COMMUNICATION

Induction of Geranyl Pyrophosphate Pyrophosphatase Activity by Cholesterol-Suppressive Isoprenoids

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ABSTRACT: Diets supplemented (1 mmol/kg) with thymol, carvacrol, and ß-ionone significantly decreased the serum cholesterol levels of cockerels. These mevalonate-derived end products of plant secondary metabolism (isoprenoids) had no impact on two cytosolic prenyl alcohol (and ethanol) dehydrogenase activities; each treatment increased microsomal geranyl pyrophosphate pyrophosphatase activity by greater than twofold. The structural diversity of the isoprenoids which suppress cholesterol synthesis may be reconciled by their ability to increase pyrophosphatase activity, thus leading to the production of the endogenous, post-transcriptional regulator of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity.
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Mevalonate-derived end products of plant secondary metabolism (isoprenoids) have a cholesterol-lowering effect and a tumor-suppressive action. Both actions may be coupled to the suppression of mevalonate synthesis (1,2). Diverse isoprenoids, including cyclic monoterpenes (3), ß-carotene (4), the tocotrienols (5), farnesyl-pyrophosphate (PP) analogues (6), and farnesol (7), down-regulate microsomal 3-hydroxy-3-methylglutaryl CoA (HMG–CoA) reductase activity by a post-transcriptional action. Mevalonic acid (8) and prenyl-PP containing one (9) to four (7) isoprene units similarly down-regulate HMG–CoA reductase activity. The regulatory actions triggered by the isoprenoids (3–7) reflect those of the physiological, mevalonate-derived nonsterol regulatory agent which acts post-transcriptionally to down-regulate HMG–CoA reductase activity (8).

A microbiological screen (10) of diverse isoprenoids revealed the potent mevalonate-suppressive activities of ß-ionone, carvacrol (2-methyl-5-isopropylphenol), and thymol (2-isopropyl-5-methylphenol) (unreported data). In a follow-up study we found that diets supplemented with 0.5–1.3 mmol/kg ß-ionone, an end-ring analog of ß-carotene, suppressed HMG reductase activities in livers of White Leghorn cockerels and pullets by 45–70% (11).

Our experiments draw on reports that cells incubated with saturating concentrations of mevalonic acid accumulate α,ω-prenyl dicarboxylic acids (12). Following the hydrolysis of prenyl-PP intermediates by a microsomal phosphatase (13), cytosolic dehydrogenase and microsomal monooxygenase activities catalyze the formation of prenyl aldehydes, α-prenoic acids, ω- and ω3 hydroxy–α-prenoic acids and α,ω-prenyl dicarboxylic acids (9,12–16). We recently reported that pentobarbital, an inducer of the microsomal P450 monooxygenase activity that catalyzes the formation of ω- and ω3 hydroxy–α-prenoic acids, totally reverses the isoprenoid-mediated suppression of HMG–CoA reductase activity (11). This finding and the postulation by Watson and colleagues that changes in the post isopentenyl-PP isopentenoids individually or in combination define the regulatory signal molecule pool (9,12,14,15) are compatible with the recent report that farnesol induces the degradation of HMG–CoA reductase in permeablized cells (7). Correll *et al.* (7) further report that the induction of protein degradation by farnesyl-PP, the putative regulator identified by Bradfute and Simoni (6), is inhibited by NaF (7). We suggest that the cyclic monoterpenes (3), ß-carotene (4), and the tocotrienols (5) that lower HMG–CoA reductase activity divert a key phosphorylated intermediate of sterol synthesis toward the synthesis of the physiological regulatory agent. These considerations led us to examine the impact of dietary isoprenoids on the reactions preceding the formation of the ω- and ω3 hydroxy–α-prenoic acids.

METHODS

Four groups (n = 8) of White Leghorn cockerels, 126 g, were fed a growing ration (control) or the same ration supplemented with 1 mmol/kg diet of one of the aforementioned isoprenoids [all from Aldrich Chemical Co. (Milwaukee, WI)]. Serum cholesterol levels and three hepatic enzyme activities, microsomal prenyl-PP pyrophosphatase and cytosolic prenyl alcohol and ethanol dehydrogenases were measured following a 21-d feeding period. Body weight gains were not influenced by the treatments. Serum cholesterol levels were measured with diagnostic kits (Sigma Chemical Co., St. Louis, MO). Enzyme activity assays were conducted using concentrations of substrates that were determined experimentally to

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Abbreviations: HMG–CoA, 3-hydroxy-3-methylglutaryl CoA; PP, pyrophosphate.

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be saturating. Microsomal prenyl-PP pyrophosphatase activity was determined by measuring the conversion of [1-^{3}H]-geranyl-PP to ^{3}H-geraniol, using a method adapted from those previously described by Christophe and Popják (13) and Croteau and Karp (17). The incubation mixture contained 50 mM Tris (pH 7.4), 100 μM geranyl-PP (Sigma), 0.03 μCi [1-^{3}H]-geranyl-PP (American Radiolabeled Chemicals, Inc., St. Louis, MO) and approximately 400 mg of microsomal protein. The reaction was carried out in a 200 μL volume at 37°C for 20 min. At the end of the incubation period, geraniol was extracted with 1 mL of hexane and counted by liquid scintillation. Results were corrected for small amounts of radioactivity extracted from heat-inactivated tissue blank samples and for geraniol extraction efficiency (90%). Reaction rates were linear with incubation time and amount of tissue extract employed. Prenyl alcohol and ethanol dehydrogenase activities were determined spectrophotometrically at 30°C by following the initial rate of reduction of NAD^+ at 340 nm. The reaction mixture contained 50 mM Tris buffer (pH 7.4), 2 mM NAD^+, 5 mM geraniol, farnesol, or ethanol, and approximately 50 μg of cytosolic protein in a total volume of 1 mL.

