Simultaneous changes in central and peripheral components of the hypothalamus–pituitary–thyroid axis in lipopolysaccharide-induced acute illness in mice

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Abstract

During illness, major changes in thyroid hormone metabolism and regulation occur; these are collectively known as non-thyroidal illness and are characterized by decreased serum triiodothyronine (T₃) and thyroxine (T₄) without an increase in serum TSH. Whether alterations in the central part of the hypothalamus–pituitary–thyroid (HPT) axis precede changes in peripheral thyroid hormone metabolism instead of vice versa, or occur simultaneously, is presently unknown. We therefore studied the time-course of changes in thyroid hormone metabolism in the HPT axis of mice during acute illness induced by bacterial endotoxin (lipopolysaccharide; LPS).

LPS rapidly induced interleukin-1β mRNA expression in the hypothalamus, pituitary, thyroid and liver. This was followed by almost simultaneous changes in the pituitary (decreased expression of thyroid receptor (TR)-β₂, TSHβ and 5’-deiodinase (D1) mRNAs), the thyroid (decreased TSH receptor mRNA) and the liver (decreased TRβ₁ and D1 mRNA). In the hypothalamus, type 2 deiodinase mRNA expression was strongly increased whereas pro-TRH mRNA expression did not change after LPS. Serum T₃ and T₄ fell only after 24 h.

Our results suggested almost simultaneous involvement of the whole HPT axis in the downregulation of thyroid hormone metabolism during acute illness.


Introduction

During illness, profound changes in thyroid hormone metabolism and regulation occur; these are collectively known as ‘non-thyroidal illness’ (NTI) or ‘sick euthyroid syndrome’. These changes include decreased serum triiodothyronine (T₃) and thyroxine (T₄) levels and increased serum reverse T₃ (rT₃) levels. Despite low serum thyroid hormone levels, serum thyrotrophin (TSH) does not increase and can actually be decreased. Several mechanisms are involved in the alterations in thyroid hormone metabolism: decreased thyrotrophin-releasing hormone (TRH) expression in the hypothalamus, decreased thyroxine hormone release by the thyroid gland, decreased transport of thyroid hormones across the plasma membrane, and a decrease in extrathyroidal (peripheral) conversion of T₄ into T₃ by 5’-deiodinase (D1), notably in the liver (Wiersinga 2000).

It has been hypothesized that during the acute phase of illness changes in thyroid hormone metabolism are predominantly caused by peripheral adaptations while anterior pituitary function remains unaltered. In prolonged critical illness, however, downregulation of the central part of the hypothalamus–pituitary–thyroid (HPT) axis plays an important role (Van den Berghe 2000). TSH secretion by the anterior pituitary decreases probably as a result of diminished hypothalamic stimulation as is evident from decreased TRH gene expression in the paraventricular nucleus (PVN) of deceased patients with documented NTI (Fliers et al. 1997). In addition, the combined administration of TRH and growth hormone-releasing factor-2 enhances pulsatile TSH secretion dramatically in patients in intensive care restoring plasma T₄ and T₃ levels, in keeping with an important role for the hypothalamus in the central downregulation of the HPT axis in prolonged illness (Van den et al. 1999).

Few animal experimental data are available on the effects of acute illness on the central part of the HPT axis. Intraperitoneal administration of bacterial endotoxin (lipopolysaccharide; LPS) results in decreased serum T₃ and T₄ levels after 24 h and inappropriately normal or low pro-TRH mRNA content in the PVN of rats (Kakucska et al. 1994). We recently showed that LPS administration in mice results in a rapid decrease of type 2 deiodinase (D2) activity in the pituitary (Boelen et al. 2004), indicating an early response of the pituitary during acute illness.
Whether alterations of the central part of the HPT axis precede changes in peripheral thyroid hormone metabolism instead of vice versa, or occur simultaneously, is presently unknown. We therefore studied the time-course of changes in thyroid hormone metabolism, characterized by mRNA expression of thyroid hormone-related genes, in the hypothalamus, pituitary, thyroid and liver of mice during acute illness induced by LPS administration.

