Review

The Roles of Nuclear Receptors CAR and PXR in Hepatic Energy Metabolism

Yoshihiro KONNO, Masahiko NEGISHI* and Susumu KODAMA
The Pharmacogenetics Section, Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, National Institutes of Health, North Carolina 27709, USA

Summary: Nuclear receptors constitutive active/androstane receptor (CAR) and pregnane X receptor (PXR) were originally characterized as transcription factors regulating the hepatic genes that encode drug metabolizing enzymes. Recent works have now revealed that these nuclear receptors also play the critical roles in modulating hepatic energy metabolism. While CAR and PXR directly bind to their response sequences phenobarbital-responsive enhancer module (PBREM) and xenobiotic responsive enhancer module (XREM) in the promoter of target genes to increase drug metabolism, the receptors also cross talk with various hormone responsive transcription factors such as forkhead box O1 (FoxO1), forkhead box A2 (FoxA2), cAMP-response element binding protein, and peroxisome proliferator activated receptor γ coactivator 1α (PGC 1α) to decrease energy metabolism through down-regulating gluconeogenesis, fatty acid oxidation and ketogenesis and up-regulating lipogenesis. In addition, CAR modulates thyroid hormone activity by regulating type 1 deiodinase in the regenerating liver. Thus, CAR and PXR are now placed at the crossroad where both xenobiotics and endogenous stimuli co-regulate liver function.

Keywords: constitutive active/androstane receptor (CAR); pregnane X receptor (PXR); cytochrome P450; drug metabolism; gluconeogenesis; lipid metabolism; thyroid hormone

Introduction

The nuclear receptors comprise a large family of ligand-activated transcription factors that control development, reproduction, energy homeostasis and other biological processes. The constitutive active/androstane receptor (CAR) and the pregnane X receptor (PXR) are expressed primarily in the liver and are defined as xenobiotic-sensing nuclear receptors. Upon exposure to xenobiotics, CAR and PXR activate transcription of their target genes that encode various enzymes and proteins involved in drug metabolism and excretion. In order to function as a xenobiotic sensor, CAR and PXR have broad ligand specificity compared with the classical steroid hormone receptors such as estrogen and glucocorticoid receptors. CAR and PXR are retained in the cytoplasm by forming a complex with HSP90 and the co-chaperon CCRP (cytoplasmic CAR retention protein). Upon xenobiotic exposures, CAR and PXR dissociate from the cytoplasmic complex to translocate into the nucleus where they form a heterodimer with the retinoid X receptor alpha (RXRα) and bind to and activate the phenobarbital-responsive enhancer module (PBREM)- and/or xenobiotic-responsive enhancer module (XREM)-bearing genes. These genes include cytochrome P450s (CYPs), UDP-glucuronosyltransferases, sulfotransferases and drug transporters. In addition to xenobiotics, CAR and PXR regulate metabolic detoxification and excretion of toxic endobiotics such as bilirubin and bile acids via the same enzymes and transporters involved in xenobiotic metabolism and excretion.

More recent works have elucidated that CAR and PXR are capable of regulating xenobiotic-induced modulation of hepatic energy metabolism. Liver is one of the major organs involved in energy production where nutrients such as carbohydrates and lipids are modified and synthesized to be used in throughout the body. Under fasting and/or starvation, the liver increases glucose production by augmenting gluconeogenesis and glycogenolysis to maintain blood glucose levels; increases fatty acid oxidation and ketogenesis to enhance synthesis of ke-
The Roles of CAR and PXR in Hepatic Energy Metabolism

To maintain energy production, the pancreatic hormones insulin and glucagon regulate transcription of the genes that encode the rate-limiting enzymes in the pathways of glucose and of lipid metabolisms. CAR and PXR are now found to interact with the hormone-responsive transcription factors to repress the transcription of these genes. In this review, we briefly describe the molecular mechanisms of nuclear receptor-mediated repression of hepatic energy metabolism.

