Biotin Biotransformation to Bisnorbiotin Is Accelerated by Several Peroxisome Proliferators and Steroid Hormones in Rats

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ABSTRACT Bisorbornbiotin and biotin sulfoxide are the major catabolites of biotin for humans, swine, and rats. Increased urinary excretion of bisnorbiotin, biotin sulfoxide, or both have been observed during pregnancy and in patients treated with certain anticonvulsants. We sought more insight into the sites and mechanisms of biotin catabolism by exposing rats in vivo to compounds known to induce classes of enzymes that were candidates to catalyze the biotransformations. Rats were treated with the anticonvulsants phenytoin, phenobarbital, and carbamazepine, the steroid hormones dexamethasone and dehydroepiandrosterone, and the peroxisome proliferators clofibrate and di(2-ethylhexyl)phthalate. [14C]Biotin was injected intraperitoneally at physiologic doses in treated rats and control rats; HPLC and radiometric flow detection were used to specifically identify and quantify [14C]biotin and its metabolites in urine. Treatment effects were assessed by the change in the urinary excretion of [14C]bisnorbiotin and [14C]biotin sulfoxide in response to administration of [14C]biotin. No significant changes resulted from treatment with any of the anticonvulsants. With the steroid hormones and the peroxisome proliferators, [14C]bisnorbiotin excretion increased significantly. These results indicate that biotin is converted into bisnorbiotin in the liver and that this conversion likely occurs in peroxisomes or mitochondria or both via β-oxidative cleavage, and, in contrast to responses in humans, the enzymes responsible for the formation of biotin sulfoxide in rats are not induced by the anticonvulsants examined here. J. Nutr. 127: 2212–2216, 1997.

KEY WORDS: • anticonvulsant • biotin • bisnorbiotin • peroxisome proliferator • rats

The water-soluble vitamin biotin acts as a coenzyme for four carboxylases in mammals. These carboxylases catalyze component steps of fatty acid biosynthesis, gluconeogenesis, amino acid metabolism, and propionate metabolism. The major catabolites of biotin for humans, swine, and rats are bisnorbiotin (BNB) and biotin sulfoxide (BSO).

Observations in two groups of patients have stimulated our interests in the catabolism of biotin. Studies from our laboratory have indicated that biotransformation of biotin to BNB and BSO is increased by long-term treatments with anticonvulsants (Mock and Dyken 1997). These studies and those from the laboratory of Krause and co-workers (1982a, 1984 and 1988) suggest that anticonvulsant therapy may also result in reduced biotin status in some individuals. We have observed significantly increased biotin biotransformation to BNB in normal pregnancy. Some of our observations suggest that about half of the women studied became marginally biotin deficient late in pregnancy and that those pregnant women with increased excretion of BNB were more likely to fall into the deficient group (Mock, D. M. et al. 1997a).

Bisorbornbiotin differs from biotin by a two-carbon reduction along the pathway for the side chain and oxidation of the sulfur of biotin in microbes was demonstrated by Ogata and co-workers (Yang et al. 1968) and by McCormick and co-workers using radiolabeled biotin as the sole carbon source (Iwahara et al. 1969, Kazarinoff et al. 1972). These oxidations were also found to occur in rats (Lee et al. 1972). Hence, the assumption that BNB is formed in mammals by enzymatic β-oxidation is reasonable. However, direct proof of the enzymatic nature of the biotransformation in mammals is minimal, and the organ site(s), the subcellular site(s), and the enzyme(s) responsible remain unknown. For example, is this biotin β-oxidation simply the result of catalysis by one or more of the mitochondrial or peroxisomal fatty acid β-oxidation enzymes? If so, the rate of biotin biotransformation would not down-regulate importantly early in the course of progressive biotin deficiency. However, our studies in which biotin deficiency was induced experimentally by egg white feeding provide evidence that the urinary excretion of BNB does decrease early in biotin deficiency (Mock, N. I. et al. 1997).

