1 Introduction

1.1 Physiological effects of thyroid hormones

1.1.1 Effects of triiodothyronine (T_3)

1.1.1.1 Effects of T_3 at nuclear receptors

The hypothesis that thyroid hormones stimulate transcription of mRNA was first advanced almost 40 years ago (Tata et al., 1963). Nuclear thyroid hormone receptors were first identified in 1986, when two groups reported the cloning of cDNA encoding proteins with the characteristics of thyroid hormone receptors. One group described the isolation of the cDNA clone from the library prepared from chick embryos (Sap et al., 1986) and the other group that from the library from human placenta (Weinberger et al., 1986). The encoded proteins had molecular weights of 50 to 55 kDa and bound T_3 with high affinity. The homology between the residue amino acid sequences for the thyroid hormone receptors and the previously reported sequences of steroid hormone receptors indicated that these proteins were members of a single family (Evans 1988). Differences in the amino acid sequences encoded by the two cDNA indicated that the receptors were the product of separate genes. The gene coding for the human placenta form was designated thyroid hormone receptor ß and that for the chicken form thyroid hormone receptor α. Alternate processing of the initial transcript of ß receptors yielded two isoforms, designated thyroid hormone receptors ß1 and ß2, and thyroid receptors α1 and α2 (for a review, see Anderson et al., 2000). While the α1, the ß1, and the ß2 forms of the receptors bind L-T_3 with 100-fold higher activity than they bind L-T_4, the α2 receptor does not bind L-T_3. The function of this receptor is unknown (Anderson et al., 2000).

The mRNAs for the thyroid hormone receptor isoforms are widely distributed among tissues and their concentrations vary widely (Chin 1991). For example, in adult rats, thyroid receptor ß1 mRNA accounts for about 80% of the total mRNA coding for T_3-

binding thyroid receptors in the liver, whereas there are approximately equal amounts of thyroid receptor ß1 mRNA and thyroid receptor α1 mRNA in the brain. On the other hand, thyroid receptor α2 mRNA is barely detectable in the liver but represents almost 80% of the total thyroid receptor mRNA in the brain (for a review, see Anderson et al., 2000).

All thyroid hormone receptors contain multiple functional domains, including a DNA-binding domain and a carboxyl terminal ligand-binding domain. The ligand domain is a series of α helices, and T_3 is buried in the hydrophobic core of this domain (Wagner et al., 1995). This finding suggests that the T_3 receptor exists in an open configuration and that it folds around T_3 after it binds to the receptor.

The ability of thyroid hormone receptors to regulate gene expression is dependent on the presence in the gene of specific thyroid hormone response elements (TRE) that bind thyroid hormone receptors and confer thyroid hormone-dependent influences on gene expression (for reviews, see Williams and Brent, 1995). Thyroid hormone receptors may function as homodimers or may bind to TRE as heterodimers with other proteins (Rosen et al., 1991). The binding of the T_3 thyroid hormone receptor complex to TRE can both activate or repress gene expression, depending on the individual gene. In the absence of T_3, thyroid receptors bind to TRE and repress the basal rate of transcription (Damm et al., 1989). The addition of T_3 not only relieves this repression but also increases gene expression to levels above basal rates in the absence of thyroid receptors. Several specific gene targets for thyroid receptors have been identified and characterized in detail: e.g., growth hormone (Brent et al., 1987), thyrotropin (Chin et al., 1985), or malic enzyme (Petty et al., 1990). With regard to the brain, it has long been accepted that thyroid hormone actions via thyroid hormone receptors have a profound influence on brain development and maturation. For example, TRE was found in the gene coding for myelin basic protein (Farsetti et al., 1992). Whether or not T_3 has physiological effects in the adult brain was long a matter of debate. Recent reviews however have acknowledged that the demonstration of thyroid hormone receptors in the adult brain, together with the multiple physiological and clinical observations of alterations in brain function in

adults with thyroid dysfunction, strongly suggests that T_3 has substantial actions also in the adult brain (Anderson et al., 2000). In the past few years various authors have reported effects of thyroid hormones on the expression of a large number of genes in the adult central nervous system (CNS); e.g., on growth factors (NGF, NT_3, BDNF; Giordano et al., 1992), on RC3/neurogranine (Iniguez et al., 1992), and on galanine (Ceccatelli et al., 1992). Yet it is still unclear whether T_3 has direct or indirect effects on these genes.

