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Influence of vitamin A status on the regulation of uridine (5′-)diphosphate-glucuronosyltransferase (UGT) 1A1 and UGT1A6 expression by L-triiodothyronine

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The uridine (5′-)diphosphate-glucuronosyltransferases (UGT) are involved in the phase II of various xenobiotics and endogenous compounds. They are responsible for glucuronidation of many substrates, especially including bilirubin (UGT1A1) and phenolic compounds (UGT1A6). We previously showed that the expression of both isoforms is regulated at the transcriptional level by thyroid hormone in rat liver. In this present study, effects of vitamin A dietary intake (0, 1·72, 69 ug retinol acetate/g food) on the regulation of UGT1A1 and UGT1A6 activity and expression by 3,5,3′ triiodo-L-thyronine (L-T3) were examined in the same organ. Activities were determined toward bilirubin and 4-nitrophenol. UGT mRNA were analysed by reverse transcription and amplification methods (reverse transcription–polymerase chain reaction) and quantified by capillary electrophoresis. In rats fed a vitamin A-balanced diet, a single injection of L-T3 (500 μg/kg body weight) increased UGT1A6 mRNA expression whereas this hormone decreased UGT1A1 mRNA expression. In addition we observed that the specific effect of L-T3 on UGT1A1 and UGT1A6 was reduced in animals receiving a vitamin A-enriched diet and disappeared in those fed a vitamin A-free diet. The modulations observed in mRNA expression are concomitant with those found for UGT activities. Our results demonstrate for the first time the existence of a strong interaction between vitamin A and thyroid hormone on the regulation of genes encoding cellular detoxification enzymes, in this case the UGT.

Vitamin A: Uridine (5′-)diphosphate-glucuronosyltransferase: Thyroid hormone

Uridine (5′-)diphosphate-glucuronosyltransferases (UGT), which are encoded by a superfamily of genes, catalyse the binding of glucuronic acid, from uridine (5′-)diphosphate-glucuronic acid, on numerous xenobiotics or endogenous compounds, including bilirubin, bile acids, steroids, thyroxine, fat-soluble vitamins and retinoids (Mackenzie *et al.* 1997). This reaction leads to water soluble, generally inactive glucuronides. UGT play a key role in the concentration of endogenous compounds important for cell differentiation and growth. Many of these compounds regulate UGT expression. We have shown that the thyroid hormones, 3,5,3′ triiodo-L-thyronine (L-T3) and L-thyroxine, which are substrates of this enzyme (Kostner *et al.* 1990; De Sandro *et al.* 1992; Visser *et al.* 1993) specifically modulate the expression and activity of some UGT isoforms (Goudonnet *et al.* 1990). In a recent study (Masmoudi *et al.* 1996), we found that treatment of rats with L-T3 differentially affected the expression of UGT1A1 and UGT1A6, which catalyse glucuronidation of bilirubin and phenols respectively. L-T3 significantly increased the mRNA encoding UGT1A6, but decreased that of UGT1A1 in rat liver, whereas the opposite situation was observed in thyroidectomised rats (Masmoudi *et al.* 1997*a*). Thyroid hormones regulate the gene expression of UGT1A1 and UGT1A6 at the transcriptional level without affecting the half-lives of their mRNA. This regulation requires a *de novo* protein biosynthesis (Masmoudi *et al.* 1997*b*). The mechanism whereby L-T3 exerts an opposite effect on the gene encoding UGT1A1 and UGT1A6 is still unknown, but previous studies revealed that, upon ligand binding, intracellular receptors for hormones act as dimeric transcriptional factors to activate or repress the expression of nuclear target genes by binding to specific DNA sequences (Bendik & Pfahl, 1994). Moreover, the thyroid hormone receptors (TR) can form heterodimers with retinoid X receptors (RXR) (Zhang *et al.* 1992). Thus,

Abbreviations: L-T3, 3,5,3′ triiodo-L-thyronine; RXR, retinoid x receptor; TR, thyroid hormone receptor; UGT, uridine (5′-)diphosphate-glucuronosyltransferase

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retinoic acids (the active metabolites of vitamin A) may be implicated in the regulating effects of L-T3 by heterodimerising TR.

In fact, little is known about the effects of vitamin A diet on UGT activity and expression (Ratanasavanh, 1990) and even less about its influence on the UGT regulation by L-T3. However, several studies revealed the importance of dietary vitamin A on the activity and the regulation of gene expression. Specifically, vitamin A deficiency led to an increase in protein kinase C activity (Pailler-Rodde *et al.* 1991*a*), whereas the gene expression of glucokinase was induced in hepatocytes treated by retinoic acid (Decaux *et al.* 1997).

Our working hypothesis was to consider that vitamin A (via retinoic acid) may be a putative regulator of UGT expression. The purpose of this present study was to investigate the role of vitamin A on the regulation of UGT1A1 and UGT1A6 expression by L-T3.

