$  SE of 3 separate experiments as described in section 2. C, control; D, deoxycoformycin ( $10 \mu$ M); A, adenosine ( $10 \mu$ M); DXA, deoxyadenosine ( $10 \mu$ M); ADA, adenosine deaminase (40 mU/ml); PIA, phenylisopropyladenosine ( $0.1 \mu$ M).

performed in triplicate. The statistical differences were calculated by Student's *t*-test: *p* values equal to or less than 0.02 were considered significant.

Table 1

Influence of cycloheximide on the stimulatory effects of insulin and adenosine on adipose tissue LPL activity

	Lipoprotein lipase activity ( $\mu\text{mol FFA} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$ wet wt)	
	No cycloheximide	With cycloheximide (36 $\mu\text{M}$ )
Control	11.3 $\pm$ 0.7	11.7 $\pm$ 1.0 <sup>a</sup>
Insulin (9 mU/ml)	23.1 $\pm$ 1.4 <sup>b</sup>	11.4 $\pm$ 1.0 <sup>a</sup>
Adenosine (10 $\mu\text{M}$ )	15.3 $\pm$ 0.8 <sup>b</sup>	11.1 $\pm$ 0.7 <sup>a</sup>

<sup>a</sup> Non-significant compared to control

<sup>b</sup> *p* < 0.001 compared to control

Incubations were performed as described in section 2. Each value represents the mean  $\pm$  SE of 3 incubations

### 3. RESULTS

As shown in fig.1 and tables 1 and 2, the magnitude of the LPL stimulation induced by insulin (9 mU/ml) was unchanged (100–125% over basal values) under all experimental conditions.

To avoid possible interference of endogenous adenosine deaminase on the effects of adenosine (10  $\mu\text{M}$ ) and DXA (10  $\mu\text{M}$ ) on LPL, these effects were studied in the presence of 2'-deoxycoformycin (10  $\mu\text{M}$ ), a potent inhibitor of adenosine deaminase [16]. As seen in fig.1, addition of 2'-deoxycoformycin slightly increased (27%) the basal LPL activity, an effect which could be due either to accumulation of endogenous adenosine [14] or to 2'-deoxycoformycin per se. Fig.1 showed that adenosine and DXA increased significantly LPL activity (34.5% with adenosine and 49.5% with DXA), but the magnitude of each of these effects was not statistically different from the other. It must be noted that the adenosine effect observed here was lower than that previously reported by us [14]. This difference could be explained by the fact that, in the latter studies, isolated fat cells were us-

Table 2

Influence of Ro 20-1724 and theophylline on the stimulatory effects of insulin, adenosine and 2'-deoxyadenosine on adipose tissue lipoprotein lipase activity

	Lipoprotein lipase activity ( $\mu\text{mol FFA} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$ wet wt)		
	No phosphodiesterase inhibitors	With Ro 20-1724 (1 mM)	With theophylline (5 mM)
Control	9.1 $\pm$ 0.37	7.4 $\pm$ 0.5 <sup>aa</sup>	4.7 $\pm$ 0.2 <sup>aa</sup>
Insulin (9 mU/ml)	20.4 $\pm$ 1.2 <sup>aa</sup>	10.5 $\pm$ 0.6 <sup>bbdd</sup>	4.6 $\pm$ 0.3 <sup>bbd</sup>
2'-Deoxycoformycin (10 $\mu\text{M}$ )	11.5 $\pm$ 0.4 <sup>aa</sup>	8.7 $\pm$ 0.4 <sup>ccd</sup>	—
2'-Deoxycoformycin (10 $\mu\text{M}$ ) + adenosine (10 $\mu\text{M}$ )	15.8 $\pm$ 1.1 <sup>cc</sup>	8.4 $\pm$ 0.6 <sup>d</sup>	—
2'-Deoxycoformycin (10 $\mu\text{M}$ ) + 2'-deoxyadenosine (10 $\mu\text{M}$ )	15.9 $\pm$ 0.6 <sup>cc</sup>	8.0 $\pm$ 0.4 <sup>d</sup>	—

<sup>a</sup> Compared to control

<sup>b</sup> Compared to insulin

<sup>c</sup> Compared to deoxycoformycin

<sup>d</sup> Compared to Ro 20-1724 or theophylline

One symbol: non-significant; two symbols: *p* < 0.001

Incubations were performed as described in section 2. Each value represents the mean  $\pm$  SE of 3 incubations

ed instead of whole adipose tissue fragments and that no adenosine deaminase inhibitor was included, allowing to consider the possibility that part of the observed effect could be due to inosine [14].

In the presence of 9 mU/ml insulin, which is a submaximal concentration [20], addition of 10  $\mu$ M adenosine or DXA did not result in a further increase in LPL activity (fig.1), a finding which suggests that a common mechanism is involved in the effects of insulin and these nucleotides on LPL.

