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The lipoprotein lipase of white adipose tissue

Changes in the adipocyte cell-surface content of enzyme in response to extracellular effectors *in vitro*

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1. An indirect labelled-second-antibody cellular immunoassay for adipocyte surface lipoprotein lipase was used to assess the changes that occurred during the incubation of cells in the presence and absence of effectors. 2. In the absence of any specific effectors, the amount of immunodetectable lipoprotein lipase present at the surface of adipocytes remained constant throughout the 4 h incubation period at 37 °C. Under such conditions total cellular enzyme activity also remained constant, with no activity appearing in the medium. 3. In the presence of heparin, cell-surface immunodetectable lipoprotein lipase increased by up to 20%, whereas in the presence of cycloheximide they decreased by up to 60%. Thus the obvious turnover of enzyme from this cell-surface site was found to be relatively rapid and dependent for its replenishment, at least in part, on protein synthesis. 4. In the presence of insulin alone, a substantial increase in cell-surface lipoprotein lipase protein occurred, only part of which was dependent on protein synthesis. The total cellular activity of lipoprotein lipase was unaffected by the presence of insulin. The insulin-dependent increase in cell-surface enzyme was potentiated somewhat in the presence of dexamethasone, which was not shown to exert any independent effect. 5. Glucagon, adrenaline and theophylline all produced a significant decline in the cell-surface immunodetectable lipoprotein lipase, which in the case examined (adrenaline) was partially additive with regard to the independent effect of cycloheximide. 6. Cell-surface immunodetectable lipoprotein lipase amounts were decreased significantly when cells were incubated in the presence of either colchicine or tunicamycin. 7. The concerted way in which cell-surface lipoprotein lipase altered during the incubations of adipocytes in the presence of effectors suggested that the translocation of enzyme to and from this cellular site was dependent on hormonal action and the integrity of intracellular protein-transport mechanisms.

INTRODUCTION

Lipoprotein lipase (EC 3.1.1.34) is the extrahepatic enzyme responsible for the hydrolysis of plasma lipoprotein triacylglycerol (Hamosh & Hamosh, 1983; Cryer, 1981). Although found sequestered at the capillary endothelium of tissues (Pedersen *et al.*, 1980; Cryer, 1983), the enzyme is synthesized, in for example adipose tissue, in the adipocyte, from which it can be released under appropriate conditions *in vitro* (Cryer, 1981). Previous studies have examined the effects of hormones and other agents on the synthesis (Robinson *et al.*, 1983; Vydelingum *et al.*, 1978, 1983) and processing (Vannier *et al.*, 1982, 1985) of the intracellular enzyme in a variety of systems. However, the role of extracellular signals in the translocation of lipoprotein lipase molecules between the known pools of intracellular (Vannier *et al.*, 1986) and extracellular enzyme has received much less detailed attention.

During nutritional change, the total activity of lipoprotein lipase recovered with adipocytes remains relatively constant (Cryer, 1981; Spencer *et al.*, 1978), whereas that activity at or near the endothelium varies dramatically (Olivecrona *et al.*, 1977; Jansen *et al.*, 1980; Cryer, 1981). Thus under different conditions the flux through the adipocyte-associated compartments must vary considerably, particularly when the rapid turnover of the endothelial-associated enzyme (Bagby, 1983) is taken into account. In this context it is clear that certain hormones, e.g. insulin (Vydelingum *et al.*, 1983) and corticosteroids (Robinson *et al.*, 1983), stimulate processes involved in lipoprotein lipase synthesis and probably help to counteract the catecholamine-stimulated intracellular degradation of the enzyme (Ashby *et al.*, 1978; Robinson *et al.*, 1983). Furthermore, heparin has been shown to stimulate the egress of lipoprotein lipase from cells *in vitro* (Cryer *et al.*, 1975*a*; Vannier *et al.*, 1985), although the possible significance of such an action *in vivo* has been questioned (Cryer *et al.*, 1984).

Evidence has indicated that, in the tissue-derived adipocyte at least, lipoprotein lipase molecules are distributed among a number of cellular pools, of which the plasma-membrane pool is a quantitatively important one (Arnaud & Boyer, 1977; Arnaud *et al.*, 1979; Verine *et al.*, 1982; Al-Jafari & Cryer, 1986). It is also clear that the activity and amount of lipoprotein lipase at this latter site vary in response to nutritional factors (Verine *et al.*, 1982; Al-Jafari & Cryer, 1986). Clearly, since enzyme could be subject to intracellular degradation from this site, which may also represent a dynamic source of enzyme for transport to the endothelium, it becomes important to assess the effects of known agents involved in the control of the lipoprotein lipase system on the size of this plasma-membrane pool of enzyme. In the current study, the presence of lipoprotein lipase molecules at the surface of isolated adipocytes during incubations in the

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presence of a variety of effectors has been investigated by using an indirect labelled-second-antibody immunoassay system for the enzyme (Al-Jafari & Cryer, 1986).

