GLIA 14:322–328 (1995)

Three Different Thyroid Hormone Receptor Isoforms Are Detected in a Pure Culture of Ovine Oligodendrocytes

JUAN FRANCISCO FIERRO-RENOY,[1,2] SARA SZUCHET,[3,4,5] MARIA FALCONE,[1,2] ENRICO MACCHIA,[1,2] AND LESLIE DEGROOT[1,2]
[1]*Thyroid Study Unit,* [2]*Department of Medicine,* [3]*Department of Neurology,* [4]*Committee on Neurobiology, and* [5]*Brain Research Institute, The University of Chicago, Chicago, Illinois 60637*

KEY WORDS Myelination, Central Nervous System, Hypothyroidism, Transcription Factors, Development

ABSTRACT Thyroid hormones are important for the normal development of the central nervous system. In humans, the period around the end of the intrauterine life and the first few months of neonatal life is critically dependent on the presence of normal amounts of thyroid hormone. There are significant events occurring during this time; myelination is one. Myelin is synthesized by oligodendrocytes. A panel of site-specific polyclonal antibodies against α-1 thyroid hormone receptor (TR), α-2 variant TR, and β-1 TR isoforms has been employed to investigate the presence of TR isoforms in a pure culture of ovine oligodendrocytes by the avidin-biotin peroxidase immunocytochemical method. Strong nuclear staining was obtained with all the anti-TR antibodies; no reaction products were detected in the cytoplasm or cellular processes. By contrast, an anti-myelin basic protein antibody gave strong cytoplasmic and process staining; no nuclear staining was seen. These latter results served to 1) confirm that the cells under study are oligodendrocytes; and 2) prove that the nuclear staining with anti-TR antibodies is specific. Preimmune sera were totally negative. Scatchard analysis of [^{125}I] T3 binding by isolated oligodendrocyte nuclei demonstrated the existence of high-affinity–low-capacity T3 binding sites with a K_a of $\approx 6 \times 10^{-9}$ M and a maximal binding capacity of $\approx$ 20 fmol/100 μg of DNA. Our results demonstrate that differentiated oligodendrocytes express α-1 and α-2 variant and β-1 isoforms of TR at the protein level and support the notion of a direct impact of thyroid hormones on oligodendrocytes in their regulation of myelin synthesis.

INTRODUCTION

Thyroid hormones are believed to act at the nuclear level, where they influence the transcriptional activity of responsive genes (DeGroot et al., 1989). This modulation is mediated by thyroid hormone receptors (TRs), which belong to a superfamily of ligand-dependent transcription factors (Evans, 1988). There are at least four isoforms of the receptor (Chin, 1992) encoded by two different genes, c-erb A α and β. Three of these isoforms are able to bind triiodothyronine (T3), but one of them, the α-2 variant, is not (Koenig et al., 1989). Interestingly, it was shown that the human variant α-2 can negatively regulate the activity of the other isoforms, i.e., the functional receptors; thus, this latter receptor effectively operates as a negative regulator of thyroid hormone action (Lazar et al., 1989). These receptor isoforms exhibit a differential tissue distribution and are distinctly regulated by T3 (Lazar, 1993).

Thyroid hormones are necessary for the normal development of the central nervous system (CNS); they seem also to be important for fetal brain development (Dussalult and Ruel, 1987). Several lines of evidence

Received February 6, 1995; accepted April 12, 1995.

Address reprint requests to Dr. Sara Szuchet, Department of Neurology, MC2030, The University of Chicago, 5841 S. Maryland Avenue, Chicago, IL 60637.

suggest that in humans, thyroid hormones are acutely required during the last weeks of intrauterine life and the first few months after birth (Fisher and Polk, 1989). During this period glial cell growth and myelination are among the important events that are taking place (Porterfield and Hendrich, 1993). Myelin is a multilamellar membrane structure that surrounds axons and facilitates nerve impulse conduction; it is synthesized, assembled, and maintained by oligodendrocytes (OLGs) (Lees and Brostoff, 1984). CNS myelination is sensitive to the presence of appropriate amounts of thyroid hormones (Legrand, 1984). The molecular basis of thyroid hormone regulation of myelin formation is a complex process that is only now beginning to be unraveled. Thyroid hormones appear to regulate the expression of several key enzymes involved in the synthesis of complex lipids and of the major protein constituents of the myelin membrane. Both transcriptional and post-transcriptional effects have been documented (Matthieu et al., 1990; Rodriguez-Peña et al., 1993; Tosic et al., 1992).

