

EXPERIMENTAL STUDY

Expression of 5'-deiodinase enzymes in normal pituitaries and in various human pituitary adenomas

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Abstract

Objective: Local 5'-deiodination of L-thyroxine (T₄) to active thyroid hormone 3,3',5-tri-iodothyronine (T₃) catalyzed by the two 5'-deiodinase enzymes (D1 and D2) regulates various T₃-dependent functions in the anterior pituitary and has been well studied in rodents. Only limited information about deiodinase expression and its cellular distribution in human anterior pituitaries is available.

Design: We examined 5'-deiodinase enzyme activities in pituitary adenomas (18 non-functioning, seven TSH-producing, one GH- and TSH-producing, five GH-producing, eight prolactin (PRL)-producing, two adenomas each from patients with Cushing's disease and Nelson's syndrome) and three normal anterior pituitaries.

Methods: Activities were measured as release of ¹²⁵I⁻ from tyrosyl-ring labeled reverse T₃ with or without propylthiouracil, a potent inhibitor of D1 which does not influence D2 activities.

Results: Most of the adenomas and normal tissues expressed both isoenzymes, with D2 activity higher than D1. In a few tissues D1 activity was higher than D2 and some tissues did not express D1 activity at all. Highest activities of both enzymes were found in TSH- and PRL-producing adenomas but absolute activities and the D1/D2 ratio were variable in the same kind of tumor in different patients.

Conclusion: The finding that all examined tissues expressed 5'-deiodinase activity, most of them expressing both isoenzymes, implies that both enzymes are still active in tumors and that local deiodination is important for the function and feedback regulation of human anterior pituitary.

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Introduction

Thyroid hormones play an important role in various functions of the anterior pituitary, e.g. thyrotropin (TSH) (1), growth hormone (GH) (2) and prolactin (PRL) gene expression. 3,3',5-tri-iodothyronine (T₃), the main active form of thyroid hormones, is generated by 5'-monodeiodination of L-thyroxine (T₄), the (pro) hormone secreted by the thyroid gland. Two distinct 5'-deiodinase enzymes (D1 and D2) which differ in reaction kinetics, substrate specificity, inhibitor sensitivity and regulation contribute to systemic and local formation of T₃. D1 is believed to be the main contributor to systemic T₃ formation. Its expression is decreased under hypothyroid conditions. D2 activity is increased in hypothyroidism and is important for providing intracellular T₃ in several tissues under these conditions. In rodents, D2 is mainly expressed in the pituitary, in brown adipose tissue, in the central nervous system (CNS) and in the heart (3). In humans, D2 mRNA and/or activity has also been demonstrated in placenta, skeletal muscle, and thyroid (4–6). The highest

expression levels of D1 are found in thyroid, liver and kidney but also in eu- and hyperthyroid pituitaries of rats (7, 8). In contrast to rodents, no D1 activity is found in human adult CNS (9, 10). Therefore, expression profiles of deiodinase enzymes in different tissues cannot be transferred from rodents to humans.

In several human tissues such as liver, kidney and thyroid the enzyme activity of D1 is decreased when the tissues undergo neoplastic transformation (3, 11, 12). Decreased activity of D2 has also been described in human papillary thyroid carcinoma compared with normal tissues (13).

It is well known that in pituitaries of euthyroid rats, half of the T₃ bound to specific T₃ receptors originates from local, intrapituitary T₄ to T₃ conversion (14) and deiodinase expression and regulation has been extensively investigated in rat pituitary *in vivo* and *in vitro*. However, there is only one report of deiodinase expression in human anterior pituitary tumors (15) and one report of deiodinase expression in the human somatomammotroph GX cell line (16). To gain more insight into the distribution of deiodinase enzymes

