Absence of Cholesterogenesis Regulation in the Liver and Prostate of the BIO 87.20 Hamster

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ABSTRACT

Normal, adult golden Syrian hamsters and the inbred strain BIO 87.20 Syrian hamsters were maintained on either control, cholesterol, candicidin or clofibrate diets for time periods of up to 4 months. The ventral prostate gland in both species was found to synthesize cholesterol at a greater rate than the liver. Also, our results show that, while hepatic cholesterol synthesis in the normal Syrian hamster is under feedback control with dietary cholesterol, hepatic cholesterol synthesis in the BIO 87.20 hamster, and prostatic cholesterol synthesis in either species, is under no such control. This apparent regulatory defect in the BIO 87.20 hamster, which results in a dramatic accumulation of cholesterol in the liver and serum, renders this animal a potentially valuable in vivo model for the study of cholesterol-related disorders.

INTRODUCTION

The inbred Syrian hamster (Mesocricetus auratus), designated as BIO 87.20, was first described by Homburger and Nixon (1) in 1970, to exhibit in the male, genetic and age-related cystic prostatic hypertrophy. After the hypocholesterolemic drug, candicidin, had been reported for its effect on benign prostatic enlargements in dogs (2) and in man (3-5), further studies in our laboratory (6) revealed that candicidin and the structurally unrelated hypocholesterolemic ion exchange resin, colestipol, both reduced the enlargement of the prostate gland in the BIO 87.20 male hamster and also reduced the overall cholesterol content of the gland. It was suggested that this effect on the prostate gland was related in some way to the known inhibitory action of candicidin and colestipol on absorption-resorption of cholesterol and bile acids, respectively, in the enterohepatic circulation. Both drugs thus effectively prevented the micellar solubilization of cholesterol in the intestinal tract.

Since the reduction of the enlarged prostate with hypocholesterolemic drugs might be related to decreased body cholesterol levels or possibly to changes in cholesterologenesis in the gland, studies were designed to compare the effects of dietary cholesterol, candicidin, and clofibrate on the content and rate of synthesis of cholesterol in the prostate gland and liver of the BIO 87.20 and normal male Syrian hamsters. In this study, the polyene macrolide antibiotic candicidin, as an inhibitor of cholesterol absorption-resorption (7) and the synthetic drug clofibrate or [ethyl-2-(4-chlorophenoxy)-2-methyl propionate], as a known inhibitor of cholesterol synthesis (8) were selected for study.

MATERIALS AND METHODS

Male BIO-RB golden Syrian hamsters as controls and male BIO 87.20 Syrian hamsters, 6 months of age, were purchased from Telaco, Bar Harbor, ME. Cholesterol was purchased from Fisher Scientific Co., Pittsburgh, PA; the polyene antifungal antibiotic candicidin (lot 671NP-7) was obtained from S.B. Penick and Co., Lyndhurst, NJ. The animals, in groups of 6 or more, were placed on control diet (Purina Lab Chow) or on control diets supplemented with either cholesterol (1%), candicidin (75 mg/kg body wt/day), or clofibrate (Atromid S, 0.3%) for a period of up to 6 months. All animals were maintained on daily rations comparable to the average for the candicidin-treated group. All animals were also maintained with water ad libitum and under an alternating 12-hr light and 12-hr dark schedule.

At 2-month intervals, the animals were sacrificed. After anesthetization with intraperitoneal injections of sodium barbital, the animals were exsanguinated and the blood used for the preparation of sera. The liver and ventral prostate, free of the fat covering, and the liver were excised for the determination of cholesterol content and rate of cholesterogenesis.

The total amount of cholesterol in sera and in the liver was determined colorimetrically by the procedure of Parekh and Jung (9). Serum and liver cholesterol concentrations were expressed as mg cholesterol/100 ml serum (mg %) and mg cholesterol/g tissue, respectively.

