APOTOPSIS IN THE DEVELOPING CEREBELLUM OF THE THYROID HORMONE DEFICIENT RAT

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1. ABSTRACT

Abstract-The mechanism underlying transient reduction of cell number in the developing cerebellum have been studied for several decades. In this study we analyzed cell death by apoptosis in the developing cerebellum of euthyroid and hypothyroid rats. Results showed that in both groups the apoptotic activity is limited to the internal granular layer from postnatal (p) day 2 to day 12 in euthyroid animals, with the peak at 8 days. No apoptotic cells were detected in the cerebellum of 22 days old euthyroid rats. The level of apoptosis in the cerebellum of hypothyroid rats also reached a peak at 8 days but was four times higher than in control animals. Apoptosis in hypothyroid animals was also observed at p22 and corresponds to the value found in the time of the apoptotic peak in euthyroid cerebellum. At the age of 42 days, no apoptotic cells were found in the cerebellum of either group. Furthermore, it appears that the hormone also plays a role in the disappearance of the external germinal layer, since its presence is still apparent in 42 day old hypothryoid cerebellum. Hence, our results suggest that the deficiency of thyroid hormone (TH) not only increases, but also extends apoptosis during rat cerebellum development and affects the disappearance of the external germinal layer.

2. INTRODUCTION

Programmed cell death (PCD) plays a major role in the differentiation and development of central nervous system (CNS) (1,2,3). Cell death is often controlled by survival promoting signals from other cells, but is executed in a cell autonomous manner (4). Apoptotic cells exhibit morphological and biochemical changes, such as, chromatin condensation, internucleosomal DNA fragmentation, cytoplasmic vacuolation, membrane blebbing and cell shrinkage (5, 6). Numerous experiments suggest that genes expression is required for neuronal death (7, 8). Most of the cell cycle regulators are involved in apoptosis, for example, cyclin D1 has been identified as an essential mediator of apoptosis of neuronal cell death (9). On the other hand, neurotrophic factors are presumably the limiting survival factors for neuronal cell types in vivo (10). Programmed death of supernumerary neurons occurring around the time when the neurons are making functional connections, abrogated by mRNA and protein synthesis inhibitors indicative of an activation of a specific genetic program (11).

Thyroid hormone (TH) influences gene expression, either positively or negatively, through binding to nuclear thyroid hormone receptors the members of steroid/thyroid hormone receptor superfamily (12, 13). Certain genes expressed in the brain have been shown to be under thyroid hormone control. These include myelin genes (14), the Purkinje cell specific gene, PCP2, (15) the transcription factor NGFI-A (16), and neuron specific enolase (NSE) (17). The lack of TH in early life has a marked effect on the development of the rat cerebellum (18, 19). In humans, the lack of adequate levels of TH during critical periods of development, results in cretinism, a syndrome of severe mental retardation often accompanied by growth retardation and/or neurological deficits (20).

The relative simplicity, structural homogeneity
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3.2 Evaluation of DNA fragmentation in cerebellar granular cells of developing euthyroid rats

The TUNEL technique was applied to probe the apoptotic DNA fragmentation characteristic of cell death in the developing cerebellum of euthyroid rats at the postnatal day 2, 8, 12, 22, and 42. Examination of the layers of coronal sections reveal that the programmed cell death after birth is limited to the IGL of cerebellum although scattered apoptotic cells could be seen in the EGL of p8 and p12 (figure 2A). Based on a statistical evaluation, results show that apoptotic cells are absent at 2, 22 and 42 days postnatally (figure 2A-B). The peak of apoptosis was observed in the IGL at p8. There is about 50% reduction in apoptotic cells at p12. In summary, as a results of postnatal cerebellum development, the number of apoptotic cells observed in the IGL increased significantly from the undetectable level at p2 reaching the highest level at p8, and gradually declined being undetectable at p22. Interestingly, DNA fragmentation was not observed in Purkinje cells layer (figure 2A-B, figure 3).

3.3 Detection of thyroid stimulating hormone (TSH)

TSH level in serum of euthyroid and hypothyroid rats was determined by radioimmunoassay and performed by CORNING Hazleton Inc., Vienna, VA 22182.

4. RESULTS

4.1 Evaluation of DNA fragmentation in cerebellar granular cells of developing euthyroid rats

The TUNEL technique was applied to probe the apoptotic DNA fragmentation characteristic of cell death in the developing cerebellum of euthyroid rats at the postnatal day 2, 8, 12, 22, and 42. Examination of the layers of coronal sections reveal that the programmed cell death after birth is limited to the IGL of cerebellum although scattered apoptotic cells could be seen in the EGL of p8 and p12 (figure 2A). Based on a statistical evaluation, results show that apoptotic cells are absent at 2, 22 and 42 days postnatally (figure 2A-B). The peak of apoptosis was observed in the IGL at p8. There is about 50% reduction in apoptotic cells at p12. In summary, as a results of postnatal cerebellum development, the number of apoptotic cells observed in the IGL increased significantly from the undetectable level at p2 reaching the highest level at p8, and gradually declined being undetectable at p22. Interestingly, DNA fragmentation was not observed in Purkinje cells layer (figure 2A-B, figure 3).
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**Figure 2A.** Detection of DNA fragmentation in the IGL of cerebellum of postnatal 8 days (p8), p12, and p22. Section of 12 µm were labeled for DNA fragmentation as described in the material and methods. At the age of 8 days, some apoptotic stained cells were detected in the euthyroid rats, whereas, more apoptotic cells were detected in the 8-day hypothyroid rats. At the age of 12 days, few apoptotic cells were found in the euthyroid rats. In contrary, lots of apoptotic cells were still stained in the 12-day hypothyroid rats. At the age of 22 days, none apoptosis were happened in the euthyoid cerebellum, relatively fewer apoptotic cells were detected in the 22-day hypothyroid rats. None apoptotic cells were Purkinje cells. Magnification, 10x except the last one of left column which is 5x.

