Expression of thyroid hormone receptor isoforms in rodents

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Summary

Thyroid hormone influences various functions in de cells by rapid non-genomic and slower genomic effects; the latter take place in the cell nucleus.

In chapter one the genomic effects of thyroid hormone are reviewed. Thyroid hormone binds to its receptor (TR) and forms a complex. This hormone receptor complex binds to specific DNA sequences called thyroid hormone response elements in the promoter region of target genes, interacts with different proteins such as co-activators and co-repressors and ultimately leads to either activation or repression of gene expression. Until now at least five TR isoforms have been identified, with marked heterogeneity in tissue distribution (e.g. TRβ1 is the predominant form in the liver and TRα1 in the heart. Studies in TR knock-out mice have revealed more insight in organ specific functions of TR. It appears that some genes are regulated by a specific TR isoform. Expression of TR isoforms differ between tissues, but differences in expression may also exist within the same tissue.

In chapter two the expression of TRβ1 at protein level in rat livers is investigated using newly developed specific polyclonal antibodies. Unexpectedly, the TRβ1 protein was expressed only in hepatocytes around the central veins in the liver lobe (zonal expression). The discovery of this pericentral zonation resulted in new insights in gene regulation by TR. The TRβ1 protein expression was also subjected to diurnal variation. In the beginning of the dark period when the rats are active, the highest level of TRβ1 protein was observed. However, the nature of this diurnal variation was not immediately clear.

In chapter three the expression of two other TR isoforms, TRα1 and TRα2, at protein level in rat livers was studied using novel specific monoclonal antibodies. The specificity of these antibodies was confirmed using Western blots and immunohistochemistry of liver sections of TR Knock-out mice. After determination of the optimal conditions to use these antibodies for immunohistochemistry, it was observed again that both isoforms were preferentially expressed around the central veins of the liver lobe although they had a slightly broader expression than the TRβ1 protein. A diurnal variation existed in the expression of the TRα2 protein, which peaked in the light period when rats mostly rest.

In chapter four the nature of the observed diurnal variation of TRs in liver was studied by two sets of experiments. In the first experiment, the biological clock was inactivated by a lesion in suprachiasmatic nucleus (SCN) of the hypothalamus. Destruction of the biological clock resulted in loss of diurnal variation of both TRα1 and TRα2 isoforms at mRNA level with no obvious changes in mRNA levels of TRβ1. In the second experiment the animals were subjected to a regular feeding regimen (by feeding them every four hours for 15 minutes). The regular feeding resulted in marked changes in diurnal variation of TRβ1 (a sharp decrease of mRNA levels at ZT18.5), whereas the diurnal variation of both TRα1 and TRα2 isoforms at mRNA level was minimally affected (reduced amplitude of their rhythms). We concluded that the biological clock in the SCN is the driving force behind a circadian rhythm in both
TR$\alpha$1 and TR$\alpha$2 isoforms, whereas food intake plays an important role in diurnal changes in TR$\beta$1 expression in rat livers.

In chapter five the regulation of three T$_3$-responsive genes ("Spot 14", 5'-deiodinase type I (5'D1) and malic enzyme) were studied at the molecular level, using TR knock-out animals (TR$\alpha$1$^-$, TR$\beta$1$^-$, TR$\alpha$1$^+$/TR$\beta$1$^-$ and TR$\alpha$2$^+$/TR$\beta$1$^-$) and wild type mice. The results were rather clear: "Spot14" was fully TR$\beta$1 dependent, 5'D1 was partly TR$\beta$1 dependent and malic enzyme was regulated by both TR$\alpha$1 and TR$\beta$ isoforms. The most impressive finding was that the zonal local expression pattern in the liver of these genes overlaps with that of TR isoforms which regulate them. The overall conclusion was that the changes in TR zonal distribution in the liver are likely the determinants of zonal expression of T$_3$-responsive genes.

In chapter six the changes in expression of TR during thyroid hormone deficiency and excess were studied. Hypothyroidism resulted generally in an increased TR$\beta$1, TR$\alpha$1 and TR$\alpha$2 mRNA and protein expression, while expression of TRs was decreased during hyperthyroidism except for TR$\beta$1 protein expression which showed a reversal in its daily variation. Both hypo- and hyperthyroid conditions did not result in changes in zonal distribution of TR in rat livers indicating that the thyroid hormone effects remain restricted to the same cells in the liver independent of ambient thyroid hormone concentrations.

In chapter seven the expression of TR in rat hearts was studied after induction of right ventricular hypertrophy by monocrotaline (which induces chronic pulmonary hypertension followed by right ventricle hypertrophy and subsequently congestive heart failure). Interestingly, the changes in TR isoform expression during congestive heart failure were ventricle specific. A decrease in TR$\beta$1 protein expression was observed in the right ventricle, whereas a fall in the expression of TR$\alpha$ isoforms was restricted to left ventricle. Changes were also found in the expression of two cardiac specific T$_3$-responsive genes (SERCA2a and the MHC isoforms) in both ventricles the SERCA2a mRNA levels showed minor changes after two weeks, but decreased after four weeks at the time of congestive heart failure whereas changes in MHC isoforms were observed after two weeks. We found no evidence of isoform specific regulation for these genes, which is in conflict with another recent publication.