In vivo regulation of canine intestinal 3-hydroxy-3-methylglutaryl coenzyme A reductase by cholesterol, lipoprotein, and fatty acids

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Abstract Thirty-Vella-isolated ileal segments in dogs were used to study the regulation of intestinal cholesterol synthesis. This excluded fistula enabled independent in vivo manipulation of luminal and vascular influences on mucosal cells. Segments were studied repeatedly and each animal served as its own control. Cholesterol synthesis rate was assessed by measuring mucosal activity of the rate-limiting enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase. Luminal cholesterol and 25-hydroxycholesterol were shown to reduce HMG CoA reductase activity to 64 ± 7% and 42 ± 4%, respectively, of control within 4 hr. Reductase activity in the excluded segment also responded to alterations in serum cholesterol produced by cholesterol or cholestyramine feeding. Similarly, in vitro studies showed that lipoprotein cholesterol inhibited HMG CoA reductase in mucosa from the excluded segment but not in mucosa from intact bowel. In contrast to sterols, fatty acids stimulated HMG CoA reductase activity by luminal contact. These findings suggest that the cholesterol needs of canine intestinal epithelial cells are acutely balanced by absorption and synthesis of cholesterol. Mucosal cells may also utilize lipoprotein cholesterol under certain conditions, perhaps via low density lipoprotein receptors. Fatty acid absorption stimulated cholesterol synthesis in the absence of luminal cholesterol, perhaps to facilitate chylomicron formation.


Supplementary key words Thirty-Vella ileal mucosa · lipoprotein receptors · cholesterol absorption · fatty acid absorption

Intestinal mucosa has been demonstrated to be second only to liver as a source of endogenously synthesized cholesterol (1, 2). In many tissues, the rate of cholesterol synthesis in cells has been shown to be regulated by cholesterol itself in a feedback manner (3, 4). This regulation occurs in most instances by modulation of the quantity or the activity of the rate-limiting microsomal enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase (EC 1.1.1.34) (5, 6). Activity of this enzyme appears to be influenced by the cholesterol content of the cell. In liver, both cholesterol synthesis and HMG CoA reductase activity appear to be principally regulated via cholesterol carried in chylomicron remnants (7, 8). These activities in fibroblasts and leukocytes are regulated primarily via cholesterol carried in low density lipoprotein (9-11).

Compared to liver, the regulation of cholesterol synthesis in intestine has been less well studied and with contradictory results. Effects of bile salts and cholesterol have been investigated most thoroughly. Biliary diversion has been shown to stimulate intestinal cholesterol synthesis rate in the rat (12), and bile salts inhibit reductase activity in cultured explants of canine intestinal mucosa (13). Luminal cholesterol appears to regulate cholesterol synthesis in cultured intestinal explants (13). In animals fed cholesterol, some studies have shown inhibition of gut synthesis (14) while other studies have not (1). Low density lipoprotein (LDL) regulation of intestinal cholesterogenesis has been suggested by the observation that cholesterol synthesis in the intestine of intact rats was stimulated by drug-induced hypolipoproteinemia (15), and subsequently suppressed by reinfusion of LDL (16). However, LDL could not be demonstrated to inhibit HMG CoA reductase activity during acute in vitro studies (15).

The current experiments were designed to evaluate the in vivo isolated ileal segment, or Thirty-Vella fistula (17), as a model for the study of intestinal HMG CoA reductase regulation. The in vivo ileal fistula allows greater independent control of luminal and humoral variables than is possible with standard feeding and drug studies in intact animals. This model was used to study the effects of luminal neutral sterol and circulating lipoprotein cholesterol on the regulation of intestinal cholesterol synthesis.

Abbreviations: HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low density lipoprotein.
tion of intestinal HMG CoA reductase. The effect of fatty acids in the intestinal lumen was also studied because cholesterol is required for chylomicron formation during fatty acid absorption and because several fatty acids have been demonstrated to influence hepatic cholesterol synthesis (18, 19).

