Thyroid hormone uptake in cultured rat anterior pituitary cells: effects of energy status and bilirubin

F W J S Wassen, E P C M Moerings, H van Toor, G Hennemann and M E Everts

Department of Internal Medicine III, Erasmus University Medical School, Rotterdam, The Netherlands

(Requests for offprints should be addressed to M E Everts, Department of Veterinary Anatomy and Physiology, Division of Physiology, Postbox 80157, 3508 TD Utrecht, The Netherlands; Email: m.everts@vet.uu.nl)

Abstract

Transport of thyroxine (T_4) into the liver is inhibited in fasting and by bilirubin, a compound often accumulating in the serum of critically ill patients. We tested the effects of chronic and acute energy deprivation, bilirubin and its precursor biliverdin on the 15-min uptake of [^{125}I]tri-iodothyronine ([^{125}I]T_3) and [^{125}I]T_4 and on TSH release in rat anterior pituitary cells maintained in primary culture for 3 days. When cells were cultured and incubated in medium without glucose and glutamine to induce chronic energy deprivation, the ATP content was reduced by 45% ($P<0{\cdot}05$) and [^{125}I]T_3 uptake by 13% (NS), but TSH release was unaltered. Preincubation (30 min) and incubation (15 min) with 10 μM oligomycin reduced ATP content by 51% ($P<0{\cdot}05$) and 53% ($P<0{\cdot}05$) under energy-rich and energy-poor culture conditions respectively; [^{125}I]T_3 uptake was reduced by 66% ($P<0{\cdot}05$) and 64% ($P<0{\cdot}05$). Neither bilirubin nor biliverdin (both 1–200 μM) affected uptake of [^{125}I]T_3 or [^{125}I]T_4. Bilirubin (1–50 μM) did not alter basal or TRH-induced TSH release. In conclusion, the absence of inhibitory effects of chronic energy deprivation and bilirubin on thyroid hormone uptake by pituitary cells supports the view that the transport is regulated differently than that in the liver.

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Introduction

Non-thyroidal illness (NTI) is associated with multiple alterations in serum thyroid hormone parameters. Most prominent are the low serum tri-iodothyronine (T_3) and increased reverse T_3 concentrations, together with inappropriately low concentrations of serum thyroid-stimulating hormone (TSH) (Wartofsky & Burman 1982, Docter *et al.* 1993). Apart from fasting, conditions that may result in NTI include chronic renal failure and liver disease (Wartofsky & Burman 1982, Kaptein 1996).

As the liver is the major site of T_3 production in humans (Hennemann 1986), the low serum T_3 during NTI can be ascribed to a reduced type I deiodinase activity (Harris *et al.* 1978, Kohrle *et al.* 1991), in addition to a reduced transport of thyroxine (T_4) into the liver (De Jong *et al.* 1992, 1994, Vos *et al.* 1995). The diminished tissue uptake of T_4 in calorie-deprived humans (Van Der Heyden *et al.* 1986) seems to be secondary to the reduction in cellular ATP (De Jong *et al.* 1994). The reduction in T_4 transport into the liver in chronic renal failure or liver disease can be explained by inhibitory effects of compounds that accumulate in the serum, such as furan fatty acids, indoxyl sulphate or bilirubin (Lim *et al.* 1993*a, b*). Conversely, despite the observed alterations in serum thyroid hormone parameters in NTI, there is no change in expression of T_3-regulated genes in the liver, indicating the maintenance of tissue euthyroidism (Chamba *et al.* 1996).

Because serum TSH does not increase, in spite of the low serum T_3 in fasting or NTI, we suggested in a previous study that T_3 transport into the pituitary is regulated differently from that in liver (Everts *et al.* 1996). Indeed, in contrast to the effects on T_4 transport observed in hepatocytes (Lim *et al.* 1993*a*), the furan fatty acid 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF) and indoxyl sulphate – compounds accumulating in serum of patients with chronic renal failure – did not affect uptake of T_3 and T_4 into pituitary cells (Everts *et al.* 1995*a*).

This hypothesis was further elaborated in the present study. We have investigated the effects of chronic energy deprivation and bilirubin – a compound often accumulating in serum of patients with liver disease (Lim *et al.* 1993*b*) – and its precursor, biliverdin, on the uptake of T_3 and T_4 and on basal and thyrotrophin-releasing hormone (TRH)-stimulated TSH release in cultured anterior pituitary cells. To induce chronic energy deprivation, we cultured anterior pituitary cells from fed rats in medium

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lacking glucose and glutamine. The effects of bilirubin and its precursor biliverdin were tested by adding the compounds to the incubation medium at concentrations (relative to albumin) in the range from those present in normal serum to greater than those present in serum of patients with liver disease.

