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Effects of compactin, mevalonate and low-density lipoprotein on 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity and low-density-lipoprotein-receptor activity in the human hepatoma cell line Hep G2

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Compactin, an inhibitor of HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase, decreased cholesterol synthesis in intact Hep G2 cells. However, after the inhibitor was washed away, the HMG-CoA-reductase activity determined in the cell homogenate was found to be increased. Also the high-affinity association of LDL (low-density lipoprotein) to Hep G2 cells was elevated after incubation with compactin. Lipoprotein-depleted serum, present in the incubation medium, potentiated the compactin effect compared with incubation in the presence of human serum albumin. Addition of either mevalonate or LDL prevented the compactin-induced rise in activities of both HMG-CoA reductase and LDL receptor in a comparable manner. It is concluded that in this human hepatoma cell line, as in non-transformed cells, both endogenous mevalonate or mevalonate-derived products and exogenous cholesterol are able to modulate the HMG-CoA reductase activity as well as the LDL-receptor activity.

In search for a suitable model for the study of cholesterol and lipoprotein metabolism in human hepatocytes, we tested the usefulness of the human hepatoma cell line Hep G2. Although transformed, these cells still possess several properties characteristic for the human hepatocyte (Knowles *et al.*, 1980; Schwartz *et al.*, 1981; Redman *et al.*, 1983; Fair & Plow, 1983). For our purpose this cell line must be able to regulate (1) its cholesterol synthesis, i.e. the activity of the rate-limiting enzyme HMG-CoA reductase (EC 1.1.1.34), (2) its LDL-receptor activity, and (3) the production of apolipoproteins. It is known that Hep G2 cells synthesize and secrete apolipoproteins (Zannis *et al.*, 1981; Rash *et al.*, 1981). We have demonstrated the occurrence of LDL-receptor activity in Hep G2 cells (Havekes *et al.*, 1983).

In the present study we investigated the effects of compactin (ML-236B), mevalonate and LDL on HMG-CoA reductase and LDL-receptor activity in the human hepatoma cell line. Compactin has been described to be a potent and specific competitive inhibitor of HMG-CoA reductase in rat liver microsomal fractions (Endo *et al.*, 1976) and to suppress cholesterol synthesis in intact liver cells (Endo *et al.*, 1977). After incubating various cells *in vitro* with compactin, several investigators measured a paradoxical increase in the HMG-CoA reductase activity in broken cell preparations (Brown *et al.*, 1978; Edwards *et al.*, 1980; Ryan *et al.*, 1981; Cohen *et al.*, 1982). Simultaneous incubation with mevalonate abolished the activity increase, indicating a feedback regulation of the reductase by mevalonate or a mevalonate-derived product (Brown & Goldstein, 1980; Cohen *et al.*, 1982).

Like HMG-CoA reductase, the LDL receptor also plays a role in providing the cell with cholesterol. Therefore common effectors may be involved in the feedback regulation of both activities. In skin fibroblasts from normal humans, compactin had only a minor effect on the LDL-receptor activity (Brown *et al.*, 1978; Filipovic & Menzel, 1981; Semenkovich *et al.*, 1982). However, in pig hepatocytes a significant increase in the high-affinity LDL receptor by compactin was found (Pangburn *et al.*, 1981).

Abbreviations used: HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; LDL, low-density lipoprotein; LPDS, lipoprotein-depleted serum; DMEM, Dulbecco's modified Eagle's medium.

Materials and methods

Materials

Compactin (ML-236B) was a gift from Dr. A. Endo, Tokyo Nōkō University, Tokyo, Japan. Sigma Chemical Co. supplied human serum albumin (fraction V, essentially fatty-acid free), DL-3-hydroxy-3-methylglutaryl-CoA and DL-mevalonolactone. Glucose 6-phosphate and glucose-6-phosphate dehydrogenase were purchased from Boehringer Mannheim. Human LPDS and LDL were prepared from freshly collected human blood as described previously (Havekes *et al.*, 1983). For experiments performed with human serum albumin (subsequently termed 'albumin'), the ^{125}I-labelled and unlabelled LDL preparations were stabilized by the addition of albumin (1%, w/v) instead of LPDS (20%, v/v).

