Post-Translational Regulation of Lipoprotein Lipase Activity in Adipose Tissue

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Changes in adipose-tissue lipoprotein lipase activity that are independent of protein synthesis were investigated in an incubation system *in vitro*. Under appropriate conditions at 25°C a progressive increase in the enzyme activity occurs that is energy-dependent. Part of the enzyme is rapidly inactivated when the tissue is incubated with adrenaline or adrenaline plus theophylline. The mechanism of this inactivation appears to be distinct from, and to follow, the activation of the enzyme. A hypothesis is presented to account for the results in terms of an activation of the enzyme during obligatory post-translational processing and a catecholamine-regulated inactivation of the enzyme as an alternative to secretion from the adipocyte.

The uptake of circulating triacylglycerol fatty acids by adipose tissue is believed to be regulated through changes in the activity of lipoprotein lipase (EC 3.1.1.3) in the tissue. Consistent with this is the fact that the enzyme activity is correlated with triacylglycerol fatty acid uptake in a variety of situations *in vivo*: for example, both are high in fed animals and both fall to low levels on starvation (Robinson, 1970). Such changes in the activity of the enzyme are probably mediated by hormones. Thus, in the rat, the activity is correlated with the plasma insulin concentration in a variety of nutritional states (Cryer *et al.*, 1976) and, in suitable incubation systems *in vitro* at 37°C, insulin causes an increase in the tissue enzyme activity, which is opposed by catecholamines (Salaman & Robinson, 1966; Wing & Robinson, 1968*b*).

The activity of lipoprotein lipase is expressed *in vivo* only at the endothelial cell surfaces of the blood capillaries (Robinson, 1970; Scow *et al.*, 1976). The enzyme is considered to be synthesized by the adipocytes and then to be secreted and transported to its site of action in the capillary bed (Schotz *et al.*, 1969; Robinson & Wing, 1970). Since inhibitors of protein synthesis cause a rapid decline in the activity of the tissue enzyme at 37°C both *in vivo* and *in vitro* (Wing *et al.*, 1967; Cunningham & Robinson, 1969; Wing & Robinson, 1968*a*), it appears that the maintenance of the activity of the enzyme at the endothelial cell surface depends on continued synthesis of new enzyme protein by the adipocyte.

Increases in the activity of the enzyme in the tissue can also occur, however, by a mechanism that is independent of protein synthesis. For example, if either intact epididymal adipose tissue from starved rats or isolated rat adipocytes are incubated in appropriate media at 25°C, there is a 2–3-fold increase in the activity of the enzyme, even when

protein synthesis is inhibited by cycloheximide (Stewart & Schotz, 1971; Cryer *et al.*, 1973). The failure to observe such increases during incubations at 37°C (see above) can probably be attributed to the greater stability of the enzyme at 25°C (Cryer *et al.*, 1973).

Both low- and high-molecular-weight forms of lipoprotein lipase have been claimed to be demonstrable in adipose tissue (Garfinkel & Schotz, 1972, 1973; Schotz & Garfinkel, 1972; Davies *et al.*, 1974). This has led to the suggestion that the protein-synthesis-independent increase of enzymic activity occurs through the conversion of the low-molecular-weight form to the higher-molecular-weight form in conjunction with the secretion of the enzyme from the adipocyte (Nilsson-Ehle *et al.*, 1976; Garfinkel *et al.*, 1976). However, we have provided evidence that the high-molecular-weight form of the enzyme is an artifact (Ashby *et al.*, 1978). This has led us to investigate further the nature of the activation of the enzyme that is independent of protein synthesis and to study the effect of possible regulatory hormones on the activation process. The results of these investigations are reported in this paper.

Materials and Methods

Materials

Glycerol dehydrogenase (EC 1.1.1.6), NAD$^+$, puromycin and cycloheximide were obtained from the Sigma Chemical Co., Kingston upon Thames, Surrey, U.K. Theophylline was obtained from BDH Chemicals, Poole, Dorset, U.K. L-[4,5-^{3}H]Leucine (specific radioactivity 48 mCi/mmol) was supplied by The Radiochemical Centre, Amersham, Bucks, U.K. Other chemicals were as specified by Davies *et al.* (1974).