Materials and Methods

Animals

All experiments were performed using male Wistar rats, weighing 200–220 g. The animals had free access to food and water and were kept in a controlled environment (21 °C) with constant day length (12 h).

Pituitary cell culture

Animals (12–16 for each experiment) were killed between 0900 and 0930 h by decapitation. The pituitary glands were removed within 5 min, the neurointermediate lobe was discarded, and the anterior lobes were collected in calcium- and magnesium-free Hank's Balanced Salt Solution, supplemented with 10 g/l human serum albumin, penicillin (10^5 U/l), amphotericin B (0·5 mg/l), and sodium bicarbonate (0·4 g/l). Anterior pituitary cells were dissociated with dispase (final concentration $2{\cdot}4 \times 10^3$ U/l), as previously described (Everts *et al.* 1993, 1994*a*). From each pituitary, $1–2 \times 10^6$ cells were obtained and the viability of the cells as determined by trypan blue exclusion was greater than 90%.

The cells were cultured at 37 °C in a water-jacketed incubator with 5% CO_2 at a density of approximately 5×10^5 cells/well in 48-well culture dishes. The cells had attached to the wells after 2 days of culture. On day 3, the cells were used for experiments. The culture medium consisted of Minimal Essential Medium with Earle's salts (MEM) supplemented with non-essential amino acids, sodium pyruvate (1 mM), 10% fetal calf serum, penicillin (10^5 U/l), amphotericin B (0·5 mg/l), glucose (6·25 mM), L-glutamine (2 mM) and sodium bicarbonate (2·2 g/l pH 7·4) (Everts *et al.* 1993, 1994*a*). For culture under energy-poor conditions, the culture medium was based on MEM without glucose, supplemented with non-essential amino acids, sodium pyruvate (1 mM), 10% fetal calf serum, penicillin (10^5 U/l), amphotericin B (0·5 mg/l) and sodium bicarbonate (2·2 g/l pH 7·4).

Cellular uptake of $[^{125}I]T_3$ and $[^{125}I]T_4$

Cellular uptake experiments were performed as described previously (Everts *et al.* 1993, 1994*a*). Briefly, cells were cultured for 3 days at a density of approximately 500 000 cells/well. Blank wells contained only culture medium. The culture medium was removed and cells were preincubated in incubation medium. This medium was identical to the culture medium, except that the fetal calf serum was replaced by 0·5% BSA when $[^{125}I]T_3$ (50 000 c.p.m.; 50 pM) was measured, and by 0·1% BSA in case of $[^{125}I]T_4$ (100 000 c.p.m.; 175 pM) uptake. Furthermore, $NaHCO_3$ (25 mM) was replaced by *N*-2-hydroxyethyl-piperazine-*N*′-2-ethanesulphonic acid (HEPES 8·9 mM), piperazine-*N*,*N*′-bis(2-ethane-sulphonic acid) (PIPES 10·6 mM), and *N*,*N*-bis(2-hydroxyethyl)-2-aminoethane-sulphonic acid (BES 11·2 mM). To study the effects of acute ATP depletion on $[^{125}I]T_3$ uptake, we carried out preincubation (0·5 ml) for 30 min in the absence or presence of oligomycin (10 μM), then the preincubation medium was discarded and cells were incubated for 15 min in 0·25 ml medium containing $[^{125}I]T_3$ without or with 10 μM oligomycin. Both pre-incubation and incubation were performed in a 37 °C incubation chamber on a rotating device. Experiments to test the effects of bilirubin (1–200 μM) and biliverdin (1–200 μM) on $[^{125}I]T_3$ and $[^{125}I]T_4$ uptake were performed as described above, except that, after preincubation (0·25 ml, 30 min) without or with bilirubin or biliverdin, incubation was started by the addition of 10 μl $[^{125}I]T_3$ or $[^{125}I]T_4$ directly to the wells.

After incubation, the medium was removed and cells were washed once with 1 ml ice-cold saline in order to remove unbound tracer. Cells were dissolved in 1 ml 0·1 M NaOH and were counted for iodine-125 activity in a 16-channel gamma-counter (NE 1600, Nuclear Enterprises, Edinburgh, Scotland). The amount of $[^{125}I]T_3$ or $[^{125}I]T_4$ taken up by the cells was expressed as a percentage of the total radioactivity added (percentage dose). The procedure described above was also applied to incubations without cells. All results were corrected for radioactivity retained in the blanks.

