Understanding the non-thyroidal illness syndrome from in vivo and in vitro studies

Kwakkel, G.J.

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Summary
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During illness thyroid hormone metabolism changes and this is collectively known as the non thyroidal illness syndrome (NTIS). The hallmarks of NTIS are decreased serum thyroid hormone levels, while pituitary thyroid stimulating hormone (TSH) and hypothalamic thyrotropin releasing hormone (TRH) expression do not increase indicating altered feedback regulation. In this thesis we aimed to study the mechanisms and cellular signaling pathways involved in the changes observed in thyroid hormone metabolism during illness. To address this question we used both animal models (in vivo studies) and cell culture models (in vitro studies).

Chapter 1 provides an introduction to the hypothalamus-pituitary-thyroid (HPT)-axis. In this thesis we primarily focus on the role of the deiodinating enzymes and thyroid hormone receptors (TR) during NTIS; therefore the deiodinases and the structure and function of the TRs are summarized. The characteristics of NTIS in humans and animal models of NTIS are discussed. Furthermore, as we investigate the involvement of the inflammatory pathways during NTIS, a simplified overview of the activation of the inflammatory pathways is given.

In chapter 2 we designed an animal model to evaluate the changes of the HPT-axis observed during illness. Administration of bacterial endotoxin (LPS) induced changes in hypothalamus, pituitary, thyroid, serum and liver. We observed a rapid simultaneous increase of the pro-inflammatory cytokine IL-1β in all compartments of the HPT-axis, which was followed by changes in genes involved in thyroid hormone metabolism. We concluded that illness induced simultaneous changes in thyroid hormone metabolism at every level of the HPT-axis.

In Chapter 3 we evaluated D2 muscle expression and D2 regulating factors in two animal models of illness (S. pneumoniae infection and turpentine induced chronic inflammation), as it was postulated that decreased muscle D2 expression might contribute to the low serum T3 levels observed during illness. In addition we measured muscle D3 expression. We observed that D2 expression was differentially regulated during illness, probably related to differences in the inflammatory response and type of pathology. During chronic inflammation muscle D2 and D3 expression both increased while after S. pneumoniae infection D2 decreased and D3 did not change. The increase of D2 and D3 during chronic inflammation coincided with the activation of the cAMP pathway. The decrease of D2 mRNA after severe bacterial infection was associated with local IL-1β mRNA expression and might also be due to diminished food-intake. Thus, muscle deiodinase expression is differentially regulated during illness, depending on severity and type of illness.
In chapters 4 and 5 we used thyroid hormone receptor knock-out mice to study the role of thyroid hormone receptors in the changes observed in peripheral thyroid hormone metabolism during illness. Our results in chapter 4 show that the LPS-induced decrease in serum T₃ and T₄ and liver D1 takes place despite the absence of TRβ. Moreover, serum T₃ decreased rapidly after LPS, followed later on by decreased liver D1, indicating that the contribution of liver D1 during NTIS may be limited with respect to decreasing serum T₃ levels.

In contrast, the decrease in serum thyroid hormones and liver D1 was attenuated in TRα–deficient mice, whereas the LPS induced fall in liver D3 mRNA was more pronounced in TRα–deficient mice (chapter 5). Muscle D2 mRNA increased similarly, whereas muscle D3 mRNA decreased less pronounced in TRα–deficient mice. From these studies we concluded that the alterations in peripheral thyroid hormone metabolism induced by LPS administration are partly regulated via TRα.

To study the illness-induced decreases of liver TRα, TRβ1 and D1 mRNA expression in detail, we used an in vitro model (human hepatoma cells) in chapters 6 and 7. We stimulated human hepatoma (HepG2) cells with the pro-inflammatory cytokine IL-1β. TRα1, TRα2, TRβ1 and D1 mRNA decreased after 4-6 hours of IL-1β stimulation, similar to liver mRNA expression after LPS administration in mice. To determine the involvement of specific inflammatory pathways, we used inhibitors of the NFκB pathway and AP-1 pathway. From these studies we concluded that the decrease of TRβ1 mRNA is exclusively mediated by the NFκB pathway, while the decrease of TRα1 and TRα2 and D1 mRNA requires inhibition of both the AP-1 and the NFκB pathway. Furthermore, using an inhibitor of promoter activity and an inhibitor of protein synthesis we observed that the IL-1β-induced decrease of TRα1 and TRα2 mRNA was independent of protein synthesis and due to reduced promoter activity.

To study the mechanisms behind the changes in muscle D2 and D3 during illness, we used the myoblast cell-line C2C12 in chapter 8. We stimulated C2C12 myoblasts and myotubes, which are differentiated myoblasts, with IL-1β, LPS, a mixture of cytokines or an activator of the cAMP pathway. We observed that activation of the cAMP pathway in myoblasts resulted in an early increase of D2 mRNA, which was followed by D3 mRNA induction, as observed in vivo during chronic inflammation. Surprisingly, stimulation of myoblasts and myotubes with inflammatory mediators, which are abundantly present during illness, did not result in any change in D2 or D3 mRNA expression, indicating that the inflammatory signalling pathways do not play a prominent role in altered deiodinase expression observed during illness.

In the general discussion, Chapter 9, our results are put in a broader perspective. For each compartment of the HPT-axis the changes during illness are summarized.
and possible mechanisms are discussed. In the concluding remarks the role of the deiodinases and TRs in the decrease of serum thyroid hormones during illness are discussed and suggestions for future research are given.