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Coordinate Regulation of Bile Acid Biosynthetic and Recovery Pathways

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Intestinal and hepatic recovery of bile acids is principally mediated by sodium dependent bile acid transporters. Of these, the ileal sodium bile acid transporter (isbt) and the hepatic sodium bile acid cotransporting polypeptide (ntcp) may be most important in determining the efficiency of bile acid recovery within the enterohepatic circulation. We used molecular probes to explore the relationship between the bile acid recovery pathway, at the level of isbt and ntcp, and the biosynthetic pathway, at the level of cholesterol 7α-hydroxylase (cyp7). Taurocholate feeding of mice suppressed ntcp, isbt, and cyp7 mRNA levels as compared to controls. Cholestyramine feeding induced both cyp7 and isbt mRNA gene expression. Cholesterol feeding induced cyp7 mRNA levels but suppressed isbt gene expression. These results suggest that in mice the bile acid recovery and biosynthetic pathways are coordinately regulated, and these pathways may be responsive to the size of the bile acid pool in the enterohepatic circulation. © 1996 Academic Press, Inc.

Intestinal recovery of liver synthesized bile acids is important for the conservation of the bile acid pool in the enterohepatic circulation. Both conjugated and unconjugated bile acids are passively recovered along the entire axis of the intestine. However, active recovery of conjugated bile acid species, which predominate in the bile acid pool, occurs in the ileum by multiple sodium dependent bile acid transport systems (1, 2). A 93-99 kDa protein has been implicated as a putative ileal bile acid transporter in both rabbits and rats (3, 4). A 38 kDa bile acid transporter, termed the ileal sodium-bile acid dependent transporter (isbt), has now been cloned from hamster, rat, and human ileal RNA (5, 6, 7). It is not clear if both proteins are encoded by the same gene. Isbt mRNA is abundantly and specifically expressed in the ileum and may determine the overall ileal bile acid uptake activity (5).

Hepatocytes recover bile acids primarily by an active process from portal circulation (8). The cloned sodium/taurocholate cotransporting polypeptide (ntcp) (9) appears to be most important for hepatic recovery of bile acids (10, 11). Expression of this transporter in hepatoma cells confers bile acid transport activity similar to isolated hepatocytes (12). Hepatocytes secrete both newly synthesized and recovered bile acids into bile. Thus, the transport of bile acids is vectorial. Bile acids repress their own synthesis by inhibiting cholesterol 7α-hydroxylase (cyp7), the rate limiting enzyme in the bile acid biosynthetic pathway (reviewed in 13). Regulation of the bile acid recovery and the biosynthetic pathways are not well understood. To explore the possible coordinate regulation of the bile acid recovery and biosynthetic pathways, we have studied the regulation of isbt, ntcp, and cyp7 gene expression under conditions that are known to perturb the bile acid pool.

MATERIALS AND METHODS

Animals and diets. Mice (C57BL/6J, female, 8 wk old) purchased from Jackson Laboratories were maintained in a reversed 12 h light and 12 h dark cycle, and given free access to chow and water for at least one week prior to the

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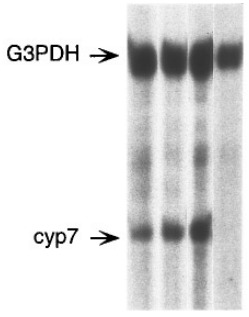

chow	+	+	+	+
1% cholesterol		+		
2% cholestyramine			+	
0.5% taurocholate				+

FIG. 1. Detection of the mouse cyp7 mRNA. Changes in cyp7 mRNA abundance in response to the indicated diets were evaluated by solution hybridization ribonuclease protection assay. The left arrows show the position of cyp7 and G3PDH mRNA fragments protected by the antisense probes from ribonuclease digestion.

initiation of the diet study. The chow diet (rodent chow 5001) was used as the base diet and supplemented with either 1% cholesterol, 2% cholestyramine or 0.5% taurocholate. The mice (n = 5 per group) were fed the diets for 2 weeks. Tissue samples (liver, intestines) were collected after an overnight fast.

