Table of contents

1 Introduction 1

1.1 Physiological effects of thyroid hormones 1
1.1.1 Effects of triiodothyronine (T_3) 1
1.1.1.1 Effects of T_3 at nuclear receptors 1
1.1.1.2 Non-nuclear effects of T_3 3
1.1.2 Physiological effects of diiodothyronines 5
1.1.2.1 Physiological effects of 3,5-diiodothyronine (3,5-T_2) 5
1.1.2.2 Physiological effects of 3,3'-diiodothyronine (3,3'-T_2) 8

1.2 Rationale for the choice of experiments performed in this study 9
1.2.1 Diiodothyronine serum concentrations in humans with thyroidal and nonthyroidal illnesses 9
1.2.2 Diiodothyronine concentrations in normal brain tissue and in brain tumors 11
1.2.3 Diiodothyronine concentrations in rat brain homogenates 11
1.2.4 Diiodothyronine concentrations in subcellular compartments 14
1.2.5 Effects of antidepressant treatment on diiodothyronine concentrations 14
1.2.6 Effects of circadian variations on diiodothyronine concentrations 15

1.3 Purpose of the study 15

2 Materials and Methods 17

2.1 Materials 17
2.1.1 Equipment for subcellular fractionation 17
2.1.2 Equipment for radiolabeling iodothyronines 17
2.1.3 Equipment for extracting, purifying, and separating iodothyronines 17
2.1.4 Equipment for RIA quantification 18
2.1.5 Chemicals and reagents for subcellular fractionation 19
2.1.6 Chemicals and reagents for radiolabeling iodothyronines 19
2.1.7 Chemicals and reagents for extracting, purifying, and separating iodothyronines 20
2.1.8 Chemicals and reagents for RIA quantification 20
2.1.9 Drugs 21

2.2 Studies in humans 21
2.2.1 Hormone determination in human serum 21
- 2.2.1.1 Healthy controls 21
- 2.2.1.2 Patients with thyroid disorders 22
- 2.2.1.3 Patients with different somatic, nonthyroidal diseases 22
  - 2.2.1.3.1 Patients with sepsis 22

- 2.2.1.3.2 Patients with head and/or brain injury....22
- 2.2.1.3.3 Patients with brain tumors and metastases....23
- 2.2.1.3.4 Patients with liver diseases....23
- 2.2.1.4 Acute stress....24
- 2.2.1.5 Sleep deprivation....24

2.2.2 Hormone determination in human brain tissue....25
- 2.2.2.1 Healthy donors....25
- 2.2.2.2 Samples of human brain tumors and metastases....26

2.3 Studies in experimental animals....26
2.3.1 Animals....26
2.3.2 Group 1: Control animals....26
- 2.3.2.1 Homogenates of different brain areas, pituitary glands, and liver of the rat....26
- 2.3.2.2 Subcellular fractions of rat brain regions....27

2.3.3 Group 2: Antidepressant treatment with desipramine....27
2.3.4 Group 3: Circadian variations of iodothyronines....27

2.4 Hormone determination....28
2.4.1 RIA buffers....28
2.4.2 Synthesis of 3,5-T_2 tracer....28
2.4.3 Synthesis of 3,3‘-T_2 tracer....28
2.4.4 Preparation of 3,5-T_2-binding antibody....30
2.4.5 Preparation of 3,3'-T_2 -binding antibody....31
2.4.6 RIA procedure for 3,5-T_2 in serum and tissue samples....31
2.4.7 RIA procedure for 3,3'-T_2 in serum and tissue samples....32
2.4.8 Serum determination of other iodothyronines and thyrotropin (TSH)....32
2.4.9 Tissue determination of other iodothyronines....33

2.5 Preparation of animal and human tissue samples....34
2.5.1 Brain dissection in the rat....34
2.5.2 Subcellular fractionation....37
- 2.5.2.1 Homogenization of the single areas of the rat brain....38
- 2.5.2.2 Centrifugation of the homogenates....38
- 2.5.2.3 Isolation of the nuclear fraction....38
- 2.5.2.4 Isolation of the mitochondrial fraction....39
- 2.5.2.5 Isolation of the synaptosomal fraction....39
- 2.5.2.6 Isolation of the myelin....39
- 2.5.2.7 Isolation of the microsomal fraction....39
- 2.5.2.8 Electron microscopic characterization of the subcellular fractions....40
- 2.5.2.9 Characterization of the subcellular fractions by biochemical markers....40

2.5.3 Extraction of iodothyronines from the subcellular fractions....43
- 2.5.3.1 Suspension of the subcellular fractions....43

