Effect of Microsomal Enzyme Inducers on the Biliary Excretion of Triiodothyronine (T₃) and Its Metabolites

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It has been postulated that inducers of UDP-glucuronosyltransferase (UGT) decrease circulating thyroid hormone concentrations by increasing their biliary excretion. The inducers pregnenolone-16α-carbonitrile (PCN), 3-methylcholanthrene (3MC), and Aroclor 1254 (PCB) are each effective at reducing serum thyroxine concentrations. However, only PCN treatment produces a marked increase in serum levels of thyroid-stimulating hormone (TSH), whereas 3MC and PCB cause little to no increase in TSH. Excessive TSH elevation is considered the primary stimulus for thyroid tumor development in rats, yet the mechanism by which enzyme induction leads to TSH elevation is not fully understood. Whereas PCN, 3MC, and PCB all increase microsomal UGT activity toward T₄, only PCN causes an increase in T₃-UGT activity in vitro. The purpose of this study was to determine whether PCN, which increases serum TSH, causes an increase in the glucuronidation and biliary excretion of T₃ in vivo. Male rats were fed control diet or diet containing PCN (1000 ppm), 3MC (250 ppm), or PCB (100 ppm) for 7 days. Animals were then given [¹²⁵I]-T₃, iv, and bile was collected for 2 h. Radiolabeled metabolites in bile were analyzed by reverse-phase HPLC with γ-detection. The biliary excretion of total radioactivity was increased up to 75% by PCN, but not by 3MC or PCB. Of the T₃ excreted into bile, approximately 75% was recovered as T₃-glucuronide, with remaining amounts represented as T₃-sulfate, T₂-sulfate, T₂, and T₂. Biliary excretion of T₃-glucuronide was increased up to 66% by PCN, while neither 3MC nor PCB altered T₃-glucuronide excretion. These findings indicate that PCN increases the glucuronidation and biliary excretion of T₃ in vivo, and suggest that enhanced elimination of T₃ may be the mechanism responsible for the increases in serum TSH caused by PCN.

Key Words: triiodothyronine (T₃); pregnenolone-16α-carbonitrile (PCN); 3-methylcholanthrene (3MC); polychlorinated biphenyl (PCB); glucuronidation; bile; rat; thyroid.
only phenobarbital and PCN produced an increase in T₃-UGT activity; 3MC and PCB did not (Hood and Klaassen, 2000a). This differential effect on T₃ glucuronidation mirrors the differential effect of these inducers on TSH, suggesting that T₃ glucuronidation may mediate increases in serum TSH produced by certain microsomal enzyme inducers. It was hypothesized, then, that induction of T₃ glucuronidation, rather than T₄, results in increased biliary excretion of T₃, thereby increasing turnover of T₃, and altering one or more factors that regulate TSH.

In the present study, the significance of increased T₃-UGT activity demonstrated in liver microsomes was assessed by determining whether an increase in the biliary excretion of T₃ occurs in vivo, and whether T₃ biliary excretion relates to serum TSH. It is known that different members of the UGT family are inducible by unique classes of microsomal enzyme inducers, and that treatment with various classes of inducers results in the increased glucuronidation of specific substrates (Arand et al., 1991; Emi et al., 1995; Fournel et al., 1987; Lilienblum et al., 1982; Ulrich and Bock, 1984; Watkins et al., 1982). It is thought that T₄ and T₃ are glucuronidated by different UGT enzymes: T₄ by bilirubin (UGT1A1) and phenol (UGT1A6) UGTs, and T₃ by androsterone UGT (2B2) (Beestra et al., 1991; van Raaij et al., 1993; Visser et al., 1993a,b). The inducibility of T₄ biliary excretion and fecal clearance by chemicals that also induce phenol and bilirubin glucuronidation may mediate increases in serum TSH produced via microsomal enzyme inducers by determining whether induction of T₃ glucuronidation and biliary excretion has not been well characterized. Thus, in this study, rats were treated with PCN, a chemical that increases UGT activity toward T₄ and T₃, as well as increasing serum TSH. Rats were also treated with 3MC and PCB, prototypical chemicals that effectively reduce serum T₄, but that increase only T₃-UGT activity and do not increase serum TSH. The biliary excretion of radiolabeled T₃ was examined, and the radioactive components of bile were determined by HPLC. It is hypothesized that because PCN treatment induces microsomal T₃-UGT activity, the same treatment will increase the biliary excretion of T₃-glucuronide, while 3MC and PCB treatments will not. This study further addresses the hypothesis that increased T₃ glucuronidation may mediate the increases in serum TSH produced via microsomal enzyme inducers by determining whether induction of T₃ glucuronidation results in the increased biliary excretion of T₃, and whether this increase coincides with elevated TSH.