In summary, at present it is generally accepted that the principle physiological actions of thyroid hormones are induced by T_3 via binding to nuclear receptors.

Whether or not the multiple and widespread functions of thyroid hormones are exclusively mediated by the action of T_3 at thyroid hormone receptors is less clear. Even staunch proponents of the importance of the genomic effects of T_3 have had to concede that "the disparate and apparently unrelated actions of T_3 in multiple species and multiple physiological domains remain unexplained" (Anderson et al., 2000). Indeed, in recent years evidence has accumulated that the "nuclear pathway" may not be the only physiological action of T_3. In addition, physiological functions of iodothyronines other than T_3 have repeatedly been demonstrated. The most important hypotheses on extranuclear T_3 actions and the functions of other iodothyronines will now be briefly discussed.

1.1.1.2 Non-nuclear effects of T_3

An increasing number of studies provide evidence for physiological actions of T_3 at the mitochondrial level (for reviews, see Soboll, 1993; Pillar and Seitz, 1997; Wrutniak et al., 1998). In the 1970s, Sterling's group characterized T_3 binding sites at mitochondria and pointed out a very rapid stimulation of oxidative phosphorylation, mitochondrial O_2 consumption, and translocation activity occurring less than 2 min in vitro or 15 min in vivo after T_3 administration (Sterling and Milch, 1975; Sterling et al., 1977; Sterling and Brenner, 1995). Furthermore, thyroid hormones induced a

decrease in mitochondrial membrane potential within several hours after treatment. This was probably the result of T_3-induced changes in the phospholipid composition of the inner membrane, leading to an increase in proton permeability (Hafner et al., 1988, Brand et al., 1992). Recently, two T_3 binding proteins, a 43 kDa protein in the matrix and a 28 kDa protein in the inner membranes, were purified in rat liver mitochondrial extracts (Wrutniak et al., 1995). The T_3-labeled 43 kDa protein was immunoprecipitated by a c-erb A antibody. Bigler et al. (1992) reported that truncated c-erb A α1 proteins are synthesized from the c-erb A mRNA encoding the T_3 nuclear receptor by using an internal AUG codon. Transfection in CV1 cells of the c-erb A α1 construct expressing a major 43 kDa protein demonstrated the mitochondrial location of this protein. Furthermore, it was shown that the 43 kDa protein specifically binds to thyroid hormone response elements in the rat mitochondrial genome (Wrutniak et al., 1995). These results suggest that a truncated c-erb A α T_3 receptor could act as a T_3-dependent mitochondrial transcription factor. Moreover, thyroid hormones affect the expression of several nuclear-encoded respiratory genes such as ADPase or cytochrome oxidase (for a review, see Pillar and Seitz, 1997). With respect to the brain, Vega-Nunez et al. (1995) demonstrated that mRNA levels of several mitochondrial genes are reduced in hypothyroid animals both during the postnatal period and in adulthood. T_3 administration restored the mRNA concentrations to control levels. In this study, the transcript levels for two nuclear-encoded mitochondrial cytochrome c oxidase subunits (IV and VI) were also decreased in the brains of hypothyroid animals. This effect was accompanied by a 40% decrease in cytochrome c oxidase activity.

In summary, all these results strongly suggest that T_3 may influence mitochondrial activity not only by binding to nuclear receptors, but also by directly binding to mitochondrial T_3 receptors. Furthermore, an effect of T_3 on membrane characteristics and proton permeability also seems likely.

