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The concentration of thyroid hormones and activities of iodothyronine deiodinases are altered in human brain gliomas

Paweł Nauman, Wiesław Bonicki, Radosław Michalik, Adam Warzecha, Zbigniew Czernicki

Department of Neurosurgery, Medical Research Centre of the Polish Academy of Sciences, Warszawa, Poland

We have determined the cellular concentration of thyroxine (T4) and triiodothyronine (T3) and the activities of two brain iodothyronine deiodinases, type II (5'-D2) and type III (5-D3), in two types of tissues — tumour (26) and non-tumour (5), derived either from human gliomas with various histological malignancies or from non-tumoural surrounding brain tissue. As it has been established, all patients before the surgery had the Non-Thyroidal Illness Syndrome (NTIS). The concentration of serum T3 was therefore significantly decreased in all the examined patients. It was over 2.5 times lower than that before surgery and 4.0 times lower at surgery than that seen in healthy controls. The serum concentration of T4 was found to be below normal range in 4/26 cases and in low levels of normal range in 6/26 cases, whereas TSH serum concentration in all patients was within normal range. The concentrations of T3 and T4 (expressed as pg of hormone/mg tissues protein) in 22/26 brain tissue samples were significantly lower in gliomas than in 5 non-tumoural brain tissue samples. As expected, the alternation in brain 5'D II activity in gliomas was seen in most cases with astrocytomas (5/8 cases), gliosarcomas (8/8 cases) and glioblastoma multiforme (10/10 cases). In general, the mean enzyme activity in tumour tissue was significantly higher than that found in non-tumoural tissue of human brain (21.79 fmol of newly generated T3/h/mg of protein vs. 4.88 fmol of T3/h/mg protein, respectively). The highest 5'D2 activity with a range from 10.82 to 45.96 (mean 23.61 fmol T3/h/mg protein) was found in gliosarcomas. The activity of 5-D3 was increased (in 8/8 cases of gliosarcoma and in 9/10 cases of glioblastoma multiforme) or decreased (in 3/3 cases of astrocytoma II, 5/5 cases of astrocytoma III) when compared to mean activity of this enzyme found in non-tumoural brain tissue. In summary, our results suggest that the concentration of brain iodothyronines and metabolism of thyroid hormones in the examined human brain tumours are altered. These changes may be related to malignant progression.

key words: human brain, gliomas, iodothyronine deiodinases, thyroid hormone

INTRODUCTION

The metabolism of peripheral thyroid hormones plays an essential role in determining the intracellular concentration of T3-receptor-active thyroid hormone complex in all human tissues including human brain [1, 17]. This thyroid hormone has not only an essential role in brain development [6], but is also crucial for the proper function of the human brain throughout life [8, 17]. The major physiological effects of thyroid hormone are exerted by its interaction with specific, triiodothyronine nuclear receptors (TRs) that regulate transcription of downstream genes in T3-dependent

Address for correspondence: Paweł Nauman, MD
Department of Neurosurgery
Medical Research Centre, Polish Academy of Sciences
ul. Cegłowska 80, 01–809 Warszawa, Poland
tel: (+48 22) 669 63 21, 0501 445 394
e-mail: paul_nauman@hotmail.com

manner [16]. By this mechanism, T3 is involved in proliferation, growth, differentiation and apoptosis of cells [18]. Several recent studies suggest [10–12, 14, 17], that deterioration in T3 control of these important physiological processes might be responsible for both initial steps of neoplastic cell transformation and subsequent tumour progression resulting in tumour malignancy. The change of T3 expression in the brain can either result from altered cellular concentration of T3, or from modified function of TRs, which are usually the consequence of mutations of TR genes. It is now generally established that at least 75% of nuclear T3 in brain is derived from local T4 monodeiodination catalysed by iodothyronine 5'-deiodinase type II (5'-D2) [5, 12, 17,] and that the expression of 5'-D2 gene (*hdio2*) in turn depends on T3 concentration in the brain [1]. The cellular concentration of T3 in the brain is also dependent on the activity of iodothyronine 5-deiodinase type III (5-D3), which catalyses monodeiodination of T3 to diiodothyronine (T2) and by this mechanism it controls the amount of T3 that can reach the brain and the nuclei in the brain cells, especially from the circulation [7]. Most recently, deterioration in iodothyronine 5' deiodinase type I and type II has been reported in human renal clear cell carcinoma and in human tumoural nervous tissues [3, 14]. In the present study, we attempted to investigate the concentrations of T3 and T4 in the brain and 5'-D2 and 5-D3 activities in different gliomas and in nontumoural brain tissues.