MATERIALS AND METHODS

Chemicals and reagents. 16-Dehydroepiandrosterone, 3-methylcholanthrene (3MC), and Aroclor 1254 (PCB) were obtained from Steraloids, Inc. (Newport, RI), Sigma Chemical Co. (St. Louis, MO), and Chem Service, Inc. (West Chester, PA), respectively. Pregnenolone-16α-carbonitrile (PCN) was synthesized from 16-dehydroepiandrosterone as previously described (Sonderfan and Parkinson, 1988). [125I]-T₃ was obtained from NEN Research Products (Boston, MA). Heparin was supplied by Elkins-Sinn, Inc. (Cherry Hill, NJ). β-Glucuronidase type IX-A was obtained from Sigma Chemical Co. (St. Louis, MO). Ultima Flo-M Scintillant was purchased from Packard Instrument Co. Inc. (Meridian, CT). All other reagents were obtained from Fisher Scientific (Pittsburgh, PA).

Animals and treatments. Male Sprague-Dawley rats (Sasco, Wilmington, MA) weighing 150–200 g were randomly assigned to 1 of 4 groups of 12 animals each. Following a 1-week acclimation period, animals were placed on either control diet or diet containing one of the following inducers: PCN (1000 ppm), 3MC (250 ppm), PCB (100 ppm). Dosages selected are those previously demonstrated to have significantly reduced serum T₄, increased T₃ glucuronidation, and in the case of PCN, increased serum TSH (Liu et al., 1995). Rats were allowed ad libitum food and water. At the end of 7 days, animals were anesthetized with sodium pentobarbital (50 mg/ml) combined 1:1 with potassium iodide (1 mg/ml) at 2 ml/kg to prevent uptake of 125I into the thyroid. The femoral vein and artery were cannulated (PE 50) and primed with either saline or heparinized saline (33 units/ml, respectively). Approximately 1 ml of blood was sampled from the artery at this time, from which serum was collected and stored at –80°C for further assay. The bile duct was cannulated (PE 10), and the animal warmed under a heat lamp to 37°C body temperature. After 10 min, the animal was given 1 ml of [125I]-T₃, iv, (10 μCi/ml in 10 mM NaOH saline plus 1% normal rat serum), and bile was collected on ice for 2 h at 30-min intervals. Fifteen min following the start of bile collection and 3 more times at intervals of 30 min, approximately 300 μl blood aliquots were collected from the femoral artery. At the end of the 2-h collection period, the urinary bladder was exposed and urine collected by puncture with a syringe and needle. The liver was removed, weighed, snap-frozen, and stored at –80°C. Bile and urine volumes were determined gravimetrically. Blood samples were centrifuged for 5 min to collect serum. Two aliquots (10 μl each) were taken from the bile samples, and 2 aliquots (15 μl each) from urine and serum samples for gamma spectroscopy. Following the addition of methanol (1:2) and storage at –20°C for 1 h to precipitate protein, bile was centrifuged at 12,000 × g (4°C) for 10 min and the supernatant collected for analysis by HPLC.

HPLC analysis. Beckman System Gold equipment and software (version 8.1), consisting of an Autosampler 507, Programmable Solvent Module 126, Radioisotope Detector 171, and 110B Solvent Delivery Module for pumping scintillation cocktail, were utilized for HPLC. Reverse-phase HPLC was performed on a 10 × 0.3-cm ChromSpher C₁₈ column in combination with both a ChromSep 10 × 2-mm reverse-phase guard column (Chrompack, Inc., Ranitan, NJ) and a 7.5 × 4.6-mm Adsorbosphere C₁₈ reverse-phase guard column (Alltech Associates, Inc., Deerfield, IL) with a 16–40% gradient of acetonitrile run against 0.02-M ammonium acetate, pH 4 (de Herder et al., 1988). The step-wise linear gradient was as follows: 6 to 18 min, 16 to 27% acetonitrile; 22 to 27 min, 27 to 40% acetonitrile; 37–47 min, 40 to 16% acetonitrile. The sample volume injected was 20 μl. To identify iodothyronine glucurononides, 100 μl bile aliquots were incubated for 4 h at 37°C with 250 units (168 μg) β-glucuronidase in 100 μl of 100 mM phosphate buffer (pH 6.8). The reaction was stopped by the addition of 50-μl methanol and cooling on ice. Samples were then concentrated to ~100 μl by vacuum centrifugation and analyzed by HPLC for the absence of glucurononide metabolites. Acid labile conjugates were identified after treatment of 100 μl bile at 80°C in 600 μl 1M HCl as previously described (Rutgers et al., 1989), again followed by cooling on ice and concentration by vacuum centrifugation.