MATERIALS AND METHODS

Details of the animals and tissues used were as described previously (Al-Jafari & Cryer, 1986). That previous paper also contains details of the preparation of rat epididymal adipocytes, the determination of cellular lipoprotein lipase activities and the immunodetection of cell-surface lipoprotein lipase.

Specialized materials were obtained from the following sources: heparin from Evans Medical, Speke, Liverpool, U.K.; medium 199, penicillin, streptomycin and glutamine from Gibco Europe, Paisley, Scotland, U.K.; cycloheximide, dexamethasone (9-fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione), adrenaline (bitartrate), colchicine, glucagon, theophylline and tunicamycin (isomer composition 7% A, 33% B, 44% C and 15% D, from *Streptomyces*) were obtained from Sigma.

For the incubation experiments described, 1 ml samples of epididymal adipocytes from either fed or 24 h-starved rats, prepared by using 0.5 mg of collagenase/ml (Al-Jafari *et al.*, 1986), were incubated at 37 °C in 3 ml of sterile medium 199 containing 100 μg each of penicillin and streptomycin/ml. After incubation, samples of cell suspension (500 μl) were removed at the specified times and the cells separated from the medium by gentle centrifugation (100 $g_{av.}$ for 1 min). The cells were then washed twice with warm Krebs–Ringer bicarbonate buffer (KRB), pH 7.4 at 37 °C (Cryer *et al.*, 1975*a*). Samples (50 μl) of cell suspension in triplicate were then incubated with 10 μl of 100-fold-diluted chicken anti-(rat lipoprotein lipase) serum (Al-Jafari & Cryer, 1986). Duplicate samples were incubated in parallel with non-immune chicken serum. After incubation, the cells were recovered by centrifugation, washed twice with KRB and then incubated in the presence of ^{125}I-labelled rabbit anti-(chicken IgG) antibody (20000–40000 c.p.m.). The binding of second antibody was then assessed, and the data were expressed as the percentage of added radioactivity (c.p.m.) that became bound specifically. The proportion of the radioactivity bound, attributable to non-specific binding after incubation with non-immune serum, was less than 20% of the total in all cases. Full details of the assay are described by Al-Jafari & Cryer (1986).

The data are given as means ± S.E.M. Student's *t* test was used to assess the significance of the differences between means (Fisher & Yates, 1957). Statistical significance was accepted when $P < 0.01$.

RESULTS AND DISCUSSION

Preliminary experiments were carried out to determine the effect of heparin on the size of the immunodetectable pool of lipoprotein lipase molecules present at the surface of intact adipocytes. Exposure to exogenous heparin has been shown previously to cause release of lipoprotein lipase activity from adipose-tissue sites *in vivo* (Anderson & Fawcett, 1950) and *in vitro* (Hollenberg, 1959) and to stimulate the release of the enzyme from adipocytes during incubation *in vitro* (Pokrajac *et al.*, 1967; Patten & Hollenberg, 1969).

Fig. 1 shows that, in the absence of heparin, when no

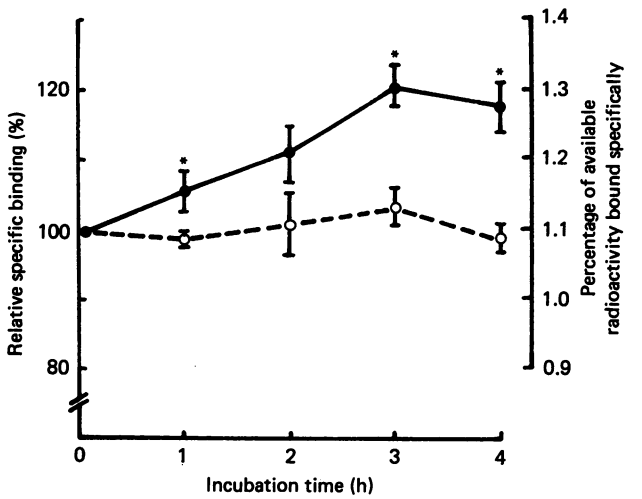

Fig. 1. Changes in immunodetectable cell-surface lipoprotein lipase on isolated adipocytes incubated in the presence and absence of heparin