MATERIALS AND METHODS

Patients and tissue samples

All patients (12 females 14 males, aged 24–70 years) were admitted to the Department of Neurosurgery (Medical Research Centre, Polish Academy of Science, Warszawa) with diagnosed primary brain tumour. None of the patients had previously undergone brain radiotherapy and all were approved for the brain surgery. All patients were given Dexavene (dexamethasone) — 8 mg/day (2 mg every 6 h) for at least three days before operation. All tumours were successfully removed during surgery. Additionally, 5 small brain non-tumour specimens (each one from the zone most distal from the tumour) were removed as the tumour was approached. Tumour samples were preferentially preserved for histopatholological examination and small parts of these tumours together with non-tumour brain tissue samples were stored for further investigations. All these tissue samples were immediately frozen on dry ice, and stored at –75°C until needed. During histopathological examinations, all tumours proved to be gliomas were finally diagnosed according to WHO classification [9, 15] as low-grade astrocytoma (AS G-II), anaplastic astrocytoma (AS G-III), gliosarcoma (GS G-IV), or glioblastoma multiforme (GBM G-IV). The tumour samples available for the present study weighed from about 70–800 mg. We had only 5 pairs of tissue samples (tumour and non-tumour) obtained from the same patients, and these 5 non-tumour tissues were generally preserved as controls. The study was carried out with the permission of the Local Ethical Committee for Human Investigations. None of the patients had the history of thyroid disorders. The thyroid status in all 26 patients was examined before the surgery by determining the concentration of TSH, T3 and T4 in the serum. The blood samples were taken on the day of admission of patients to the Department and before they were given Dexavene (dexamethasone). In 10 cases blood samples for these determinations were also taken from these patients during surgery.

The activities of 5'-D2 and 5-D3 were finally evaluated in 26 gliomas: 3 tumours diagnosed as astrocytoma (AS GII) (WHO grade II), in 5 tumours diagnosed as astrocytoma III anaplasticum (WHO grade III), 8 tumours of poorly differentiated tumour gliosarcoma (GS GIV) (WHO grade IV), and in 10 tumours representing glioblastoma multiforme (GBM IV) (WHO grade IV). In 5 cases (1 AS GII, 1 AS GIII, 1 GS GIV, and 2 GBM G-IV), we were able to investigate the activity of 5'D2 and 5-D3 enzymes in paired tissue samples — tumour and non-tumour. The tissue concentration of both iodothyronines total T4 and total T3 were determined in all gliomas and non-tumour samples.

Reagents

Radiolabelled 3,5,3',5'[^{125}I]-T4 and 3,3'5'[^{125}I]-rT3 (1200 Ci/mmol) were purchased from New England Nuclear (Boston, MA). AG 50W-X2 resin and protein assay kit were obtained from Bio-Rad Laboratories, Inc. LH-Sephadex from Pharmacia, Sweden. Highly purified standards of T3 and T4 iodothyronines were purchased from Henning GmBH (Berlin, Germany). All other chemicals of highest quality and purity were obtained from Sigma (St Louis, MO).

Determination of plasma iodothyronines and TSH concentrations

Serum concentrations of total thyroxine (TT4), total triiodothyronine (TT3) and thyroid stimulating hormone (TSH) in sera of patients were measured in duplicate in the same one series of determination by enzyme im-

munoassay using automatic Abbott system (MEIA). In addition, pooled sera from patients were used to evaluate the intra-assay coefficient of variation of T4, T3 and TSH measurements, which was estimated as less than 5% for each assay.