Determination of serum T₃, T₄, and TSH. The concentrations of total (free and protein-bound) serum T₃ and T₄ at Day 7, as well as TSH, prior to the collection of bile, were determined by radioimmunoassay (RIA) kits (total T₃ and T₄; Diagnostic Products Corp., Los Angeles, CA; rat TSH; Amersham Life Science, Inc., Arlington Heights, IL). Limits of detection for these kits were 7 ng/dl, 0.25 μg/dl, and 0.50 ng/ml, respectively.

Data analysis. All results are expressed as the mean ± SE. For analysis of individual T₄ metabolites, the percent total peak area of each biliary metabolite as determined by HPLC was multiplied by the total biliary radioactivity to determine the amount for each metabolite. Means were compared by one-way ANOVA and Student’s t-test.
ANOVA followed by Dunnett’s test. Statistical significance is reported at the $p < 0.05$ level.

RESULTS

After 7 days of treatment, each of the 3 inducers produced an increase in liver to body weight ratio (Fig. 1). PCN and PCB produced statistically significant increases of 48% and 27%, respectively. 3MC increased liver to body weight ratio by 15% greater than control, but was not statistically significant.

Concentrations of serum thyroid hormones after 7 days of treatment with each of the inducers are shown in Figure 2. Serum T4 concentrations were decreased 45% by PCN treatment, 49% by 3MC treatment, and 67% by PCB treatment (upper panel). Serum concentrations of T3 were not decreased by 3MC, but were reduced to a similar extent by PCN and PCB, 23% and 21%, respectively (middle panel). Serum TSH concentrations were increased by 90% above control, only by PCN treatment.

Following surgical implantation of the biliary cannula, animals were administered $^{125}$I-T3, iv, and during the collection of bile, the disappearance of $^{125}$I-T3 from the serum was measured (Fig. 3). 3MC treatment had no effect on serum concentrations of $^{125}$I-T3. At 15 min following the injection of $^{125}$I-T3, serum concentrations in PCN-treated animals were reduced by 22%, although this was not statistically significant. Serum $^{125}$I-T3 concentrations in PCB-treated animals were significantly lower than in control rats at both 15 and 45 min after administration (by 36% and 33%, respectively).

The rate of biliary excretion of total $^{125}$I-T3 (T3 and metabolites) is depicted in the upper panel of Figure 4. Neither 3MC nor PCB treatments produced an increase in the total biliary excretion of T3 and its metabolites. However, PCN treatment increased the biliary excretion of T3 and its metabolites at all time points after the first 30 min, with the peak rate of excretion occurring at 60 min (58% above control). At 90 min, biliary excretion rates in PCN-treated animals increased to 63% above control rates, and reached double that of control rats at 120 min. The cumulative biliary excretion of total $^{125}$I-T3, over the 2-h collection period is shown in the lower panel. Again, PCN treatment increased the cumulative amount of T3 and metabolites excreted into bile by 56, 75, and 67%.

CON, control. Effect of pregnenolone-16α-carbonitrile (PCN), 3-methylcholanthrene (3MC), and Aroclor 1254 (PCB) on serum concentrations of total T4, T3, and TSH after 7 days of inducer treatment. Values are means ± SE. *Significantly different from control ($p < 0.05$).
Neither 3MC nor PCB affected the excretion of total $[^{125}\text{I}]-\text{T}_3$ in bile.

The chemical form of $[^{125}\text{I}]-\text{T}_3$ excreted into bile of control rats was primarily in the form of $\text{T}_3$-glucuronide (74%), followed by $\text{T}_3$ sulfate (16%), non-conjugated $\text{T}_2$ (6%), $\text{T}_2$ sulfate (3%), and nonconjugated $\text{T}_3$ (2%). Each metabolite was quantified by HPLC, and its cumulative excretion in control rats and rats treated with the 3 microsomal enzyme inducers is depicted in Figure 5. PCN treatment produced 66% and 55% increases in the cumulative excretion of $\text{T}_3$-glucuronide at 90 and 120 min, respectively (top panel), and 112, 164, and 138% increases in the cumulative excretion of $\text{T}_3$ sulfate at 60, 90, and 120 min, respectively (Fig 5, second from bottom). PCN had no effect on the biliary excretion of $\text{T}_3$ sulfate, $\text{T}_3$, or $\text{T}_3$.