TSH release and cellular content

The culture medium was removed, centrifuged ($2000 \times g$) and frozen until required for hormone determination. The incubation medium was identical to the culture medium, except that the fetal calf serum was replaced by 0·5% BSA. Cells were preincubated for 2 h in the absence or presence of bilirubin (1–200 μM). Thereafter, this medium was discarded and cells were incubated for 2 h with the same concentrations bilirubin without or with TRH (100 nM). After incubation, the medium was removed, centrifuged ($2000 \times g$) and stored at − 20 °C.

To determine the TSH content of the cells, 0·5 ml incubation medium was added to the wells. The cells were scraped from the wells with a rubber policeman and the wells were sonicated for 2×30 s. The extracts were removed and the wells were washed with 0·5 ml medium. The two fractions were combined (volume of cell extract 1·0 ml), centrifuged ($2000 \times g$) at room temperature and the supernatant was frozen until further analysis.

Table 1 Effects of chronic or acute energy depletion on ATP content, [^{125}I]T_3 uptake and DNA content of anterior pituitary cells

	ATP content (μg/well)	**[^{125}I]T_3 uptake** (% dose/well)	**DNA content** (ng/well)	**[^{125}I]T_3 uptake** (% dose/μg DNA)
Experimental conditions				
Normal culture conditions				
Controls	0·495 ± 0·031 (9)	1·49 ± 0·07 (12)	2475 ± 35 (3)	0·60 ± 0·03 (12)
+10 μM T_3	0·431 ± 0·027 (8)	0·76 ± 0·06 (12)*	2476 ± 158 (3)	0·31 ± 0·02 (12)*
+10 μM oligo	0·244 ± 0·042 (9)*	0·51 ± 0·08 (12)*	2791 ± 165 (3)	0·18 ± 0·03 (12)*
Culture without glucose and glutamine				
Controls	0·272 ± 0·013 (6)†	1·29 ± 0·07 (12)	2133 ± 99 (3)†	0·61 ± 0·03 (12)
+10 μM T_3	0·291 ± 0·034 (8)†	0·55 ± 0·05 (12)*†	2014 ± 59 (3)	0·27 ± 0·03 (12)*
+10 μM oligo	0·128 ± 0·064 (9)	0·47 ± 0·14 (12)*	1718 ± 59 (3)*†	0·27 ± 0·08 (12)*

Data represent the means ± S.E. (number of observations). ATP content and [^{125}I]T_3 uptake were measured in three or four experiments and DNA content in one experiment. Fifty percent of the anterior pituitary cells (500 000 cells/well) were cultured for 3 days in medium containing 10% fetal calf serum (normal culture conditions) and 50% were cultured in medium lacking glucose and glutamine but with 10% fetal calf serum. Cells were preincubated (30 min) in the absence or presence of oligomycin (oligo), and incubation (15 min) was performed with [^{125}I]T_3 without or with 10 μM T_3 or oligomycin. *P<0·05 compared with respective controls; †P<0·05 compared with normal culture conditions.

TSH determination

TSH in incubation media and cell extracts was measured by RIA (Everts *et al.* 1993). Addition of bilirubin (5 and 200 μM) to the TSH standards did not alter the standard curve.

Free hormone concentration

Calculation of the free T_3 (fT_3) or free T_4 (fT_4) concentrations was based on determination of the free hormone fractions by equilibrium dialysis (Sterling & Brenner 1966). As shown previously (Everts *et al.* 1993), the fT_3 fraction was not altered by 10 μM T_3 or oligomycin.

ATP and DNA determination

The cellular ATP content was determined in perchloric acid (0·2 M) extracts with the Lumac AEC Kit (Lumac, Landgraaf, The Netherlands). The DNA content of the cells was determined using a modification (Everts *et al.* 1994*a*) of the fluorescence technique described by Downs & Wilfinger (1983).

Chemicals and isotopes

All solutions used for cell isolation and cell culture were obtained from GIBCO Europe (Breda, The Netherlands), with the exception of human serum albumin (Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), dispase (grade II; Boehringer, Mannheim, Germany) and amphotericin B (Fungizone, Bristol-Myers Squibb, Woerden, The Netherlands). Culture dishes (48 wells) were from Costar (Cambridge, MA, USA). PIPES, HEPES, BES, BSA (fraction V), oligomycin, bilirubin and biliverdin were all from Sigma (St Louis, MO, USA). L-T_3 (T_3) was from Henning Berlin Gmbh (Berlin, Germany) and TRH was from Hoechst AG (Frankfurt am Main, Germany). [3′,5′-^{125}I]T_4 (1500 μCi/μg) and [3′-^{125}I]T_3 (3070 μCi/μg) were obtained from Amersham International (Aylesbury, Bucks, UK). Reagents for the rat TSH RIA were kindly provided by the NIDDK (Bethesda, MD, USA). Sephadex LH-20 and G-25 were from Pharmacia (Uppsala, Sweden). All other reagents were of the highest purity available.