Considering the pivotal role of thyroid hormones in the assembly of myelin membranes, surprisingly little is known about the type and number of thyroid hormone receptors in OLGs. The availability of pure cultures of OLGs capable of in vitro synthesis of myelin structures—myelin palingenesis (Szuchet et al., 1986; Yim et al., 1986)—affords an opportunity to address this issue. In the present study, we have utilized pure primary cultures of OLGs in conjunction with a panel of antibodies (Abs) directed specifically against each of the TR isoforms to establish the type and number of TRs in these cells. Our results confirm the presence of TRs in OLGs and show that each TR isoform is expressed at the protein level, thus giving additional support to the concept that TH effects upon myelination occur through their interaction with OLG-specific genes.

MATERIALS AND METHODS

Isolation and Culture of Oligodendrocytes

OLGs were isolated from 3- to 6-month-old lamb brains by a previously described procedure (Szuchet et al., 1980). For culture, OLGs were plated at a density of $\approx 3.0 \times 10^6$ cells/ml and kept at 37°C and 5% CO_2 according to our standard procedure (Szuchet and Yim, 1984). For binding studies, cells were grown on tissue culture plates to which they did not adhere and were used after 3 days. For immunocytochemistry, non-adhered OLGs were transferred onto polylysine-coated coverslips on which they adhered and with time developed an extensive network of processes. OLGs were examined after 2–3 weeks in culture.

Immunocytochemistry

Cells attached to polylysine-coated coverslips were washed with phosphate-buffered saline (PBS), 10 mM, pH 7.4, fixed with cold 4% paraformaldehyde for 10 min and washed with PBS. Cells were then incubated with PBS containing 1% bovine serum albumin (BSA), 10% normal goat serum, and 0.05% Triton X-100 for 45 min followed by incubation for 2 h with the specific antiserum, at a 1:200 dilution, made in PBS with 0.1% BSA and 0.05% Triton X-100. The coverslips were washed for 1 h by immersion in a beaker containing 50 ml of PBS buffer, OLGs were incubated with a biotinylated goat anti-rabbit immunoglobulin for 1 h, washed by immersion, and incubated wtih an avidin-horseradish peroxidase complex for 45 min and washed again for 1 h. Color was developed with 0.02% H_2O_2 and 0.1% diaminobenzidine tetrahydrochloride in PBS. The coverslips were left to dry at room temperature and mounted with Permount (Fisher Scientific Co).

Antibodies

A panel of site-specific Abs against several TR isoforms was used. Antibodies were raised in rabbits against peptide sequences taken from nonidentical areas of the proteins. The following antibodies were employed (corresponding position of the peptide in the receptor protein is stated in parentheses): α-1 403 Ab (403–410); α-2 431 Ab (431–451); β-62 Ab (62–81); and α-144 Ab (144–162 in α-1 TR). The first three antibodies are specific for α-1, α-2, and β-1 TR, respectively. α-144 Ab recognizes a peptide sequence common to α-1 and hTR variant α-2. These Abs have been characterized and studies showing their specificity have been published (Falcone et al., 1992; Macchia et al., 1990). The Abs react in enzyme-linked immunosorbent assays (ELISA) against the immunizing peptide or the whole recombinant protein; this reactivity is blocked by either form of the antigen. Western blot experiments using the rTR as antigen showed that each Ab uniquely detected its antigen without cross-reactivity. Binding of the Ab in Western blots was prevented when the antiserum was blocked with excess amounts of the specific TR protein but not with a nonspecific TR. α-1 403 and α-144 antibodies have been used successfully to immunoprecipitate [^{125}I] α-1 TR at a $1{:}10^6$ and $1{:}5 \times 10^5$ dilution, respectively. β-62 Ab is able to precipitate a [^{125}I]-labeled β 62–81 peptide (data not shown). These Abs were also able to recognize and to retard the complex formed by the specific protein bound to a specific DNA probe (TRE) in a gel mobility shift assay (Falcone et al., 1992). Normal preimmune rabbit serum was used as a negative control at a 1:200 dilution. For a positive control, an Ab against the 18,500 isoform of myelin basic protein (MBP) (Sternberger et al., 1985) was used at a dilution of 1:100.

Binding Assays

L-[^{125}I]T3 binding studies were carried out on isolated nuclei by a modification of a described procedure (Samuels and Tsai, 1973). Three-day-old non-adhered OLGs were harvested and centrifuged at 500*g* for 5

