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Colchicine Inhibition of the Heparin-Stimulated Release of Clearing-Factor Lipase from Isolated Fat-Cells

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When isolated fat-cells are incubated at 25°C in serum-based media containing glucose, insulin and heparin, the rise that occurs in the clearing-factor lipase activity of the incubation medium is inhibited by colchicine. The rise in the fat-cell clearing-factor lipase activity that occurs during similar incubations in the absence of heparin is not affected by colchicine.

The function of the enzyme clearing-factor lipase (lipoprotein lipase) is to hydrolyse the plasma triglycerides and thereby facilitate the uptake of their constituent fatty acids by the extrahepatic tissues of the body. It normally exerts this action on the plasma triglycerides at the luminal surfaces of the endothelial cells lining the blood capillaries of such tissues (see Robinson, 1970). However, in adipose tissue the enzyme is also present in association with the fat-cell component of the tissue, and current evidence, reviewed previously (Robinson & Wing, 1971; Cryer *et al.*, 1975*a*), suggests that the fat-cell enzyme is the precursor of the functional enzyme at the endothelial cell surface. Moreover, it seems that hormonal control of the activity of the enzyme in adipose tissue is normally exerted on this precursor (see Robinson & Wing, 1971).

Although the nature of this hormonal control has received considerable attention (Robinson & Wing, 1971; Cryer *et al.*, 1975*a*), the mechanism of transport of the enzyme from the fat-cell to the endothelial cell in adipose tissue remains unknown. The enzyme is, however, released by heparin from tissues in which it is present both *in vivo* and *in vitro* (Robinson, 1970), and Chajek *et al.* (1975*a*,*b*) have shown that this release is inhibited by colchicine. Because colchicine is known to interfere with the secretion of a number of cell proteins (see under 'Discussion'), these authors have suggested that it may act in such a situation either directly by interfering with the release of the enzyme from the endothelial cell or indirectly through an effect on the transport of the enzyme from its precursor site to the endothelial cell. On present evidence this precursor site in adipose tissue is the fat-cell, and it has already been shown that increases in clearing-factor lipase activity occur when isolated fat-cells are incubated in media containing glucose and insulin. Moreover, in appropriate circumstances, these increases are entirely due to enzyme that is released into the incubation medium (see Robinson & Wing, 1971; Stewart & Schotz, 1974; Cryer *et al.*, 1975*b*). It was therefore decided to investigate the effects of colchicine in such incubation systems. The present paper reports the results of such a study.

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Materials and methods

Animals and tissue preparations. Male albino rats of the Wistar strain, maintained on Oxoid pasteurized diet 41B (H. Styles Ltd., Bewdley, Worcs., U.K.), were used throughout. All the animals weighed 170–190g in the fed state, and, before use, they were starved for 24h from between 9:00 and 10:00h the previous day. After starvation, the epididymal fat-pads were removed while the animals were under light ether anaesthesia. Fat-cells were isolated from the fat-pads as described previously (Cryer *et al.*, 1975*b*).

Incubation of fat-cells. In all the experiments, the cells were incubated in the serum-based medium described previously (Cryer *et al.*, 1975*b*). In cases where heparin was omitted from the medium, the volume of water present was adjusted accordingly. When present, freshly prepared solutions of cycloheximide and colchicine also replaced equivalent volumes of water. The colchicine was present in the medium at a concentration (0.1mM) that previous work has shown is generally without effect on protein synthesis (Ehrlich *et al.*, 1974; Redman *et al.*, 1975). The sources of all other materials were as described previously (Cryer *et al.*, 1975*b*). Colchicine was obtained from Sigma Chemical Co., London S.W.6, U.K.

Assay of clearing-factor lipase. Assays of the enzyme in fat-cells and in incubation media were carried out as described previously (Cryer *et al.*, 1975*b*). The assays of the enzyme in the medium were performed immediately after the end of the incubations. Those of the fat-cell enzyme were carried out in homogenates of acetone–ether-dried preparations made at the end of the incubations and then stored overnight at −20°C. The final heparin concentration in all the assays was 0.7 unit/ml. Colchicine had no detectable effect on the assay of enzyme activity.