Functional effects of T_3 directly at the plasma membrane level have also been reported. For example, Segal's group demonstrated an increase in the intracellular calcium concentration of thrombocytes 15 to 30 seconds after T_3 application (Segal

and Ingbar, 1989). With respect to the CNS, it was recently shown that T_3 inhibits the function of $GABA_A$ receptors (Martin et al., 1996). However, the doses necessary to induce these effects were several orders of magnitude higher than the physiological T_3 levels in the brain. Taken together, the direct effects of T_3 at the level of the plasma membrane are as yet the least well characterized actions of these hormones. Future studies will have to clarify whether such effects do indeed exist under physiological conditions, whether they are mediated by a specific receptor, and whether second messenger systems are involved in the hormonal responses.

1.1.2 Physiological effects of diiodothyronines

1.1.2.1 Physiological effects of 3,5-diiodothyronine (3,5-T_2)

In the past decade, numerous reports from several different study groups have described physiological effects of 3,5-T_2. Horst et al. (1989) reported that 3,5-T_2 stimulated oxygen consumption in isolated perfused livers from hypothyroid rats at concentrations as low as 1 pmol. Compared with T_3 or T_4 application, 3,5-T_2 application resulted in a faster stimulation. The inhibition of deiodinase activity by PTU abolished this effect, demonstrating that 3,5-T_2 is the only active hormone for the rapid stimulation of hepatic oxygen consumption. In 1992 O'Reilly and Murphy showed that injections of 3,5-T_2 into hypothyroid rats increase the respiration rate of liver mitochondria. This stimulation occurred in the presence of cycloheximide and was therefore independent of protein synthesis. Goglia's group demonstrated specific binding sites for 3,5-T_2 in rat liver mitochondria (Goglia et al., 1994). The same group also reported several effects of 3,5-T_2 at the mitochondrial level; e.g., an increase in cytochrome oxidase activity of rat liver mitochondria (Lanni et al., 1992). The administration of 3,5-T_2 resulted in faster stimulation of cytochrome oxidase activity than that achieved with T_3. Similar results were reported by the same study group in further experiments (Lanni et al., 1993, 1994). Cimmino et al. (1996) demonstrated an effect of 3,5-T_2 on lipid ß-oxidation and leucine metabolism in hypothyroid rats.

The same group also showed that 3,5-T_2 improves the cold tolerance of hypothyroid rats (Lanni et al., 1998) and has an inhibiting effect on TSH as well as a stimulating effect on growth hormone levels in hypothyroid rats (Moreno et al., 1998). A suppressive effect of 3,5-T_2 on TSH serum concentrations and TSH levels in the pituitary fragment had already been reported by Horst et al. (1995). Baur et al. (1997) investigated the effects of 3,5-T_2 on pituitary 5' deiodinase (5'DI) activity in vivo in male rats. 5'DI activity in the anterior pituitary was transiently increased after a single injection of 3,5-T_2, whereas serum TSH levels declined. In pituitary cell cultures, 3,5-T_2 stimulated 5'DI activity 24 hours after application in a dose-dependent manner. Likewise, 5'DII activity was decreased by 3,5-T_2. Finally, Kvetny (1992) showed a rapid increase in oxygen consumption in human mononuclear blood cells by 3,5-T_2, which was not inhibited by 6-n-propyl-2-thiouracil (PTU).

These reports notwithstanding, a physiological role of 3,5-T_2 is still not generally accepted. One reason for persisting doubt is the relatively high concentrations required to achieve 3,5-T_2 effects in vivo. Goglia's group, for example, reported effects of 3,5-T_2 in rats after administration of 2.5 µg/100 g body weight. This group however used the same amount of T_3 to demonstrate effects of the latter hormone, although the serum concentrations of 3,5-T_2 in rats are approximately 160-fold lower than those of T_3 (see below). In none of the investigations mentioned above, which reported effects of 3,5-T_2 administration in rats, were serum or tissue levels of this hormone ever measured. It therefore cannot be ruled out that the reported effects were due to unphysiologically high doses of 3,5-T_2 and that such effects do not occur under physiological conditions. Both Horst et al. (1995) and Baur et al. (1997) reported that doses of 3,5-T_2 had to be 5- to 10-fold higher than doses of T_3 in order to induce the same suppression of TSH levels at the pituitary.