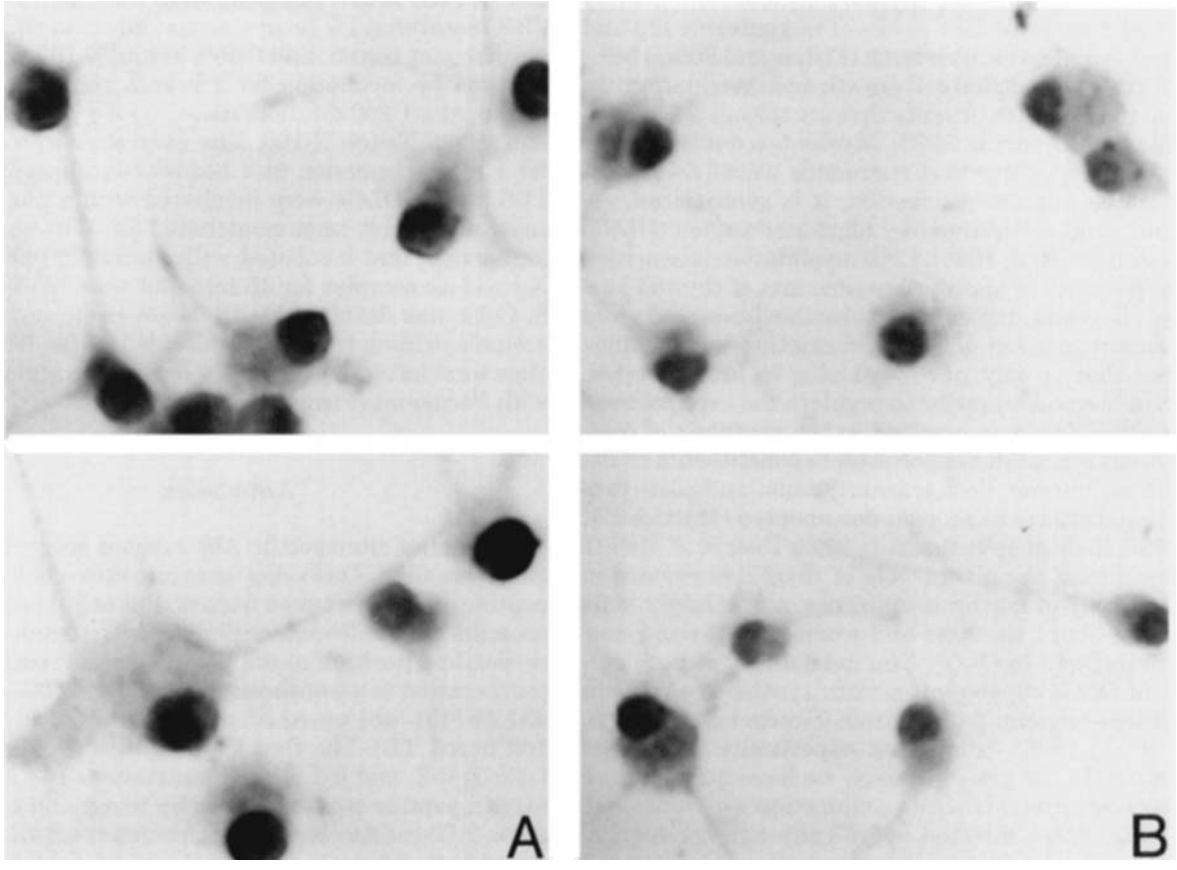

Fig. 1. Immunocytochemical staining of cultured oligodendrocytes with site-specific anti-thyroid hormone receptor antibodies. Pure cultures of oligodendrocytes after 2–3 weeks in vitro were exposed to antibodies raised against specific peptide sequences of the thyroid hormone receptor isoforms. Staining was detected by the avidin-biotin peroxidase method. For details see Materials and Methods. A: Oligodendrocytes stained wtih anti-α-1 403 antibody. B: Oligodendrocytes stained with anti-β-1 62 antibody. Notice the strong nuclear staining with both antibodies. Cytoplasm and cellular processes reveal no reactivity.

min. The cell pellet was washed twice with serum-free Ham's F-10, suspended in STM-Triton buffer (0.25 M sucrose, 20 mM Tris.Cl, pH 7.4, 1 mM $MgCl_2$, 0.5% Triton X-100), and centrifuged; the nuclear pellet was resuspended in STM buffer. Scatchard analysis of T3 binding was performed after incubating nuclei ($\sim 10^6$ nuclei/ml) with increasing amounts of [^{125}I]T3 (SA 2200 Ci/mmol, Dupont) for 2 h at 37°C. Nonspecific binding was estimated from reaction mixtures containing a 200-fold molar excess of nonradioactive T3. DNA in the nuclear pellet was measured by the method of Burton (1956).

RESULTS

A well-characterized pure culture of ovine OLGs was used to document the presence of several isoforms of TR employing a panel of site-specific polyclonal Abs. The characterization of these cells as OLGs has been described in detail (Massa et al., 1984; Szuchet and Stefansson, 1980; Wollmann et al., 1981). More than 98% of these cells can be classified as OLGs by current immunological, morphological, and biochemical criteria. Adherence of these OLGs to a substratum transduces a signal that turns on a myelinogenic metabolism (Szuchet et al., 1983; Yim et al., 1986) and over time in culture, the cells assemble multilamellar membranes with the ultrastructure and biochemical characteristics of myelin (Szuchet et al., 1986; Szuchet, 1987).