For each experiment, 1 ml of isolated adipocytes prepared from fed rats as described in the Materials and methods section was incubated in 3 ml of medium 199 at 37 °C in either the absence (○) or the presence (●) of heparin (1 unit/ml). Samples of cells were removed, washed and subjected to the cellular immunoassay for cell-surface lipoprotein lipase as described in the Materials and methods section. The binding of ^{125}I-labelled rabbit anti-(chicken IgG) antibody is expressed either as a percentage of the added radioactivity (c.p.m.) which became bound specifically or as a percentage of the specific binding observed with cells at 0 h. The data are means ± S.E.M. for three independent experiments. Asterisks indicate a significant difference between the means as defined in the Materials and methods section. The labelled-second-antibody radioactivity added in each case was approx. 40000 c.p.m.

enzyme release occurred (results not shown; see also Vannier & Ailhaud, 1986), the immunodetectable lipoprotein lipase at the surface of the adipocytes did not change. However, in the presence of heparin (1 unit/ml), the assay revealed a progressive increase in antibody binding during the first 3 h of incubation. Clearly, in the presence of heparin, under conditions previously shown to produce a release of lipoprotein lipase activity without producing a decline in cellular activity (Stewart & Schotz, 1971, 1974; Cryer *et al.*, 1975*a*; Kornhauser & Vaughan, 1975; Ashby *et al.*, 1978), the amount of enzyme protein measurable at the adipocyte surface did not decline. Indeed, a small but significant increase in the immunodetectable lipoprotein lipase at this site was observed (Fig. 1). It is clear therefore that enzyme which is released from the cell surface by the action of heparin may be replaced rapidly from an intracellular pool (Vannier & Ailhaud, 1986). Whether such a putative intracellular movement of enzyme is stimulated by heparin itself or is a response to the removal of cell-surface enzyme is unknown.

Fig. 2 illustrates the effect of insulin or cycloheximide or combinations thereof on the size of the immunodetectable adipocyte-surface lipoprotein lipase pool when adipocytes from 24 h-starved rats were incubated at 37 °C in medium 199. Under the conditions described,