Materials and methods

Animals and diets

The official French regulations for the care and use of laboratory animals were followed. Male Wistar rats weighing 40 g were obtained from Iffa-credo (L'Arbresle, France). They were housed in an air-conditioned room at a temperature of 21°C, with a 12 h light–day cycle. Animals had free access to drinking water and to semi-purified diets prepared in our laboratory according to Potier *et al.* (1989). The vitamin A-free diet contained (g/kg dry diet): vitamin-free casein 180, sucrose 220, corn oil 50, cellulose 20, maize starch 480, salt mixture 40, vitamin mixture depleted of vitamin A 10 (Atelier de Préparation d'Aliments Expérimentaux, INRA, Jouy-en-Josas, Domaine de Vilvert, France; ref. no.102).

For vitamin A-balanced and vitamin A-overloaded diets, the diet was supplemented with 1·72 ug or 69 ug retinol acetate (Merck, Nogent sur Marne, France) per g diet respectively.

Seventy-two animals were randomly divided into three groups of twenty-four animals designed as vitamin A-deficient, vitamin A-overloaded, and vitamin A-adequate. Five weeks after the beginning of the experiment, rats of each group were given a single dose of L-T3 (sodium salt, T 2752; 500 μg/kg body weight; Sigma, Saint Quentin Fallavier, France) by intraperitoneal injection, and killed by decapitation 6, 12, 16, 20, 24, 36, 48 h after injection (three different rats per kinetic point). Three control rats per diet were injected with the vehicle alone (9 g NaCl/l).

Assays

Vitamin A concentrations in serum and liver homogenates were assayed by HPLC (Millennium 2010; Waters, Saint Quentin en Yvelines, France), according to a technique described by Jezequel-Cuer *et al.* (1995), using tocol (Roche, Basel, Switzerland) as an internal standard. Concentration was expressed as ng retinol/mg phospholipid. Phospholipids were measured with a fast centrifuge analyser (Cobas Bio; Roche, Basel, Switzerland) with the PAP 150 kit from Biomerieux (Marcy l'étoile, France). Liver microsomes were prepared according to Hogeboom (1955). Their protein content was measured by the technique of Lowry *et al.* (1951) with the fast centrifuge analyser using bovine serum albumin as a standard. UGT activity toward bilirubin was determined according to Heirweigh *et al.* (1972), and that toward 4-nitrophenol by the method of Mulder & Van Doorn (1975) as modified by Colin-Neiger *et al.* (1984).

Quantification of mRNA

mRNA was quantified by reverse transcription and amplification by the polymerase chain reaction. The amounts of the different UGT isoform mRNA were evaluated by comparison with those of an internal standard, β-actin mRNA (A_{UGT}/A_{act}), which is known to be insensitive to nutritional and hormonal conditions (Mitsuhashi & Nikodem 1989; Ma *et al.* 1990). The standard sequence controls for variables such as amount and 'amplifiability' of DNA, and for tube-to-tube variation in amplification efficiency. β-Actin mRNA was simultaneously co-amplified in the same test-tube, and semi-quantitative analysis was performed as previously described (Masmoudi *et al.* 1996, 1997*b*). Extraction of total RNA was performed using RNAXEL kit (Eurobio, Les Ulis, France) according to the protocol suggested by the supplier. cDNA was made by reverse-transcribing 1 μg total RNA for 60 min at 37°C using random primers. 10 μl cDNA were then used for amplification in a Minicycler MJ Research (Watertown, MA, USA). The reaction mixture contained 20 mM-Tris-HCl buffer (pH 8·5), 16 mM-$(NH_4)_2SO_4$, 150 μg bovine serum albumin/ml, 0·2 mM each dNTP, 2U Taq polymerase (Eurobio, Les Ulis, France), 1·5 mM-$MgCl_2$, 25 pmol each β-actin primer and 50 pmol UGT1A1 or UGT1A6 primer in a total volume of 50 μl. The annealing temperatures were optimised at 60°C for bilirubin UGT and 55°C for 4-nitrophenol UGT. The number of cycles were determined to obtain the values of target sequences and β-actin before the amplification reactions reached the plateau phase. Absence of genomic contamination was verified by using a control tube supplemented with 10 μg RNase. The sequence of the primers used is indicated in Table 1. Both primers were 100 % homologous with the sequences described by Sato *et al.* (1990) and Iyanagi *et al.* (1986) and were chosen in

Table 1. Sequences of oligonucleotide primers for β-actin, uridine (5′-)diphosphate-glucuronosyltransferase (UGT) 1A1 and UGT1A6