Another unanswered question is the biochemical mechanism underlying a possible physiological effect of 3,5-T_2. It remains unclear whether this may involve nuclear receptors or non-nuclear receptor-mediated direct mechanisms at the level of the cell membrane, mitochondria, or plasma membrane. Arnold et al. (1998) reported specific

binding of labeled 3,5-T_2 to subunit Va of cytochrome c oxidase from bovine heart. 3,5-T_2 abolished the allosteric inhibition of ascorbate respiration of cytochrome c oxidase by ATP. This effect reportedly resulted in an increase in cytochrome oxidase activity. Again, however, a relatively high dose of 1 µmol 3,5-T_2 was applied. This dose is much higher than even physiological concentrations of T_3 in different rat tissues (see below). It is therefore not certain whether the effects reported by Arnold et al. (1998) may also appear under physiological conditions or whether they are artifacts due to the high 3,5-T_2 doses applied.

While a few studies have measured 3,5-T_2 concentrations in human serum, tissue levels of this hormone have never been reported either in humans or in experimental animals. The concentrations of 3,5-T_2 in human serum ranged from 4 to 10 ng/dl, which correspond to a range of 76 to 190 pmol/L (Meinhold and Schürnbrand, 1978; Maciel et al., 1979; Pangaro et al., 1980; Kirkegaard et al., 1981; Nishikawa et al., 1981; Nishikawa et al., 1983). However, the results of these early studies on 3,5-T_2 serum concentrations were somewhat contradictory. For example, some studies found 3,5-T_2 concentrations to be elevated in hyperthyroidism (Meinhold and Schürnbrand, 1978; Pangaro et al., 1980; Kirkegaard et al., 1981) and reduced in hypothyroidism (Pangaro et al., 1980). Surprisingly, others reported completely normal concentrations in both thyroid diseases (Maciel et al., 1979; Nishikawa et al., 1983) or in hypothyroidism alone (Kirkergaard et al., 1981). These contradictory results may be due to the difficulties of accurately measuring the very low serum concentrations of 3,5-T_2. Until now, it has been rather difficult to produce labeled 3,5-T_2 with a specific radioactivity high enough to permit accurate measurement of low levels of this hormone.

As mentioned above, most of the studies that found 3,5-T_2 effects applied relatively high doses. To evaluate whether the reported effects may indeed occur under physiological conditions, it would seem necessary to measure 3,5-T_2 concentrations not only in serum but also in tissue. Yet this has never been done.

One purpose of the present study, therefore, was to measure 3,5-T_2 concentrations in two different tissues (brain and liver) in experimental animals (rat). Furthermore,

3,5-T_2 levels were also investigated in different subcellular compartments (nuclei, mitochondria, myelin, synaptosomes, and microsomes) of rat brain tissue in order to determine whether 3,5-T_2 is particularly highly bound to any of these fractions; e.g., to the mitochondria. In addition, 3,5-T_2 serum levels were measured in healthy humans as well as in rats. Finally, this study investigated whether different pathological conditions such as disease, stress factors, or pharmacological treatment affect serum and tissue concentrations of 3,5-T_2 in humans and rats, respectively. A detailed explanation for the different experiments conducted in this study is given below (1.2).

1.1.2.2 Physiological effects of 3,3'-diiodothyronine (3,3'-T_2)

Only a few studies have reported physiological effects of 3,3'-T_2 and all of these have come from one study group. In 1994, Goglia et al. reported mitochondrial binding sites for 3,3'-T_2. The same group claimed that this hormone stimulates rat liver cytochrome oxidase activity (Lanni et al., 1992, 1993, 1994).

