

4. Discussion

4.1 3,5-T₂ serum and tissue concentrations in humans

The mean concentration of 3,5-T₂ in serum of healthy humans was 16.2 ± 6.4 pmol/l. This value is between 5- and 10-fold lower than the results reported in previous studies, which ranged from 76 to 109 pmol/l (Meinhold and Schürnbrand, 1978; Maciel et al., 1979; Pangaro et al., 1980; Kirkegaard et al., 1981; Nishikawa et al., 1981; Jaedig and Faber, 1982; Nishikawa et al., 1983). The low 3,5-T₂ serum concentrations reported in this study may be due to the improved sensitivity of the RIA technique used. A new 3,5-T₂ RIA was applied that uses 3-bromo-5-[¹²⁵I]-L-thyronine (3-Br-5-[¹²⁵I]T₁) as tracer. The 3-Br-5-[¹²⁵I]T₁ tracer was obtained by a newly developed method in which one atom of bromine in the 3,5-dibromo-L-thyronine (3,5-Br₂T₀) substrate is replaced with one atom of ¹²⁵I. The lower limits of detection for 3,5-T₂ with this new RIA technique are 1.0 fmol/g and 0.8 pmol/l 3,5-T₂ in tissue and in serum, respectively. These threshold limits are considerably lower than those in all reports published to date (for references, see above). Therefore, the RIA methods available in the early 1980s, when all of the above cited studies were performed, were probably not sufficiently sensitive to measure 3,5-T₂ serum concentrations accurately.

The main finding of this part of the study is the elevation of serum concentrations of 3,5-T₂ in four patient groups with widely different forms of NTI. To date, serum levels of 3,5-T₂ in patients with NTI have been measured in only two studies, both of which reported subnormal levels of this hormone in patients with liver cirrhosis (Maciel et al., 1979; Kirkegaard et al., 1981). These studies also reported normal concentrations of 3,5-T₂ in hyperthyroidism (Maciel et al., 1979) and hypothyroidism (Maciel et al., 1979; Kirkegaard et al., 1981). In the present study, 3,5-T₂ serum concentrations were clearly elevated in patients with hyperthyroidism and decreased in those with hypothyroidism. Therefore, the differences between our results and

those of previous studies may again have been due to differences in the sensitivity of the RIA method employed.

In interpreting the origin of the elevated 3,5-T₂ serum concentrations in patients with NTI, it has to be considered that most of the patients were receiving a variety of different medications for treatment of underlying disorders. Many of these medications, such as heparin, norepinephrine, and dopamine, have previously been reported to affect serum thyroid hormone levels (cf. Kaptein et al., 1980; Wenzel, 1981; Davies and Franklyn, 1991). But these drugs seem to affect serum levels of thyroid hormones in very different ways. It would therefore seem highly unlikely that the elevated serum levels of 3,5-T₂ found in all four diagnostic groups were predominantly a drug-induced phenomenon. Yet it is still unclear by which enzymatic pathway 3,5-T₂ is produced from T₃ or from any other iodothyronine. As long as no specific deiodinase isoenzyme or other metabolizing pathway for the production of 3,5-T₂ has been characterized, it cannot be completely ruled out that the medications administered to severely ill patients may have a stimulating influence on this pathway.

The high 3,5-T₂ serum concentrations in the four patients groups with NTI are also not due to an assay artifact, as we failed to find any relevant effect of the medication on the 3,5-T₂ assay procedure. It therefore seems likely that the high serum 3,5-T₂ concentrations in four very different groups of patients with NTI are somehow related to the low T₃ syndrome seen in these four patients groups. As briefly outlined in the introduction, it is not yet clear why patients with NTI with markedly reduced serum levels of T₃, sometimes over prolonged periods of time, still appear clinically euthyroid (Chopra, 1996; De Groot, 1999; Wiersinga, 2000). The few study groups investigating this issue have reasonably focused on the hypothesis that a change in the intracellular thyroid hormone metabolism -or function- (e.g., enhanced T₃ nuclear binding capacity) may compensate for low serum levels of T₃, thus maintaining the cell in euthyroid condition. However, these study groups have reported inconsistent results (Tibaldi and Surks, 1985; Williams et al., 1989; Kvetny and Matzen, 1990; Chamba et al., 1996). Alternatively, clinical euthyroidism may be maintained by an as