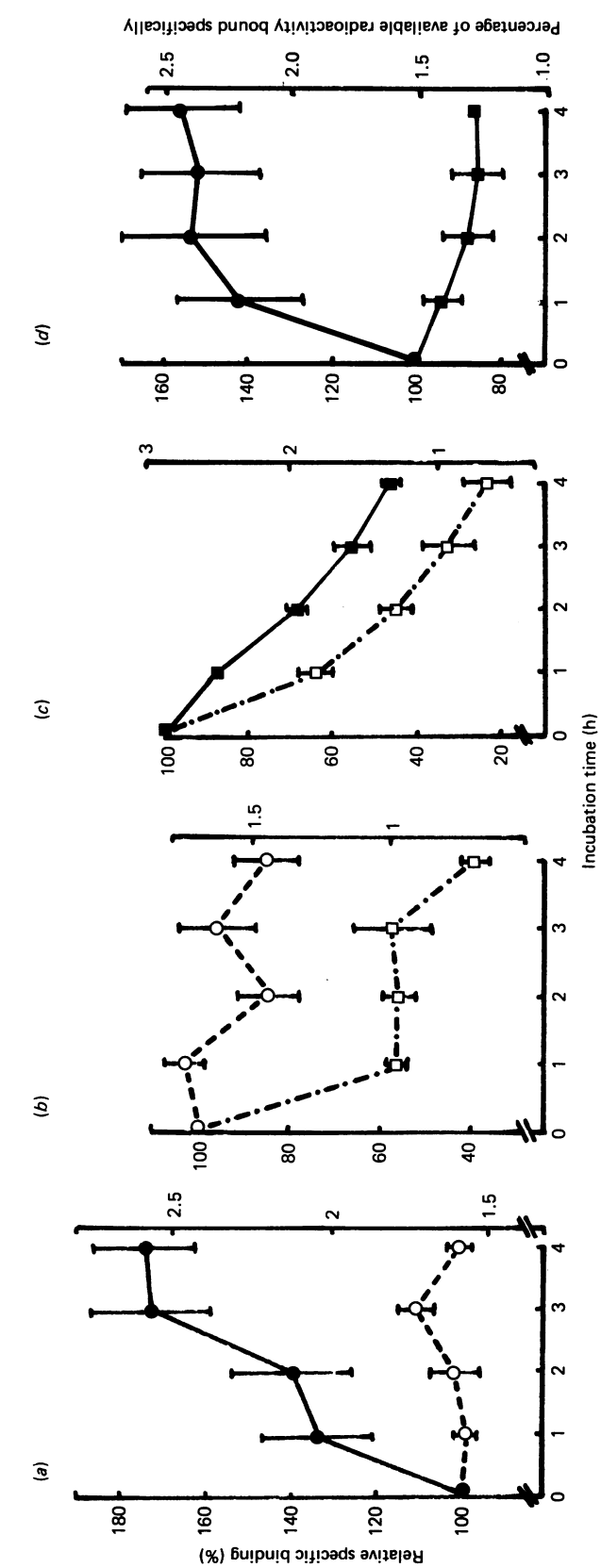

Fig. 2. Changes in immunodetectable cell-surface lipoprotein lipase on isolated adipocytes incubated in medium 199 or in medium 199 containing (*a*) insulin, (*b*) cycloheximide, or (*c*) and (*d*) combinations thereof

For each experiment 1 ml of isolated adipocytes prepared from 24 h-starved rats was incubated in 3 ml of medium 199 at 37 °C: (*a*) in the absence (○) or presence (●) of insulin (12 m-units/ml); (*b*) in the absence (○) or presence (□) of cycloheximide (10 μg/ml); (*c*) in the presence (□) of cycloheximide (10 μg/ml) or the presence (■) of cycloheximide (10 μg/ml) and insulin (12 m-units/ml); (*d*) in the presence (●) of insulin (12 m-units/ml) or the presence (■) of insulin (12 m-units/ml) and cycloheximide (10 μg/ml). The specific binding of labelled second antibody is expressed either as a percentage of available radioactivity or as a percentage of that detected at 0 h. The values are given as means ±S.E.M. of data derived from eight (*a*) or three (*b*, *c* and *d*) independent comparative experiments. The difference between comparative means was significant, as defined in the Materials and methods section, in all cases. The radiolabelled-second-antibody radioactivity added was approx. (*a*) 45000 c.p.m., (*b*) 29000 c.p.m., (*c*) 29000 c.p.m. and (*d*) 35000 c.p.m. for each series of experiments.