Animals

Specific pathogen-free male rats of the Wistar strain and of body weight 170–190g were used (A. Tuck and Son, Rayleigh, Essex, U.K.). They were maintained as described by Ashby *et al.* (1978) and were starved for 24h from 09:00h before the start of the experiments.

Incubation of epididymal fat-bodies

In each experiment, the required number of rats was killed and their fat-bodies were removed as described by Ashby *et al.* (1978). The fat-bodies were collected in Krebs–Henseleit bicarbonate buffer solution, pH7.3–7.5 (Krebs & Henseleit, 1932) gassed with O_2/CO_2 (19:1) at 25°C. They were blotted on filter paper to remove surplus buffer and distributed randomly in groups of four to incubation flasks at 25°C containing 10ml of the gassed buffer solution, supplemented with the amino acid mixture used for HeLa-cell culture (Eagle, 1955) and bovine serum albumin that had been dialysed against water (final concentration, 2%, w/v). To this basic medium, other additions were made as specified in the text. The time from the killing of the animals to the distribution of the fat-bodies to the incubation flasks never exceeded 25min and was usually completed in 10–15min.

Preparation and incubation of adipocytes

Adipocytes were prepared as described by Spencer *et al.* (1978) and were incubated in the basic medium described above but with dialysed rat serum (48%, v/v) in the place of the bovine serum albumin. Additions to the basic medium were as stated in the text.

Assay of lipoprotein lipase

Acetone/ether-dried preparations of the fat-bodies and the incubation medium combined were made as described by Ashby *et al.* (1978). Since the fat-bodies were homogenized in the incubation medium, the medium albumin served as the carrier protein. The dried preparations were homogenized in aq. 50mM-NH_3 adjusted to pH8.1 with HCl (2.5ml/fat-body equivalent). The homogenates were filtered through glass wool to remove coarse tissue fragments, and the assay of lipoprotein lipase in the filtered homogenate was carried out as described by Ashby *et al.* (1978).

In some experiments, acetone/ether-dried preparations of adipocytes and of adipocyte incubation medium were made. The procedure described by Spencer *et al.* (1978) was followed, except that the cells were resuspended in 2% (w/v) bovine serum albumin solution after separation from the incubation medium.

One unit of lipoprotein lipase activity is defined as the amount of enzyme that releases 1 μmol of fatty acid/h in the assay.

Assay of glycerol

Glycerol in the incubation media was assayed as described by Hagen & Hagen (1962) using glycerol dehydrogenase. The determination was carried out on samples (0.2ml) of medium deproteinized with 10% (v/v) $HClO_4$ and then neutralized with K_2CO_3.

Incorporation of radioactive amino acid into protein

During the incubation of the fat-bodies, [^{3}H]-leucine (0.33μCi/ml) was routinely present in the medium. The incorporation of [^{3}H]leucine into the protein of the fat-bodies was determined by the method of Mans & Novelli (1961) on samples (150μl) of the homogenates of the acetone/ether-dried preparations. Radioactivity was measured in a Beckman LS-230 liquid-scintillation counter with toluene containing 4g of butyl-PBD [5-(4-biphenylyl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole]/l as the scintillant.

Statistical analysis

The lipoprotein lipase activity of fat-bodies from starved rats can vary considerably from one consignment of animals to the next. In the present study with 24h-starved animals it varied between 3 and 24 units/fat-body. However, within a single consignment, the activity is very consistent. For example, the mean (±S.D.) initial activity of seven groups of four fat-bodies was typically 6±0.4 units/fat-body. In our studies of the effects of hormones on the activation of lipoprotein lipase during the incubation of fat-bodies we have therefore carried out each experiment with groups of fat-bodies taken from animals from a single consignment and with body weights within the range given above. Estimation of the significance of a given effect required the pooling of data from separate experiments, each of which used a different batch of rats. The data were therefore analysed for significance by the paired sample *t* test, which eliminates the effect of the variation between consignments (Campbell, 1974).

Results

Incubations of epididymal fat-bodies with cyclo-heximide at 25°C

All of the incubation media contained cyclo-heximide (36μM) but no heparin. The incorporation

of L-[4,5-^{3}H]leucine into the protein of the fat-bodies was inhibited by 94–97%, and, during the incubations, there was no significant extraction of enzyme into the incubation medium.