- 2.5.3.2 Extraction of the subcellular fractions 43
- 2.5.4.3 Preparation of the subcellular fractions and homogenate extracts for HPLC 45

2.5.5 Extraction of diiodothyronines from serum 45
2.5.6 Protein quantification 45

2.6 HPLC 46
2.6.1 Preparation of the tissue sample extracts for the autosampler 46
2.6.2 Purification and separation of the extracted iodothyronines by HPLC 47
2.6.3 Iodothyronine collection for RIA quantification 47

2.7 Data analysis 47

3 Results 49

3.1 Method validation 49
3.1.1 HPLC 49
- 3.1.1.1 Separation of iodothyronines by HPLC 49
- 3.1.1.2 Recovery of iodothyronines after extraction and HPLC 50

3.1.2 RIA for 3,5-T_2 50
- 3.1.2.1 RIA sensitivity for 3,5-T_2 50
- 3.1.2.2 Cross-reactivity of 3,5-T_2 antibody with iodothyronines 50
- 3.1.2.3 Cross-reactivity of 3,5-T_2 antibody with drugs 52
- 3.1.2.4 Recovery of “cold” 3,5-T_2 53
- 3.1.2.5 Inter- and intra-assay coefficients of variation 53

3.1.3 RIA for 3,3‘-T_2 54
- 3.1.3.1 RIA sensitivity for 3,3‘-T_2 54
- 3.1.3.2 Cross-reactivity of 3,3‘-T_2 antibody with iodothyronines 55
- 3.1.3.3 Cross-reactivity of 3,3‘-T_2 antibody with drugs 56
- 3.1.3.4 Recovery of “cold“ 3,3‘-T_2 57
- 3.1.3.5 Inter- and intra-assay coefficients of variation 57

3.2 Clinical Studies 58
3.2.1 Serum concentrations of 3,5-T_2 58
- 3.2.1.1 Healthy controls 58
- 3.2.1.2 Patients with thyroid disorders 59
- 3.2.1.3 Patients with different somatic, nonthyroidal diseases 60
- 3.2.1.4 Acute stress 60
- 3.2.1.5 Sleep deprivation 61

3.2.2 Serum concentrations of 3,3'-T_2: 61
- 3.2.2.1 Healthy controls 61
- 3.2.2.2 Patients with thyroid disorders 62

- 3.2.2.3 Patients with different somatic, nonthyroidal diseases 62
- 3.2.2.4 Acute stress 63
- 3.2.2.5 Sleep deprivation 63

3.2.3 Serum concentrations of other iodothyronines and thyrotropin (TSH)..... 64
3.2.4 Tissue levels of 3,5-T_2 65

- 3.2.4.1 Human brain areas of healthy donors 65
- 3.2.4.2 Human brain tumors and metastases 67

3.2.5 Tissue levels of 3,3'-T_2 67

- 3.2.5.1 Human brain areas of healthy donors 67
- 3.2.5.2 Human brain tumors and metastases..... 68

3.2.6 Tissue levels of other iodothyronines in human brain areas of healthy donors..... 68
3.2.7 Tissue levels of other iodothyronines in human brain tumors and metastases..... 69

3.3 Animal studies 71
3.3.1 Serum concentrations of 3,5-T_2 and 3,3'-T_2 in control animals 71
3.3.2 Tissue levels of 3,5-T_2 71

- 3.3.2.1 Homogenates of various brain areas of the rat 71
- 3.3.2.2 Subcellular fractions of brain areas of the rat..... 73

3.3.3 Tissue levels of 3,3'-T_2 74

- 3.3.3.1 Homogenates of various brain areas of the rat 74
- 3.3.3.2 Subcellular fractions of brain areas of the rat..... 74

3.3.4 Tissue levels of other iodothyronines..... 76

- 3.3.4.1 Homogenates of various brain areas of the rat 76
- 3.3.4.2 Subcellular fractions of brain areas of the rat..... 78

3.3.5 Effects of antidepressant drugs on brain subcellular concentrations of 3,5-T_2 and 3,3‘-T_2..... 79
3.3.6 Effects of circadian variation on concentrations of 3,5-T_2 and other iodothyronines..... 82

4. Discussion 87

4.1 3,5-T_2 serum and tissue concentrations in humans..... 87
4.2 3,3'-T_2 serum and tissue concentrations in humans..... 91
4.3 3,5-T_2 serum and tissue concentrations in rats 94
4.4 3,3'-T_2 serum and tissue concentrations in rats..... 100

5 Summary..... 103

6 Reference List..... 106

Curriculum Vitae 117

List of Publications 119

Poster and Slide Presentation 121

Acknowledgment 124