Determination of brain T4 and T3 concentrations

T4 and T3 in the studied brain samples (tumour and non-tumour) were measured by the specific method described by Pachucki et al. [11] during one series of determination. Brain samples of about 50 mg each were used. Each sample was then homogenised in 1 ml of methanol using Teflon-glass homogeniser. For protein measurements, a 50 μl aliquot of each homogenate was solubilised by adding 10 μl of 1 M NaOH. The solubilised proteins were diluted in water and concentrations of protein was measured as described by Bradford, using bovine albumin (BSA) as a standard [2]. To assess the recovery of T4 or T3, approximately 500 cpm of high specific activity [^{125}I]T4, or [^{125}I]T3 (NEN Life Science Products) were added to the rest of each homogenate respectively and counted. Homogenates were then spun for 20 min at 5,000 × g and the supernatant mixed with 2 ml of chloroform. Thyroid hormones were extracted into aqueous solution by two successive 0.5 ml aliquots of 0.4 M NH4OH. The supernatants were pooled after centrifuging for 20 min at 5,000 × g. Any possible tracers of chloroform in pooled supernatants were removed by adding 1 ml of ethyl ether and gravity separation. Samples were then evaporated, lyophilised and re-dissolved in 400 μl of 0.01 M NaOH. Each sample was again counted to determine the iodothyronines recovery, which ranged from 60–70%. Duplicate samples of solubilised T4 or T3 were assayed in sodium salicylate (0.2 M glycine-acetate buffer, pH 8.6) using specific and sensitive rabbit polyclonal T4 or T3 antibodies. In addition, the same aliquot of each sample and standards were used to determine non-specific binding of [^{125}I]T4 or [^{125}I]T3 in the absence of T3 or T4 antibodies. Linearity of measurements was confirmed by assay of four serial 2-fold dilutions of T3 or T4 extracts from the brain.

Deiodinase activity assays

The 5'-deiodinase type 2 (5'-D2) and 5-deiodinase type 3 (5-D3) activities were measured by quantification of the released radioactive iodide as previously described [11]. Purification of [^{125}I]T4 and [^{125}I]T3 was performed on Sephadex LH 20-columns just before use by eluting with 75% ethanol in several 0.5 ml fractions. From the fraction with the highest activity (30,000 cpm to 50,000 cpm/μl) 0.5 to 2 μl aliquots were added to each reaction tube. This amount of ethanol was confirmed not to affect the reaction. The amount of protein used for assay was set to keep the percent of deiodination below 30%. Incubation mixture for 5'-D type II assays contained 50–100 μg protein in 0.1 M potassium phosphate buffer (pH 7.0), 1 mM EDTA with approximately 50,000 cpm of ^{125}I-labeled T4, cold 2 nM T4, 20 mM dithiothreitol (DTT) in the presence or absence of 1 mM 2-N-propyl-6-thiouracyl (PTU). The final volume of incubation mixture was 200 μl, which was incubated for 60 min at 37°C. Putting the samples into the ice bath stopped the reactions. 5'-^{125}I was separated from T4 by precipitating trichloroacetic acid by adding 2/3 volume of horse serum and 1/3 volume of 50% TCA (trichloric acid). Samples were vortexed for at least 2 minutes and centrifuged (15,000 × g, 10 minutes). Two thirds of the supernatant volume containing iodine, but not thyroid hormone, was counted in a gamma counter (Wallac, Pharmacia). Assays were carried out in triplicate and the activity of the enzyme expressed as femtomoles of released ^{125}I per mg of protein per hour. Incubation mixture for 5-D type III assays contained 50–100 μg of tissue proteins, 5-[^{125}I]-T3 (approximately 50,000 cpm), in 0.1 M phosphate buffer (pH 7.4), 1mM EDTA, cold 25 nM T3, 20 mM DTT and 1 mM PTU in final volume of 200 μl. Incubation was performed for 60 min at 37°C. The 5-D III activity was calculated from % of deiodination of T3. Activity of 5-D type III was expressed as fmoles of released ^{125}I per mg of protein per hour.