Treatment with 3MC had no effect on biliary excretion of $\text{T}_3$-glucuronide, but did increase significantly the amount of $\text{T}_3$ sulfate in bile by 68, 64, 60, and 51% at 30, 60, 90, and 120 min, respectively (Fig 5, second from top). 3MC treatment had no effect on the biliary excretion of any other $\text{T}_3$ metabolite. PCB treatment did not affect the biliary excretion of any $\text{T}_3$ metabolite.

The amount of total $[^{125}\text{I}]-\text{T}_3$ in urine was determined as well, but individual metabolites were not quantified because of their low urinary excretion. Figure 6 compares the cumulative biliary excretion (top panel) of $[^{125}\text{I}]-\text{T}_3$ after 2 h to that in urine (bottom panel). In control animals, the radioactivity in urine was only 5% that in bile. The total $[^{125}\text{I}]-\text{T}_3$ in bile was increased 67% by PCN, and although PCN tended to increase total $[^{125}\text{I}]-\text{T}_3$ in urine, this was not statistically significant. PCB treatment tended to decrease the cumulative urinary excretion of $\text{T}_3$ and its metabolites, but it was not statistically significant either.

**DISCUSSION**

In the present study, the effects of microsomal enzyme inducers on $\text{T}_3$ glucuronidation *in vivo* are consistent with those previously demonstrated using *in vitro* liver microsomal preparations (Hood and Klaassen, 2000a). Of the inducers tested, only PCN increased the biliary excretion of $\text{T}_3$, whereas 3MC and PCB did not (Fig. 4). When bile from control and inducer-treated rats was analyzed by HPLC, the primary component was $\text{T}_3$-glucuronide, as previously reported (de Herder et al., 1988), and the excretion of $\text{T}_3$-glucuronide in bile was increased solely by PCN treatment (Fig. 5). This finding is consistent with the increase in $\text{T}_3$-UGT activity shown in microsomal preparations from PCN-treated rats. In the present study, urinary excretion was minor in comparison to biliary excretion of $\text{T}_3$ and its metabolites in both control and inducer-treated animals (Fig. 6).

Total $\text{T}_3$ serum concentrations were reduced after 7 days by all 3 inducer treatments (Fig. 2), findings that are consistent with previous studies (Hood et al., 1999a; Liu et al., 1995). It has been shown that PCN, 3MC, and PCB reduce serum $\text{T}_4$ by an extrathyroidal mechanism. This mechanism is believed to be the increased hepatic elimination of $\text{T}_4$ (Barter and Klaassen, 1992). The increases in liver weight produced by inducer treatment (Fig. 1) also support an increase in liver enzyme activity. In the present study, PCN and PCB treatments reduced total $\text{T}_3$ serum concentrations as well. Previous reports of microsomal enzyme-inducer treatments reducing serum $\text{T}_3$ are variable (Bastomsky, 1977; Gorski and Rozman, 1987; Liu et al., 1995; Masubuchi et al., 1997; Semler et al., 1989). Though serum $\text{T}_3$ concentrations in this study appear to be maintained compared to serum $\text{T}_4$, both PCN and PCB treatments significantly reduced serum total $\text{T}_3$. It was recently reported that activity of outer-ring deiodinase (ORD), which catalyzes the conversion of $\text{T}_4$ to $\text{T}_3$, was reduced by microsomal inducer treatment (Hood and Klaassen, 2000b); therefore, it is possible that the reduced deiodination may contribute to reduced serum $\text{T}_3$. However, the effects on ORD activity were similar for PCN, 3MC, and PCB, not differential, as are the effects on serum $\text{T}_3$. PCB and PCN treatments do appear to increase the clearance of $\text{T}_3$ from serum (Fig. 3), suggesting an increase in the tissue uptake of serum $\text{T}_3$. Future studies are required to further elucidate this mechanism. Despite reductions in serum $\text{T}_4$, 3MC had no effect on serum $\text{T}_3$, consistent with findings in previous studies that have examined effects of 3MC on serum
Reduction of total T3 serum concentrations by both PCN and PCB seems to be inconsistent with the overall increase in T3 biliary excretion, which occurred only following PCN treatment. However, it is important to note that total T3 serum concentrations, not free T3 serum concentrations, were measured in the present study. In general, free T3 concentrations are maintained following microsomal enzyme inducer treatment by mechanisms that are not well understood (Hood and Klaassen, 2000b). Neither free nor total T3 serum concentrations appear to reflect the status of the pituitary-thyroid axis (i.e., thyroid activation by TSH), and exactly when and how the pituitary senses changes in serum T3 are not clearly defined. Changes in rodent total T4 and total T3 serum concentrations following treatment with PCN, 3MC, and PCB have been well characterized, and were compared in the present study at a single time point (day 7) to verify reproducibility against previous studies using these compounds.

