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BRAIN-SPECIFIC PROSTAGLANDIN D2 SYNTHETASE mRNA IS DEPENDENT ON THYROID HORMONE DURING RAT BRAIN DEVELOPMENT

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Summary: We have previously described several cDNA clones whose expression is affected by thyroid hormone during rat brain development. We now report the identification of one of these, the E2 clone, as the brain-specific prostaglandin D_2 (PGD_2) synthetase gene. Sequence comparison shows a nearly complete identity between the 356 nucleotides of the E2 clone and nucleotides 403 to 759 of PGD_2 synthetase cDNA. The pattern of E2 expression corresponds to that expected for brain specific PGD_2 synthetase gene, i.e. the corresponding mRNA is not detected in any other tissue analyzed apart of the brain, and it was present at different levels in all brain regions. Hypothyroidism decreased E2 mRNA concentrations in cerebral cortex and cerebellum. Control of the level of expression of PGD_2 synthetase gene may contribute the complex effects of thyroid hormone on brain development and function.

Thyroid hormones are essential for normal mammalian brain development. In humans, lack of adequate levels of thyroid hormones may lead to severe and irreversible mental deficiency, and other neurological abnormalities (1). In experimental animals such as the rat the deleterious effects of thyroid hormone deprivation during the fetal and neonatal periods on brain maturation have also been extensively documented (2). The action of thyroid hormone is exerted through the interaction of triiodothyronine (T3) with nuclear receptors identified as different isoforms of the c-erbA proto-oncogene, the cellular homologue of the viral v-erbA oncogene (3, 4). T3 receptors, as other members of the nuclear receptor superfamily, which includes retinoid and steroid hormone receptors function as ligand-dependent transcription factors. Since T3 receptor isoforms are specially abundant in

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most regions of the brain (5,6), it would be expected that the actions of thyroid hormone on brain development are due to the controlled expression of a large set of brain genes. Despite this, only a few genes have been shown to be dependent on thyroid hormone at the pretranslational level. Using neonatally hypothyroid rats as a paradigm, we performed a systematic search for thyroid hormone regulated mRNAs employing subtractive and differential hybridization techniques (7). Among the mRNA sequences found to be altered by hypothyroidism the identity of one of them, E2 remained unknown. E2 mRNA (0.75 kb) expression was lowered by hypothyroidism in whole cerebrum of 15-day old rats. Sequencing of the available E2 cDNA clone has now allowed its identification as the brain-specific prostaglandin D_2 synthetase gene (8). This enzyme is involved in the synthesis of prostaglandin D_2 from its precursor, prostaglandin H_2 and it is believed to play an important role in the regulation of body temperature, sleep-wake cycle control, sensitivity to pain, neurotransmitter release, and other functions of the central nervous system (9-12). Our results raise the question of whether thyroid hormone contributes to the modulation of these physiological processes through the controlled expression of the PGD_2 synthetase gene.

Materials and Methods

Preparation of hypothyroid animals : Wistar rats raised in our animal facilities were used. The rules of the European Community concerning maintenance and handling of animals were followed. Hypothyroidism was induced by a combination of chemical and surgical thyroidectomies, as previously described (7). 0.02% methyl-mercapto-imidazol (MMI) was administered in the drinking water to the dams from the 9th day after conception. In addition to the MMI treatment, the newborns were surgically thyroidectomized when they were 5 days old. This protocol results in profound hypothyroidism, as shown by very low thyroid hormone concentrations in the brain, and by obvious physiological landmarks of hypothyroidism.

Northern blots: For RNA analysis, animals were killed at different times during the first month of life (as indicated) and the brain and other tissues were quickly removed and cool down on ice. Different brain regions were dissected out and frozen on liquid nitrogen. Total RNA was prepared using the guanidinium isothiocyanate-phenol-chloroform procedure (13). Northern blots were performed on nylon membranes (Nytran, Schleicher & Schuell, Inc., Keene, NH) according to standard protocols. As controls for the amount and integrity of RNA present on the filters, the blots were stained for ribosomal RNAs (14) in a 0.02% methylene blue solution made in 0.3M sodium acetate and rehybridized with a cyclophilin cDNA probe (15). Cyclophilin mRNA is constant during postnatal development and it is not influenced by thyroid hormone (7). Specific probes were labeled by the random priming method with [^{32}P]dCTP (Amersham, England) to specific activities of around 10^8 cpm/µg DNA. Hybridizations were carried out overnight at 65ºC in 7% SDS, 500 mM sodium phosphate buffer, pH 7.2 and 1 mM EDTA, according to the method of Church and Gilbert (16). Filters were washed twice for 30 min each in 1% SDS, 40 mM sodium phosphate buffer, pH 7.2 at 65ºC. Before

rehybridizing the membranes with probes for others genes, the radioactive probe was stripped off the membrane by placing it in a 75ºC water bath for 5 min.