MATERIALS AND METHODS

Materials

Fatty acids, cofactors, enzyme substrates, and highly purified cholesterol were purchased from Sigma (St. Louis, MO). Cholesterol purity was greater than 99% as judged by thin-layer chromatography and was confirmed by gas–liquid chromatography. Synthetic dipalmitoyl-DL-α-phosphatidylcholine was obtained from Sigma and was 99% pure. 25-Hydroxycholesterol was obtained from Steraloids (Wilton, NH) and showed less than 5% impurities by gas–liquid chromatography. DL-[5-3H]Mevalonic acid, [4-14C]cholesterol (purified by thin-layer chromatography), [1-14C]oleic acid, and [5-14C]HMG CoA of high specific activity were obtained from New England Nuclear. The specific activity of the [14C]HMG CoA was adjusted to 12 nCi/nmol using nonradioactive HMG CoA from Sigma.

Mylar-backed Silica Gel G chromatography sheets were obtained from Eastman Kodak. Organ culture dishes were obtained from Falcon Plastics (Oxnard, CA) and Roswell Park Memorial Institute (RPMI) 1640 tissue culture media was purchased from Difco Labs. Mucosal biopsies were obtained using the Quinton-Rubin multipurpose biopsy tube from the Quinton Instrument Company (Seattle, WA).

Delipidated bovine serum albumin (BSA) was obtained from Miles Laboratory (Elkart, IN). Fetal calf serum was obtained from Gibco (Grand Island, NY). The bile acid-binding resin cholestyramine was purchased from Mead Johnson Laboratories (Evansville, IN).

In vivo studies

Healthy, adult mixed-breed dogs, fed a standard diet of Wayne Dog Chow (Allied Mills, Inc., Chicago, IL), were utilized. Five animals underwent formation of an isolated ileal segment fistula (Thiry-Vella fistula) under pentothal anesthesia. A 45-cm segment of ileum approximately 30 cm proximal to the ileocecal valve was isolated in each case. The remaining bowel was anastomosed to reestablish luminal continuity. The isolated segment retained its vascular relationships but was removed from luminal continuity by creating an enterocutaneous stoma at each end. Thus, access to the lumen of each animal’s isolated ileal segment was chronically maintained for perfusion and mucosal biopsy. Animals gained weight postoperatively and remained healthy on the standard diet, except during specific diet changes, for more than 12 months.

Isolated ileal segments were luminally irrigated with saline or solutions of gelatin or albumin multiple times weekly. Following irrigation, mucosal biopsies for HMG CoA reductase determinations were obtained from the distal end of the segments using the Quinton-Rubin biopsy tube. Biopsies were obtained at 10 AM and two to four specimens were pooled to minimize sampling variability. Perfusion studies were performed over a 4-hr period between 10 AM and 2 PM. Unanesthetized dogs stood quietly in Pavlov stands while the segments were perfused with control or experimental solutions. Mucosal biopsies from the isolated segments were obtained before and after perfusion. Mucosal reductase activity was measured in a paired fashion for each animal and animals served as their own controls for experimental and control perfusions. Because of variability between animals’ initial HMG CoA reductase activity, values were best expressed as percent change over the 4-hr period (or HMG CoA reductase at 4 hr/HMG CoA reductase initial × 100%).

The control perfusion solution consisted of saline containing 0.1% gelatin with or without 0.5% ethanol. Experimental perfusion solutions consisted of 1) fatty acids (hexanoic acid, octanoic acid, lauric acid, palmitic acid, stearic acid, oleic acid, and linoleic acid) dissolved in ethanol and sonicated in normal saline as 2-mM suspension with 0.1% gelatin and a final concentration of 0.5% ethanol; 2) 25-hydroxycholesterol at a concentration of 100 μg/ml in control solution with 0.5% ethanol; or 3) cholesterol-containing suspensions or liposomes which were made by a modification of the method of Rothblatt, Arbogast, and Ray (20). Suspensions of cholesterol and dipalmitoyl-DL-α-phosphatidylcholine in molar ratios of either 3.5:1 or 0.5:1 were sonicated for 20 min in saline with 5% BSA. Following centrifugation, the high cholesterol preparation yielded liposomes with a molar ratio of 2.8:1 (15.5 μmol cholesterol/ml), while in the low cholesterol liposomes the ratio was 0.5:1.