Results

When fat-cells are incubated in the presence of glucose and insulin in a serum-based medium at 25°C, the total clearing-factor lipase activity of the incubation system rises progressively over a period of several hours (Robinson & Wing, 1971). If heparin is also a component of the incubation medium, all of the rise in total activity is accounted for by the activity of enzyme that appears in the incubation medium and the cell activity remains essentially unchanged. In the absence of heparin, on the other hand, although the extent of the rise in the total enzyme activity of the incubation system is similar, much less enzyme appears in the medium and most of the increase in activity takes place in the fat-cells (Cryer *et al.*, 1975*b*).

The results in Fig. 1(*a*) show that there is a marked diminution in the rise in the enzyme activity in the medium that occurs during incubations carried out in the presence of heparin when colchicine is also present in the incubation medium. The effect is evident within 1 h and persists for at least 3 h. Although the results of only a single experiment are shown in Fig. 1, the findings have been confirmed in additional experiments carried out under similar incubation conditions. Thus in four separate experiments, each of which involved the incubation of fat-cells from 12 fat-pads in the presence of heparin, the enzyme activity present in the medium after 3 h at 25°C was 4.4 ± 0.33 (mean ± S.D.) units (μmol of free fatty acid/h)/fat-pad equivalent when colchicine was absent and 1.3 ± 0.28 units/fat-pad equivalent in its presence. The diminution in the rise in enzyme activity in the medium when colchicine was present was significant at the $P = 0.001$ level.

Experiments have also been carried out that are identical with those in Fig. 1(*a*) except for the absence of heparin from the incubation medium. In these the much lower activity appearing in the medium was not affected in the presence of colchicine, activities in the medium after 3 h being respectively 1.4 ± 0.2 and 1.3 ± 0.2 units/fat-pad equivalent in the presence of colchicine (mean ± S.D. for three separate experiments).

At concentrations that have been shown to inhibit protein synthesis in the fat-cell by more than 90%, cycloheximide inhibits the rise in total clearing-factor lipase activity that occurs during fat-cell incubations such as those described above (Cryer *et al.*, 1975*b*). However, its action is restricted to an effect on the activity of the enzyme in the fat-cell. Thus, during

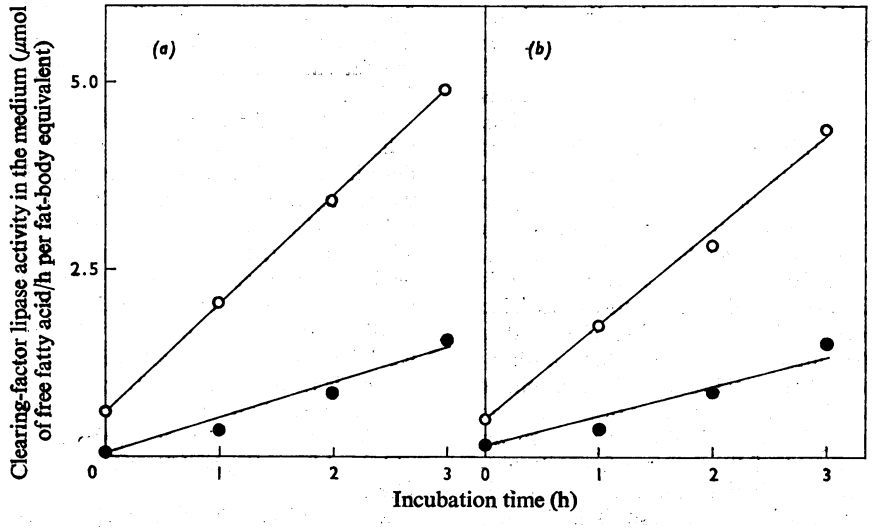

Fig. 1. *Effects of colchicine on the increases in clearing-factor lipase activity in the medium occurring during fat-cell incubations at 25°C in the absence and in the presence of cycloheximide*

All the incubation media contained glucose (13.3 mM), insulin (12 m-i.u./ml), heparin (2.4 units/ml) and dialysed serum (48%, v/v). The composition has been described in detail previously (Cryer *et al.*, 1975*b*). Colchicine (0.1 mM) was either absent from (○) or present in (●) the media. Figs. 1(*a*) and 1(*b*) respectively refer to incubations carried out in the absence and in the presence of cycloheximide (10 μg/ml). In each incubation, fat-cells derived from 12 fat-pads were used and the volume of the medium was 36 ml. Portions of the media were removed at intervals, and the clearing-factor lipase activity was determined after removal of the fat-cells by flotation in the centrifuge (1 min at 1000*g*). The values shown are the means of duplicate assays in each case. Figs. 1(*a*) and 1(*b*) show the results of a single set of incubations which constituted a single experiment. The numbers are of experiments carried out described under 'Results'.