As already described for 3,5-T_2, there is a dearth of information on serum and tissue concentrations of 3,3'-T_2 as well. Only a few studies have measured 3,3'-T_2 serum concentrations in humans (Wu et al., 1976; Burger and Sakoloff, 1977; Burman et al., 1977; Gavin et al., 1978; Faber et al., 1979; 1981; Nishikawa et al., 1981). To date, no study group has ever investigated tissue 3,3'-T_2 concentrations either in humans or in experimental animals. Therefore, in a second part of this study, 3,3'-T_2 serum and tissue concentrations were measured under physiological, pharmacological, and pathological conditions. The rationale for the experiments performed in the present study will now be given in more detail.

1.2 Rationale for the choice of experiments performed in this study

1.2.1 Diiodothyronine serum concentrations in humans with thyroidal and nonthyroidal illnesses

As already outlined above, the few studies that measured 3,5-T_2 serum concentrations in hyper- or hypothyroid patients have yielded conflicting results. To clarify the physiological roles of the diiodothyronines, it is of interest to determine whether serum concentrations of these hormones change in hyper- or hypothyroidism. If, for example, 3,5-T_2 indeed has some physiological functions at mitochondria, an increase in these hormone levels in serum as well as in tissue in hyperthyroid patients could contribute to some of the symptoms associated with this disease. The same is also true for hypothyroidism.

For the same reasons, serum concentrations of diiodothyronines are of interest in nonthyroidal illnesses (NTI). Many systemic NTI are associated with specific changes in serum concentrations of thyroid hormones; namely, decreases in T_3 ("low T_3 state") and in T_4 ("low T_3 and T_4 state") and increases in reverse T_3 levels (Wartowsky and Burman, 1982; Chopra, 1996; De Groot, 1999; Wiersinga, 2000).

In a mild illness, this syndrome is characterized by decreases in serum T_3 levels. However, as the severity of the illness increases, there is a drop in both serum T_3 and T_4 levels. This decrease in thyroid hormone levels is seen in starvation, sepsis, surgery, myocardial infarction, bypass, and other serious diseases (for a review, see Wiersinga, 2000). The syndrome has also been called "euthyroid sick syndrome", as patients with NTI are not clinically hypothyroid despite low hormone levels in blood. However, most authors consider these patients as biochemically hypothyroid, which they see as a beneficial physiological response in severe somatic illness as it would reduce energy consumption. Other authors hypothesize that tissue hypothyroidism is not beneficial to these patients and therapy should be initiated if not only serum T_3 but also serum T_4 is severely depressed (for a review of the different standpoints, see De Groot, 1999). As yet only one study has provided significant data on thyroid

hormone concentrations in tissues of patients with NTI (Arem et al., 1993). The overall finding was one of dramatically reduced T_3 levels in all tissues. However, some patients with NTI showed sporadically and inexplicably high levels of T_3 in certain tissues, especially in skeletal muscle and in the heart. These findings suggest a deposition of T_3 in select tissues during NTI.

The biochemical mechanisms underlying the development of a low T_3 syndrome in NTI is also unclear. An inhibition of 5' deiodinase activity, for example by cytokines, as well as a reduction of T_4 cellular uptake and other mechanisms has been discussed for a long time. No final consensus, however, has been achieved. Some evidence suggests that an alteration in the hypothalamic and pituitary function may cause the low production of thyroid hormones. In rats, for example, starvation reduces hypothalamic mRNA for TRH, portal serum TRH, and pituitary TSH content (Blake et al., 1991). Recent studies have documented low TRH mRNA in hypothalamic paraventricular nuclei in NTI patients (Fliers et al., 1997). Yet it is unclear why both hypothalamic and pituitary production of TRH should be diminished in the presence of low serum thyroid hormone levels. One possible explanation is that active iodothyronine metabolites other than T_3 may be involved in the control of pituitary responsiveness. As already described above, two study groups have shown that 3,5-T_2 inhibits TSH secretion (Horst et al., 1995; Baur et al., 1997). Likewise, the fact that NTI patients with even severe depression of serum T_3 and T_4 levels do not appear to be clinically hypothyroid may hypothetically be explained by the fact that any physiologically active iodothyronine metabolite may maintain these patients in a euthyroid condition. Again, 3,5-T_2 is a possible candidate.