Detection of mRNA. Total RNA was isolated from tissues using standard procedures (14). Mouse ntcp and isbt mRNA were detected by RNA blot analysis of total RNA (10 μg) using cDNA probes containing the translated sequences of hamster isbt (5), rat ntcp (9), and porcine ileal binding protein (ilbp) mRNA (15). The [^{32}P]-labeled probes were prepared by random priming and hybridized to the blots overnight in a solution containing 0.25 M sodium phosphate (pH 7.2), 7% SDS, 0.1% pyrophosphate and 2 mM EDTA at 60°C. The blots were washed twice with a solution containing 2× SSC (1× SSC = 0.15 M NaCl, 15 mM sodium citrate) and 0.1% SDS for 15 min each at 25°C and once with a solution containing 0.5× SSC and 0.1% SDS for 30 min at 55°C. The amount of radioactivity associated with the hybridizing bands was quantitated by phosphorimaging using the Fuji-X BAS1000 phosphorimager. The blots were stripped and reprobed with a cDNA encoding mouse glyceraldehyde-3-phosphate dehydrogenase (G3PDH) and the bands were quantitated as above. A solution hybridization ribonuclease protection assay was used to detect the mouse cyp7 mRNA. [^{32}P]-labeled antisense riboprobes complementary to the mouse cyp7 and G3PDH mRNA were synthesized and hybridized with 20 μg of total liver RNA. Unhybridized probes were digested with RNAse One (Promega Biotech) and the digestion products were analyzed on polyacrylamide sequencing gels. The radioactivity associated with the protected bands was quantitated by phosphorimaging. The abundance of the tested mRNA is expressed relative to the abundance of G3PDH mRNA.

RESULTS AND DISCUSSION

The abundance of the mouse cyp7 mRNA was markedly increased by feeding 1% cholesterol or 2% cholestyramine as compared to feeding chow alone (Fig. 1). In contrast, feeding the chow diet supplemented with a bile acid (0.5% taurocholate) decreased cyp7 mRNA abundance to undetectable levels (Fig. 1). These results indicate that cyp7 gene expression responds to alterations in the bile acid pool. Changes in cyp7 mRNA abundance correlate with changes in cyp7 enzymatic activity (13). The regulation of cyp7 gene expression by cholesterol and bile acids appears to be mediated predominantly at the transcriptional level (13).

To determine the responsiveness of the recovery pathway to alterations in the bile acid pool, we first determined if existing molecular probes for ntcp or isbt were suitable for detection and quantitation of their corresponding mouse mRNA species. The hamster isbt cDNA probe detected a single mRNA species of ~4.0 kb in total mouse ileal RNA (Fig. 2). Likewise, the rat ntcp cDNA detected a single transcript (~2.0 kb) in total mouse liver RNA (Fig. 2). The sizes of the mouse mRNA detected in the blots are consistent with their respective counterparts in rat and hamster RNA (9, 5).

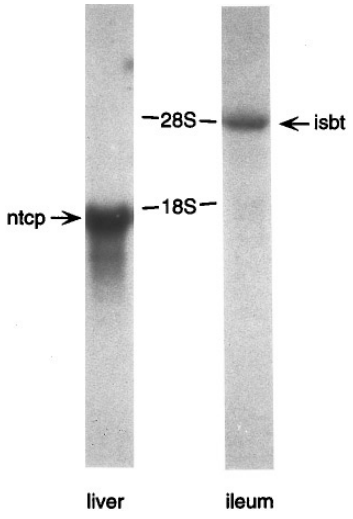

FIG. 2. Detection of the mouse ntcp and isbt mRNA. The mouse ntcp and isbt mRNA were detected (left and right arrows, respectively) using the rat ntcp and hamster isbt cDNA probes. The positions of the 28S and 18S ribosomal RNA are also shown.