Statistics

Results are presented as means ± S.E. The statistical significance of the effects of the compounds tested and of glucose deprivation on TSH release, [^{125}I]T_3 and [^{125}I]T_4 uptake, fT_3 and fT_4 fractions, and cellular ATP and DNA content was evaluated by Student's *t*-test for unpaired observations or by one-way analysis of variance and Duncan's test for a repeated measure design. Differences were considered significant at P<0·05.

Results

Effects of energy deprivation

Culture of pituitary cells for 3 days without glucose and glutamine did not affect TSH release (22·44 ± 0·85 ng, n=44, compared with 22·60 ± 1·11 ng, n=44). However, the cellular ATP content was reduced by 45% (P<0·05) compared with that in cells cultured in the normal culture medium (Table 1).

As further shown in Table 1, chronic energy deprivation did not alter the 15-min uptake of [^{125}I]T_3. Exposure to 10 μM unlabelled T_3 significantly reduced [^{125}I]T_3 uptake under both normal (49%; P<0·05) and energy-poor (57%; P<0·05) culture conditions. Chronic

energy deprivation only slightly affected the DNA content of the wells. When [^{125}I]T_3 uptake was expressed per μg DNA, the uptake values for normal and energy-deprived cells were precisely the same.

Preincubation (30 min) and incubation (15 min) with 10 μM oligomycin reduced the cellular ATP content by 51% ($P<0·05$) when added under energy-rich culture conditions and by 53% ($P<0·05$) when the conditions were energy-poor. This was associated with significant reductions in [^{125}I]T_3 transport: of 66% ($P<0·05$) and 64% ($P<0·05$) respectively (Table 1). Under energy-poor culture conditions only, oligomycin slightly reduced the DNA concentrations (Table 1).

Effects of bilirubin and biliverdin

Free hormone fraction The fT_3 and fT_4 fractions were determined in medium with 0·5% and 0·1% BSA, respectively. The added concentrations of the compounds were expressed relative to albumin (molar ratios of from 0·06 to 2·40) because bilirubin and biliverdin both bind to albumin. The fT_3 fraction was 3·57 ± 0·07% ($n=8$), and the fT_4 fraction was 3·39 ± 0·26% ($n=8$) – values in good agreement with previous findings (Everts *et al.* 1995*a*). Greater concentrations of bilirubin had no effect on the fT_3 fraction, whereas the fT_4 fraction was increased at bilirubin:albumin ratios of 0·60 and 2·40 respectively (Fig. 1*a*). In contrast, biliverdin did not affect the fT_3 or the fT_4 fraction (Fig. 1*b*).

[^{125}I]T_3 and [^{125}I]T_4 uptake The effects of bilirubin and biliverdin on plasma membrane uptake of [^{125}I]T_3 and [^{125}I]T_4 were determined in short-term incubations (15 min). Neither the uptake of [^{125}I]T_3 nor that of [^{125}I]T_4, both expressed per pM free hormone, were altered in presence of bilirubin (Fig. 2*a*). The effect of biliverdin on [^{125}I]T_3 and [^{125}I]T_4 uptake was determined in a similar way. The uptake of the two tracers was not altered by biliverdin (Fig. 2*b*).

Basal TSH release The effect of bilirubin on basal TSH release was tested in experiments with 2 h preincubation, followed by 2 h incubation. The results of the incubation period are shown in Fig. 3, in which data are expressed as percentages of the control value. The concentrations of bilirubin tested (1, 5, 50 and 200 μM) correspond to molar ratios with albumin of 0·01, 0·06, 0·60 and 2·40 respectively. The lower bilirubin concentrations (1–50 μM) did not affect basal TSH release, but 200 μM bilirubin resulted in a significant increase (38%; $P<0·05$). DNA content after 4 h of incubation with 200 μM bilirubin was significantly lower than that of the controls (1·31 ± 0·03 μg DNA/well, $n=3$, compared with 1·80 ± 0·05 μg DNA/well, $n=6$, respectively; $P<0·001$).