Human hepatoma cell line Hep G2

The Hep G2 cells were cultured in multiwell dishes (2 or 10cm^2; Costar) as described by Havekes *et al.* (1983). At 18h before the assays mentioned below were performed, the medium was replaced with DMEM supplemented with either 10 or 20% (v/v) LPDS or 1% (w/v) albumin and different amounts of compactin, mevalonate or LDL as specified in the Results section.

Assays

The rate of cholesterol synthesis in Hep G2 cells grown in 10cm^2 wells, as well as the high-affinity association of ^{125}I-LDL to cells grown in 2cm^2 wells, were determined essentially as described by Havekes *et al.* (1983).

The assay of HMG-CoA reductase activity, as described by Shapiro *et al.* (1974), was adjusted for the determination in Hep-G2-cell homogenate. After the 18h incubation, cells, cultured in 10cm^2 wells, were washed twice with DMEM and incubated in DMEM for another 15min at 37°C, to remove intracellular compactin. Cells were washed three times with cold phosphate-buffered saline (0.15M-NaCl/10mM-Na_2HPO_4/1.5mM-KH_2PO_4, pH7.4) and once with 0.1M-potassium phosphate/0.1M-NaCl (pH7.4), and were harvested by scraping them into 200μl of assay buffer (0.1M-potassium phosphate/0.1M-NaCl/10mM-EDTA, pH7.4). The cell suspension was frozen in liquid N_2 before storage at −70°C. After thawing and rupture of the cells by sonication (Branson sonifier B-12, 70W output, for 5s at 0°C), four 50μl samples (80–160μg of protein) for duplicate determinations in the presence or absence of cofactors were preincubated for 25min at 37°C. The enzyme reaction was performed for 40min at 37°C after addition of cofactors and substrate. The 100μl assay mixture contained: 0.5mmol-[^{14}C]HMG-CoA (sp. radioactivity 2500–3000d.p.m./nmol), 5mM-$NADP^+$, 50mM-glucose 6-phosphate, 0.7 unit of glucose-6-phosphate dehydrogenase, 50mM-potassium phosphate (pH7.4), 50mM-NaCl, 10mM-EDTA, 5mM-dithiothreitol and 0.8–1.6mg of Hep-G2-cell protein/ml. The substrate [^{14}C]HMG-CoA, purchased from Amersham, was purified before use by t.l.c. (Philipp & Shapiro, 1979). Preliminary experiments showed that the formation of [^{14}C]mevalonic acid was linear with incubation time up to 50min and with protein concentrations up to 6mg/ml. Reductase activity is expressed in pmol of mevalonic acid formed/min per mg of cellular protein. The values given are the averages of duplicate determinations in two homogenates of identically treated cells. The average values for the individual homogenates agreed within 10%.

Protein concentrations were determined in the cell homogenates as described by Lowry *et al.* (1951). The data presented in the Figures and Table are representative of at least two separately performed experiments.

Results

Inhibition of the cholesterol synthesis in Hep G2 cells by compactin

Preliminary experiments were performed to assess the effect of compactin in Hep G2 cells and homogenate. In the presence of 0.2μM- or 2.0μM-compactin the rate of cholesterol synthesis in the cells was inhibited to 48 or 17% of the control rate (10.1ng-atoms of ^{3}H incorporated into cholesterol/h per mg of cell protein) respectively. Adding comparable amounts of compactin to a Hep-G2-cell homogenate gave a similar degree of inhibition of HMG-CoA reductase (Fig. 1), suggesting that compactin is active at concentrations of the same magnitude in the cell.

Increase in HMG-CoA reductase activity and high-affinity LDL association by incubation of Hep G2 cells with compactin

Hep G2 cells were incubated in the presence of different concentrations of compactin for 18h. After the drug was washed away, the reductase activity was determined in the cell homogenate. As shown in Fig. 2(*a*) there was a marked increase in reductase activity corresponding to the increasing compactin concentrations present during the cell incubation. A similar effect was found on the high-affinity LDL association, as shown in Fig. 2(*b*).