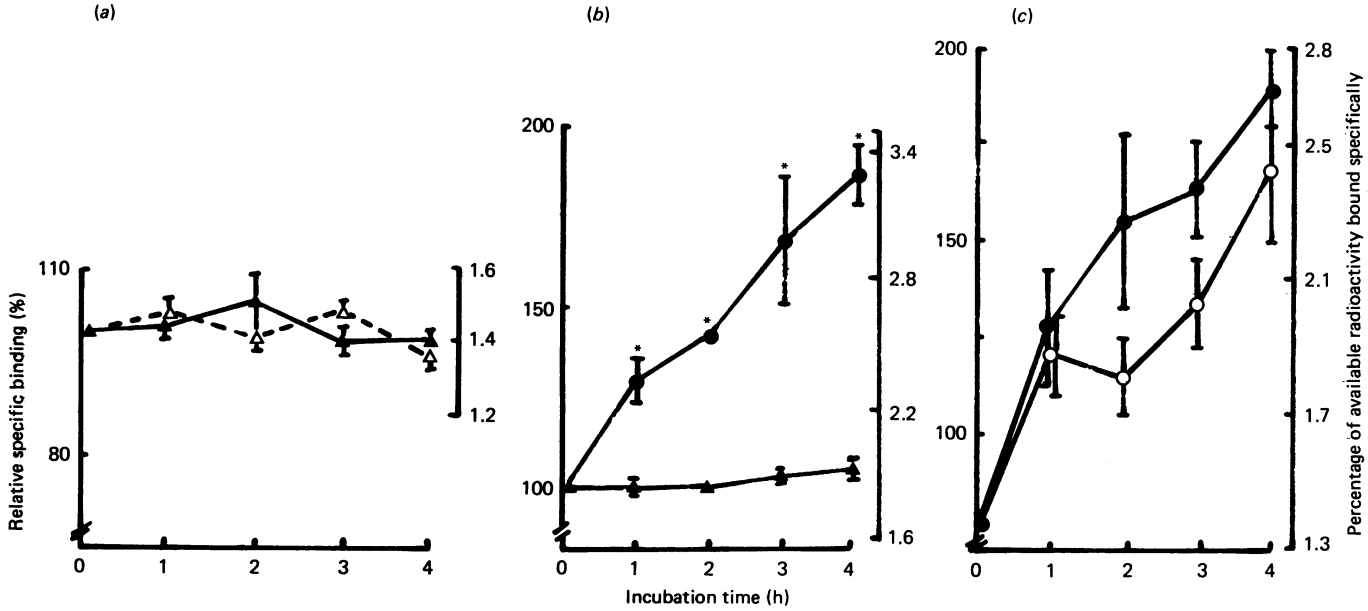

Fig. 3. Changes in immunodetectable cell-surface lipoprotein lipase on isolated adipocytes incubated in medium 199 or in medium 199 containing (*a*) dexamethasone or (*b*) and (*c*) combinations of dexamethasone and insulin

For each experiment 1 ml of isolated adipocytes isolated from 24 h-starved rats was incubated in 3 ml of medium 199 at 37 °C: (*a*) in the absence (△) or presence (▲) of dexamethasone (400 nM); (*b*) in the presence (▲) of dexamethasone (400 nM) or the presence (●) of dexamethasone (400 nM) and insulin (12 m-units/ml); (*c*) in the presence (○) of insulin or the presence (●) of dexamethasone (400 nM) and insulin (12 m-units/ml). The specific binding of labelled second antibody is expressed either as a percentage of available radioactivity or as a percentage of that detected at 0 h. The values given are means ± S.E.M. for data derived from three independent comparative experiments in each case. Asterisks indicate statistical significance at $P < 0.01$. The labelled-second-antibody radioactivity added was approx. (*a*) 41000 c.p.m., (*b*) 42000 c.p.m. and (*c*) 35000 c.p.m. for each series of experiments.

the cellular activity of lipoprotein lipase determined in acetone/diethyl ether-dried powders did not change in three independent experiments, neither was there any detectable release of activity into the medium in the presence or absence of insulin, although pilot experiments showed the soluble enzyme to have limited stability at 37 °C in the media used. However, a progressive and significant increase in immunodetectable enzyme was detected at the cell surface (Fig. 2*a*) when insulin was present, which did not occur in the absence of the hormone. The increase seen in the presence of insulin was prevented by the inclusion of cycloheximide (10 μg/ml) in the medium (Fig. 2*d*). However, a comparison of the effect of cycloheximide alone on the immunodetectable surface enzyme (Fig. 2*b*) with that seen when insulin and cycloheximide (Fig. 2*d*) were present suggests that the relative maintenance of enzyme amounts seen in the latter case may have involved a protein-synthesis-independent component. This possibility was confirmed directly in experiments (Fig. 2*c*) in which cells in the presence of cycloheximide were incubated in the presence and absence of insulin. In this case too, insulin had a significant protein-synthesis-independent effect on immunodetectable lipoprotein lipase at the adipocyte surface. These data are consistent with a number of other observations made on adipocytes incubated *in vitro*. Firstly, the protein-synthesis-dependent component seen here is consistent with the stimulatory effect of insulin on adipocyte protein and lipoprotein lipase synthesis (Krahl, 1972; Vydelingum *et al.*, 1978, 1983). Secondly, the protein-synthesis-independent effect of insulin may be related to the movement of the polypeptide through specific intracellular compartments (Spooner *et al.*, 1979*a*,*b*), during which the enzyme may also undergo a change in specific activity. Thirdly, in that turnover of cell-surface enzyme under the conditions of incubation described here must occur, as indicated by the decline in cell-surface enzyme in the presence of cycloheximide (Fig. 2*b*) and the known capacity of adipocytes *in vitro* to internalize 20% of their cell surface per hour by fluid-phase endocytosis (Gibbs & Lienhard, 1984), a balance between synthesis, activation and translocation may need to be invoked to integrate the effects of insulin on the lipoprotein lipase system of adipocytes. That insulin may stimulate the translocation of adipocyte lipoprotein lipase from an intracellular to a cell-surface location may be plausible in that such an action in the translocation of glucose transport units has already been suggested (Cushman & Wardzala, 1980; Suzuki & Kono, 1980; Karnieli *et al.*, 1981; Simpson *et al.*, 1983; Czech, 1984).