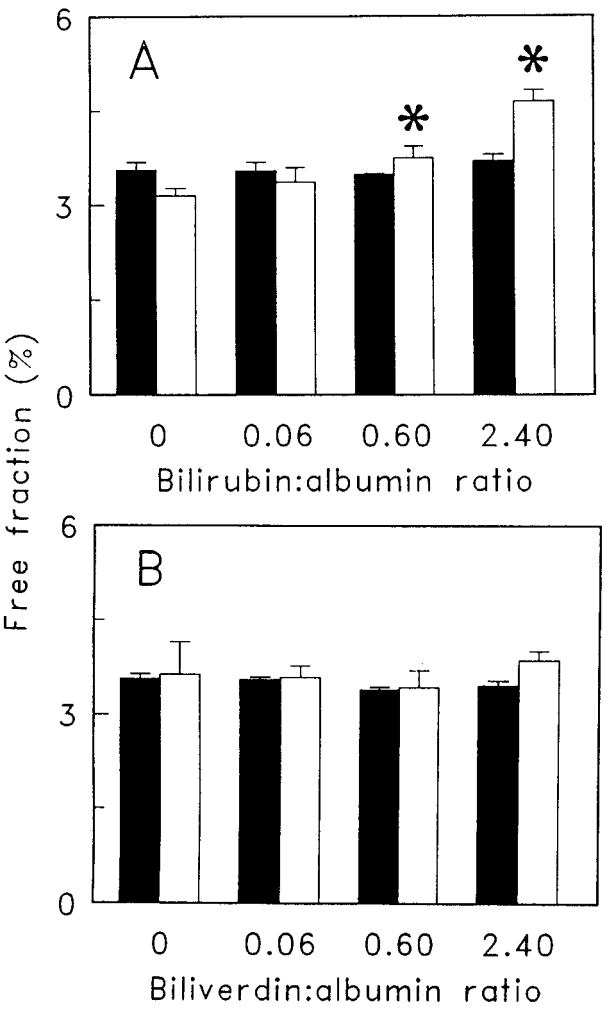

Figure 1 Effects of bilirubin (*a*) and biliverdin (*b*) on the fT_3 (■) and fT_4 (□) fractions, determined in medium with 0·5% BSA for [^{125}I]T_3 and with 0·1% BSA for [^{125}I]T_4. The fT_3 and fT_4 fractions were determined by equilibrium dialysis. The added bilirubin and biliverdin concentrations are expressed relative to albumin (bilirubin:albumin ratio). Bars show the mean of four observations from two experiments. *$P<0·05$ compared with no additions.

TRH-induced TSH release The effect of bilirubin on TRH-induced TSH release was determined during short (2 h) exposure to TRH after preincubation for 2 h without or with bilirubin (5–200 μM) (Table 2). Incubation with TRH alone stimulated TSH release by 200% ($P<0·05$). TRH-induced TSH release was not altered significantly by the lower bilirubin concentrations (5–50 μM), but 200 μM bilirubin produced a large increase ($P<0·05$; $n=6$). Cellular TSH content was not altered after addition of TRH and 5 or 50 μM bilirubin, but in the presence of 200 μM bilirubin a significant decline was observed. The total TSH content showed no significant change with any of the additions.