For the determination of the in vitro incorporation of radiolabeled acetate into cholesterol, the excised ventral prostate and the liver were minced and weighed in tared Teflon test tubes. Approximately 30-mg and 200-mg samples of minced prostate and liver tissues on a
wet wt basis, respectively, were used to study the incorporation of acetate into cholesterol. The minced tissues were kept in ice until further use. Subsequently, the tissues were incubated with 2 ml Hank's Balanced Salt solution supplemented with 0.2% glucose and 1 μCi/ml of 2-[14C]acetate, sp act 50 mCi/mmol, New England Nuclear, Boston, MA, (pregassed with 95% O2 and 5% CO2) at 37°C for 2 hr on a constant speed shaker. At the end of the incubation period, the reaction was terminated by instant freezing of the tubes in a Dry Ice/acetone bath.

For the analysis of radioactivity in cholesterol, the tissues were saponified by the addition of alcoholic KOH to a final concentration of 10% KOH and 50% ethanol (95%) at 75°C for 75 min. Nonsaponified lipids were pooled by repeated extractions with n-hexane. The hexane extracts were evaporated under nitrogen and diglucosin precipitation was done according to the procedure of Sperry (10). The cholesterol-diglucoside complex was dissolved in 1 ml of methanol and 0.1-ml aliquots were counted in duplicate for [14C] activity in a Packard Scintillation Counter. The rates of cholesterol synthesis were expressed as counts/min/g tissue x 10^3.

RESULTS

In a preliminary study with the BIO 87.20 male Syrian hamster, the effects of dietary cholesterol and candicidin on serum cholesterol concentration was determined over a period of 6 months. Compared to animals on a control diet, it was immediately apparent that 1% cholesterol feeding produced a dramatic and marked hypercholesterolemia in this animal model. With an initial 0-time serum cholesterol level of 93.6 ± 7.7 mg % whereas the control animals exhibited levels of 112.0 ± 5.2, 105.2 ± 7.0 and 95.7 ± 3.5 mg %, respectively, after 2, 4 and 6 months, the cholesterol-treated animals exhibited levels of 177.6 ± 30.9, 286.3 ± 32.5, and 476.6 ± 35.0, respectively. Animals on a candicidin diet (75 mg/kg body weight) exhibited, in contrast, serum cholesterol levels of 85.9 ± 9.5, 67.3 ± 5.0, and 45.9 ± 4.3, respectively, after 2, 4 and 6 months of treatment. Examination of the liver of the cholesterol-treated animals also revealed a dramatic accumulation of cholesterol compared to the livers of control and candicidin-treated animals. In fact, 1% cholesterol feeding to these animals over a period of 6 months resulted in a ca. 50% mortality rate. For this reason, all further studies were terminated at 4 months.

This initial study was followed by a second study in which the effects of dietary cholesterol, candicidin and clofibrate on the serum and liver cholesterol content of BIO 87.20 and normal male Syrian hamsters were compared. In this study, male BIO 87.20 Syrian hamsters and golden Syrian hamster controls were treated, respectively, with control, cholesterol, candicidin and clofibrate diets over a period of 4 months. In all groups, no significant differences in food intake were noted. The serum cholesterol levels for these animals after 2 and 4 months of treatment are given in Table 1. In these results, there is no significant difference in serum cholesterol levels between the BIO 87.20 and control Syrian hamsters.

TABLE 1

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Normal Syrian hamster—serum cholesterol (mg %)</th>
<th>BIO 87.20 hamster—serum cholesterol (mg %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Time</td>
<td>2 Months</td>
</tr>
<tr>
<td>Control diet</td>
<td>81.42 ± 6.88**(6)**</td>
<td>87.55 ± 7.56(6)</td>
</tr>
<tr>
<td>Cholesterol diet</td>
<td>101.42 ± 9.33(6)</td>
<td>90.17 ± 8.48(6)</td>
</tr>
<tr>
<td>Candicidin diet</td>
<td>51.38 ± 5.18(6)**</td>
<td>46.72 ± 7.13(6)**</td>
</tr>
<tr>
<td>Clofibrate diet</td>
<td>71.88 ± 6.42(6)</td>
<td>67.32 ± 6.13(6)</td>
</tr>
</tbody>
</table>

*Mean ± SE.
**Number of animals/group.
Significance: p value a<0.05; b<0.01.