**Figure 2B.** Coronal sections of euthyroid and hypothyroid cerebellar at p2 and p42 stained for apoptotic cells as described in the material and methods. Magnification, 10x.
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5. DISCUSSION

Although it is not arguable that TH is crucial for the cerebellum development, it has not been clearly established whether the arrested development of Purkinje cells dendrites, the primary synaptic target of granular cells, in the hormone deficient cerebellum, leads to the increased apoptotic activity of granular cells or whether the reduced number of granular cells connections with Purkinje cells affects Purkinje cell maturation. Indeed, the molecular basis for TH during brain development are poorly understood. Hormone action is mediated by thyroid hormone receptors especially the ß1, whose level reaches its highest during the first 2-3 weeks after birth. Thus, at the level of transcription, TH can regulate expression of variety of factors known to play an important role in the regulation of neuronal survival during development (28,29,30,31). Lindholm et al (32) reported that expression of neurotrophin 3 is under control of TH. Recently, Neveu et al (27) showed that neurotrophin 3 and brain derived neurotrophin factor prevent induced cell death of granular cells in hypothyroid cerebellum in vivo. Muller et al (33) reported that thyroid hormone also promotes expression of the bcl-2 protooncogene, the programmed cell death suppressor, and prevents apoptosis of early differentiating cerebellar granular neuron in vitro.

It is well established that programmed cell death plays an important role during maturation of the central nervous system. In this study, we have chosen to investigate the effect of the TH on the development of rat cerebellum since its maturation takes place postnatally. We have shown that the lack of TH increases and extends the apoptosis in the developing cerebellum and affects disappearance of the EGL.

In the rat, the cerebellum granular cells, a source of major excitatory afferents to the Purkinje cells, are generated from EGL (34). From p3 to p30, granular cells migrate to the IGL, by translocation of the cell body through the descending portion of the growing parallel fiber, leaving their axons (parallel fibers) in the ML forming synapse with the dendrites of Purkinje cells (21). Thus, it seems likely that growth of the descending portion of the parallel fiber is an important and perhaps rate-limiting aspect of granular cell migration and cerebellum maturation. It has been shown that the proper levels of TH are necessary to secure the growth of parallel fibers (35,36). Furthermore, hypothyroid rats show retardation in the proliferation, migration, and differentiation of cerebellar granular cells. Although a number of Purkinje cells do not appear to be affected by the lack of the hormone, there is, however, deficiency in the elaboration of Purkinje cell dendrite trees, spines and the synapses (18).

Using a sensitive method for detecting DNA fragmentation, a hallmark of apoptotic activity, our data showed that no apoptotic cells were detected in the euthyroid cerebellum at p2, 22 and p42. In agreement
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with previous results reported by Wood et al (37), the apoptosis reach its maximal value at p7 and then declined. It is during this period that most of the histogenesis of the cerebellum occurs. Thus, granular cell death may be a key factor in regulating the final number of neurons. Indeed, in the stage where the maximal rate of migration is finished, the apoptotic activity is descended and becomes undetectable at p22. This developmental stage is also marked by the absence of the EGL. In both the euthyroid and hypothyroid rats, the p8 apoptosis reached the peak level, however, there was a greater proportion of apoptotic cells in the IGL of hypothyroid rats than that in the control. Surprisingly, the granular cells were still undergoing apoptosis at p22 in the IGL hypothyroid cerebellum, contrary to the control. Thus, it appears that the deficiency in TH during postnatal cerebellum development promotes and also extends the event of apoptosis. The presence of the EGL in hypothyroid cerebellum at p 42, the time when brain maturation should be completed, indicates that the hormone also plays a role in morphological changes accompanying brain development. Another striking morphological change in hypothyroid cerebellum is the impaired dendritic arborization of Purkinje cell in ML. Previous results showed that in hypothyroidism, retardation in the morphological maturation of Purkinje cells is the most apparent during the second postnatal week (22,38). We have shown that this period coincides with the peak of aggressive apoptosis augmented by the absence of the hormone. It is now important to uncover the mechanism whereby TH is able to control the cell cycle so as to limit the extent and time of apoptosis in the developing cerebellum.

6. ACKNOWLEDGMENTS

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7. REFERENCES


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**Key Works:** Apoptosis, Thyroid Hormone, Developing Cerebellum, Hypothyroid Rat, Brain

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