It is therefore of interest to measure the serum concentrations of diiodothyronine in patients with different forms of NTI in whom low T_3 syndrome can be demonstrated.

1.2.2 Diiodothyronine concentrations in normal brain tissue and in brain tumors

As outlined above, tissue thyroid hormone levels in patients with NTI have been reported by only one study thus far. In this study (Arem et al., 1993), T_3 tissue concentrations were significantly decreased in most tissues of patients with NTI. No study has ever measured tissue concentrations of diiodothyronines in patients with NTI. Furthermore, it would be of interest to measure thyroid hormone concentrations not only in different tissues of patients with different NTI, but also in that specific tissue that is directly affected by the NTI. Therefore, diiodothyronine concentrations were determined in brain tumors and metastases and compared with those measured in brain tissue from patients who died from diseases not directly affecting the brain. T_4 and T_3 tissue concentrations were also measured in samples in order to determine whether the tumor tissue exhibited a "low T_3 syndrome" compared with tissue from donors who did not suffer from a brain disease.

1.2.3 Diiodothyronine concentrations in rat brain homogenates

Thyroid hormone metabolism in the rat CNS is subject to a highly specific regulation mechanism that differs substantially from the one described in tissues of organs such as the liver or kidney. In these organs, most of the active iodothyronine compound T_3 is taken up directly from the blood, whereas the brain's T_3 supply depends mainly on cellular uptake and intracellular deiodination of thyroxin (Crantz et al., 1982). Furthermore, the mechanisms of deiodination in the CNS are very different from those described in the liver or kidney. In peripheral tissues of the rat, type I 5' iodothyronine deiodinase (5'D-I) catalyzes both phenolic and tyrosyl ring deiodination of both T_4 and T_3. In the CNS, two other isoenzymes catalyze the production and metabolization of T_3 (fig 1). 5' iodothyronine deiodinase (5'D-II) catalyzes the 5' deiodination of T_4 and rT_3 to T_3 and 3,3'-T_2, respectively. Type III 5 iodothyronine deiodinase (5D-III) catalyzes the tyrosyl ring deiodination of T_4 to rT_3 and that of T_3

to 3,3'-T_2, thereby inactivating T_3 (Kaplan and Yaskoski, 1980; Silva et al., 1982; Visser et al., 1982; for a review, see Leonard and Köhrle, 2000).

