Sterol biosynthesis: Effect of compactin and its derivatives

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The common objective of therapeutic attempts to inhibit cholesterogenesis, whether by nutritional or pharmacological means, is to limit the availability of cholesterol in order to treat hyperlipidaemia, a risk factor for the development of atherosclerosis and coronary heart disease. Consideration of this role of cholesterol must take account of modern theories of lipoprotein transport and function. In particular, the identification of HDL* as a negative risk factor for atherosclerosis, because of its function in centripetal transport, has stimulated recent research to concentrate on ways of specifically lowering the levels of LDL and VLDL.

Certain of the common types of hyperlipidaemia may be characterized by elevated cholesterogenesis but even basal rates of synthesis can contribute more than 50% of the daily input so that an inhibition, if consistently effective and safe, has clinical potential. Various classes of compound have been identified as inhibitors of individual steps of the sterol biosynthetic pathway (Fears, 1983) but action at a late stage leads to the accumulation of deleterious intermediates. The reactions catalysed by mevalonate kinase and prenyl transferase have been discussed as possible sites for pharmacological intervention (Gibons et al., 1982) but most attention has been devoted to the control of HMG-CoA reductase (EC 1.1.3.4); the enzyme usually considered to be rate limiting for the pathway.

Compaction and mevinolin: comparison of species

Studies on the regulation of the pathway have been advanced by the use of compactin (ML-236B, mevastatin), which was isolated from the culture broth of Penicillium brevicompactum and comprises a hexahydronaphthalene skeleton substituted with a β-hydroxy-δ-lactone (Fears, 1981). Compaction (0.1 μm) competitively inhibits HMG-CoA reductase activity in rat liver microsomes (0.70 ± 0.03 nmol/30 min incubation per mg of protein compared with the control rate of 4.8 ± 0.59 nmol/30 min per mg of protein; n = 5, P < 0.01) but the rate is restored to the control level (4.0 ± 0.25 nmol/30 min per mg of protein) when compactin is washed out of the microsomes. Equivalent inhibition by compactin in vitro is also observed for that relatively small proportion of HMG-CoA reductase believed to be active in vitro (measured in microsomes prepared using 50 mM NaF) and for the elevated enzyme activity induced in cholestyramine-treated rats and also in microsomes isolated from rat small intestine.

Daily oral dosing of compactin to rats also transiently inhibits cholesterogenesis in liver and small intestine in vivo (Fears et al., 1980) when rates are measured by using 1H2O, a method considered to give the most meaningful results (Fears, 1978) but there is no decrease in the serum concentration of cholesterol (Table 1). In fact, there is little net change in the mass of cholesterol synthesized during the 24h-period after dosing because an early inhibition of HMG-CoA reductase leads to subsequent induction of enzyme synthesis with expression of increased activity. A similar response is observed in cell cultures, possibly with concomitant induction of HMG-CoA synthase synthesis (Luskey et al., 1982).

However, hypercholesterolaemic activity is observed in dogs (Table 1) in association with decreases in serum triacylglycerol and phospholipid levels. Studies in dogs on mevinolin, the 6α-methyl analogue of compactin (Kovanen et al., 1981), have demonstrated that inhibition of HMG-CoA reductase activity can lead not only to impaired VLDL synthesis but also to an increase in the rate of LDL catabolism by inducing the hepatic apoprotein B/E receptor, presumably in order to satisfy sterol requirements in the liver by uptake from the circulation. It is not established whether induction of the receptor is confined to the liver.

Fig. 1. Dose–response relationship for effect of compactin on HMG-CoA reductase activity in microsomes prepared from rat and dog liver

Results are means of four assays at each concentration. Control levels of HMG-CoA reductase activity (nmol/30 min incubation per mg of protein) were for rat liver, 3.98 ± 0.20 (72% apparently phosphorylated in vitro) and for dog liver, 1.21 ± 0.04 (85% apparently phosphorylated in vitro). Compaction sodium salt (pH 7.5) was pre-incubated with microsomes at 37°C for 15 min before addition of substrate.

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; HMG-CoA, δ-hydroxy-β-methylglutaryl-CoA.

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<th>Table 1</th>
<th>Response of male rats and female dogs to compactin by daily oral dosing</th>
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| | Animals, maintained on stock diets ad libitum, were given compactin (25 mg/kg body wt.) by oral dosing (twice daily, at 09:00h and 16:00h), for 7 days. Blood samples were taken 2h after the last dose at 09:00h on day 8. Results are means ± S.E.M.; the number of animals in each group is given in parentheses. Significant difference (P < 0.02) from control is indicated by *.