Experiments were also carried out in the presence of dexamethasone, an effector thought to stimulate adipocyte lipoprotein lipase gene expression specifically (Ashby & Robinson, 1980; Robinson *et al.*, 1983). Fig. 3(*a*) indicates that, in the presence of dexamethasone, no change in cell-surface immunoreactive lipoprotein lipase occurred. With dexamethasone present the previously demonstrated (Fig. 2*a*) stimulation in cell-surface immunoreactivity by insulin was re-affirmed (Fig. 3*b*). In order to determine whether the presence of dexamethasone modified this effect of insulin on cell-surface lipoprotein lipase, adipocytes were incubated with insulin in either the presence or the absence of the synthetic glucocorticoid.

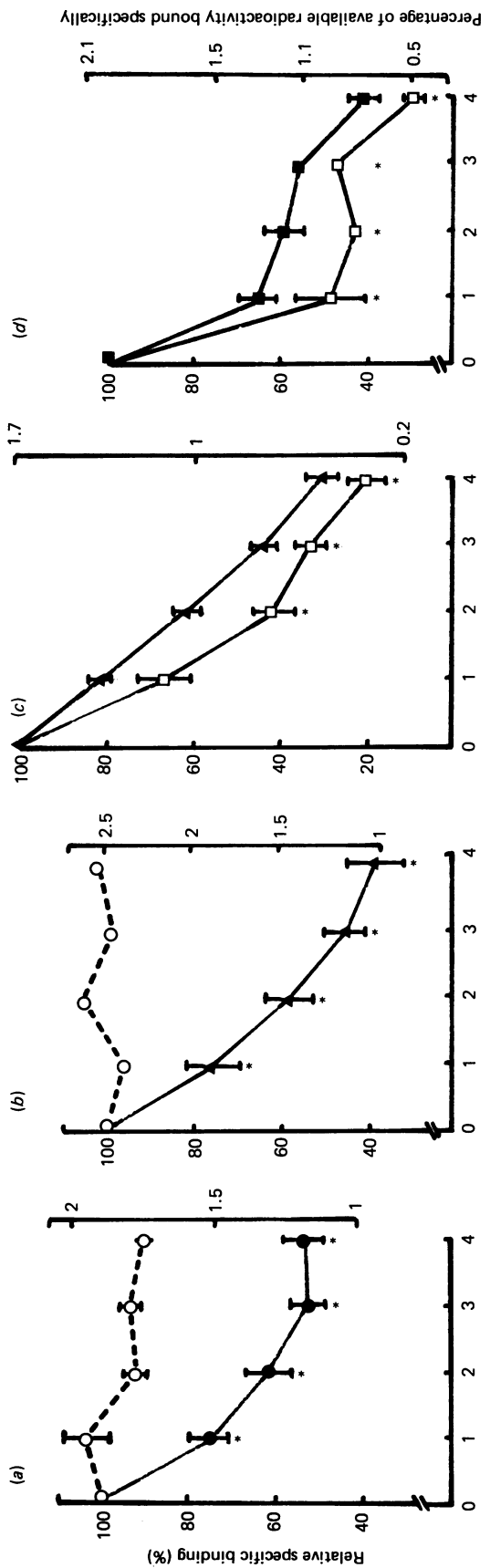

Fig. 4. Changes in immunodetectable cell-surface lipoprotein lipase on isolated adipocytes incubated in medium 199 or in medium 199 containing (*a*) glucagon, (*b*) adrenaline, or (*c*) and (*d*) combinations of adrenaline and cycloheximide

For each experiment 1 ml of isolated adipocytes isolated from 24 h-starved rats was incubated in 3 ml of medium 199 at 37 °C: (*a*) in the absence (○) or presence (●) of glucagon (0.2 μM); (*b*) in the absence (○) or presence (▲) of adrenaline (10 μM); (*c*) in the presence (▲) of adrenaline (10 μM) or the presence (□) of adrenaline (10 μM) and cycloheximide (10 μg/ml); (*d*) in the presence (■) of cycloheximide (10 μg/ml) or in the presence (□) of cycloheximide (10 μg/ml) and adrenaline (10 μM). The specific binding of labelled second antibody is expressed either as a percentage of available radioactivity or as a percentage of that detected at 0 h. The values given are means ± S.E.M. for data derived from three independent comparative experiments in each case. Asterisks indicate statistical significance at $P < 0.01$. The labelled-second-antibody radioactivity added was approx. (*a*) 35000 c.p.m., (*b*) 40000 c.p.m., (*c*) 43000 c.p.m. and (*d*) 41000 c.p.m. for each series of experiments.