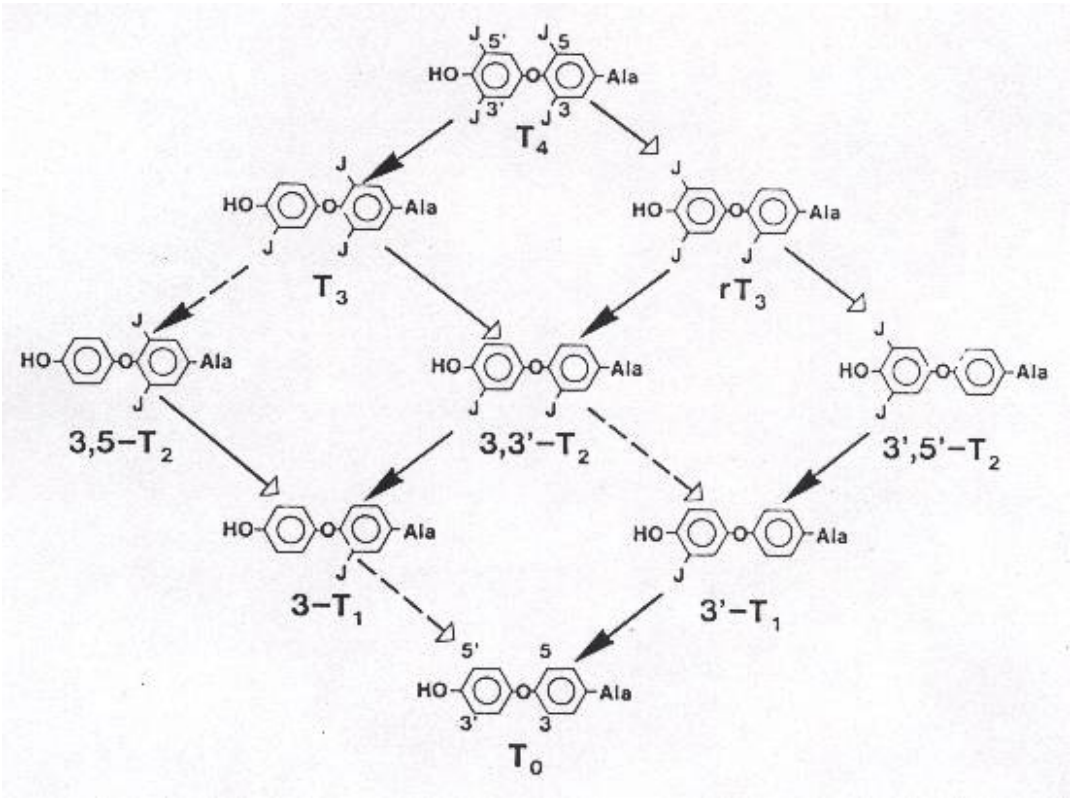

Fig 1. Pathway of the sequential monodeiodination cascade of T_4 to T_0 (from Hesch and Köhrle, 1986).

All three deiodinases have recently been cloned in rats and in humans and have proven to be seleno-proteins (Berry et al., 1991; Salvatore et al., 1995; Croteau et al., 1996). Subsequent investigation has revealed that 5'D-II and 5D-III are also expressed in several tissues other than CNS tissue; e.g., the placenta, skeletal muscle, ovaries, testes, and thyroid gland (Salvatore et al., 1996; Bates et al., 1999). As regards the regulation of 5'D-II and 5D-III activities in the CNS, there is evidence that the activity of 5'D-II is inhibited and that of 5D-III stimulated by different iodothyronine compounds, whereas in hypothyroidism the opposite occurs (Leonard et al., 1981; Burmeister et al., 1997). It is believed that the purpose of this

"autoregulatory mechanism" is to protect the CNS against unphysiological changes in T_3 concentrations in the case of hypo- or hyperthyroidism.

Recent evidence suggests that concentrations of T_3 in the CNS may vary substantially following pharmacological interventions and even under physiological conditions. For example, 5'D-II deiodinase activity in the rat CNS exhibits a relevant circadian rhythm with significant variations in tissue levels of T_3 (Campos-Barros et al., 1997). Our group recently reported the surprising finding that even mild forms of stress, such as handling a rat, cause dramatic increases in 5'D-II activity and T_3 concentrations in specific regions of the rat brain (Baumgartner et al., 1998). Furthermore, acute or subchronic treatment with different pharmacological drugs such as antidepressants, neuroleptics, or anticonvulsants affected the activities of deiodinase isoenzymes and in most cases T_3 or T_4 concentrations as well (Campos-Barros et al., 1994, 1995; Baumgartner et al., 1994a, 1997; Eravci et al., 2000). It seems likely that these often highly complex and specific changes in thyroid hormone homeostasis in the CNS are functionally important, as in recent years effects of thyroid hormones on the expression of a large number of genes have also been reported in the adult CNS (see above). Furthermore, it has been demonstrated that thyroid hormones have a large number of effects on a variety of other parameters in the adult CNS. These effects range from numerous influences on the characteristics of many kinds of G protein coupled neurotransmitter receptors (e.g., Tejani-Butt et al., 1993) to morphological changes (e.g., Gould et al., 1990).

In the light of this complex regulation of thyroid hormone metabolism and the multiple functions of these hormones in the brain, it may be of interest whether diiodothyronine concentrations are measurable in such a tissue. As outlined above, tissue levels of 3,5-T_2 and 3,3'-T_2 have never before been measured in tissues of humans or rats. Therefore, I attempted to measure the concentrations of these hormones in homogenates of different rat brain areas. Furthermore, I determined diiodothyronine concentrations in the liver, where iodothyronine metabolism is very different from and less complicated than that in the brain.

