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Synergistic acceleration of thyroid hormone degradation by phenobarbital and the PPARα agonist WY14643 in rat hepatocytes

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ABSTRACT

Energy balance is maintained by controlling both energy intake and energy expenditure. Thyroid hormones play a crucial role in regulating energy expenditure. Their levels are adjusted by a tight feedback-controlled regulation of thyroid hormone production/incretion and by their hepatic metabolism. Thyroid hormone degradation has previously been shown to be enhanced by treatment with phenobarbital or other antiepileptic drugs due to a CAR-dependent induction of phase II enzymes of xenobiotic metabolism. We have recently shown, that PPARα agonists synergize with phenobarbital to induce another prototypical CAR target gene, *CYP2B1*. Therefore, it was tested whether a PPARα agonist could enhance the phenobarbital-dependent acceleration of thyroid hormone elimination. In primary cultures of rat hepatocytes the apparent half-life of T3 was reduced after induction with a combination of phenobarbital and the PPARα agonist WY14643 to a larger extent than after induction with either compound alone. The synergistic reduction of the half-life could be attributed to a synergistic induction of CAR and the CAR target genes that code for enzymes and transporters involved in the hepatic elimination of T3, such as *OATP1A1*, *OATP1A3*, *UGT1A3* and *UGT1A10*. The PPARα-dependent CAR induction and the subsequent induction of T3-eliminating enzymes might be of physiological significance for the fasting-induced reduction in energy expenditure by fatty acids as natural PPARα ligands. The synergism of the PPARα agonist WY14643 and phenobarbital in inducing thyroid hormone breakdown might serve as a paradigm for the synergistic disruption of endocrine control by other combinations of xenobiotics.

Introduction

In the long run, any disparity between food intake and energy expenditure will lead to either wasting or accumulation of excessive energy stores, mainly as adipose tissue. Obesity and the closely associated metabolic syndrome, as well as type 2 diabetes, are major health problems in industrialized and developing countries. Both food intake and energy expenditure are under tight hormonal control. Normally, energy expenditure can be adjusted to variations in food supply: it decreases during fasting or voluntary weight loss, and it is up-regulated if food is provided in excess of the actual demand or during voluntary weight gain (Leibel et al., 1995). It has been estimated that energy expenditure can vary by about 8% to 10%, depending on the fuel supply (Grun and Blumberg, 2007). Thereby, a surplus of calories may be dissipated to a considerable extent as heat, although the relevance of this mechanism is still a matter of controversy (Joosen and Westerterp, 2006). On the other hand, reduction of the metabolic rate allows limiting weight loss as a result of calorie restriction, and a failure of this adaptive response leads to reduced fasting tolerance (Maglich et al., 2004). The thyroid hormones are key controllers of energy metabolism. They act via nuclear thyroid hormone receptors that induce a number of genes in various tissues, collectively increasing the metabolic rate. The level of thyroid hormones is adjusted in a dual manner: the liberation of thyroid hormones from the thyroid gland is regulated by thyrotropin-releasing hormone (TRH) and thyroid stimulating hormone (TSH) of the hypothalamic–pituitary axis. The release of TRH and TSH is feedback-inhibited by thyroid hormones. The thyroid gland predominantly releases thyroxin (T4) which is converted into the about 100-fold more active triiodothyronine (T3) by deiodination of the outer ring, primarily in the liver (Bianco et al., 2002). The outer ring deiodination as well as the elimination of T3 and T4 provide a second level of control. Deiodination and elimination are modulated by hepatic phase II

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sulfotransferases and glucuronosyltransferases. Outer ring deiodination is inhibited while inner ring deiodination, which yields inactive metabolites, is stimulated by sulfation of the phenolic hydroxyl group of T4 (Visser, 1994). Glucuronidation of T3 and T4 by UDP-glucuronosyltransferases (UGT), especially by the UGT1A family, results in inactive, water soluble products that are excreted into urine and bile (Vansell and Klaassen, 2002; Visser et al., 1993; Findlay et al., 2000). Thus, induction of enzymes of phase II of xenobiotic metabolism in the liver may result in a decrease in active thyroid hormone. The genes of both UGT1A and sulfotransferases are targets of the nuclear receptor CAR, which plays a pivotal role in mediating the induction of xenobiotic metabolizing enzymes by phenobarbital-like inducers. As a consequence, long term treatment with phenobarbital-like inducers (e. g. with antiepileptic drugs such as phenobarbital, phenytoin or carbamazepin) may result in up regulation of thyroid hormone inactivating enzymes, decreasing thyroid hormone levels as it has been shown to occur in long term treatment of epilepsy (Rootwelt et al., 1978; Benedetti et al., 2005). As a consequence, development of adiposity may be promoted. We have recently shown that PPARα agonists induced CAR and thereby enhanced the phenobarbital-dependent induction of a prototypical CAR target gene, *CYP2B1*, in primary rat hepatocytes (Wieneke et al., 2007). Therefore, the question was addressed, whether the observed synergism between PPARα agonists and phenobarbital extends to other CAR target genes and might result in a synergistic stimulation of thyroid hormone degradation in hepatocytes.

Materials and methods

Materials. All chemicals were purchased from commercial sources indicated throughout the text. Oligonucleotides were custom-synthesized by MWG Biotech AG (Ebersberg, Germany).

Animals. Male Wistar rats (200–300 g) were purchased from Charles River (Sulzfeld, Germany) and kept on a 12 h day/night rhythm (light from 06:00 to18:00 h) with free access to water and the standard rat diet 1320 (Altromin, Gesellschaft für Tierhaltung, Lage, Germany). Treatment of the animals followed the German Law on the Protection of Animals and was performed with permission of the state animal welfare committees.

Hepatocyte preparation and culture. Rat hepatocytes were isolated as described previously (Püschel et al., 1993). Hepatocytes (1.2×10^5 cells/cm^2) were plated on plastic tissue culture plates (Sarstedt, Nürnbrecht, Germany) in Williams E medium containing 0.5 nM insulin, 100 nM dexamethasone (all Sigma-Aldrich, Deisenhofen, Germany), 10 mg/ml penicillin/streptomycin (Biochrom, Berlin, Germany) and for the first 4 h of culture 4% newborn calf serum (v/v) (Biochrom, Berlin, Germany). Culture was then continued in serum-free medium supplemented with 5 mg/l (870 nM) insulin at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. The medium was replaced daily. After 24 h phenobarbital was added, and after 48 h phenobarbital and either WY 14643, GW 7647 or the solvent DMSO were added to final concentrations of 1 mM, 10 μM, 1 μM or 0,1%, respectively. The culture was continued for a total of 72 h. Hepatocytes were either used to determine T3 degradation or washed twice with PBS (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, $Na_2HPO_4\times2\ H_2O$, pH 7.3) and then either homogenized in lysis buffer for subsequent determination of enzyme activity or shock frozen in N_2 and stored at −70 °C for subsequent RNA preparation.

CYP2B, SULT and UGT enzyme assays. Hepatocytes were scraped into 100 μl lysis buffer per 3.5 cm diameter plate (20 mM Tris/HCL pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 2.5 mM $Na_4P_2O_7$, β-glycerol phosphate, 50 mM NaF, 200 μM pefabloc (Roche Applied Science, Mannheim, Germany), 10 μg/ml leupeptin and 10 μg/ml trypsin inhibitor). Suspensions were then sonified. The cell homogenates were centrifuged for 15 min at 10,000 *g*. The collected supernatant was stored at −20 °C. Prior to further use protein content was determined (Lowry et al., 1951).Pentoxyresorufin O-dealkylation activity was measured by direct fluorometric detection of resorufin formation (Lubet et al., 1985) as described in detail previously (Wieneke et al., 2007). Sulfotransferase activity was measured by a fluorometric assay determining the decrease of resorufin fluorescence essentially as described by Beckmann (1991) with minor adaptations for micro plate reader use. UDP-glucuronosyl transferase activity was determined as conversion of the fluorescent substrate 4-methylumbelliferone (4-MU) to a non fluorescent glucuronide as described by Collier et al. (2000).