Reductions in serum thyroid hormones produced by treatment with microsomal enzyme inducers are considered important because of the associated increases in serum TSH that follow, due to the decreased negative feedback effect at the hypothalamus and pituitary. It has been well documented that constant stimulation of the thyroid gland in rats with elevated levels of TSH causes goiter, thyroid hyperplasia, adenomas, and carcinomas (Curran and DeGroot, 1991). McClain et al. (1988) originally hypothesized that T4 glucuronidation mediates increases in serum TSH of microsomal enzyme inducer-treated rats. Many thyroid endocrine disruptors have been shown to reduce serum T4; however, their effect on TSH is, at best, variable. For example, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), polychlorinated biphenyls (PCB), and 3-methylcholanthrene (3MC) produce marked to dramatic reductions in serum T4 in rats, with no change in serum TSH and little stimulation of the thyroid gland (Henry and Gasiewicz, 1987; Hood et al., 1999a; Liu et al., 1995; Schuur et al., 1997).

Recently, a poor correlation between the ability of microsomal enzyme inducers to increase T4 glucuronidation and increase serum TSH has been demonstrated (Hood and Klaassen, 2000a). In the aforementioned study, PCN increased T4-UGT activity and serum TSH, while 3MC and PCB increased T4-UGT activity but had no effect on serum TSH, despite reducing serum T4. PCN also increased T3-UGT activity whereas 3MC and PCB had no effect on T3-UGT activity. Because only microsomal enzyme inducers that induce T3-UGT activity increase serum TSH, the suggestion is that glucuronidation of T3, and Aroclor 1254 (PCB) treatment. Biliary excretion of radioactive T3 metabolites was determined by HPLC at 30-min intervals following iv administration of [125I]-T3. Data points represent the mean cumulative excretion of each metabolite in fmol/kg body weight over 2 h ± SE. The excretion of five metabolites is depicted from top to bottom: T3-glucuronide; T3-sulfate; T2-sulfate; T2. *Significantly different from control (p < 0.05).
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rather than T₄, mediates the increase in serum TSH. This was the first association made between increases in serum TSH and induced T₃ glucuronidation in rats treated with microsomal enzyme inducers. The present study has demonstrated that these differential effects of PCN, 3MC, and PCB are maintained when examining in vivo glucuronidation of T₃. The present data suggest that increased glucuronidation and biliary excretion of T₂-glucuronide may be the mechanism by which PCN treatment increases serum TSH; 3MC and PCB, which do not increase serum TSH, did not increase the biliary excretion of T₃ and its metabolites.

In addition to glucuronidation, iodothyronines may also be sulfated to facilitate their excretion, though this is a relatively minor pathway of T₃ metabolism compared to glucuronidation. Treatment with both 3MC and PCN produced increases in the sulfated metabolites of T₃, namely T₃S, and T₂S, respectively (Fig. 5). There is known to be an increase in the mRNA for the sulfotransferase enzyme SULT1B1 following treatment of rats with the same dose of PCN used in the present study (Dunn et al., 1999). SULT1B1 has been shown to conjugate thyroid hormones, including T₂ (Fujita et al., 1999). Therefore, the increase in T₃S biliary excretion following PCN treatment can likely be attributed to an increase in sulfotransferase activity.