1.2.4 Diiodothyronine concentrations in subcellular compartments

The determination of thyroid hormone concentrations in tissue homogenates is unsatisfactory in so far as these hormones are probably bound to very different subcellular organelles with different physiological functions. A much better understanding of the role of thyroid hormones in a given tissue would be gained if the hormones could be directly measured at their target organelles; for example, in the nuclei, mitochondriae, and at the plasma membrane level. Our study group therefore developed a method for measuring thyroid hormone concentrations in subcellular compartments of brain tissue, such as nuclei, mitochondria, synaptosomes, myelin, and microsomes (Pinna et al., 1999; Prengel et al., 2000; Pinna and Baumgartner, in preparation). As several studies have reported effects of 3,5-T_2 on mitochondrial functions, an attempt was made to determine concentrations of diiodothyronines not only in homogenate but also in subcellular fractions of different brain areas.

1.2.5 Effects of antidepressant treatment on diiodothyronine concentrations

Several studies by our group have showed that antidepressant drugs affect the activity of the deiodinase isoenzyme and also affect T_4 and T_3 concentrations in rat brain (Campos-Barros et al., 1994, 1995; Baumgartner et al., 1994a, 1997; for a review, see Baumgartner et al., 2000). For example, it was shown that subchronic treatment with the tricyclic antidepressant desipramine enhanced T_3 concentrations in homogenates of cortical areas (Campos-Barros et al., 1995). Our group also investigated whether antidepressants enhance T_3 tissue concentrations in specific subcellular compartments. Prengel et al. (2000) were the first to report that desipramine treatment induced increases in T_3 concentrations specifically in the mitochondria, but not in the nuclei or the synaptosomes of the amygdala. As both T_3 and 3,5-T_2 have been implicated in mitochondrial functions, a further purpose of this study was to measure 3,5-T_2 tissue concentrations in subcellular compartments of rat brain areas after antidepressant treatment.

1.2.6 Effects of circadian variations on diiodothyronine concentrations

As already mentioned above, Campos-Barros et al. (1997) reported a circadian variation in T_4 and T_3 serum and tissue concentrations. With respect to the brain, 5'D-II activities increase during the dark (active) phase and decrease during the light (resting) phase. As result the T_3 concentrations peaked in the light phase. Thus another goal of the present study was to determine whether or not diiodothyronine concentrations also exhibit a circadian rhythm in brain areas.

1.3 Purpose of the study

In the last 15 years, a multiplicity of studies from different laboratories reported physiological effects of 3,5-T_2 and, to a lesser extent, of 3,3'-T_2. These effects range from stimulation of mitochondrial activity to suppression of TSH secretion at the pituitary level. However, most of these effects were significant only after in vivo or in vitro application of relatively high doses of 3,5-T_2 or 3,3'-T_2. At present, it is unclear whether the claimed physiological effects of diiodothyronines do indeed exist or whether they represent artifacts due to the use of supraphysiological doses. To clarify this issue, the physiological serum and tissue concentrations of these diiodothyronines would have to be determined. However, concentrations have never been measured in any tissue of humans or experimental animals, and the few studies that have reported serum concentrations of these hormones are contradictory.

The purpose of the present study was therefore to develop highly sensitive RIA methods for measuring 3,5-T_2 and 3,3'-T_2 concentrations in serum and tissue of humans and experimental animals.

The main questions addressed by the present study are:

- **a**) Are 3,5-T_2 and 3,3'-T_2 measurable in serum and tissue of healthy humans?

- **b**) If yes, are these concentrations affected by different thyroidal and nonthyroidal illnesses?

- **c**) Are both iodothyronines measurable in rat serum and tissue, particularly in the brain?

- **d**) If yes, are they enriched in any specific subcellular compartment (e.g., mitochondria)?

- **e**) Do different physiological (e.g., circadian rhythm) or pharmacological (e.g., antidepressant drugs) influences affect diiodothyronine concentrations in brain tissue homogenates and in subcellular fractions?