Northern blot analysis. Total RNA was isolated from hepatocyte cultures by guanidinium thiocyanate phenol extraction (Chomczynski and Sacchi, 1987), subjected to electrophoresis on formaldehyde-agarose gels, and subsequently transferred to Hybond-N nylon membranes as described previously (Aubrecht et al., 1993). RNA blots were hybridized to the oligonucleotide probe 5′-GGTTGGTAGCCGGTGTGA-3′ for the rat *CYP2B1* gene (bases 49–66 of exon 7 region; GeneBank L00318) which had been 5′end-labeled by T4-polynucleotide kinase utilizing [γ-32P]ATP (Omiecinski et al., 1985). RNA expression was quantified by an imaging system (Fujix BAS 1500 Bio-Imaging Analyzer, Fujix, Tokio, Japan). Control hybridizations were performed using a GAPDH antisense oligonucleotide probe (Hirsch-Ernst et al., 2001).

Real time RT-PCR. RNA was isolated using the SV total RNA kit (Promega) and reverse transcribed into cDNA using a mixture of oligo dT and random nucleotide primers and an H Minus M-MuLV Reverse Transcriptase (Fermentas, St. Leon Rot). Hot start real-time PCR for the quantification of each transcript was carried out in a reaction mixture of 15 μl of MESA GREEN qPCR MasterMix Plus (Eurogentec, Seraing, Belgium) and 5 μl of cDNA. PCR was performed with an initial enzyme activation step at 95 °C for 10 min, followed by 50 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min in a real-time DNA thermal cycler (iCycler™, BIO-RAD; Munich). The oligonucleotides used are listed in Table 1. The expression level was calculated as an n-fold induction of the gene of interest (int) in treated versus control cells with *β-actin* (act) as a reference gene. The calculation is based on the differences in the threshold cycles between control (c) and treated (t) groups according to the formula: fold induction $=2^{(c-t)int}/2^{(c-t)act}$.

Determination of T3 degradation. Hepatocytes were cultured and treated as described above. At the end of the culture time they were washed extensively with Williams E medium supplemented with 100 nM dexamethasone, 0.5 nM insulin and 10 mg/ml penicillin/streptomycin and culture was continued in this medium. T3 (Sigma Deisenhofen) was added to a final concentration of 10 nM. Aliquots of the supernatant were removed immediately after addition as well as 30, 60 and 120 min after addition. Free T3 was determined by chemiluminescence immunoassay (Immulite2000, Siemens Medical Solutions Diagnostics GmbH, Bad Nauheim, Germany) according to the instructions of the provider.

T3 degradation and the appearance of glucuronides were also determined by LC-MS. Briefly, cells were washed 3 times with a Hepes-buffered salt solution (15 mM Hepes pH 7.4, 140 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 2.2 mM $CaCl_2$ and 5 mM glucose). Then T3 was added to a final concentration of 2 μM in the same buffer. Cells were incubated at 37 °C for 2 h. T3 and its metabolites were extracted from the buffer using SPE (solid phase extraction, OASIS HLB 1cc, Waters, Milford, USA). The SPE was conditioned with 1 ml methanol, equilibrated with 1 ml water, loaded with 0.9 or 1.8 ml sample preparation, washed with 2 ml water and eluted with 1 ml methanol. The sample was evaporated under a stream of nitrogen and then

Table 1
Oligonucleotide primers for qPCR.

Gene (Acc. no.)	Forward	Reverse
CAR (AB204900)	5′-CATGTAGGGTTCCAGTACGAG	5′-GACTGTTGCTCCATAATGTGG
CPT-1 (NM_031559)	5′-CCAAGCTGTGGCCTTCCAGT	5′-GGACGCCGCTCACAATGTTC
DIO1 (NM_021653)	5′-GCCAAAAGACCGGAATGACCAGG	5′-TCTACGAGTCTCTTGAACTGGTC
DIO3 (NM_017210)	5′-CCTGCTGCTTCACTCTCTGAGGC	5′-ACACGGCCTTGAGAGAGGCCAGG
GSTA2 (NM_017013)	5′-GCAGCAGGAGTGGAGTTTGATGAG	5′-AGGCGGTCTTGGCTTCTTTTTG
MCT8 (NM_147216)	5′-TGCGCTACTTCACCTATGGGATTC	5′-GAGGGGCCGGTAGGTGAGTGAGAG
MRP-1 (AY170916)	5′-GTTTCCCCCTCTACTTCCTCTATC	5′-GAATCTCGAAACATGTCCACTTGA
MRP-2 (X96393)	5′-GGCCACCCCAGTCTTCGCTATCAT	5′-ACGTCCCCGGTTAAGGTTTTTCT
MRP-3 (AF072816)	5′-GACCGCTTGTGCGGCTCCGGGGA	5′-GTAGAACAGGTCCACCCATGAGAT
OATP1A1 (NM_017111)	5′-CTGGGTTGGTGCTTGGTGGATT	5′-CCGAGGCATATGGGAGGTAAGC
OATP1A4 (NM_131906)	5′-GCCTCGGATATTTAATTGGTGGTT	5′-CTGCAGATGAGTTTCCCGATGAC
SULT1A1 (BC072468)	5′-CCGCTAGTGCATGTGAAGGGTAT	5′-AGGGAGCAAGGACAGGGGCAGATG
UGT1A3 (NM_201424)	5′-GGCTCTCTGGCGAGACTTGCG	see *UGT1A10*
UGT1A8 (AF461735)	5′-GAAGGAGCGTGCATTTTGCCC	see *UGT1A10*
UGT1A10 (NM_201425)	5′-CTCAAAATCCGCAGACACCAT	5′-TCCCAAAGAGAAAACCACGAT

reconstituted in 300 µl 30% ethanol. 100 µl of this sample preparation was analyzed using a Shimadzu LC system with a SPD-M20A photodiode array detector and a 2010-EV single mass analyzer. Components were separated on a PerfectSil Target ODS-3 column (Octadecyl-bonded silica column, 250×3 mm, 3 µm particle size, MZ-Analysentechnik GmbH Mainz, Germany). The column was eluted with a gradient of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile/methanol (1:1 v:v) (B). The mobile phase B was held at 30% for the first 5 min, linearly increased from 30% to 55% over 10 min, to 100% over 10 min, held for 2 min, decreased at starting conditions and reequilibrated for 7 min. The flow rate was 0.3 ml/min. The temperature of the column oven was set at 40 °C.

The chromatograms were recorded in the selected ion-monitoring (SIM) mode at m/z 652 and 828, which corresponds to the molecular ions $[M+H]^+$ of T3 and T3 glucuronides, respectively. The analytical conditions for the LC-MS QP-2010 (Liquid chromatographic-mass spectrometry quadrupole-2010) were the following: probe (interface) voltage, 4.5 kV (ESI (Electrospray) positive mode); nebulizing gas flow, 1.5 l/min; drying gas pressure, 0.2 MPa, CDL (Curved desolvation line) temperature, 120°C; CDL voltage 0 V; Q-array DC (Direct Current) voltage, 10 V; Q-array RF (Radio Frequency), 150 V; and block heater temperature, 120 °C.

Results

T3 degradation in hepatocytes treated with phenobarbital and/or the PPARα agonist WY14643

Hepatocytes were cultured for a total of 72 h. During the last 48 h of culture, a part of the hepatocytes was treated with phenobarbital, and for the last 24 h with phenobarbital and the PPARα agonist WY14643, as indicated, to induce the enzymes of xenobiotic metabolism. At the end of the culture period cells were washed extensively to remove the inducers. Then triiodothyronin (T3) was added to a final concentration of 10 nM with fresh medium. Aliquots of the medium were withdrawn immediately after addition as well as after 30, 60 and 120 min (Fig. 1). T3 was rapidly degraded in control hepatocyte cultures with an apparent half-life of about 110 min. In hepatocytes treated with either phenobarbital or WY14643, T3 degradation was accelerated. After 2 h, the remaining T3 concentration in the supernatant was significantly lower than in control cells. The apparent half-life was reduced to around 80 min. The rate of T3 degradation was further enhanced in hepatocytes that were incubated with both phenobarbital and WY14643 prior to the addition of T3. After 2 h the residual T3 concentration was significantly lower than in hepatocytes treated with either phenobarbital or WY14643 alone. The apparent half-live of T3 was reduced to about 65 min (Fig. 1).