Assay for HMG CoA reductase activity

HMG CoA reductase was measured in whole mucosal homogenates as previously described (13). Tissue fragments were homogenized in 0.1 M potassium phosphate buffer (pH 7.2) containing 0.2 M
sucrose and 40 mM EDTA. Assay conditions consisted of 0.1 to 0.2 mg of mucosal homogenate protein with 25.5 mM glucose-6-phosphate, 3 mM NADP, 15 mM diethytoiol, 70 mM NaCl, and 1 unit/ml of glucose-6-phosphate dehydrogenase. Samples were preincubated at 37°C for 5 min prior to addition of [14C]HMG CoA to a final concentration of 31 μM. This concentration of HMG CoA has previously been shown to represent substrate excess for these conditions (13). Incubations were terminated after 15 min by addition of 0.025 ml of concentrated HCl followed by 2 pmol of d-[5-3]H]mevalonic acid (20,000 dpm/pmol). After allowing time for mevalonic acid to lactonize, samples were centrifuged and a portion of the supernatant was applied to activated Silica Gel G thin-layer chromatography sheets and developed in benzene-acetone 1:1. The Rf region 0.5 to 0.9 was removed, placed in 10 ml of Aquasol and radioactivity was measured. [3H]Mevalonate was utilized to assess recovery of counts and reductase activity was expressed as picomoles of [14C]mevalonate formed/mg tissue protein per min. Protein was determined by the method of Lowry et al. (21).

Organ culture

For some experiments, mucosa was obtained from ileum in luminal continuity at time of surgery or from isolated ileal segments using the Quinton-Rubin biopsy tube. Mucosal organ culture was performed as previously described (13). For some cultures, RPMI 1640 media was enriched with lipoprotein prepared from dog serum by the method of Havel, Eder, and Bragdon (22). The lipoprotein fraction of density 1.006–1.063 g/ml, containing LDL, was utilized. After washing, the cholesterol to protein ratio of this fraction was 0.6 while the triglyceride to cholesterol ratio was 1.8. These values indicate that this preparation is predominantly canine LDL as characterized by Mahley and Weisgraber (23). At the end of 6-hr cultures, tissue was removed, homogenized, and assayed for HMG CoA reductase activity.

RESULTS

HMG CoA reductase activity in isolated ileal segment mucosa

Fig. 1 illustrates the changes in activity of mucosal HMG CoA reductase at representative times after formation of the Thiry-Vella fistula in five dogs. Initial activity was measured in biopsy mucosa taken from ileum in continuity with the fecal stream at the time of surgery. Subsequently, activity was measured in mucosal biopsy samples from the isolated ileal segments. All mucosal biopsies, including the initial tissue, were obtained at 10 am. Mucosal reductase activity increased significantly over the initial 1–2 weeks following removal of the ileal segment from the fecal stream. After 2 weeks, however, reductase activity fell to levels near or below the initial values. Basal activity then remained at these levels for subsequent experiments except during periods of altered diet.

The initial rise in reductase activity most likely reflected the fact that neutral steroids and bile acids were removed from contact with the isolated loop mucosa. As a consequence of this luminal deprivation, synthesis of endogenous cholesterol may have been stimulated in order to meet the cholesterol needs of mucosal cells. The subsequent fall in activity may be accounted for in at least two ways. Mucosal cholesterol need may have decreased because of villous hypoplasia and changes in epithelial cell mitotic index which have been reported to occur in Thiry-Vella segments (24). Alternatively, the cells may have become better able to utilize cholesterol from another source, such as circulating serum lipoprotein.

