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Determining the enzymatic activities of iodothyronine 5′- deiodinases in renal medulla and cortex

Określenie aktywności enzymatycznej 5′-dejodynazy jodotyroninowej w rdzeniu i korze nerek

Stanisław Niemczyk[1, 2], Marta Dudek[3, 4], Zbigniew Bartoszewicz[5], Katarzyna Szamotulska[6], Łukasz Woźniacki[1], Dorota Brodowska-Kania[1], Longin Niemczyk[2], Władysław Małek[7], Joanna Matuszkiewicz-Rowińska[2]

[1]*Department of Internal Medicine, Nephrology and Dialysotherapy , Military Institute of Medicine, Warsaw, Poland*
[2]*Department of Nephrology, Dialysotherapy and Internal Medicine, Medical University of Warsaw, Poland*
[3]*Centre of New Technologies, University of Warsaw, Poland*
[4]*Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Poland*
[5]*Department of Internal Medicine and Endocrinology, Medical University of Warsaw, Poland*
[6]*Department of Epidemiology, Institute of Mother and Child, Warsaw, Poland*
[7]*Department of Urology, Ministry of Interior and Administration, Warsaw, Poland*

Abstract

Introduction: Thyroid hormone disorders in patients with chronic kidney disease (CKD) are a result of impaired conversion of T4 to T3. The importance of kidneys in thyroid hormones conversion is not fully understood. The activities of different types of iodothyronine deiodinases in the kidney structures have not been determined yet.
The aim of this study was to determine the activity of deiodinase type 1 (D1) and type 2 (D2) in renal cortex and medulla in renal cancer patients.
Material and methods: Samples of renal cortex and medulla (ten patients) or renal cortex alone (13 patients) were taken from kidneys resected because of malignant cancer, from a site opposite to the cancer. Resections were performed in the 23 patients (seven female and 16 male) who were 52–82 years old. The material was stored at –72 °C.
Results: Activity of D1 in renal cortex was 3.785 ± 2.041 fmol ^{125}I/mg protein/minute and activity of D2 was 0.236 ± 0.125 fmol ^{125}I/mg protein/minute. There was a strong positive correlation between D1 and D2 activities in renal cortex ($r = 0.890$, $p < 0.001$). Activity of D1 in renal medulla was 2.157 ± 2.176 fmol ^{125}I/mg protein/minute, and activity of D2 was 0.168 ± 0.095 fmol ^{125}I/mg protein/minute. A positive correlation between D1 and D2 in renal medulla ($r = 0.661$, $p = 0.038$) was observed as well. Activities of D1 in cortex and medulla were strongly and positively associated ($r = 0.794$, $p = 0.006$), whereas there was no correlation between the activities of D2 in cortex and medulla ($r = 0.224$, $p = 0.553$).
Conclusions: Results presented in this study suggest that both cortical and medullary D1 and D2 may be involved in thyroid hormone metabolism. This finding could be of clinical relevance in patients with impaired renal function. **(Endokrynol Pol 2013; 64 (3): 182–185)**

Key words: *deiodinase, iodothyronine 5′-deiodinase, thyroid hormones, kidney, cortex, medulla*

