FEBS Letters 354 (1994) 110–112

FEBS 14699

Expression of type II thyroxine 5′-deiodinase from rat Harderian gland in *Xenopus laevis* oocytes

Juan F. Garcia-Macias, Patrocinio Molinero, Juan M. Guerrero, Carmen Osuna*

Department of Medical Biochemistry and Molecular Biology, The University of Seville School of Medicine and Virgen Macarena Hospital, Avda Sanchez Pizjuan 4, 41009-Seville, Spain

Received 9 August 1994; revised version received 15 September 1994

Abstract The presence of isoenzymes mediating the conversion of thyroxine to 3,5,3′-triiodothyronine has been studied according to characteristic kinetics and physiological regulation. In this paper, we report the expression of type II 5′-deiodinase (5′D) activity in oocytes of *Xenopus laevis*. Oocytes injected with total RNA extracted from rat Harderian gland, and then incubated up to five days demonstrated a progressive increase in 5′D activity, reaching a maximal value at 24 h; then, 5′D activity remained almost stable for an additional period of four days. Characteristics of the enzyme activity expressed by oocytes included its inhibition by iopanoic acid, but not by propylthiouracil, and its increase during β-adrenergic agonist treatment and hypothyroidism. The expressed activity manifests characteristics typical of the type II isoenzyme. Deiodinating activity in oocytes also exhibited diurnal variations. In this study, 5′D activity expressed in oocytes exhibited low values when animals were killed during the day, and high values when animals were killed at night. Maximal values were reached 3–4 h before the nocturnal peak of 5′D activity in Harderian gland crude homogenates. Results suggest that the in vivo activation of 5′D by isoproterenol, hypothyroidism, or dark exposure may be caused by an increase in the synthesis and/or maturation of the RNA expressing the enzyme.

Key words: Deiodinase; Thyroxine; Harderian gland; *Xenopus laevis*; RNA expression

1. Introduction

In man and experimental animals most triiodothyronine (T_3) is produced by extrathyroidal 5′-monodeiodination of thyroxine (T_4) [1]. This process is catalyzed by enzymes found in microsomes of several tissues. At least two types of thyroxine 5′-deiodinase (5′D) have been studied and classified according to kinetic characteristics and physiological regulation. This includes a high K_m 5′D in the range of 1–5 micromolar which is mainly present in liver [2] and kidney [3] (type I isoenzyme), and plays an important role in maintaining serum T_3 levels. Another isoenzyme is present in specific tissues including anterior pituitary [4], brain [5], brown adipose tissue [6], and epidermal keratinocytes [7]. This isoenzyme exhibits a lower K_m in the range of 1–5 nM (type II isoenzyme) when dithiothreitol is used as cofactor, is sensitive to inhibition by iopanoic acid (IOP), and increases its activity during hypothyroidism [8]. Thus, it is believed to have an important role in maintaining intracellular levels of T_3 and could serve as a defense against thyroid hormone deficiency. During the last few years, the presence of type II 5′D activity was also identified in pineal [9–11] and Harderian glands [12,13]. In both glands, the activity of the deiodinating enzyme increases with hypothyroidism and exhibits a marked nyctohemeral profile reaching maximal values late in the dark period. Since no specific reagents for quantitation of the enzyme have been developed, it has not been possible to confirm that these activity changes are due to alterations in the enzyme content or, if so, whether they are transcriptional or post-transcriptional.

Several attempts have been made to isolate the type I 5′D isoenzyme, but have been unsuccessfuyl, apparently because there is substantial inactivation of 5′D activity during the isolation procedures used [14,15]. A growing number of proteins, including intracellular enzymes and cell surface receptors, have been found to amenable to study after expression in *Xenopus laevis* oocytes. Using this system, the expression of type I 5′-deiodinase has been reported [16], providing new information concerning the biochemical characteristics of this isoenzyme. However, little is known about the properties of the type II isoenzyme.

The aim of this work is to characterize the expression of the type II 5′D isoenzyme using *Xenopus laevis* oocytes as an in vivo translational assay system to identify total RNA. Results show that the type II isoenzyme can be induced in *Xenopus laevis* oocytes by the injection of total RNA from rat Harderian gland.