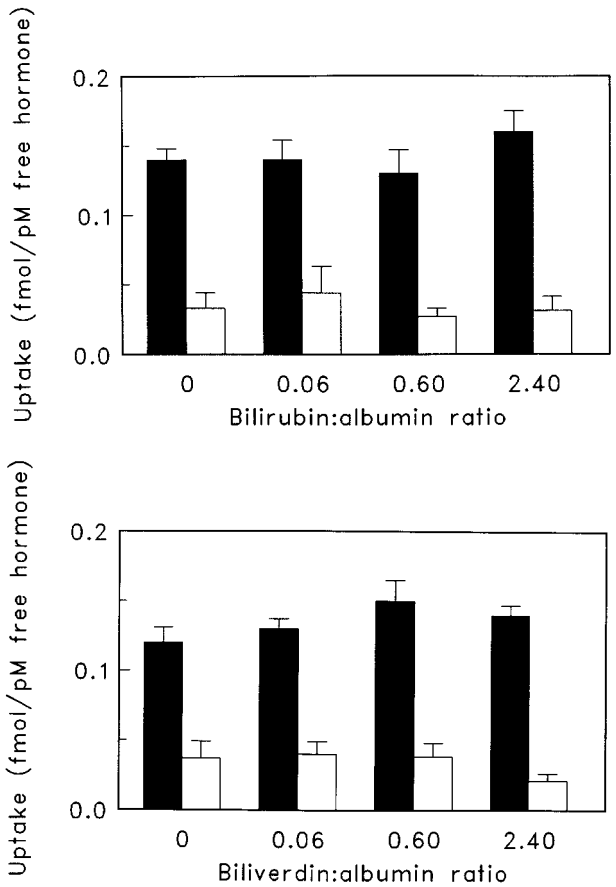

Figure 2 Uptake of [^{125}I]T_3 (■) and [^{125}I]T_4 (□) by anterior pituitary cells in the presence of bilirubin (*a*) or biliverdin (*b*). Cells were cultured for 3 days at a density of 500 000 cells/well. Thereafter, culture medium was removed and cells were preincubated for 30 min in incubation medium containing 0·5% BSA when [^{125}I]T_3 uptake was measured and 0·1% BSA in the case of [^{125}I]T_4, in the absence or presence of bilirubin or biliverdin (1–200 μM). Incubation was started by the addition of 10 μl [^{125}I]T_3 (50 000 c.p.m.) or [^{125}I]T_4 (100 000 c.p.m.) directly to the preincubation medium. The 15-min uptake of the two tracers is expressed per pM free hormone and the concentrations bilirubin and biliverdin are expressed relative to albumin. Bars represent the mean ± S.E. of from five to nine observations from two experiments for [^{125}I]T_4 and three for [^{125}I]T_3 uptake.

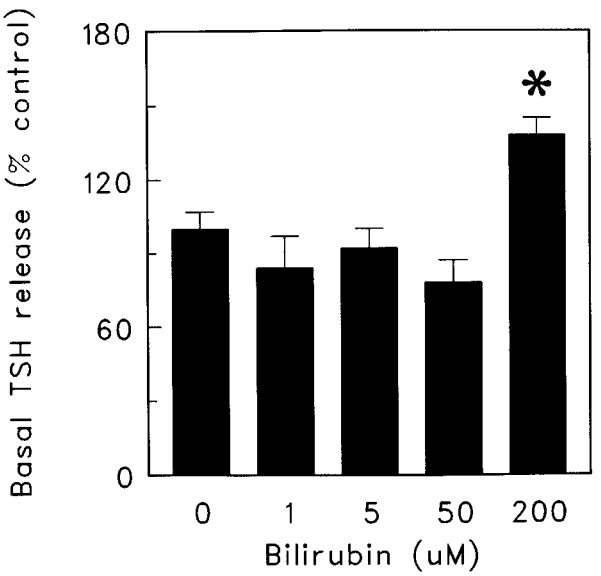

Figure 3 Effect of bilirubin (1–200 μM) on basal TSH release, expressed as a percentage of the control value. Anterior pituitary cells were cultured for 3 days at a density of 400 000 cells/well. Thereafter, culture medium was removed and cells were preincubated for 2 h in incubation medium containing 0·5% BSA. This medium was discarded and incubations were performed for 2 h in medium without or with various concentrations of bilirubin. Bars show the mean ± S.E. of nine observations from three experiments. *P<0·05 compared with no additions.

Discussion

We have previously evaluated the role of the liver and pituitary in the maintenance of the low serum T_3 and normal TSH concentrations in NTI and fasting (Everts *et al.* 1996). In that review, we postulated that the plasma membrane thyroid hormone transport mechanism is regulated differently in these two tissues, and we provided experimental evidence for this idea from both *in vivo* and *in vitro* experiments. The hypothesis is further elaborated in the present study on thyroid hormone transport in cultured rat anterior pituitary cells. Although this experimental system does not reflect the physiological situation because of the absence of pituitary control factors such as dopamine, it is an appropriate model to study the role of each compound separately. Our major conclusions are: (1) T_3 uptake into the pituitary is reduced by acute ATP depletion induced by oligomycin, but not by chronic energy depletion induced by energy-poor culture conditions; (2) chronic energy depletion is not associated with changes in TSH release; (3) neither bilirubin nor biliverdin alter T_3 and T_4 transport into the pituitary; and (4) bilirubin does not alter basal or TRH-induced TSH release.

The effects of fasting on thyroid hormone uptake into the liver can be investigated *in vivo* (Van der Heyden *et al.* 1986, De Jong *et al.* 1994) or in the perfused liver (De Jong *et al.* 1992, 1994), and experiments with hepatocytes can be performed 4 h after plating (Lim *et al.* 1993*a, b*, Vos *et al.* 1995), but it is more complicated to study the effects of fasting on thyroid hormone uptake into the pituitary. *In vivo* measurements and adequate perfusion experiments are excluded for practical reasons, and studies in cultured cells require at least 2 days to permit attachment of the cells (Everts *et al.* 1993, 1994*a,* 1995*a, b*); consequently, effects on the pituitary induced by *in vivo* treatment may disappear during prolonged culture of the cells.