Streszczenie

Wstęp: Zaburzenia hormonów tarczycy u pacjentów z przewlekłą chorobą nerek (PChN) są wynikiem zaburzeń konwersji T4 do T3. Znaczenie nerek w konwersji hormonów tarczycy nie jest w pełni poznane. Działania różnych typów dejodynaz jodotyroninowych w strukturach nerek, nie zostały jeszcze określone.
Celem badań było określenie aktywności dejodynazy typu 1 (D1) i typu 2 (D2) w korze i rdzeniu nerek u chorych z rakiem nerki.
Materiał i metody: Próbki kory i rdzenia nerek (10 pacjentów) lub tylko samej kory (13 pacjentów) były pobrane z przeciwnego bieguna tej samej nerki do guza , z nerek usuniętych z powodu raka. Resekcje wykonano u 23 chorych (7 kobiet i 16 mężczyzn) w wieku 52–82 lat.
Wyniki: Aktywność D1 w korze nerki wynosiła 3,785 ± 2,041 fmol ^{125}I/mg białka/min., a aktywność D2 wynosiła 0,236 ± 0,125 fmol ^{125}I/mg białka/min. Znaleziono silną, dodatnią korelację pomiędzy aktywnością D1 i D2 w korze nerki ($r = 0,890$, $p < 0,001$). Aktywność D1 w rdzeniu nerek wynosiła 2,157 ± 2,176 fmol ^{125}I/mg białka /min., a aktywność D2 wynosiła 0,168 ± 0,095 fmol ^{125}I/mg białka/min. Zaobserwowano również dodatni związek pomiędzy aktywnością D1 i D2 w rdzeniu nerek ($r = 0,661$, $p = 0,038$). Stwierdzono silną dodatnią korelację aktywności D1 w korze i rdzeniu ($r = 0,794$, $p = 0,006$) oraz brak korelacji aktywności D2 w korze i rdzeniu ($r = 0,224$, $p = 0,553$).
Wnioski: Wyniki przedstawionej pracy sugerują, że aktwność dejodynaz 1 i 2 zarówno w korze jak i w rdzeniu nerki może miec wpływ na metabolizm hormonów tarczycy. To ustalenie może mieć znaczenie kliniczne dla chorych z upośledzoną funkcją nerek. **(Endokrynol Pol 2013; 64 (3): 182–185)**

Słowa kluczowe: *dejodynaza, 5′-dejodynaza jodotyroninowa, hormony tarczycy, nerka, kora, rdzeń*

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dr hab. n. med. Stanisław Niemczyk, Department of Internal Medicine, Nephrology and Dialysotherapy , Military Institute of Medicine, Warsaw, Szaserów St. 128, 04–141 Warszawa, tel.: +48 22 681 70 72, fax: +4822 681 68 11, e-mail snemczyk@wim.mil.pl

Introduction

Thyroid hormone disorders in patients with chronic kidney disease (CKD) result from impaired conversion of T4 to T3 leading to decreased T3 levels. The importance of kidneys in thyroid hormones conversion is not fully understood. In adult mammals, the activity of deiodinases has been identified in the liver, kidney, thyroid, pituitary gland, intestines, placenta and gonads. The activities of different types of iodothyronine deiodinases in kidney structures have not been determined yet. The disorders may be partly explained by the absence of renal tissue and decreased activity of deiodinases connected with this matter. There are significant differences in the course of 'sick euthyroid syndrome' in patients with CKD and with normal renal function in other severe diseases. The concentration of rT3 is normal in CKD patients as opposed to other patients with this syndrome.

Determination of deiodinases activity in various kidney structures may be useful in explaining thyroid hormone disorders and differences between patients with CKD and other patients. In various diseases, the presence of pro-inflammatory cytokines and acidosis may be a key factor affecting hormonal conversion and hormonal state [1–4].

The purpose of the study was to determine the activity of deiodinase type 1 (D1) and type 2 (D2) in renal cortex and medulla in renal cancer patients.

Material and methods

Specimens of renal cortex and medulla (ten patients) or renal cortex alone (13 patients) were taken from kidneys resected because of malignant cancer. The differentiation of tissues (cortex or medulla) was performed macroscopically by a urologist during kidney resection by taking samples. The collection of material from both structures was not possible in all patients. Collecting cortex, an outer part of the kidney, is easy and has always been possible (23 specimens). Examining the renal medulla was performed only when there was a macroscopical certainty that it was a proper material (ten specimens). The samples were taken from a site opposite to the cancer. Resections were performed in 23 patients (seven females and 16 males) aged 52–82 years. None of the patients was pharmacologically treated for cancer. The material was stored at –72°C.

Apparatus

Tissues were homogenised using an Ultra-Turrax T25 Homogeniser. Radioactivity was measured on a Wallac WIZARD Automatic Gamma Counter. The protein concentration was measured on Pharmacia Biotech Ultraspec 3000 spectrophotometer.

Reagents

All chemicals were purchased from Sigma (EDTA, PTU, [^{125}I] rT3, [^{125}I] T4, rT3, T4, Sephadex LH-20), Merck (column for the purification of radioactive probes, DTT, TCA, BSA, EtOH, K_2PO_4), Pierce (BCA reagent), Gibco and Biomed (horse serum).