Effectors of the activation of lipoprotein lipase

The effects of different additions to the basic incubation medium were studied through direct comparisons in incubations lasting 3 h. The results in Table 1 show that activation is promoted by glucose (13.3 mM), and that insulin (12 m-i.u./ml) has no significant effect at this glucose concentration. However, adrenaline and, in particular, adrenaline and theophylline together, oppose the activation. Incubation with theophylline alone under the conditions stated for Table 1 had no significant effect. In four experiments theophylline (3 mM) decreased the mean ±S.E. enzyme activity of the tissue by 0.5±0.3 unit/fat-body from that of tissue incubated with glucose (13.3 mM) and insulin (12 m-i.u./ml). The difference is not significant. In these experiments adrenaline (10 µM) lowered the mean±S.E. enzyme activity by 1.25±0.16 units/fat-body ($P<0.0025$) and adrenaline (10 µM) with theophylline (3 mM) decreased it by 2.23±0.29 units/fat-body ($P<0.0025$).

Previous evidence has implicated insulin in the regulation of the activity of adipose-tissue lipoprotein lipase (see the introduction). Further experiments were therefore carried out with incubation media containing zero, 2.8 mM- or 13.3 mM-glucose. The plasma glucose concentration in rats starved for 48 h is approx. 2.8 mM (Hawkins et al., 1971), and any effects due to an action of insulin on glucose transport would be expected to be evident at this concentration.

Cycloheximide has been reported to have insulin-like effects on glucose uptake and utilization by adipose tissue (Moskowitz & Fain, 1970; Garcia-Sainz et al., 1977). In separate experiments, therefore, puromycin (100 µM) and cycloheximide (36 µM) were used to inhibit protein synthesis. No significant effect of insulin on the increase in enzyme activity was observed in any of the experiments. Only at a concentration of glucose of 2.8 mM and in the presence of puromycin was the mean (±S.E.M.) increase higher in the presence of insulin (4.3±0.6 versus 2.4±0.7 units/fat-body in four experiments), and this difference was not significant ($0.1>P>0.05$).

Time course of the activation of lipoprotein lipase

The increases in lipoprotein lipase activity that occur when epididymal fat-bodies from starved rats are incubated at 25°C in the presence of glucose, insulin, cycloheximide and heparin are complete within 2 h (Cryer et al., 1973). The results in Fig. 1 are consistent with these observations in showing that activation of the enzyme at 25°C in the absence of heparin is also complete within 2 h.

When adrenaline and theophylline are present in the incubation medium, activation initially follows the same time course as when they are absent (Fig. 1). In seven experiments, each involving incubations of groups of four fat-bodies for 30 min, the mean increases in the absence and the presence of adrenaline and theophylline were respectively 2.3 and 2.7 units/fat-body. Although both these increases were significant ($P<0.001$), the difference between the two means was not. After a period of 30 min to 1 h, however, the enzyme activity ceases to rise in the incuba-

Table 1. Effectors of the activation of lipoprotein lipase

Groups of four fat-bodies were incubated in 10 ml of the basic incubation medium described in the Materials and Methods section. Cycloheximide (36 µM) was present in all the media and the other additions were as shown. The concentrations of substances added were: glucose, 13.3 mM; insulin, 12 m-i.u./ml; adrenaline, 10 µM; theophylline, 3 mM. Acetone/ether-dried preparations were made from the fat-bodies and the medium combined at 0 and at 3 h for lipoprotein lipase assay. The increases in enzyme activity shown represent the mean differences (±S.E.M.) between the 0 and the 3 h activities of these preparations. The number of experiments signifies the number of direct comparisons, and the significance of the differences between the means was calculated with the paired sample t test (see under 'Statistical analysis' in the Materials and Methods section).

