Metabolism of thyroid hormones in cultured cardiac fibroblasts of neonatal rats

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Abstract

We have investigated the potential role of fibroblasts in local thyroid hormone metabolism in neonatal rat heart. Incubation of cardiac fibroblasts with thyroxine (T4) or 3,5,3'-tri-iodothyronine (T3) resulted in the appearance of water-soluble metabolites, whereas incubation of cardiomyocytes under the same conditions did not or did so to a much lesser extent. Time-course studies showed that production is already evident after 1–5 h of exposure and that the process equilibrates after 24–48 h. Analysis of the products revealed both the T4 and the T3 metabolites to be glucuronides. These results were corroborated

by the detection of uridine diphosphate (UDP)-glucuronyltransferase activity in cardiac fibroblasts. We found no indication for outer ring deiodination in fibroblasts, cardiomyocytes or heart homogenates.

From these results we have concluded that cardiac fibroblasts, but not cardiomyocytes, are able to glucuronidate T4 and T3 and secrete the conjugates. This could play a role in local metabolism, e.g. to protect the heart tissue from high levels of thyroid hormones.

Journal of Endocrinology (2002) **174**, 111–119

Introduction

The main secretory product of the thyroid gland is thyroxine (T4). Deiodination of T4 to form 3,5,3'-tri-iodothyronine (T3) is an important metabolic pathway, as are glucuronidation and sulfation pathways to form glucuronated and sulfated thyroid hormone conjugates respectively. Both conjugates have an increased water solubility, facilitating urinary and biliary excretion. Thyroid hormone glucuronides, which are excreted in bile, are hydrolyzed by bacterial glucuronidases in the gut and are partly reabsorbed as native thyroid hormones in the enterohepatic cycle (Visser 1996).

A major target organ for thyroid hormones is the heart. The genomic actions of T3 are well described, and it is through regulation of the transcription of genes coding for e.g. sarcoplasmic reticulum Ca^{2+} -ATPase, subunits of the plasma membrane sodium pump (Na^+/K^+)-ATPase, and α - and β -isoforms of the contractile protein myosin heavy chain (Kessler-Icekson 1988, Rohrer & Dillmann 1988, Dillmann 1990, Horowitz *et al.* 1990, Muller *et al.* 1997) that T3 affects cardiac inotropic and chronotropic performance. It is not yet clear whether this occurs because of uptake of T3 from the blood or uptake of T4 that is locally converted to T3 in the heart (van Doorn *et al.* 1985).

On a protein basis, cardiomyocytes compose about 90% of the heart. Measured by cell numbers, however, the

fibroblast is the most abundant cell type in the heart, constituting an estimated two-thirds of the total cardiac cell population (Nair *et al.* 1968, Agocha & Eghbali-Webb 1997). The thyroid status of an animal not only affects cardiomyocyte function, but also that of cardiac fibroblasts. Rats in which ventricular hypertrophy is induced by injections of T4 have a decreased expression of collagen type-I and an increased collagen turnover in ventricular tissue (Klein *et al.* 1996). The inhibitory effect of T4 on cardiac collagen synthesis has been observed in rat, mouse and dog (Edgren *et al.* 1976, Bonnin *et al.* 1983, Yao & Eghbali 1992a,b, Kim *et al.* 1996, Klein *et al.* 1996), and it has been postulated that this negatively affects the supporting properties of the myocardial connective tissue (Edgren *et al.* 1976). Fibroblasts from human skin biopsies express a thyroid hormone receptor (Mixon *et al.* 1993), and it is possible that, besides the well-investigated collagen genes, novel thyroid hormone-responsive genes will be identified in (cardiac) fibroblasts. Indeed, the recently discovered *ZAKI-4* gene, with an as yet unknown function, was identified in fibroblasts and found to be transcribed in heart and several other organs (Miyazaki *et al.* 1996a,b).