Similar results were obtained when T3-degradation was determined by LC-MS. In order to reach sufficient sensitivity to detect T3 metabolites, the initial concentration in the medium was 2 µM. Also an additional, structurally different PPARα agonist (GW 7647) was included into the experiment. In control medium incubated in the absence of hepatocytes for 120 min, a single peak with a mass of 652 corresponding to T3 was detected, which eluted with a retention time of 22.07 min (Fig. 2A). In medium incubated with control hepatocyte cultures the area under the curve of this peak was reduced to about 60% (Fig. 2B), indicating that about 40% of the T3 was degraded after 2 h. The apparent rate of T3 degradation was higher in phenobarbital-induced hepatocytes and was further enhanced by a combined induction with phenobarbital and either of the two PPARα agonists WY14643 or GW7647 (Fig. 2B). After incubation of T3-containing medium with hepatocytes a peak with a retention time of 20.09 min and a mass of 828 appeared, which was absent from the control

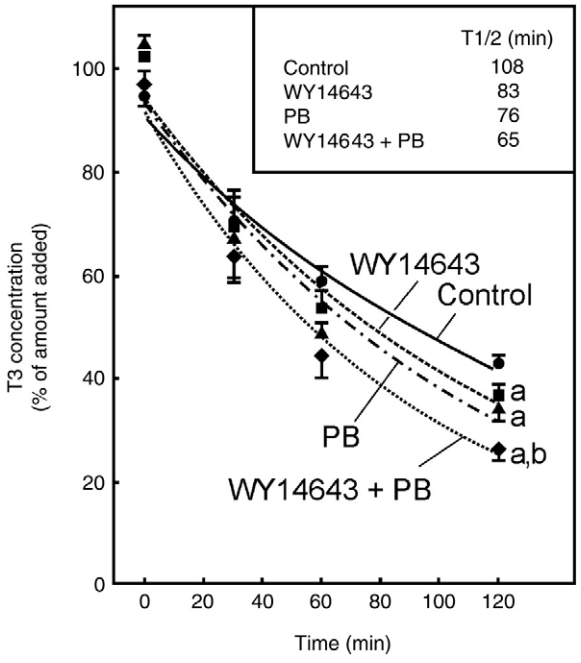

Fig. 1. Synergistic induction of T3 elimination by phenobarbital and the PPARα agonist WY14643 in hepatocytes. Hepatocytes were cultured in the presence or absence of 1 mM phenobarbital (PB) and 10 µM WY14643 as described in Materials and methods. At the end of the culture time medium was replaced by fresh medium and T3 was added to a final concentration of 10 nM. At the times indicated medium samples were drawn and free T3 was determined by radioimmuno assay. Values are means ± SEM of three independent experiments. The average concentration at $t=0$ min of all samples in each experiment was set to 100%. The apparent half-life was deduced from curves fitted into the data points as $y=X^{-\lambda t}$ with $\lambda=\ln(2)/t1/2$. Statistics: Student's *t*-test for unpaired samples. a: significantly lower than control ($p<0.05$); b: significantly lower than with phenobarbital or WY14643 alone ($p<0.05$).

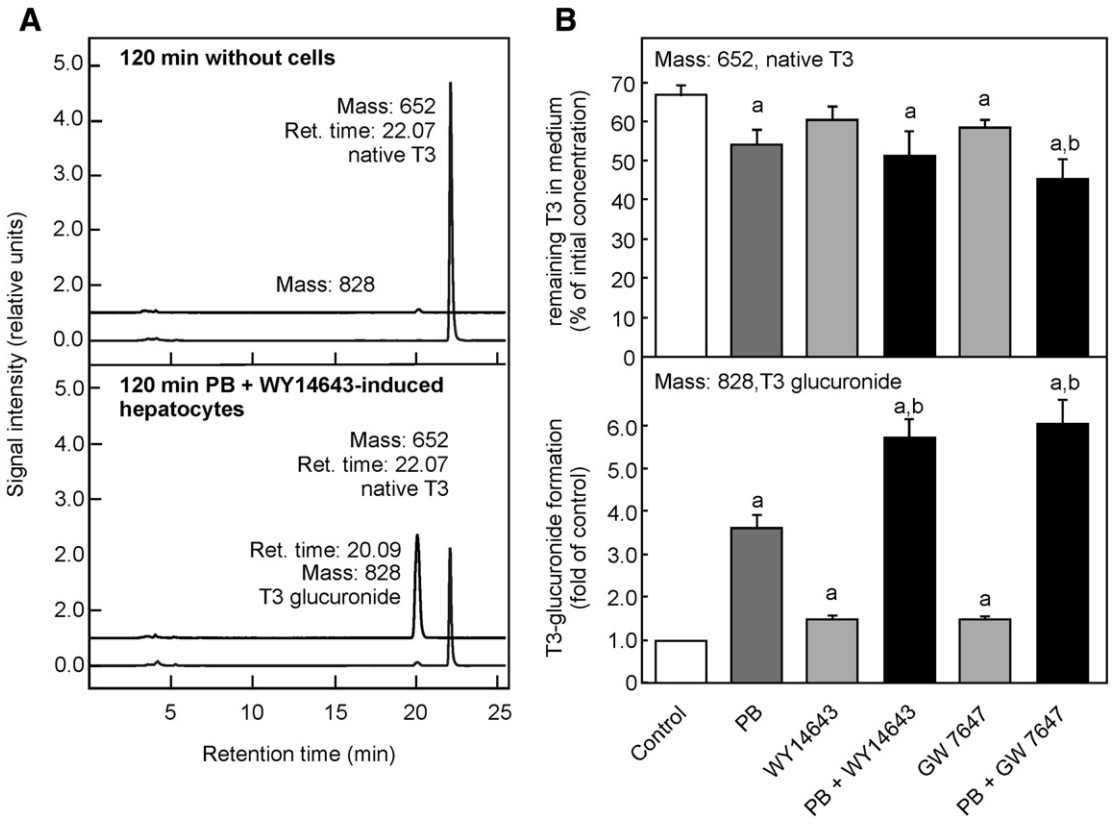

Fig. 2. Determination of T3 degradation and glucuronidation by LC-MS. Hepatocytes were cultured in the presence or absence of 1 mM phenobarbital (PB) and 10 μM WY14643 or 1 μM GW7647 as described in Materials and methods. At the end of the culture time medium was replaced by Hepes-buffered saline containing 2 μM T3. Cells were incubated for 2 h at 37 °C. T3 and its metabolites were then extracted from the medium by solid phase extraction. Components in the extract were separated, identified and quantified by LC-MS as detailed in Materials and methods. (A) Example traces for medium incubated in the absence of hepatocytes or with hepatocyte cultures induced with PB and WY14643. (B) T3 and T3-glucuronide were quantified by comparing the areas under the respective mass peaks. For T3, the area under the curve of medium incubated at 37 °C for 2 h in the absence of cells was set to 100%; for the glucuronide, which was not detectable in medium incubated without cells, the peak area in the medium incubated with control hepatocytes was set to 1. Values are means ± SEM of 4 independent cell preparations. Statistics: Student's *t*-test for paired samples, *a* = significantly different from control, $p<0.05$; *b* = significantly different from hepatocytes treated with phenobarbital only, $p<0.05$.

medium (Fig. 2A). This peak corresponded to T3 glucuronide. Treatment of hepatocytes with either PPARα agonists alone enhanced T3-glucuronide formation only slightly. The area under the curve of the T3-glucuronide peak was more than 3 times larger in medium incubated with phenobarbital-induced hepatocytes than in medium incubated with control hepatocytes. The glucuronide formation was further enhanced in hepatocytes treated with a combination of phenobarbital and either of the two PPARα-agonists WY14643 or GW7647. In medium incubated with these hepatocytes the area under the glucuronide peak was almost twice as large than in medium incubated with hepatocytes that were induced with phenobarbital alone (Fig. 2B). Apparently, the impact of PPARα agonists and phenobarbital on T3-glucuronide formation by hepatocytes was more than additive.

