Tissue-specific coordinate regulation of enzymes of cholesterol biosynthesis: sciatic nerve versus liver

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Abstract Exposure of weanling rats to a diet containing the element tellurium results in specific inhibition of squalene epoxidase, an obligate enzyme in cholesterol biosynthesis. Liver responds to the resulting intracellular sterol deficit by up-regulating, in parallel and to the same extent, expression of mRNA for squalene epoxidase and for HMG-CoA reductase, the major rate-limiting enzyme in the pathway. This increased mRNA expression, coupled with additional translational and posttranslational activation of the pathway, allows normal levels of cholesterol synthesis in liver despite tellurium-induced inhibition of squalene epoxidase. The response to tellurium challenge in sciatic nerve is very different. In this tissue, cholesterol synthesis is prominent because of the large amount of cholesterol required for synthesis and maintenance of myelin. Although nerve shows an initial (at 1 day) up-regulation of mRNA expression for both enzymes in response to tellurium exposure, this is followed quickly by parallel down-regulation of both enzymes, in concert with down-regulation of mRNA expression for myelin proteins. We suggest that the tellurium-induced deficit in sterols leads to a coordinate down-regulation of synthesis of myelin components. The initial early up-regulation of cholesterol biosynthesis in sciatic nerve due to the cholesterol deficit is countered by down-regulation which is coordinated with overall control of the program of myelin assembly. This tissue-specific control of cholesterol synthesis in sciatic nerve is a point of vulnerability to toxicants, and may be related to the need for coordinate synthesis of all components of myelin.


Supplementary key words HMG-CoA reductase • squalene epoxidase • demyelination • gene expression • peripheral neuropathy • liver • sciatic nerve

A compound may be considered a “neurotoxicant” if its adverse effects, although possibly present on a systemic basis, are disproportionately apparent as damage to the central or peripheral nervous systems. Presumably, some metabolic process preferentially relevant to nervous system function is targeted. This may involve a toxic action on neurons and/or their processes. However, most cells of the nervous system are glial cells, and these can be preferential targets for neurotoxicants as well, with resulting insults also leading to nervous system dysfunction (see ref. 1 for review). In the peripheral nervous system (PNS), Schwann cells are the glial cells responsible for synthesis and maintenance of myelin, the compact multilamellar extension of the Schwann cell plasma membrane required for efficient and rapid impulse conduction in axons.

Inclusion of tellurium (element #52) in the diet of weanling rats leads to rapid inhibition of the synthesis of cholesterol (2) by specifically blocking squalene epoxidase (3, 4), an obligate enzyme in the cholesterol biosynthetic pathway. Although inhibition of this enzyme by a metabolite of tellurium is systemic, the Schwann cell–myelin unit of the PNS is preferentially affected by this insult (see ref. 5 for review). This reflects the quantitative significance of cholesterol in PNS myelin, which requires that its synthesis be especially prominent during the period of rapid PNS myelin accumulation occurring immediately following weaning. The block in the synthesis of cholesterol, a molecule required for formation and maintenance of a stable myelin structure, leads to a highly synchronous demyelination of up to one-fourth of the myelin internodes in sciatic nerve. This demyelination, in turn, results in a peripheral neuropathy characterized by hindlimb paralysis and paralysis. When tellurium exposure is discontinued, there is rapid and synchronous remyelination, with consequent recovery of normal neurological function.

Despite inhibition of squalene epoxidase by tellurium, a normal level of circulating cholesterol is maintained. That under these conditions the sciatic nerve demyelinites suggests that the PNS cannot utilize this

Abbreviations: HMG-CoA, hydroxymethylglutaryl-coenzyme A; PNS, peripheral nervous system; LDL, low density lipoprotein; PCR, polymerase chain reaction; SRE, sterol regulatory element.