DNA sequencing: E2 nucleotide sequence was obtained after double-stranded DNA sequencing according to the method of Sanger et al. (17) using commercial kits (Sequenase 2.0, United States Biochemical, Cleveland, Ohio).

Results and Discussion

Previous work in our laboratory led to the isolation of E2 as a brain cDNA corresponding to a mRNA whose concentration in whole cerebrum was altered in hypothyroid animals on neonatal day 15 (7). The E2 clone was isolated from a normal rat brain cDNA library probed with a normal rat brain cDNA probe subtracted with an excess cDNA insert from a hypothyroid brain library using the phenol-enhanced rehybridization technique described by Travis and Sutcliffe (18). The size of E2 cDNA was about 350 bp and hybridized to a 0.75 kb brain mRNA. The E2 cDNA sequence was obtained

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RPGDs- CCTCAGGCTCAGACACCTGCTCTACTCCAAGCAAATGGCTGCTCTTCCAATGCTGTGGAC - 60
RPGDs- CGGGCTGGTCCTCTTGGGTCTCTTGGGATTTCCACAGACCCCAGCCCAGGGCCATGACAC -120
RPGDs- AGTGCAGCCCAACTTTCAACAAGACAAGTTCCTGGGGCGCTGGTACAGCGCGGGCCTCGC -180
RPGDs- CTCCAATTCAAGCTGGTTCCGGGAGAAGAAAGAGCTACTGTTTATGTGCCAGACAGTGGT -240
RPGDs- AGCTCCCTCCACAGAAGGCGGCCTCAACCTCACCTCTACCTTCCTAAGGAAAAACCAGTG -300
RPGDs- TGAGACCAAGGTGATGGTACTGCAGCCGGCAGGGGTTCCCGGACAGTACACCTACAACAG -360
RPGDs- CCCCCACTGGGGCAGCTTCCACTCCCTCTCAGTGGTAGAAACCGACTACGATGAGTACGC -420
                                                  ************** *
                                                  GACTACGATGAGTANGN - 17

RPGDs- GTTCCTGTTCAGCAAGGGCACCAAGGGCCCAGGCCAGGACTTCCGCATGGCCACCCTCTA -480
       ************ ***** ** **** ************** **************
E2     - GTTCCTGTTCAGNAAGGGNACNAAGGCCCCAGGCCAGGACTTCGGCATGGCCACCCTCTA - 77

RPGDs- CAGCAGAGCCCAGCTTCTGAAGGAGGAACTGAAGGAGAAATTCATCACCTTTAGCAAGGA -540
       ************************************************************
E2     - CAGCAGAGCCCAGCTTCTGAAGGAGGAACTGAAGGAGAAATTCATCACCTTTAGCAAGGA -137

RPGDs- CCAGGGCCTCACAGAGGAGGACATTGTTTTCCTGCCCCAACCGGATAAGTGCATTCAAGA -600
       ************************************************************
E2     - CCAGGGCCTCACAGAGGAGGACATTGTTTTCCTGCCCCAACCGGATAAGTGCATTCAAGA -197

RPGDs- GTAAACACAGGTGAGAGAAGTCAGTCACAGGTAACACATGGTGATGTGGCCTCAGGACTC -660
       ************************************************************
E2     - GTAAACACAGGTGAGAGAAGTCAGTCACAGGTAACACATGGTGATGTGGCCTCAGGACTC -257

RPGDs- CCGTGCTCTGTCACTCTTGAGACCCAAGCCCTGGCTCCCCAAAGACCTTCTCCGCCCTCC -720
       ************************************************************
E2     - CCGTGCTCTGTCACTCTTGAGACCCAAGCCCTGGCTCCCCAAAGACCTTCTCCGCCCTCC -317

RPGDs- AGCTTTGCCTTGGTGGAGAAATAAAATCCAAAGCAAGTC                      -759
       ***************************************
E2     - AGCTTTGCCTTGGTGGAGAAATAAAATCCAAAGCAAGTC                      -356
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Fig.1. Nucleotide sequence of E2 cDNA aligned with that of prostaglandin D_2 synthetase.

and used to perform a database search. We found that it corresponded to the nucleotide sequence of brain specific prostaglandin D_2 (PGD_2) synthetase (Fig1). The E2 sequence showed a >98 % homology to nucleotides 403 - 759 of PGD_2 synthetase cDNA sequence (8). As described for PGD_2 synthetase (8, 19), E2 mRNA was brain specific, since it could not be detected in other rat organs, and was readily detected in all the brain regions studied. Cerebral cortex and cerebellum contained particularly high concentrations of E2 mRNA (Fig 2).

