Renal and hepatic distribution of type I and type III iodothyronine deiodinase protein in chicken

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Abstract

Iodothyronine deiodinase in vitro activity studies in the chicken showed the presence of type I and type III iodothyronine deiodinase in both liver and kidney. Due to the lack of a specific antiserum the cellular localization of the deiodinase proteins could not be revealed until now. In the present study, specific antisera were used to study the renal and hepatic distribution of type I and type III iodothyronine deiodinase protein in the chicken. Immunocytochemical staining of liver tissue led to an immunopositive signal in the hepatocytes in general. Moreover, a zonal distribution could be detected for both enzymes. Maximum protein expression was shown in a thin layer of hepatocytes bordering the blood veins.

Although pericentral localization of type I deiodinase protein has been previously reported in the rat, no data were given concerning type III deiodinase protein. In the present study, we report the co-localization of both enzymes in the chicken. Co-expression of the deiodinases was also found in the kidney. Expression of both proteins was associated with the tubular epithelial cells and with the transitional epithelium, and the inner longitudinal and outer circular muscle layers of the ureter. No staining could be detected in the lamina propria or in the fat tissue surrounding the ureter.

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Introduction

In most vertebrates, the thyroid gland mainly secretes the relatively inactive prohormone, thyroxine. By means of a process called outer ring deiodination this prohormone is converted into its active form, namely 3,5,3’-tri-iodothyronine (T3). T3 can be degraded into an inactive hormone, 3,3’-di-iodothyronine, by means of inner ring deiodination (Visser 1990). The enzymes responsible for these reactions are the iodothyronine deiodinases. Three different deiodinases have been characterized, namely type I, type II and type III deiodinase. Type I deiodinase is able to exert both outer and inner ring deiodination, while type II and type III deiodinase only catalyse outer or inner ring deiodination respectively. Deiodinases are selenoproteins, containing the rare amino acid selenocysteine in their active site (St Germain & Galton 1997). The cloning of the first deiodinase cDNA in the rat by Berry et al. (1991) led to a very rapid revelation of deiodinase sequences in other species such as the human (Mandel et al. 1992, Salvatore et al. 1995, 1996), the mouse (Maia et al. 1995), the dog (Toyoda et al. 1995), the chicken (Van der Geyten et al. 1997, Gereben et al. 1999), tilapia (Lee et al. 1993, Sanders et al. 1997, 1999), Rana catesbeiana (Becker et al. 1995, Davey et al. 1995) and Xenopus laevis (St Germain et al. 1994).

Although the in vitro deiodinase activities as well as the tissue-related expression of the deiodinases have been studied extensively (Darras et al. 1992, Bates et al. 1999, Van der Geyten et al. 2002), little is known about the cellular distribution of the enzymes within a given tissue because of the lack of good antisera. Only within the past 2 years some reports become available on the use of antisera in iodothyronine deiodinase protein localization studies. Zandieh Doulabi et al. (2002) showed that, in the rat, hepatic type I deiodinase protein is associated with the hepatocytes that circumscribe the central blood veins. They found a co-localization of the enzyme with the thyroid hormone receptor isoform β1. Our group (Verhoelst et al. 2002) demonstrated the presence of type III deiodinase in the Purkinje cells of the chicken cerebellum. This was the first report on the presence of a deiodinase protein in a neuronal cell type. Just recently, Diano et al. (2003) described the localization of rat type II deiodinase immunolabelled cells in the hypothalamus. The strongest signal was found in the arcuate nucleus/median eminence and in the periventricular region of the third ventricle.

Since the few immunocytochemical data on iodothyronine deiodinase proteins are mostly limited to the brain, except for the study of Zandieh Doulabi et al. (2002), the present work was performed to reveal protein expression...
patterns in some peripheral tissues, namely the liver and the kidney, since these are known to contain relatively high deiodinase activities.