All reagents were of the highest quality and the procedures are widely available in publications.

Tissue homogenate solution

Tissue fragments of about 100 mg were homogenised in a buffer containing 1 M K_2PO_4, 10 mM DTT, 0.25 M glucose and 1 mM EDTA in a teflon-glass homogeniser. The homogenate solution was made fresh and stored at –20°C.

Determination of protein concentration

Protein concentration was determined by BCA method, using appropriate dilutions of homogenates and bovine serum albumin (BSA) as a standard. Incubation was performed at 37°C for 30 min and the absorbance measurements were carried at wavelength $\lambda = 562$.

Tissue preparation

Tissues were homogenised in cold DI homogenisation solution at a 1:21 dilution. Homogenates were centrifuged (1,500 x g) for 10 min at 4°C. Floating brush was removed by aspiration and the supernatant was aliquotted to 2 mL screw-capped tubes. Homogenates were recentrifuged (20,000 x g) for 5 min at 4°C, floating brush was removed and the supernatant decanted into 2 mL plastic tubes and used for the DI-I assay.

Calculations

The method assumes homogeneous distribution of ^{125}I-labelled and nonlabelled rT3 in the incubation solution. Calculations were performed as follows, where: CPM — counts per minute; Bo — blank counts; Total — total counts; FV — final volume (μL); MV — measurements volume (μL); PC — protein concentration (g/μL); PV — protein volume; IT — incubation time (min).

Determination of activity of deiodinase type 1 (5'D1) was performed on the basis of amounts of radioactive iodine released from the [^{125}I]-rT3 in deiodination reaction, in which 5'D1 is a catalysator, according to the method described in [5]. On the day of the experiment, labelled rT3 was purified on a Sephadex LH 20, eluted with 75% ethanol into a series volumes of 600 μL each. The probe with the highest activity (50,000-60,000 cpm/ul) was the source of [^{125}I]-rT3 necessary to the deiodination reaction.

The composition of the reaction mixture was: fraction of kidney homogenate as a source of deiodinase, [^{125}I]-rT3, 1 nM rT3, 20 mM DTT, 0.1 M K_2PO_4 buffer + 1 mM EDTA,

1 mM PTU. Reactions were carried out at 37°C/30 min and were stopped by immersing the samples in ice.

The separation of labeled iodine released from the iodothyronines was achieved by precipitation with 100 μL of horse serum and 50 μL of 50% trichloroacetic acid. After vortexing, the probes were centrifuged and 150 μL of supernatant was collected for measurements of gamma radiation in the gamma counter.

Deiodinases specific activity was determined in femtomoles of released ^{125}I per 1 mg of protein per 1 minute [fmol ^{125}I/mg protein/minute].

The enzymatic activity was assayed as described previously [5]. D1 activity was determined by quantification of the radioiodine released by (^{125})I-rT3 in deiodination reaction catalysed by 5'DI according to the method described by Larson et al. [5]. D2 activity was determined by quantification of the radioiodine released by 3,3',5, ^{125}I 5' tetraiodothyronine (T4) in deiodination reaction. The procedure was identical as for D1. In statistical analysis, Spearman's correlation coefficients were used.

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Results

Activity of D1 in renal cortex was 3.785 ± 2.041 fmol ^{125}I/mg protein/minute, and activity of D2 was 0.236 ± ± 0.125 fmol ^{125}I/mg protein/minute. There was a strong positive correlation between D1 and D2 activities in renal cortex ($r = 0.890$, $p < 0.001$).

Activity of D1 in renal medulla was 2.157 ± 2.176 fmol ^{125}I/mg protein/minute, and activity of D2 was 0.168 ± 0.095 fmol ^{125}I/mg protein/minute. A positive correlation between D1 and D2 in renal medulla ($r = 0.661$, $p = 0.038$) was observed as well.

Activities of D1 in cortex and medulla were strongly and positively associated ($r = 0.794$, $p = 0.006$), whereas there was no correlation between activities of D2 in cortex and medulla ($r = 0.224$, $p = 0.553$).