Treatment of cultured OLGs at various time points with Abs α-1-403 and β-1-62 targeted, respectively, against peptides of α-1 TR and β-1 TR revealed strong nuclear staining, little to no cytoplasmic staining, and virtually no reaction products were seen in the cellular processes (Fig. 1). Similar patterns of reactivity were obtained with Ab α-2-431, which recognizes α-2 variant hTR (Fig. 2A) and Ab α-1-144, which identifies epitopes on both α-1 TR and α-2 variant hTR (Fig. 2B). Of these TR isoforms only α-2 variant hTR does not belong to the ligand-binding class; all the others are ligand-binding receptors. Taken together, these results indicate that: 1) OLGs have several TR isoforms; 2) all receptors are translated as proteins; and 3) the extent of staining seems to be independent of the function of the receptor, e.g., whether or not it binds ligand. Staining with these

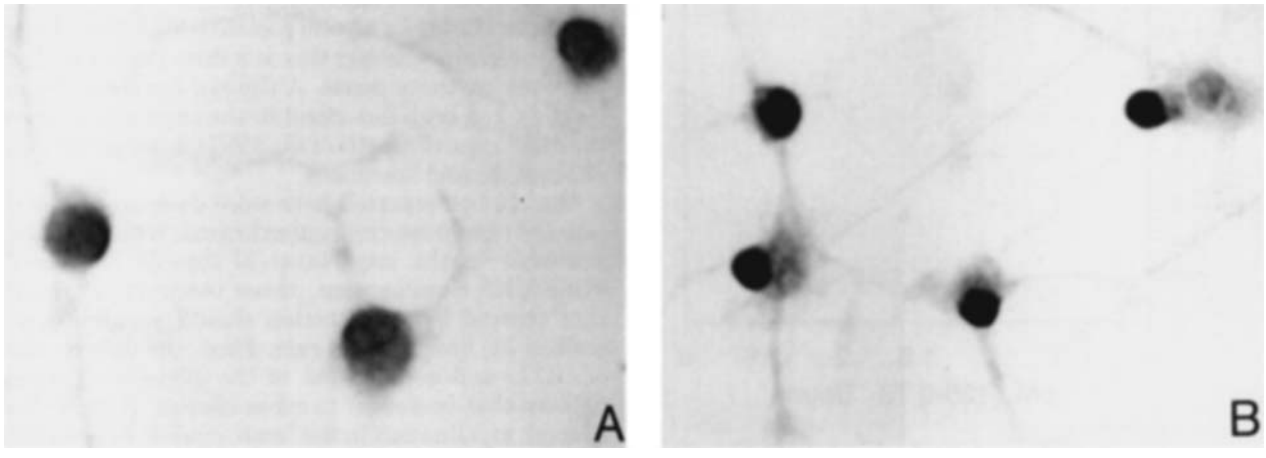

Fig. 2. Immunocytochemical staining of cultured oligodendrocytes with site-specific anti-thyroid hormone receptor antibodies. A: Staining wtih anti α-2 431 antibody. B: Staining with anti-α 144 antibody, which recognizes an epitope common to α-1 and the human variant α-2. The patterns of staining are very similar to those illustrated in Figure. 1. The intensity of staining is also comparable.