Materials and Methods

Polyclonal antisera

For the production of polyclonal antisera against type I (D1) and type III iodothyronine deiodinase (D3), specific synthetic peptides were chosen to consider the hydropathy profile of the entire chicken enzyme amino acid sequence: D1 peptide (NH₂-(235)YHPQEIRAVLEKLK(250)-COOH) and D3 peptide (NH₂-(41)TAGEGPPPDDP PV(53)-COOH). New Zealand White rabbits were injected every 3 weeks with the conjugates (D1 – keyhole limpet haemocyanine; D3 – bovine serum albumin). The antisera contained 150 000 c.p.m. [3'-125I]T3, 10 nM T3, 1 µM reverse T3, 50 µg protein/ml, 0·1 mM PTU 6-propyl-2-thiouracil and 50 mM dithiothreitol in sodium phosphate buffer (pH 7·2) and 2 mM EDTA. Reaction products were analysed by HPLC (Eelkman-Rooda et al. 1989). Incubation was stopped by the addition of 300 µl ice-cold methanol. After centrifugation (3500 g, 10 min), 200 µl supernatant aliquots were transferred to vials containing 250 µl ammonium acetate (0·02 M, pH 4·0). Labelled iodothyronines and I⁻ were separated by injection of 80 µl of this mixture onto a C18 column (Nucleosil 100–5 C18; Filter Service, Eupen, Belgium) and elution with a 47/53% (v/v) mixture of methanol and ammonium acetate buffer at a flow rate of 1 ml/min. Radioactivity was assessed with an on-line radioactivity monitor (LB 506 C-1; Perkin Elmer, Norwalk, CT, USA), and peaks were integrated using the winflow program (JBMS, Grenoble, France). All activities were calculated as the amount of substrate deiodinated/mg protein per min.

ECL Western analysis

Starting from 250 mg liver (saline- versus DEX-treated animals), hepatic membrane proteins were separated from the soluble protein fraction using the proteoprep universal extraction kit (Sigma). Protein concentrations were determined according to the method of Bradford (1976). Samples were diluted to a final concentration of 7 mg/ml in distilled water. Laemmli buffer was added in a 1:3 ratio. Ten microlitres of sample were loaded onto a 12.5% acrylamide/bisacrylamide gel. After electrophoresis, the gels were blotted onto a PVDF membrane. The D3 antiserum was added in a dilution of 1:100. The secondary antibody (GAR-HRP) was used in a dilution of 1:1000. Detection of the antiserum occurred using the ECL Western blotting detection system of Amersham.
Pharmacia Biotech. The blots were exposed to an autoradiographic Hyper ECL film and exposed for approximately 10 s.

All experimental procedures were approved by the Katholieke Universiteit Leuven ethical experimental animal committee.

Results

As shown in Fig. 1A hepatocytes were stained using the type I deiodinase antiserum. Although overall staining of the liver was found, a very strong signal could be detected in the epithelial cells around the hepatic blood vessels in general. Figure 1C represents one of the negative controls, namely incubation with preimmune serum. Clearly, no immunopositive cells were detected in the latter. For D3, the overall staining of the hepatocytes seemed to be much more intense. Therefore, the staining pattern around the blood veins was not as local as for D1, but was nevertheless present (Fig. 1B). The hepatic distribution for DEX-treated animals is shown in Fig. 1D. It can be seen that in this case D3 protein expression is restricted to the area circumscribing the blood vessels, comparable with D1 protein expression. D3 activity levels and Western blot analysis are represented in Fig. 2. DEX-treated chicken
embryos showed a significantly lower D3 activity level when compared with saline-injected animals (P<0.001). Furthermore, the D3 protein expression itself was decreased in these animals as was determined by Western analysis. The 30 kDa band representing the D3 enzyme showed a much lower intensity than that of control chicks.

Immunocytochemistry performed on kidney tissue revealed staining of the tubular epithelial cells for both D1 and D3 protein (Fig. 3A and B). Figure 3C represents a detail of the tubuli stained with anti-D3. Furthermore, a very clear positive result was found in the ureter with both antisera. Figure 3D and E represent the staining of the ureter for D1 and D3 respectively. In both cases, the transitional epithelium as well as the inner longitudinal and the outer circular muscle layer of the ureter were stained. No staining was found in the lamina propria or in the fat tissue surrounding the ureter. Figure 3F represents an overview of tissue slices incubated with preimmune serum. The negative control remained blank.
Discussion

In the present report we describe the co-localization of D1 and D3 protein in liver and kidney. Renal and hepatic D1 and D3 activity have been extensively studied in the past, but only a few data are available on the distribution of the deiodinase proteins in general. Bates et al. (1999) previously showed the co-expression of D3 mRNA, together with D1 and D2 mRNA in several rat tissues. The fact that the enzymes tend to be expressed in the same tissue suggests the need to tightly regulate the intracellular T3 levels within these tissues at certain critical stages in development. The co-expression that Bates et al. (1999) found was similar to earlier published results in tadpoles at the point of metamorphosis (Becker et al. 1995).

