5 Summary

It is widely accepted that the principle physiological actions of thyroid hormones are induced by the binding of T_3 to its nuclear receptors. However, in recent years, a number of reports from several independent study groups have suggested that diiodothyronine 3,5-T_2 and, to a lesser extent, 3,3'-T_2 may also have physiological effects, for example on mitochondrial activity. Until now, no radioimmunoassay methods has been available to accurately measure serum concentrations of these diiodothyronines in humans, and these hormones have never been measured in the tissues of humans or experimental animals. The purpose of this study was to develop sensitive radioimmunoassays for the measurement of these hormones in blood and in tissues of humans and rats under physiological and pathological conditions.

Using 3-Br-5-[^{125}I]T_1 as tracer, a 3,5-T_2 RIA was developed with lower limits of detection that allowed the measurement of 1.0 fmol/g and 0.8 pmol/l 3,5-T_2 in tissue and in serum, respectively. The detection limits of a newly developed 3,3'-T_2 RIA allowed the determination of 1.8 fmol/g and 1.5 pmol/l in tissue and in serum, respectively.

The mean serum levels of 3,5-T_2 in healthy humans were 16.2 $\pm$ 6.4 pmol/l, those of 3,3'-T_2 were 46.6 $\pm$ 20 pmol/l. Serum concentrations of both hormones were increased in hyperthyroid patients and decreased in hypothyroid subjects.

Serum concentrations of both hormones were also measured in four groups of patients with different NTI. The most interesting results were significant increases in 3,5-T_2 serum concentrations of all four patients groups with NTI. Hypothetically, the increased 3,5-T_2 production in patients with NTI could explain why these patients are clinically euthyroid in spite of sometimes dramatically decreased serum and tissue levels of T_3. An enhanced metabolization pathway of T_4 to T_3 to 3,5-T_2 in patients with NTI may also explain why not only serum T_3 but also serum T_4 concentrations decrease in patients with advanced NTI. 3,5-T_2 concentrations in brain tumors and metastases were considerably higher than those measured in tissue from patients

who died from causes other than brain disorders. This result confirms the enhanced production of 3,5-T_2 in NTI also on the tissue level.

3,3'-T_2 serum concentrations were either increased, normal, or decreased in the groups of patients with different NTI. These results do not suggest an involvement of this hormone in the maintenance of euthyroidism in patients with NTI.

Both 3,5-T_2 and 3,3'-T_2 were detectable in rat brain homogenates, where their concentrations varied widely among the brain areas.

On the subcellular level, 3,5-T_2 was not bound to any of the subcellular fractions (nuclei, mitochondria, myelin, synaptosomes, and microsomes) investigated in control rats. However, after subchronic treatment with three different doses of the antidepressant desipramine, 3,5-T_2 tissue levels were detectable in the mitochondria and myelin fractions of the amygdala, but not in those of the parieto-occipital cortex. The effects of desipramine on 3,5-T_2 paralleled those on T_3, which was also increased in the myelin fraction of the amygdala but not in that of the parieto-occipital cortex.

These results suggest that 3,5-T_2 may be produced and bound to specific cellular compartments only under certain circumstances (such as after antidepressant administration), which need to be investigated further. The well-known interaction between thyroid hormones and depressive illness, as well as the specific role of the amygdala in the modulation of emotions, indicates that the effects of desipramine on thyroid hormone levels may be related to the as yet unknown mechanism of action of this drug as an antidepressant.

In summary, this study reported, for the first time, reliable serum and tissue concentrations of 3,5-T_2 and 3,3'-T_2 in humans and in rats. Specific changes in their concentrations could be demonstrated under pathological conditions (e.g., NTI), following pharmacological treatment (e.g., with antidepressants), or even under physiological conditions (e.g., circadian variations). The detection of clearly measurable tissue levels of these hormones, which are affected by different physiological and pathological circumstances, makes it plausible that these

hormones may indeed have physiological functions and provides a reasonable basis for the further investigation of the biochemical mechanisms underlying these effects. The data indicate that biochemical and physiological effects of 3,5-T_2 are much more likely than those of 3,3'-T_2.