Additions to medium	Number of experiments	Mean increase in enzyme activity (units/fat-body)	Significance of increase (P)	Significance of difference between means (P)
None	10	3.2±0.3	<0.001	<0.005
Glucose, insulin		7.3±1.1	<0.001	
Glucose	5	8.9±3.0	<0.05	N.S.
Glucose, insulin		8.8±2.4	<0.05	
Glucose, insulin	4	6.4±0.5	<0.001	<0.05
Glucose, insulin, adrenaline		4.1±0.8	<0.02	
Glucose, insulin	7	6.8±1.1	<0.001	<0.001
Glucose, insulin, adrenaline, theophylline		−0.8±0.7	N.S.	

tions with adrenaline and theophylline, and, in fact, falls until a new stable activity is reached (Fig. 1). Moreover, when adrenaline and theophylline are added later than 30 min after the initiation of incubations in the presence of glucose and insulin, the increase in enzyme activity stops immediately and
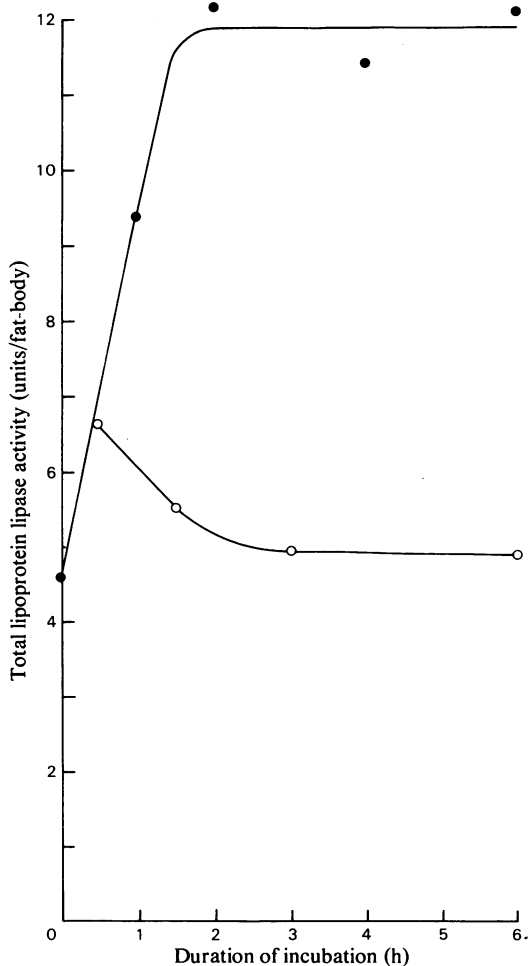
there is again a fall in activity to a new stable level (Fig. 2a). The stable level of activity is higher, the longer the period between the start of the incubations and the addition of the adrenaline and theophylline. This progressive rise has been confirmed in seven experiments and, in one of these, there was no loss of activity when the adrenaline and theophylline were added after 4.5 h.

The time lag in the effect of adrenaline and theophylline in inhibiting the activation when present
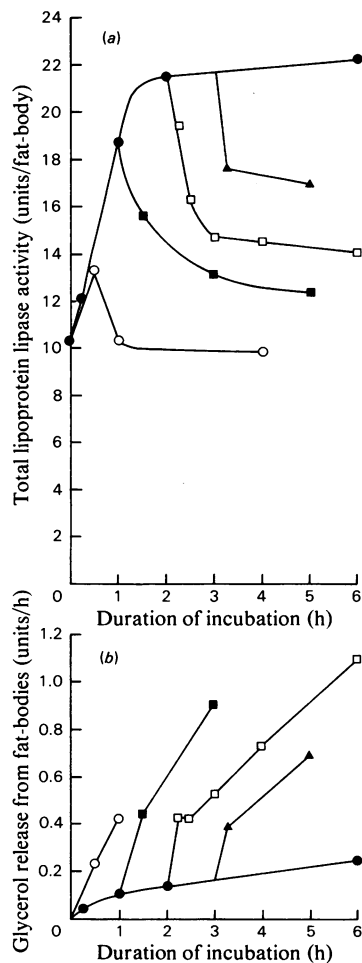

Fig. 1. *Time-course of changes in lipoprotein lipase activity during incubations of epididymal fat-bodies*

Groups of four fat-bodies were incubated in 10 ml of the basic incubation medium described in the Materials and Methods section to which cycloheximide (36μM), glucose (13.3 mM) and insulin (12 m-i.u./ml) had been added (●). Some of the incubation media also contained adrenaline (10μM) and theophylline (3 mM) (○). At intervals, acetone/ether-dried preparations were made from the fat-bodies and the medium combined, and the lipoprotein lipase activity of these was determined. The results shown are typical of seven such experiments.