Primers	Sequences 5′3′	Complementary site
β-Actin*	TGCAGAAGGAGATTACTGCC	2818 to 2837
	CGCAGCTCAGTAACAGTCC	3153 to 3135
UGT1A1†	GAAGAATATCAGCGGGAAATA	250 to 270
	CGGACATTGTGTAGCCTCA	555 to 537
UGT1A6‡	TTGCCTTCTTCCTGCTGC	6 to 23
	TCTGAAGAGGTAGATGGAAGGC	513 to 492

* From rat cytoplasmic β-actin gene according to the sequence of Nudel *et al.* (1983).
† From rat liver gene according to the sequence of Sato *et al.* (1990).
‡ From rat liver gene according to the sequence of Iyanagi *et al.* (1986).

order to avoid any crosslinking with other known sequences recorded in data bank. The specificity of polymerase chain reaction products obtained was verified by restriction site analysis: the 303 base pairs bilirubin UGT and the 507 base pairs nitrophenol were digested with *Alu*I-*Eco*RI and *Hin*dIII-*Pvu*II respectively. Co-amplified fragments were separated by capillary electrophoresis and quantified by laser-induced fluorescence.

Capillary electrophoresis conditions

Capillary electrophoresis is a useful method to separate amplicons generated by reverse transcription–polymerase chain reaction. Thus, much research has focused on the analysis of polymerase chain reaction products (Heiger *et al.* 1990; Schwartz *et al.* 1991; Nesi *et al.* 1994) in the size range 100–1000 base pairs. This technique was used for that purpose with a P/ACE System 5010 (Beckman Instruments, Inc., Fullerton, CA, USA). The amplicons were detected by a laser-induced fluorescence detector at excitation and emission wavelengths of 488 and 530 nm respectively. Injection was carried out electrokinetically for 70 s at 8 kV. Separations were carried out at a constant voltage (10 kV, reverse polarity). The fused-silica capillary (50 μm internal diameter, and 47 cm total length, eCap™ capillary tubing; Beckman Instruments Inc.) was kept constantly at 45°C. The capillary was conditioned with eCAP dsDNA 1000 gel buffer which contained 10 μl Lifluor dsDNA 1000 EnhanceCE intercalator/20 ml (Beckman, Gagny, France). The capillary was rinsed with the gel buffer for 2 min before each injection. A B8 ladder (Boerhinger, Mannheim, Germany) was used to correlate migration time with DNA size. The peak areas were calculated using the Gold Chromatography data software 8.10 from Beckman.

Statistical data

For statistical comparisons, the data obtained in triplicate for groups of twenty-four animals were analysed by Student's *t* test. Results with $P < 0{\cdot}05$ were considered as significant.

Results

Concentration of retinol in serum and liver microsomes

As expected, rats fed a diet deficient in vitamin A for 5 weeks presented a serum retinol level 60 % lower than animals fed a balanced vitamin A diet (control diet). Treatment with L-T3 did not modify this concentration in rats fed the vitamin A-enriched diet (results not shown).

In liver microsomes, vitamin A-overloaded diet led to a 5-fold increase in the levels of retinol in comparison with results obtained with the control diet. Treatment by L-T3 increased the retinol concentration by 80 % in animals fed the balanced diet, and by 50 % in those receiving the vitamin A-enriched diet. Maximal effects appeared between 16 and 20 h after the injection of the hormone. Retinol was detected in very low amounts in microsomes from vitamin A-deficient rats (Table 2).

Effects of vitamin A intake and 3,5,3′ triiodo-L-thyronine administration on UDP-glucuronosyltransferase isoforms

Glucuronidation activity toward 1-naphthol and bilirubin. Fig. 1 presents the UGT activities measured with 1-naphthol and bilirubin as substrates in liver microsomes of rats fed the different vitamin A diets. We observed that rats fed a balanced or enriched diet exhibited no significant change on the glucuronidation of 1-naphthol. By contrast, this activity was enhanced in animals receiving the vitamin A-free diet. Glucuronidation of bilirubin was not significantly affected by the vitamin A status of the diets (Fig. 1).

Figs 2 and 3 indicate kinetics of UGT activities toward 1-naphthol and bilirubin, after an intraperitoneal single dose of L-T3 (500 μg/kg body weight). As shown in Fig. 2, the activity measured with 1-naphthol UGT depended on the vitamin A status. A tendancy increase in 1-naphthol conjugation was observed 12 h after the treatment in rats fed the balanced diet and 20 h in animals fed the vitamin A-overloaded diet. Maximal effect was observed at 36 h with both diets. After L-T3 injection, the activity was markedly increased by 2- and 3-fold in balanced and vitamin A-overloaded diets

Table 2. Effect of vitamin A concentration in the diet on retinol concentration in liver microsomes of rats‡
(Mean values with standard errors of the means for three independent determinations)