For these reasons, we sought more insight into the process of biotin catabolism in mammals by exposing rats in vivo to compounds known to induce classes of enzymes that are candidates to catalyze the biotin biotransformation. The effect

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5 Abbreviations used: BSO, biotin sulfoxide; BNB, bisnorbiotin; DHEA, dehydroepiandrosterone; DEHP, di(2-ethylhexyl)phthalate.
on biotin biotransformation was assessed by measuring changes in the urinary metabolite profile.

MATERIALS AND METHODS

Chemicals and reagents. Clofibrate, phenobarbital, and dehydroepiandrosterone (DHEA) were purchased from Sigma Chemical (St. Louis, MO). Phenytoin, carbamazepine, and di(2-ethylhexyl)phthalate (DEHP) were purchased from Aldrich Chemical (St. Louis, MO). Dexamethasone sodium phosphate was obtained in injectable form at a concentration of 100 mmol/L (American Reagent Laboratory, Shirley, NY). D-([4-14C]-carbonyl)biotin with a specific radioactivity of 2 GBq/mmol was obtained from Amersham (Arlington Heights, IL). D-[8,9-3H(N)]Biotin with a specific radioactivity of 1.7 TBq/mmol was purchased from DuPont NEN (Boston, MA). The synthesis and characterization of radiolabeled biotin analogs have been described elsewhere (Wang et al. 1996).

Animals and diets. Male Sprague-Dawley rats (Harlan, Madison, WI) were provided a biotin-sufficient diet (PROLAB R-M-H 3000, Agway, Syracuse, NY), for at least 1 wk to stabilize their biotin nutritional status. The diet contains 1.6 mmol biotin/g. The same diet was provided during the experimental period. The proximate analysis of the diet is 22.5% protein, 5.5% fat, 4.5% fiber, 3.5% ash, 10% moisture and 52% nitrogen-free extract. Animal protocols were approved by the University of Arkansas for Medical Sciences Animal Care and Use Committee.

Response to anticonvulsants, steroid hormones and peroxisome proliferators. The treatment, doses, duration, and route of administration for each compound tested are listed in Table 1. A single control group was used for the comparison to all the treated groups. Data of the control group have been reported in the cited reference (Wang et al. 1996).

RESULTS

Urinary excretion of total radiolabeled biotin plus metabolites. Depicted in Figure 1 are the cumulative urinary radioactivity data for the various treatment groups and control group. The cumulative excretions at 24 h varied from 55 to 75% of the injected radioactivity, but a consistent trend to increase or decrease with treatment was not apparent. The curve shapes were similar in all groups; the major portion of administered radioactivity was excreted within 24 h of the injection. Analysis of variance of the cumulative excretion at 24 h revealed no significant differences.

Effect of treatment on urinary biotin metabolite profiles. Depicted in Figure 2 are the metabolite excretion data for BNB...
and co-workers (Yang et al. 1968) and by McCormick and co-
workers (Iwahara et al. 1969, Kazarinoff et al. 1972). More-
over, the presence of bisnorbiotin methyl ketone in urine of
human subjects (Zempleni et al. 1997b) provides additional
support of the hypothesis that BNB is formed by an enzymatic
β-oxidation, because this methyl ketone probably arises from
a less stable β-keto acid intermediate in a fashion analogous
to the enzymatic β-oxidation of fatty acids (Lee et al. 1972,
Kazarinoff et al. 1972). Notwithstanding these pioneering ob-
servations, little is known about the primary organ sites, sub-
cellular organelle sites, identities, characteristics, or regulation
of the enzymes responsible for biotin catabolism to BNB.

This study provides evidence that the biotin biotransforma-
tion to BNB is inducible by certain peroxisome proliferators
and steroid hormones. Of course, this conclusion is based in
part on the assumption that urinary excretion of BNB re¯ects
formation of BNB that is in turn directly related to the enzyme
activity induced by each treatment. Separate studies from our
laboratory detect very little biliary excretion of biotin or me-
tabolites (Zempleni et al. 1997a), con®rming that urine is the
primary route for excretion of biotin and its metabolites.