Discussion

It has been well proven that kidneys are able to catalyse the conversion of T4 to T3 [5–8]. However, the roles of deiodinases D1 and D2 in the human kidneys remain undetermined. Deiodinases are the enzymes that catalyse the deiodination of thyroid hormone molecules (particularly T4 to T3 and T4 to rT3). As a result, they affect the balance of thyroid hormones that have different biological activities. There has been no agreement as to the intracellular localisation of the enzymatic activity of 5'-deiodinases in human kidney in the presented studies. There have been suggestions of partial microsomal contribution [9, 10]. The half-life of D2 in normal cells is 20–30 minutes in the presence of T4, whereas the D1 protein has a longer half-life (> 12 hours).

Type 1 iodothyronine 5'-deiodinase is an integral membrane protein catalysing the phenolic ring deiodination of thyroxine. The type 1 enzyme was present exclusively in tubular epithelial cells of the outer renal cortex and co-purified with basolateral plasma membranes; the renal medulla lack of activity [11]. The results of our studies prove that there is an activity of type 1 deiodinase in renal medulla. It is approximately 1.8 times lower than in renal cortex (respectively 1.4 for D2). In rat kidney studies, the activity of type 1 deiodinase in the proximal convoluted tubule cells and collagenases-dispersed rat kidney cells has been found [12, 13].

Type 1 iodothyronine 5'-deiodinase (5'D-1) is a protein with a M_r of 29 kDa. It catalyzes the 5'-deiodination reaction of thyroxine. It takes part in the synthesis of more than 75% of the biologically active thyroid hormone in the circulation [14]. The biochemical and molecular characteristics of D2 seem to be reflecting extrathyroidal T3 production. Its activity is strictly limited by the concentration of its preferred substrate, T4, since it catalyses the ubiquitination of this enzyme, inactivating it and quickening its degradation in proteasomes [15].

It is important to determine the cellular location of iodothyronine deiodinases since they play a significant role in the regulation of the available intracellular T3 concentration. In our study, we evaluated the activity of type 1 and type 2 deiodinases. However, the methodology used in this study does not allow us to determine whether the activity is strictly microsomal or generally intracellular. In the kidney, the presence of type 3 iodothyronine deiodinase (D3) seems to be crucial to maintain homeostasis of T3 levels. To date, no cellular localisation pattern of D3 protein has been determined in human kidneys.

It is very difficult to compare the activity of type 1 and 2 deiodinases by performing laboratory examinations, because of the different activity of their product (e.g. T3 *v.* rT3).

Therefore, comparisons are approximate. It seems that the activity of deiodinases in kidneys established in our studies is lower than in liver and muscles indicated in other studies, as there are methodological differences [16, 17]. In chronic severe illness, a decrease is also supplemented by an increase in the inner-ring deiodination of T4 and T3 by type 3 iodothyronine deiodinase in the liver and skeletal muscle [18].

There have been no previous studies addressing the roles of human renal iodothyronine deiodinases in the peripheral conversion of T4 to T3. A number of

studies have indicated that the expression of deiodinases is altered in several types of cancers, suggesting that they may represent a useful cancer marker and/or could play a role in modulating cell proliferation [19, 20]. In our study, we cannot fully exclude the influence of renal cancer on the activity of deiodinases, despite the fact that material was collected from a site opposite to the cancer assessed by a urologist to be healthy tissue.

In this study, we determined the activities of D1 and D2 in renal cortex and medulla. The activity of D1 is many times higher than D2, both in cortex and medulla. The positive correlation of D1 and D2 is particularly high in renal cortex. We suggest that the study should be enhanced and performed on a larger amount of material with methodology including histological differentiation of kidney structure and the analysis of influence of various clinical situations including inflammation, severity of renal failure, presence or absence of neoplasm, primary and secondary nephropathy and influence of immunosuppressive therapy.

It seems that loss of kidney tissue in CKD may lead to reported thyroid hormone disorders in CKD patients manifested mainly by impaired conversion of T4 to T3 [4].

Conclusions

The results presented in this study suggest that both cortical and medullary D1 and D2 may be involved in thyroid hormone metabolism. This finding could be of clinical relevance in patients with impaired renal function.

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