	Vitamin A concentration in liver microsomes (ng retinol/g phospholipid)					
Vitamin A diet…	Deficient		Balanced		Enriched	
Time (h)	Mean	SEM	Mean	SEM	Mean	SEM
0	0*	0	288	12	1558*	75
6	7	5	204	20	1302	56
12	0	0	211	17	1196	76
16	0	2	244	21	2169	92
20	2	1	390	22	2303†	57
24	0	3	519†	45	1286	65
36	8	5	230	25	1562	74
48	0	0	189	19	1422	83

Mean values were significantly different from rats fed the vitamin A-balanced diet (Student's *t* test):*$P < 0{\cdot}01$.
Mean values were significantly different from control rats (0 h) in the same group (Student's *t* test): †$P < 0{\cdot}01$.
‡ For details of diets and procedures see p. 290. The rats were treated at time 0 with a single dose of 3,5,3′ triiodo-L-thyronine (500 μg/kg body weight) and killed at the time indicated.

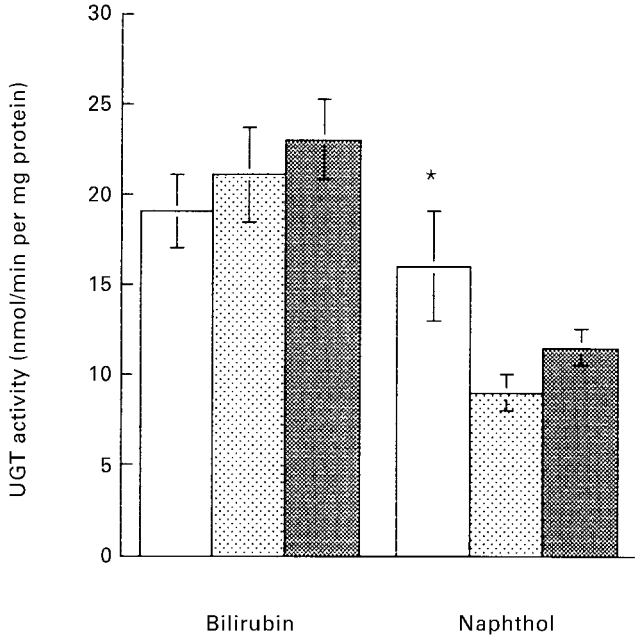

Fig. 1. Bilirubin and naphthol basal uridine (5′-)diphosphate-glucuronosyltransferase (UGT) activities in liver microsomes of rats fed vitamin A-free (□), vitamin A-balanced (▨) or vitamin A-rich (■) diets. For details of diets and procedures see p. 290. Values are means of three independent experiments with standard errors of the means shown by vertical bars. Mean value was significantly different from rats fed the vitamin A-balanced diet: $^{*}P < 0{\cdot}05$.

respectively. In vitamin A-deficient animals, L-T3 injection had no effect on 1-naphthol glucuronidation.

Similarly, UGT activity toward bilirubin was not modified in response to the administration of L-T3 in animals receiving the vitamin A-free diet, whereas it decreased by 52 % and 40 % in animals fed the balanced and enriched diets respectively (Fig. 3). The decrease was maximal 24 h after L-T3 treatment for both diets.

Quantitative analysis of polymerase chain reaction products. The levels of mRNA encoding UGT1A6 and UGT1A1 isoforms in rat liver were estimated in comparison to those of β-actin, a protein whose expression remains unmodified by the diet or the treatment by thyroid hormone. The amount of UGT1A1 mRNA was 35 % lower in animals receiving the vitamin A-free diet, when compared with that of animals fed the balanced diet. It did not differ, however, between rats fed the balanced and supplemented diets (Fig. 4). Conversely, the amount of UGT1A6 mRNA strongly depended on the presence or the absence of vitamin A. Thus, a deficiency in vitamin A increased by 3-fold the level of mRNA encoding UGT1A6 (Fig. 4).

After L-T3 administration (20 h), the amount of mRNA encoding the UGT1A1 isoform decreased 2·0- and 1·6-fold in rats fed the balanced diet and the vitamin A-enriched diet respectively (Fig. 5). During the same time period, UGT1A1 expression was not significantly modified in animals fed a diet deficient in vitamin A (Fig. 5). By contrast, a gradual and strong increase in level of mRNA