PPARα-dependent induction of CAR expression

As described recently (Wieneke et al., 2007), treatment of hepatocytes with the PPARα agonist WY14643 resulted in an induction of CAR mRNA (Fig. 3A). This induction was strongly enhanced by simultaneous treatment with phenobarbital. In contrast to our previous findings, phenobarbital itself caused a slight increase in CAR expression. The combined action of phenobarbital and the PPARα agonist was clearly more than additive (Fig. 3A). To exclude that the induction of CAR by WY14643 could be attributed to its xenobiotic character, the experiment was repeated with a different, structurally unrelated PPARα agonist, namely GW7647. GW7647 induced CAR to a similar extent as WY14643. The induction was further enhanced by the simultaneous presence of phenobarbital. The induction of the prototypical PPARα target gene *CPT1* (carnitine palmitoyltransferase 1) was studied in parallel. As expected, both WY14643 and GW7647 strongly induced CPT1 mRNA while phenobarbital had no effect on CPT1 expression (Fig. 3B). Surprisingly, phenobarbital slightly enhanced WY14643-induced but not the GW7647-induced CPT1 expression. The former result contrasts with previous *in vivo* findings in mouse where CPT1 expression was repressed in response to phenobarbital in livers of wild type but not of CAR-deficient mice (Ueda et al., 2002).

Potentiation by PPARα activation of phenobarbital-dependent induction of CAR target genes

In order to test, whether the induction of CAR had an impact on the expression of CAR-dependent genes, the induction of the prototypical CAR-regulated gene *CYP2B1* was determined (Table 2). In control hepatocytes, no CYP2B activity was detectable. mRNA levels were barely detectable by Northern blot analysis. Treatment with phenobarbital resulted in a significant induction of CYP2B activity and mRNA. This phenobarbital-dependent induction of CYP2B1 was potentiated by the PPARα agonists WY14643 and GW7647 which by themselves did not affect *CYP2B1* expression. Similarly, the mRNA of another typical CAR target gene, *GSTA2*, was induced roughly 20-fold by phenobarbital (Table 2). Simultaneous treatment with WY14643 enhanced this induction to about 40-fold, while treatment of hepatocytes with WY14643 alone affected GST-A2 mRNA levels only marginally. Neither *GSTA2* nor *CYP2B1* is involved in thyroid hormone

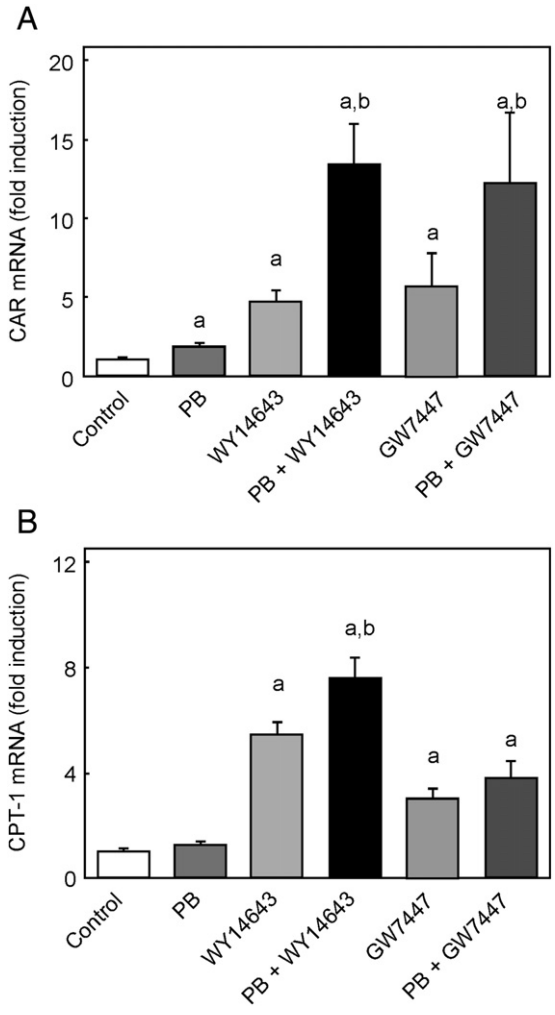

Fig. 3. Induction of CAR and the prototypical PPARα target gene *CPT1* by the PPARα agonists WY14643 and GW7647. Hepatocytes were cultured as described in the legend to Fig. 1. After 72 h cells were harvested and the mRNA content was determined by qPCR as described in Materials and methods. Values are means ± SD of 12 to 32 determinations with three to six independent cell preparations. Statistics: Student's *t*-test for unpaired samples. a: significantly higher than control, ($p<0.05$); b: significantly higher than in cultures with either inducer alone ($p<0.05$).

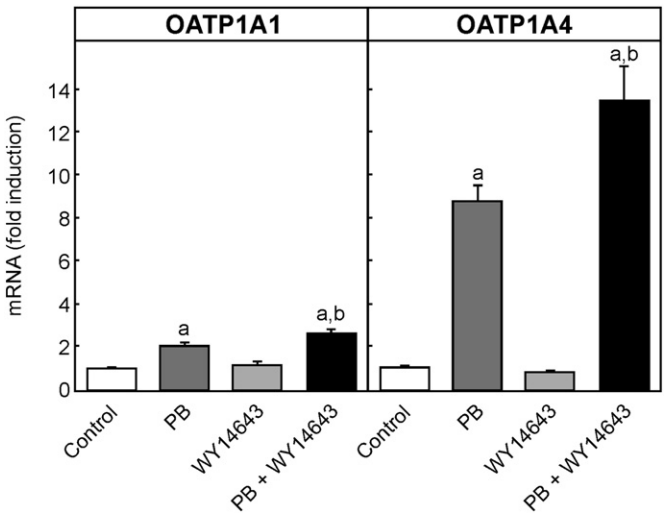

Fig. 4. Enhancement by the PPARα agonist WY14643 of the phenobarbital-dependent induction of transporters involved in hepatic thyroid hormone uptake. Hepatocytes were cultured as described in the legend to Fig. 1. After 72 h cells were harvested and the mRNA content was determined by qPCR as described in Materials and methods. Values are means ± SD of 16 to 18 determinations with three independent cell preparations. Statistics: Student's *t*-test for unpaired samples. a: significantly higher than control, ($p<0.05$); b: significantly higher than in cultures with either inducer alone ($p<0.05$).

metabolism. Therefore, it was tested, whether phenobarbital and WY14643 synergize to induce other CAR target genes presumably involved in the degradation and inactivation of thyroid hormone.