Fig. 2. *Effects of addition of adrenaline and theophylline during the incubation of epididymal fat-bodies in the presence of glucose and insulin on (a) increases in lipoprotein lipase activity and (b) glycerol release*

The experiment was carried out as described in the legend to Fig. 1. Adrenaline and theophylline were either absent throughout the incubations (●) or were added at 0 h (○), 1 h (■), 2 h (□) or 3 h (▲). The results shown are typical of seven such experiments.

from the beginning of the fat-body incubations might have been due to a delay in their entry into the tissue. However, as the results in Fig. 2(b) show, no delay in their ability to increase the rate of glycerol release is evident. It may be concluded that they are immediately effective in activating mobilizing lipase in the tissue (Butcher *et al.*, 1965; Corbin *et al.*, 1970).

Effects of respiratory poisons

In order to investigate whether the activation of lipoprotein lipase that occurs in the presence of glucose is an energy-dependent process, incubations of fat-bodies were carried out in media containing, besides glucose and insulin, dinitrophenol and arsenate as uncouplers of oxidative phosphorylation (Slater, 1967). Three such experiments were carried out. In each, the activation was completely abolished and instead there was an exponential decay of the enzyme activity with a half-life of 4–5h (Fig. 3).

Incubations of isolated adipocytes with cycloheximide at 25°C

All the activation of lipoprotein lipase that occurred during the incubations of intact epididymal fat-bodies at 25°C was due to rises in the activity of the enzyme within the tissue, because of the absence of heparin from the medium. However, the possibility could not be excluded that it was associated with the secretion of the enzyme from the adipocyte to extracellular sites in the tissue (Nilsson-Ehle *et al.*, 1976; Garfinkel *et al.*, 1976). To test this possibility, isolated adipocytes were incubated in the presence of glucose, insulin and cycloheximide. If activation were an intracellular event, rises in both the adipocyte and the medium activity might be expected, whereas, if it was associated with enzyme secretion, only the medium activity would increase. The results (Table 2) support our previous studies (Cryer *et al.*, 1975) in indicating the intracellular activation of the enzyme, and confirm that, as during the incubations of intact
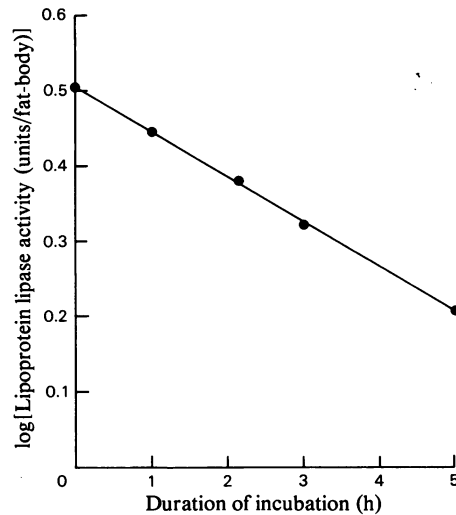

Fig. 3. *Changes in the activity of lipoprotein lipase during the incubation of epididymal fat-bodies in the presence of respiratory poisons*

Groups of four fat-bodies were incubated in the basic incubation medium described in the Materials and Methods section to which glucose (13.3 mM), insulin (12 m-i.u./ml), dinitrophenol (1 mM) and arsenate (40 mM) had been added. At intervals, acetone/ether-dried preparations were made from the fat-bodies and the medium combined; the lipoprotein lipase activity of these was determined. The results shown are typical of three such experiments.

epididymal fat-bodies, the process is opposed by adrenaline and theophylline.

Although the possibility of some activation during the secretory process cannot yet be rigorously excluded, there is no compelling evidence for it. Thus all of the experiments quoted in support of such a view were carried out in media that contained heparin. However, heparin is known to increase the rate of extraction of the enzyme from adipocytes

Table 2. *Activation of lipoprotein lipase during incubation of isolated adipocytes at 25°C*
Isolated adipocytes were incubated at 25°C in the basic incubation medium described in the Materials and Methods section. Cycloheximide (36 μM), glucose (13.3 mM) and insulin (12 m-i.u./ml) were also present in all the media. Adrenaline (10 μM) and theophylline (3 mM) were present as indicated. The adipocytes and the medium were separated from each other for lipoprotein lipase assay at either 0 or 3 h. The mean ($\pm$ S.E.M.) lipoprotein lipase activities for three experiments are shown. The significance of the increases over the 3 h period of incubation were as follows: $*P < 0.02$; $**P < 0.01$; $***P < 0.001$.