Materials and Methods

Animals
Female, random cycling Balb/c mice (Sprague–Dawley; Harlan, Horst, The Netherlands) were used at 6–12 weeks of age. The mice were kept in 12 h light:12 h darkness, in a temperature-controlled room (22 °C) and received food and water ad libitum. One week before the experiments the mice were housed in groups according to the experimental set-up. The study was approved by the local animal welfare committee. We performed two experiments.

Experiment 1 Acute illness was induced by an intraperitoneal injection of 150 µg LPS (endotoxin; E.coli 127:B8; Sigma Chemical Co., St Louis, MO, USA) diluted in 0·5 ml sterile 0·9% NCl. Control mice received 0·5 ml saline (control) and water ad libitum. Control mice were injected with isoflurane and killed. The liver, pituitary and hypothalamus were obtained. In both experiments, blood was taken by cardiac puncture and serum was stored at −20 °C until analysed. All tissues were stored immediately in liquid nitrogen.

Thyroid hormones
Serum T₃ and T₄ were measured with in-house RIAs (Wiersinga & Chopra 1982). To prevent interassay variation, all samples for one experiment were measured within the same assay.

RNA isolation and real-time PCR
mRNA was isolated from the hypothalamus, pituitary, thyroid and 10 mg liver tissue of mice using the Magna Pure apparatus and the Magna Pure LC mRNA isolation kit II (tissue) (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer’s protocol and cDNA synthesis was performed with the 1 strand cDNA synthesis kit for RT–PCR (AMV) (Roche Molecular Biochemicals). Published primer pairs were used to amplify hypoxanthine phosphoribosyl transferase (HPRT), a housekeeping gene (Sweet et al. 2001) and interleukin (IL)-1β (Bouaboula et al. 1992). We designed primer pairs for D₁, D₂, type 3 deiodinase D₃, thyroid receptor (TR)-β1, TRβ2, TSHβ and preproTRH (D₁ forward: CATTCTACTCCCTCTACCA and reverse: GCACTT TCCCCGACATT; D₂ forward: GATGCTCCCAAT TCAAGTGT and reverse: AGTGAAGGTGTCAG TGGG; D₃ forward: CTACGTCATCCAGAGTGGCA and reverse: CTTTCATCATAGGCTCCTCA; TRβ1 forward: CACCTGATCCTGACGATGT and reverse: ACAGGTGTAGTCACGGATG; TRβ2 forward: GTGAATCAGCCTTATACCTG and reverse: AGAGGGTGTAGTCACGGATG; D₁ forward: TCAACAC CACCCTCTGTGCT and reverse: TTGGCACA CTTGACCTAC; preproTRH forward: TCGTGTCATA CTTGATCCCC and reverse: CCCCACATCCCCCT CTCCTTC). Real-time PCR was performed for the quantitative estimation of the above-mentioned mRNAs. Standards for the different mRNAs were prepared from RNA of murine liver or lung. For each mRNA assayed, a standard curve was generated using tenfold serial dilutions of this target standard PCR product and the same primers used to amplify the cDNA. For each gene the standard protocol was optimized by varying MgCl₂ concentrations. PCR reactions were set up with cDNA, MgCl₂ (25 mM), SybrGreenI (Roche Molecular Biochemicals), forward and reverse primer and H₂O. The reactions were then cycled in the LightCycler (Roche Molecular Biochemicals) with the following parameters: pre-denaturation for one cycle at 95 °C for 10–30 s, amplification for 35–45 cycles (temperature transition of 20 °C/s), which consists of denaturation for 0–5 s at 95 °C, annealing at various temperatures for 10 s and elongation for 15 s at 72 °C (annealing temperature: D₁, 52 °C; D₂, 55 °C; D₃, 62 °C; TRβ1, 54 °C; TRβ2, 55 °C; TSHβ, 55 °C; preproTRH, 55 °C; IL-1β, 60 °C; and HPRT, 54 °C). The LightCycler software generated a standard curve (measurements taken during the exponential phase of the amplification) which enabled the amount of each gene in each test sample to be determined. All results were corrected for their mRNA content using HPRT mRNA.