During the measurement of activity 5'D-II, we considered the random distribution of ^{125}I$^-$ between the 3' and 5' position of T4 by multiplying the percent of deiodination by a factor of 2. The intra-assay variability was about 7% and inter-assay variability about 14%.

Statistical analysis

Statistical analysis was done using the computer program — ANOVA. The data were presented as mean values ± standard deviation (SD), median (M.), and T-test for Equality of means (statistics of paired samples, correlations of paired samples).

RESULTS

Serum concentration of TT4, TT3, and TSH before surgery

In all 26 patients, the total T4 serum concentration (TT4) was within normal range and varied between 4.4–7.8 μg/dl with a mean value of 6.2 ± 1.3 μg/dl. The serum total T3 (TT3) concentration was below normal range in all patients and varied from 46 ng/dl to

78 ng/dl with a mean of 61.4 ± 18.6 ng/dl. The serum TSH concentration in all patients was within the normal range (0.92–3.0 mIU/L) with a mean of 1.45 ± ± 1.5 mIU/L.

Serum concentration of T4, T3 and TSH during surgery

The normal range of concentration of T4 in serum is 4.5–12.5 μg/dl. The concentration T4 in serum from patients during surgery was close or slightly below the normal range in 2/10 cases (12.9, 14.7 μg/dl). In remaining 8/10 patients, it was close to the lowest value of the normal range (3.5–4.6 μg/dl with a mean of 4.1 ± 0.7). Serum T3 concentrations in all patients with gliomas significantly decreased and varied from 20.5 to 55.2 ng/dl with a mean of 38.6 ± 15.6 ng/dl, which was lower than the normal range (135–185 ng/dl). Serum TSH concentration was in all cases within the normal range, including patients with slightly decreased T4 concentration.

T4 and T3 concentration in tumour and non-tumour tissues

The tissue iodothyronines concentration was determined in 26 tumour tissues and 5 non-tumour tissue samples. Unfortunately, we had only 5 pairs of tissue samples (tumour and non-tumour) obtained from the same patients, and these 5 non-tumour tissues were generally preserved as controls. Concentrations of T4 (pg/mg protein) in 19/26 tumour tissues were lower than that determined in non-tumour tissues. In 2/26 tumour tissues, the concentration was higher than observed in "control" tissue sample. And the concentration of T4 in 5/26 tumour tissues was in the same range as that of control tissues.

In 3 cases (AS GII) T4 concentration was from 175.4 to 190.4 pgT3/mg of protein (mean 182.16 ± ± 7.60, M. = 180.7), in 5 cases (AS GIII) T4 concentration was from 103.2 to 218.2 pgT3/mg of protein (mean 171.46 ± 57.2, M. = 198.0), in 8 cases (GS GIV) T4 concentration was from 61.4 to 160.2 pgT3/mg of protein (mean 111.3 ± 32.29, M. = 106.25), in 10 cases (GBM GIV) T4 concentration was from 23.2 to 176.5 pgT3/mg of protein (mean 107.27 ± 43.5, M. = 102.10). In non-tumour tissue, T4 concentration was from 163.0 to 211.2 pgT3/mg of protein (mean 189.22 ± 19.1, M. = 187.8).

The mean value of T4 concentration in all (26) cases of glioma was 143.05 ± 35.16 pgT3/mg of protein, M .= 164.58, vs. 189.22 ± 19.1, M. =187.8 pgT3/mg of protein in non-tumour tissue.