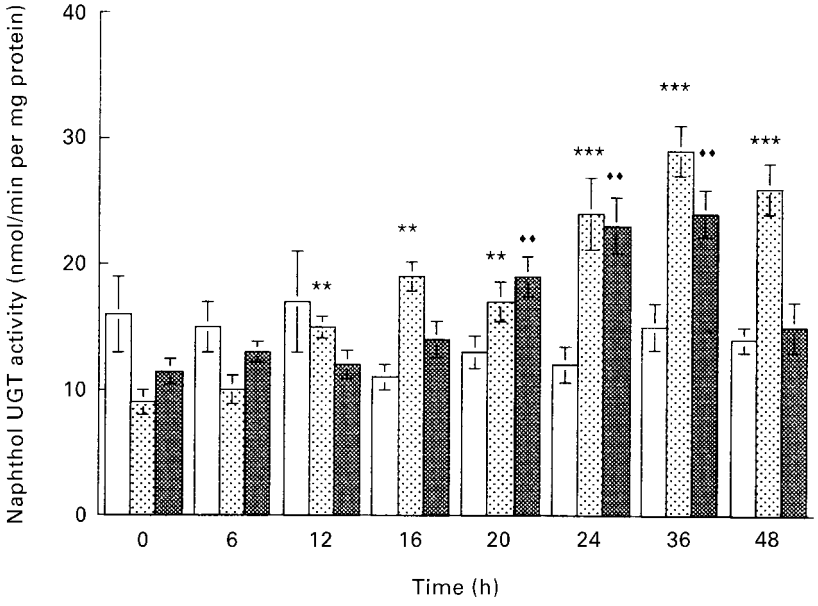

Fig. 2. Naphthol uridine (5′-)diphosphate-glucuronosyltransferase (UGT) activity in liver microsomes of rats fed vitamin A-free (□), vitamin A-balanced (▨) or vitamin A-rich (■) diets, and treated intraperitoneally with a single dose of 3,5,3′-triiodo-L-thyronine at time 0. For details of diets and procedures see p. 290. Values are means for three separate measurements of three rats with standard errors of the means shown by vertical bars. Mean values were significantly different from corresponding values measured at 0 h in vitamin A-balanced group: $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$. Mean values were significantly different from corresponding values measured at 0 h in vitamin A-overloaded group: $\dagger P < 0{\cdot}01$.

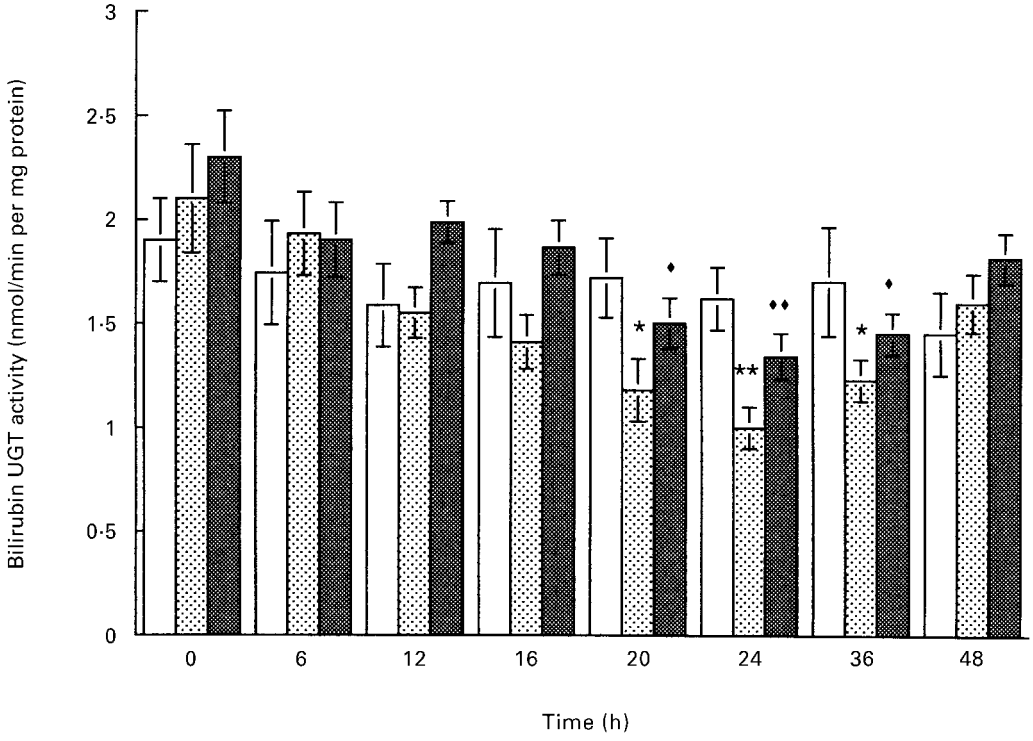

Fig. 3. Bilirubin uridine (5′-)diphosphate-glucuronosyltransferase (UGT) activity in liver microsomes of rats fed vitamin A-free (□), vitamin A-balanced (▨) or vitamin A-rich (■) diets, and treated intraperitoneally with a single dose of 3,5,3′-triiodo-L-thyronine at time 0. For details of diets and procedures see p. 290. Values are means for three separate measurements of three rats with standard errors of the means shown by vertical bars. Mean values were significantly different from corresponding values measured at 0 h in vitamin A-balanced group: $*P < 0{\cdot}05$, $**P < 0{\cdot}01$. Mean values were significantly different from corresponding values measured at 0 h in vitamin A-overloaded group: $\dagger P < 0{\cdot}05$, $\ddagger P < 0{\cdot}01$.