In vivo treatment with 3MC did not have an effect on SULT1B1, and has even been reported to suppress mRNA for another sulfotransferase, SULT1A1 (Runge-Morris, 1998). SULT1C1 has also been reported to be important for sulfation of thyroid hormones in male rat liver, but again, this enzyme is not inducible (Fujita et al., 1999; Visser et al., 1998). In addition, examination of T₃ sulfation in cytosolic fractions from inducer-treated rats has found it to be unaffected (Hood and Klaassen, 2000b). It does not appear that, the increased biliary excretion of T₃S following 3MC treatment is due to an increase in T₃ sulfation. Rather, the accumulation of T₃S in the bile of 3MC-treated rats is most likely due to an inhibition of inner-ring deiodinase (IRD) activity. T₃ is the outer ring deiodination product of T₄, while 3,3′-T₂ is the inner ring deiodination (Visser, 1996). Sulfation is known to facilitate deiodination, and studies have demonstrated that T₃S is rapidly deiodinated in rat liver (Moreno et al., 1994; Visser et al., 1983, 1984). This activity is carried out in liver by Type I iodothyronine deiodinase (ID-I) and by Type III iodothyronine deiodinase (ID-III), mainly in rat brain and placenta (Visser, 1990; Visser and Schoenmakers, 1992). T₃S is a substrate for ID-I in rat hepatocytes, and this activity is subject to physiological regulation and inhibition by propylthiouracil (Roorda et al., 1989). If IRD activity were inhibited, one would expect T₃S to accumulate in bile and urine, as demonstrated in vivo following PTU inhibition of ID-I (de Herder et al., 1988). To date, no investigations into 3MC inhibition of IRD activity exist. The inhibition of Type I ORD activity following microsomal enzyme inducer treatment has been reported, but IRD activity was not examined (Hood and Klaassen, 2000b). It is important to recall that accumulation of T₃S in bile following 3MC treatment was minor in the present study, and did not affect the total amount of T₃ excreted in bile or urine (Fig. 6).

The present study demonstrates an increase in the biliary excretion of T₃ produced by PCN, and proposes that this increase is due primarily to increased glucuronidation of T₃. In order to further understand this mechanism, it is important to investigate which UDP-glucuronosyltransferase (UGT) is responsible for the glucuronidation of T₃, and whether this enzyme is induced by PCN treatment. UGT2B2 is the UGT that has been suggested to glucuronidate T₃, because (1) Beetstra et al. (1991) showed that LA Wistar rats have reduced ability to glucuronidate androsterone and T₃, and (2) UGT2B2 glucuronidates androsterone (Haque et al., 1991). However, the presence of UGT2B2 mRNA and protein has not been directly examined for inducibility by PCN.

In the future, it will also be important to consider the role of other processes that are involved in the disposition of T₃, including hepatic uptake and transport of T₄G into bile. Herein, PCN tended to decrease the concentration of T₃ in the serum within 15 min of administration (Fig. 3). This suggests enhanced disposition of T₃ from serum to some tissue(s) following PCN treatment, which may be due to increased hepatic T₃ uptake. Thyroid hormones contain a polar alanine side chain making their passage through membranes by diffusion a difficult process. Evidence now suggests that thyroid hormones are actively transported across plasma membranes (Hennemann et al., 1998; Hennemann and Visser, 1997; Hood and Klaassen, 2000a). However, the mechanism by which the liver takes up
T_3_ and makes it available for glucuronidation, and the chemical inducibility of this process is yet unresolved. Recently, several proteins that transport chemicals into the liver have been identified and cloned from human and rat. Rat proteins that have been shown to transport thyroid hormones include oatp1, oatp2, and oatp3 (Abe et al., 1998; Docter et al., 1997; Eekelman Rooda et al., 1989; Rondeel et al., 1995), which have fairly similar affinities for T_3_. Currently, investigations into the chemical inducibility of these transporters are limited, but it has been reported that oatp1 is regulated by testosterone and estrogen in rat kidney (Lu et al., 1996). It has also been determined that oatp2 mRNA and protein are induced by PCN treatment in rat liver (Rausch-Derra et al., 2001). The increased uptake of T_3_ into liver by oatp2 may also, then, contribute to the increased biliary excretion of T_3_ following PCN treatment.

In conclusion, these data provide evidence that PCN treatment induces the glucuronidation and biliary excretion of T_3_, whereas 3MC and PCB treatments do not. This increased biliary excretion is accompanied by an increase in serum TSH, which does not occur following treatment of rats with 3MC and PCB. These in vivo findings are in agreement with those demonstrated in liver microsomes (Hood and Klaassen, 2000a), and support the mechanism for increased TSH following treatment with microsomal enzyme inducers to be the result of increases in T_3_ metabolism and excretion.

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