It has been shown that cultured fibroblasts possess a saturable and energy-dependent T4 uptake mechanism with a K_m value 15 times lower than that for T3 (Dochter *et al.* 1987). Other studies, performed with skin and

cardiac fibroblasts, confirm the observations of T4 and T3 uptake and also provide indications for a temperature- and verapamil-sensitive thyroid hormone extrusion mechanism in these cells, possibly mediated through P-glycoprotein, a member of the multi-drug resistance protein family (Ribeiro *et al.* 1996, Benvenha & Robbins 1998). Investigations in other organs have shown that fibroblasts play a local, paracrine role for subepithelial fibroblasts in the intestine (Berschneider & Powell 1992, Kandil *et al.* 1994).

Based on these experimental data we propose the hypothesis that fibroblasts are equipped to fulfil a potential role in local thyroid hormone metabolism in the heart. We investigated this hypothesis in cultured fibroblasts and myocytes from neonatal rats. After exposure to T4 or T3 the iodothyronine metabolites in the culture medium were determined using Sephadex LH-20 chromatography.

Materials and Methods

Materials

All reagents used for cell isolation and cell culture were obtained from Life Technologies BV (Breda, The Netherlands), with the exception of trypsin (Boehringer, Mannheim, Germany). Polystyrene 24-well culture dishes were from Corning Costar Europe (Badhoevedorp, The Netherlands). Iodothyronines (T3, 3,3',5'-tri-iodothyronine (rT3) and T4), 6-n-propyl-2-thiouracil (PTU), β -D-glucuronidase, DL-dithiothreitol (DTT), uridine 5'-diphosphoglucuronic acid (UDPGA), *p*-nitrophenol (*p*NP) and bovine serum albumin (BSA) (fraction V) were purchased from Sigma Chemical Co. (St Louis, MO, USA). [125 I]T3 (81.4 TBq/mmol), [125 I]rT3 (29.9 TBq/mmol) and [125 I]T4 (4.3 TBq/mmol) were obtained from NEN Life Science Products, Inc. (Boston, MA, USA). Sephadex LH-20 was purchased from Amersham Pharmacia Biotech Benelux (Roosendaal, The Netherlands). All other chemicals were analytical grade and obtained from commercial supplies.

Animals

Three-day-old Wistar rats were obtained from laboratory stock (Utrecht University, The Netherlands). They were killed by decapitation and hearts were quickly dissected and processed as described below. All experiments were approved by the local ethical committee.

Cell culture

Primary cultures of neonatal rat cardiac fibroblasts and cardiomyocytes were prepared using a trypsin digestion method as described previously (Everts *et al.* 1996). Hearts were digested using trypsin to obtain a cell suspension. Cardiac fibroblasts were obtained by preplating the cell

suspension for 2 h in culture flasks and cultured at 37 °C in a 5% CO₂ atmosphere in 4:1 (w/w) Dulbecco's modified Eagle's medium/medium 199 supplemented with 5% horse serum, 5% fetal calf serum and 2% penicillin/streptomycin. Cardiomyocytes were separated from the fibroblasts by aspiration of the culture medium and seeded into 24-well culture dishes at a density of 1.0×10^6 cells/well. Two days after isolation the medium of both fibroblast and myocyte cultures was replaced by fresh medium. Fibroblasts were grown for 6 days, after which they were harvested with 0.25% trypsin in Ca²⁺-free buffer and seeded into 24-well culture dishes at a density of 1.0×10^6 cells/well.

Deiodinase assays

D1 After 6 days of culture, culture medium was removed and fibroblasts were quickly washed with 2×2 ml 150 mM KCl. Cells were harvested by trypsinization, suspended in 1 ml 150 mM KCl and rinsed three times in 100 μ l 150 mM KCl. Cells were disrupted in three cycles of snap freezing in liquid nitrogen and rapid thawing at 37 °C. Trypan blue exclusion tests showed that virtually 100% was disrupted. Cellular protein content was measured according to Lowry *et al.* (1951), using BSA as a reference. Cells were incubated for 15 min at 37 °C with 1 μ M rT3 and tracer ([125 I]rT3, 10^6 c.p.m./ml) in incubation medium (100 mM phosphate buffer, 2 mM EDTA, 10 mM DTT) in the presence or absence of 100 μ M PTU. The reaction was quenched by adding 100 μ l 5% ice-cold BSA followed by 500 μ l 10% trichloroacetic acid for deproteinization. Samples were centrifuged (15 min at 4 °C, 1500 g) and 500 μ l of the supernatant was acidified with 500 μ l 1.0 M HCl. Liberated iodide was analyzed by Sephadex LH-20 column chromatography as described below, collecting the first three 1 ml 0.1 M HCl eluates. A crude rat liver homogenate served as a positive control. The specific D1 activity was thus measured as the PTU-sensitive outer ring deiodination of rT3.