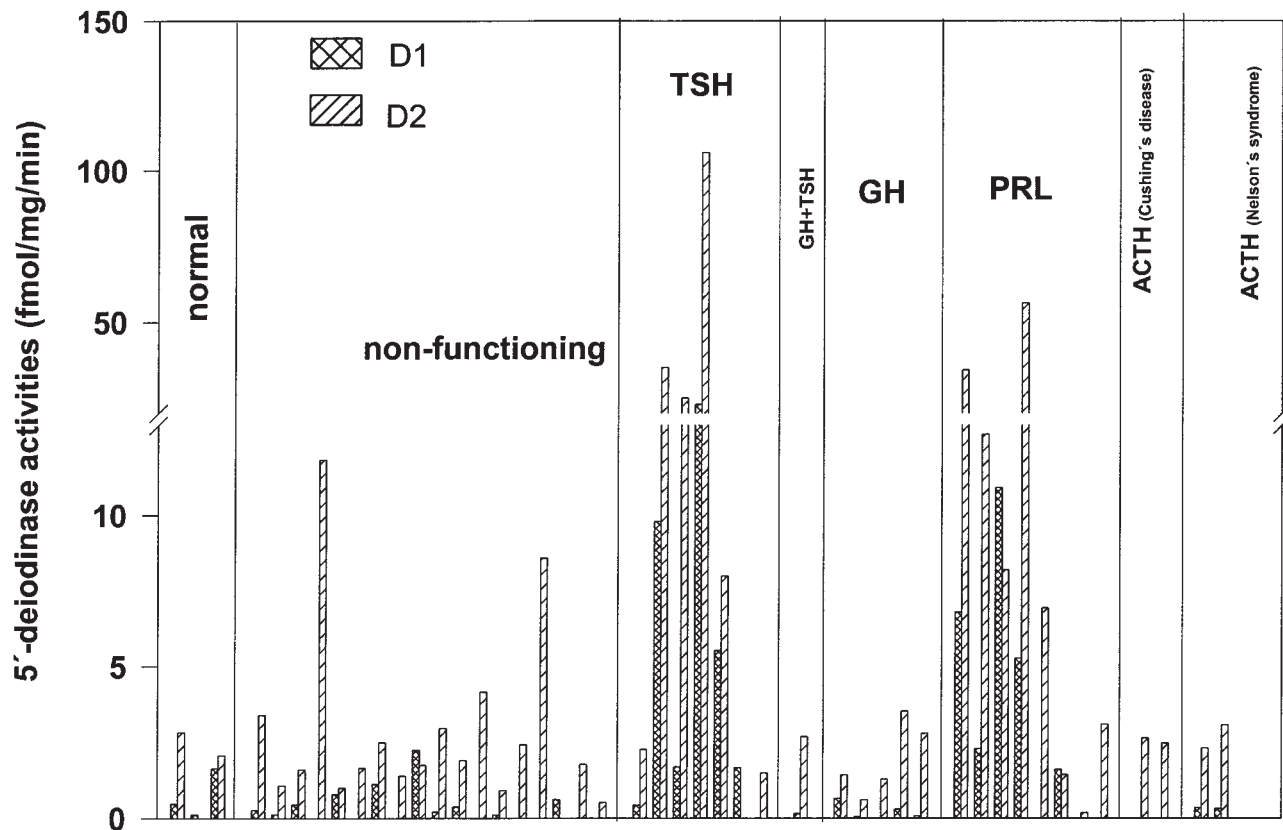


Figure 1 Specific D1 and D2 activities in human pituitary tissues. Activities were determined as described in Materials and methods and are given in fmol released iodide per mg protein per minute.

among various pituitary cell types and to elucidate if deiodinase activities are altered by neoplastic transformation, possibly dependent on the endocrine type of adenoma, we examined the activity of D1 and D2 in various pituitary adenomas and 3 normal pituitary tissues.

Materials and methods

Tissue preparations

Human pituitary tissue aliquots were obtained from 43 patients with pituitary adenomas during trans-sphenoidal surgeries; in addition, three normal pituitary tissue aliquots which had to be removed during neurosurgical procedures were analyzed. The neurosurgeon attempted to obtain only solid tumorous material and avoided contamination with hemorrhagic and necrotic regions. Immunocytochemistry confirmed that the tissues consisted either of normal pituitary or adenomas. Ethical approval was obtained by the local committee. The tissues were immediately frozen in liquid nitrogen and stored at -80°C until use. The frozen tissues were then homogenized in ice-cold homogenization buffer (250 mmol/l sucrose, 20 mmol/l HEPES, 1 mmol/l ethylenediamine tetraacetic acid, 1 mmol/l dithiothreitol

(DTT), pH 7.0) by sonication (0.5 s, 100 W, 10 times). The protein contents of the homogenates were determined by a modified Bradford protein assay (Biorad, Munich, Germany) using gamma-globulin as protein standard (17).