<table>
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<tr>
<th>Expt. 1 (rat)</th>
<th>Expt. 2 (dog)</th>
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<tbody>
<tr>
<td>Serum lipid</td>
<td>Control (8)</td>
</tr>
<tr>
<td>Cholesterol (mg/100 ml)</td>
<td>80 ± 7</td>
</tr>
<tr>
<td>HDL cholesterol (mg/100 ml)</td>
<td>55 ± 3</td>
</tr>
<tr>
<td>Triacylglycerol (mg/100 ml)</td>
<td>70 ± 8</td>
</tr>
<tr>
<td>Phospholipid (μmol/100 ml)</td>
<td>215 ± 19</td>
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liver but this response is not essential for some expression of LDL-lowering activity because compactin is also hypcholesterolaemic in the WHL rabbit with a genetic receptor defect. It is not clear why compactin is hypolipidaemic in dogs but not rats. The receptor for apoprotein B can be induced in rats by other pharmacological agents that deplete sterol pools and the dose-response relationship in vitro for HMG-CoA reductase activity is similar (Fig. 1). It seems likely that the species-specificity for compactin can be explained by relative rates of drug metabolism, and hence expression of homeostatic response, rather than by qualitative differences in the control of lipid metabolism.

Compactin lowers the serum concentration of cholesterol in patients and mevinolin is active in volunteers (Table 2). The action appears to be specific for LDL in contrast with the response in dogs (Table 1), where HDL is also lowered, presumably because dog HDL, is rich in apoprotein E and so is recognized and bound by the induced hepatic receptor. The lack of a dose-response relationship in the published studies (Table 2) suggests that the optimum dose may be low; there were no serious side effects reported and in particular steroid hormone production requiring cholesterol precursor, was maintained.

**Inhibition of HMG-CoA reductase in vitro**

Studies in vitro have identified potentially deleterious consequences of markedly inhibiting mevalonate production, particularly in cells with a rapid rate of turnover. Thus, inhibition of cholesterolgenesis in the small intestine can affect the motional freedom of lipids in the microvillus membrane (Brasitus & Schachter, 1982), also decreasing crypt-cell renewal and villous-cell differentiation, perhaps consistent with impaired enterohepatic recirculation (Fears, 1983).

Compactin at very high concentrations affects the function of other cells in culture, possibly by interrupting the supply of key non-sterol intermediates, e.g. squalene, dolichol, ubiquinone, isopentenyl adenine, rather than cholesterol itself (Fears, 1981). Thus compactin inhibits concanavalin A induction of DNA synthesis in mouse spleen lymphocytes (Perkins et al., 1982) and this inhibition is reversed by mevalonate but not by cholesterol or by isopentenyl adenine; the latter observation is in contrast with the restoration of DNA synthesis in synchronized cultures of BHK-21 cells (Quescne-Hunelecus et al., 1980). The existence of a key, unidentified, mevalonol metabolite is supported by studies with human peripheral blood lymphocytes (Larson et al., 1982) and Swiss 3T3 cells (Schmidt et al., 1982).

In conclusion, using compactin and its analogues we are now able to evaluate in detail the beneficial as well as the possible toxicological consequences of manipulating HMG-CoA reductase. Whether this approach has clinical use is still too early to say but for the biochemist these pharmacological tools may enable significant advances to be made both as regards our understanding of the control of this enzyme and also with respect to the comparison of possible interspecies differences in lipid metabolism.

**Recent chemical developments**

Inhibitors related to compactin and mevinolin are obtained from other micro-organisms and simpler, less active, mevalonolactone derivatives have also been synthesized (Fears, 1983). Approaches to the total synthesis of (+)-compactin have recently been published, the first by Wang and co-workers (Wang et al., 1981) and if these routes can be adapted for the production of analogues then valuable information may be obtained on the attributes required for optimal activity. It is noteworthy that in addition to binding at the active site, compactin and mevinolin may bind to other regions of HMG-CoA reductase, inducing a change in protein conformation that is essentially irreversible (Rogers & Rudney, 1982).

**Other applications for compactin and related compounds**

In addition to the potential therapeutic use of inhibitors of HMG-CoA reductase as hypolipidaemic agents and to their employment as tools to study the detailed control of mevalonate flux, the wide phylogenetic occurrence of HMG-CoA reductase suggests a possible use in the control of metabolism of certain plants and insects. For example, compactin inhibited HMG-CoA reductase in the corpora allata of the tobacco hornworm Manduca sexta (Monger et al., 1982), the K (0.9nm) being similar to that measured in mammalian cells. Furthermore, injection of compactin into larvae induced darkening of the cuticle, indicative of a deficiency of the isoprenoid juvenile hormone. The aphid, Schizaphis graminium, uses mevalonate by the trans-methylglutaconate shunt pathway for the synthesis of long-chain fatty alcohols in cuticular wax (Nes et al., 1982), which suggests another type of control based on inhibition of HMG-CoA reductase.