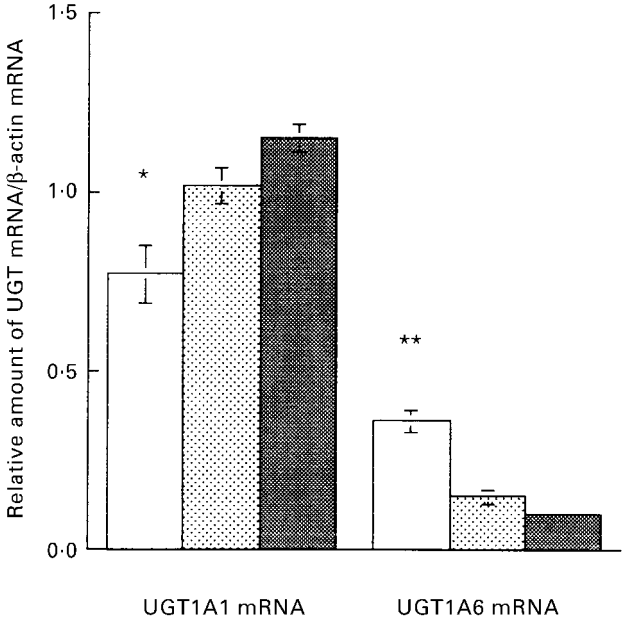

Fig. 4. Relative basal amount of uridine (5′-)diphosphate-glucuronosyltransferase (UGT) 1A1 and UGT1A6 in liver of rats fed vitamin A-free (□), vitamin A-balanced (▨) or vitamin A-rich (■) diets, and treated intraperitoneally with a single dose of 3,5,3′-triiodo-L-thyronine at time 0. For details of diets and procedures see p. 290. Values are means for three independent experiments with standard errors of the means shown by vertical bars. Mean values were significantly different from rats fed vitamin A-balanced diet: $*P < 0{\cdot}05$, $**P < 0{\cdot}01$.

encoding UGT1A6 was observed in animals fed the balanced or supplemented diet, reaching a maximal value 36 h after treatment with L-T3 (Fig. 6). No variation in mRNA was observed in rats fed the vitamin A-free diet. The increase in UGT1A6 was sooner in animals receiving normal diet than in animals fed vitamin A-rich diet.

Discussion

In this present work we provide evidence that dietary vitamin A intake modulates the effects of L-T3 on the expression of two UGT isoforms, UGT1A6 and UGT1A1 in rat liver. We found that, in the absence of L-T3 administration, a vitamin A-free diet enhanced both UGT activity toward 1-naphthol and the corresponding mRNA, whereas compared with vitamin A-balanced diet, a diet rich in vitamin A did not significantly affect these variables. The opposite situation was observed with bilirubin UGT: UGT1A1 mRNA level was decreased by the vitamin A-free diet, whereas the decrease of activity toward bilirubin was not significant. The implied mechanism is unknown, but we can exclude a direct mechanism, which implies the involvement of membrane modifications. The phospholipid concentrations estimated in liver microsomes of rats fed the three diets did not differ (results not shown). Therefore, mRNA variations observed in the vitamin A-free diet suggest that the vitamin A effect might depend on a transcriptional or post-transcriptional process, which could