D2 The methods were the same as in our D1 assays, using 0.5 μ M T4 as a substrate and 20 mM DTT. PTU was omitted from the incubation medium.

Exposure of fibroblasts to thyroid hormones

Fibroblasts grown in a 24-well culture dish were washed with 1 ml saline, and then incubated in 1.5 ml culture medium to which 100 nM T3 or 100 nM T4 was added (using 10^6 c.p.m./ml [125 I]T3 or [125 I]T4 respectively as tracer). This was done to prevent substrate depletion during the 2-day incubation period. 125 I-labeled iodothyronines were purified on Sephadex LH-20 before use. Cells were incubated at 37 °C in a 5% CO₂ atmosphere, and 500 μ l samples of the incubation medium were taken

at regular time-intervals. Iodothyronine metabolites were resolved by Sephadex LH-20 column chromatography as described below.

Analysis of thyroid hormone metabolites

Acid hydrolysis Water fractions from Sephadex LH-20 columns were pooled and evaporated to dryness under a stream of N₂. The residue was dissolved in 2 ml 1.0 M HCl and divided into two equal aliquots. One aliquot was incubated at 80 °C for 1 h, the other remained on ice and served as a control.

β-Glucuronidase digestion Water fractions were collected and evaporated to dryness as described above. The residue was dissolved in 2 ml 50 mM phosphate-buffered saline, pH 6.8, and divided into two equal aliquots. To one aliquot 50 µg β-D-glucuronidase was added, the other received vehicle only, and both were incubated at 37 °C overnight.

The effects of both acid hydrolysis and the β-glucuronidase digestion were assessed by Sephadex LH-20 column chromatography as described below.

Sephadex LH-20 column chromatography

Columns consisted of Sephadex LH-20 (10%, w/v) with a 1 ml bed volume in a glass pipette and were equilibrated with 3 × 1 ml 0.1 M HCl before use. For the post-analysis following acid hydrolysis and β-glucuronidase digestion treatments, a bed volume of 2.5 ml was used. Samples were acidified with one sample volume 1.0 M HCl before adding to the column. Chromatography was performed according to Mol & Visser (1985), collecting the reaction products and metabolites, i.e. iodide, water-soluble conjugates and native hormones in 3 × 1 ml 0.1 M HCl, 8 × 1 ml H₂O and 3 × 1 ml 0.5 M NH₃ in 96% ethanol respectively. The fractions were analyzed for ¹²⁵I-activity in a γ-counter (Packard Cobra II; Packard Instruments Co., Meriden, CT, USA).

Synthesis of iodothyronine sulfates and glucuronides

Sulfate synthesis T4 or [¹²⁵I]T3 were evaporated to complete dryness under a stream of N₂ at 40 °C. Chlorosulfonic acid was added to ice-cold dimethylformamide (1:4, v/v) and the mixture was added to the dry residues. The mixture was incubated for 2 h at 37 °C and the reaction was stopped by adding 4 sample volumes ice-cold water. Sulfates thus synthesized separated in a single, well-defined peak on Sephadex LH-20. The final purified preparations contained virtually no contamination from free iodide or native hormone.