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TABLE 2

Cholesterol Content of the Liver of Normal Syrian and BIO 87.20 Male Hamsters

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Normal Syrian hamster—liver cholesterol (mg/g)</th>
<th>BIO 87.20 hamster—liver cholesterol (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Time</td>
<td>2 Months</td>
</tr>
<tr>
<td>Control diet</td>
<td>1.72 ± .24*(6)**</td>
<td>2.01 ± .31(6)</td>
</tr>
<tr>
<td>Cholesterol diet</td>
<td>2.94 ± .22(6)a</td>
<td>3.07 ± .38(6)a</td>
</tr>
<tr>
<td>Candicidin diet</td>
<td>2.48 ± .36(6)</td>
<td>2.71 ± .34(6)</td>
</tr>
<tr>
<td>Clofibrate diet</td>
<td>1.26 ± .10(6)b</td>
<td>1.43 ± .13(6)</td>
</tr>
</tbody>
</table>

*Mean ± SE.
**Number of animals/group.
Significance: p value a<0.05; b<0.01.

Hamsters after 2 months on the cholesterol diet. On the other hand, serum cholesterol levels in the BIO 87.20 hamsters increased by 130% after 4 months. The whole blood upon removal also appeared quite milky. In contrast, both the candicidin and clofibrate treatments decreased serum cholesterol levels in the normal Syrian hamsters and the BIO 87.20 hamsters after 2 and 4 months of treatment.

The cholesterol content of the livers of both groups of hamsters fed control diet and diets containing cholesterol, candicidin and clofibrate was also measured and is given in Table 2. On a cholesterol diet, the liver cholesterol content after 2 and 4 months increased by 46 and 67%, respectively, in the control hamsters compared to ca. 500 and 1500% in the BIO 87.20 hamsters. This dramatic increase of liver cholesterol content in the BIO 87.20 hamsters resulted in a wholly abnormal greyish-white liver. With the clofibrate diet, the liver cholesterol content of the normal hamsters decreased by 37 and 22%, respectively, compared to animals on control diet after 2 and 4 months. In the BIO 87.20 hamster, the decrease of liver cholesterol content was ca. 60% after both intervals of time. There was no significant change in liver cholesterol levels in all animals maintained on a candicidin-containing diet.

In order to determine the effects of the different dietary treatments on rates of cholesterol synthesis in both groups of animals after 2 and 4 months, the liver and prostate gland were specifically selected for study here. The incorporation of carbon-14 labeled acetate into cholesterol was specifically measured in minced tissues of excised liver and prostate gland. The results obtained with the livers of both groups of Syrian hamsters are presented in Table 3.

In the normal control hamster maintained on a cholesterol diet for 2 and 4 months, the rate of cholesterol synthesis in the liver decreased by 88 and 82%, respectively, compared to that of the animals on a control diet. In the BIO 87.20 hamsters on a cholesterol diet, there was no apparent decrease in the rate of cholesterol synthesis compared to those animals on a control diet. It is also notable that the rate of liver cholesterol synthesis in the BIO 87.20 hamster was significantly lower than in the normal control hamster. Also quite apparent is that the synthesis of cholesterol in the liver of the BIO 87.20 Syrian hamster is not under feedback regulation as it is in the Syrian hamster control. For all animals on the candicidin diet, the rate of cholesterol synthesis approximately doubled after 2 and 4 months of treatment. In contrast, on a clofibrate diet, the rates of cholesterol synthesis in the liver of the control hamsters decreased 78 and 69%, respectively, after 2 and 4 months whereas in the BIO 87.20 hamster, the decrease in synthesis was 30 and 50%, respectively, compared to that of the animals on a control diet.