incubations in the presence of heparin and cycloheximide, the fat-cell activity falls instead of remaining constant, but the rise in the activity in the incubation medium is unchanged. The results in Fig. 1(*b*) show that this rise in activity in the medium, like that which occurs in the absence of cycloheximide (Fig. 1*a*), is also considerably diminished when colchicine is present in the medium. Thus for three separate experiments, carried out essentially as above, but in the presence of cycloheximide and heparin, colchicine diminished the activity in the medium from 4.0 ± 0.4 to 1.1 ± 0.3 units/fat-pad equivalent ($P < 0.001$).

During incubations in the absence of heparin, cycloheximide again affects only the cell activity. Thus it diminishes the increase in cell activity that occurs in its absence, but is without effect on the much lower activity that appears in the incubation medium (Cryer *et al.*, 1975*b*). This low activity is also unchanged when colchicine is present. For example, in experiments identical with those in Fig. 1(*b*) except for the omission of heparin, the activities in the medium at 3h were respectively 1.3 and 1.1 units/fat-pad equivalent in the presence and in the absence of colchicine (mean values of two separate experiments).

The clearing-factor lipase activities of the fat-cells were also determined in the above experiments. In all cases colchicine had no marked effect on these. Thus the rise in the fat-cell activity that occurred during incubations in the absence of heparin also took place when colchicine was present in the incubation medium, the cell activities after 3h incubations in the absence and in the presence of colchicine being respectively 133 and 138% of the initial values. During incubations in the presence of heparin, the cell activity remained constant whether colchicine was present or absent, the activities after 3h being respectively 104 and 100% of the initial ones. Colchicine also had no effect on the cell activities during incubations in the presence of cycloheximide. In the absence of heparin they remained constant, and in the presence of heparin they fell after 3h incubations in the absence and in the presence of colchicine respectively to 85 and 87% of the initial values.

Discussion

The inhibition by colchicine of the secretion of a variety of proteins from cells is widely attributed to its ability to interfere with intracellular transport mechanisms involving the microtubular system of the cells (Lacy *et al.*, 1968; Butcher & Goldman, 1972; Rossignol *et al.*, 1972; Le Marchand *et al.*, 1973, 1974; Ehrlich *et al.*, 1974; Stein *et al.*, 1974; Redman *et al.*, 1975). Recent work has shown the existence of such systems in isolated fat-cells (Soifer *et al.*, 1971). The present findings that the release of clearing-factor lipase from the fat-cell that occurs during incubations carried out in the presence of heparin is inhibited by colchicine is therefore consistent with microtubule involvement in this process. Moreover, the possibility that the action of colchicine is secondary to an effect on protein synthesis is excluded by its persistence during incubations carried out under conditions where protein synthesis is almost entirely inhibited.

Recent studies have shown that in a variety of tissues colchicine may interfere with intracellular processes other than those involving microtubules (Mizel & Wilson, 1972; Orr *et al.*, 1972; Douglas & Sorimachi, 1972; Trifaro *et al.*, 1972). However, no marked intracellular metabolic effects have been observed in work with fat-cells at colchicine concentrations similar to those used here (Soifer *et al.* 1971; Loten & Jeanrenaud, 1974; Schimmel, 1974; Cheng & Katsoyannis, 1975). Moreover, in the present study colchicine was without effect on the increase in cellular clearing-factor lipase activity that occurred during fat-cell incubations in the absence of heparin. Although further evidence for direct microtubule involvement in the release of the enzyme from the fat-cell in response to heparin is clearly required, therefore, it does appear that the effect of colchicine now observed is specifically on the transport of the enzyme out of the fat-cell. This would not be inconsistent with the work of Schimmel (1974) and Cheng & Katsoyannis (1975), which focuses attention on the fat-cell plasma membrane as the site of colchicine's actions.

The present findings also suggest that the action of colchicine in diminishing the release of clearing-factor lipase from the endothelial cell by heparin (see the introduction) is an indirect one that is exerted on the transport of the enzyme from its cellular precursor site. Whether the release of clearing-factor lipase from the fat-cell and from the endothelial cell by heparin occurs by identical mechanisms is not clear. However, it is noteworthy that there is already good evidence that heparin has a general affinity for the enzyme (Robinson, 1970).