Glucuronide synthesis T4 and [¹²⁵I]T3 were evaporated to complete dryness under a stream of N₂ at 40 °C. Microsomes were used from livers of polychlorinated

biphenyl-treated rats (van Birgelen *et al.* 1997). Mixtures of iodothyronine (1 µM) and tracer (10⁶ c.p.m. [¹²⁵I]iodothyronine), 5 mM UDPGA and microsomes (1 mg/ml) in Tris-HCl (100 mM Tris-HCl, 10 mM MgCl₂, pH 7.8) were incubated at 37 °C for 2 h. The reaction was stopped by adding 1 sample volume ice-cold methanol and the mixtures were centrifuged (15 min, 2200 g). The supernatants were acidified with one sample volume 1.0 M HCl before Sephadex LH-20 chromatography as described above. Glucuronides thus obtained separated in a single, well-defined peak of water eluates from Sephadex LH-20 columns. Water fractions were collected, acidified and chromatographed, using 3 × 1 ml 0.1 M HCl, 1 × 1 ml H₂O and 3 × 1 ml 0.5 M NH₃ in 96% ethanol. Fractions 5–7, containing purified glucuronides with virtually no contamination from free iodide or native iodothyronines, were pooled and evaporated to dryness before use in our assays.

Uridine diphosphate (UDP)-glucuronyltransferase assay

Cells were harvested and protein content was measured as described earlier. Cells, in an amount of 1 mg/ml protein, were incubated with 1 µM pNP in incubation medium (100 mM Tris-HCl, 5 mM MgCl₂, 0.05% Brij56) in the presence or absence of 5 mM UDPGA (30 min at 37 °C). The reaction was quenched by adding 3.8 ml 0.1 M ice-cold NaOH and absorbance was measured at 405 nm.

Free fraction analysis

Free hormone fractions, from which free hormone concentrations were calculated, were measured by equilibrium dialysis as previously described (Sterling & Brenner 1966). In culture medium containing 9% serum and 100 nM T4 or 100 nM T3, the free fractions were 1.2 ± 0.06% and 3.9 ± 0.28% (means ± S.D. of one triplicate determination) respectively.

Statistics

All data are shown as means ± S.E.M. unless indicated otherwise. Statistical significance was evaluated by Student's *t*-test, non-parametric Mann-Whitney test or Welch's approximate alternate *t*-test where appropriate. Significance was accepted at *P* < 0.05. The number of observations (*n*) is shown in parentheses.

Results

We were not able to detect any outer ring deiodinating activity of either D1 or D2 in either neonatal cardiac fibroblasts or neonatal cardiomyocytes (data not shown). Figures 1A and 2A show that, upon 48-h exposure to 100 nM T4 and 100 nM T3 respectively, cardiac fibroblasts secreted a water-soluble [¹²⁵I]metabolite in their

Figure 1 (A) Profile of Sephadex LH-20 elution of thyroid hormone metabolites produced by cardiac fibroblasts (●) and cardiomyocytes (○) exposed to 100 nM T4 with 9% serum at $t=48$ h (typical result for 12 experiments). The inserts in (A) and (B) show the relevant water fractions in detail. (B) Profile of Sephadex LH-20 eluates of thyroid hormone metabolites produced by cardiac fibroblasts (●) exposed to 100 nM T4 without serum at $t=48$ h (typical result for 12 experiments). Wells without cells (○) served as a control.

Figure 2 (A) Profile of Sephadex LH-20 eluates of thyroid hormone metabolites produced by cardiac fibroblasts (●) and cardiomyocytes (○) exposed to 100 nM T3 with 9% serum at $t=48$ h (typical result for 12 experiments). The inserts in (A) and (B) show the relevant water fractions in detail. (B) Profile of Sephadex LH-20 eluates of thyroid hormone metabolites produced by cardiac fibroblasts (●) exposed to 100 nM T3 without serum at $t=48$ h (typical result for 12 experiments). Wells without cells (○) served as a control.

culture medium, whereas cardiomyocytes did not. No increase in ^{125}I -activity in the HCl eluates, containing free iodide, was found, indicating that no appreciable outer ring deiodination of thyroid hormones occurred, thus corroborating the negative results from our deiodinase assays. Figures 1B and 2B show that, upon 48-h exposure to 100 nM T4 and 100 nM T3 respectively without serum, the water-soluble [^{125}I]metabolite fraction secreted by cardiac fibroblasts was almost twice the amount of that found in the presence of serum, indicating a dependence on free hormone concentration.