2. Materials and methods

2.1. Animals

Wistar rats of both sexes born in our animal facilities were used. Animals received food and water ad libitum and were exposed to an automatically regulated light/dark cycle of 14:10 h; the lights were turned off daily from 20.00 through 06.00 h. When required, animals were rendered hypothyroid by adding 20 mg/100 ml methimazole to the drinking water; this treatment was maintained for 10 days before the animals were used for the experiments. On the day of the experiment, animals were killed by decapitation at the times indicated, and Harderian glands were quickly collected, frozen on solid CO_2, and stored at −80°C until used.

2.2. Preparation of total RNA

Total RNA was isolated from rat Harderian gland using the method of Chomczynski and Sacchi [17]. Harderian glands (1 g) were homogenized with 10 ml of 4 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7, 0.5% sarcosyl, 0.1 M 2-mercaptoethanol, and after both the extraction and precipitation with isopropanol, the resulting RNA pellet was dissolved in water for injection into oocytes. The yield of total RNA was measured by the optical density at 260 nm.

2.3. Microinjection of total RNA into Xenopus laevis oocytes

Xenopus laevis oocytes were microinjected with RNA according to the general method of Coleman [18]. Female *Xenopus laevis* frogs (Centre d'Elevage de Xenopes du CRBM, Montpellier, France) were anesthetized by hypothermia and ovarian lobes removed through an abdominal incision and placed into 5 mM HEPES buffer, pH 7.5, containing 82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$ (OR2 solution).

*Corresponding author. Fax: (34) (5) 490 7048.

SSDI 0014-5793(94)01086-2

Oocytes were dissected from the ovarian tissue and incubated for 45 min in collagenase (type II, Sigma); then, washed extensively in OR2 solution and incubated overnight in 5 mM HEPES medium, pH 7.5, containing 96 mM NaCl, 2 mM KCl, 1.8 mM KCl_2, 1 mM $MgCl_2$ (ND96 medium). Oocytes were injected with 50 nl (1 ng/nl) of a solution of total RNA in sterile water. Typically, 12 oocytes were injected with each RNA preparation; then, they were incubated in ND96 medium containing 25 mg/ml streptomycin with daily medium changes. After incubation, oocytes were frozen at −80°C until the quantitation of 5'D activity.

2.4. Type II thyroxine 5'-deiodinase assay

The measurement of type II 5'D activity was based on the release of radioiodine from [3',5'-^{125}I]T_4. This method, commonly used in Harderian gland studies [12,13], is sensitive enough for Harderian 5'D determinations and is specific for 5'D deiodinase since the substrate contains ^{125}I only in position 5'. Other deiodinating activities, i.e. conversion of T_4 to rT_3, would release only nonradioactive iodide. Type II 5'D activity was determined in groups of 12 oocytes. Each group was disrupted by ultrasound in cold 0.05 M phosphate buffer, pH 6.8; then, 50 μl were incubated in the presence of 40 mM DTT and 2 nM [3',5'-^{125}I]T_4 as substrate (200 μl final volume). The reaction was started by the addition of the substrate and continued for 60 min at 37°C. Control incubations were performed by omission of the homogenate. The reaction was terminated by the addition of 100 μl cold BSA (2%) and 750 μl trichloroacetic acid (10%). Samples were centrifuged for 30 s at 10,000 rpm and 500 μl of the supernatant were placed onto a 0.5 ml column packed with Dowex-50W ion-exchange resin and washed with 500 μl of 10% glacial acetic acid. Radioactivity in the eluate, corresponding to the ^{125}I released, was counted in a gamma counter as an index of 5'D activity. The recovery of ^{125}I in this process was better than 95%. Specific activity was determined by subtracting the control value, which usually amounted to less than 1% of the radioactivity added. 5'D activity is referred to as fentomoles ^{125}I released/100 oocytes/h or fentomoles ^{125}I released/mg protein/h. When required, proteins were determined by the method described by Lowry et al. [19]. Results are expressed as means ± standard errors (S.E.). Data were statistically analyzed using an ANOVA followed by a Student–Newman–Keuls multiple range test.