yet unknown active thyroid hormone metabolite other than T_3 . As mentioned in the introduction, several groups have shown, independently of each other, that 3,5- T_2 may have effects on mitochondria, such as an increase in oxygen consumption, respiratory rate, and cytochrome oxidase activity (cf. Horst et al., 1989; Lanni et al., 1994). However, most of these studies employed pharmacological doses of 3,5- T_2 . Whether the slight elevations in serum concentrations of 3,5- T_2 seen in our patients with NTI are somehow involved in the maintenance of clinical euthyroidism in these patients is therefore unclear. To clarify this question, 3,5- T_2 concentrations need to be measured in the affected tissues, such as in brain tumors. It seems noteworthy that levels of 3,5- T_2 in the different brain tumors and metastases were between two- and four-fold higher than tissue concentrations of this hormone in the brain areas of donors who died from diseases not directly affecting the brain. On condition that 3,5- T_2 is not equally distributed over all subcellular compartments but rather is concentrated in specific organelles such as the mitochondria, physiologically relevant increases in concentrations of 3,5- T_2 at its target organelles may well appear in tissues of patients with NTI. If one also considers that the 3,5- T_2 measured in serum most likely originates from the intracellular conversion of T_3 in tissues (see below), than increases in the intracellular production and function of this hormone may well be much greater than is evidenced by measurement of the fraction that is transported into the blood circulation. It is particularly striking that serum 3,5- T_2 concentrations were elevated in patients with brain tumors whose T_3 and T_4 concentrations were hardly measurable at all.

Several causes have been discussed as the origin of low T_3 syndrome in NTI (for a review, see Wiersinga 2000). An inhibition of 5' deiodinase type I activity is generally regarded as the most likely explanation. To our knowledge, however, direct evidence of an inhibition of the conversion of T_4 to T_3 in NTI has yet to be furnished. Even studies that found increases in the serum concentration of T_3 in patients with NTI after administration of T_4 that were slightly lower than those determined in healthy controls (Nomura et al., 1975) cannot exclude the possibility that this may have been

due to enhanced metabolism of T_3 rather than a fall in its production. Furthermore, an inhibition of 5'D-I activity does not explain the falls in T_4 serum concentrations in patients with more severe forms of NTI. Interestingly, enhanced metabolism of T_3 to T_3 sulfate may indeed occur in NTI, as recently reported by two study groups (Chopra et al., 1992; Santini et al., 1996). If the elevated serum levels of 3,5- T_2 found in our patients with NTI are also taken into account, one should consider the possibility that the low levels of T_3 seen in NTI may be at least partially due to an increase in the metabolism of T_3 to various metabolites. Such a possibility would also explain why T_4 levels also decrease in more seriously ill patients.

Horst et al. (1985) reported that 3,5- T_2 may suppress TSH levels in the serum of euthyroid rats. Similar findings were reported by Baur et al. (1997). Our findings of an elevation of 3,5- T_2 serum concentrations in NTI and the presence of 3,5- T_2 in human pituitary gland post-mortem could be of relevance for the as yet unexplained finding that TSH concentrations remain normal (or are even decreased) in patients with NTI despite marked and prolonged reduction in the serum levels of T_3 and sometimes of T_4 .