In order to assess this latter possibility, the effect of dietary alterations on HMG CoA reductase activity in the mucosa of the isolated segment was examined. At least 6 weeks following formation of the Thiry-Vella segment, dogs were fed cholestyramine (12 g/day) for 2 weeks. Cholestyramine feeding resulted in a significant rise in activity of HMG CoA reductase in the mucosa of the isolated segments (Fig. 1). This increase
TABLE 1. Lipoprotein effect on reductase activity in mucosa removed from intact ileum and from Thiry-Vella ileum

<table>
<thead>
<tr>
<th>Tissue Cultured</th>
<th>0.07 mg/ml of Lipoprotein</th>
<th>0.2 mg/ml of Lipoprotein</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Cholesterol Added</td>
<td>Cholesterol Added</td>
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<tr>
<td>Intact ileal mucosa (n = 12)</td>
<td>95 ± 3</td>
<td>96 ± 8</td>
</tr>
<tr>
<td>Thiry-Vella ileal mucosa (n = 12)</td>
<td>75 ± 4</td>
<td>75 ± 3</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for 12 culture pairs.

Lipid and cholesterol-content of mucosa from ileum in the luminal stream and mucosa from the isolated bowel were maintained for 6 hr in organ cultures as described in Methods. Culture has been shown to induce a 4- to 6-fold rise in HMG CoA reductase activity (13). Each culture pair consisted of tissue cultured without lipoprotein and tissue cultured with lipoprotein (d 1.006–1.063 g/ml) added to media. Tissue reductase was measured in each pair and lipoprotein effect was expressed as:

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\frac{\text{activity in the presence of lipoprotein}}{\text{activity in the absence of lipoprotein}} \times 100\%.
\]

occurred in association with a mean serum cholesterol decrease from 135 mg/dl to 107 mg/dl. Following the cholestyramine feeding, dogs were fed cholesterol in the form of a 1% supplementation with free cholesterol in the normal diet. One week of feeding resulted in a fall in HMG CoA reductase activity in the segment to 48% of the control value (Fig. 1). This fall in reductase activity occurred concomitant with an increase in serum cholesterol levels to a mean of 189 mg/dl. Both of these diet-related changes in reductase activity occurred in the Thiry-Vella mucosa, isolated from luminal contact with the fed substances. This suggests that the principle stimulus for change in the HMG CoA reductase activity under these conditions originated from the serosal side of the epithelial cells.

To test the idea that ileal mucosa isolated from luminal contents may become responsive to lipoprotein cholesterol, the in vitro organ culture methods of Browning and Trier were utilized (25). We have previously shown that culture conditions produce an increase in mucosal HMG CoA reductase activity over 6 hr (13). The extent to which canine lipoprotein fraction of density 1.006–1.063 g/ml inhibits this increase was assayed by measuring reductase in paired 6-hr cultured mucosa with and without lipoprotein added to the media. Mucosal biopsies were obtained from intact ileum at time of surgery and from Thiry-Vella segments at least 6 weeks after their preparation. As previously reported (13), LDL had no effect on reductase activity in mucosa from intact ileum (Table 1). In contrast, LDL significantly inhibited the rise in reductase activity in mucosa from the Thiry-Vella segment. These findings are consistent with the hypothesis that ileal cells are capable of responding to lipoprotein cholesterol.

**Luminal sterol effect**

The lumen of the Thiry-Vella loops were regularly and repeatedly perfused via a balloon catheter introduced into the proximal stoma. Perfusate effluxed from the distal stoma and mucosal biopsies were taken from this stoma before and after 4-hr perfusions. Control perfusions with saline containing 0.1% gelatin, 0.5% alcohol, or BSA had no significant effect on reductase activity over this 4-hr period; initial reductase activity for 21 control perfusions was 32.5 ± 2.3 pmol mevalonate/mg protein per min, while post-perfusion activity was 32.7 ± 2.8 pmol/mg per min. The luminal effect of other substances was then studied in comparison to these control perfusions. Control and test perfusion studies were carried out in isolated segments at least 6 weeks old.