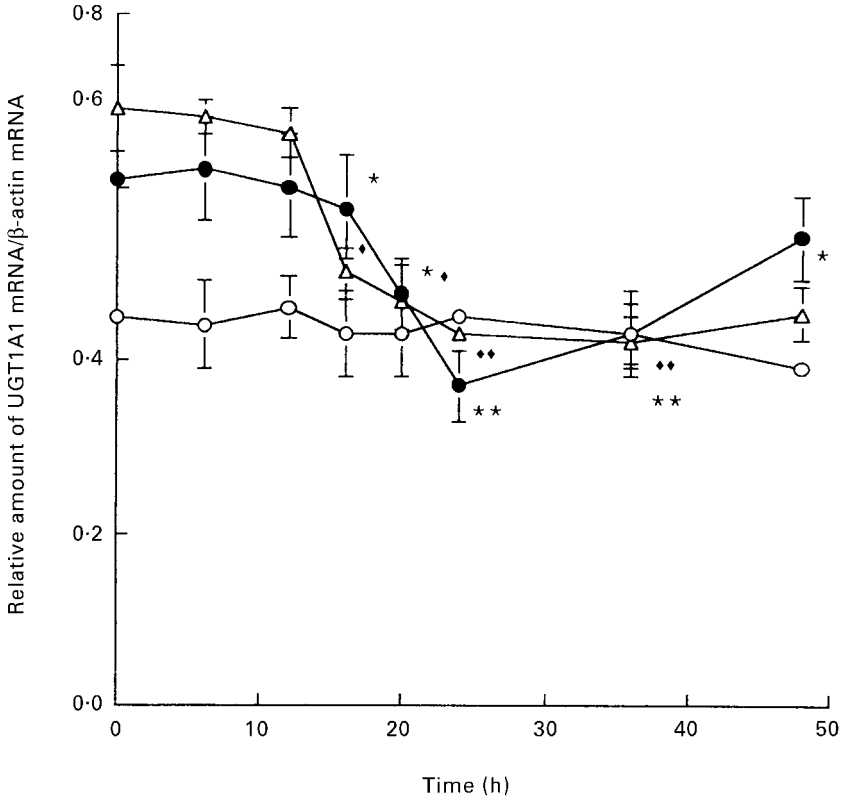

Fig. 5. Kinetics of uridine (5′-)diphosphate-glucuronosyltransferase (UGT) 1A1 mRNA accumulation in liver of rats fed vitamin A-free (○), vitamin A-balanced (●) or vitamin A-rich (Δ) diets, and treated intraperitoneally with a single dose of 3,5,3′-triiodo-L-thyronine at time 0. For details of diets and procedures see p. 290. Values are means for three independent experiments with standard errors of the means shown by vertical bars. Mean values were significantly different from corresponding values measured at 0 h in vitamin A-balanced group: $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$. Mean values were significantly different from corresponding values measured at 0 h in vitamin A-overloaded group: $\dagger P < 0{\cdot}05$, $\ddagger P < 0{\cdot}01$.

imply transcriptional co-regulator and co-repressor (Mano *et al.* 1994).

As previously reported, pharmacological doses of L-T3 significantly decreased the glucuronidation of bilirubin, whereas that of 1-naphthol was increased in the liver of rats receiving a vitamin A-balanced diet (Masmoudi *et al.* 1996, 1997*b*). The unique dose of L-T3 given to the different rats to elicit the responses corresponds to a dose which is able to saturate the thyroid hormone nuclear receptors, and thus to induce a maximal pharmacological effect, without any thyrotoxicity (Oppenheimer *et al.* 1977). In this present study, we show that a vitamin A-free diet induced the same effect as L-T3. However, these effects were not additive in rats fed vitamin A-free diet and simultaneously treated by L-T3. We also show that vitamin A deprivation inhibited these effects of L-T3: L-T3 had no effect on UGT in the absence of vitamin A. This suggests that both vitamin A and L-T3 could modify UGT activity. In order to get insight into the molecular mechanisms implicated, we measured the level of mRNA encoding UGT1A1 and UGT1A6 responsible for the glucuronidation of bilirubin and 1-naphthol, following a single injection of L-T3. Similar results were obtained. L-T3 enhanced mRNA encoding UGT1A6, but reduced UGT1A1 mRNA level with a vitamin A-sufficient diet. These results are in agreement with those reported by Masmoudi *et al.* (1997*b*), which showed that UGT1A1 and UGT1A6 genes were regulated by L-T3 at transcriptional level, and required a *de novo* protein synthesis.

Samuels *et al.* (1988) observed that responses elicited by L-T3 were mediated by nuclear TR, which require the cooperation of other transcription factors such as peroxisome proliferator-activated receptors, retinoic acid receptors or RXR. The general lack of responsiveness to the different hormonal signals may reflect altered DNA–protein and protein–protein interactions between the nuclear hormone receptors, other factors, and proteins of the basal transcription system (Tzameli & Zannis, 1996). Retinoic acid (the active metabolite of vitamin-A) is able to increase mRNA levels of RXR, whereas retinol deficiency causes the decrease of retinoic acid receptor mRNA (Haq & Chytil, 1992). Moreover, Pailler-Rodde *et al.* (1991*a*) showed that retinol deficiency reduced the amount of c-erb-A mRNA, a gene encoding for a thyroid hormone receptor (Sap *et al.* 1986), of which RXR is a heterodimerisation partner (Nagaya *et al.* 1998). Furthermore, findings by