**Gluconeogenesis**

Insulin and glucagon control glucose production by regulating transcription of the genes that encode the key enzymes in gluconeogenesis and glycogenolysis such as glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase 1 (PEPCK1). Glucagon increases glucose production by up-regulating the transcription of these genes: stimulates PKA to phosphorylate the cAMP-response element binding protein (CREB) that binds to and activates the transcription of CRE-bearing genes such as G6Pase and PEPCK1.7,8) Insulin decreases glucose production by repressing the transcription of the G6Pase and PEPCK1 genes.8,9) In the absence of insulin, the forkhead transcription factor 1 (FoxO1) binds to the insulin response sequence (IRS) and activates IRS-bearing genes such as G6Pase and PEPCK1.9,10) Insulin activates the phosphatidylinositol 3-kinase (PI3K)-Akt pathway9,11) to phosphorylate FoxO1 to exclude it from the nucleus, resulting in the insulin-dependent repression of the G6Pase and PEPCK1 genes.

It has long been known that chronic treatment with phenobarbital (PB) decreases plasma glucose levels and improves insulin sensitivity in diabetic patients.12) Moreover, treatments with CAR activators such as PB and 1, 4-bis[2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP) were found to repress hepatic levels of PEPCK1 and G6Pase in mouse liver13) and rat hepatocytes14). Recent studies with Car−/− and Pxr−/− mice clearly have indicated that these receptors are primary regulators of the drug-dependent regulation of hepatic glucose metabolism.15) Chronic treatment of PB resulted in the decrease of hepatic Pepck1 mRNA in Car+/+ mice, but not Car−/− mice, and the increase of glucose level only in Car−/− mice. PXR activator pregnenolone 16α-carbonitrile (PCN) treatment decreased serum glucose levels by PXR activation in fasting Pxr+/+ but not in Pxr−/− mice. Given these facts, we investigated the molecular mechanisms by which CAR and/or PXR regulate the G6Pase and PEPCK1 genes.10,15) Using GST-pull down, mammalian-two hybrid and gel-shift assays, we found that both CAR and PXR directly bind to FoxO1, preventing FoxO1 binding to its response element IRS. Cell-based transient transfection assays showed that CAR and PXR repress FoxO1-mediated transcription of IRS-bearing promoter. Thus, the direct interaction of CAR and PXR with FoxO1 appears to be the underlying mechanism repressing the G6Pase and PEPCK1 genes in response to xenobiotics. As a result, although the molecular mechanism differs from that by insulin, CAR and PXR cross talk with the same insulin-responsive transcription factor FoxO1 to repress gluconeogenesis.

Phosphorylated CREB binds to its response element CRE to activate transcription of CRE-bearing genes such as G6Pase.7)

Co-immunoprecipitation, gel-shift and cell-based transient transfection assays revealed that PXR forms a complex with
phosphorylated CREB in a ligand-dependent manner to prevents CREB binding to the CRE and represses CREB-mediated transcription of the G6Pase promoter.10) ChiP assays revealed decreased CREB binding to the G6Pase promoter in fasted Pxr−/− mice after PCN treatment but not in Pxr+/+ mice. Similar to the cross talk with FoxO1, PXR interacts with CREB to repress G6Pase, antagonizing glucagon activation. The peroxisome proliferator-activated receptor γ co-activator 1 (PGC1) α is also a glucagon-activated gene and binds to and co-activates HNF-4α–mediated transcription.16) Drug-activated PXR and/or CAR are shown to dissociate PGC1α from the HNF-4α complex, thus repressing transcription of PEPCK1 and G6Pase.16) The underlying molecular mechanism of how CAR and PXR repress glucose production is the direct binding of the nuclear receptors to the transcription factors that activate gluconeogenic genes such as FoxO1, CREB and PGC-1α (Fig. 2).

While drug treatments modulate insulin- and glucagon-controlled hepatic glucose metabolism, these hormones and other endogenous signals also regulate hepatic drug-metabolism. Insulin reduces PB induction of CYP2B in rat primary hepatocytes.17) Hepatic levels of CYP2B, CYP3A and CYP4A are increased in the experimentally generated diabetic rats and mice, and are reduced to normal levels by insulin treatment.18) We found that FoxO1 co-activates CAR and PXR, augmenting the expression of target genes such as CYP2B.15) By removing FoxO1 from the nucleus, insulin effectively abrogates the co-activation of CAR and PXR, repressing the genes that encode drug-metabolizing enzymes. In fact, the CAR-regulated genes such as Cyp2b10 and Cyp2c37 are up-regulated in the liver of FoxO1-transgenic mice.19) Is there any physiological meaning in the drug-induced repression of gluconeogenesis? NADPH is essential for cytochrome P450-dependent monoxygenase activity well as for glutathione recycling. In the liver, the pentose phosphate pathway converts glucose 6-phosphate to ribose 5-phosphate by glucose 6-phosphate dehydrogenase to generate NADPH. Increase of hepatic glucose 6-phosphate, resulting from the repression of the G6Pase gene, could enhance NADPH generation. Thus, repression of gluconeogenesis by drug activated CAR and PXR might help liver cells maintain sufficient NADPH levels for drug metabolism.