**DISCUSSION**

The inactive metabolite BNB differs from biotin by a two-
carbon reduction in the length of the valeric acid side chain.
This two-carbon reduction is a common motif for the enzy-
matic β-oxidation of saturated straight-chain fatty acids
of various lengths. Thus, it would be reasonable to assume that
the biotransformation of biotin to BNB is an enzyme-catalyzed
process. Indeed, the presence of a β-oxidation pathway for the
side chain of biotin was demonstrated in microbes by Ogata
and co-workers (Yang et al. 1968) and by McCormick and co-
workers (Iwahara et al. 1969, Kazarinoff et al. 1972). Moreover,
the presence of bisnorbiotin methyl ketone in urine of human subjects (Zempleni et al. 1997b) provides additional
support of the hypothesis that BNB is formed by an enzymatic
β-oxidation, because this methyl ketone probably arises from
a less stable β-keto acid intermediate in a fashion analogous
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**FIGURE 1** Cumulative urinary excretion of total radioactivity
([14C]biotin plus [14C]biotin metabolites) in rats treated with anticonvul-
sants [phenytoin (PHT) (n = 3), phenobarbital (PB) (n = 3), and carba-
mazepine (CBZ) (n = 6)], steroid hormones [dexamethasone (DEX) (n = 6)
and dehydroepiandrosterone (DHEA) (n = 5)], or peroxisome prolif-
erators [clofibrate acute high dose (CFa) (n = 6), clofibrate therapeutic
dose (CFc) (n = 6), and di[2-ethylhexyl] phthalate (DEHP) (n = 5)] and
the control group (n = 6). Data shown are means. Error bars were
omitted because the analytical variability is generally smaller than the
symbols used. All rats received a constant weight normalized dose of
[14C]biotin = 57 pmol/g body wt (Wang et al. 1996).

(2A) and BSO (Fig. 2B) after treatment with anticonvul-
sants (phenytoin, phenobarbital, and carbamazepine), steroid
hormones (dexamethasone and DHEA), or peroxisome prolif-
erators (clofibrate and DEHP).

Neither the absolute excretion (data not shown) nor the
relative proportion of BNB changed signi®cantly in response to
the treatment of rats with the three anticonvulsants. Likewise,
neither the absolute nor relative excretion of BSO changed
in response to anticonvulsants.

In response to treatment with the two steroid hormones or
the two peroxisome proliferators, BNB excretion in the treated
groups was higher than that in the control group. Biotin sulfox-
ide excretion was not signi®cantly different between either
the hormone-treated groups or the peroxisome proliferator±
treated groups and the control group.

Our initial study with clofibrate used an acute high dose
treatment regimen (CFa) commonly used in enzyme induction
studies in rats (Grafnetter et al. 1971, Sundseth and Waxman
1992). We wondered whether longer treatment with doses
similar to those used for treatment of hyperlipidemia (CFc)
would also produce signi®cant changes in BNB excretion. The
increased excretion of BNB was similar to the increase seen
with the acute dose; again no signi®cant differences were seen
in BSO excretion.

**FIGURE 2** Percentage of urinary bisnorbiotin (BNB, panel A) and
biotin sulfoxide (BSO, panel B) in rats treated as described in Figure
1. Data are expressed as the percentage of the sum of biotin plus BNB
plus BSO and are depicted as means ± SD. Signi®cances of differences
in the means of the relative urinary excretion of BNB and BSO of the
drug-treated groups vs. the control group were tested using ANOVA
with Dunnett’s post-hoc procedure. An asterisk indicates P < 0.0001
vs. control group.
Both the steroid hormones and the peroxisome proliferators studied here belong to the steroid hormone receptor superfamily of ligand-activated transcription factors and exert their effects through a receptor-mediated mechanism involving peroxisome proliferator–activated receptors (Anderson et al. 1996, Green 1995, Issmann and Green 1990). In general and for the particular peroxisome proliferators studied here, treatment increases both the number and volume of peroxisomes in rodent liver and causes a marked induction of lipid-metabolizing enzymes, including cytochrome P450–dependent fatty acid ω-hydroxylases (Hawkins et al. 1987), the enzymes of the peroxisomal fatty acid β-oxidation (Lazarow and De-Duwe 1976), and some mitochondrial enzymes (Hawkins et al. 1987) including mitochondrial fatty acid β-oxidases, where examined. As indicated in Table 2, the induction of fatty acid β-oxidation by the steroid hormones and peroxisome proliferators used here cannot be categorized as organelle specific. Indeed, the similarity in β-oxidases of fatty acid in peroxisomes and mitochondria might reflect some degree of coordinate regulation. Thus we cannot reasonably exclude either peroxisomes or mitochondria as the source of the induced enzyme activity. Likewise, the known hepatic effects of peroxisome proliferators do not exclude the possibility that other organs besides liver participate in biotin β-oxidation and are responding to the β-oxidase inducers.