In the experiments depicted in Fig. 3, HMG-CoA reductase activity and LDL-receptor activity were determined after incubation of the Hep G2 cells in DMEM supplemented with either LPDS (10 or 20%, v/v) or albumin (1%, w/v) in the

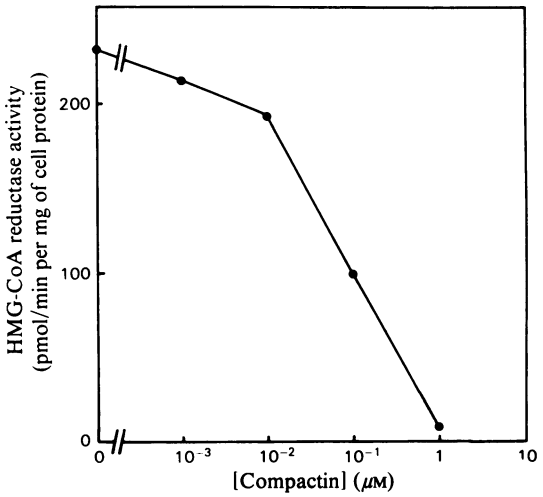

Fig. 1. *Inhibition of HMG-CoA reductase in Hep-G2-cell homogenate by compactin*

Cells grown in DMEM supplemented with 10% heat-inactivated foetal bovine serum were washed twice with phosphate-buffered saline and scraped in the same buffer. Cells were collected by centrifugation and sonified in HMG-CoA reductase assay buffer as described in the Materials and methods section. HMG-CoA reductase activity was determined in the homogenate after addition of compactin to final concentrations as indicated in the Figure.

absence or presence of 2 μM-compactin. Incubation in LPDS gave a slight increase in the reductase activity compared with incubation in the presence of albumin. In all cases the addition of compactin resulted in an increase in the measured activity, as observed above. However, the compactin-induced increase was larger when the incubation was performed in LPDS than in albumin (Fig. 3*a*). The high-affinity LDL association was markedly elevated in the presence of LPDS. As seen with the reductase activity, the stimulating effect of compactin on the LDL-receptor activity was much stronger when 20% LPDS was used instead of 1% albumin during the incubation (Fig. 3*b*).

Effect of mevalonic acid and of LDL on the compactin-induced increase in the HMG-CoA reductase activity and high-affinity LDL association

Cells were incubated with 2 μM-compactin, 10% LPDS and increasing amounts of mevalonate. As depicted in Fig. 4(*a*), mevalonate decreased the compactin-induced rise in HMG-CoA reductase activity proportionally to the amount added to the medium. Furthermore, in the absence of compactin, mevalonate also decreased the reductase activity (Fig. 4*a*).

The effect of mevalonate on the high-affinity association of LDL is shown in Fig. 4(*b*). When the