Alteration of the bile acid pool by dietary manipulation induced specific changes on the abundance of both isbt and ntcp mRNA (Fig. 3). Taurocholate feeding suppressed isbt mRNA abundance by 67% ($p<0.02$) whereas cholestyramine induced isbt mRNA by 342% ($p<0.05$) as compared to chow alone (Figs. 3A). In rats, changes in isbt mRNA abundance have been associated with parallel changes in enzyme mass (6). Feeding studies using rats and guinea pigs also show contrasting effects of bile acids and cholestyramine treatment on active ileal bile acid recovery (16). Thus, it appears that the isbt gene responds to bile acids in a manner that parallels cyp7. However, opposing effects of bile acids on ileal active bile acid uptake have been observed (16, 17, 18). Although the basis for the discrepancy is not clear, the observed decrease in mouse isbt mRNA after taurocholate feeding is compatible with the idea

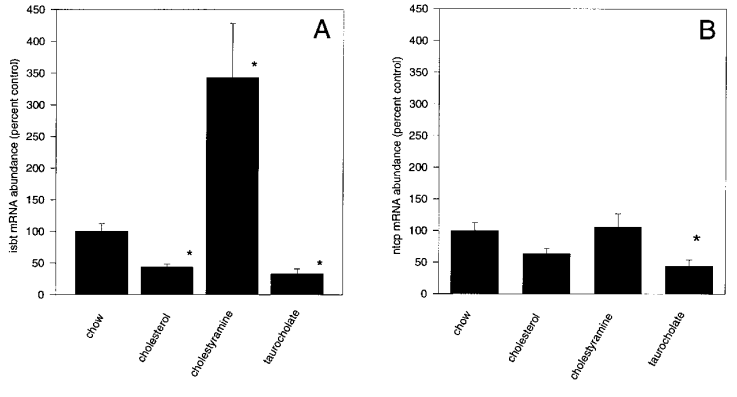

FIG. 3. Regulation of the mouse ntcp and isbt mRNA abundance. Mice were fed a chow diet or the chow diet supplemented with 1% cholesterol, 2% cholestyramine or 0.5% taurocholate. After 2 weeks on the diet, total RNA isolated from the liver and ileum were analyzed for ntcp and isbt mRNA. Panel A, isbt mRNA abundance. Panel B, ntcp mRNA abundance. The mean ± S.E. are indicated. Differences in ntcp and isbt abundance in the absence (chow) or presence of supplements were evaluated using Student's *t* test. *Denotes significant difference ($P<0.05$).

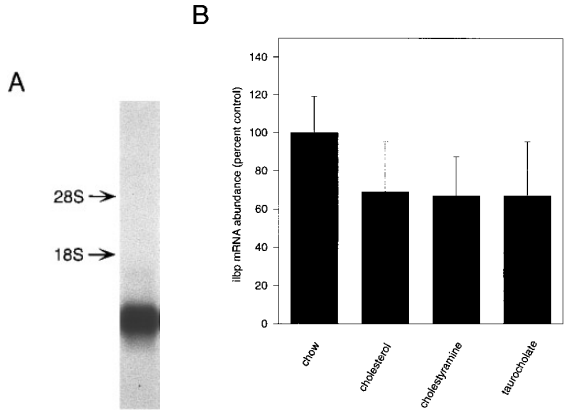

FIG. 4. Detection and regulation of ilbp mRNA. Panel A shows a blot of mouse ileal RNA probed with the porcine ilbp cDNA. The positions of the 28S and 18S ribosomal RNA are shown on the left and the mouse ilbp mRNA on the right arrow. Ilbp mRNA abundance (Panel B) was measured in total RNA isolated from the ileum of mice fed chow or chow with the indicated supplements. The mean ± S.D. are indicated.

that a reduction of isbt activity prevents the excessive increase of the enterohepatic bile acid pool. Ileal bile acid recovery is also decreased in starved rats and common bile duct ligation models of cholestasis (17, 18). It is also possible that isbt gene expression responds to changes in bile acid flux in the enterohepatic circulation.