2.5. Reagents

All reagents were of analytical grade and obtained from commercial sources. T_3, D,L-dithiothreitol (DTT), methimazole, 6-*n*-propyl-2-thiouracil (PTU), (−) isoproterenol, and Dowex-50W were purchased from Sigma (St. Louis, MO); iopanoic acid (IOP; Telepaque, 3-[3-amino-2,4,6-triiodophenyl]-2-ethylpropionic acid) was generously supplied by Dr. Thielking, Sterling-Winthrop Research Institute (Rensselaer, NY). Na^{125}I was purchased from Amersham (Amersham, Bucks, UK). ^{125}I was bound to T_3 using the chloramine-T method, as described by Nakamura et al. [20]. [3',5'-^{125}I]T_4 was purified on a 3-ml Sephadex LH-20 column, containing the purified tracer and less than 2% free iodine, and immediately used for 5'D analyses.

3. Results and discussion

Results demonstrate, for the first time, that type II thyroxine 5'D can be induced in *Xenopus laevis* oocytes by the injection of total RNA isolated from rat Harderian gland. The expressed activity manifests characteristics typical of the type II enzyme. The time course of expression of 5'D activity in oocytes after injection of RNA total shows that the activity can be detected at 12 h, but the maximal value is reached at 24 h and, after this time, the expression is maintained almost stable (Fig. 1, top). Therefore, further incubations were performed for 24 h to provide maximal deiodinating activity. Other authors investigated the expression in *Xenopus laevis* of type I thyroxine 5'D, and have demosntrated a progressive increase in this activity for up to 5 days [16]. The differential timing of the induction of both isoenzymes supports the critical importance of extrathyroidal 5' monodeiodination of T_4 in different tissues.

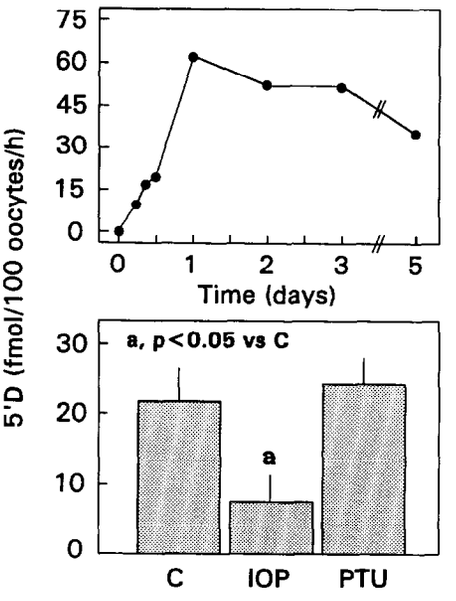

Fig. 1. (Top) Time course of expression of 5'D activity in *Xenopus laevis* oocytes. Oocytes were injected with 50 ng of total RNA extracted from Harderian gland of hypothyroid rats. Groups of 12 oocytes were incubated for the times indicated. Results are the mean of 2 experiments performed in triplicate. (Bottom) Oocytes were injected with 50 ng of total RNA extracted from Harderian gland of hypothyroid rats. After 24 h incubation, oocytes were sonicated and 5'D activity was determined in the absence (C) or presence of 1 mM IOP or 1 mM PTU. Results are the mean ± S.E. of 3 experiments performed in triplicate.

A characteristic of the type II 5'D isoenzyme is the different sensitivity of the enzyme to inhibition by PTU and IOP acid. As shown in Fig. 1 (bottom), the enzyme activity was insensitive to PTU, but was inhibited by IOP acid (70%). Because these characteristics define the type II thyroxine deiodinating pathway in other tissues [21], we suggest that 5'D expressed by oocytes also shares this pathway.