Transporters involved in thyroid hormone uptake into hepatocytes

A prerequisite for hepatic degradation of thyroid hormones is their uptake into hepatocytes. Transporters of the OATP family, particularly OATP1A1 and OATP1A4 (OATP1 and OATP2, respectively, according to the old nomenclature) have been implicated in this transport in rat liver (Jansen et al., 2005). OATP1A1 mRNA was induced about 2-fold by treatment with phenobarbital (Fig. 4). WY14643 had no effect on the basal OATP1A1 expression but significantly enhanced the phenobarbital-dependent induction by about 25%. The induction of OATP1A4 by phenobarbital was much more pronounced, treatment of hepatocytes with phenobarbital induced OATP1A4 about 8-fold. WY 14643 did not affect basal expression of OATP1A4, yet enhanced the phenobarbital-dependent induction by about 60%. Apparently the PPARα-dependent induction of CAR augmented the phenobarbital-elicited induction of both transporter mRNAs. The phenobarbital-induced OATP1A4 expression was dependent on the level of CAR. This is in line with a previous report in which OATP1A4 expression was reduced as a consequence of a reduced CAR expression in a mouse model of chronic arthritis (Kawase et al., 2007). The results, however, contrast with previous findings in mice in which phenobarbital-like inducers rather suppressed OATP1A1 and OATP1A4 expressions while PPARα agonists did not affect expression at all (Cheng et al., 2005; Moffit et al., 2006). The expression of the putative T3 transporter MCT-8 (Jansen et al., 2005) was not affected by either treatment (not shown).

Phase II enzymes and deiodinases involved in hepatic thyroid hormone inactivation

Members of the UGT1A family, which catalyze the glucuronidation of thyroid hormones, have previously been identified as potential CAR

Table 2
Enhancement by the PPARα agonist WY14643 of the phenobarbital-dependent induction of CAR target genes.

	CYP2B activity (pmol mg protein^{-1} min^{-1})	CYP2B1 mRNA (% of PB)	GSTA2 mRNA (% of PB)
Control	n.d.	28 ± 15	5.8 ± 0.8
PB	21.1 ± 5.99[a]	100 ± 0[a]	100 ± 0[a]
Wy 14643	n.d.	37 ± 17	8.4 ± 1.1
PB+ Wy 14643	42.76 ± 11.76[a,b]	292 ± 85[a,b]	214 ± 29[a,b]
GW 7647	n.d.	29 ± 14	n.t.
PB+ GW 7647	44.97 ± 11.12[a,b]	249 ± 92[a,b]	n.t.

Primary rat hepatocytes were isolated and cultured for 72 h as described in Materials and methods. Culture medium contained 1 mM phenobarbital for the last 48 h of culture and 10 μM WY 14643 or 1 μM GW7647 for the last 24 h of culture, as indicated. At the end of the culture period cells were harvested. CYP2B activity was determined fluorometrically, CYP2B1 mRNA by Northern blot and GSTA2 mRNA by qPCR as described in Materials and methods. Values are means ± SEM of at least 3 independent cell preparations. Statistics: Student's *t*-test, a: significantly above control ($p<0.05$); b: significantly above PB-treated cells ($p<0.05$).

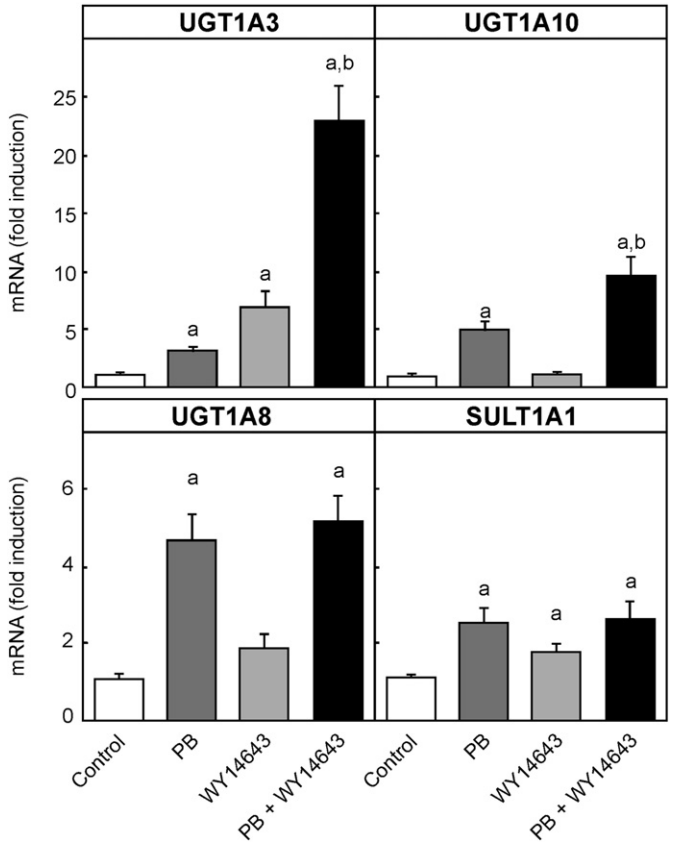

Fig. 5. Enhancement of the phenobarbital-dependent induction of UGT1A3 and UGT1A10 but not UGT1A8 and SULT1A1 mRNA by the PPARα agonist WY14643. Hepatocytes were cultured as described in the legend to Fig. 1. After 72 h cells were harvested and the mRNA content was determined by qPCR as described in Materials and methods. Values are means ± SD of 16 to 18 determinations with three independent cell preparations. Statistics: Student's *t*-test for unpaired samples. a: significantly higher than control, ($p<0.05$); b: significantly higher than in cultures with either inducer alone ($p<0.05$).

target genes (Sugatani et al., 2001). Of these, *UGT1A3*, *UGT1A8* and *UGT1A10* appear to be particularly relevant in T3 glucuronidation, according to a study with recombinant human enzymes (Tong et al., 2007). In rat hepatocytes *UGT1A3* was induced about 3-fold by phenobarbital and about 7-fold by the PPARα agonist WY14643 (Fig. 5). When both agonists were combined, the effect was clearly more than additive and resulted in an over 20-fold induction of *UGT1A3*. Similar results were obtained with the structurally unrelated PPARα agonist GW7447, yet the induction was somewhat weaker (GW7647 3.7 ± 1.0-fold, GW7647 + phenobarbital 11.0 ± 1.4-fold, not shown). *UGT1A10* was induced by phenobarbital about 5-fold. This induction was enhanced about two-fold by the simultaneous presence of the PPARα-agonist WY14643, while WY14643 did not induce *UGT1A10* by itself (Fig. 5). The expression of *UGT1A10* was thus regulated in a manner similar to the prototypical CAR target gene *CYP2B1*. By contrast, the phenobarbital-dependent induction of *UGT1A8* was not enhanced by WY14643, which by itself induced *UGT1A8* only marginally. In an UDP-glucuronosyl transferase assay that does not discriminate between the different isoforms, phenobarbital-induced UGT-activity about 1.5-fold. While neither WY14643 nor GW7647 induced UGT-activity by themselves, both PPARα agonists significantly enhanced the phenobarbital-dependent induction (Fig. 6).

The mRNA of SULT1A1, which catalyzes the sulfation of T4 and prevents its conversion into T3 by outer ring deiodination, was induced by both phenobarbital and WY14643 about two-fold and 1.5-fold, respectively. However, when present simultaneously, the two substances did not synergize to cause a stronger induction than either substance alone (Fig. 5). In contrast with the mRNA data sulfotransferase activity was enhanced neither by phenobarbital nor by the PPARα agonists nor by a combination of both (not shown).

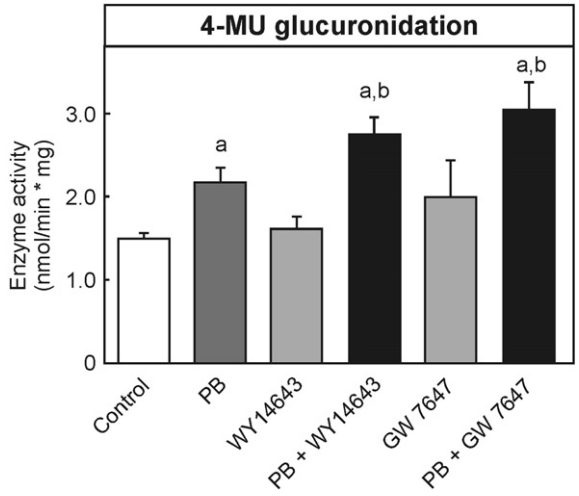

Fig. 6. Enhancement of the phenobarbital-dependent induction of UDP-glucuronosyl transferase activity by the PPARα agonists WY14643 and GW7647. Hepatocytes were cultured in the presence of 1 mM phenobarbital, 10 μM WY14643 1 μM GW7647 alone or in combination as described in the legend to Fig. 1. After 72 h cells were harvested and a crude microsomal fraction was prepared as detailed in Materials and methods. UDP-glucuronosyl transferase activity was determined fluorometrically with the substrate 4-methylumbelliferone (4-MU). Values are means ± SEM of 3 to 6 independent cell preparations. Statistics: Student's *t*-test for unpaired samples. a: significantly higher than control, ($p<0.05$); b: significantly higher than in cultures with either inducer alone ($p<0.05$).