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circulating cholesterol, an hypothesis which we have verified (6). How does liver maintain circulating cholesterol levels in the face of inhibition of the biosynthetic pathway? We (7) have shown that in sciatic nerve, expression of mRNA for both HMG-CoA reductase, the major rate-limiting enzyme in cholesterol biosynthesis, and for myelin proteins is down-regulated in parallel. We interpret this as down-regulation of the program for synthesis and assembly of myelin components, secondary to the tellurium-induced deficit in required cholesterol. This is in contrast to liver, which responds to tellurium challenge by up-regulation of HMG-CoA reductase. The latter response is as expected from knowledge of the well-characterized regulatory mechanism involving intracellular sterol levels and the LDL-receptor (see refs. 8–10 for review). We have now examined in more detail these differences in tissue-specific responses to tellurium challenge. Of interest was whether the difference in response of liver and sciatic nerve was specific for HMG-CoA reductase, or whether squalene epoxidase, another key enzyme of cholesterol biosynthesis located subsequent to reactions in the pathway that provide isoprenoid units, was coordinately controlled with HMG-CoA reductase. In addition, we were able to assay in vivo synthesis of cholesterol in these tissues. Correlation of this parameter with levels of mRNA for the two enzymes of interest gives insight into organ-specific pathophysiology associated with tellurium toxicity.

EXPERIMENTAL PROCEDURES

Animal models

All animal use procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of North Carolina Institutional Animal Care and Use Committee. At 20 days of age, male Sprague-Dawley rats were placed on a diet containing 1.5% elemental tellurium powder (60 mesh, Aldrich, Milwaukee, WI) in milled Purina rodent chow (very low cholesterol content), with 12% (w/w) corn oil added to prevent separation of the mixture (11). Control animals were maintained on the same milled rodent chow plus corn oil, but without tellurium. Exposure to tellurium was continued for 7 days, after which all rats were placed on regular pelleted rodent chow ad lib. Rats were killed at times ranging from 1 to 30 days following beginning of the 7-day tellurium exposure period. Sciatic nerves and a 30–50-mg sample of liver were removed, quickly frozen on dry ice, and stored at −80°C until RNA was isolated.

The procedure for sciatic nerve transection was as described previously (12). Rats were anesthetized with ketamine and xylazine and subjected to unilateral transection of the sciatic nerve at the level of the sciatic notch. The proximal portion of the nerve was sutured into an adjacent muscle to inhibit axonal regeneration. At times ranging from 1 to 10 days after nerve transection, the distal stumps were removed, quickly frozen on dry ice, and stored at −80°C until RNA was isolated.

RNA isolation and Northern blot analyses

Total RNA was isolated from frozen nerve and liver samples by homogenization in guanidine isothiocyanate and purified by centrifugation through cesium chloride and subsequent ethanol precipitation (13; see also 11). RNA species were separated according to molecular weight on denaturing 0.8% agarose gels containing formaldehyde (14) and transferred to Zeta-Probe nylon blotting membranes (Bio-Rad Laboratories, Richmond, CA). Filters were hybridized with 32P-labeled cDNA probes, synthesized using either double-stranded polymerase chain reaction (PCR) methodology (15) for amplification of squalene epoxidase cDNA (16), or single-stranded PCR methodology (17) for HMG-CoA reductase (18) and Pa (19) cDNA. Filters were washed and then exposed to X-ray film to obtain a visual pattern of mRNA levels. mRNA levels were quantitated using a Packard Instant Imager electronic autoradiography imaging system. To control for variability in sample handling, values obtained were normalized to the amount of 28S ribosomal RNA in each lane, as assayed with a 32P-end-labeled oligonucleotide (20) specific for bases 2673–2692 and 3361–3383 of the published sequence (21).

Measurement of cholesterol synthesis rates in vivo

A detailed description of this methodology and its theoretical basis have been presented (6, 22, 23). Systematically injected [3H]water rapidly equilibrates with the body pool of water. The biosynthetic pathway between acetate (primarily derived from glycolysis and β-oxidation) and cholesterol involves addition of hydrogens equivalent to 11 moles of water (22). After injection of labeled water, the metabolic intermediates of cholesterol synthesis rapidly approach a steady-state of specific radioactivity with respect to the pool of body water. Simple calculations, based on the assumption that newly synthesized cholesterol will have a specific radioactivity 11 times that of body water, allow for determination of the actual rate of synthesis of cholesterol. The number of molecules of newly synthesized squalene present at any given time can also be determined with the assumption that half of the water-equilibrated protons in cholesterol entered the metabolic pathway at, or prior to, squalene formation.