**Lipid metabolism**

Hepatic lipid metabolism plays a major role in survival during fasting and/or prolonged exercise. When blood glucose levels are low, the liver increases fatty acid oxidation and ketogenesis to provide extra-hepatic tissues with ketone bodies through β-oxidation and ketogenesis. At the same time, the liver decreases lipogenesis to attenuate hepatic storage of triglycerides. Under these conditions, carnitine palmitoyltransferase 1A (CPT1A) and mitochondrial 3-hydroxy-3-methylglutarate-CoA synthase 2 (HMGC2S), the key enzymes in β-oxidation and ketogenesis, respectively, are up-regulated,20,21) while stearoyl-CoA desaturase 1 (SCD1), key enzyme in the synthesis of unsaturated fatty acids, is up-regulated by glucose and fructose.22) In the absence of insulin, FoxA2, a winged-helix/forkhead transcription factor, activates CPT1A and HMGC2S.23) Insulin represses these two genes by inactivating the FoxA2 through the Akt-dependent signal pathway.24) Insulin also increases the transcription of SCD1, in part, by activating the sterol regulatory element-binding protein (SREBP).25)
The Roles of CAR and PXR in Hepatic Energy Metabolism

Our recent studies revealed that treatment with PXR activator PCN down-regulates the mRNA levels of Cpt1a and Hmgs2 in Pxr+/+ mice, but not Pxr−/− mice, while up-regulating Scd1 mRNA level only in Pxr+/+ mice.26) Consistent with this pattern of gene expression, the levels of serum 3-hydroxybutyrate and hepatic triglycerides were decreased and increased, respectively, in PCN-treated Pxr+/+ mice only. We investigated the molecular mechanisms by which PXR regulates the Cpt1a and Hmgs2 genes. Using gel shift, GST-pull down, CHIP, and cell-based reporter assays, we found that drug-activated PXR directly binds to FoxA2, preventing FoxA2 binding to the Cpt1a and Hmgs2 promoters and repressing the activation of these genes. The direct interaction of PXR with FoxA2 appears to be the underlying mechanism repressing Cpt1a and Hmgs2. On the other hand, the molecular mechanism of the PXR-mediated up-regulation of the Scd1 gene remains unclear. Thus, drug-activated PXR acts like insulin and represses hepatic energy metabolism by increasing triglyceride synthesis and decreasing β-oxidation and ketogenesis, although the molecular mechanism differs from that of insulin (Fig. 3). Since fatty acid β-oxidation produces and supplies chemical energy such as ATP and NADH to gluconeogenesis in the liver, the repression of β-oxidation by PXR could lead to down-regulation of gluconeogenesis.

Consistent with our conclusion of the role of PXR in hepatic lipid metabolism, Zhou et al., found increased hepatic deposit of triglycerides and the concomitant up-regulation of the Scd1 and Cd36 (free fatty acid transporter) genes in constitutively active PXR transgenic mice.27) In both Zhou’s and our studies, PXR did not regulate SREBP-1c and its primary lipogenic target enzymes, such as fatty-acid synthase and acetyl-CoA carboxylase 1. Intriguingly, the Scd1 and Cpt1a genes were found to be up-regulated and down-regulated, respectively, in the liver of untreated Pxr−/− mice to similar levels as observed in the PCN treated wild type mice, developing severe hyperglycemia and high hepatic triglycerides.26) Both Cd36 and Scd1 genes are up-regulated in primary hepatocytes prepared from untreated Pxr−/− mice compared to Pxr+/+ mice.27) Since these phenotypes are correlated with high level of serum insulin in Pxr−/− mice,26) there is the possibility that endogenous PXR is capable of controlling serum insulin level. However, the molecular mechanism of how the endogenous PXR regulates the expression of these genes and the level of serum insulin is virtually unknown at the present time.