Deiodinases (DIO) are involved in a dual manner in thyroid hormone metabolism in hepatocytes. DIO-1 can remove an iodine from the outer ring of T4 to yield the more active T3. It may also remove an iodine from the inner ring to yield the inactive rT3 or degrade inactive rT3 to T2. Inner ring deiodination is favored by prior sulfation of the phenolic OH-group of T4 by sulfotransferases. DIO-3 in turn, which is also expressed in extrahepatic tissues, can inactivate T3 by deiodination to yield the inactive T2 or can convert T4 to the inactive rT3. mRNAs of both enzymes were detected in rat hepatocytes. In line with recent *in vivo* findings in regenerating mouse liver (Tien et al., 2007) DIO-1 expression was induced about 2-fold by

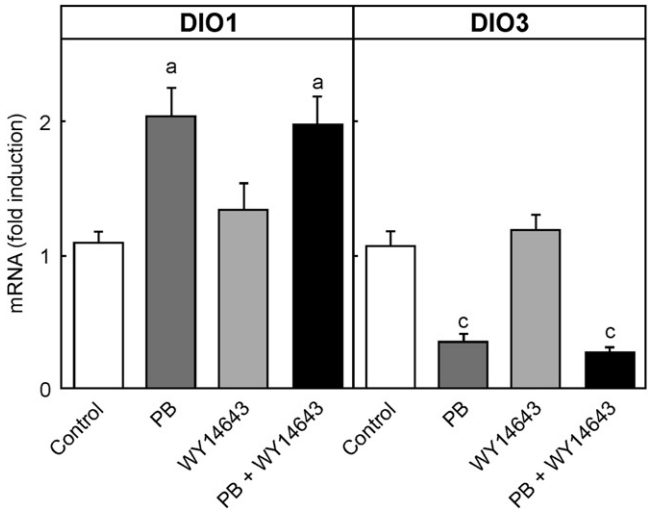

Fig. 7. Antidromic regulation of the expression of deiodinase-1 and deiodinase-3 by phenobarbital in hepatocytes. Hepatocytes were cultured as described in the legend to Fig. 1. After 72 h cells were harvested and the mRNA content was determined by qPCR as described in Materials and methods. Values are means ± SD of 16 to 18 determinations with three independent cell preparations. Statistics: Student's *t*-test for unpaired samples. a: significantly higher than control, ($p<0.05$); c: significantly lower than control ($p<0.05$).

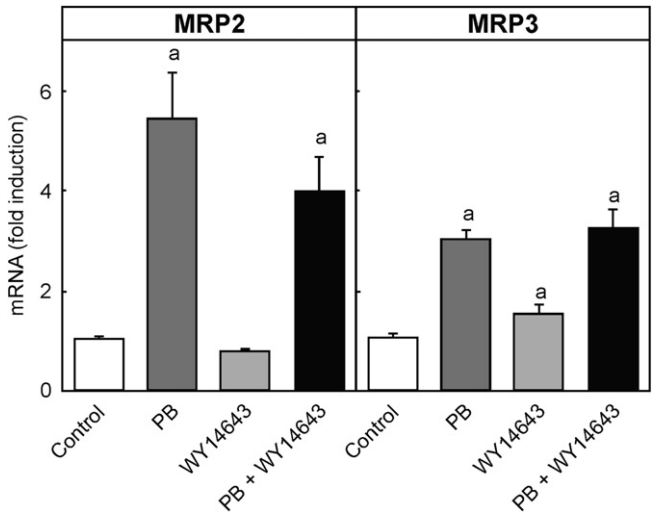

Fig. 8. Induction of conjugate export pumps in hepatocytes by phenobarbital and WY14643. Hepatocytes were cultured as described in the legend to Fig. 1. After 72 h cells were harvested and the mRNA content was determined by qPCR as described in Materials and methods. Values are means ± SD of 16 to 18 determinations with three independent cell preparations. Statistics: Student's *t*-test for unpaired samples. a: significantly higher than control, ($p<0.05$).

phenobarbital. By contrast, DIO-3 mRNA was suppressed to about 25% of the basal expression by phenobarbital (Fig. 7). WY 14643 did not affect the mRNA level of either enzyme, nor did it impact the phenobarbital-dependent modulation of the expression.

Transporters involved in excretion of thyroid hormone conjugates

Thyroid hormone conjugates are exported from the hepatocyte by export carriers of the MRP family. While MRP2 is responsible for the biliary excretion of conjugates, MRP1 and MRP3 are located at the basolateral membrane and mediate export into the blood for subsequent renal excretion. The expression of MRP1 was not affected by phenobarbital or WY14643 either alone or in combination (not shown). MRP2 mRNA was up-regulated about 5-fold by phenobarbital, whereas WY14643 did not affect its expression. Though there was a tendency towards an inhibition of the phenobarbital-dependent induction of MRP2 by the PPARα agonist, this was not significant. MRP3 mRNA was induced about 3-fold by phenobarbital and about 1.5-fold by the PPARα agonist (Fig. 8). There was, however, no synergism between the two inducers.

Discussion

The current study showed that the PPARα agonist WY14643 induced the nuclear receptor CAR and potentiated the phenobarbital-dependent induction of a number of enzymes and transporters involved in hepatic thyroid hormone inactivation and elimination, thereby reducing the half-life of T3 synergistically with phenobarbital. While both phenobarbital-like inducers or PPARα-agonists have previously been reported to affect the thyroid hormone status in rodents (Hood and Klaassen, 2000), dogs (Gaskill et al., 1999), pigs (Luci et al., 2006) and humans (Benedetti et al., 2005; Castro-Gago et al., 2007), to our knowledge this is the first time that a synergism between PPARα-agonists and phenobarbital-like inducers in the induction of thyroid hormone elimination has been reported.

Possible physiological consequences of the observed synergism for CAR-dependent fasting adaptation