Additions to medium	Time (h) ...	Cell enzyme activity (units/fat-body equivalent)		Medium enzyme activity (units/fat-body equivalent)	
		0	3	0	3
None		3.9 ± 0.2	$9.0 \pm 0.5*$	0.7 ± 0.1	$4.4 \pm 0.2***$
Adrenaline, theophylline		3.9 ± 0.2	3.7 ± 0.6	0.7 ± 0.1	$2.4 \pm 0.06**$

(Stewart & Schotz, 1974), and, because of this, it is difficult to resolve activation before secretion from activation during secretion.

Discussion

The results of the present study confirm that adipose-tissue lipoprotein lipase can be activated within the adipocyte by processes that are independent of protein synthesis. The activation is energy-dependent and is complete in 2 h at 25°C. [An energy-dependent activation of lipoprotein lipase has already been reported by Stewart & Schotz (1974). However, these workers related the process to the energy-dependence of secretion of the enzyme from the adipocyte, whereas the activation studied here appears to occur within the cell.] It may be limited by the availability of glucose but does not appear to be influenced directly by insulin. Adrenaline and theophylline do not oppose the activation of lipoprotein lipase for about 30 min when present in the medium from the start of the incubations, although they are immediately effective in increasing the activity of mobilizing lipase in the tissue. However, they do cause a decline in the lipoprotein lipase activity after the first 30 min. This indicates that they do not directly oppose or inhibit the activation process, but suggests rather that they inactivate the enzyme through an effect on a separate process.

A model that could account for the above observations is presented in Fig. 4. Lipoprotein lipase is a protein that is secreted by the adipocyte and, as such, it is likely to follow the general path for the transport of such proteins from the ribosomes through the channels of the rough and smooth endoplasmic reticulum to the Golgi apparatus and, thence, probably in vesicles, to the adipocyte surface. As a secreted protein, it could be modified during these transport processes in a number of ways, for example, by glycosylation, by phosphorylation or by the removal of part of the peptide chain (Campbell & Blobel, 1976). In Fig. 4 it is suggested that it is such a modification that is associated with a rise in the assayable enzyme activity during incubations in the presence of cycloheximide at 25°C. As released from the ribosomes, the enzyme could be either partially active or
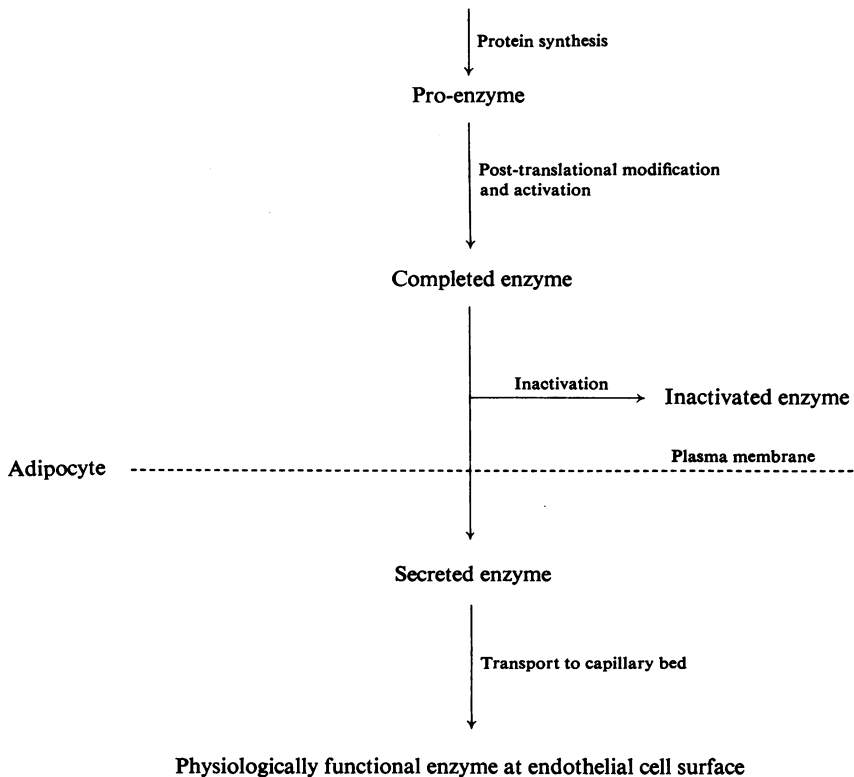
Protein synthesis