As an approach to examining the role of cellular energy status in T_3 transport and TSH release in the pituitary, we

Table 2 Effects of bilirubin (5–200 μM) on TRH-induced TSH release and cellular TSH content

	TSH content (ng)			
	Preincubation medium	Incubation medium	Cells	Total§
Incubation conditions				
No additions	3·81 ± 0·65	1·59 ± 0·24	44·06 ± 3·13	49·46 ± 3·45
100 nM TRH	3·15 ± 0·47	4·83 ± 0·12*	36·15 ± 5·82	44·13 ± 5·82
TRH +5 μM bilirubin	3·22 ± 0·51	4·45 ± 0·17*	41·36 ± 4·83	49·03 ± 4·65
TRH +50 μM bilirubin	3·21 ± 0·52	4·93 ± 0·09*	41·63 ± 4·89	49·77 ± 4·81
TRH +200 μM bilirubin	8·15 ± 0·95*	12·41 ± 0·72*†	23·94 ± 2·06*	44·50 ± 3·52

Data represent the means ± S.E. of six observations in two independent experiments. Anterior pituitary cells (400 000 cells/well) were cultured for 3 days in medium containing 10% fetal calf serum. Thereafter, cells were preincubated for 2 h in medium containing 0·5% BSA without or with bilirubin (5–200 μM). Incubations were performed either in the absence of any additions or with added TRH (100 nM) alone, or TRH (100 nM) and bilirubin (5–200 μM). TSH was measured in medium and cells by RIA. §Calculated as the sum of TSH in the two media and the cells. *$P<0·05$ compared with no additions; †$P<0·05$ compared with 100 nM TRH.

compared the effects of long-term energy deprivation achieved by culture and incubation of the cells in the absence of glucose and glutamine with those of acute energy depletion induced by exposure to oligomycin, an inhibitor of oxydative phosphorylation. The effect of these treatments was validated by a 45–50% reduction in cellular ATP. T_3 uptake was not altered under long-term energy-deprived conditions, but it was significantly reduced by exposure to oligomycin. Both in cells cultured in the normal medium and in those cultured in energy-poor medium, 10 μM unlabelled T_3 decreased [^{125}I]T_3 uptake by around 65%, without affecting the ATP content of the cells. In previous experiments (Everts *et al.* 1994*b*) in which we studied the effect of 10 μM oligomycin on T_3 and tri-iodothyroacetic acid transport, we found that oligomycin did indeed reduce the cellular ATP level, but that T_3 transport was much more reduced than that of tri-iodothyroacetic acid transport. As the experimental conditions differed only with regard to the thyroid hormone analogue added, it is unlikely that non-specific toxicity could account for the observed effect. This is in agreement with our present observation that DNA content was not altered in presence of oligomycin.

The relation between thyroid hormone uptake and TSH release during starvation has previously been studied *in vivo*: in spite of the decreases in plasma T_3 and T_4 concentrations, nuclear T_3 content in the pituitary was only slightly reduced and plasma TSH did not increase (St Germain & Galton 1985). The nuclear-bound T_3 in the pituitary is partly derived from the plasma T_3 and partly from intracellular conversion of T_4 to T_3 by type II deiodinase (Van Doorn *et al.* 1984), and the presence of type II but not type I deiodinase activity in cultured anterior pituitary cells has been reported (Everts *et al.* 1995*b*). Because type II deiodinase activity was diminished even in the pituitary of fasted rats (St Germain & Galton 1985), a normal intracellular T_3 concentration could be maintained only if transport of thyroid hormone into the pituitary were enhanced.

Our experiments did not show a stimulation of T_3 transport under energy-poor culture conditions. In view of the concept that T_3 and T_4 share a common transporter in the pituitary (Everts *et al.* 1994*a,* 1996), T_4 uptake will be similarly unaffected by long-term energy deprivation. The observation that T_3 transport was not altered complements our *in vitro* observation that TSH release remained the same under long-term energy-deprived conditions. Conversely, the fact that TSH release during energy-deprived culture did not change also implies that the uptake of [^{125}I]T_3 into the thyrotrophic cells was unaltered. This is important to note, because our measurements of [^{125}I]T_3 uptake reflect uptake in all cell types of the pituitary.