During the last week of embryonic development the chicken embryo displays a high hepatic D1 activity, whereas the D3 activity is very high until days 18–19 and decreases by 100-fold thereafter. These changes explain the strong increase in plasma T3 on E20, which is the point at which the embryo switches to lung respiration (Darras et al. 1992, Van der Geyten et al. 1997). The current study describes the presence of D1 and D3 protein in the hepatocytes in general with a maximal expression of the enzymes around the central blood veins, especially in the case of D1. For D3 deiodinase we suggest that, given the data on enzyme activity, the amount of protein is so high on day 18 of embryonic development that no difference can be seen at this stage between the hepatocytes in general and those surrounding the blood vessels. Zandieh Doulabi et al. (2002) recently reported on the presence of D1 protein in rat liver. They described a preferential pericentral expression of both the thyroid hormone receptor B1 and D1. The expression of both proteins decreased from the pericentral zone towards the periphery as found in the rat.

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The strong increase in plasma T3 on E20, which is the point of metamorphosis, suggests the need to tightly regulate the intracellular T3 levels. Given the local activation of the deiodinases is not only able to comply with the intracellular need for active thyroid hormone, but T3 can also be easily transported through the blood circulation. Furthermore, local action of active T3 is suggested, given the presence of thyroid hormone receptor pericentrally as found in the rat.

With regard to the kidney, Van der Geyten et al. (2002) recently reported the differential expression of iodothyronine deiodinases in chicken tissues during the last week of embryonic development. In kidney, D1 and D3 activity and mRNA are both present during the last week of development and they both gradually increase towards hatching. Taking into account the high expression of D1 in kidney, the authors suggest that it is very unlikely that D3, given its relatively low activity levels, plays a major role in the regulation of the renal T3 exported to plasma. However, considering the fact that D3 protein expression might be limited to a specific cell type, it might play a role in regulating the intracellular T3 levels that are optimal for tissue development. We have described the expression of D1 and D3 in renal tubuli and several layers of the ureter. Leonard et al. (1991) performed affinity labelling and catalytic activity studies to determine the regional distribution of D1 in rat kidney. They also found D1 activity in the tubular epithelial cells of the outer renal cortex. Lee et al. (1993) also reported the presence of D1 mRNA in the rat kidney tubuli. Given the fact that the source of the substrate for both D1 and D3 is the circulation, finding their distribution pattern in close proximity to blood reservoirs was expected. The presence of D1 and D3 in the urerter could be related to development. Thyroid hormones play a crucial role in the regulation of different developmental processes. They are important for growth in general, thermogenesis, and several metabolic and differentiation processes (Decuyper et al. 1990, Beckett & Arthur 1994). Therefore the presence of D1 and D3 in the urerter may be associated with the local provision of the essential levels of active thyroid hormone needed for development. Localization of the deiodinases in the tubuli, on the other hand, might rather be related to the distribution of the thyroid hormones towards the renal cells for appropriate differentiation of these cells at this stage.

Further comparative studies between embryonic and post-hatch chickens should be performed to confirm this.

In conclusion, this study showed the distribution of D1 and D3 deiodinase protein in the chicken liver and kidney. In accordance with previous results in the rat, D1 could be detected in hepatocytes in general and the expression pattern was most intense in the area surrounding the blood vessels. For D3, a very strong staining was found throughout the whole liver. Comparison of DEX-treated animals...
with control animals revealed that the D3 protein showed the same distribution as does D1 when it is downregulated. In the kidney, again co-localization of both enzymes was found in the epithelial cells of the renal tubuli and in several layers of the ureter. Comparison of the staining signals for both deiodinases strongly suggests that they are indeed expressed in the same cell type, although this may need additional confirmation.

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References

Bates JM, St Germain DL & Galton VA 1999 Expression profiles of the three iodothyronine deiodinases D1, D2 and D3, in the developing rat. Endocrinology 140 844–851.

Becker KB, Schneider MJ, Davey JC & Galton VA 1995 The type III 5'-deiodinase in Rana catesbeiana tadpoles is encoded by a thyroid hormone-responsive gene. Endocrinology 136 4424–4431.


Lee WS, Berry MJ, Hediger MA & Larsen PR. 1993 The type I iodothyronine 5'-deiodinase messenger ribonucleic acid is localized to the S3 segment of the rat kidney proximal tubule. Endocrinology 132 2136–2140.


St Germain DL & Galton VA 1997 The deiodinase family of selenoproteins. Thyroid 7 655–668.


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