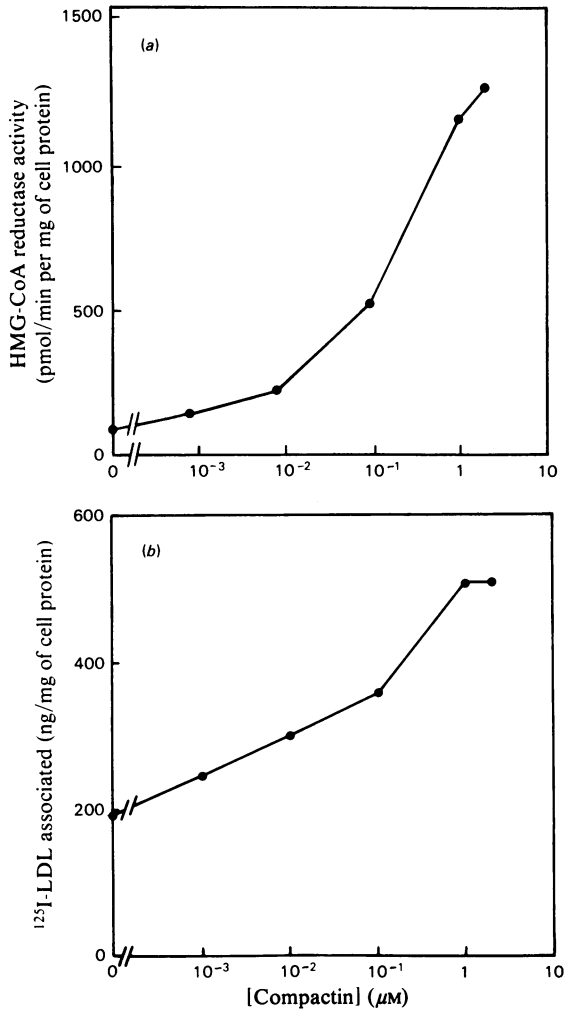

Fig. 2. *Effect of compactin on HMG-CoA reductase activity (a) and high-affinity LDL association (b) in Hep G2 cells*

At 18 h before the assay, the medium of the Hep G2 cells was replaced by DMEM containing 10% (*a*) or 20% (*b*) LPDS and the indicated concentrations of compactin. After incubation at 37°C HMG-CoA reductase activity (*a*) and high-affinity LDL association (*b*) were determined as described in the Materials and methods section.

cells had been incubated in the presence of 1 μM-compactin and 20% LPDS, the high-affinity LDL association decreased with increasing concentrations of mevalonate. In contrast with the effect on the HMG-CoA reductase activity, mevalonate had virtually no decreasing effect on the LDL association in the absence of compactin.

The addition of LDL (200 μg of protein/ml) to the incubation medium decreased both the basal and compactin-induced activities of HMG-CoA

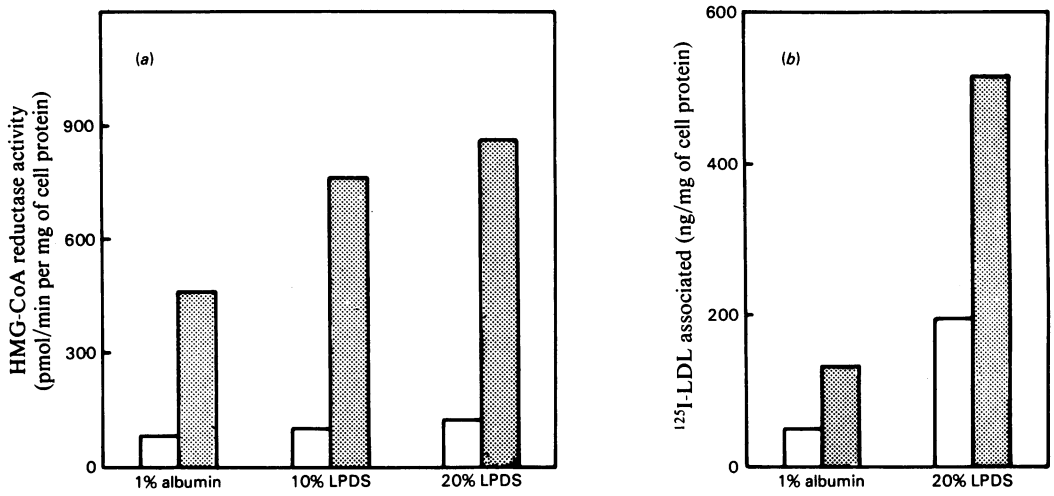

Fig. 3. *Effect of LPDS on the compactin-induced increase in the HMG-CoA reductase activity (a) and the high-affinity LDL association (b)*

Hep G2 cells were incubated for 18h in DMEM supplemented with 1% albumin, 10% LPDS or 20% LPDS in the absence (□) or presence (▨) of 2 μM-compactin. The subsequent measurements of the HMG-CoA reductase activity (*a*) and the high-affinity LDL association (*b*) were performed as described in the Materials and methods section.