Biochemical assays

Specific activities of Type I and Type II 5'-deiodinase (D1 and D2) were determined in parallel by the release of $^{125}\text{I}^{-}$ from 3,3',5'-[^{125}I]tri-iodothyronine (rT₃, DuPont, Bad Homburg, Germany, specific activity: 35–45 MBq/ μg) in the absence or presence of 1 mmol/l PTU (6-n-propyl-2-thio-uracil) using 10 nmol/l non-radioactive rT₃, 20 mmol/l DTT and 20–60 μg protein (18). Homogenates were incubated with the tracer for 1 h in a total volume of 100 μl at pH 7.0. The fraction of iodide release blocked by 1 mmol/l PTU was assigned to D1 activity, the residual activity not inhibited by PTU was ascribed to D2 activity. Deiodinase activities of each sample were determined in triplicate and expressed as fmol $^{125}\text{I}^{-}$ released per min per mg protein. The limit of detection for D1 activity was 0.05 fmol/mg/min and for D2 activity it was 0.18 fmol/mg/min.

Table 1 Specific D1 and D2 activities in human pituitary tissues and corresponding serum levels of free (f) T₃, fT₄ and TSH in the patients. Activities were determined as described in Materials and methods and given in fmol released iodide per mg protein per minute.

Patient no.	Diagnosis	Sex	Tumor diameter (mm)	Age	Drugs	D1 (fmol/mg/min)	D2 (fmol/mg/min)	fT ₃ (ng/dl)	fT ₄ (ng/dl)	TSH (μU/ml)
1	Normal pituitary	f	∅	73	Iodine	0.47	2.83	0.3	1.45	0.42
2	Normal pituitary	m	∅	35	∅	0.12	<I.d.	0.42	1.22	1.02
3	Normal pituitary	m	∅	13	Quinagolide	1.64	2.07	0.4	0.71	2.27
4	Non-secreting	m	20	80	∅	0.27	3.4	0.29	1.04	0.84
5	Non-secreting	m	20	50	Carbimazole, thyroxine, hydrocortisone	0.13	1.07	0.23	0.45	4.84
6	Non-secreting	f	20	63	∅	0.45	1.6		0.86	0.65
7	Non-secreting	m	19	61	∅	<I.d.	11.85	0.33	1.16	0.07
8	Non-secreting	m	20	73	∅	0.79	1	0.30	1.24	0.02
9	Non-secreting	m	29	70	∅	<I.d.	1.65	0.28	0.53	1.11
10	Non-secreting	m	18	50	∅	1.13	2.50	0.38	1.08	2.14
11	Non-secreting	f	18	67	Thyroxine	<I.d.	1.4	0.39	2.14	0.66
12	Non-secreting	f	25	64	∅	2.25	1.75	0.28	1.14	1.14
13	Non-secreting	m	19	77	∅	0.21	2.96	0.31	0.87	0.99
