

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 (T_3) and thyroxine (T_4) 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)– β_2 , TSH β and 5'-deiodinase (D1) mRNAs), the thyroid (decreased TSH receptor mRNA) and the liver (decreased TR β_1 and D1 mRNA). In the hypothalamus, type 2 deiodinase mRNA expression was strongly increased whereas prepro-TRH mRNA expression did not change after LPS. Serum T_3 and T_4 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.

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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 (T_3) and thyroxine (T_4) levels and increased serum reverse T_3 (r T_3) 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 thyroid hormone release by the thyroid gland, decreased transport of thyroid hormones across the plasma membrane, and a decrease in extrathyroidal (peripheral) conversion of T_4 into T_3 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 T_4 and T_3 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 T_3 and T_4 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% NaCl. Control mice received 0.5 ml sterile 0.9% NaCl. At different time-points after LPS injection ($t=0, 4, 8$ and 24 h) four to five mice were anaesthetized with isoflurane and killed. The liver, pituitary and hypothalamus were obtained.

Experiment 2 In this experiment, LPS was administered as described above and mice were killed at $t=0, 1, 2, 3, 4$ and 6 h ($n=5$). The liver, thyroid (two in each group), 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 T_3 and T_4 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 1st 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)-1 β (Bouaboula *et al.* 1992). We designed primer pairs for D1, D2, type 3 deiodinase D3, thyroid receptor (TR)- β 1, TR β 2, TSH β and preproTRH (D1 forward: CATTCTACTCCCTCTACCA and reverse: GCATCT TCCCGACATTT; D2 forward: GATGCTCCCAAT TCCAGTGT and reverse: AGTGAAAGGTGGTCAG GTGG; D3 forward: CTACGTCATCCAGAGTGGCA and reverse: CTGTTTCATCATAGCGCTCCA; TR β 1 forward: CACCTGGATCCTGACGATGT and reverse: ACAGGTGATGCAGCGAT AGT; TR β 2 forward: GTGAATCAGCCTTATACCTG and reverse: ACAGG TGATGCAGCGATAGT; TSH β forward: TCAACAC CACCATCTGTGCT and reverse: TTGCCACACTT GCAGCTTAC; preproTRH forward: TCGTGCTAAC TGGTATCCCC and reverse: CCCAAATCTCCCCT CTCTTC). 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 $MgCl_2$ concentrations. PCR reactions were set up with cDNA, $MgCl_2$ (25 mM), SybrGreenI (Roche Molecular Biochemicals), forward and reverse primer and H_2O . 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; TR β 1, 54 °C; TR β 2, 55 °C; TSH β , 55 °C; preproTRH, 55 °C; IL-1 β , 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 Polytron (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