Table 1. *Effect of LDL on basal and compactin-induced activities of HMG-CoA reductase and LDL receptor*

Hep G2 cells were incubated for 18h in albumin (1%)-containing DMEM supplemented with compactin (2 μM) and/or LDL (200 μg of protein/ml). Subsequently HMG-CoA reductase and LDL-receptor activities were determined as described in the Materials and methods section. Each value represents the mean of duplicate incubations.

		HMG-CoA reductase activity (pmol/min per mg)		High-affinity LDL association (ng of ^{125}I-LDL associated/mg)	
LDL present	Compactin ...	−	+	−	+
−		82	456	96	219
+		35 (43)*	128 (28)*	43 (45)*	49 (22)*

* Percentage of values without addition of LDL.

reductase and LDL receptor (Table 1). In the absence of compactin, the effect of LDL on both activities was relatively smaller than in its presence.

Discussion

Both HMG-CoA reductase activity and high-affinity LDL association are concentration-dependently elevated by an 18h incubation of the cells with compactin. This effect is prevented by simultaneous addition of either mevalonate or LDL. Incubation in the presence of LPDS resulted in a further increase in the compactin-induced activities, compared with incubations with albumin as the sole serum component. This LPDS effect was not merely additive to the compactin stimulation, but was synergistic with the latter for both measured activities.

We conclude from these observations that Hep G2 cells are capable of feedback regulating their HMG-CoA reductase activity and LDL-receptor activity. To our knowledge, this is the first time that this has been described for a human hepatoma cell line. For a rat hepatoma cell line the existence of regulation of the reductase activity was reported by Beirne *et al.* (1977).

Further, it is concluded that mevalonate or mevalonate-derived products are effectors in the regulation of the HMG-CoA reductase activity as well as in the regulation of the LDL-receptor activity. The addition of LDL to the cells could compensate for the blocked formation of these effectors in the presence of compactin (Table 1). So

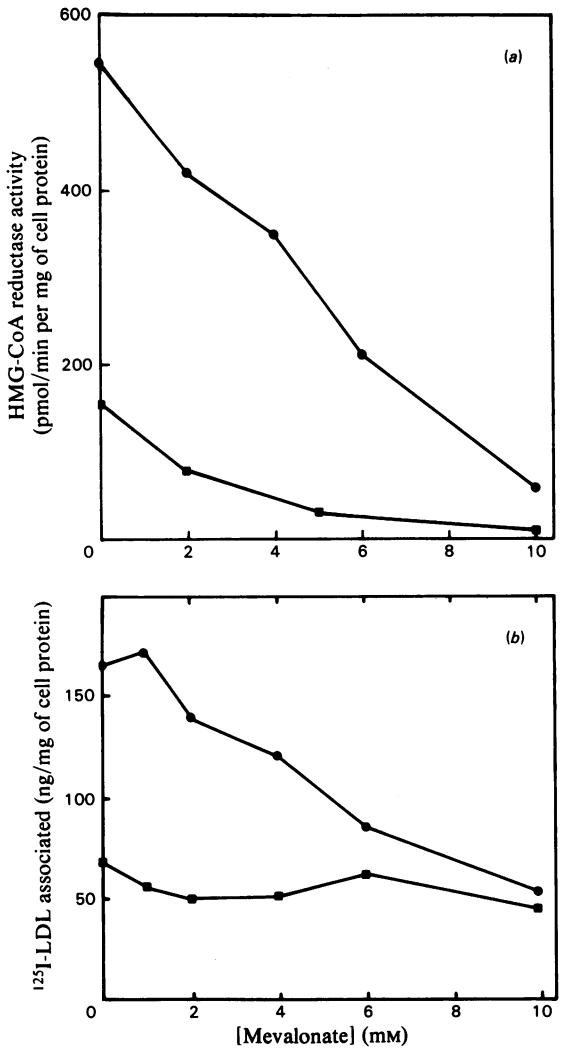

Fig. 4. *Effect of mevalonate on the compactin-induced increase of the HMG-CoA reductase activity (a) and the high-affinity LDL association (b)*