Cholesterol per se has an extremely low solubility and its absorption in the absence of bile salts and other solubilizers is minimal. Therefore, the lumen of Thiry-Vella segments was perfused with sonicated cholesterol-phospholipid liposomes at a molar ratio of 2.8:1. Cholesterol-rich liposomes have previously been shown to be able to transfer cholesterol into red blood cell membranes (26) and liver cells (27). In preliminary experiments using purified [14C]cholesterol in these liposomes, cholesterol uptake by mucosa was shown by the fact that 14C-labeled cholesteryl ester (by thin-layer chromatography) appeared in epithelial cells. The effect of 4-hr luminal perfusion with cholesterol-rich liposomes on mucosal reductase activity is shown in Fig. 2. Contact with high cholesterol liposomes reduced mucosal HMG CoA reductase activity to 64 ± 7% of control values over 4 hr. In contrast, luminal perfusion with a cholesterol-deficient suspension (cholesterol:phospholipid molar ratio of 0.5:1) caused mucosal reductase activity to increase to 133 ± 8% of the initial control value. Liposomes with low cholesterol content may remove cholesterol from cell membranes (28) and therefore increase the cholesterol requirements of the cell, a stimulus for synthesis. This in vivo evidence that luminal cholesterol may regulate mucosal reductase activity and thus cholesterol synthesis is consistent with previous in vitro findings (13). Also consistent with in vitro data was the finding that mucosal reductase activity was significantly inhibited by perfusing the lumen of the isolated segment with 25-hy-
Effect of fatty acids

The process of fatty acid absorption by intestinal mucosa utilizes cholesterol as a component of chylomicron formation. For this reason, mucosal HMG CoA reductase activity was measured during perfusion of fatty acids through the isolated ileal segment. Seven fatty acids of varying saturation and chain length were sonicated into 2-mM suspensions with normal saline, 0.1% gelatin, and 0.5% alcohol. As before, mucosal biopsy samples were obtained from the distal stoma before and after a 4-hr perfusion with 100 ml of the fatty acid solution. Activity of HMG CoA reductase in the mucosal homogenates was measured and the percent change in the paired samples was determined. Ileal segments in the animals were repeatedly studied with all fatty acid perfusates as well as with the control solution in an alternating fashion. Fatty acid absorption under these conditions was demonstrated using \([^{14}\text{C}]\)oleic acid in the perfusate. Label appeared in peripheral blood within 10 min, and 10% of the label was present in the vascular space at 2 hr. Fig. 3 illustrates the effect of perfusion of these fatty acids on mucosal HMG CoA reductase activity in isolated ileal mucosa. Except for hexanoic acid, all of the fatty acids studied had a marked stimulatory effect on reductase activity. The degree of stimulation appeared to be unrelated to the saturation of the fatty acid or to the length of longer chain fatty acids.

DISCUSSION

Previous studies of the regulation of intestinal cholesterol synthesis and HMG CoA reductase activity have utilized intact animal models or isolated in vitro cell or mucosal preparations. Intact animal feeding or infusion studies have the disadvantage of allowing poor control over a large variety of interdependent variables. For example, feeding bile salts will affect cholesterol absorption and metabolism as well as having their own direct effect. Similarly, sterol or drug feeding and infusions are likely to affect multiple aspects of metabolic and hormonal balance. The precise basis for observed changes during in vivo feeding studies may be obscure. In
vitro mucosal preparations, in contrast, allow precise manipulation of isolated variables. However, cell viability in vitro limits the duration of studies. For cells with the well-defined polarity of the intestinal epithelium, it is also difficult to determine whether a substance is acting on the luminal or the serosal side of the cell.

The Thiry-Vella loop model offers several advantages. First, animals serve as their own controls for repeated studies or to provide tissue for in vitro techniques. Second, evaluation of a substance placed in the lumen of these segments is not complicated by the complex luminal contents of intact gut or by diverse alterations in circulating blood substances resulting from absorption. Finally, influences on the serosal side of the cell can be assessed by feeding or injection studies without affecting content of the study segment lumen.