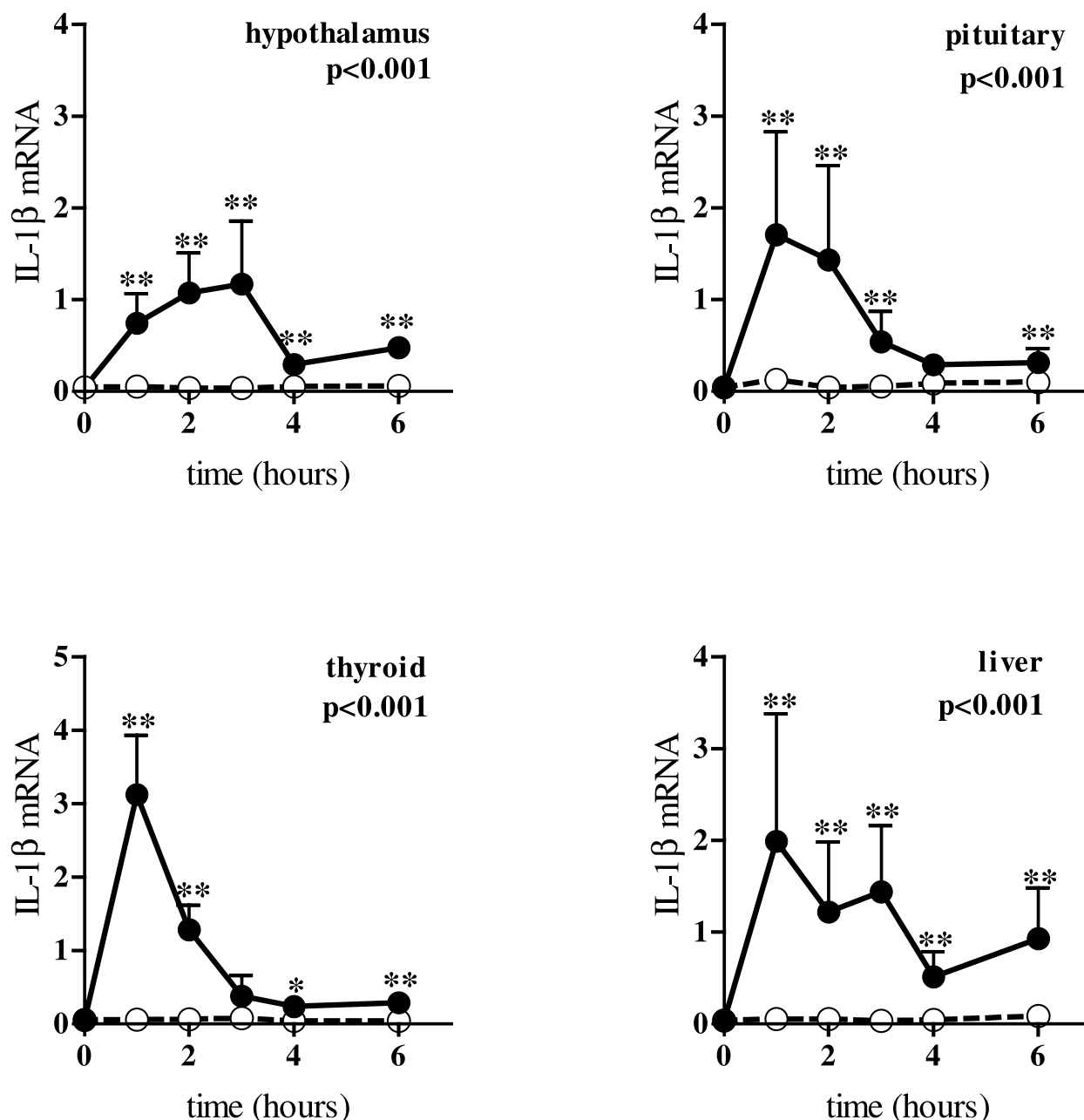


Figure 1 Relative expression of IL-1 β mRNA in the hypothalamus, pituitary, thyroid and liver of mice after administration of LPS (●) or saline (○). Mean values \pm S.E.M. are shown. P values indicate differences between groups by ANOVA. * $P < 0.05$ and ** $P < 0.01$, differences between groups at a single time-point.

incubations of homogenates (10 μ g protein) for 30 min at 37 °C with 0.1 μ M [3',5'- 125 I]rT $_3$ (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. Follow-

ing centrifugation, 125 I $^-$ was isolated from the supernatant by chromatography on Sephadex LH-20 minicolumns.

Statistics

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

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 (Horn 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 T_3 and T_4 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-1 β 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 TR β 1 mRNA expression was also not influenced by LPS administration. TR β 2 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-1 β mRNA expression, maximal at 1 h after administration (Fig. 1). LPS also resulted in a significant decrease in TSH β , TR β 2 and D1 mRNA expression within 24 h, with the lowest mRNA expression at 4 h after LPS administration. D2 mRNA expression and TR β 1 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-1 β 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). TR β 1 mRNA expression was not