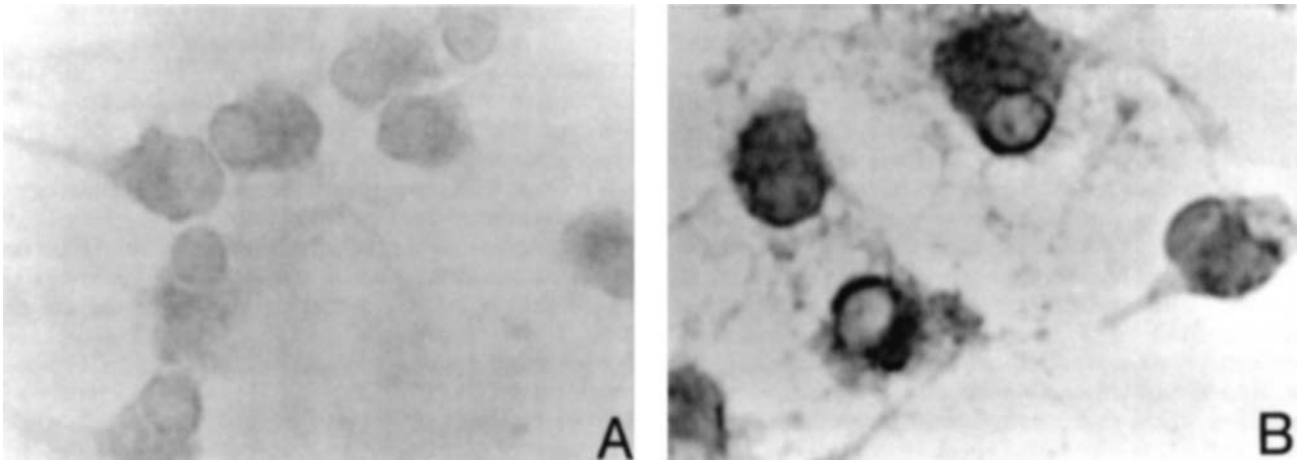

Fig. 3. A: Immunocytochemical staining of cultured oligodenrocytes with control sera. Representative photomicrograph of oligodendrocytes stained with a preimmune serum. No reaction was detected. B: Immunocytochemical staining of cultured oligodendrocytes with an anti-myelin basic protein antibody. Notice that the staining is confined to the cytoplasm, plasmalemma, and processes. This is in sharp contrast to Figures 1 and 2. These results fulfill two functions: 1) they attest to the oligodendrocytic nature of the cells under study; and 2) they confirm that the nuclear staining obtained with the anti-thyroid hormone receptor antibodies is specific.

Abs brings out the characteristic morphology of OLGs with their eccentric nuclei.

Controls in which the antisera were replaced by preimmune sera proved totally negative (Fig. 3A). As an additional control OLGs were reacted with an anti-MBP serum, a specific marker for OLGs and one of the major myelin proteins. As illustrated in Figure 3B, the cells stained intensely with this Ab; however, here the staining was confined to the cytoplasm, plasmalemma, and cellular processes.

Ligand binding studies performed on 3-day-old cultures of nonattached OLGs demonstrated the existence of high-affinity–low-capacity T3 binding sites in the nuclei of the cells. Scatchard plot analysis of binding by isolated OLGs nuclei, done in duplicate, and in four different experiments, revealed the existence of nuclear binding sites with a K_a for T3 of approximately 6 nM with a maximal binding capacity of $\approx$ 20 fmol/100 μg of DNA. Figure 4 presents a typical Scatchard plot. Having shown that upon adhesion to a substratum, these OLGs reenact the ontogenic development of myelin, it would be of interest to determine whether there is a change in the number and/or affinity of T3 binding sites in the adhered cells. Such work is in progress.

DISCUSSION

We have utilized a panel of site-specific Abs that recognize individual TR isoforms in conjunction with well-

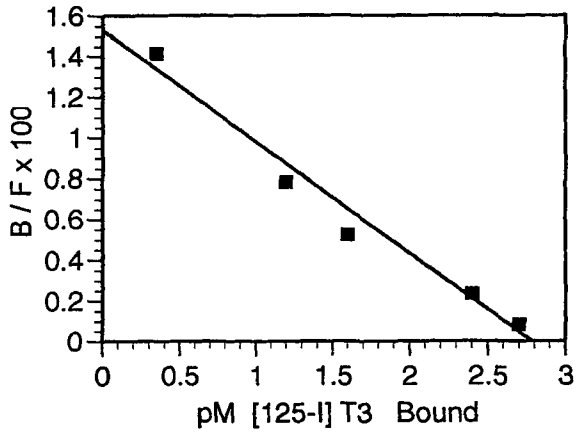

Fig. 4. Scatchard plot. Isolated oligodendrocyte nuclei were treated with increasing concentrations of [^{125}I]T3 for 2 h at 37°C and the data were subjected to Scatchard analysis. The result revealed the existence of high-affinity–low-capacity T3 binding sites with a K_a of 6×10^{-9} M and a maximal binding of 20 fmol/100 μg of DNA. B/F: bound/free ligand.

characterized pure cultures of OLGs to define the types and properties of TR present in OLGs. In this communication we present evidence for the presence of α-1 TR, α-2 TR, and β-1 TR in OLGs. These receptors are expressed as proteins and are localized in the nucleus. Little to no staining was detected in the cytoplasm or cellular processes. Because these receptors are present in OLGs that have been in culture for 2–3 weeks, a time when the cells have developed an extensive network of processes and begun to assemble myelin membranes, these results have interesting implications. First, they reaffirm previous suggestions that OLGs are targets for thyroid hormone action. Second, they indicate that the effect of thyroid hormones on myelination might occur at the transcriptional level.

An important impact of thyroid hormone on brain development and maturation in humans and in rodents occurs during the perinatal period and during the first few months of postnatal life. In the rat, hypothyroidism induces detectable divergence from normal brain development in the first 2 weeks postnatally (Schwartz, 1983). The normal brain development achieved by infants with congenital hypothyroidism when treated soon after birth with an appropriate dose of thyroid hormone, together with the irreversible damage that occurs if the replacement starts later than the first few months after birth, suggests that this is a critical period in terms of thyroid hormone requirements (Fisher and Polk, 1989).