Protein phosphorylation and the hormonal control of hepatic cholesterol synthesis

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Cholesterol synthesis in mammalian liver is controlled by a variety of factors, including the hormones insulin and glucagon (Ingebritsen, 1983; Rodwell et al., 1976). The stimulation of cholesterol synthesis by insulin and the inhibition of the pathway by glucagon are in line with the concerted effects of these two hormones on other metabolic pathways in the liver, such as glycogen synthesis and breakdown, gluconeogenesis and fatty acid synthesis. A wide variety of enzymes involved in the control of these pathways are regulated by reversible phosphorylation, and this mechanism underlies the acute control of liver intermediary metabolism in response to insulin and glucagon (Cohen, 1982).

Regulation of HMG-CoA by a Bicyclic Phosphorylation System

A microsomal enzyme, termed HMG-CoA reductase (EC 1.1.1.34), is the primary site for regulating cholesterol biosynthesis (Ingebritsen, 1983; Rodwell et al., 1976). In view of the involvement of protein phosphorylation in the hormonal control of other metabolic pathways in the liver, it is perhaps not surprising that this regulatory mechanism has now been shown to play an important role in the control of HMG-CoA reductase. Studies carried out over the past 10 years in several laboratories have shown that HMG-CoA reductase is regulated by a bicyclic phosphorylation system (Fig. 1), which involves two protein kinases and three protein phosphatases (Ingebritsen, 1983).

An initial indication that HMG-CoA reductase was regulated by reversible phosphorylation came from studies by Beg and Gibson (Beg et al., 1973), which showed that HMG-CoA reductase was inactivated in a time-dependent fashion after incubation of microsomal membranes with MgATP and ATP. Subsequently it was found that the enzyme could be fully re-activated by further incubating the membranes with a highly purified liver protein phosphatase, indicating that a protein-phosphorylation event was associated with the MgATP-dependent inactivation of HMG-CoA reductase (Ingebritsen et al., 1978, 1981).

Further experiments in several laboratories demonstrated that inactivation of HMG-CoA reductase was due to the phosphorylation of the enzyme itself, rather than an associated regulatory protein (reviewed by Ingebritsen, 1983). In these studies, microsomal or solubilized HMG-CoA reductase was incubated with [γ-32P]ATP under conditions which led to inactivation of the enzyme. The enzyme was then either precipitated with a specific antibody or purified to homogeneity and shown to be covalently labelled with 32P radioactivity. Further experiments in which homogeneous HMG-CoA reductase was inactivated by incubation with [γ-32P]ATP demonstrated that stoichiometric amounts (0.9 mol/mol of M, 50,000 subunit) of phosphate were incorporated into the enzyme (Beg et al., 1980).

The enzyme, termed HMG-CoA reductase kinase, that phosphorylates HMG-CoA reductase is localized primarily in the cytosol, in contrast with HMG-CoA reductase itself, which is firmly bound to the endoplasmic reticulum. A small amount (10-15%) of HMG-CoA reductase kinase is also present in unwashed microsomal membranes, but this activity is readily extracted under conditions that do not solubilize HMG-CoA reductase (Ingebritsen et al., 1981).

The first evidence that HMG-CoA reductase kinase was also regulated by reversible phosphorylation came from studies by Ingebritsen and co-workers (Ingebritsen et al., 1978, 1981). In these experiments, it was noted that the activity of the protein kinase in the microsomal fraction was decreased in a time-dependent fashion after preincubation at 37°C in imidazole buffer (pH 7.4). The inactivation was partially inhibited in the presence of 50 mm NaF and completely blocked in the presence of 50 mm NaF. Since both compounds are potent inhibitors of protein phosphatase (EC 3.1.3.16) activity, these results suggested that the inactivation of HMG-CoA reductase kinase resulted from dephosphorylation of the enzyme. Further evidence supporting this hypothesis came from studies on the inactivation of HMG-CoA reductase kinase in microsomal extracts. In these preparations, the rate of inactivation of the enzyme was increased by incubation with MgATP and ATP, and the activity of the kinase was decreased in a time-dependent fashion.

The relative contributions of protein phosphatases 1, 2A and 2C to the total potential protein phosphatase activity in liver extracts towards HMG-CoA reductase and HMG-CoA reductase kinase have been estimated (Ingebritsen et al., 1983). These studies indicate that protein phosphatase 2C is the major activity towards these two substrates, accounting for at least 70% of the total activity. Protein phosphatases 1 and 2A accounted for the remainder. Abbreviations: HMGR, HMG-CoA reductase; RK, HMG-CoA reductase kinase; RKK, HMG-CoA reductase kinase; PrP-1, -2A and -2C, protein phosphatases 1, 2A and 2C.