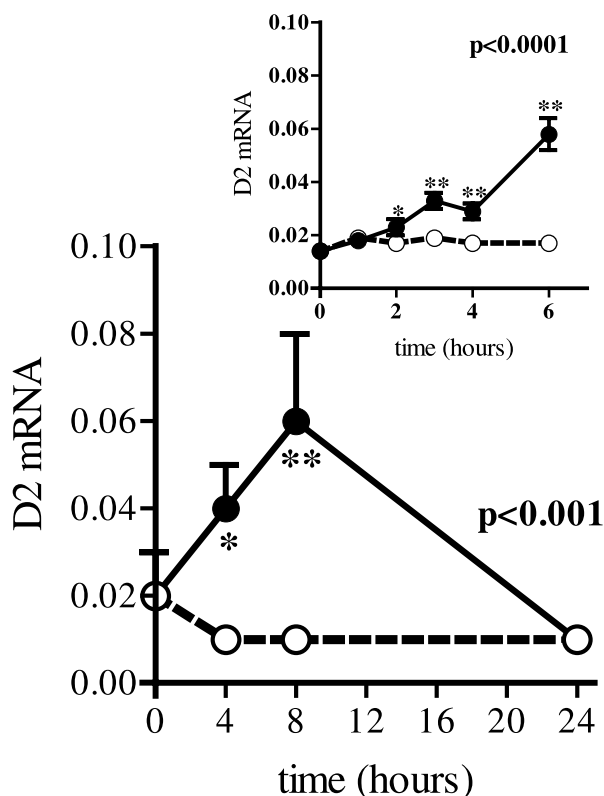


Figure 2 Relative expression of hypothalamic D2 mRNA in mice after administration of LPS (●) or saline (○) within 24 h (lower graph) and shortly after LPS administration (inset). Mean values \pm S.E.M. are shown. *P* values indicate differences between groups by ANOVA. **P*<0.05 and ***P*<0.01, differences between groups at a single time-point.

different between LPS-treated and control mice (data not shown).

Liver and circulation

LPS administration resulted in strongly elevated IL-1 β mRNA expression in the liver, which was maximal at 1 h and remained elevated until 6 h (Fig. 1). LPS decreased liver TR β 1 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 TR β 1 mRNA preceded the decrease of D1 mRNA expression which was, in turn, followed by decreased serum T_3 and T_4 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