The fact that, when the release from the fat-cell is inhibited by colchicine during incubations in the presence of heparin, there is no build-up of enzyme in the cell, such as occurs during incubations in heparin's absence, also requires explanation. It may be that the changes in the activities in the cell and in the medium are not directly related, as has previously been suggested (Cryer *et al.*, 1975*b*), and in this connexion it is important to emphasize that different forms of clearing-factor lipase have been identified both in intact adipose tissue (Schotz & Garfinkel, 1972; Garfinkel & Schotz, 1973; Davies *et al.*, 1974) and in isolated fat-cells (Cryer *et al.*, 1975*b*). The possibility that the form of the enzyme that is capable of being released by heparin may be different from that which accumulates in the cell can clearly not be excluded (Cryer *et al.*, 1975*a*).

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Butcher, F. R. & Goldman, R. H. (1972) *Biochem. Biophys. Res. Commun.* **48**, 23–29

Chajek, T., Stein, O. & Stein, Y. (1975*a*) *Biochim. Biophys. Acta* **380**, 127–131

Chajek, T., Stein, O. & Stein, Y. (1975*b*) *Biochim. Biophys. Acta* **388**, 260–267

Cheng, K. & Katsoyannis, P. G. (1975) *Biochem. Biophys. Res. Commun.* **64**, 1069–1075

Cryer, A., Davies, P. & Robinson, D. S. (1975*a*) in *Blood and Arterial Wall in Atherogenesis and Arterial Thrombosis* (Hautvast, J. G. A. J., Hormus, R. J. J. & van den Haar, F., eds.), pp. 102–110, E. J. Brill, Leiden

Cryer, A., Davies, P., Williams, E. R. & Robinson, D. S. (1975*b*) *Biochem. J.* **146**, 481–488

Davies, P., Cryer, A. & Robinson, D. S. (1974) *FEBS Lett.* **45**, 271–275

Douglas, W. W. & Sorimachi, M. (1972) *Br. J. Pharmacol.* **45**, 129–132

Ehrlich, H. P., Ross, R. & Bornstein, P. (1974) *J. Cell Biol.* **62**, 390–405

Garfinkel, A. S. & Schotz, M. C. (1973) *Biochim. Biophys. Acta* **306**, 128–133

Lacy, P. E., Howell, S. L., Young, D. A. & Fink, C. J. (1968) *Nature (London)* **219**, 1177–1179

Le Marchand, Y., Singh, A., Assimacopoulos-Jeannet, F., Orci, L., Rouiller, C. & Jeanrenaud, B. (1973) *J. Biol. Chem.* **248**, 6862–6870

Le Marchand, Y., Patzelt, C., Assimacopoulos-Jeannet, F., Loten, E. G. & Jeanrenaud, B. (1974) *J. Clin. Invest.* **53**, 1512–1517

Loten, E. G. & Jeanrenaud, B. (1974) *Biochem. J.* **140**, 185–192

Mizel, S. B. & Wilson, L. (1972) *Biochemistry* **11**, 2573–2578

Orr, T. S. C., Hall, D. E. & Allison, A. L. (1972) *Nature (London)* **236**, 350–351

Redman, C. M., Banerjee, D., Howell, K. & Palade, G. E. (1975) *J. Cell Biol.* **66**, 42–59

Robinson, D. S. (1970) *Compr. Biochem.* **18**, 51–116

Robinson, D. S. & Wing, D. R. (1971) *Biochem. Soc. Symp.* **33**, 123–135

Rossignol, B., Herman, G. & Keryer, G. (1972) *FEBS Lett.* **21**, 189–194

Schimmel, R. J. (1974) *J. Lipid Res.* **15**, 206–210

Schotz, M. C. & Garfinkel, A. S. (1972) *Biochim. Biophys. Acta* **270**, 472–478

Soifer, D., Braun, T. & Hechter, O. (1971) *Science* **172**, 269–271

Stein, O., Sanger, L. & Stein, Y. (1974) *J. Cell Biol.* **62**, 90–103

Stewart, J. E. & Schotz, M. C. (1974) *J. Biol. Chem.* **249**, 904–907

Trifaro, J. M., Collier, B., Lastowecka, A. & Stern, D. (1972) *Mol. Pharmacol.* **8**, 264–267