Liver D₁
Liver D₁ activity was determined as described previously (Peeters et al. 2003). Briefly, mouse liver samples were homogenized on ice in 10 volumes of PE buffer (0·1 M phosphate and 2 mM EDTA (pH 7·2)) using a Polytorn (Kinematica AG, Lucerne, Switzerland). Homogenates were snap frozen in aliquots and stored at −80 °C until further analysis. Protein concentration was measured with the Bio–Rad protein assay using bovine serum albumin (BSA) as the standard following the manufacturer’s instructions. D₁ activity was measured by duplicate
incubations of homogenates (10 µg protein) for 30 min at 37°C with 0.1 µM [3,5,125I]-rT3 (100 000 c.p.m.) in a final volume of 0.1 ml PED10 buffer (PE+10 mM dithiothreitol). Reactions were stopped by the addition of 0.1 ml 5% (w/v) BSA in water on ice. The protein-bound iodothyronines were precipitated by the addition of 0.5 ml ice-cold 10% (w/v) trichloroacetic acid in water. Following centrifugation, 125I was isolated from the supernatant by chromatography on Sephadex LH-20 minicolumns.

Statistics
Data are presented as the means ± S.E.M. Variations between LPS-treated and saline-treated mice were evaluated

Figure 1 Relative expression of IL-1β mRNA in the hypothalamus, pituitary, thyroid and liver of mice after administration of LPS (●) or saline (○). Mean values ± S.E.M. are shown. P values indicate differences between groups by ANOVA. *P<0.05 and **P<0.01, differences between groups at a single time-point.
by two-way ANOVA with two grouping factors (time and treatment). Post-hoc analysis (Tukey test) was performed in order to make multiple comparisons between the groups (Hochberg & Tamhane 1987). If the data were abnormally distributed or variances between groups were unequal, we first ranked the data, then performed an ANOVA (Hora 1984) and used a Tukey test for the post-hoc analyses (all analysed in SPSS 11·5·1 (SPSS Inc., Chigaco, IL, USA)). The differences in serum T₃ and T₄ at 24 h and in pituitary D₂ mRNA expression at 4 h were analysed by the Student’s t-test or by the Mann–Whitney U test where appropriate. P values less than 0·05 were considered as statistically significant.

Results

Hypothalamus

LPS administration resulted in strongly elevated IL-1β mRNA expression in the hypothalamus reaching a maximum after 3 h (Fig. 1). Hypothalamic preproTRH mRNA expression did not change compared with control mice 1–24 h after LPS administration. Hypothalamic TRβ1 mRNA expression was also not influenced by LPS administration. TRβ2 mRNA expression could hardly be detected (data not shown). By contrast, LPS administration induced a significant threefold increase in D₂ mRNA expression (maximal at 6–8 h) (Fig. 2). Hypothalamic D₃ mRNA expression was low and not different between LPS–treated and control mice (data not shown).

Pituitary

In the pituitary, LPS induced high IL-1β mRNA expression, maximal at 1 h after administration (Fig. 1). LPS also resulted in a significant decrease in TSHβ, TRβ2 and D₁ mRNA expression within 24 h, with the lowest mRNA expression at 4 h after LPS administration. D₂ mRNA expression and TRβ1 mRNA expression tended to decrease after LPS administration compared with control mice but this difference was not statistically different (Fig. 3). However, pituitary D₂ mRNA expression at 4 h was significantly lower in LPS-treated animals compared with controls.

Thyroid

Thyroidal IL-1β mRNA expression was induced shortly after LPS administration (maximal expression at 1 h) (Fig. 1). LPS resulted in a significant decrease in thyroidal TSH receptor (TSH-R) mRNA expression; a reduction in D₁ mRNA expression did not reach statistically significant difference (Fig. 4). TRβ1 mRNA expression was not different between LPS-treated and control mice (data not shown).

Liver and circulation

LPS administration resulted in strongly elevated IL-1β mRNA expression in the liver, which was maximal at 1 h and remained elevated until 6 h (Fig. 1). LPS decreased liver TRβ1 and D₁ mRNA expression within 8 h of administration. Liver D₁ activity was also significantly decreased after LPS administration, which was in agreement with mRNA expression. The decrease of TRβ1 mRNA preceded the decrease of D₁ mRNA expression which was, in turn, followed by decreased serum T₃ and T₄ levels after 24 h (see Fig. 5).