Thyroid hormone synthesis

Thyroid hormone (TH) has a well-established role in liver regeneration and energy usage. Levels of TH are controlled by a balance of its synthesis, metabolism and secretion. Thyroid stimulating hormone (TSH) enhances synthesis of inactive 3,5,3′,5′-tetraiodothyronine (T4) in the thyroid gland which is subsequently converted to various forms of TH by deiodinases in the peripheral target tissues, such as liver and kidney.28) Three deiodinases are known; type 1 deiodinase (D1, gene name Dio1), type 2 deiodinase (D2, Dio2), and type 3 deiodinase (D3, Dio3).29) D1 is the major enzyme responsible for the conversion of T4 into the active 3,3′,5′-triiodothyronine (T3) in the liver. D2 also converts T4 into T3 in the extra-hepatic tissues, while D3 converts T4 into the lesser active 3,3′,5′-triiodothyronine (reverse T3, rT3) and converts T3 into T2. D1 also catalyzes the conversion of rT3 into T2 for clearance. T3 has been suggested to decrease T3 activity by competing with T3 for its binding to thyroid hormone receptor (TR) and transporters.30,31)

Chronic treatment with PB is known to promote thyroid hypertrophy in humans and rats.12,13) Given this fact, we investigated serum levels of total T4, total T3, free T3 and rT3 level in the Car+/+ and Car−/− mice treated with PB after partial hepatectomy (PH). No change in the total T4, total T3 and free T3 levels was observed in the Car+/+ or Car−/− mice.31) Unexpectedly, the rT3 level was increased in both mice only after PH and was recovered to the normal level by PB treatment only in Car+/+ mice. Moreover, we identified the Dio1 gene as the CAR-regulated gene and correlated its expression with these changes of rT3 levels in the regenerating liver (Fig. 4).31) D3 was expressed in the livers of all mice tested while D2 was barely expressed in the liver. Thus, the decrease of level relative to D3 could increase the rT3 level over T3 in the PH liver. Our finding that the repression of the Dio1 gene resulted in the increase of serum rT3 levels is consistent with a recent report showing higher rT3 level in the Dio1−/− mice.34) Even when T3 levels are maintained, increase of relative levels of rT3 over T3 could result in the attenuation of TH activity, which was substantiated by the fact that T3-targets such as tyrosine aminotransferase gene were down-regulated after PH in the liver of wild type and Car−/− mice. PB induced the Dio1 expression and decreased rT3 levels, up-regulating the TH-target genes to return to their normal levels in the Car+/+ mice only. Through regulation of the Dio1 gene, CAR modulates TH activity in the
Fig. 4. Proposed model for the role of CAR and D1 in the thyroid hormone synthesis after PH

Serum rT3 level is increased through the repression of hepatic D1 after PH and is recovered to the normal level by the activation of CAR. Serum T3 level is not changed after PH, because D1 preferentially catalyzes the deiodination of rT3 rather than T4 and D2 could compensate for the lost hepatic T3 production after PH.

regenerating liver.

Two different laboratories have recently reported that drug activation of CAR decreases serum level of total T4, but not of total T3 in the Car+/- mice only, but did not measure serum rT3 levels.35,36) Moreover, one of the two concluded that this decrease of T4 levels is associated with the increase of TSH, which resembles with hypothyroidism. Chronic treatment with another CAR activator phenytoin decreases serum levels of total T4, promoting thyroid hypertrophy.35,37) Since thyroid hormone is known to be sulfated or glucuronidated for its clearance and excretion,39) these two reports implicated UDP-glucuronosyltransferase UGT1A1 and sulfotransferase SULT1A1 for the determining factors responsible for T4 decrease35,36). However, it remains elusive whether UGT1A1 and SULT1A1 are the major enzymes conjugating TH and how these enzymes conjugate more effectively T4 over T3. We also examined TH in normal adult mice and found no change in serum levels of total T4 and TSH. Thus, in our hand, CAR does not play any role in the regulation of TH activity in normal adult mice.constitutive androstane receptor (CAR) and pregnane X receptor (PXR) regulate not only drug metabolism but also hepatic energy metabolism; gluconeogenesis, glycogenolysis, fatty acid β-oxidation, ketogenesis, lipogenesis and thyroid hormone activity. The underlying molecular mechanisms regulating energy metabolism is the cross-talk of CAR and PXR with insulin- or glucagon-responsive transcription factors such as FoxO1, FoxA2 and CREB. These nuclear receptors directly bind to these transcription factors and repress their activity and the genes encoding the key enzymes in energy metabolism. The repression of energy metabolism by the nuclear receptors may have importance in understanding and treating liver diseases such as diabetes. In insulin-resistance type 2 diabetes, high blood glucose is often complicated by hyperglycemia. Drug-activated PXR is able to decrease blood 3-hydroxybutyrate as well as glucose levels, possibly improving diabetic condition. However PXR may also become a factor in negatively affecting this disease, because activation of PXR led mice to increase hepatic triglycerides, a complication that is associated with Type 2 diabetes. Once some of the negative factors are eliminated, PXR may become a potential therapeutic target of Type 2 diabetes.