Another open question concerns the as yet unknown origin of 3,5- T_2 . The results of several studies have all showed that this hormone is probably produced by peripheral deiodination from circulating T_3 , whereas production controlled by the thyroid gland is extremely unlikely (Maciel et al., 1979; Pangaro et al., 1980). I do not know of any study published to date that has investigated which type of deiodinase catalyzes the reaction by which T_3 or any other iodothyronine metabolite is converted to 3,5- T_2 . We have, however, been able to measure 3,5- T_2 in different regions of the human brain and in the pituitary gland. Although exact comparison between concentration units of serum (picomoles per l) and of brain tissue (femtomoles per g) is not possible, a rough estimate reveals that brain concentrations of 3,5- T_2 are many times higher than serum concentrations of this hormone. Again, this would argue in favor of a local production of the hormone in the human CNS.

In conclusion, our study has showed, for the first time, that 3,5-T₂ serum concentrations are significantly elevated in patients with very different nonthyroidal diseases. Likewise, 3,5-T₂ tissue concentrations were elevated in different brain tumors and metastases. In the light of increasing reports on physiological functions of 3,5-T₂ at the mitochondrial level, it may be hypothesized that the elevated levels of this hormone in patients with NTI may be at least one factor in maintaining these patients clinically euthyroid despite sometimes even dramatically decreased T₃ and T₄ serum concentrations. Clarification of this hypothesis will require further investigation into the exact mechanism of action of 3,5-T₂, as well as into specific, 3,5-T₂-mediated functions in tissues of patients with NTI and the correlation of these functions to the respective 3,5-T₂ tissue levels.

4.2 3,3'-T₂ serum and tissue concentrations in humans

Serum concentrations of 3,3'-T₂ were significantly increased in patients with hyperthyroidism and decreased in patients with hypothyroidism. This is in agreement with the results of two previous reports (Wu et al., 1976; Burman et al., 1977).

As regards serum levels of 3,3'-T₂ in the four patient groups with different NTI, the results are complex. In two of the four groups, 3,3'-T₂ concentrations were significantly enhanced (liver diseases and brain tumors), and another group showed increases that failed to reach statistical significance (sepsis). In the fourth group, levels of 3,3'-T₂ were significantly reduced (brain injury). The few reports on this issue in the literature showed either normal (Gavin et al., 1978; Faber et al., 1979) or reduced (Faber et al., 1979) serum concentrations of 3,3'-T₂ in patients with different NTI. Gavin et al. (1978) reported normal serum levels of 3,3'-T₂ in ten patients with NTI, whereas Faber et al. (1979) found normal concentrations of 3,3'-T₂ in patients with liver cirrhosis but low levels in patients with uremia, malignancies, and myocardial infarction. The latter study group, however, also reported reduced 3,3'-T₂ concentrations in patients with liver cirrhosis (Faber et al., 1979). Low serum levels of

3,3'-T₂ have also been measured in patients with anorexia nervosa (Burger and Sakoloff, 1977), and elevated hormone concentrations have been found in umbilical cord sera of newborns (Wu et al., 1976; Burman et al., 1977; Geola et al., 1979).

While concentrations of T₃ are subnormal in patients with NTI, and those of rT₃ and 3,5-T₂ are higher than normal, changes in the concentrations of 3,3'-T₂ seem to be disease specific as both elevated and reduced concentrations may be found depending on the illness concerned. Why the levels of 3,3'-T₂ should behave in this manner is not immediately clear. The difference in the levels of 3,3'-T₂ could depend on the severity of the NTI. In two of our patient populations (those with brain tumors and liver diseases), we measured lower than normal serum concentrations of T₄. This may indicate that these patients had a rather severe form of NTI. By contrast, in the two other diagnostic groups (sepsis and head injury), serum levels of T₄ were normal, indicating that these patients had a milder form of NTI. The patients with brain tumors had by far the lowest serum levels of T₄ and T₃ but the highest level of 3,3'-T₂. Patients with liver diseases had the second lowest serum levels of T₃ and T₄ and also the second highest level of 3,3'-T₂. Conversely, the patients with brain injury who had normal serum levels of T₄ and only modestly reduced serum concentrations of T₃ had low levels of 3,3'-T₂. It would therefore seem that the more severe the NTI, the lower the serum concentrations of both T₃ and T₄ and the higher the serum levels of 3,3'-T₂. Further studies will be required to confirm this observation.