The mechanisms involved in regulating Harderian gland 5'D have been studied in vivo [12,13]. In Harderian gland, besides the thyroid status, 5'D activity is regulated by the light–dark cycle, exhibiting a progressive rise in activity after the onset of the dark period and reaching a peak value 5–6 hours later [12,22]. This nocturnal increase in 5'D activity seems to be dependent on the sympathetic noradrenergic input [22]. In addition, in vivo studies have shown that isoproterenol, a β-adrenergic agonist, activates 5'D activity, while propranolol, a β-adrenergic blocker, inhibits it [23,24]. The mechanisms involved in this regulation are not well understood. In this paper we support the hypothesis that synthesis and/or maturation of RNA can be involved in the regulation of 5'D activity. Thus, we studied the induction of type II 5'D activity in *Xenopus laevis* oocytes from rats maintained under different treatments. Both euthyroid and hypothyroid animals were injected with isoproterenol, and the isolated total RNA from Harderian gland, confirmed that isoproterenol treatment and/or hypothyroid status induced an increase in the expression of the enzyme (Fig. 2).

Additional experiments were performed to investigate the diurnal variations in the expression of 5'D activity in *Xenopus laevis* oocytes. Harderian glands of hypothyroid rats were col-

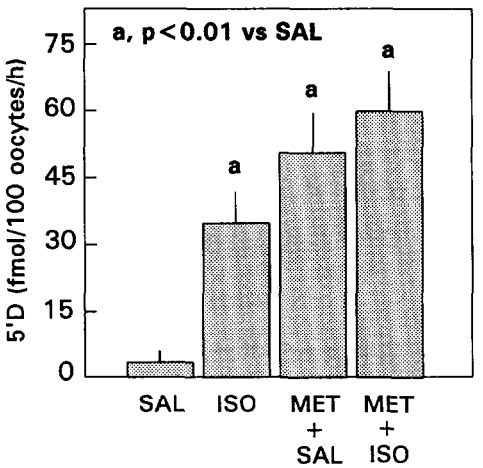

Fig. 2. Expression of 5'D activity in *Xenopus laevis* oocytes injected with total RNA under different conditions. Oocytes were injected with 50 ng of total RNA extracted from Harderian gland of rats maintained under the following conditions. Two groups of euthyroid animals were injected with either saline (SAL) or isoproterenol (1 mg/kg BW) (ISO). Two additional groups were rendered hypothyroid administering methimazole in the drinking water for 10 days, and also injected with saline (MET + SAL) or isoproterenol (MET + ISO); animals were injected with drugs at 12.00 and 14.00 h; then animals were killed at 16.00 h and Harderian glands were quickly removed and stored at −80°C until used to extract total RNA. Results are the mean ± S.E. of 3 experiments performed in triplicate.

lected at the times indicated, and the extracted total RNA was injected in oocytes to measxure 5'D activity expression. 5'D activity was determined in parallel in the Harderian gland crude homogenates. As shown in Fig. 3, the enzyme activity in crude homogenates exhibited low values during the daytime; then, activity increased after entering the dark period, and reached the maximal values at 04.00 h. Type II 5D activity expressed by total RNA injected in oocytes exhibited a similar pattern, with low values during the day and high values at night. However, the nocturnal peak value was reached between 00.00 and

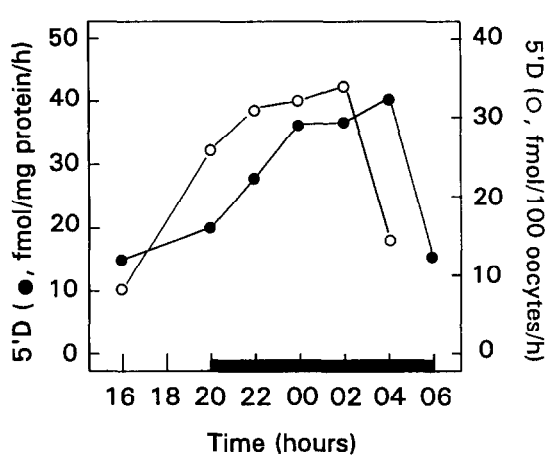

Fig. 3. Diurnal variations in the expression of 5'D activity in *Xenopus laevis* oocytes. Harderian glands of hypothyroid rats were collected at the indicated times; then, the extracted total RNA was injected in oocytes and 5'D activity was determined (○). Simultaneously, 5'D activity was also determined in Harderian gland crude homogenates (●). Data are the mean of 2 experiments performed in triplicate.