To further confirm the dependence of E2 mRNA of thyroid status we analyzed cerebral cortex and cerebellum RNAs from normal control rats and from hypothyroid rats at different times during development. The result is shown in figure 3. Both in the cerebral cortex and in the cerebellum E2 mRNA was more abundant in normal animals than in hypothyroid animals from day 15 onwards. The effect of hypothyroidism was more evident in the cerebellum at days 20 and 25 after birth.

These results clearly show that PGD_2 synthetase mRNA is regulated by thyroid hormone. Other mRNAs which have been so far shown to be dependent of thyroid hormone include those encoding myelin protein (7), Purkinje cell specific mRNAs (20), and the protein kinase C substrate RC3 (21). With the exception of the latter adult hypothyroidism did not result in altered concentrations of the same mRNAs which are regulated by thyroid hormone during the postnatal period. Interestingly, E2 mRNA was also found not to be affected by hypothyroidism in adult rats (result not shown). This might be related to the fact that PGD_2 synthase is expressed in neurons in

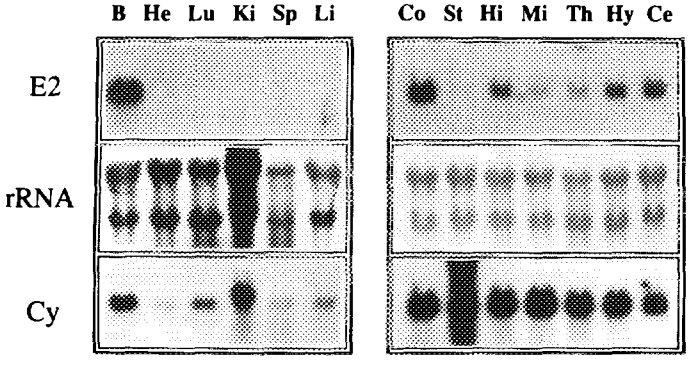

Fig. 2. Expression of E2 mRNA in normal animals. Total RNA was blotted onto nylon membranes and hybridized with a E2 cDNA probe. The filters were stained with methylene blue to visualize the ribosomal RNAs and also rehybridized with a cyclophilin control cDNA probe. Expression of E2 mRNA was restricted to the brain (B) and it was not present in heart (He), lung (Lu), kidney (Ki), spleen (Sp) or liver (Li). Among brain regions, E2 mRNA was clearly present in cerebral cortex (Co), hippocampus (Hi), hypothalamus (Hy) and cerebellum (Ce). Lower levels were found in striatum (St), midbrain (Mi), and thalamus (Th).

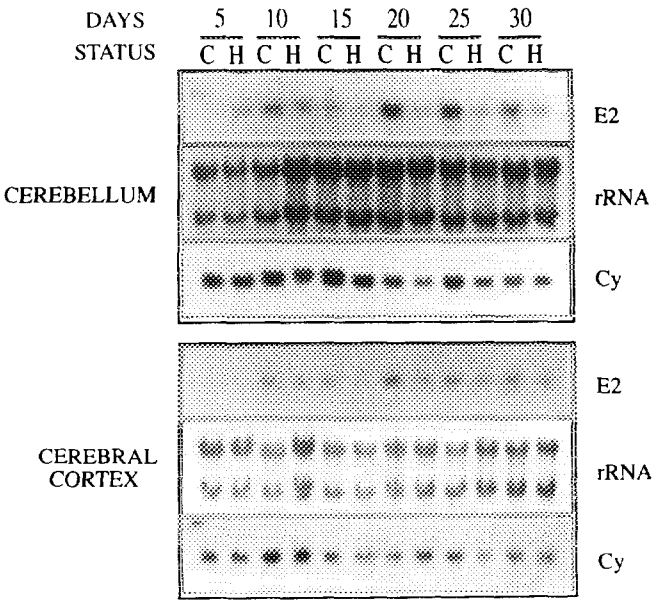

Fig. 3. Effect of hypothyroidism on developmental expression of E2 mRNA in the cerebral cortex and cerebellum. Individual samples from normal or hypothyroid rats at different ages after birth were pooled in groups of three before total RNA isolation, and blotting onto Nytran nylon filters. The filters were stained with methylene blue to control for the amount of ribosomal RNA present in the filters, and rehybridized with a cyclophilin control cDNA probe.

developing animals, whereas in adult animals it is present mainly in oligodendrocytes (22) which are not a target of thyroid hormones in mature animals.