14	Non-secreting	f	25	60	Bromocriptine	0.38	1.91	0.35	1.32	1.0
15	Non-secreting	m	19	53	∅	<I.d.	4.16	0.32	1.71	1.53
16	Non-secreting	m	15	62	Thyroxine	0.12	0.92	0.29	1.01	0.81
17	Non-secreting	f	25	43	∅	<I.d.	2.42	0.31	0.83	1.75
18	Non-secreting	m	37	61	Hydrocortisone, thyroxine, testosterone, fludrocortisone	<I.d.	8.58	0.29	1.12	0.09
19	Non-secreting	m	20	59	∅	0.63	<I.d.	0.24	0.65	0.82
20	Non-secreting	m	37	65	∅	<I.d.	1.78	0.39		0.48
21	Non-secreting	m	21	48	∅	<I.d.	0.52	0.40	0.76	1.19
22	TSHoma	f	18		Propranolol, carbimazole	0.43	2.27		0.91	8.41
23	TSHoma	f	15	25	Carbimazole, somatostatin analogs	9.8	35.2		0.98	13.3
24	TSHoma	f	15		Thyroxine	1.7	25.1		1.73	53.1
25	TSHoma	f	9	54	Carbimazole, propranolol	22.89	105.92	0.22	0.77	15.0
26	TSHoma	m	20	59	Somatostatin analogs, thyroxine	5.52	7.98	0.39	1.51	0.81
27	TSHoma	f	17	34	Somatostatin analogs	1.68	<I.d.	0.36	1.94	0.07
28	TSHoma	m	8	36	Carbimazole	<I.d.	1.94	0.75	3.53	7.48
29	TSH- and GHoma	f	25		Carbimazole	0.15	2.7		1.6	0.6
30	GHoma	m	17	38	∅	0.66	1.44	0.45	1.26	0.51
31	GHoma	m	9	39	∅	0.05	0.62	0.38	0.85	0.71
32	GHoma	m	17	33	Somatostatin analogs	<I.d.	1.29	0.33	1.3	1.14
33	GHoma	f	14	54	Somatostatin analogs, thyroxine	0.3	3.53	0.3	1.15	1.7
34	GHoma	m	30	48	Somatostatin analogs	0.08	2.8	0.3	1.08	0.97
35	PRLoma	f	17	40	Cabergoline	6.78	34.28	0.26	0.82	1.46
36	PRLoma	f	12	24	∅	2.3	12.7		1.22	2.1
37	PRLoma	f	16	40	Bromocriptine	10.92	8.19	0.32	1.06	1.5
38	PRLoma	f	9	20	∅	5.26	56.19	0.44	1.44	2.89
39	PRLoma	f	28	61	∅	<I.d.	6.93	0.20	0.67	1.2
40	PRLoma	f	23	38	∅	1.61	1.44	0.29	1.46	1.15
41	PRLoma	m	17	48	∅	<I.d.	0.18	0.33	0.89	0.62
42	PRLoma	m	38	48	Hydrocortisone, quinagolide	<I.d.	3.1	0.28	1.10	0.48
43	ACTHoma (Cushing's disease)	m	10	69	Thyroxine	<I.d.	2.65	0.35	2.69	0.03
44	ACTHoma (Cushing's disease)	f	9	57	∅	<I.d.	2.48	0.34	1.66	0.52
45	ACTHoma (Nelson's syndrome)	f	11	58	Thyroxine, hydrocortisone	0.35	2.31	0.30	1.26	0.37
46	ACTHoma (Nelson's syndrome)	f	17	56	Hydrocortisone fludrocortisone	0.32	3.07	0.39	1.29	1.55

I.d.: limit of detection; ∅: no drugs.