The rates of cholesterol synthesis in the prostate gland of all animals were also determined and are given in Table 4. It is immediately apparent that the rate of cholesterol synthesis in the prostate gland of all animals was 2- to 3-fold higher than in the liver. It is also apparent that the rate of prostate cholesterol synthesis...
TABLE 3
In Vitro Incorporation of [14C] Acetate into Cholesterol in the Liver of Normal Syrian and BIO 87.20 Male Hamsters

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Normal Syrian hamster—rate of synthesis (cpm/g tissue × 10^4)</th>
<th>BIO 87.20 hamster—rate of synthesis (cpm/g tissue × 10^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Time</td>
<td>2 Months</td>
</tr>
<tr>
<td>Control diet</td>
<td>0.523 ± 0.021*(6)**</td>
<td>0.658 ± 0.016(6)</td>
</tr>
<tr>
<td>Cholesterol diet</td>
<td>0.976 ± 0.111(6)b</td>
<td>1.13 ± 0.023(6)b</td>
</tr>
<tr>
<td>Candidin diet</td>
<td>1.527 ± 0.173(6)b</td>
<td>1.463 ± 0.168(6)b</td>
</tr>
<tr>
<td>Clofibrate diet</td>
<td>1.47 ± 0.033(6)b</td>
<td>1.463 ± 0.168(6)b</td>
</tr>
</tbody>
</table>

*Mean ± SE.
**Number of animals/group.
Significance: p value <0.05; b<0.01.

synthesis was not affected by cholesterol feeding in either the normal hamster control or the BIO 87.20 hamster, reflecting the lack of negative feedback regulation. On the candidin diet, the rate of prostate cholesterol synthesis increased by ca. 60-70% in both Syrian hamster groups whereas on the clofibrate diet, the inhibitory effect on cholestero genesis was more pronounced in the BIO 87.20 hamsters than it was in the normal hamster controls. After 2 and 4 months of clofibrate treatment, the rates of cholesterol synthesis decreased by 45 and 49%, respectively, in the normal hamster whereas in the BIO 87.20 hamster, the decline was 82 and 86%, respectively.

**DISCUSSION**

In the current studies with the male BIO 87.20 Syrian hamster, it was our original intention to determine the effect of dietary cholesterol on cholesterol metabolism of the prostate gland as possibly related to the age-dependent and spontaneous cystic prostatic hypertrophy exhibited by this animal model (1). Since our earlier investigations (6) had revealed that the chemically unrelated hypocholesterolemic drugs, candidin and colestipol, both prevented this enlargement and reduced the cholesterol content of the prostate gland, the exact role, if any, of cholesterol in this animal disease process remained obscure.

It was immediately apparent from the results of the first study presented here that cholesterol feeding to the BIO 87.20 hamster produced an accumulation of cholesterol in the liver and hypercholesterolemia over a period of 6 months of treatment.

Although significant increases of cholesterol in serum and liver of hamsters on a cholesterol diet have been previously observed by Ho (11), it is well known that cholestero genesis in the liver of common laboratory rodents is usually quite responsive to dietary cholesterol (12). The presence of moderate amounts of cholesterol in the diet leads to a rapid and, depending on the species, near total cessation of liver cholesterol synthesis. This is achieved by an efficient negative feedback control system with the rate-limiting enzyme 3-hydroxy-3-methyl glutaryl CoA reductase (13,14).

Considering the marked increase of cholesterol in the serum and liver of the BIO 87.20 hamsters on a cholesterol diet, it became important to determine the presence of feedback control of cholestero genesis in the liver of this animal disease model compared to normal Syrian hamsters. While the BIO 87.20 hamsters on a cholesterol diet after 4 months revealed significant increases in serum cholesterol levels and most surprising increases in liver content, the control Syrian hamsters exhibited no significant increases in serum cholesterol and only slight increases in liver content.

The difference in both hamster groups could be readily explained from the present results obtained in the stereogenesis studies. The determination of the rates of cholesterol synthesis in the liver and prostate of the BIO 87.20 hamster clearly revealed the lack of

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negative feedback control of endogenous cholesterol synthesis in either organ. While the normal Syrian hamster on a cholesterol diet clearly exhibited feedback control of liver cholesterol synthesis, it also lacked such control in the prostate gland.