During this critical postnatal period, cerebral neurogenesis is essentially complete in both humans and rats (Zamenhof and VanMarthens, 1971). This is, however, an important period for glial cell growth and neuronal myelination as well as neuronal maturation and synapse formation (Porterfield and Hendrich, 1993). These events, particularly myelination, are known to be sensitive to the presence of thyroid hormones. Myelin is synthesized by differentiated OLGs. Neonatal hypothyroidism in the rat reduces the accumulation of mRNAs coding for the major myelin proteins (Muñoz et al., 1991). It is uncertain whether this is a direct effect of thyroid hormone on these genes. A thyroid hormone response element has been described in the regulatory region of the MBP gene (Farsetti et al., 1991), supporting a direct effect of thyroid hormones.

The studies reported here were done on ovine OLGs isolated from postmyelination brains. While no data are available on the importance of thyroid hormones on ovine CNS development, three observations indicate that thyroid hormone action should parallel that described for humans and rats. First, the differentiation of OLGs and myelination of the ovine fetus follow a pattern that is similar to other species (Barlow, 1969). Second, myelination in the lamb appears to be biphasic, with maxima occurring pre- and post-natally (Patterson et al., 1971). Third, though we isolate OLGs from post-myelination brains, the cells regenerate in vitro, reenacting the ontogenic development of myelin (Yim et al., 1986).

A key development in the understanding of thyroid hormone action at the nuclear level has been the discovery of the existence of several TR isoforms. At least four different forms have been described (Chin, 1992), and they appear to have a differential tissue distribution and expression during development (Lazar, 1993). The reasons for this diversity of TRs are not clear, but it may well be that they have different roles in gene regulation. Interestingly, a recent observation suggests that the differentiation of glial progenitor cells into OLGs or astrocytes is accompanied by a loss of β-1 TR expression in cells that acquired the astrocyte phenotype (Farwell et al., 1993). However, the in situ significance of this observation is questionable. A preferential β-1 TR transactivation of the MBP-thyroid response element has also been suggested (Farsetti et al., 1992).

In view of the importance of thyroid hormones on myelination, the conclusive demonstration of thyroid hormone receptors in OLGs is of relevance. Observations made on nuclei isolated by gradient centrifugation from brains of several species, in which OLG nuclei were identified only by morphology, have given conflicting results (Gullo et al., 1987; Haidar et al., 1983; Yokota et al., 1986). This is in part because of the difficulty of ascertaining the exact origin of the nuclei being investigated. Some studies found no evidence for T3 receptors in these cells, implying that OLGs were not a target for thyroid hormones (Kolodny et al., 1985; Ruel et al., 1985).

Primary cultures of glial cells from several species have also been used with relative success (Luo et al., 1986; Pascual et al., 1986). The main problem with these systems has been that they are usually composed mainly of astrocytes and thus it is difficult to derive information specifically related to OLGs (Sarlieve et al., 1989). The first unequivocal report of the existence of nuclear T3 receptors in this type of cell was made in a pure secondary culture of OLGs (Yusta et al., 1988). This study has shown that OLGs contain a number of

T3 binding sites per cell approximately similar to that observed in neurons. In addition, these authors have shown that these receptors are less abundant in astrocytes.

We considered it of interest to know whether the different TR isoforms are expressed in OLGs. Here we report that OLGs expressed α-1 TR, α-2 TR, and β-1 TR isoforms. The role played by each of these isoforms in the process of OLG differentiation and myelination remains a subject for further research.

ACKNOWLEDGMENTS

We thank Ms. G. Deibler from the National Institute of Mental Health for providing us with the anti-MBP antibody. The expert assistance of Ms. Michelle Losekamp and Mr. Paul Polak is gratefully acknowledged. The work was supported by United States Public Health grants DK 13377 and DK 27384, The March of Dimes Foundation, the David Wiener Research Fund, and in part by NIH grant PO1-NS24575-06(SS).

REFERENCES

Barlow, R.M. (1969) The foetal sheep: Morphogenesis of the nervous system and histochemical aspects of myelination. *J. Comp. Neurol.*, 135:249–262.

Burton, K. (1956) A study on the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxiribonucleic acids. *Biochemistry*, 62:315–323.

Chin, W.W. (1992) Current concepts of thyroid hormone action. *Thyroid Today*, 3:1–9.

DeGroot, L.J., Nakai, A., Sakurai, A., and Macchia, E. (1989) The molecular basis of thyroid hormone action. *J. Endocrinol. Investi.*, 12:843–861.

Dussault, J.H. and Ruel, J. (1987) Thyroid hormones and brain development. *Annu. Rev. Physiol.*, 49:321–334.

Evans, R. (1988) The steroid and thyroid hormone receptor superfamily. *Science*, 140:889–895.

Falcone, M., Miyamoto, T., Fierro-Renoy, F., Macchia, E., and DeGroot, L.J. (1992) Antipeptide polyclonal antibodies specifically recognize each human thyroid hormone receptor isoform. *Endocrinology*, 131:2419–2429.