Pro-enzyme

Post-translational modification and activation

Completed enzyme

Inactivation → Inactivated enzyme

Adipocyte -   Plasma membrane

Secreted enzyme

Transport to capillary bed

Physiologically functional enzyme at endothelial cell surface

Fig. 4. *Scheme to account for the protein synthesis independent changes in adipose-tissue lipoprotein lipase activity*

entirely inactive. In either case, the epididymal fat-bodies, when removed from the animal, would be expected to contain a pool of enzyme chains requiring modification before their full enzymic activity could be expressed. Lipoprotein lipase is known to be a glycosylated enzyme (Bensadoun et al., 1974; Iverius & Östlund-Lindquist, 1976) and, therefore, does undergo post-translational processing. However, there is no evidence that it is this modification, rather than any other, that is associated with an increase in the specific activity of the enzyme.

The inactivation process that we think is necessary to explain the findings with adrenaline and theophylline is also shown in Fig. 4. We believe that the delay before the effect of these substances becomes evident in incubations in which they are present initially can be accounted for if the enzyme has first to be transported to the region where inactivation occurs. We have, therefore, represented it as occurring at a relatively late stage. The higher levels of stable enzyme activity observed in the experiments in which the additions of adrenaline and theophylline to the incubation medium were delayed would then be explained by the fact that in these cases some of the enzyme would have been transported past the in-activation site, and may even have been secreted from the adipocyte.

The nature of the inactivation process can only be a subject for speculation at present. It could be a cyclic AMP-mediated process in view of the adrenaline and theophylline effects. However, efforts so far to demonstrate a direct role of protein kinases in the regulation of the enzyme's activity have not been successful (Steinberg & Khoo, 1977). Moreover, indirect evidence for the existence of forms of the enzyme of similar molecular weight (Davies et al. 1974) cannot easily be related to the present studies. At the present time, we have no evidence that the inactivation is reversible. If it is not, then it could be due to accelerated degradation of the enzyme in the adipocyte. The activities of a number of enzymes, including acetyl-CoA carboxylase and fatty acid synthetase, are now thought to be subject to some degree of regulation by this means (Numa, 1974; Schimke, 1975). Moreover, it has been shown that the rate of degradation of mammary-gland fatty acid synthetase is decreased by insulin, prolactin and cortisol in combination, and that dibutyryl cyclic AMP and theophylline oppose this stabilizing hormonal effect (Speake et al., 1976).

The activity of lipoprotein lipase against triacylglycerol can only be expressed at the capillary endothelial cell surface where the enzyme can associate with a specific protein (apoprotein CII) that is present at the surface of the plasma lipoproteins that are its natural substrates (Osborne & Brewer, 1978). The activation of the enzyme studied here cannot, therefore, lead to any expression of activity within

the adipocyte and it does not appear to be subject to any significant hormonal regulation. Regulation of the enzyme's activity by hormonal action seems likely to occur at the level of protein synthesis (see the introduction). However, hormonal control in the short term could also have physiological significance if it was exercised on the process of inactivation of the enzyme within the adipocyte. Thus the enzyme at the capillary endothelial cell surface is probably normally turning over extremely rapidly (Robinson, 1970). If its activity against the plasma triacylglycerol is to be maintained, this functional enzyme will need to be continually replenished from within the adipocyte. Inactivation within that cell immediately before enzyme secretion, particularly if it was brought about rapidly through adrenergic stimuli, could prevent such replenishment and quickly alter the functional activity at the endothelial cell surface. Such changes could be important in response to stress situations such as sudden exercise when the uptake of triacylglycerol fatty acids by adipose tissue needs to be decreased quickly and where a delayed response through an alteration in the rate of enzyme synthesis would be less appropriate.

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