00.02 h, 3–4 h before the nocturnal peak of 5'D activity in Harderian gland crude homogenates.

In conclusion, we have shown that type II thyroxine 5'D can be induced in *Xenopus laevis* oocytes by the injection of total RNA from rat Harderian gland. The expressed activity manifests characteristics typical of the type II isoenzyme. Results also suggest that, in addition to other possible mechanisms, the in vivo activation of 5'D by isoproterenol, hypothyroidism, or dark exposure may be caused by an increase in the synthesis and/or maturation of the RNA expressing the enzyme.

Acknowledgements: Supported by grants from CICYT (PB91-0616) and FISSS (93/0210).

References

[1] Larsen, P.R., Silva, J.E. and Kaplan, M.M. (1981) Endocr. Rev. 2, 87–102.
[2] Visser, T.J., Fekkes, D., Docter, R. and Hennemann, G. (1978) Biochem. J. 174, 221–229.
[3] Leonard, J.L. and Rosenberg, I.N. (1978) Endocrinology 103, 2137–2144.
[4] Kaplan, M.M. (1980) Endocrinology 106, 567–576.
[5] Crantz, F.R. and Larsen, P.R. (1980) J. Clin. Invest. 69, 935–938.
[6] Leonard, J.L., Mellen, S.A. and Larsen, P.R. (1982) Endocrinology 112, 1153–1155.
[7] Kaplan, M.M., Pan, C., Gordon, P.R., Lee, J. and Gilchrest, B.A. (1988) J. Clin. Endocrinol. Metab. 66, 815–822.
[8] Dratman, M.B., Crutchfield, F.L., Gordon, J.T. and Jennings, A.S. (1983) Am. J. Physiol. 245, E184–E193.
[9] Tanaka, K., Murakami, M. and Greer, M.A. (1986) Biochem. Biophys. Res. Commun. 137, 863–868.
[10] Guerrero, J.M., Puig-Domingo, M. and Reiter, R.J. (1988) Endocrinology 122, 236–241.
[11] Molinero, P. and Guerrero, J.M. (1993) Neurosci. Lett. 151, 130–133.
[12] Guerrero, J.M., Puig-Domingo, M., Vaughan, G.M. and Reiter, R.J. (1987) Life Sci. 41, 1179–1185.
[13] Guerrero, J.M., Gonzalez-Brito, A., Santana, C. and Reiter, R.J. (1989) Proc. Soc. Exp. Biol. Med. 190, 186–189.
[14] Mol, J.A., van der Berg, T.P. and Visser, T.J. (1988) Mol. Cell. Endocrinol. 55, 159–166.
[15] Boye, N., Laurberg, P. and Jorgenson, P.L. (1988) Mol. Cell. Endocrinol. 56, 99–106.
[16] St. Germain, D.L. and Morganelli, C.M. (1989) J. Biol. Chem. 264, 3054–3056.
[17] Chromczynski, P. and Sacchi, N. (1987) Analyt. Biochem. 162, 156–159.
[18] Coleman, A. (1984) in: Transcription and Translation (Hamed, D. and Higgins, S.J., Eds.) pp. 271–302, IRL Press, Washington, DC.
[19] Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[20] Nakamura, Y., Chopra, I.J. and Solomon, D.H. (1977) J. Nucl. Med. 18, 1112–1115.
[21] Leonard, J.L. (1990) in: The Thyroid Gland (Greer, M.A., Ed.) pp. 285–305, Raven Press, New York.
[22] Osuna, C., Jimenez, J., Reiter, R.J., Rubio, A. and Guerrero, J.M. (1992) Am. J. Physiol. 263, E884–E889.
[23] Osuna, C., Rubio, A., Goberna, R. and Guerrero, J.M. (1990) Life Sci. 46, 1945–1951.
[24] Rubio, A., Guerrero, J.M., Gonzalez, M.A., Lopez-Gonzalez, M.A. and Osuna, C. (1991) Life Sci. 49, 1523–1530.