Both CAR activation with phenobarbital and PPARα activation with WY14643 resulted in an increase in the T3 elimination rate in rat hepatocyte cultures (Fig. 1). With the notable exception of deiodinase 3, phenobarbital induced the mRNAs of all enzymes involved in the thyroid hormone degradation and conjugation (Figs. 5 and 7) as well as two uptake and two export carriers involved in thyroid hormone metabolism (Figs. 4 and 8). By contrast, the PPARα agonist induced only UGT1A3 and SULT1A1 as well as the export carrier MRP3. This might explain why the reduction of T3 half-life was somewhat less pronounced in WY14643-treated than in phenobarbital-treated hepatocytes (Fig. 1). WY14643 enhanced the phenobarbital-dependent induction of a number of enzymes. Importantly, the mRNA levels of several of these enzymes were not affected by the PPARα agonist alone (i. e. OATP1A1, OATP1A4 and UGT1A10), indicating that the promoters of these enzymes do not have a PPRE, and the synergism was rather a consequence of the PPARα-dependent induction of CAR (Wieneke et al., 2007). For UGT1A3, which was induced by either phenobarbital or WY14643 alone, the combination of both agonists resulted in an over-additive induction, also pointing to the possibility that in addition to the local cooperation of PPARα and CAR at the promoter, the PPARα-dependent induction of CAR might contribute to the synergistic induction. Notably, total UGT-activity was enhanced stronger in hepatocytes treated with a combination of phenobarbital and PPARα agonists than with either agent alone (Fig. 6). This resulted in an apparently more efficient glucuronidation of T3 in these hepatocyte cultures (Fig. 2). The apparent rate of T3 glucuronidation was inversely correlated with the apparent T3 half-life. The synergistic induction of the thyroid hormone-metabolizing enzymes resulted in a faster rate of T3 degradation than observed after either agonist alone (Fig. 1). This might be of physiological significance: the nuclear receptor CAR has recently been discussed as an important player in the control of energy metabolism (Konno et al., 2008). CAR-deficient mice suffered a more pronounced weight loss upon calorie restriction than their wild type sibling littermates. Among other mechanisms CAR appeared to act via the induction of enzymes involved in the thyroid hormone degradation (Maglich et al., 2004). Although the endogenous activator for fasting-induced CAR-dependent induction of phase II and III enzymes that confer thyroid hormone inactivation and excretion is still enigmatic, fasting has been shown to induce a number of these enzymes in a CAR-dependent manner. Fasting is also accompanied by an increase in the concentration of free fatty acids, which are endogenous ligands for PPARα. We have recently shown that CAR was induced by fasting in mice in a PPARα-dependent manner (Wieneke et al., 2007). Thus, the synergistic induction of thyroid hormone inactivating enzymes by CAR activators and PPARα agonists described here might be the basis for an efficient induction of thyroid hormone-metabolizing enzymes during fasting.

Interference between xenobiotic and thyroid hormone metabolism

In several animal models chronic treatment with phenobarbital or related drugs resulted in a CAR-dependent reduction of circulating T4 and an increase in TSH levels (Qatanani et al., 2005), while only some studies also observed a decrease in T3. Similarly, chronic treatment with antiepileptic drugs that induce phase II enzymes resulted in a mild hypothyroidism in humans (Benedetti et al., 2005; Eiris-Punal et al., 1999; Yuksel et al., 1993). Apparently, an activation of the hypothalamic–pituitary axis could only partially compensate for the enhanced thyroid hormone elimination. While an elevation of TSH in response to CAR activation has clearly been shown in mice (Qatanani et al., 2005), conflicting results have been obtained in patients under antiepileptic therapy (Benedetti et al., 2005; Cunha and van Ravenzwaay, 2005). In general, the impact of an increase in thyroid hormone degradation on circulating thyroid hormone level appears to be less pronounced in humans than in rodents (Wu and Farrelly, 2006). This issue is of considerable importance since elevated TSH levels, as a consequence of enzyme induction with phenobarbital-like

inducers, may elicit a hyperproliferation of thyroid tissue and promote the development of thyroid tumors in rodents (McClain, 1992; Finch et al., 2006) while the relevance of enzyme induction in thyroid tumor development has been disputed for humans (Wu and Farrelly, 2006). The synergism between phenobarbital and PPARα agonists in the induction of thyroid hormone-metabolizing enzymes reported here might, however, be of significance as drug–drug interaction in patients receiving both e.g. fibrates and CAR-activating drugs, although species differences in the sensitivity towards PPARα-agonists clearly limit the feasibility of such an extrapolation.

Possible toxicologic implications of the observed synergism

The observed synergism between phenobarbital and the PPARα agonist WY14643 can serve as a paradigm for the interaction of other xenobiotics that act as activators of CAR and PPARα, respectively. Thus, the environmental pollutant 2,2′,4,4′-tetrabromodiphenyl ether (BDE47), a widely used fire retardant that can be detected in many human samples, has been reported to reduce circulating thyroid hormone levels in mice, likely by inducing genes involved in thyroid hormone metabolism in a CAR-dependent manner (Richardson et al., 2008). However, doses required to interfere with thyroid hormone metabolism far exceed the general pollution-derived burden. Yet pollutants are not normally ingested as pure compounds but rather in a complex mixture. Pthalates and organotins are two other major groups of xenobiotics to which humans are frequently exposed. Both have been described as ligands for PPARs. As an example metabolites of di-(2-ethylhexyl)-phthalate (DEHP), which is an industrial plasticizer found in significant levels in human blood or urine samples, have been shown to act as PPARα agonist, to activate both human and rodent PPARα (Maloney and Waxman, 1999) and to induce PPARα-dependent genes (Ito et al., 2007). It seems possible that a similar synergism as observed for phenobarbital and WY14643 exists between environmental pollutants that act as phenobarbital-like inducers and those that act as PPARα agonists. As a consequence, much lower doses of both pollutants in combination might be needed to elicit endocrine disruption than if either compound acts alone.