Discussion

The aim of the present study was to evaluate the time-course of changes in central and peripheral thyroid hormone metabolism during acute illness. To this end we
Figure 3. Relative expression of TSHβ, TRβ1, TRβ2, D1 and D2 mRNA in the pituitary of mice after administration of LPS (●) or saline (○). Mean values ± S.E.M. are shown. P values indicate differences between groups by ANOVA. *P<0.05 and **P<0.01, differences between groups at a single time-point.
injected mice with LPS, which is a very potent activator of the immune system and induces several proinflammatory cytokines via the Toll-like receptor-4 (Lohrer et al. 2000). These cytokines are produced by a variety of cells in many organs. We measured IL-1β mRNA expression in the HPT axis as a representative of the inflammatory response and observed a strong induction in the hypothalamus, pituitary, thyroid and liver. IL-1β mRNA expression peaked very rapidly (within 1 h) in the pituitary, thyroid and liver while hypothalamic IL-1β mRNA expression was maximal at 3–4 h after LPS administration. Hypothalamic D2 mRNA expression increased following the rise in IL-1β mRNA, reaching peak values at 6–8 h after LPS. This did not however result in a change of preproTRH mRNA which – by the presumed rise in local T3 generation via enhanced D2 activity – indicates altered thyroid hormone feedback. The reason that we could not detect TRβ2 mRNA in the hypothalamus might be that TRβ2 is present only in specific highly localized areas of the hypothalamus (Cook et al. 1992, Lechan et al. 1994). Since we isolated RNA from the complete hypothalamus in the present experiment, it is very possible that this led to a dilution of the specific signal.

The time-course of events in the various organs suggests that LPS induces changes almost simultaneously in the central (hypothalamus and pituitary) and peripheral (thyroid and liver) part of the HPT axis. In particular, the early changes in the pituitary gland cannot be explained from prior changes in the hypothalamus. One may argue that the changes in the thyroid gland may be to some extent secondary to a fall in pituitary TSH release. This seems unlikely however since downregulation of the TSH-R has been described as a result of higher ambient TSH concentrations (Shimura et al. 1997), whereas we observed downregulation in thyroidal TSH-R mRNA expression associated with low TSHβ mRNA expression. Our results are consequently best interpreted as having independent effects of LPS on the various components of the HPT axis during the early stages of induced illness. In more advanced stages, downregulation at the hypothalamic level may enhance downregulation at the pituitary level, which may then further contribute to decreased secretion of thyroid hormones by the thyroid.

Some of the alterations in the HPT axis that we observed have been described previously. The decrease of pituitary D1 and D2 mRNA expression (only after 4 h) confirms our recent observations (Boelen et al. 2004). The decrease in liver D1 mRNA expression, which precedes the decrease in serum thyroid hormones, has also been described previously by us (Boelen et al. 1995). Unaltered hypothalamic preproTRH mRNA expression in rats after LPS administration has been reported by Kakucska et al. (1994), and the increase in hypothalamic D2 expression is consistent with a recent observation of an increase in D2 activity in the mediobasal hypothalamus (MBH) of the rat during acute LPS-induced illness (Fekete et al. 2003). Recently, Diano et al. (1998) showed that 3 days of fasting also induced an increase in D2 mRNA expression in the hypothalamus of rats, possible resulting from decreased plasma leptin or elevated glucocorticoids levels. The effect of food deprivation as a result of illness can be excluded in our study because D2 mRNA expression started to rise shortly after LPS administration. At that time, the effect
of food deprivation on thyroid hormone metabolism is negligible (Boelen et al. 1995).