Rats exposed to tellurium for 1, 3, or 5 days and their
age-matched controls were injected intraperitoneally with [3H]water (100 mCi/ml; ICN Radiochemicals, Irvine, CA) at a dosage level of 0.5–1.0 mCi/g body weight. After a 2-h labeling period, rats were killed and a blood sample was collected to obtain serum for determination of specific radioactivity of the body water pool. Sciatic nerves and livers were removed and lipids were extracted from these tissues by a modification (24) of the method of Folch, Lees, and Sloane Stanley (25). Aliquots of these lipid extracts were saponified with methanolic KOH, and sterols were separated from the other nonsaponifiable lipids by reverse-phase HPLC using a C18 column. The mass of free cholesterol and radioactivity associated with cholesterol were determined, and the absolute rate of cholesterol synthesis was calculated from these data and the specific radioactivity of the body water pool. Appropriate controls to account for the time needed for cholesterol pathway intermediates to equilibrate with body water were included (see ref. 6 for details).

RESULTS

mRNA expression in liver and sciatic nerve

After the first day of weaning, there was a significant decline in mRNA expression for HMG-CoA reductase in livers of control animals (the considerable up-regulation of this message when animals are weaned from cholesterol-rich milk onto low-cholesterol rat chow has already taken place during the first 24 h and is not a factor in these experiments). In contrast, if upon weaning rats are administered tellurium, the resulting inhibition of cholesterol synthesis leads to up-regulation of mRNA expression for HMG-CoA reductase (maximal increase about 6-fold over controls after 3 days of tellurium; Fig. 1 and Fig. 2). mRNA expression for squalene epoxidase, the enzyme inhibited by tellurium, was also upregulated, with a virtually identical temporal pattern (Figs. 1 and 2). After about 3 weeks of recovery from tellurium exposure (30 day time point), mRNA levels for both enzymes had returned to control levels.

The temporal pattern of mRNA expression for HMG-CoA reductase in sciatic nerves of control animals resembled liver in that there was a gradual decrease following weaning (Figs. 1 and 2). However, the response to tellurium exposure was very different in sciatic nerve. There was a moderate (approx. 1.5-fold) initial up-regulation after 1 day of tellurium exposure, but this was followed by a marked down-regulation. After tellurium exposure was terminated, there was a gradual return to control levels. As in liver, temporal changes in steady-state mRNA levels for squalene epoxidase were parallel to those for HMG-CoA reductase. Except for the initial up-regulation seen after 1 day of tellurium exposure, changes in mRNA expression for these two cholesterol biosynthetic enzymes in sciatic nerve were similar to those seen for P0, the major protein of PNS myelin (Fig. 2), and for other myelin proteins (11, 26).

Both squalene epoxidase and HMG-CoA reductase were also down-regulated distal to a nerve transection (Fig. 3). In these nerve segments, there is axonal degeneration with accompanying secondary demyelination,
Fig. 2. Temporal changes in steady-state mRNA levels in liver (upper panel) and sciatic nerve (lower panel) for HMG-CoA reductase, squalene epoxidase, and myelin P₀ protein (sciatic nerve only) after exposure of developing rats to tellurium. After hybridization of sciatic nerve and liver RNA samples with ³²P-labeled cDNA probes, radioactivity in each lane was quantitated using an electronic autoradiography imaging system. Values obtained were normalized to a constant amount of ribosomal RNA in each lane, as described in Experimental Procedures. Data are means ± SEM for 3–4 separate sets of samples, each consisting of 4–6 animals for each time point. Te, tellurium; Con, control; HMG-CoAR, HMG-CoA reductase; SOX, squalene epoxidase.

and myelin-producing Schwann cells revert to a more primitive nonmyelinating phenotype (see refs. 27, 28 for details). The pattern of down-regulation for squalene epoxidase and HMG-CoA reductase was similar to that seen for P₀ protein, although perhaps not quite as severe for the former, presumably because some cholesterol synthesis is required for basal maintenance of cells remaining in the nerve.

In vivo cholesterol synthesis in liver and sciatic nerve

Tellurium-induced alterations in mRNA expression for HMG-CoA reductase and squalene epoxidase in liver and sciatic nerve were compared to changes in the rates of cholesterol synthesis in these tissues. Utilizing an experimental design in which rats were systematically labeled with [³H]water allows for determination of the absolute rate of cholesterol biosynthesis in various organs and tissues in vivo (22, 23). An extension of this methodology has been developed to examine the relationship between cholesterol biosynthesis and accumulation of cholesterol during development in various tissues, including sciatic nerve (6) and brain (29).

Cholesterol synthesis in liver was initially inhibited by tellurium exposure (50% inhibition after 1 day), but
by 5 days after beginning exposure, synthesis levels had increased to slightly above normal (Fig. 4, upper panel). Cholesterol synthesis in sciatic nerve was also inhibited after 1 day of tellurium exposure (56% of control). In contrast to liver, however, the rate of synthesis remained severely depressed (13% of control after both 3 and 5 days of tellurium exposure; Fig. 4, lower panel).