The fistula model was used to study the regulation of intestinal HMG CoA reductase activity in the dog. In all instances, total reductase activity was measured rather than state of enzyme activation. Results confirm that intestinal HMG CoA reductase activity is in part regulated by absorption of cholesterol from the bowel lumen. Regulation by cholesterol in the lumen was demonstrated by the fact that mucosal reductase activity decreased when unesterified cholesterol liposomes were placed in the bowel lumen. Regulation by cholesterol in the lumen was demonstrated by the fact that mucosal reductase activity decreased when unesterified cholesterol liposomes were placed in the bowel lumen. The degree of inhibition by cholesterol in vivo was greater than has been observed in vitro (13). Cellular cholesterol content appears to be a principle factor in the control of reductase activity and endogenous cholesterol synthesis in many tissues. This is likely to be the case for enterocytes as well. Since intestinal mucosal cells absorb cholesterol from the bowel lumen, it is not surprising that luminal cholesterol inhibited reductase activity. 25-Hydroxycholesterol, a cholesterol analogue which inhibits reductase activity and cholesterol synthesis in hepatocytes (29, 30) and many other cells, also inhibited mucosal reductase activity when placed in the lumen.

Andersen and Dietschy (15, 16) have reported the regulation of intestinal cholesterogenesis by serum LDL cholesterol in fasted rats. Stange et al. (31) have reported LDL regulation of reductase activity in cultured rabbit mucosa. Three findings in the current study also suggest that canine mucosal reductase can be influenced by serum lipoprotein cholesterol. Following formation of the ileal segment isolated from luminal contents, reductase activity of mucosa increased to meet cell needs, but then fell. The fall in activity may have been due to cells obtaining cholesterol from a source other than the lumen. The second evidence for LDL regulation was the finding that reductase activity in isolated bowel inversely correlated to serum cholesterol levels during cholestyramine and cholesterol feeding periods. McNamara, Davidson, and Fernandez (32) have shown increased cholesterogenesis in mononuclear cells of patients fed cholestyramine. They suggested that the rate of cholesterol flux into and out of cells may regulate synthetic rate. Changes in plasma cholesterol levels would alter cellular cholesterol flux if the enterocytes were capable of utilizing lipoprotein. Such utilization would account for the changes in reductase activity observed. The final piece of evidence for lipoprotein influence on reductase activity came from the in vitro studies. LDL significantly inhibited reductase activity in cultured mucosa from the isolated segment but not from intact ileum. The quantity of luminal cholesterol absorbed by intact canine mucosa may be large enough to minimize mechanisms for utilization of lipoproteins. Enterocytes removed from luminal contents, however, may synthesize LDL receptors and increase lipoprotein uptake. Similarly, fibroblasts cultured in the absence of cholesterol are found to synthesize LDL receptors to facilitate binding and entry of LDL into the cell (33).

These studies have shown an additional dietary factor to be capable of affecting bowel cholesterol synthesis. Fatty acids placed in the lumen of the isolated segment resulted in increased activity of intestinal HMG CoA reductase. During long-chain fatty acid absorption, apoprotein and cholesterol are contributed by mucosal cells for chylomicron formation. Exogenous cholesterol was absent from the Thiry-Vella segment lumen, so that cholesterol synthesis may have been necessary to meet demands. Consistent with this premise, the C-6 shorter chain fatty acid had no effect on HMG CoA reductase activity. However, the C-8 acid, which transported in large measure without chylomicron production, did produce moderate stimulation. It may be that intrinsic properties of the fatty acids such as solubility or uptake are involved in the observed stimulation. Fatty acids have been demonstrated to influence hepatic cholesterogenesis and reductase activity. However, both stimulation (18) and inhibition (19) have been reported.

Fatty acids with variable degrees of saturation were studied because a diet containing unsaturated fatty acids has been observed to reduce serum cholesterol levels in man. These studies revealed that fatty acids of the C-18 series all stimulated intestinal HMG CoA reductase to approximately the same extent. This finding is also consistent with the hypothesis that the cholesterol requirement for packaging and transport
of fatty acid was the mechanism producing the rise in reductase activity. Alternatively, the lack of correlation between stimulation and degree of saturation could be species specific, since serum cholesterol in the dog has been shown to increase following ingestion of fats containing fatty acids of varying saturation (34). While it appears that fatty acid absorption is capable of influencing intestinal cholesterol synthesis, the quantitative importance of this synthesis warrants further study.

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