In contrast to the effects of long-term energy deprivation (3 days), acute energy deprivation (i.e. exposure to oligomycin for a total of 45 min) resulted in a significant reduction in T_3 uptake, indicating energy-dependence of the transport process itself. Although it has been questioned, whether oligomycin can be used to deduce information about the ATP-dependence of transport processes in intact cells (Blondeau *et al.* 1988), previous studies with anterior pituitary cells (Everts *et al.* 1993) have provided additional evidence for ATP-dependence of the T_3 uptake process by its dependence on the Na^+ gradient (Pedersen & Carafoli 1987) and the temperature. In hepatocytes, energy-dependence of T_3 uptake was demonstrated using other metabolic inhibitors besides oligomycin (potassium cyanide and dinitrophenol; Docter & Krenning 1990).

Whereas long-term and acute energy deprivation resulted in a similar decline in total cellular ATP content, only acute energy deprivation resulted in a reduction in T_3 uptake in pituitary cells. In perfused liver, both acute

energy depletion (De Jong *et al.* 1994) and prolonged fasting (De Jong *et al.* 1992) resulted in a decrease in thyroid hormone transport. These observations support the view that the thyroid hormone uptake mechanism is energy-dependent both in the pituitary and in the liver, whereas the long-term regulation of the thyroid hormone transport capacity in the two organs is different under conditions associated with a reduction in plasma T_3 (see also Everts *et al.* 1996).

The concentration of bilirubin is increased in serum of critically-ill patients and was suggested as one of the factors causing the low T_3 plasma concentrations in these patients, by the inhibition of T_4 transport into the liver (Lim *et al.* 1993*b*). In another study, bilirubin and bilirubin derivatives were found to interfere with thyroid hormone transport in astrocytes (Chantoux *et al.* 1993). However, in cultured pituitary cells of the rat, neither bilirubin nor biliverdin affected T_3 or T_4 uptake. This again supports the view of tissue-dependent regulation of thyroid hormone transport in different cell types during NTI, and is in agreement with the concept of a common carrier for T_3 and T_4 in the pituitary.

In addition, we questioned whether bilirubin also could account for the inappropriately low serum TSH level observed in critically ill patients. The presence of bilirubin (1–50 μM) did not affect basal or TRH-induced TSH release; only exposure to the greatest concentration of bilirubin (200 μM) resulted in a significant increase in basal and TRH-induced TSH release. The presence of 200 μM bilirubin resulted in a molar ratio of bilirubin:albumin of 2·4 – a ratio considerably greater than that measured in normal individuals (0·012) and in patients with critical illness (0·42) (Lim *et al.* 1993*b*). Therefore, the observed effect of a high bilirubin concentration could be due to some unspecific effect. Nevertheless, the observation that bilirubin does not affect pituitary function although it certainly affects liver function (Lim *et al.* 1993*b*) is in accordance with earlier reported differences concerning the effects of interleukins in liver and pituitary (Lim *et al.* 1993*b*, Wassen *et al.* 1996).

As the uptake of thyroid hormones is the first step in the mediation of thyroid hormone action, one could postulate that other mechanisms are involved in the maintenance of normal TSH values with low serum T_3. Recently, a differential expression of thyroid hormone receptor isoforms has been found in thyrotropin-secreting pituitary tumours that could account for the altered negative feedback (Gittoes *et al.* 1998). This could also be the case in NTI, although in NTI patients with chronic liver disease thyroid receptor expression in the liver was not altered (Chamba *et al.* 1996). In a more recent paper, decreased TRH gene expression in the hypothalamus of patients with NTI was observed (Fliers *et al.* 1997). This may in the long run account for a reduction in the TSH concentration in NTI, but the immediate decline in TSH seems to occur before the reduction in TRH (Van Haasteren *et al.* 1994). Together with a previous report of a direct effect of cytokines on TSH secretion by pituitary cells (Wassen *et al.* 1996), these observations lead to the conclusion that maintenance of normal TSH concentrations in NTI is achieved through an action at several levels.

In conclusion, the present study shows that thyroid hormone uptake into cultured anterior pituitary cells is not affected by long-term energy deprivation, bilirubin or its precursor biliverdin. These observations lend further support to the view that thyroid hormone transport into the pituitary is regulated differently than that in the liver. However, the fact that T_3 uptake is not stimulated under chronic energy-deprived conditions or by bilirubin cannot account for the absence of an increase in serum TSH that is observed during starvation or hyperbilirubinaemia.

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