Figure 3 shows time-courses of water-soluble iodothyronine metabolite accumulation in culture media. The production of the water-soluble T4 metabolites by cardiac fibroblasts was already measurable at our first 1-h time-point and increased further in time (Fig. 3A). After 48 h, the percentage was 2.9 ± 0.7 of the total ^{125}I -activity in the incubation medium. Figure 3B shows the production

of the water-soluble T3 metabolites by cardiac fibroblasts, which was clearly present after 5 h of incubation. After 48 h the percentage was 2.2 ± 0.3 of total ^{125}I -activity.

Analysis of the metabolites in the water-soluble fraction of the column chromatography

Having collected the water-soluble fractions containing the thyroid hormone metabolites, we investigated the characteristics of the product. Known water-soluble thyroid hormone conjugates are sulfates and glucuronides and we used the commonly employed acid hydrolysis and β -glucuronidase digestion to distinguish between them.

β -Glucuronidase digestion β -Glucuronidase treatment resulted in a significant decrease ($P < 0.01$) of the ^{125}I -activity in the two water-soluble fractions (Fig. 4) and a concomitant increase in the native iodothyronine fraction,

Figure 3 (A) Time-course of water-soluble metabolite produced by cardiac fibroblasts (●) after exposure to 100 nM T4. Wells without cells (○) served as a control. (B) Time-course of water-soluble metabolite produced by cardiac fibroblasts (●) after exposure to 100 nM T3. Wells without cells (○) served as a control. Values are means $\pm$ S.E.M.

as analyzed by Sephadex column chromatography (results not shown). These results indicated that the water-soluble metabolites were glucuronides, and we therefore expected the conjugates to be insensitive to acid hydrolysis.

Acid hydrolysis Figure 5 shows that the water-soluble T3 metabolite was resistant to acid hydrolysis, which corroborated the results of the β -glucuronidase digestion. In contrast, 58% of the water-soluble T4 metabolite was sensitive to acid hydrolysis, indicating that either T4 sulfate is a major component of this fraction or the T4 metabolite is a glucuronide which is sensitive to acid hydrolysis.

We validated our analytical methods using synthetic iodothyronine sulfates and glucuronides. Figure 6A shows that, as predicted, virtually 100% of the T4 glucuronide and T3 glucuronide were hydrolyzed by β -glucuronidase, whereas T4 sulfate and T3 sulfate were not. Figure 6B shows that T4 sulfate and T3 sulfate were sensitive to acid hydrolysis, T3 glucuronide was not and, surprisingly, that

Figure 4 Analysis of the water fraction by digestion with 50 μ g β -glucuronidase at 37 °C overnight of the eluate of cardiac fibroblasts (open bars) exposed to 100 nM T4 and 100 nM T3 respectively (T4, $n=2$; T3, $n=3$). Incubation without β -glucuronidase (solid bars) served as a control. Total ¹²⁵I-activity is expressed relative to control. Values are means $\pm$ S.E.M. ** $P \leq 0.01$, *** $P \leq 0.001$.

Figure 5 Analysis of the water fraction by acid hydrolysis with 1.0 M HCl for 1 h at 80 °C of the eluate of cardiac fibroblasts (open bars) exposed to 100 nM T4 and 100 nM T3 respectively ($n=5$). Incubation at 0 °C (solid bars) served as a control. Total ¹²⁵I-activity is expressed relative to control. Values are means $\pm$ S.E.M. ** $P \leq 0.01$.

circa 40% of the synthetic T4 glucuronide was sensitive to this treatment. The pattern of these results concurred with the results of the analyses of the water-soluble fractions, which indicated that (1) T4 glucuronide is sensitive to acid hydrolysis and (2) cardiac fibroblasts glucuronidate iodothyronines.

Figure 6 (A) Analysis of synthetic thyroid hormone sulfates (T4S and T3S) and thyroid hormone glucuronides (T4G and T3G) by digestion with 50 µg β-glucuronidase at 37 °C overnight (open bars) (all bars *n*=1, except for T4 glucuronide *n*=2). Incubation without β-glucuronidase (solid bars) served as a control. (B) Analysis of synthetic thyroid hormone sulfates and thyroid hormone glucuronides by acid hydrolysis with 1.0 M HCl for 1 h at 80 °C (open bars) (all bars *n*=1, except for T4 glucuronide *n*=2). Incubation at 0 °C (closed bars) served as a control.