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References

Aubrecht, J., Kahl, G.F., Hohne, M.W., 1993. Regulation of fibronectin mRNA expression in primary hepatocytes in response to EGF and phenobarbital. Biochem. Biophys. Res. Commun. 190, 1023–1028.
Beckmann, J.D., 1991. Continuous fluorometric assay of phenol sulfotransferase. Anal. Biochem. 197, 408–411.
Benedetti, M.S., Whomsley, R., Baltes, E., Tonner, F., 2005. Alteration of thyroid hormone homeostasis by antiepileptic drugs in humans: involvement of glucuronosyltransferase induction. Eur. J. Clin. Pharmacol. 61, 863–872.
Bianco, A.C., Salvatore, D., Gereben, B., Berry, M.J., Larsen, P.R., 2002. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocr. Rev. 23, 38–89.
Castro-Gago, M., Novo-Rodriguez, M.I., Gomez-Lado, C., Rodriguez-Garcia, J., Rodriguez-Segade, S., Eiris-Punal, J., 2007. Evolution of subclinical hypothyroidism in children treated with antiepileptic drugs. Pediatr. Neurol. 37, 426–430.
Cheng, X., Maher, J., Dieter, M.Z., Klaassen, C.D., 2005. Regulation of mouse organic anion-transporting polypeptides (Oatps) in liver by prototypical microsomal enzyme inducers that activate distinct transcription factor pathways. Drug. Metab. Dispos. 33, 1276–1282.
Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162, 156–159.
Collier, A.C., Tingle, M.D., Keelan, J.A., Paxton, J.W., Mitchell, M.D., 2000. A highly sensitive fluorescent microplate method for the determination of UDP-glucuronosyl transferase activity in tissues and placental cell lines. Drug. Metab. Dispos. 28, 1184–1186.
Cunha, G.C., van Ravenzwaay, B., 2005. Evaluation of mechanisms inducing thyroid toxicity and the ability of the enhanced OECD Test Guideline 407 to detect these changes. Arch. Toxicol. 79, 390–405.
Eiris-Punal, J., Del Rio-Garma, M., Del Rio-Garma, M.C., Lojo-Rocamonde, S., Novo-Rodriguez, I., Castro-Gago, M., 1999. Long-term treatment of children with epilepsy with valproate or carbamazepine may cause subclinical hypothyroidism. Epilepsia. 40, 1761–1766.
Finch, J.M., Osimitz, T.G., Gabriel, K.L., Martin, T., Henderson, W.J., Capen, C.C., Butler, W.H., Lake, B.G., 2006. A mode of action for induction of thyroid gland tumors by pyrethrins in the rat. Toxicol. Appl. Pharmacol. 214, 253–262.
Findlay, K.A., Kaptein, E., Visser, T.J., Burchell, B., 2000. Characterization of the uridine diphosphate-glucuronosyltransferase-catalyzing thyroid hormone glucuronidation in man. J. Clin. Endocrinol. Metab. 85, 2879–2883.
Gaskill, C.L., Burton, S.A., Gelens, H.C., Ihle, S.L., Miller, J.B., Shaw, D.H., Brimacombe, M.B., Cribb, A.E., 1999. Effects of phenobarbital treatment on serum thyroxine and thyroid-stimulating hormone concentrations in epileptic dogs. J. Am. Vet. Med. Assoc. 215, 489–496.
Grun, F., Blumberg, B., 2007. Perturbed nuclear receptor signaling by environmental obesogens as emerging factors in the obesity crisis. Rev. Endocr. Metab. Disord. 8, 161–171.
Hirsch-Ernst, K.I., Schlaefer, K., Bauer, D., Heder, A.F., Kahl, G.F., 2001. Repression of phenobarbital-dependent CYP2B1 mRNA induction by reactive oxygen species in primary rat hepatocyte cultures. Mol. Pharmacol. 59, 1402–1409.
Hood, A., Klaassen, C.D., 2000. Differential effects of microsomal enzyme inducers on in vitro thyroxine (T(4)) and triiodothyronine (T(3)) glucuronidation. Toxicol. Sci. 55, 78–84.
Ito, Y., Yamanoshita, O., Kurata, Y., Kamijima, M., Aoyama, T., Nakajima, T., 2007. Induction of peroxisome proliferator-activated receptor alpha (PPARalpha)-related enzymes by di(2-ethylhexyl) phthalate (DEHP) treatment in mice and rats, but not marmosets. Arch. Toxicol. 81, 219–226.
Jansen, J., Friesema, E.C., Milici, C., Visser, T.J., 2005. Thyroid hormone transporters in health and disease. Thyroid. 15, 757–768.
Joosen, A.M., Westerterp, K.R., 2006. Energy expenditure during overfeeding. Nutr. Metab. (Lond). 3, 25.
Kawase, A., Tsunokuni, Y., Iwaki, M., 2007. Effects of alterations in CAR on bilirubin detoxification in mouse collagen-induced arthritis. Drug. Metab. Dispos. 35, 256–261.
Konno, Y., Negishi, M., Kodama, S., 2008. The roles of nuclear receptors CAR and PXR in hepatic energy metabolism. Drug. Metab. Pharmacokinet. 23, 8–13.
Leibel, R.L., Rosenbaum, M., Hirsch, J., 1995. Changes in energy expenditure resulting from altered body weight. N. Engl. J. Med. 332, 621–628.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.
Lubet, R.A., Mayer, R.T., Cameron, J.W., Nims, R.W., Burke, M.D., Wolff, T., Guengerich, F.P., 1985. Dealkylation of pentoxyresorufin: a rapid and sensitive assay for measuring induction of cytochrome(s) P-450 by phenobarbital and other xenobiotics in the rat. Arch. Biochem. Biophys. 238, 43–48.
Luci, S., Kluge, H., Hirche, F., Eder, K., 2006. Clofibrate increases hepatic triiodothyronine (T3)-and thyroxine (T4)-glucuronosyltransferase activities and lowers plasma T3 and T4 concentrations in pigs. Drug. Metab. Dispos. 34, 1887–1892.
Maglich, J.M., Watson, J., McMillen, P.J., Goodwin, B., Willson, T.M., Moore, J.T., 2004. The nuclear receptor CAR is a regulator of thyroid hormone metabolism during caloric restriction. J. Biol. Chem. 279, 19832–19838.
Maloney, E.K., Waxman, D.J., 1999. trans-Activation of PPARalpha and PPARgamma by structurally diverse environmental chemicals. Toxicol. Appl. Pharmacol. 161, 209–218.
McClain, R.M., 1992. Thyroid gland neoplasia: non-genotoxic mechanisms. Toxico. Lett. 65, 397–408.
Moffit, J.S., Aleksunes, L.M., Maher, J.M., Scheffer, G.L., Klaassen, C.D., Manautou, J.E., 2006. Induction of hepatic transporters multidrug resistance-associated proteins (Mrp) 3 and 4 by clofibrate is regulated by peroxisome proliferator-activated receptor alpha. J. Pharmacol. Exp. Ther. 317, 537–545.
Omiecinski, C.J., Walz Jr., F.G., Vlasuk, G.P., 1985. Phenobarbital induction of rat liver cytochromes P-450b and P-450e. Quantitation of specific RNAs by hybridization to synthetic oligodeoxyribonucleotide probes. J. Biol. Chem. 260, 3247–3250.
Püschel, G.P., Kirchner, C., Schröder, A., Jungermann, K., 1993. Glycogenolytic and antiglycogenolytic prostaglandin E2 actions in rat hepatocytes are mediated via different signalling pathways. Eur. J. Biochem. 218, 1083–1089.
Qatanani, M., Zhang, J., Moore, D.D., 2005. Role of the constitutive androstane receptor in xenobiotic-induced thyroid hormone metabolism. Endocrinology. 146, 995–1002.
Richardson, V.M., Staskal, D.F., Ross, D.G., Diliberto, J.J., DeVito, M.J., Birnbaum, L.S., 2008. Possible mechanisms of thyroid hormone disruption in mice by BDE 47, a major polybrominated diphenyl ether congener. Toxicol. Appl. Pharmacol. 226, 244–250.
Rootwelt, K., Ganes, T., Johannessen, S.I., 1978. Effect of carbamazepine, phenytoin and phenobarbitone on serum levels of thyroid hormones and thyrotropin in humans. Scand. J. Clin. Lab. Invest. 38, 731–736.
Sugatani, J., Kojima, H., Ueda, A., Kakizaki, S., Yoshinari, K., Gong, Q.H., Owens, I.S., Negishi, M., Sueyoshi, T., 2001. The phenobarbital response enhancer module in the human bilirubin UDP-glucuronosyltransferase *UGT1A1* gene and regulation by the nuclear receptor CAR. Hepatology. 33, 1232–1238.
Tien, E.S., Matsui, K., Moore, R., Negishi, M., 2007. The nuclear receptor constitutively active/androstane receptor regulates type 1 deiodinase and thyroid hormone activity in the regenerating mouse liver. J. Pharmacol. Exp. Ther. 320, 307–313.

Tong, Z., Li, H., Goljer, I., McConnell, O., Chandrasekaran, A., 2007. In vitro glucuronidation of thyroxine and triiodothyronine by liver microsomes and recombinant human UDP-glucuronosyltransferases. Drug. Metab. Dispos. 35, 2203–2210.

Ueda, A., Hamadeh, H.K., Webb, H.K., Yamamoto, Y., Sueyoshi, T., Afshari, C.A., Lehmann, J.M., Negishi, M., 2002. Diverse roles of the nuclear orphan receptor CAR in regulating hepatic genes in response to phenobarbital. Mol. Pharmacol. 61, 1–6.

Vansell, N.R., Klaassen, C.D., 2002. Increase in rat liver UDP-glucuronosyltransferase mRNA by microsomal enzyme inducers that enhance thyroid hormone glucuronidation. Drug. Metab. Dispos. 30, 240–246.

Visser, T.J., 1994. Role of sulfation in thyroid hormone metabolism. Chem. Biol. Interact. 92, 293–303.

Visser, T.J., Kaptein, E., van Toor, H., van Raaij, J.A., van den Berg, K.J., Joe, C.T., van Engelen, J.G., Brouwer, A., 1993. Glucuronidation of thyroid hormone in rat liver: effects of in vivo treatment with microsomal enzyme inducers and in vitro assay conditions. Endocrinology. 133, 2177–2186.

Wieneke, N., Hirsch-Ernst, K.I., Kuna, M., Kersten, S., Puschel, G.P., 2007. PPARalpha-dependent induction of the energy homeostasis-regulating nuclear receptor NR1i3 (CAR) in rat hepatocytes: potential role in starvation adaptation. FEBS. Lett. 581, 5617–5626.

Wu, K.M., Farrelly, J.G., 2006. Preclinical development of new drugs that enhance thyroid hormone metabolism and clearance: inadequacy of using rats as an animal model for predicting human risks in an IND and NDA. Am. J. Ther. 13, 141–144.

Yuksel, A., Kartal, A., Cenani, A., Yalcin, E., 1993. Serum TH and pituitary response to thyrotropin-releasing hormone in epileptic children receiving anti-epileptic medication. Acta. Paediatr. Jpn. 35, 108–112.