While reduced serum levels of 3,3'-T₂ may simply result from a decrease in the availability of T₃, the mechanisms leading to a rise in 3,3'-T₂ production are unclear at present. 3,3'-T₂ may be produced from T₃ by inner-ring deiodination or from rT₃ by outer-ring deiodination. The activity of 5'I-deiodinase is said to be inhibited in NTI (for a review, see Wiersinga 2000), which should result in a lower, rather than higher, production of 3,3'-T₂ from both rT₃ and T₃. Enhanced availability of rT₃ as substrate could nevertheless contribute to an increase in the production of 3,3'-T₂. Furthermore, inner-ring deiodination of T₃ may be catalyzed also by 5 D-III deiodinase. To our knowledge, nothing definitive is known about the activities of this

enzyme in the tissues of patients with NTI. The possibility that the activity of the inner-ring deiodinase isoenzyme is enhanced in affected tissues cannot yet be ruled out. Moreover, T_3 sulfate is easily deiodinated to $3,3'$ - T_2 (Rutgers et al., 1987), and serum concentrations of T_3 sulfate are enhanced in patients with NTI (Chopra et al., 1992; Santini et al., 1996). As desulfation may occur in human tissue (Kung et al., 1988), elevated levels of $3,3'$ - T_2 may be induced by T_3 sulfate via deiodination and desulfation.

Finally, at present it cannot be excluded that specific changes in the metabolism or clearance rate of $3,3'$ - T_2 in patients with different NTI may affect its serum concentrations.

Concentrations of $3,3'$ - T_2 in different brain tumors and metastases do not seem to differ substantially from those measured in non-tumorous human brain homogenates. Therefore, in contrast to the results reported for $3,5$ - T_2 , serum concentrations of $3,3'$ - T_2 do not seem to reflect concentrations of this hormone in pathological tissue. A few studies, conducted by the same study group, have reported physiological effects of $3,3'$ - T_2 on rat liver cytochrome oxidase activity and respiratory rate (Lanni et al., 1993, 1994). Yet it is somewhat hard to believe that a hormone that is the product of both an active (T_3) and an inactive iodothyronine (rT_3) could itself be physiologically active. The contradictory changes in $3,3'$ - T_2 serum concentrations in different patient groups with NTI as well as the normal concentrations of this hormone in brain tumors suggest that $3,3'$ - T_2 does not play a specific functional role in patients with NTI.

NTI are associated with different forms of stress such as pain or fear. To evaluate whether stress effects are involved in the changes in diiodothyronines in NTI we measured $3,5$ - T_2 and $3,3'$ - T_2 after two different forms of acute stress. Both during sleep deprivation and while delivering a lecture, the serum concentrations of T_3 and T_4 rose, but those of $3,5$ - T_2 and $3,3'$ - T_2 remained unchanged. Rises in the serum levels of T_3 and T_4 and also of TSH during sleep deprivation have frequently been reported (cf. Parker et al., 1976; Baumgartner et al., 1993). Although TSH concentrations on the stress day were not significantly elevated compared with those

on the control day, the fact that the TSH levels were significantly higher before the lecture than they were afterwards militates in favor of an involvement of TSH in the rises in the serum concentrations of T_4 and T_3 during this kind of stress. As these increases in T_3 and T_4 occur in parallel, they are most likely due to enhanced secretion from the thyroid gland. These results, therefore, suggest that 3,5- T_2 and 3,3'- T_2 are not directly secreted by the thyroid after the experience of an acutely stressful stimulus and are not sensitive to acute psychological stress, at least up to 24 hours' duration.

4.3 3,5- T_2 serum and tissue concentrations in rats

No studies have previously measured serum or tissue concentrations of 3,5- T_2 in experimental animals. In this study, 3,5- T_2 was detected in rat serum, the mean concentration being 11.4 ± 0.8 pmol/l. This is approximately 160-fold lower than the respective T_3 serum concentrations in rats.