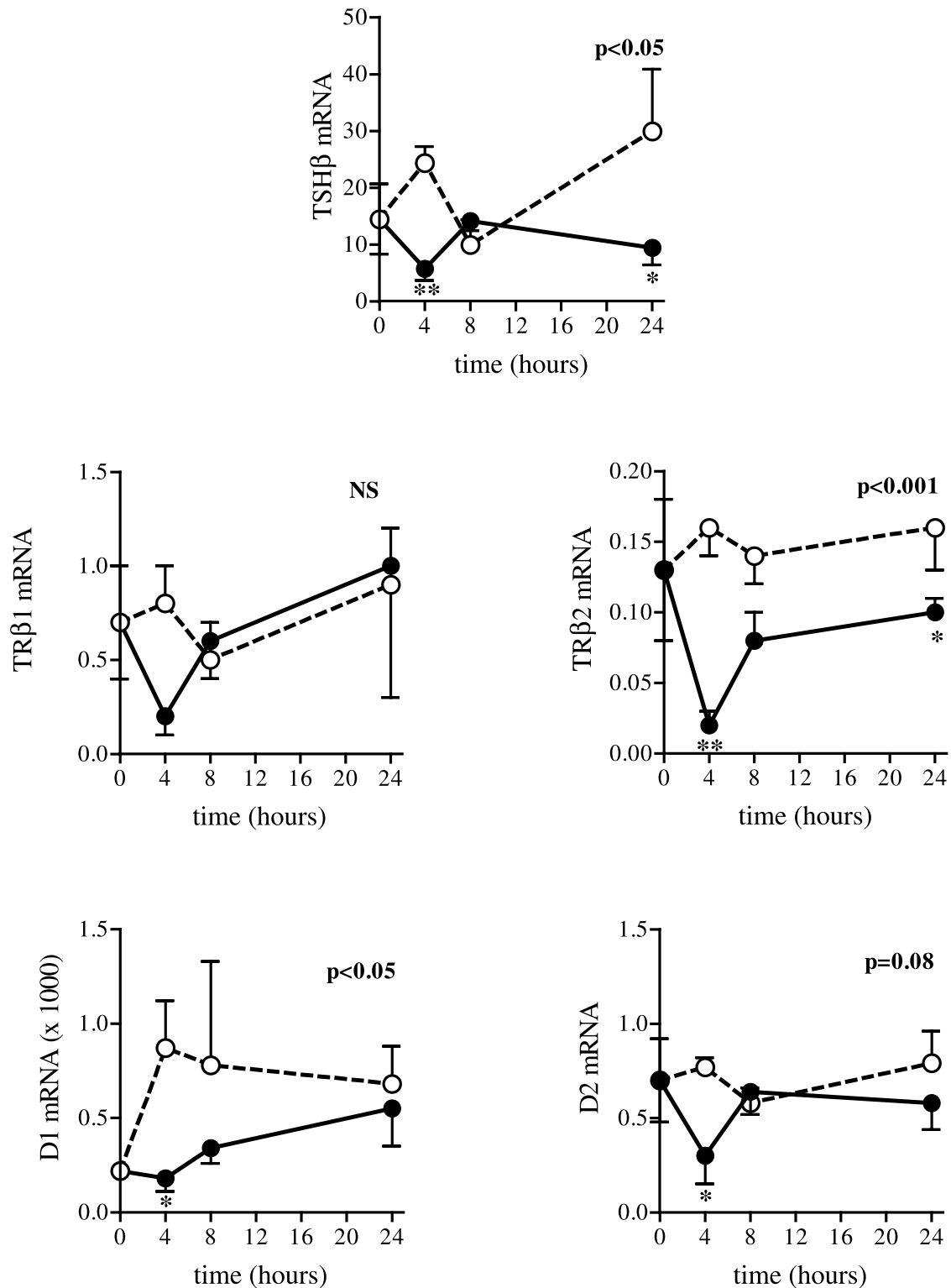


Figure 3 Relative expression of TSHβ, TRβ1, TRβ2, D1 and D2 mRNA in the pituitary of mice after administration of LPS (●) or saline (○). Mean values ± S.E.M. are shown. *P* values indicate differences between groups by ANOVA. **P*<0.05 and ***P*<0.01, differences between groups at a single time-point.

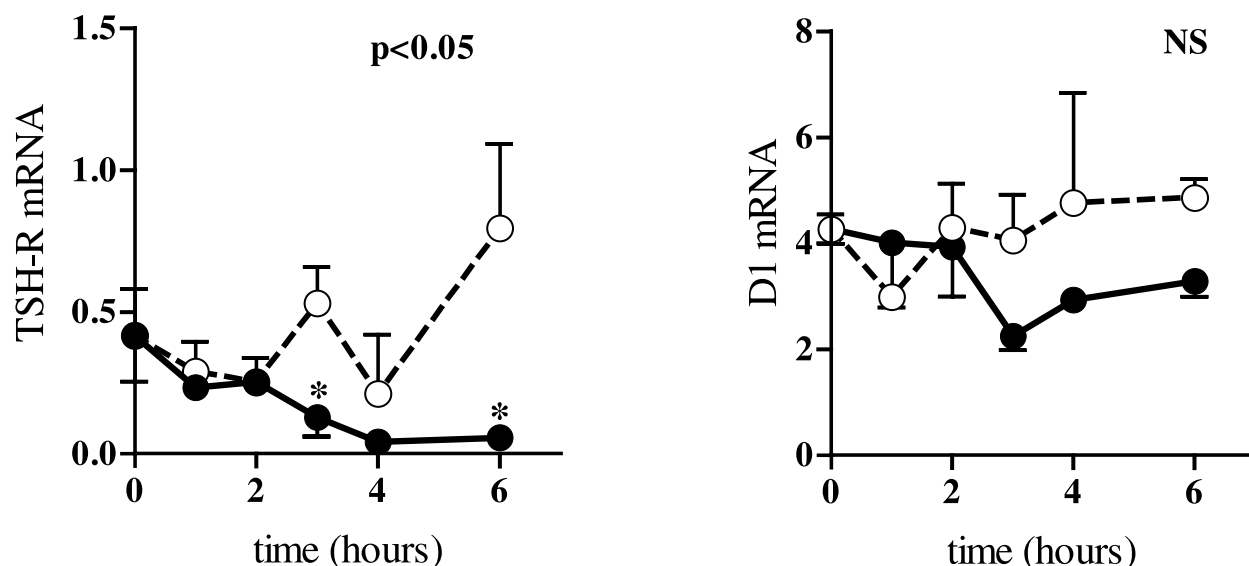


Figure 4 Relative expression of TSH-R and D1 mRNA expression in the thyroid of mice after administration of LPS (●) or saline (○). Mean values \pm S.E.M. are shown. *P* values indicate differences between groups by ANOVA. **P* < 0.05, differences between groups at a single time-point. NS, not significant.