The fatty acid β-oxidation in peroxisomes is similar to the four-step mitochondrial fatty acid oxidation; however, the initial and rate-limiting dehydrogenation step is catalyzed by an acyl-CoA dehydrogenase (Reddy and Mannuerts 1994) rather than by acyl-CoA dehydrogenase. Increased peroxisomal β-oxidation is often used as a biochemical indicator of the peroxisome proliferation effect and as a rapid and sensitive method to identify hypolipidemic drugs (Anderson et al. 1996, Lazarow et al. 1982). We speculate that these steroids and peroxisome proliferators induce biotin biotransformation enzymes in a manner similar to induction of fatty acid β-oxidation enzymes. Is “biotin β-oxidase” one (or more) of the fatty acid β-oxidase systems? We have observed that the production of NBN in rats was increased by the administration of steroids and peroxisome proliferators that induce fatty acid β-oxidation, suggesting that this might be true. However, the one published observation has reached the opposite conclusion. In 1953, Baxter and Quastel (1953) examined the oxidation of biotin by measuring evolved \(^{14}\)CO\(_2\) (or radioactivity remaining in Warburg vessels) after incubating slices of guinea pig kidney cortex with carboxyl-labeled \[^{14}\]C\)biotin. Their work examined the inhibitory effects caused by various fatty acids added to the medium on biotin oxidation as well as inhibitory effects of biotin and biotin analogs on butyrate oxidation. They concluded that separate enzyme systems were involved in biotin and fatty acid oxidation. However, these inhibitory interactions could have occurred 1) at the level of transport into the cell, 2) via effects on the energy supply, 3) at the level of conversion of biotin to biotinyl-CoA, 4) at the level of the transport into the mitochondria or peroxisomes or 5) at the level of competition for the substrate binding site on one or more of the fatty acid or biotin β-oxidation systems. Thus it is not reasonable to conclude from the study of Baxter and Quastel (1953) (or from our current study) whether there are discrete systems for biotin β-oxidation and fatty acid β-oxidation. However, it is clear from our studies of patients treated with anticonvulsants, from studies in which biotin deficiency was induced experimentally, and from the biotransformation studies presented here that biotin catabolism can be influenced by biotin status and by factors such as medication and drug therapy that are not directly related to biotin status.

Long-term treatment with anticonvulsants has been reported to increase biotin catabolism (Mock and Dyken 1997) to NBN and BSO and to lead to marginal biotin deficiency (Krause et al. 1982a, 1982b and 1984, Mock and Dyken 1997). In the current study in rats, none of the anticonvulsants accelerated the formation of BNB or BSO. This failure to reproduce the increased BSO excretion seen in humans may relate to species differences in inducibility of the various P450 isoenzymes and their related substrate specificities. Other candidates for the sulfoxidation reaction include the flavin monoxygenase. Likewise, the failure to reproduce anticonvulsant-accelerated BNB production may be species related. Whether BNB production is accelerated in humans by steroid hormones or peroxisome proliferators is not known.

In conclusion, the fact that biotin β-oxidation can be increased by steroid hormones and peroxisome proliferators that also induce fatty acid β-oxidation suggests that biotin is catabolized by an enzymatic pathway that is similar to that of fatty acids and that pathway may include one or more of the fatty acid oxidases. Failure of the three anticonvulsant drugs to alter biotin metabolism suggests that the microsomal mixed-function enzymes that are inducible by these drugs in rats are not rate-limiting in biotin sulfoxidation or β-oxidation in rats.
LITERATURE CITED