3,5- T_2 was detectable in only six of the 12 brain areas. The highest levels were found in the amygdala, the lowest measurable levels in the cerebellum. The "pattern" of 3,5- T_2 distribution across the different brain areas was unexpected as it did not correspond to the distribution of T_4 or T_3 tissue concentrations in the respective areas. For example, T_3 concentrations are measurable in all brain areas, being highest in the midbrain and lowest in the amygdala. 3,5- T_2 was highest in the amygdala and lower in the midbrain. The T_3 concentrations of the frontal cortex are approximately twice as high as those measured in the parieto-occipital cortex. By contrast, 3,5- T_2 concentrations were measurable only in the parieto-occipital cortex and undetectable in the frontal cortex. These data indicate that there is no linear relationship between T_3 and 3,5- T_2 concentrations in the brain areas. As already mentioned above, the enzymatic mechanism by which 3,5- T_2 is produced is currently not known. Both 5'D-I and 5'D-II are outer-ring deiodinases and operate in all rat brain areas. However, the regional distribution of their activities does not correspond

to the respective 3,5-T₂ tissue concentrations. The activity of the 5'D-I, for example, is highest both in cortical areas and in the hippocampus, lower in the amygdala, and even lower in the striatum and the medulla (Baumgartner et al., 1994a; Campos-Barros et al., 1994). 5'D-II activity was also lower in the parieto-occipital cortex than in the frontal cortex and even lower in the striatum and the medulla (Baumgartner et al., 1994a; Campos-Barros et al., 1994). These results indicate that there is no linear correlation between the activity of either of the two outer-ring deiodinases and 3,5-T₂ tissue concentrations.

5D-III is an inner deiodinase that catalyzes the inactivation of T₃ to 3,3'-T₂. Theoretically, this enzyme could indirectly influence the production of 3,5-T₂ from T₃, as the higher its activity, the more T₃ would be deiodinated to 3,3'-T₂. By far the highest 5D-III activity of all brain areas was measured in the amygdala, where 5D-III activity is approximately three-fold more active than in the other brain areas (cf. Baumgartner et al., 1994a; Campos-Barros et al., 1994). There is, indeed, a close relationship between the high 5D-III activity and the T₃ tissue levels in the amygdala, which are the lowest among all the brain areas. But the 3,5-T₂ concentrations in the amygdala are the highest among all brain areas. Moreover, the 3,5-T₂ levels of the medulla are also relatively high although no 5D-III activity could be detected in this area (Campos-Barros et al., 1994; for a review, see Leonard and Köhrle, 2000). Therefore, it seems unlikely that the 5D-III deiodinase isoenzyme is critically involved in the production or degradation of 3,5-T₂.

In conclusion, the lack of any obvious correlation between the 3,5-T₂ levels in the different rat brain areas on the one hand and the distribution of T₃ and T₄ as well as that of the deiodinase isoenzyme on the other suggest that other, as yet unknown enzymatic mechanisms may be involved in the production and degradation of 3,5-T₂ in the brain. For example, Visser's group convincingly showed in a series of studies that T₃ sulfate is desulfated and deiodinated in rat liver as well as in human liver (see Rutgers et al., 1987). The product of desulfation and deiodination of T₃ sulfate would, however, be 3,5-T₂. If such a mechanism is also operating in the brain, then not only

5'D-I deiodinase activity, but also the activity of sulfotransferases and sulfatases may be determining factors in the production of 3,5-T₂. Further studies are needed to characterize whether T₃ is a substrate for outer-ring deiodination and whether iodothyronine sulfates are measurable in the brain.

Whether 3,5-T₂ does indeed exist in those brain areas where we did not find measurable 3,5-T₂ concentrations or whether the sensitivity of our RIA was too low to detect 3,5-T₂ in those brain areas, is unclear.

On the other hand, the widely varying tissue levels of 3,5-T₂ between brain areas make it unlikely that 3,5-T₂ is exclusively derived from the blood. There is no evidence of a particularly high blood supply to the amygdala, nor were relevant differences found between the blood supply of the frontal cortex and that of the parieto-occipital cortex. A local production of 3,5-T₂ within the tissue seems more likely.