The tumour tissues concentration of T3 in 22 out of 26 cases decreased, 3/26 cases had higher concentration than that observed in control tissues and one had not changed in comparison with the concentration observed in non-tumour tissues. In 3 cases (AS GII) T3 the concentration was from 7.0 to 9.55 pgT3/mg of protein (mean 8.22 ± 1.27, M. = 8.13), in 5 cases (AS GIII) T3 concentration was from 5.1 to 12.4 pgT3/mg of protein (mean 7.64 ± 2.91, M. = 6.5), in 8 cases (GS GIV) T3 concentration was from 4.3 to 8.5 pgT3/mg of protein (mean 6.48 ± 1.7, M. = 6.95), in 10 cases (GBM GIV) T3 concentration was from 3.8 to 15.2 pgT3/mg of protein (mean 7.61 ± 3.6, M. = 6.40), vs. in non-tumour tissue T3 concentration was from 9.3 to 14.7 pgT3/mg of protein (mean 11.22 ± 2.21, M. = 10.9). The results are summarised in Table 1.

The iodothyronine deiodinases activity in tumour and non-tumour human brain tissues

5'-D type 2 and 5-D type 3 activities were finally determined in 26 brain gliomas and in 5 non-tumour tissue samples.

5'-D2 activity

Using the preferred substrate for 5-'D2 [^{125}I-T4], all brain tissues tested had clearly detectable enzyme activity, which was not suppressed by 1 mM PTU. In addition, the 5'-D2 activities in some tissues were different in T4 monodeiodination at low substrate levels of 2 nM (high substrate levels, 100 nM). In general the mean value of 5'-D2 activity in all evaluated tumours was higher than that determined in non-tumour brain tissue samples.

In samples of astrocytomas (n = 3), AS GII the 5'-D2 activities from 4.27 to 9.86 fmol I^-/h/mg protein, with a mean of 6.96 ± 2.80, M. = 6.75 fmol I^-/h/mg protein. In samples of astrocytomas anaplasticum (n = 5), AS GIII the 5'-D2 activities from 6.01 to 18.4 fmol I^-/h/mg protein, with a mean of 9.96 ± 5.0, M. = 8.11 fmol I^-/h/mg protein. In samples of gliosarcoma (n = 8), GS GIV the 5'-D2 activities from 10.82 to 45.96 fmol I^-/h/mg protein, with a mean of 23.61 ± 13.51, M. = 20.05 fmol I^-/h/mg protein. In GBM GIV (n = 10), the 5'-D2 activities ranged from 9.59 to 40.32 fmol I^-/h/mg protein, with a mean of 24.26 ± 12.24, M. = 23.30 fmol I^-/h/mg protein. In 5 non-tumour brain tissue samples 5'-D II activity varied from 2.15 to 6.57 fmol I^-/h/mg of protein with a mean of 4.88 ± 1.79, M. = 5.55 fmol I^-/h/mg protein. Similar differences were found when 5'-D2 activities were evaluated in paired tumour and non-tumour tissues.

Table 1. The tissue concentration of total thyroxine (TT4) and total triiodothyronine (TT3) in glia-derived tumour (T) and non-tumour tissue (NT)

Type of tissue Grading — G Number of cases — n	Total T4 pg/mg protein			Total T3 pg/mg protein		
	Range	Mean ± SD	Median	Range	Mean ± SD	Median
T — AS GII **(n = 3)**	175.4–190.4	182.16 ± 7.60	180.70	7.0–9.55	8.22 ± 1.27	8.13
T — AS GIII **(n = 5)**	103.2–218.2	171.46 ± 57.2	198.00	5.1–12.4	7.64 ± 2.91	6.50
T — GS GIV **(n = 8)**	61.4–160.2	111.3 ± 32.29	106.25	4.3–8.5	6.48 ± 1.70	6.95
T — GBM GIV **(n = 10)**	23.2–176.5	107.27 ± 43.5	102.10	3.8–15.2	7.61 ± 3.6	6.40
NT (control group) **(n = 5)**	163.0–211.2	189.22 ± 19.1	187.80	9.3–14.7	11.22 ± 2.21	10.90

T — tumour, NT — non-tumour tissue, G (II–IV) — WHO classification of grade of neoplasm, n — number of cases, AS — astrocytoma, GS — gliosarcoma, GBM — glioblastoma multiforme.