The relationship of the absence of negative feedback control of cholesterogenesis in the liver and prostate gland of the male BIO 87.20 hamster to the other pathological conditions in this inbred hamster line, viz., development of cystic prostatic hypertrophy and susceptibility to cholesterol feeding, may be more than coincidental. Compared to normal Syrian hamsters, it was quite evident that the BIO 87.20 hamster did not fare well on the 1% cholesterol diet exhibiting marked hypercholesterolemia and greyish-white mottled livers after a relatively short period of feeding.

Other pathological conditions of the liver such as hepatomas are also associated with the loss of negative feedback regulation of cholesterol synthesis seen in normal liver (15). Neoplasmas, in general, are also characteristically associated with the absence of this control of cholesterogenesis (16). Derangements in cholesterol metabolism have also been observed in some leukemias (17). In this particular case, aside from this loss of regulation in cholesterogenesis, the gross appearance of the livers of the BIO 87.20 hamsters on a normal diet appear to be, otherwise, quite normal and similar to those of the normal control Syrian hamsters.

With both the BIO 87.20 and normal Syrian hamsters, the diets supplemented with the hypocholesterolemic drugs candidicin and clofibrate produced significant reductions in serum cholesterol levels. While clofibrate reduced the liver cholesterol content in both groups of animals, candidicin produced no such significant changes.

Clofibrate and candidicin clearly exhibited opposing effects on the rates of cholesterol synthesis in the liver and prostate gland of both hamster lines. Treatment with clofibrate, which illicits its hypocholesterolemic effect by inhibiting cholesterol synthesis, clearly displayed this inhibition in both organs of both hamster lines. Candidicin is believed to lower serum cholesterol levels by preventing the absorption-resorption of cholesterol from the intestinal tract, thus interfering with the enterohepatic circulation of cholesterol. In both the normal Syrian and BIO 87.20 hamsters, dietary candidicin clearly stimulated cholesterol synthesis in the liver to a significant extent, but less so in the prostate gland. In the normal Syrian hamster, for which hepatic cholesterol synthesis is under feedback control by exogenous cholesterol, the stimulation of cholesterol synthesis by candidicin treatment is understandable. In the BIO 87.20 hamster, for which hepatic cholesterol synthesis is not under the usual negative feedback regulation by exogenous cholesterol, candidicin treatment, in sharp contrast to the effects of dietary cholesterol, illicits a marked increase in the hepatic synthesis of cholesterol. This suggests the presence of some sort of positive feedback regulation of cholesterogenesis in the liver of both hamster
lines. When the cholesterol pool of the enterohepatic circulation is lowered by inhibitory action of candidicidin on cholesterol absorption-resorption in the small intestine, the synthesis of endogeneous cholesterol in the liver and prostate gland is stimulated.

Another strain of golden hamster maintained on a standard hamster chow was reported (18) to exhibit a marked increase in liver cholesterol ester content. Here, in comparison to normal control animals, the disease model animals exhibited a marked increase in the esterification of newly synthesized cholesterol rather than any alteration in the synthesis of cholesterol from mevalonate. The animals on a normal diet also exhibited hypercholesterolemia as well as hyperlipidemia involving elevated serum triglyceride levels.

The marked accumulation of cholesterol in the serum and livers of the BIO 87.20 male hamster maintained on a 1% cholesterol diet, resulting in death, is now understandable in light of the finding that cholestogenesis in the liver of this animal disease model is defective in its lack of negative feedback regulation. These animals on a normal hamster chow diet were otherwise quite normal in respect to their serum and liver cholesterol contents and their rates of cholestogenesis in the liver and prostate gland were reduced compared to normal Syrian hamster controls. It is now evident that the BIO 87.20 male hamster is an important animal disease model for studies involving both the prostate gland and cholesterol metabolism of the liver.

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