The origin of the high concentrations of 3,5-T₂ in the liver is unclear. Whether the liver 3,5-T₂ content is entirely derived from the blood or is locally produced from another iodothyronine metabolite remains to be investigated.

The investigation of the circadian variations of tissue concentrations of 3,5-T₂ showed that this hormone has a circadian variation similar to that of T₃ in the brain areas and in the liver. As already shown for T₃ in a previous study (Campos-Barros et al., 1997), concentrations of 3,5-T₂ decrease during the light phase until the beginning of the dark (active) phase, and increase during the dark phase reaching maximum values at the early stage of the light phase. The study by Campos-Barros et al. (1997) also reported that T₄ serum levels peaked during the light phase and decreased in the dark phase. No circadian variation was reported for T₃ serum concentrations. These data suggest that the metabolization of T₄ to T₃ and, by an unknown pathway, to 3,5-T₂ is enhanced at the end of the active period (night) of the animals. As very similar results were obtained for both the liver and the brain tissues, it is not likely that 5'D-II activity is the major mechanism mediating these circadian

variations, as this enzyme does not operate in the liver (see Leonard and Köhrle, 2000). The finding that the circadian variations of T_3 and 3,5- T_2 were essentially parallel both in the brain areas investigated and in the liver indicates that 3,5- T_2 may operate in concert with T_3 to exert its actions; for example, by modulating the expression of mitochondrial target genes. However, this interpretation is merely speculative at this stage. Again, only further investigation of the enzyme catalyzing 3,5- T_2 production may help to clarify the mechanisms underlying the circadian variation of this hormone's concentration in different tissues.

An attempt was made to measure 3,5- T_2 concentrations in different subcellular fractions of rat brain areas. The technique of subcellular fractionation was originally developed by our study group to measure the effects of different pharmacological treatments on T_3 concentrations in different subcellular compartments. As shown in figure 14, T_3 was measurable in all subcellular fractions. The highest concentrations by far were found in the nuclei. This result may reflect the covalent binding of T_3 molecules with their nuclear receptors. The detection of T_3 levels in synaptosomes and mitochondria was also not completely unexpected, as several study groups had previously reported physiological effects of T_3 at both subcellular compartments (see introduction). Furthermore, Dratman's group also detected T_3 in synaptosomes in several earlier studies (cf. Dratman et al., 1976, 1978, 1982). It was however surprising that T_3 concentrations were measured in microsomes and myelin. No studies had ever attempted to measure T_3 in these fractions. Whether the detection of T_3 concentrations in subcellular compartments such as myelin and microsomes is a valid finding or represents a methodological artifact remains to be investigated (for further discussion, see below).

3,5- T_2 concentrations were not measurable in any subcellular fraction in the amygdala or in the parieto-occipital cortex of control animals. These areas were chosen because relatively high 3,5- T_2 concentrations had been measured in their homogenates. We were therefore surprised to reliably detect 3,5- T_2 in mitochondria