RESULTS AND DISCUSSION

Cockerels fed diets supplemented (1 mmol/kg diet) with carvacrol ($P < 0.005$), thymol ($P < 0.05$), or ß-ionone ($P < 0.001$) had a reduced serum cholesterol level as compared to the controls; differences between treatment groups were not significant (Table 1). The isoprenoid-mediated depression of serum cholesterol concentration ranged from 9 to 18%. Under the conditions employed in this study, that is, birds fed an isoprenoid-supplemented, cholesterol-free diet, a 5% suppression of HMG–CoA reductase activity was associated with a 2% decrease in serum cholesterol ($P < 0.004$) (18).

Microsomal geranyl-PP pyrophosphatase activities in livers of birds fed carvacrol ($P < 0.01$), thymol ($P < 0.005$), and ß-ionone ($P < 0.005$) were >2.2-fold greater than the control activity (Table 1). The induction of microsomal prenyl-PP pyrophosphatase activity may represent a key common site of action for the diverse plant isoprenoids that alter cholesterol metabolism in animals. Although this activity diverts prenyl-PPs from the sterologenic pathway, the diversion is not of sufficient magnitude to suppress the incorporation of mevalonate into cholesterol (5,6,19). More likely this activity increases the intracellular concentration of the post isopentenyl-PP metabolite(s) that accelerates the degradation of HMG–CoA reductase (6,7,9,12,14,15). The finding that the farnesol-induced degradation of HMG–CoA reductase, unlike that of farnesyl-PP (7) and farnesyl acetate (6), occurred with no apparent lag time led Correll *et al.* (7) to suggest that farnesol has a more direct role in enzyme degradation.

It should be noted that none of the studies leading to the identification of farnesol as the physiological regulator of HMG–CoA reductase activity (5–7) employed an inhibitor of the prenyl dehydrogenase activity. It remains possible that an inhibitor targeted to the constitutive prenyl alcohol dehydrogenases (Table 1) might potentiate or attenuate the HMG–CoA reductase-suppressive action of farnesol depending on whether farnesol or a farnesol metabolite proves to be the physiological regulator.

Human liver prenyl alcohol dehydrogenase exists in multiple forms with Kms for geraniol and farnesol ranging from 0.2 to 73 and <0.1 to 3 μM, respectively (16). Keung (16) has suggested that genetic determinants of the prenyl alcohol de-

TABLE 1
Effect of Feeding Carvacrol, Thymol, or ß-Ionone on Serum Cholesterol and Hepatic Ethanol and Prenyl Alcohol Dehydrogenase and Geranyl-Pyrophosphate Pyrophosphatase Activities[a]

	Control	Carvacrol	Thymol	ß-Ionone	P[b]
Serum cholesterol (mmol/L)	4.69 ± 0.29	3.85 ± 0.65	4.28 ± 0.35[c]	4.03 ± 0.33[f]	<0.01
Geranyl-PP pyrophosphatase (nmol/min/mg, microsomal protein)	0.124 ± 0.051	0.283 ± 0.073[d]	0.276 ± 0.058[e]	0.270 ± 0.082[e]	<0.005
Geraniol dehydrogenase (nmol/min/mg, cytosolic protein)	17.5 ± 4.7	21.3 ± 5.1	18.3 ± 4.0	15.3 ± 3.7	n.s.
Farnesol dehydrogenase (nmol/min/mg, cytosolic protein)	1.17 ± 0.84	0.87 ± 0.58	0.85 ± 0.48	1.25 ± 0.55	n.s.
Ethanol dehydrogenase (nmol/min/mg, cytosolic protein)	1.24 ± 0.50	1.00 ± 0.46	0.88 ± 0.54	1.05 ± 0.45	n.s.

[a]Cockerels were fed either a growing ration (Control) or the Control ration supplemented with 1 mmol/kg of carvacrol, thymol, or ß-ionone for 21 d; mean ± S.D. n.s., Not significant.
[b]F-test of treatment effects, one-way analysis of variance.
[c–f]Pairwise comparisons with control; [c]$P < 0.05$; [d]$P < 0.01$; [e]$P < 0.005$; [f]$P < 0.001$.

hydrogenase isozymes influence the disposition of products diverted from the sterologenic pathway; differences in prenyl alcohol dehydrogenase isozyme characteristics thus may contribute to differences in serum cholesterol levels among various populations.

This study, to our knowledge, is the first to show the induction of a prenyl-PP pyrophosphatase activity. The disparity between this activity and the prenyl alcohol dehydrogenase activities is consistent with our suggestion that the microsomal prenyl-PP pyrophosphatase reaction is more important in the synthesis of a physiological regulator of HMG–CoA reductase activity.

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