Table 2a. Type II 5'- iodothyronine deiodinase (5'D2) activity in brain tumour and non-tumour tissue

Type of tissue Grading — G Number of cases — n	5'-D2 activity (fmol/h/mg protein)		
	Range	Mean ± SD	Median
AS GII **(n = 3)**	4.27–9.86	6.96 ± 2.80	6.75
AS GIII **(n = 5)**	6.01–18.4	9.96 ± 5.0	8.11
GS GIV **(n = 8)**	10.82–45.96	23.61 ± 13.51	20.05
GM GIV **(n = 10)**	9.59–40.32	24.26 ± 12.24	23.30
NT **(n = 5)**	2.15–6.57	4.88 ± 1.79	5.55

Table 2b. 5'-D2 activity (expressed fmol/h/mg protein) in paired samples from tumour and non-tumour tissues of brain obtained from the same patient

Type of tissue Grading — G Number of cases — n	T 5'D2 protein	NT 5'D2 protein	T/NT ratio
AS GII	9.86	2.15	4.58
AS GIII	10.45	4.05	2.58
GS GIV	45.96	6.10	7.53
GBM GIV	27.40	6.57	4.17
GBM GIV	15.30	5.55	2.75
Mean	**21.79**	**4.88**	**4.46**

T — tumour, NT — non-tumour, AS — tastrocytoma, GS — tgliosarcoma, GBM — glioblastoma multiforme.

The results obtained from the analysis of different gliomas are summarised in Table 2a and 2b.

5-D3 activity

The enzyme activity was detectable in both tumour and non-tumour brain tissue samples and showed striking differences.

In samples of AS GII (n = 3) the 5-D3 activities were from 65.3 to 134.5 fmol I^-/h/mg protein, with a mean of 106.8 ± 36.6, M. = 120.6 fmol I^-/h/mg protein. In AS GIII (n = 5), the 5-D3 activities from 85.2 to 142.4, fmol I^-/h/mg protein, with a mean of 118.26 ± 24.8, M. = 121.5 fmol I^-/h/mg protein. In samples of GS GIV (n = 8), the 5-D3 activities from 202.7 to 431.8 fmol I^-/h/mg protein, with a mean of 311.35 ± 85,33, M. = 311.5 fmol I^-/h/mg protein. In GBM GIV (n = 10), the 5-D3 activities ranged from 180.4 to 415.0 fmol I^-/h/mg protein, with a mean of 307.34 ± 84.21, M. = = 303.15 fmol I^-/h/mg protein. In 5 non-tumour brain tissue samples 5-D3 activity varied from 105.8 to 230.4 fmol I^-/h/mg of protein with a mean of 187.34 ± ± 50.10, M. = 195.5 fmol I^-/h/mg protein.

Table 3a. Type III 5-iodothyronine deiodinase (5-D3) activity in brain tumour and non-tumour tissues

Type of tissue Grading — G Number of cases — n	5-D3 activity (fmol/h/mg protein)		
	Range	Mean ± SD	Median
AS GII **(n = 3)**	65.3–134.5	106.8 ± 36.6	6.75
AS GIII **(n = 5)**	85.2–142.4	118.26 ± 24.8	8.11
GS GIV **(n = 8)**	202.7–431.8	311.35 ± 85.33	20.05
GBM GIV **(n = 10)**	180.4–415.0	307.34 ± 84.21	23.30
NT **(n = 5)**	105.8–230.4	187.34 ± 50.10	5.55

Table 3b. 5-D3 activity (median value - expressed fmol/h/mg protein) in paired samples from tumour and non-tumour tissue of brain obtained from the same patient

Type of tissue Grading — G	T 5-D3 fmol/h/mg protein	NT 5-D3 fmol/h/mg protein	T/NT ratio
AS GII	65.3	180.0	0.362
AS GIII	121.5	195.5	0.620
GS GIV	431.8	230.4	1.874
GBM GIV	310.5	225.0	1.380
GBM GIV	407.8	105.8	3.854

T — tumour, NT — non-tumour, AS — astrocytoma, GS — gliosarcoma, GBM — glioblastoma multiforme.