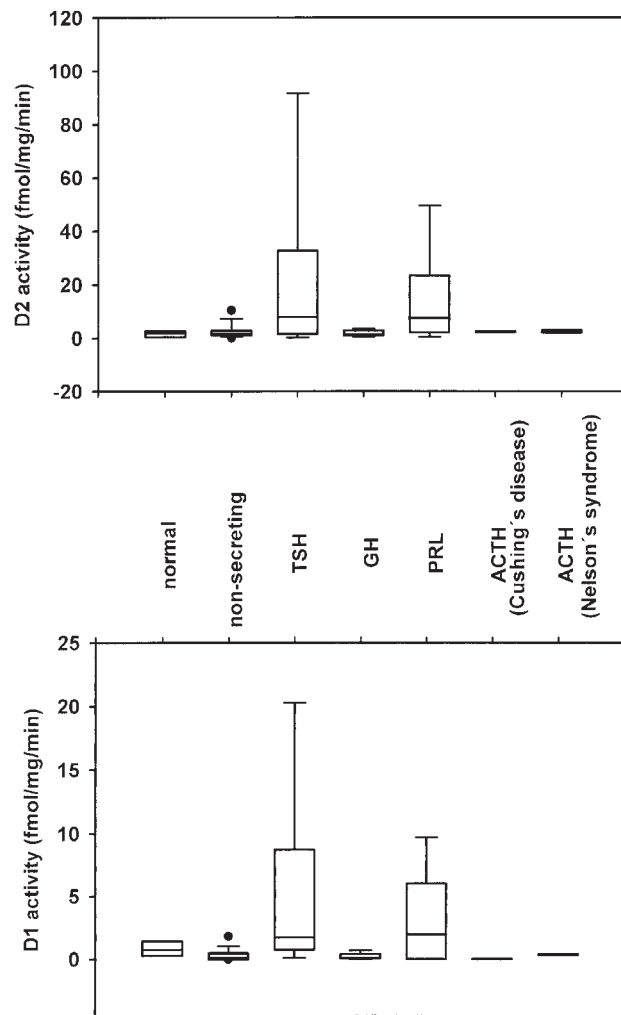


Figure 2 Box plot illustrating median (—) specific activities of D1 and D2 in human pituitary tissues. Boxes show 5th to 95th percentiles. Symbols (●) indicate extreme datapoints outside the 5th and 95th percentiles and bar caps give 10th and 90th percentiles.

Results

All pituitary tumors expressed either one or both of the 5'-deiodinase activities (Fig. 1 and Table 1). The highest activities were found in some TSH- and PRL-producing tumors. In most of the tissues D2 activity was higher than D1, but there were also 3 tumors (2 non-functioning, 1 prolactinoma) and one normal tissue where D1 activity was higher than D2. Some of the tumors showed no D1 activity at all (8 non-functioning tumors, 1 TSH-producing, 1 GH-producing, 3 PRL-producing and 2 adrenocorticotropin (ACTH)-producing). The relative activities of D1 and D2 and the D1/D2 ratio varied in pituitaries of different patients with the same type of tumor and even in normal tissues. A correlation between the gender of the patients and deiodinase activities was not observed. There was also no significant correlation between

deiodinase activities and TSH levels in patients with TSH-secreting adenomas. Figure 2 shows the median activities of D1 and D2 in the different types of adenomas.

Discussion

In the present study we demonstrated for the first time that not only functional D2 but also D1 activity is expressed in human pituitary tissues, both in adenomas of different secretory activities and also in normal tissues. Itagaki *et al.* found only D2 activity in 12 human pituitary adenomas and one normal pituitary (15). This difference might be due to different enzyme substrates that were employed for the measurement of deiodinase activity. Itagaki *et al.* used radiolabeled T_4 as substrate whereas we performed the assay with radiolabeled rT_3 together with 10 nmol/l nonradioactive rT_3 , the substrate that is preferred by D1. As a control we also analyzed activity using T_4 as substrate (data not shown). These experiments revealed that D2 activity with T_4 as substrate was elevated in most cases ($413 \pm 78\%$) compared with the assays with rT_3 as substrate, whereas D1 activity was underestimated or not measurable with T_4 as substrate (15%).

All examined tissues showed deiodinase activity. In most cases D2 activity was higher than D1, but there were also three adenomas and one normal tissue where D1 was higher than D2. In human tissues expressing high levels of functional D1 activity, such as liver, kidney and thyroid, the enzyme activity is decreased or even absent when the tissues undergo neoplastic transformation (3, 11, 12, 19). It is unlikely that the difference in the relative expression profile of the two 5'-deiodinases in most of the human adenomas is due to such a dysregulation in neoplastic tissues compared with normal, euthyroid rat anterior pituitaries, where D1 activity is an order of magnitude higher than D2 (7), because a higher specific D2 activity was also found in two of the three normal anterior pituitaries. It is more likely that this represents the normal conditions in the human anterior pituitary with higher activities of D2 than D1. Such a difference of the relative expression levels of the two 5'-deiodinase isoenzymes between human and rat tissues has also been described for the CNS. In contrast to rat CNS, no or only negligible D1 activity was found in the human CNS (9, 10). D2 activity was not changed in tumors compared with normal tissues when expressed in relation to DNA content, but was significantly increased when expressed in relation to protein content (10). A high activity of D2 has recently been described in the human mesothelioma cell line MSTO-211H, whereas cells derived from normal mesothelium do not express D2 activity (20). Therefore, D2 seems to be induced by neoplastic transformation in some tissues but this observation cannot be extrapolated to all