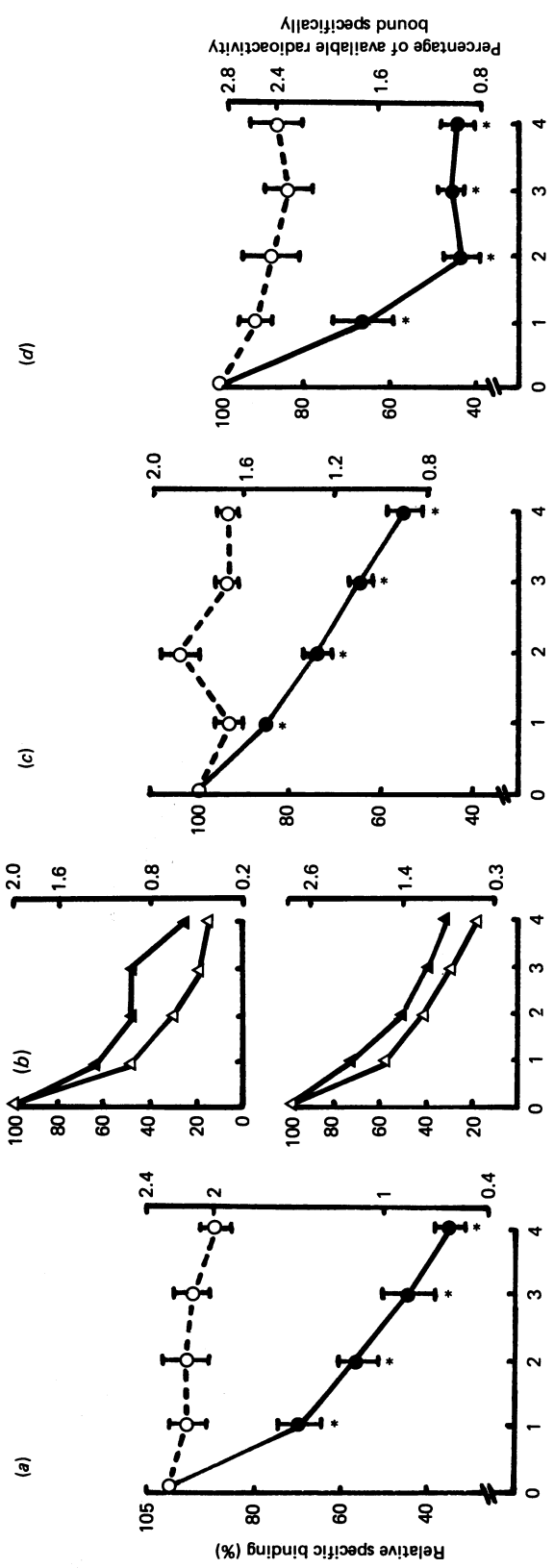

Fig. 5. Changes in immunodetectable cell-surface lipoprotein lipase on isolated adipocytes incubated in medium 199 or in medium 199 containing (*a*) theophylline, (*b*) theophylline and adrenaline, (*c*) tunicamycin and (*d*) colchicine

For each experiment 1 ml of isolated adipocytes from 24 h-starved rats was incubated in 3 ml of medium 199 at 37 °C: (*a*) in the absence (○) or presence (●) of theophylline (3 mM); (*b*) in the presence (▲) of adrenaline (10 μM) or in the presence (△) of adrenaline (10 μM) plus theophylline (3 mM); (*c*) in the absence (○) or presence (●) of tunicamycin (2.5 μg/ml); (*d*) in the absence (○) or presence (●) of colchicine (0.1 mM). The specific binding of labelled second antibody is expressed either as a percentage of available radioactivity or as a percentage of that detected at 0 h. For panels (*a*), (*c*) and (*d*) the values given are means ± S.E.M. for data derived from three independent comparative experiments in each case. Panel (*b*) shows the results from each of two independent comparative experiments. Asterisks indicate statistical significance at $P < 0.01$. The labelled-second-antibody radioactivity added was approx. (*a*) 35000 c.p.m., (*b*) 30000 c.p.m. (*c*) 35000 c.p.m. and (*d*) 43000 c.p.m. for each series of experiments.