Feeding cholesterol decreased isbt mRNA abundance by 54% ($p<0.01$) as compared to chow (Fig. 3A). This observation is in contrast with the stimulatory effect of cholesterol on cyp7 gene expression (Fig. 1). Since induction of cyp7 increases bile acid biosynthesis, the increased production of bile acids may mediate the repression of isbt mRNA abundance. On the other hand, it is possible that cholesterol itself may directly regulate isbt gene expression in a manner that is analogous to the regulation of the low density lipoprotein receptor gene (19). Nonetheless, the repression of isbt gene expression may represent a response to prevent the overexpansion of the bile acid pool in the enterohepatic circulation following the induction of cyp7. This idea also provides a mechanistic explanation for the observed increase of bile acid excretion in some species after intake of a diet containing high levels of cholesterol (20, 21). In this regard, isbt may play a significant role in both bile acid and cholesterol homeostasis.

The ileal lipid binding protein (ilbp) is a 14 kDa protein that is predominantly and abundantly expressed in the ileum and is thought to partake in binding and transport of bile acids across enterocytes (22, 23). In contrast to isbt, the dietary supplements tested did not affect the mRNA abundance of mouse ilbp (Fig. 4). Thus, the changes in isbt mRNA abundance observed in response to the dietary supplements reflect specific regulation of the mouse isbt gene.

The level of ntcp gene expression was not affected by cholestyramine or cholesterol feeding (Fig. 3B). These observations suggest that the efficiency of bile acid recycling can be effectively controlled at the level of isbt. However, feeding bile acids significantly repressed ntcp mRNA abundance by 55% ($p<0.02$) (Fig. 3B). In isolated hepatocytes, ntcp mRNA abundance is also positively correlated with enzymatic activity (24). Consistent with this notion, the decrease in sinusoidal sodium dependent bile acid uptake in bile duct ligation models of extrahepatic cholestasis occurs in parallel with the decrease of both ntcp mRNA abundance and protein mass (25). In a preliminary report, taurocholate was shown to suppress the expression of reporter genes under the control of the ntcp promoter (26). In addition, it appears that ntcp gene expression may also be regulated posttranscriptionally (25). As suggested by the present study, ntcp function may be regulated by increased flux of bile acids through hepatocytes.

TABLE I
Summary of Changes in isbt, ntcp and cyp7 mRNA Abundance

	Dietary supplement		
	1% cholesterol	0.5% taurocholate	2% cholestyramine
isbt mRNA	decrease	decrease	increase
ntcp mRNA	no change	decrease	no change
cyp7 mRNA	increase	decrease	increase

Since cholestyramine feeding (i.e., bile acid removal) did not appear to affect ntcp mRNA abundance, suppression of bile acid transport function may be a cytoprotective response in situations when there is very high flux of bile acids through the liver.

In summary, taurocholate feeding suppressed ntcp, isbt, and cyp7 mRNA levels as compared to chow alone (Table I). On the other hand, cholestyramine treatment induced the expression of both cyp7 and isbt mRNA genes, but had no effect on the ntcp gene. Feeding bile acids causes an expansion of the bile acid pool (27) whereas feeding cholestyramine, a bile acid sequestrant, decreases the size of the bile acid pool (28). Thus, these observations suggest that the bile acid transporters involved in the recovery pathway and the rate limiting enzyme of bile acid biosynthetic pathway (cyp7) are responsive to alterations of levels of bile acids in the enterohepatic circulation. Feeding cholesterol also causes an expansion of the bile acid pool (27). However, cholesterol feeding increased cyp7 mRNA levels but suppressed isbt gene expression. This contrasting effect may simply reflect an opposing compensatory mechanism responding to an increase in the bile acid pool, due to increased bile acid synthesis. We conclude that in C57/BL6 mice, bile acid recovery and biosynthetic pathways are coordinately regulated and these pathways may be responsive to alterations in the size of the enterohepatic bile acid pool.

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