Hep G2 cells were incubated for 18h with DMEM containing 10% (*a*) or 20% (*b*) LPDS, 1 μM- (*b*) or 2 μM- (*a*) compactin and the indicated concentrations of mevalonate (●). Control incubations were performed without compactin (■). The subsequent determinations of the HMG-CoA reductase activity (*a*) and the high-affinity LDL association (*b*) were performed as described in the Materials and methods section.

it is likely that cholesterol or cholesterol-derived products are effectors in the regulation of both activities. This may also explain the observed effect of LPDS, because LPDS is thought to potentiate the efflux of cholesterol from cells (Stein *et al.*, 1976; St. Clair & Leight, 1983). Together with the finding that Hep G2 cells regulate their apolipoprotein synthesis (Rash *et al.*, 1981), the results of the investigations described here underline the usefulness of this human cell line as a model for the study of lipoprotein metabolism in human liver.

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References

Beirne, O. R., Heller, R. & Watson, J. A. (1977) *J. Biol. Chem.* **252**, 950–954

Brown, M. S. & Goldstein, J. L. (1980) *J. Lipid Res.* **21**, 505–517

Brown, M. S., Faust, J. R., Goldstein, J. L., Kaneko, I. & Endo, A. (1978) *J. Biol. Chem.* **253**, 1121–1128

Cohen, D. C., Massoglia, S. L. & Gospodarowicz, D. (1982) *J. Biol. Chem.* **257**, 11106–11112

Edwards, P. A., Lemongello, D., Kane, J., Schechter, I. & Fogelman, A. M. (1980) *J. Biol. Chem.* **255**, 3715–3725

Endo, A., Kuroda, M. & Tanzawa, K. (1976) *FEBS Lett.* **72**, 323–326

Endo, A., Tsujita, Y., Kuroda, M. & Tanzawa, K. (1977) *Eur. J. Biochem.* **77**, 31–36

Fair, D. S. & Plow, E. F. (1983) *J. Lab. Clin. Med.* **101**, 372–384

Filipovic, I. & Menzel, B. (1981) *Biochem. J.* **196**, 625–628

Havekes, L., van Hinsbergh, V., Kempen, H. J. & Emeis, J. (1983) *Biochem. J.* **214**, 951–958

Knowles, B. B., Howe, C. C. & Aden, D. P. (1980) *Science* **209**, 497–499

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Pangburn, S. H., Newton, R. S., Chang, C.-M., Weinstein, D. B. & Steinberg, D. (1981) *J. Biol. Chem.* **256**, 3340–3347

Philipp, B. W. & Shapiro, D. J. (1979) *J. Lipid Res.* **20**, 588–593

Rash, J. M., Rothblat, G. H. & Sparks, C. E. (1981) *Biochim. Biophys. Acta* **666**, 294–298

Redman, C. M., Anellino, G. & Yu, S. (1983) *J. Biol. Chem.* **258**, 3446–3452

Ryan, J., Hardeman, E. C., Endo, A. & Simoni, R. D. (1981) *J. Biol. Chem.* **256**, 6762–6768

Schwartz, A. L., Fridovich, S. E., Knowles, B. B. & Lodish, H. F. (1981) *J. Biol. Chem.* **256**, 8879–8881

Semenkovich, C. F., Ostlund, R. E., Levy, R. A. & Osa, S. R. (1982) *J. Biol. Chem.* **257**, 12857–12865

Shapiro, D. J., Nordstrom, J. L., Mitschelen, J. J., Rodwell, V. W. & Schimke, R. T. (1974) *Biochim. Biophys. Acta* **370**, 369–377

St. Clair, R. W. & Leight, M. A. (1983) *J. Lipid Res.* **24**, 183–191

Stein, O., van der Hoek, J., Friedman, G. & Stein, Y. (1976) *Biochim. Biophys. Acta* **450**, 367–378

Zannis, V. I., Breslow, J. L., San Giacomo, T. R., Aden, D. P. & Knowles, B. B. (1981) *Biochemistry* **20**, 7089–7096