Conclusion

CAR and PXR regulate not only drug metabolism but also hepatic energy metabolism; gluconeogenesis, glycogenolysis, fatty acid β-oxidation, ketogenesis, lipogenesis and thyroid hormone activity. The underlying molecular mechanisms regulating energy metabolism is the cross-talk of CAR and PXR with insulin- or glucagon-responsive transcription factors such as FoxO1, FoxA2 and CREB. These nuclear receptors directly bind to these transcription factors and repress their activity and the genes encoding the key enzymes in energy metabolism. The repression of energy metabolism by the nuclear receptors may have importance in understanding and treating liver diseases such as diabetes. In insulin-resistance type 2 diabetes, high blood glucose is often complicated by hyperglycemia. Drug-activated PXR is able to decrease blood 3-hydroxybutyrate as well as glucose levels, possibly improving diabetic condition. However PXR may also become a factor in negatively affecting this disease, because activation of PXR led mice to increase hepatic triglycerides, a complication that is associated with Type 2 diabetes. Once some of the negative factors are eliminated, PXR may become a potential therapeutic target of Type 2 diabetes.

References

9) Nakae, J., Kitamura, T., Silver, D. L. and Accill, D.: The for-
khead transcription factor FoxO1 (FKhr) confers insulin sensitiv-
ity onto glucose-6-phosphatase expression. J. Clin. Investig.,
10) Kodama, S., Moore, R., Yamamoto, Y. and Negishi, M.: Human
nuclear pregnane X receptor cross-talk with CRBP to repress
AMP activation of the glucose-6-phosphatase gene. Biochem. J.,
11) Matsuok, H., Daitoku, H., Hatta, M., Tanaka, K. and
Fukamizu, A.: Insulin-induced phosphorylation on FKHR (Fox-
USA, 100: 11285–11290 (2003).
12) Lahtela, J. T., Arranto, A. J. and Sotanaient, E. A.: Enzyme in-
ducers improve insulin sensitivity in non-insulin-dependent dia-
13) Manenti, G., Draghi, T. A. and Della Porta, G.: Effects of
phenobarbital and 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene on
4 differentiated functions in mouse liver. Chem. Biol. Interact.,
14) Argaud, D., Halimi, S., Catelloni, F. and leveve, X. M.: Inhibi-
tion of glucogenogenesis in isolated rat hepatocytes after chronic
Receptors CAR and PXR cross talk with FOXO1 to regulate
genes that encode drug-metabolizing and glucogenenic en-
16) Miao, J., Fang, S., Bae, Y. and Kemper J. K.: Functional inhibi-
tory cross-talk between constitutive androstanerceptor and
hepatic nuclear factor-4 in hepatic lipogenesis/glucose metabolism is
mediated by competition for binding to the DR1 motif and to
the common coactivators, GRIP–1 and PGC–1a. J. Biol. Chem.,
17) Sidhu, J. S. and Omiecinski, C. J.: Insulin-mediated modulation
of cytochrome P450 gene induction profiles on primary rat
18) Yamazoe, Y., Murayama, N., Shimada, M., Yamauchi, K. and
Kato, R.: Cytochrome P450 in livers of diabetic rats: regulation
of cytochrome P450 gene induction profiles on primary rat
hepatocytes cultures. Arch. Biochem. Biophys., 268:
19) Zhang, W., Patil, S., Chauhan, B., Guo, S., Powell, D. R., Le, J.,
Klotzas, A., Matika, R., Xiao, X., Franks, R., Heidenreich, K. A.,