Table 1 Specific UDP-glucuronyltransferase activities in different preparations from the neonatal rat (means ± S.D.)

	pNP (µmol/h per mg)
Whole liver homogenate (<i>n</i> =9)	0.75 ± 0.37
Whole heart homogenate (<i>n</i> =6)	0.30 ± 0.32
Cardiac fibroblasts (<i>n</i> =3)	0.90 ± 0.26
Cardiomyocytes (<i>n</i> =4)	0.44 ± 0.39

UDP-glucuronyltransferase assay

Table 1 shows that cardiac fibroblasts possess a phenol UDP-glucuronyltransferase activity of 0.90 ± 0.26 µmol pNP/h per mg. Compared with cardiomyocytes and whole heart homogenates, fibroblasts had the highest specific activity.

Discussion

Our results showed that cultured cardiac fibroblasts produce and secrete two different glucuronidated iodothyronine conjugates upon exposure to T4 or T3, whereas cultured cardiomyocytes do not or do so to a much lesser extent. These findings support our hypothesis that cardiac fibroblasts are equipped to fulfil a potential role in local thyroid hormone metabolism in the heart.

The results from our analyses indicate that the water-soluble iodothyronine metabolites produced *in vitro* by cardiac fibroblasts are glucuronidated conjugates, and this conclusion is supported by our observation of the presence of UDP-glucuronyltransferase activities in these cells. To the best of our knowledge this is the first evidence of iodothyronine glucuronidation in the heart. Furthermore, our fibroblast cultures have no outer ring deiodinating activity, as judged from the absence of the appearance of free iodide in the culture medium upon exposure to phenolic (outer) ring ¹²⁵I-labeled T4 or T3. Moreover, in our hands, cardiac fibroblasts from neonatal rats did not show a D1 activity. Inner ring deiodination can be conferred both by D1 and D3. However, the expression of D3 is most prominent in placenta and fetal tissues, declining rapidly with gestational age and after birth (Richard *et al.* 1998, Bates *et al.* 1999, Hernández *et al.* 1999). Indeed, in adult rat hearts, D3 activity is low to undetectable (Schiel *et al.* 2001). We therefore concluded that the glucuronidated conjugates produced by cardiac fibroblasts do not constitute deiodinated metabolites derived from T4 and T3, and that they most likely represent T4 glucuronide and T3 glucuronide respectively.

The analytical methods we used (i.e. acid hydrolysis and enzyme digestion) have also been employed by others to distinguish between sulfated and glucuronidated iodothyronine conjugates (Mol & Visser 1985, Finnson & Eales 1996). The conjugates produced upon T4 and T3 exposure behaved differently in our analyses, and this again indicated that our cultured cardiac fibroblasts indeed produced two different metabolites from the two iodothyronines. We also observed, initially to our surprise, that T4 glucuronide produced *in vitro* by fibroblasts was sensitive to acid hydrolysis, a result that was replicated for a T4 glucuronide synthesized from T4 using rat liver microsomes. Thus, an unknown conjugate that is sensitive to acid hydrolysis is not necessarily a sulfate, and we feel that the results from acid hydrolysis analyses should be interpreted cautiously.

In general, conjugation pathways such as glucuronidation are associated with detoxification processes in liver and bile excretion. The UDP-glucuronyltransferases (UDPGTs) constitute a family of isozymes. It has been demonstrated that, in rat liver microsomes, T4 and rT3 are the preferred substrates for phenol-UDPGT, whereas T3 is preferentially glucuronated by androsterone-UDPGT (Beetstra *et al.* 1991, Visser *et al.* 1993a,b,c). It remains to

be established which UDPGT isozymes are responsible for T4 and T3 glucuronidation in cardiac fibroblasts. Our results suggested that a phenol-UDPGT is involved in T4 glucuronidation in fibroblasts. However, although cardiomyocytes possess a considerable phenol-UDPGT activity, they did not secrete conjugated iodothyronines in the culture medium. This would suggest that, in cardiomyocytes, phenol-UDPGT is not the major enzyme for thyroid hormone glucuronidation.