Additional insight into regulation of the cholesterol synthesis pathway is available from analysis of the incorporation of label into squalene (intermediate immediately above the tellurium-induced block) and cholesterol. Taken together, these are indicative of the total amount of intermediates committed to the sterol biosynthesis pathway, and are a measure of the flux of intermediates through HMG-CoA reductase (there are no other pools of intermediates large enough to contain significant amounts of label). In liver, tellurium exposure greatly enhanced flux of precursors committed to this pathway (over 8-fold after 5 days of tellurium exposure; Fig. 5, upper panel). This indicates a compensatory up-regulation of the cholesterol synthesis pathway in response to the tellurium insult. Because of the block in squalene epoxidase, there was a marked increase in liver squalene levels resulting from this up-regulation (Table 1). We assume that this pile-up of precursor somehow drives the conversion to sterols, despite inhibition of the squalene epoxidase step by tellurium. In contrast to liver, the combined synthesis of cholesterol and squalene in sciatic nerve was only slightly increased (0.6-fold) after 3 and 5 days of tellurium (Fig. 5, lower panel). This relatively moderate increase in flux of precursors committed to sterol synthesis is not sufficient to compensate for the inhibition of squalene epoxidase in sciatic nerve.

Relative response of liver and sciatic nerve to tellurium

The response of liver to tellurium challenge included an almost 4-fold elevation in levels of mRNA for HMG-CoA reductase after 3 and 5 days of tellurium exposure (see Fig. 2). This is reflected in an 8-fold increase in flux through the cholesterol synthesis pathway (Fig. 5), indicating that translational and posttranslational mechanisms may account for up to half of the increase in activity of HMG-CoA reductase. The situation in sciatic nerve was similar in that, although the actual amount of message for HMG-CoA reductase was decreased to about half of control levels at 3 and 5 days.
Fig. 5. Synthesis of squalene plus cholesterol in liver and sciatic nerve. The combined synthesis of squalene (intermediate immediately above the tellurium-induced block) plus cholesterol is indicative of the total amount of intermediates committed to the sterol biosynthesis pathway, and provides a measure of the flux of intermediates through HMG-CoA reductase. Levels of synthesis were calculated as described in the legend to Fig. 4. Values are means of two separate sets of animals, and times are days after beginning exposure to a diet containing 1.5% tellurium. Error bars show average deviations of individual values from the mean.

(see Fig. 2), translational and posttranslational activity increased (about 3-fold) so that flux through the pathway was actually somewhat increased (Fig. 5).

In addition to analyses of tellurium-induced perturbations, our data allow for some comparisons of cholesterol synthesis in liver and sciatic nerve of control animals. The concentration of total cellular RNA is very different in these two tissues (0.97 ± 0.04 and 6.2 ± 0.8 ng/mg tissue for 21-day sciatic nerve and liver, respectively). This 6-fold difference presumably reflects the role of liver in the synthesis and export of a vast variety of proteins. The ratio of HMG-CoA reductase mRNA to total cellular RNA is about 3-fold greater in sciatic nerve than in liver (calculated from experiments in which samples from both tissues were hybridized on the same filter, thus controlling for any differences in probe specific radioactivity and other hybridization variables). These data allow for comparison of levels of cholesterol synthesis in control animals as a function of mRNA levels for HMG-CoA reductase. Cholesterol synthesis per unit of HMG-CoA reductase mRNA was approximately 3 times greater in control sciatic nerve than in liver, suggestive of greater reserve capacity for cholesterol synthesis by liver.

DISCUSSION

Exposure to dietary tellurium results in a rapid, highly specific inhibition of squalene epoxidase in all tissues (3, 4). Inhibition of this obligate enzyme in the cholesterol biosynthesis pathway results in decreased sterol production. In liver, this intracellular sterol deficit leads to up-regulation of mRNA expression for HMG-CoA reductase, the major rate-limiting enzyme for cholesterol biosynthesis. This is as expected in view of the well-documented regulatory mechanism whereby cholesterol feed-back inhibits its own synthesis, acting primarily at the level of HMG-CoA reductase (see ref. 8 for review).