Saín, M. P., Farese, R. V., Stolz, D. B., Tso, P., Koo, S. H., Mon-
tmny, M. and Unterman, T. G.: FoxO1 regulates multiple meta-
lolic pathways in the liver: effects on glucogenenic, glycolytic,
and lipogenic gene expression. J. Biol. Chem., 281:
20) Hegardt, F. G.: Mitochondrial 3-hydroxy-3-methylglutaryl-CoA
synthase: a control enzyme in ketogenesis, Biochem. J., 338:
569–582 (1999).
21) Louet, J. F., Le May, C., Pegorier, J. P., Decaux, J. F. and Girard,
J.: Regulotion of liver carmine palmitoyltransferase I gene ex-
berson by hormones and fatty acids, Biochem. Soc. Trans.,
22) Dobrzyn, A. and Ntambi, J. M.: The role of stearoyl-CoA
desaturase in the control of metabolism. Prostaglandins Leukot.
23) Wolfrum, C., Asilmaz, E., Luca, E., Friedman, J. M. and Stoffel,
M.: Foxa2 regulates lipid metabolism and ketogenesis in the
(2004).
24) Wolfrum, C., Besser, D., Luca, E. and Stoffel, M.: Insulin regu-
lates the activity of forkhead transcription factor Hnf-3beta/Fox-
a-2 by Akt-mediated phosphorylation and nuclear/cytosolic
(2003).
Identification of conserved cis-elements and transcription fac-
tors required for sterol-regulated transcription of stearoyl-CoA
26) Nakamura, K., Moore, R., Negishi, M. and Suyoshi, T.: Nuclear
pregnane X receptor cross-talk with FoxA2 to mediate drug-in-
duced regulation of lipid metabolism in fasting mouse liver. J.
27) Zhou, J., Zhai, Y., Yu, M., Gong, H., Uppal, H., Toma, D., Ren,
S., Evans, R. M. and Xie, W.: A novel pregnane X receptor-
mediated and sterol regulatory element-binding protein-in-
dependent lipogenic pathway. J. Biol. Chem., 281:
28) Bianco, A. C., Salvatore, D., Gereben, B., Berry, M. J. and Larsen,
P. R.: Biochemistry, cellular and molecular biology, and
physiological roles of the iodothyronine selenodeiodinases.
29) Hutija, M. and Joss, J. M.: Thyroid hormone deiodinases revisited:
insights from lungfish: a review. J. Comp. Physiol[B], 176:
31) Tien, E. S., Matsu, K., Moore, R. and Negishi, M.: The nuclear
receptor constitutively active/androstanerceptor regulates type I deiodinase and thyroid hormone activity in the regenerat-
32) Curran, P. G. and DeGroot, L. I.: The effect of hepatic enzyme-
inducing drugs on thyroid hormones and the thyroid gland. End-
t of thyroid and hepatocarcinogenesis by 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene in rats at doses that cause
34) Schneider, M. J., Fiering, S. N., Thai, B., Wu, S. Y., St. Germain,
knockout of the type 1 selenodeiodinase gene (Dio1) results in
marked changes in thyroid hormone economy in mice. Endocrino-
35) Maglich, J. M., Watson, J., McMillen, P. J., Goodwin, B.,
Willson, T. M. and Moore, J. T.: The nuclear receptor CAR is a
regulator of thyroid hormone metabolism during caloric restric-
36) Qatanani, M., Zhang, J. and Moore, D. D.: Role of the constitut-
ive androstanerceptor in xenobiotic-induced thyroid hormo-
37) Hegedu, L., Hansen, J. M., Lubdorf, K., Perrild, H., Feldt-Ras-
mussen, U. and Kampmann, J. P.: Increased frequency of goitre
in epileptic patients on long-term phenytoin or carbamazepine
38) Visser, T. J., Kaptein, E., Glatt, H., Bartsch, I., Hagen, M. and
Coughtrie, M. W.: Characterization of thyroid hormone sul-