tissues; for example, in human papillary thyroid carcinoma D2 is decreased compared with normal controls (13). A decrease in D1 activity in tumors compared with normal tissue has been described for several tissues (thyroid, kidney etc.) (11, 12) whereas in human intestine no differences between D1 activity in tumors and normal tissue are found (3). Hence tumor-dependent regulation of the two 5'-deiodinase enzymes differs between different tissues.

The expression pattern of 5'-deiodinases in human anterior pituitary tumors depended on the endocrinological type of tumor since the highest activities were found in most of the TSH- and PRL-producing adenomas although the absolute activities and the D1/D2 ratio varied among patients with the same type of tumor. This is in accordance with the early report of Itagaki *et al.* who also described substantial variability of the D2 activity among individuals with the same kind of tumor (15). During the last few years it has been clearly demonstrated that most pituitary tumors are monoclonal in origin with a mutation in a single cell that subsequently undergoes clonal expansion (21, 22). This could be an explanation for the variability of the deiodinase activities in the same kind of tumor since endocrine cells which produce the same hormone are a heterogenous population with some cells differing in function and regulation (23). Whether the cytokine expression pattern, known to be changed in pituitary adenoma, or tumor-induced alterations in cellular communication and interaction between hormone producing and folliculo-stellate cells (24–27) leads to individual tumor-specific changes in the expression of deiodinases remains to be analyzed.

Another component of thyroid hormone action, the thyroid hormone receptors are also cell type-dependently expressed in human pituitary adenoma. It has been shown by RT-PCR that mRNAs for thyroid hormone receptors (TR) β 1, α 1 and α 2 were expressed in nonfunctioning adenomas, TR β 1 and TR α 1 in prolactinomas, TR β 1 in GHomas but there was no expression of these three isoforms in one TSHoma and in ACTH-producing adenomas (28). Gittoes *et al.* (29) also reported abnormal expression of thyroid hormone receptors in TSH-producing adenoma compared with normal pituitaries and they postulated that this is the reason for the defective negative feedback of thyroid hormones on TSH production from human TSHomas. Nevertheless, the expression of both 5'-deiodinase isoenzymes, D1 and D2, in human thyrotroph cells furthers our understanding of TSH feedback regulation. The expression of D1 and its stimulation by T₃ helps to explain the observations that TSH secretion is not only correlated with plasma T₄ but also with plasma T₃ levels (30, 31), thus formulating a logical TSH feedback regulation.

Besides TSH production and release, several other functions of the anterior pituitary are regulated by T₃

and have been well studied in rodents. Chomczynski *et al.* demonstrated that T₃ stimulates GH gene transcription in the deiodinase expressing human somatomammotroph GX cell line (32) compatible with *in vivo* observations in humans that GH production and release is influenced by thyroid hormone levels (2).

The expression of 5'-deiodinases in ACTH-producing adenomas indicates that thyroid hormones are also important for the function of corticotroph cells. In rat anterior pituitaries pro-opiomelanocortin mRNA is not influenced by thyroid hormones. However, the prohormone processing enzymes, PC1 and PC2, are regulated by thyroid hormone levels (33), and in mouse corticotroph AtT 20 cells thyroid hormone receptors are expressed and PC1 mRNA is decreased by T₃ (our own unpublished data). Furthermore, cell proliferation and/or differentiation of pituitary cells can be influenced by thyroid hormones (34).

These data, together with our findings that all examined adenomas and normal pituitary tissues expressed either one or both of the two 5'-deiodinases, indicate that thyroid hormones and the local conversion of T₄ to the active T₃ is involved in the regulation and function of all hormone-producing cells in the human anterior pituitary even in neoplastic pituitary adenoma.

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