From Fig. 3(*c*) it appears that the presence of dexamethasone potentiated somewhat the independent effect of insulin. Although the effect of dexamethasone was not significant statistically when data from the three experiments of this kind were combined (as in Fig. 3*c*), when the data in the series were analysed on the basis of paired comparisons a statistically significant ($P < 0.05$) effect was seen at the later incubation times. Ashby & Robinson (1980) have shown that dexamethasone was without an independent effect on adipose-tissue lipoprotein lipase activity *in vitro*, but that the glucocorticoid potentiated the effect of insulin on the activity. In the present context dexamethasone was also without effect on the amount of lipoprotein lipase protein detected at the cell surface, but was able to increase the otherwise insulin-dependent effect on the pool of enzyme at this site. These observations may also be consistent with the suggestions that insulin may act at the post-transcriptional level both to enhance translation of the protein and to stimulate its intracellular translocation (Fig. 2).

It has been indicated elsewhere (Cryer, 1981; Hamosh & Hamosh, 1983) that additional hormonal regulation of lipoprotein lipase in adipose tissue is probably exerted by adrenaline (Wing *et al.*, 1966; Davies *et al.*, 1974). Such control is short-term, and it has been suggested (Ashby *et al.*, 1978) that adrenaline and other lipolytic agents lower the activity of the enzyme by stimulating degradation, possibly from a transport-vesicle pool (Vannier *et al.*, 1985). Evidence in favour of a stimulation of lipoprotein lipase degradation in the presence of adrenaline has come also from pulse–chase experiments carried out by Parkin *et al.* (1985). Although the β-adrenergic effect is mimicked by dibutyryl cyclic AMP, there is no evidence that it involves a protein-kinase-mediated phosphorylation of the enzyme (Steinberg & Khoo, 1977; Robinson *et al.*, 1983) or whether such adrenergic stimulation is involved in the control of the enzyme *in vivo* (Hansson *et al.*, 1981). Fig. 4 shows that, in the presence of either glucagon or adrenaline, an extensive decline in cell-surface lipoprotein lipase occurred during the 4 h of incubation (Figs. 4*a* and 4*b*). This decline was even more extensive when cycloheximide was added to cell incubations in the presence of adrenaline. Fig. 5 shows that not only does theophylline mimic the action of adrenaline in producing an extensive decline in adipocyte surface lipoprotein lipase protein (Fig. 5*a*), but it potentiates the decline produced by adrenaline alone (Fig. 5*b*). Taking these data (Figs. 4 and 5) it would seem possible that a proportion of the β-adrenergic-induced decline in enzyme activity may be due to accelerated rates of degradation from the plasma-membrane-associated pool of enzyme.

Previous studies have indicated that the release of lipoprotein lipase from adipocytes incubated with heparin was not only energy-dependent (Stewart & Schotz, 1974) but was also dependent on microtubular function (Cryer *et al.*, 1975*b*). This situation has also been illustrated by using various models for the control of cardiac lipoprotein lipase (Borensztajn *et al.*, 1975; Chajek *et al.*, 1975*a*,*b*, 1978; Cryer *et al.*, 1981). Fig. 5(*d*) shows that, even in the absence of heparin, colchicine, at concentrations thought not to interfere with protein synthesis in adipocytes (Cryer *et al.*, 1975*b*; Stein *et al.*, 1974), but which block the intracellular movement/secretion of a variety of proteins by interference with intracellular transport mechanisms involving microtubular function (Lacy *et al.*, 1968; Ehrlich *et al.*, 1974), caused a decline in cell-surface immunodetectable lipoprotein lipase protein. Thus, whatever other mechanisms contribute to the translocation of lipoprotein lipase to the cell-surface site, microtubular function is also involved.

Tunicamycin also caused a significant decline in cell-surface immunodetectable lipoprotein lipase, which did not occur in its absence (Fig. 5*c*). It is well known that lipoprotein lipase from a number of sources (Parkin *et al.*, 1982) is a glycoprotein and, in common with our findings on the heparin-stimulated secretion of enzyme from cardiac cells (Cryer *et al.*, 1981), the current data indicate that glycosylation of the enzyme protein (Vannier *et al.*, 1985) is also necessary for its location at the adipocyte cell surface.

The present data are complemented by that of Vannier *et al.* (1986) who, using differentiated Ob17 adipocytes grown in culture, have shown that lipoprotein lipase has a subcellular distribution which is consistent with it being a secretory product of these cells. In this latter system, however, it is possible that, although all the intracellular lipoprotein lipase is sequestered in closed membrane structures, quantitatively most of it is associated with the Golgi apparatus.

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