The appearance of glucuronidated thyroid hormone metabolites in the culture medium, as we observed in our cardiac fibroblast cultures, is the net result of a pathway consisting of an uptake and extrusion step and a glucuronidating mechanism, the latter almost certainly being a UDPGT activity. Although there are only a few experimental data on transport processes of iodothyronines in fibroblasts, it has been shown that fibroblasts possess temperature- and energy-dependent mechanisms for the uptake and extrusion of thyroid hormones (Docter *et al.* 1987, Benvenge & Robbins 1990, Ribeiro *et al.* 1996). It is interesting to note that cultured fibroblasts and rat ventriculocytes possess a verapamil-sensitive thyroid hormone extrusion mechanism, possibly P-glycoprotein, a member of the multi-drug resistance protein family (MRP) (Ribeiro *et al.* 1996, Cavaliere *et al.* 1999). MRP glycoproteins have been reported to transport anionic conjugates (Keppler *et al.* 1999), and we will investigate whether these transporters are involved in the protein-mediated secretion of glucuronidated iodothyronines.

Experimental data on the cellular uptake of iodothyronines in cardiomyocytes are very scarce. Our previous results have shown that cardiomyocytes possess a temperature- and energy-dependent uptake mechanism for T3 (Everts *et al.* 1996, van der Putten *et al.* 2001, Verhoeven *et al.* 2001) and possibly also for T4 (van der Putten *et al.* 2001). In one of these studies (Verhoeven *et al.* 2001), a direct comparison between T3 uptake in fibroblasts and cardiomyocytes showed that T3 uptake (expressed per μg protein) was at least tenfold higher in the cardiomyocyte. The absence of glucuronated thyroid hormones in the cardiomyocyte culture medium is thus probably not due to negligible uptake of native hormones from the incubation medium.

It has been tentatively suggested that, e.g. during fetal development, in non-thyroidal illness or during treatment with outer ring deiodinating inhibitors, T4 and T3 sulfates are stored as inactive conjugates from which rapid mobilization of active hormone by sulfatase activity can occur (Visser 1996). A similar potential role in the inactivation and storage of hormone can be envisaged for glucuronidation of iodothyronines by cardiac fibroblasts. T3 sulfate, but not T4 sulfate, was sensitive to digestion by sulfatase; the resistance of T4 sulfate to sulfatase activity was probably due to steric hindrance of the two iodide atoms on the phenolic ring. In contrast, T3 glucuronide and T4 glucuronide were both deconjugated by β -glucuronidase.

Thus, glucuronidation of T3 as well as T4 is reversible, and this could be a mechanism for storing thyroid hormones. β -Glucuronidase activities have been amply demonstrated in lysosomal fractions derived from mammalian heart (Johnson *et al.* 1986, Ambrosio *et al.* 1990), and it would be interesting to investigate whether these activities are indeed located in cardiomyocytes and whether these cells possess a mechanism for the uptake of glucuronidated iodothyronines.

Others have shown that fibroblasts are involved in mediating the trophic response of the neonatal rat heart to angiotensin II, probably by releasing transforming growth factor- β (Kim *et al.* 1995, Lee *et al.* 1995), and the secretory response of the intestine to inflammation by releasing prostaglandins (Berschneider & Powell 1992, Kandil *et al.* 1994). We are currently investigating whether cardiac fibroblasts fulfil a similar role in local thyroid hormone action in the heart, using animal models of experimentally induced hyper- and hypothyroidism.

Acknowledgements

The authors are grateful to Pieter Looijmans and Miranda Niessen for competent analytical assistance and to Brian Joosten, Hans van Toor and Ellen Kaptein for expert technical support. The study was supported in part by The Netherlands Heart Foundation (NHS, The Hague, The Netherlands) (grant 96.175) and The Netherlands Organisation for Scientific Research (NWO, The Hague, The Netherlands) (grant 15.17.039).

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Received 31 December 2001

Accepted 19 March 2002