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-1 β 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-1 β mRNA expression peaked very rapidly (within 1 h) in the pituitary, thyroid and liver while hypothalamic IL-1 β mRNA expression was maximal at 3–4 h after LPS administration.

Hypothalamic D2 mRNA expression increased following the rise in IL-1 β 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 TR β 2 mRNA in the hypothalamus might be that TR β 2 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 TSH β 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

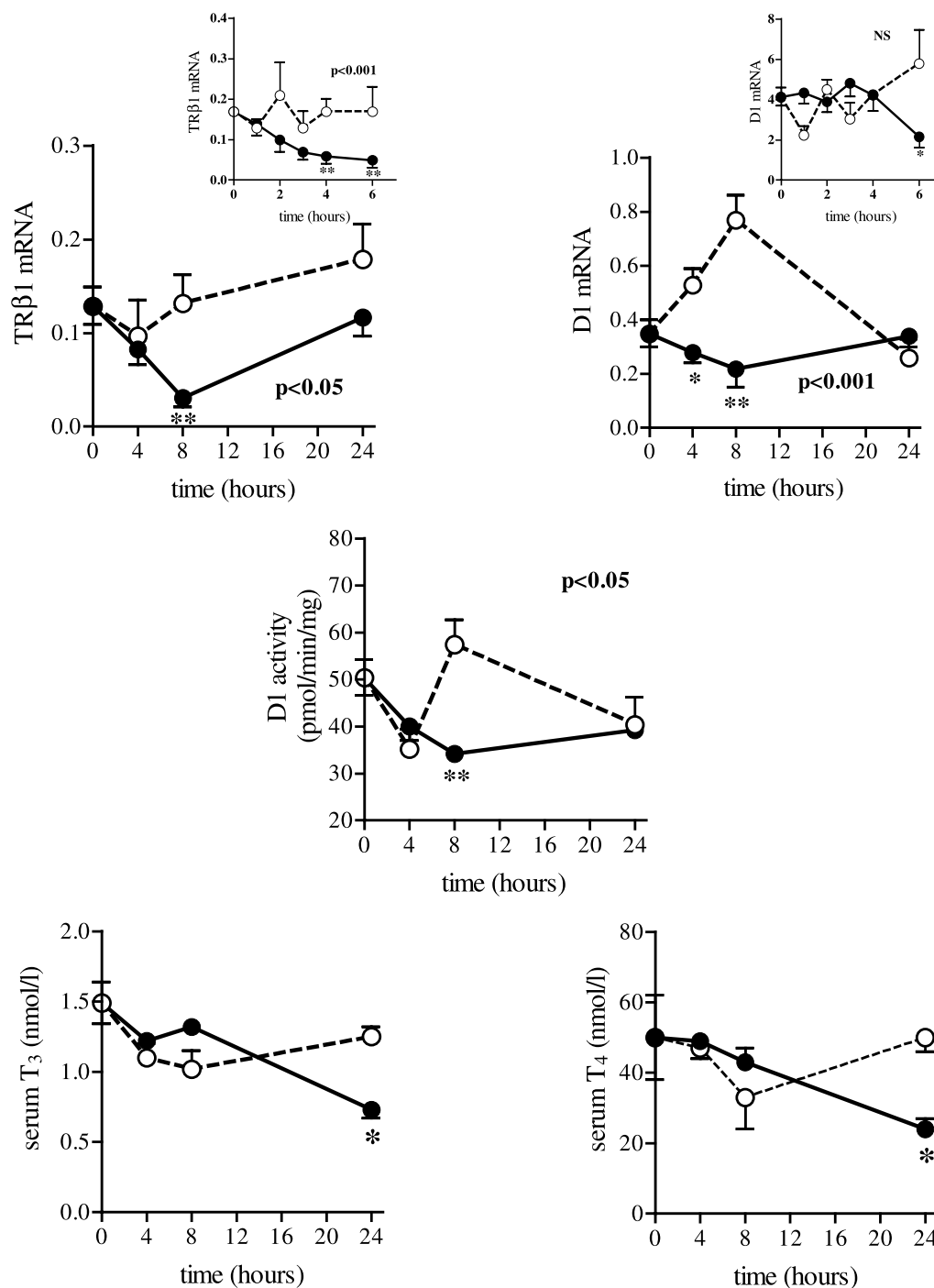


Figure 5 Relative expression of liver TRβ1 and D1 mRNA, liver D1 activity and serum T₃ and T₄ levels in mice after administration of LPS (●) or saline (○). Mean values ± S.E.M. are shown. *P* values indicate differences between groups by ANOVA. **P*<0.05 and ***P*<0.01, differences between groups at a single time-point.

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

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 neurones 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- κ B (NF- κ B) and TR β 1, might be responsible for decreased liver D1 mRNA expression because of competition between cytokine-induced NF- κ B and the TR β 1/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 TR β 2 and TSH β mRNAs that we observed suggests a shortage of a common factor needed for the expression of both TR β 2 and TSH β . 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 TR β 1 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.