Farsetti, A., Mitsuhashi, T., Desvergne, B., Robbins, J., and Nikodem, V.M. (1991) Molecular basis of thyroid hormone regulation of myelin basic protein gene expression in rodent brain. *J. Biol. Chem.*, 266: 23226–23232.

Farsetti, A., Desvergne, B., Hallenbeck, P., Robbins, J., and Nikodem, V.M. (1992) Characterization of myelin basic protein thyroid hormone response element and its function in the context of native and heterologous promoter. *J. Biol. Chem.*, 267:15784–15788.

Farwell, A.P., Trauter, M.P., and Leonard, J.L. (1993) Introduction of the functional thyroid hormone receptor (beta-1 TR) into rat C6 astrocytomas cells results in the expression of both oligodendrocytes and neural cell proteins. In; *75th Annual Meeting of The Endocrine Society*, p. 531 (Abstract).

Fisher, D.A. and Polk, D. (1989) Maturation of thyroid hormone actions. In: *Research in Congenital Hypothyroidism*. F.D. Delange and D. Gilnoer, eds. Plenum Press, New York, pp. 61–77.

Gullo, D., Sinha, A.K., Woods, R., Pervin, K., and Ekins, R.P. (1987) T3 binding in adult rat brain: Compartmentation of receptor populations in purified neuronal and glial nuclei. *Endocrinology*, 120:325–331.

Haidar, M.A., Dube, S., and Sarkar, P.K. (1983) Thyroid hormone receptor of developing chick brain are predominately in neurons. *Biochem. Biophys. Res. Commun.*, 112:221–227.

Koenig, R.J., Lazar, M.A., Hodin, R.A., Brent, G.A., Larsen, P.R., Chin, W.W., and Moore, D.D. (1989) Inhibition of thyroid hormone action by a non-hormone binding c-erbA protein generated by alternative splicing. *Nature*, 337:659–661.

Kolodyln, J.M., Larsen, P.R., and Silva, J.E. (1985) In vitro T3 binding to rat cerebrocortical neuronal and glial cells suggest the presence of binding sites unavailable in vivo. *Endocrinology*, 116:2019–2028.

Lazar, M. (1993) Thyroid hormone receptors: Multiple forms, multiple possibilities. *Endocrinol. Rev.*, 14:184–193.

Lazar, M.A., Hodin, R.A., and Chin, W.W. (1989) Human carboxyl-terminal variant of alpha type c-erbA inhibits transaction by thyroid hormone receptor without binding thyroid hormone. *Proc. Natl. Acad. Sci. USA*, 86:7771–7774.

Lees, M.B. and Brostoff, S.W. (1984) Proteins of Myelin In: *Myelin*. P. Morell, ed. Plenum Publishing Corp., New York, pp. 197–224.

Legrand, J. (1984) Effect of thryoid hormones on central nervous system. In: *Neurobehavioral Teratology*. J. Yamai, ed. Elsevier, Amsterdam, pp. 331–362.

Luo, M., Faure, R., and Dussault, J.H. (1986) Ontogenesis of nuclear T3 receptors in primary cultured astrocytes and neurons. *Brain Res.*, 381:275–280.

Macchia, E., Nakai, A., Janiga, A., Sukurai, A., Fisfalen, M.E., Gardner, P., Soltani, K., and DeGroot, L.J. (1990) Characterization of site-specific polyclonal antibodies to c-erbA peptides recognizing human thyroid hormone receptors alpha-1, alpha-2 and beta and study of tissue distribution of the antigen. *Endocrinololy*, 126:3232–3239.

Massa, P.T., Szuchet, S., and Mugnaini, E. (1984) Cell-cell interactions of isolated and cultured oligodendrocytes: Formation of linear occluding junctions and expression of peculiar intramembrane particles. *J. Neurosci.*, 4:3128–3139.

Matthieu, J.M., Roch, J.M., Torch, S., Tosic, M., Carpano, P., Insirello, L., Stella, A.M.G., and Honegger, P. (1990) Triiodothyronine increase the stability of myelin basic protein mRNA in aggregating brain cell cultues. In: *Regulation of Gene Expression in the Nervous System*. G. Stella, A.M. de Vellis, and J.R. Perez-Polo, eds. Wiley-Liss, New York, pp. 109–121.

Muñoz, A., Rodriguez-Peña, A., Castillo-Perez, A., et al. (1991) Effects of neonatal hypothyroidism on rat brain gene expression. *Mol. Endocrinol.*, 5:273–280.

Pascual, A., Aranda, A., Fettet-Sena, V., Gabellec, M.M., Rebel, G., and Sarlieve, L.L. (1986) T3 receptor in developing mouse neuronal and glial cell cultures and in check-cultured neurons and astrocytes. *Dev. Neurosci.*, 8:89–101.