Squalene epoxidase, the enzyme targeted by tellurium, is also up-regulated with a pattern virtually identical to that for HMG-CoA reductase, indicating a close coordinate control of these two enzymes. This is so even though HMG-CoA reductase catalyzes an early committed step in cholesterol biosynthesis, while squalene epoxidase catalyzes a reaction following production of isoprenoid units required for dolichol, ubiquinone, and isopentyl tRNA synthesis, and for polyprolpylalkylation of p21 and some GTP binding proteins (8, 30, 31). Studies examining regulation of squalene epoxidase mRNA expression (32) and enzyme activity (33, 34) suggest it also functions as a regulatory enzyme, and our current findings, as well as our previous metabolic data (4), clearly support this conclusion.

A number of recent studies have examined the coordinate transcriptional control of various cholesterol biosynthetic enzymes, including HMG-CoA reductase, HMG-CoA synthase, mevalonate kinase, farnesyl di-
enzymes is markedly down-regulated, to the same extent observed for both HMG-CoA reductase and squalene epoxidase in liver in response to tellurium challenge (about 4-fold) accounts for about half of the 8-fold increase in incorporation of \(^{3}H\)water into cholesterol. Thus, the compensatory responses of liver to tellurium challenge occur not only at the level of up-regulation of the steady-state mRNA levels, but also at the levels of translational and/or posttranslational modification. Sciatic nerve also responds to tellurium challenge with an initial coordinate up-regulation of mRNA expression for HMG-CoA reductase and squalene epoxidase. This presumably results from the tellurium-induced deficit in intracellular sterols, with the mechanism being the same as that in liver. In contrast to liver, however, after one day the mRNA expression for both enzymes is markedly down-regulated, to the same extent seen for myelin proteins. We note that even though mRNA levels for HMG-CoA reductase and squalene epoxidase are somewhat down-regulated by 5 days, and are only half of control values by 5 days of tellurium exposure, the flux of precursors committed to the cholesterol synthesis pathway (assayed as synthesis of squalene and cholesterol) is actually still somewhat elevated. Despite this up-regulation of material flowing through the HMG-CoA reductase step, up-regulation is apparently not sufficient to build up enough squalene to force the required level of cholesterol synthesis. The ratio of labeled cholesterol to labeled squalene subsequent to tellurium challenge is similar in nerve and liver (0.09 ± 0.01, overall mean ± SEM for sciatic nerve and liver at 3 and 5 days; calculated from data used to prepare Fig. 5). Thus, without an 8-fold or greater increase in flux of intermediates through the synthesis pathway in sciatic nerve, normal levels of cholesterol biosynthesis cannot be maintained.

Why is the cholesterol pathway in sciatic nerve not more up-regulated in response to tellurium challenge? One possibility is a generalized down-regulation in expression of myelin components due to the deficit in the supply of cholesterol, a major myelin membrane component. If this is the case, there may be an additional level of control of cholesterol synthesis in Schwann cells related to the program for myelination. Cholesterol is the major lipid component of myelin, accounting for about 30% of the total lipid dry weight (35), and virtually all of the cholesterol synthesized in these rapidly myelinating Schwann cells is destined for myelin assembly. It seems reasonable that some mechanism might be operational to coordinate the supply of cholesterol with that of other required myelin lipids and the myelin proteins. The parallel down-regulation of both HMG-CoA reductase and squalene epoxidase with myelin protein gene expression in degenerating stumps of transected nerves further supports such "myelin-specific" coordinate regulation in sciatic nerve.

An explanation alternative to that proposed above is that the down-regulation of cholesterol synthesis in Schwann cells results from increased intracellular levels of sterols derived from degenerating myelin. However, in situ hybridization experiments examining P_{60} expression during tellurium exposure have shown that myelin synthesis is down-regulated in all myelinating Schwann cells, and not just in those undergoing demyelination (36). Thus, in sciatic nerve, the initial up-regulation of the cholesterol synthesis pathway due to sterol deprivation is countered by an overriding down-regulation coordinated with down-regulation of the program of myelin assembly.

The very marked differences in the response of liver and sciatic nerve to the tellurium-induced inhibition of cholesterol synthesis suggest that in sciatic nerve, the entire cholesterol biosynthesis pathway is regulated in parallel with the synthesis of other components required for myelin (P_{60} and other myelin proteins). This coordinate regulation may involve a common controller element that coordinates mRNA expression for all required myelin lipid and protein components (for review, see 37, 38), and further studies will be needed to determine whether genes for synthesis of myelin lipids contain regulatory sequences in common with those of myelin proteins.

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