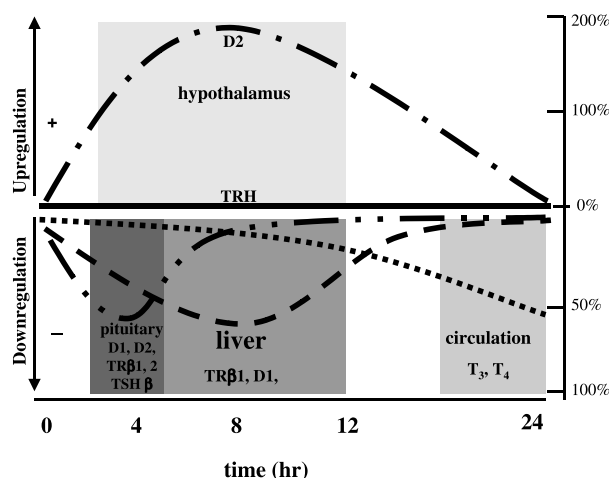


Figure 6 Model of the time-course of central and peripheral thyroid hormone metabolism during acute illness in mice induced by LPS administration. LPS rapidly induces IL-1 β mRNA expression in all organs of the HPT axis (see Fig. 1) which is followed by almost simultaneous involvement of the hypothalamus, pituitary, thyroid and liver in the downregulation of thyroid hormone metabolism during illness.

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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, Platvoet-ter Schiphorst MC, Bakker O & Wiersinga WM 1995 The role of cytokines in the lipopolysaccharide-induced sick euthyroid syndrome in mice. *Journal of Endocrinology* **146** 475–483.
- Boelen A, Maas MA, Lowik CW, Platvoetter 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.
- Boelen A, Kwakkel J, Platvoet-ter Schiphorst MC, Baur A, Kohrle J & Wiersinga WM 2004 Contribution of interleukin-12 to the pathogenesis of nonthyroidal illness. *Hormone and Metabolic Research* **36** 101–106.
- Bouaboula M, Legoux P, Pessegue B, Delpech B, Dumont X, Piechaczyk M, Casellas P & Shire D 1992 Standardization of mRNA titration using a polymerase chain reaction method involving co-amplification with a multispecific internal control. *Journal of Biological Chemistry* **267** 21830–21838.
- Cook CB, Kakucska I, Lechan RM & Koenig RJ 1992 Expression of thyroid hormone receptor beta 2 in rat hypothalamus. *Endocrinology* **130** 1077–1079.