and in the myelin of the amygdala of rats treated with three different doses of the antidepressant desipramine. We then addressed the question whether the undetectable levels of 3,5-T₂ in all subcellular fractions of the control animals was due to insufficient sensitivity of our RIA procedure. For this purpose, 12 amygdalae and four parieto-occipital cortices, respectively, were pooled and 3,5-T₂ was measured in subcellular fractions of four pools of each brain area. Again, 3,5-T₂ was not detectable in any subcellular compartment but it was measurable in the pooled homogenate aliquots. These results suggest that, under physiological conditions, 3,5-T₂ is not bound to any of the subcellular compartments under investigation. However, as yet undefined conditions such as subchronic antidepressant treatment may induce a binding of 3,5-T₂ to the mitochondria and the myelin. This would seem to be valid as 3,5-T₂ was detectable in both the mitochondria and the myelin fractions after treatment with all three different doses of desipramine. These results are all the more interesting because, at least in part, they paralleled the respective findings for T₃. After subchronic treatment with desipramine, T₃ concentrations were dramatically enhanced in the myelin fraction of the amygdalae but not in any of the fractions of the parieto-occipital cortices. The same results were obtained after treatment with two chemically different antidepressant drugs; namely, MAO inhibitor tranylcypromine and serotonin reuptake inhibitor paroxetine (Prengel et al., 2000; Pinna et al., in preparation). The results are somewhat surprising since both the amygdala as a brain region and the mitochondria or the myelin as such have only rarely been considered in connection with the investigation of the mechanisms of action of antidepressant drugs. This relative lack of interest in the amygdala seems surprising in the light of current knowledge on the possible function of this brain structure. In brief, the amygdala seems to assign affective values to the incoming information from all sensory systems (for reviews, see LeDoux, 1992; Kandel et al., 1995). One of the key symptoms of depressive disorders is a disturbance of the effects associated with all kinds of sensory impressions. It is therefore very likely that the amygdala is at least partly involved in the pathogenesis of depression. Indeed, the few studies that have investigated a possible role of this brain region in the affective disorders have also confirmed a specific role of the amygdala either in the

pathogenesis of depression or in the mechanisms of action of antidepressant treatment (Duncan et al., 1986; Ordway et al., 1991; Drevets et al., 1992; Sheline et al., 1998). An increase in the concentrations of physiologically active thyroid hormones in relevant brain areas after antidepressant treatment may well be involved in the as yet unknown mechanisms of action of these treatments for the following reasons. Thyroid disorders such as severe hypothyroidism may well mimic the symptoms of major depression (for a review, see Hall et al., 1986). On the other hand, both T₃ and T₄ have repeatedly been reported to enhance the action of antidepressant drugs, particularly in treatment-resistant depressed patients (cf. Joffe et al., 1993; Baumgartner et al., 1994b; Bauer et al., 1998). Whether or not the increase in 3,5-T₂ concentrations in the mitochondria and myelin fractions of the amygdala is of any relevance for the mechanism of action of desipramine is still unclear. The relevance of this finding, particularly with respect to the myelin, remains to be investigated in further studies.

One potential methodological pitfall of the measurement of hormones in subcellular fractions should also briefly be discussed. Theoretically, it cannot be ruled out that, during the procedure of subcellular fractionation and during different centrifugation steps, any thyroid hormone, which is originally located in the cytosol or even outside the cells, may bind to any subcellular fraction by non-covalent binding mechanisms. On the other hand, it is possible that non-covalently bound hormones may loosen from specific binding compartments and be retained in the supernatants.

Yet it does not seem likely that the measurement of 3,5-T₂ in the mitochondria and myelin fractions of the amygdala after subchronic desipramine treatment is due to such an artifact. First, we did not measure any increase in 3,5-T₂ concentrations in the homogenate of the amygdala after desipramine treatment. However, only on condition that 3,5-T₂ is strongly increased in the cytosol, which should be reflected in the homogenates, it seems plausible that 3,5-T₂ is artificially "caught" by the mitochondria and myelin fractions during centrifugation. Second, it is not apparent why an enhanced cytosolic 3,5-T₂ concentration should result in artificially high concentrations of this hormone only in mitochondria and myelin but no tendency

toward an increase in 3,5-T₂ levels should be seen in synaptosomes, microsomes, and nuclei.

For these reasons, the measurements of 3,5-T₂ in the myelin and the mitochondria after desipramine treatment seem to be a valid finding. As mentioned above, this was also a new and surprising finding, whose potential functional significance is completely unknown at present and has to be characterized in future studies.

4.4 3,3'-T₂ serum and tissue concentrations in rats

This study reported for the first time the measurement of serum and tissue concentrations of 3,3'-T₂ in experimental animals. 3,3'-T₂ could be detected in rat serum, the mean concentration being 15.7 ± 5.7 pmol/l. This is approximately 120-fold lower than the mean T₃ serum concentrations in rats.