The consequence of thyroid hormone regulation of PGD_2 synthase is unclear. PGD_2 has been shown to induce sleep and hypothermia when administered intracerebrally (9,11,12). Hypothyroid animals have a tendency to drowsiness and hypothermia, the contrary that should be expected from a decreased activity of PGD_2 synthase. One explanation for this discrepancy is that the enzyme is not regulated by thyroid hormone in regions where PGD_2 have these actions, such as the preoptic area. However, be believe that thyroid hormone control of PGD2 synthetase expression could be more related to developmental processes. Early developmental expression in specific neuronal groups in different parts of the brain has been shown by immunochemical methods (23). The enzyme was localized in neuronal somata, dendritic processes and proximal parts of the axons. Since addition of PGD_2 to neuroblastoma cell cultures induced morphological signs of neuronal differentiation (24), it has been proposed that PGD_2 in developing animals contribute to dendrite development and formation of neuronal networks, which agree with the proposed physiological roles of thyroid hormone during brain maturation (1).

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References

1. Porterfield, S. P., and Hendrich, C. E. (1993) Endocrine Rev. 14, 94-106.
2. Legrand, J. (1984) In: J Yanai, Editor, Neurobehavioral Teratology. Elsevier Science Publishers, Amsterdam. pp331-363.
3. Sap, J.,Muñoz, A.,Damm, K.,Goldberg, Y.,Ghysdael, J., Lentz, A.,Beug, H., and Vennström, B. (1986) Nature 324, 635-640.
4. Weinberger, C.,Thompson, C. C.,Ong, E. S.,Lebo, R.,Gruol, D. J., and Evans, R. M. (1986) Nature 324, 641-646.
5. Mellström, B.,Naranjo, J. R.,Santos, A.,Gonzalez, A. M., and Bernal, J. (1991) Mol. Endocrinol. 5, 1339-1350.
6. Bradley, D. J.,Towle, H. C., and Young III, W. S. (1992) J. Neurosci. 12, 2288-2302.
7. Muñoz, A.,Rodriguez-Peña, A.,Perez-Castillo, A.,Ferreiro, B.,Sutcliffe, J. G., and Bernal. J. (1991) Mol Endocrinol 5, 273-280.
8. Urade, Y.,Nagata, A.,Suzuki, Y.,Fujii, Y., and Hayaishi, O. (1988) J. Biol. Chem. 264, 1041-1045.
9. Horiguchi, S.,Ueno, R.,Hyodo, M., and Hayaishi, O. (1986) Eur. J. Pharmacol. 122, 173-179.
10. Hayaishi, O. (1988) J. Biol. Chem. 263, 14593-14596.
11. Hayaishi, O. (1991) FASEB J. 5, 2575-2581.
12. Ueno, R.,Ishikawa, Y.,Nakayama, T., and Hayaishi, O. (1982) Biochem. Biophys. Res. Comm. 109, 576-582.
13. Chomczynski, P., and Sacchi, N. (1987) Anal Biochem 162, 156-159.
14. Herrin, D. L. (1988) BioTechniques 6, 196-200.
15. Danielson, P. E.,Forss-Peter, S.,Brow, M. A.,Calavetta, L.,Douglass, J.,Milner, R. J., and Sutcliffe, J. G. (1988) DNA 7, 261-267.
16. Church, G. M., and Gilbert, W. (1984) Proc. Nat. Acad. Sci. USA 81, 1991-1995.
17. Sanger, F.,Nicklen, S., and Coulson, A. R. (1977) Proc. Nat. Acad. Sci. USA 74, 5463-5467.
18. Travis, G. H., and Sutcliffe, J. G. (1988) Proc Natl Acad Sci USA 85, 1696-1700.
19. Islam, F.,Urade, Y.,Watanabe, Y., and Hayaishi, O. (1990) Arch. Biochem. Biophys. 277, 290-295.
20. Strait, K. A.,Zou, L., and Oppenheimer, J. H. (1992) Mol Endocrinol 6, 1874-1880.
21. Iñiguez, M. A.,Rodriguez-Peña, A.,Ibarrola, N.,Morreale de Escobar, G., and Bernal, J. (1992) J. Clin. Invest. 90, 554-558.
22. Urade, Y.,Fujimoto, N.,Kaneko, T.,Konishi, A.,Mizuno, N., and Hayaishi, O. (1987) J. Biol. Chem. 262, 15132-15136.
23. Ueno, R.,Osama, H.,Urade, Y., and Hayaishi, O. (1985) J. Neurochem. 45, 483-489.
24. Miwa, N.,Miwa, T.,Sugino, H.,Hayashi, Y.,Ueno, R., Hayaishi, O., and Amano, T. (1985) Neurosci. Res. S32.