5-D3 activity in tumour and non-tumour tissues of the same patients

The 5-D3 activity in samples of AS GII tumour there was 65.3 fmol I^-/h/mg protein, vs. 180.0 fmol I^-/h/mg protein in non-tumour tissues, in AS GIII tumour there was 142.4 fmol I^-/h/mg protein, vs. 195.5 fmol I^-/h/mg protein in non tumour tissue, in GS GIV (n = 2) there were: 220.3 and 431.8, vs. 105.8 and 230.4 fmol I^-/h/mg protein in non-tumour tissues, respectively. In GBM GIV there was 310.5 fmol I^-/h/mg protein in tumour, vs. 225.0 fmol I^-/h/mg protein in non-tumour. The results of this evaluation are summarised in Table 3a and 3b.

DISCUSSION

The determinations of total T4, T3 and TSH in serum have shown that almost all patients have had moderate Non-Thyroidal Illness Syndrome (NTIS). Although NTIS can be present in both acute and chronic disorders including brain tumours [4, 13], the effect of administration of dexamethasone on the metabolism of thyroid hormones is well recognised [4], and it also applies to our investigated patients during operation. However, the fact that during surgery, despite the possible action of dexamethasone, NTIS further significantly worsened, strongly suggests that tumours themselves and surgery, at least in part, were both responsible for the deterioration of homeostasis of thyroid hormones. It is well known that although several humoral and cellular factors can be responsible for the development of NTIS, they all lead first to the decrease in the activity and concentration of 5'-deiodinase type I, which is mostly present in the liver, kidney and thyroid [1, 4] and has a very limited role in maintaining the concentrations of T4 and T3 in the brain [6, 9]. Apart from the general thyroid status in the patients with different types of gliomas, our main interest and the aim of this study was to investigate whether the concentration of biologically active thyroid hormone had changed in brain tumours. If so, we wanted to know whether the change was as a consequence of alteration in activity of the brain iodothyronine deiodinases. We successfully attempted to measure the concentrations of T4 and T3 in both tumour and non-tumour brain tissue samples. Although in general the knowledge about the concentration of both thyroid hormones in human brain is very much limited, the results from our study on brain non-tumour tissues are within the same range as reported by others who studied similar tissues [3, 7], or results from studies on the developing foetal brain [5]. Moreover, our finding that both thyroxine and triiodothyronine concentrations in tumour tissue are lower than in non-tumour tissue samples correspond to recently published data [3]. It is generally recognised that the major role of 5'-D type II in human brain is to maintain T3 homeostasis by catalysing adequate production of intracellular T3 and to ensure all T3 dependent cellular functions [1, 5]. On the other hand, 5-D type III in brain serves as a guardian of T3 brain concentration and catalyses the 5 monodeiodination of an excess of T3, especially of the hormone that reach the brain from the periphery [7]. The present data on the activity of both enzymes in human brain is the third report [3, 7] ever published and our results are in agreement with those recently reported by Calvo et al. [3]. In general the mean activities of both deiodinases were increased in glioma tissues although the results from studies on tumours, even those of the

same type and grade, differed. There is growing evidence that both the metabolism of thyroid hormones and the expression of T3 and its nuclear receptors are all altered in cancers and that these alterations may be different in tumours of different organs. The expression of the gene for 5' deiodinase type I was deeply altered in human clear cell carcinoma leading to significant decrease in the enzyme activity in cancer and non-tumour tissue surrounding the cancer [14]. The expression of genes for 5'-D II and 5-D III in human brain tumours has never been investigated. But the present results suggest that both genes in gliomas might be overexpressed. As preliminary studies on the expression of *TRA and TRB* in different gliomas have shown that both genes, especially *TRB,* are changed and probably mutated. The present results strongly suggest that these alterations also concern intracellular metabolism of thyroid hormones in glia-tumours.

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