3,3'-T₂ concentrations were detectable in ten of the 12 brain areas. The concentrations showed a relatively wide variation between the areas, ranging from 15.2 fmol/g in the medulla to 197 fmol/g in the cerebellum. The distribution of 3,3'-T₂ concentrations across the different brain areas may be at least partly explained by the activity of the 5'D-I isoenzyme. For example, 5'D-I activity is approximately four to five times higher in the cerebellum than in the medulla. This could result in a higher conversion of rT₃ to 3,3'-T₂ in the cerebellum. The other isoenzyme that deiodinates rT₃ to 3,3'-T₂, the 5'D-II, is also approximately three- to four-fold higher in the cerebellum than in the medulla (Baumgartner et al., 1994a; Campos-Barros et al., 1994). As no type III deiodinase isoenzyme operates in the cerebellum, the inner deiodination of T₃ to 3,3'-T₂ is catalyzed also by the 5'DI deiodinase. Therefore, the different patterns of the 5'D-I and 5'D-II enzymes in the cerebellum and in the medulla could well be one reason for the more than 10-fold higher levels of 3,3'-T₂ in the cerebellum than in the medulla. The midbrain provides another example for a relatively close correlation between the activity of the different deiodinase isoenzymes and 3,3'-T₂ tissue levels. 3,3'-T₂ was not detectable in the midbrain, which has by far the lowest activity of all three isoenzymes (Campos-Barros et al.,

1994; Eravci et al., 2000). The fact that the midbrain has the highest T_3 tissue concentrations among all brain areas may be due to the very low activity of type III deiodinase isoenzyme in this area. The low activity of type III deiodinase isoenzyme would also result in very low conversion of T_3 to $3,3'$ - T_2 and, therefore, in relatively low $3,3'$ - T_2 tissue levels. As 5'D-I and 5'D-II deiodinase activity is also low in the midbrain, the conversion of rT_3 to $3,3'$ - T_2 is also lower here than in other brain areas. In summary, the low activity of all three deiodinase isoenzymes may be responsible for the undetectable levels of $3,3'$ - T_2 . The undetectable levels in the hypothalamus may be explained in a similar manner. Among all brain areas, the hypothalamus has the second lowest activity of the three deiodinase isoenzymes (Campos-Barros et al., 1994).

This relatively close correlation between deiodinase activities on the one hand and $3,3'$ - T_2 tissue concentrations on the other hand suggests that $3,3'$ - T_2 is mainly produced locally in the tissues and is derived from the serum to a lesser extent, if at all.

It was surprising that $3,3'$ - T_2 was not detected in the liver, as concentrations of T_4 and those of $3,5'$ - T_2 were much higher in this tissue than in the 12 brain areas examined. Further studies will need to be conducted in order to clarify the exact mechanisms of production and metabolization of $3,3'$ - T_2 in the liver. It is conceivable that pathways different from those of deiodinase activities, such as sulfatation and glucuronization, which have been characterized in the liver, may be responsible for the metabolization of T_3 to compounds other than $3,3'$ - T_2 .

$3,3'$ - T_2 was also measured in subcellular fractions of the cortex and amygdala and was found exclusively in the synaptosomes. The physiological relevance of this finding is unclear. It may indicate that T_3 is deiodinated to $3,3'$ - T_2 by type III deiodinase in synaptosomal membranes. But the exact subcellular location of type III deiodinase isoenzyme is not yet known (see Leonard and Köhrle, 2000). Whether or not $3,3'$ - T_2 has any physiological effect at synaptosomal membranes is also unknown. We found no binding of $3,3'$ - T_2 to the mitochondria. These data, therefore,

do not support the hypothesis that 3,3'-T₂ has physiological functions at the mitochondrial level (Lanni et al., 1993, 1994).

Finally, there was no evidence of effects of the antidepressant desipramine on synaptosomal 3,3'-T₂ content in the amygdala or in the frontal cortex.