Data on changes in the human hypothalamus in critical illness are sparse. Arem et al. (1993) reported a decreased T₃/T₄ ratio in the post-mortem human hypothalamus of patients who died after protracted illness as compared with patients who died acutely, suggesting decreased deiodination of T₄ in human illness. However, D2 activity was not

Figure 5 Relative expression of liver TRβ1 and D1 mRNA, liver D1 activity and serum T₃ and T₄ levels in mice after administration of LPS (●) or saline (○). Mean values ± S.E.M. are shown. P values indicate differences between groups by ANOVA. *P<0.05 and **P<0.01, differences between groups at a single time-point.
assessed by Arem et al. (1993) in their study. Furthermore, decreased deiodination is hard to reconcile with decreased TRH mRNA expression in the PVN of patients with critical illness as reported in our earlier studies (Fliers et al. 1997), or with true unaltered hypothalamic TRH expression in the present study. At least two factors should be taken into account in interpreting these findings. First, LPS-induced increased D2 expression in the MBH as reported by Fekete et al. (2003) in the rat and replicated by the present study in the mouse probably represents a more acute disease model than the patients studied by Arem et al. (1993) and Fliers et al. (1997). Secondly, only a small proportion of hypothalamic TRH-expressing neurons is involved in HPT axis feedback regulation (Segerson et al. 1987). This may therefore obscure the effects of LPS that are restricted to selected hypothalamic nuclei such as the PVN when studying hypothalamic tissue blocks, probably explaining the unaltered hypothalamic TRH mRNA expression found in the present study.

Our results suggest simultaneous involvement of the hypothalamus, pituitary, thyroid and liver in the down-regulation of thyroid hormone metabolism during acute illness. It is logical to assume a general mechanism inducing these changes in various organs, since the changes are viewed as part of the acute phase response during illness. Cytokines have been implicated in the pathogenesis of altered thyroid hormone metabolism during illness (Boelen et al. 1996). It has been hypothesized recently that limiting amounts of steroid receptor coactivator-1 (SRC-1), a coactivator for both nuclear factor-κB (NF-κB) and TRβ1, might be responsible for decreased liver D1 mRNA expression because of competition between cytokine-induced NF-κB and the TRβ1/D1 complex for SRC-1 (Yu & Koenig 2000). A similar mechanism could also play a role in the pituitary, because the simultaneous decrease of pituitary TRβ2 and TSHβ mRNAs that we observed suggests a shortage of a common factor needed for the expression of both TRβ2 and TSHβ. One candidate could be the pituitary specific transcription factor Pit-1, which is necessary for the expression of both genes (Shupnik 2000).

The early decrease in liver TRβ1 mRNA expression, which occurs prior to and probably contributes to the decrease of liver D1 mRNA, might be caused by other mechanisms, for instance, the recently described participation of the ubiquitine proteasome in TR degradation (Englebienne et al. 2003).

Our results, presented schematically in Fig. 6, suggest involvement of the hypothalamus, pituitary, thyroid and peripheral tissues (liver) in the downregulation of thyroid hormone metabolism during acute illness. Although the mechanisms remain to be established, competition for limiting amounts of nuclear factors involved in both immune response and thyroid hormone metabolism seems an attractive possibility to explain, at least partly, the central and peripheral changes in this condition.

References
Arem R, Wiener GJ, Kaplan SG, Kim HS, Reichlin S & Kaplan MM 1993 Reduced tissue thyroid hormone levels in fatal illness. *Metabolism* 42 1102–1108.
Boelen A, Maas MA, Loewik CW, Platvoeter Schiphorst MC & Wiersinga WM 1996 Induced illness in interleukin-6 (IL-6) knock-out mice: a causal role of IL-6 in the development of the low 3,5,3'-triiodothyronine syndrome. *Endocrinology* 137 5250–5254.
Changes in the HPT axis during acute illness · A BOELEN and others 323


Engelbienne P, Verhas M, Herst CV & De Meirleir K 2003 Type I interferons induce proteins susceptible to act as thyroid receptor (TR) corepressors and to signal the TR for destruction by the proteasome: possible etiology for unexplained chronic fatigue. *Medical Hypotheses* **60** 175–180.


Shupnik MA 2000 Thyroid hormone suppression of pituitary hormone gene expression. *Reviews in Endocrine and Metabolic Disorders* **1** 35–42.


Yu J & Koenig RJ 2000 Regulation of hepatocyte thyroxine 5'-deiodinase by T3 and nuclear receptor coactivators as a model of the sick euthyroid syndrome. *Journal of Biological Chemistry* **275** 38296–38301.

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