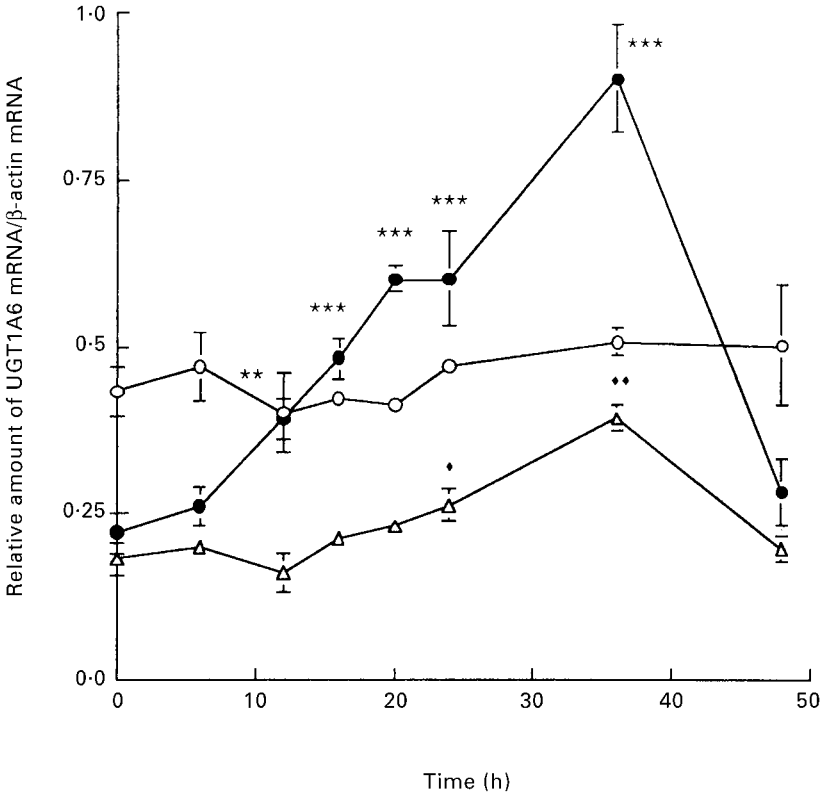

Fig. 6. Kinetics of uridine (5′-)diphosphate-glucuronosyltransferase (UGT) 1A6 mRNA accumulation in liver of rats fed vitamin A-free (○), vitamin A-balanced (●) or vitamin A-rich (Δ) diets, and treated intraperitoneally with a single dose of 3,5,3′-triiodo-L-thyronine at time 0. For details of diets and procedures see p. 290. Values are means for three independent experiments with standard errors of the means shown by vertical bars. Mean values were significantly different from corresponding values measured at 0 h in vitamin A-balanced group: $^{**}P < 0{\cdot}05$, $^{***}P < 0{\cdot}01$. Mean values were significantly different from corresponding values measured at 0 h in vitamin A-overloaded group: $\dagger P < 0{\cdot}05$, $\ddagger P < 0{\cdot}01$.

Verma *et al.* (1992) indicate that vitamin A nutritional status influences the expression of nuclear receptors and retinoic acid-responsive gene. If we consider that L-T3 effect on UGT expression is mediated through TR–RXR heterodimers, the decrease of receptors observed in the vitamin A-deficient diet could explain that, in our study, the specific effect of L-T3 disappeared in animals fed a vitamin A-free diet.

Interestingly, the variations of UGT1A1 mRNA levels were the same in rats fed a vitamin A-balanced diet as in animals fed the vitamin A-overloaded diet. By contrast, the variations in UGT1A6 mRNA levels were larger in rats fed a vitamin A-balanced diet than in animals fed the vitamin A-overloaded diet. We showed previously that L-T3 differently affects UGT1A1 and UGT1A6 mRNA level. Li *et al.* (1999) suggest the involvement of a different molecular mechanism resulting from the diversity of promoter elements. The interaction of the nuclear TR with other transcription factors including co-activators or co-repressors may also be involved. However, a possible mechanism implied in the different UGT1A6 inductions is the formation of fewer TR–RXR heterodimers. Thus, the amount of c-erb A mRNA, which is reduced by a vitamin A-excess diet (Pailler-Rodde *et al.* 1991*b*) could be implicated in the mechanism leading to increases in the UGT1A6 mRNA level, which is less pronounced than in rats fed vitamin A-supplemented diet. In addition, Lehman *et al.* (1993) observed that 9-*cis* retinoic acid induced RXR homodimer formation that causes reduced amount of the RXR monomer to be available for TR–RXR heterodimer formation. This reduction of heterodimerising receptor formation involved a reduction of the T3 response (Lehman *et al.* 1993). On the other hand, recent studies suggested the involvement of a nuclear receptor co-repressor, which promotes chromatin condensation in the absence of ligand (Park *et al.* 1999).

In summary, the modification of the effects of thyroid hormone on UGT mRNA levels depending upon vitamin A status suggests that TR–RXR is in some way involved in the action of L-T3. This hypothesis, which is being investigated in our laboratory, could bring further insight in the knowledge of the molecular mechanisms of gene regulation of the UGT1 family by thyroid hormones and retinoic acids.

This work demonstrates that: (1) the specific effect of L-T3 on UGT1A1 and UGT1A6 is not observed in animals

fed a vitamin A deficient diet; (2) vitamin A and L-T3 exert opposite effects on the level of UGT mRNA. Dietary vitamin A differentially affects UGT1A1 and UGT1A6 expression; (3) the presence of high amounts of vitamin A could negatively modulate the effects of thyroid hormone on UGT activity and mRNA level. Molecular mechanisms remain to be elucidated.

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