Patterson, D.S.P., Sweasey, D., and Herbert, C.N. (1971) Changes occurring in the chemical composition of the central nervous system during foetal and post-natal development of the sheep. *J. Neurochem.*, 18:2027–2040.

Porterfield, S.P. and Hendrich, C.E. (1993) The role of thyroid hormones in perinatal and neonatal neurological development. *Endocrinol. Rev.*, 14:99–106.

Rodriguez-Peña, A., Ibarrola, N., Iniguez, M., Muñoz, A., and Bernal, J. (1993) Neonatal hypothyroidism affects the timely expression of myelin-associated glycoprotein in the rat brain. *J. Clin. Invest.*, 91: 812–818.

Ruel, J., Faure, R., and Dussault, J.H. (1985) Regional distribution of nuclear T3 receptors in rat brain and evidence for preferential localization in neurons. *J. Endocrinol. Invest.*, 8:343–348.

Samuels, H.H. and Tsai, T.R. (1973) Thyroid hormone action in cell culture: Demonstration of nuclear receptors in intact cells and isolated nuclei. *Proc. Natl. Acad. Sci. USA*, 70:3488–3492.

Sarlieve, L.L., Besnard, F., Labourdette, G., Yusta, B., Pascual, A., Aranda, A., Luo, M., Puymirat, J., and Dussault, J.H. (1989) Investigation of myelinogenesis in vitro: Transient expression of T3 nuclear receptors in secondary cultures of pure rat oligodendrocytes. In: *Iodine and the Brain*. R. DeLong, J., Robbins, and P. Condilife, eds. Plenum Press, New York, pp. 113–139.

Schwartz, H.L. (1983) Effects of thyroid hormone in growth and development. In: *Molecular Basis of Thyroid Hormone Action*. J.H. Oppenheminer and H.H. Samuels, eds. Academic Press, New York, pp. 413–440.

Sternberger, N.H., del Cerro, C., Kies, M.W., and Herndon, R.M. (1985) Immunocytochemistry of myelin basic proteins in adult rat oligodendroglia. *J. Neuroimmunol.*, 7:355–363.

Szuchet, S. (1987) Myelin palingenesis: The reformation of myelin by mature ligodendrocytes in the absence of neurons. In: *Glial-Neuronal Communication in Development and Regeneration*. H.H. Althaus and W. Seifert, eds. Springer-Verlag, Berlin, pp. 755–777.

Szuchet, S. and Stefansson, K. (1980) In vitro behavior of isolated oligodendrocytes. In: *Advances in Cellular Neurobiology*. S. Federoff and L. Hertz, eds. Academic Press, New York, pp. 313–346.

Szuchet, S., Arnason, B.G.W., and Polak, P. (1980) Separation of ovine oligodendrocytes into two distinct bands on a linear sucrose gradient. *J. Neurosci. Methods*, 3:7–19.

Szuchet, S., Yim, S.H., and Monsma, S. (1983) Lipid metabolism of isolated oligodendrocytes maintained in long-term culture mimics

events associated wtih myelinogenesis. *Proc. Natl. Acad. Sci. USA*, 80:7019–7023.

Szuchet, S., Polak, P.E., and Yim, S.H. (1986) Mature oligodendrocytes cultured in the absence of neurons recapitulate the ontogenic development of myelin. *Dev. Neurosci.*, 8:208–221.

Tosic, M., Torch, S., Comte, V., Dolive, M., Honegger, P., and Matthieu, J.M. (1992) T3 has diverse and multiple stimulating effects on expression of the major myelin protein genes. *J. Neurochem.*, 59: 1770–1777.

Wollmann, R.L., Szuchet, S., Barlow, J., and Jerkovic, M. (1981) Ultrastructural changes accompanying the growth of isolated oligodendrocytes. *J. Neurosci. Res.*, 6:757–769.

Yim, S.H., Szuchet, S., and Polak, P.E. (1986) Cultured oligodendrocytes: A role for cell-substratum interaction in phenotypic expression. *J. Biol. Chem.*, 261:11808–11815.

Yokata, T., Nakamura, H., Akamizu, T., Mori, T., and Imura, H. (1986) Thyroid hormone receptor in neuronal and glial nuclei from mature rat brain. *Endocrinology*. 118:1770–1776.

Yusta, B., Besnard, F., Ortiz-Caro, J., Pascual, A., Aranda, A., and Sarlieve, L. (1988) Evidence for the presence of nuclear, 3,5,3′-triiodothyronine receptors in secondary cultures of pure rat oligodendrocytes. *Endrocrinology*, 122:2278–2284.

Zamenhof, S. and VanMarthens, E. (1971): Hormonal and nutritional aspects of prenatal brain development. In: *Cellular Aspects of Neuronal Growth and Differentiation*. D.C. Pease, ed. University of California Press, Berkeley, pp. 329–355.