- Diano S, Naftolin F, Goglia F & Horvath TL 1998 Fasting-induced increase in type II iodothyronine deiodinase activity and messenger ribonucleic acid levels is not reversed by thyroxine in the rat hypothalamus. *Endocrinology* **139** 2879–2884.
- Englebienne 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.
- Fekete C, Gereben B, Doleschall M, Harney JW, Miguel DJ, Bianco AC, Sarkar S, Liposits Z, Rand W, Emerson C, Kacsokovics I, Reed LP & Lechan RM 2004 Lipopolysaccharide induces type 2 iodothyronine deiodinase in the mediobasal hypothalamus: implications for the nonthyroidal illness syndrome. *Endocrinology* **145** 1649–1655.
- Fliers E, Guldenaar SE, Wiersinga WM & Swaab DF 1997 Decreased hypothalamic thyrotropin-releasing hormone gene expression in patients with nonthyroidal illness. *Journal of Clinical Endocrinology and Metabolism* **82** 4032–4036.
- Hochberg Y & Tamhane A 1987 *Multiple Comparison Procedures*. New York: Wiley & Sons.
- Hora SCW 1984 The F-statistic in two-way layout with rank-scored transformed data. *Journal of the American Statistical Association* **79** 668–673.
- Kakucska I, Romero LI, Clark BD, Rondeel JM, Qi Y, Alex S, Emerson CH & Lechan RM 1994 Suppression of thyrotropin-releasing hormone gene expression by interleukin-1-beta in the rat: implications for nonthyroidal illness. *Neuroendocrinology* **59** 129–137.
- Lechan RM, Qi Y, Jackson IM & Mahdavi V 1994 Identification of thyroid hormone receptor isoforms in thyrotropin-releasing hormone neurons of the hypothalamic paraventricular nucleus. *Endocrinology* **135** 92–100.
- Lohrer P, Gloddek J, Nagashima AC, Korali Z, Hopfner U, Pereda MP, Arzt E, Stalla GK & Renner U 2000 Lipopolysaccharide directly stimulates the intrapituitary interleukin-6 production by folliculostellate cells via specific receptors and the p38 alpha mitogen-activated protein kinase/nuclear factor-kappaB pathway. *Endocrinology* **141** 4457–4465.
- Peeters RP, Wouters PJ, Kaptein E, Van Toor H, Visser TJ & Van den BG 2003 Reduced activation and increased inactivation of thyroid hormone in tissues of critically ill patients. *Journal of Clinical Endocrinology and Metabolism* **88** 3202–3211.
- Segerson TP, Kauer J, Wolfe HC, Mobtaker H, Wu P, Jackson IM & Lechan RM 1987 Thyroid hormone regulates TRH biosynthesis in the paraventricular nucleus of the rat hypothalamus. *Science* **238** 78–80.
- Shimura MA, Haraguchi K, Endo T & Onaya T 1997 Regulation of thyrotropin receptor gene expression in 3T3-L1 adipose cells is distinct from its regulation in FRTL-5 thyroid cells. *Endocrinology* **138** 1483–1490.
- Shupnik MA 2000 Thyroid hormone suppression of pituitary hormone gene expression. *Reviews in Endocrine and Metabolic Disorders* **1** 35–42.
- Sweet MJ, Leung BP, Kang D, Sogaard M, Schulz K, Trajkovic V, Campbell CC, Xu D & Liew FY 2001 A novel pathway regulating lipopolysaccharide-induced shock by ST2/T1 via inhibition of Toll-like receptor 4 expression. *Journal of Immunology* **166** 6633–6639.
- Van den Berghe G 2000 Novel insights into the neuroendocrinology of critical illness. *European Journal of Endocrinology* **143** 1–13.
- Van den Berghe G, Wouters P, Weekers F, Mohan S, Baxter RC, Veldhuis JD, Bowers CY & Bouillon R 1999 Reactivation of pituitary hormone release and metabolic improvement by infusion of growth hormone-releasing peptide and thyrotropin-releasing hormone in patients with protracted critical illness. *Journal of Clinical Endocrinology and Metabolism* **84** 1311–1323.
- Wiersinga WM 2000 Nonthyroidal illness. In *The Thyroid*, edn 7, pp 281–295. Eds LE Braverman & RD Utiger. Philadelphia: Lippincott.
- Wiersinga WM & Chopra IJ 1982 Radioimmunoassay of thyroxine (T4), 3,5,3'-triiodothyronine (T3), 3,3',5'-triiodothyronine (reverse T3, rT3), and 3,3'-diiodothyronine (T2). *Methods in Enzymology* **84** 272–303.
- 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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