As recent experiments showed that the insulin effect on adipose tissue LPL resulted, at least in part, from an increased post-transcriptional synthesis of the enzyme, the LPL responses to adenosine and insulin were compared in the presence of cycloheximide (36  $\mu$ M). As shown in table 1, cycloheximide completely prevented the effect of adenosine on LPL and, as expected from previous experiments from this laboratory [11], the effect of insulin.

In the next experiments, the effects of the  $R_i$ -site analog of adenosine, PIA (0.1  $\mu$ M), on LPL were studied under the same experimental conditions as above, except that 2'-deoxycoformycin was replaced by adenosine deaminase to prevent the possible interference of endogenous adenosine (fig.1). Confirming previous observations [14], addition of adenosine deaminase alone induced a 34% increase in the basal LPL activity, an effect which, as shown previously, can be accounted for by inosine [14]. Addition of PIA resulted in a further increase of the LPL activity (24% over the activity found in the presence of adenosine deaminase alone,  $p < 0.001$ ), but, like adenosine and DXA, did not further enhance the magnitude of the LPL stimulation induced by insulin under these conditions (fig.1).

To investigate the possible role of cyclic AMP in the effects described above, the influence of insulin, adenosine and DXA on LPL was reexamined in the presence of a selective inhibitor of cyclic AMP phosphodiesterase, Ro 20-1724 [21], which does not interact with the binding of adenosine to its  $R_i$ -sites [7]. As shown in table 2, addition of Ro 20-1724 alone (1 mM) resulted in a 26% decrease in the basal LPL activity. Interestingly, when insulin was added in combination with Ro 20-1724, the ability of insulin to stimulate LPL was considerably reduced, falling from 125% stimulation in the absence to 45% stimulation in

the presence of Ro 20-1724. When another phosphodiesterase inhibitor, theophylline [15], was added in place of Ro 20-1724, about the same results were found except that no stimulation at all was observed in the presence of the hormone. As also shown in table 2, addition of Ro 20-1724 also completely suppressed the ability of either adenosine or DXA to stimulate adipose tissue LPL activity.

#### 4. DISCUSSION

It is now well established that in several tissues, including adipose tissue, the purine substituted adenosine analog, PIA, binds to specific sites located on the cell surface and called the  $R_i$ -sites, whereas the ribose-modified adenosine analog, DXA, binds to intracellular sites, the P-sites [7]. Binding of these analogs to their respective sites results in adenylate cyclase inhibition [7] and, consequently, in a reduction of the intracellular cyclic AMP level. Like insulin which also decreases cyclic AMP level in adipose tissue [4], adenosine and its  $R_i$ - and P-site analogs, PIA and DXA, were found to increase adipose tissue LPL activity. Since cyclic AMP has been suggested to play a role in the regulation of LPL in adipose tissue [12], these findings raise two questions. (i) Is LPL stimulation induced by insulin, adenosine, PIA and DXA the consequence of cyclic AMP reduction induced by these compounds? (ii) Because adenosine exerts other insulin-like effects and sensitizes the fat cells to some actions of insulin [1,22], does endogenous adenosine play a role in the LPL stimulation induced by insulin?

The present data showing that the insulin- and the adenosine- (or adenosine analog-) effects on LPL are suppressed or severely reduced in the presence of potent inhibitors of cyclic AMP phosphodiesterase strongly suggest that cyclic AMP is indeed involved in the mechanism by which both insulin and adenosine promote LPL activity in adipose tissue. However, the fact that the LPL response to adenosine is suppressed by cycloheximide also suggests that adenosine, like insulin [23], promotes the post-transcriptional biosynthesis of LPL. It cannot be excluded that this effect on the enzyme biosynthesis may be indirectly linked to decreased cyclic AMP consider-

ing the important role played by phosphorylation/dephosphorylation processes in the control of protein synthesis initiation [24].

On the other hand, this study suggests that endogenous adenosine has probably no significant influence on the insulin action on LPL. In fact the magnitude of the LPL stimulation induced by a submaximal concentration of insulin [20] was (i) unaltered whether studied in the absence or presence of adenosine deaminase or deoxycofornycin and (ii) not additive with the effects of exogenous adenosine or analogs.

The importance of adenosine in the control of adipose tissue metabolism has already been documented in several previous reports. In fact, adenosine has been shown to increase the sensitivity of the glucose transport system and of lipid biosynthesis to insulin in fat cells [22], two effects which do not seem to be entirely accounted for by a reduction in cyclic AMP biosynthesis. Moreover, adenosine, through binding to  $R_i$ -sites, inhibits, like insulin [4], adenylate cyclase and lipolysis [1,7] and, as recently shown by one of us [25], completely masks the antilipolytic effect of agents inhibiting adenylate cyclase such as the  $\alpha_2$ -adrenergic component of catecholamines. Finally, like insulin again [5], adenosine stimulates cyclic AMP phosphodiesterase activity in adipose tissue [6], an effect which seems to result from the selective stimulation of the low  $K_m$  membrane-bound enzymic isoform [26]. The present findings of increased LPL activity by adenosine and its  $R_i$ - and P-site analogs provide an additional argument in favour of a potential role played by adenosine in the control of adipose tissue metabolism.

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