Progressive Somatofugal Activation of Cerebroside Sulfotransferase in the Developing Chick Optic Fibers

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Abstract. A myelination parameter of the chick optic pathway has been investigated: the activity of cerebroside sulfotransferase. Evidence is presented for a somatofugal progression of the enzyme activity peak along the optic nerve, chiasm, optic tract, tectum anterior, and tectum posterior. The enzyme activity appears on the fifteenth embryonic day and reaches a maximum in the nerve two days before hatching. One day after hatching, the peak is found in the chiasm, and three days after that in the optic tract. The peak is found in the tectum anterior on the tenth day. The results suggest a somatofugal progression of myelination.

INTRODUCTION

Myelination in the nervous system is age-dependent (14). The formation of the myelin sheaths depends upon the synthesis of myelin components, e.g., myelin lipids. One of these lipids, sulfatide, has been studied in this connection, and the synthesis of sulfatide correlates definitely with myelination (1). One of the key enzymes of sulfatide synthesis, cerebroside-sulfotransferase (EC 2.8.2.11) (CST), shows an age-dependent activity pattern paralleling the rate of myelin formation (6). Nothing is known about the regulation of the enzyme activation pattern. In order to investigate this question we chose the chick optic pathway, which shows post-hatching myelination (13). In this system we attempted to determine whether there is a temporal activation pattern of CST along the optic pathway, especially in the optic nerve, optic tract, optic chiasm, and the tectum anterior and tectum posterior. We found a somatofugal sequence of CST peak activities. The shift of the peak activities along the optic pathway can be correlated with the speed of the slow axonal flow (5).

MATERIALS AND METHODS

The embryos or chicks were decapitated and the eyes and brains were dissected at 0° under a stereomicroscope. The optic nerve was cut between the exit from the bulb and the confluence with the chiasm. The optic chiasm was cut between this point and the emergence of the tract. The optic tract was then detached from the mesencephalon and cut at the level of the groove delimiting the tectum. The optic lobe was cut into two pieces along the vertical frontal meridian, each half freed from its core along the ventricle and the deep tectal layers, and defined as anterior and posterior tectum. In some experiments, the samples were lyophilized and weighed before CST determination. Each point is based on 2 to 4 samples. The samples were homogenized with 10 times the volume of 0.32 M sucrose, containing EDTA and Na₂HPO₄ (12). One hundred μl of the homogenate were then placed in a plastic tube and incubated for 20 minutes at 30°C,

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after the addition of 200 µl 250 mM imidazol/HCl buffer, pH 7.0, containing 20 mM CaCl$_2$, 100 µg cerebrosides in 100 µl 0.01% deoxycholate, 100 µl 150 mM NaCl, and 500,000 dpm PAPS as a precursor, in order to determine the activity of cerebroside-sulfotransferase. The incubation was stopped by the addition of 5 ml chloroform/methanol 2:1, and partitioning was provided by 1.0 ml 0.74% KCl. The samples were centrifuged for 10 minutes at 6000 × g, the upper phase discarded, the lower phase washed twice with chloroform/methanol water 4:48:47 (4), transferred to a counting vial, dried with a hair drier, and counted in a liquid scintillation counter. Proteins were determined by the method of Lowry et al. (8).

RESULTS

The results can be seen in Figure 1. There is a small increase in specific activity of CST in all segments between the fifteenth and eighteenth embryonic days. Two days before hatching, the highest CST activity is found in the nerve. One day after hatching, peak CST activity is in the chiasm and three days after hatching is in the optic tract. In the anterior tectum, the peak activity is found eight days after hatching and in the posterior tectum, thirteen days after hatching. To check whether these specific CST activity patterns are due to changes in the protein denominators, we measured total enzyme activity of every single preparation. The results in Figure 2 show a percentage presentation of total peak activity of the different regions investigated. The peak activity of CST is considered as 100%. The peaks in both specific and total CST activity are found

![Graph](http://jnen.oxfordjournals.org/)

**Fig. 1.** Time course of the specific activity of cerebroside-sulfotransferase in the optic nerve (○), optic chiasm (●), optic tract (■), and anterior and posterior tectums (▲, △) of the chick during development. Values are means of three experiments ± SEM.
on the same day. This shows that the age-dependent changes of specific activity in the different regions are not due to changes in their protein content.

This pattern reveals, first, that the CST activity rises within a few days along the optic fibers and, second, that the peak of CST activity follows a temporal
sequence, like a wave in the proximodistal axis of these fibers. As the length of the optic fibers increases during the developmental period studied, it is difficult to calculate the exact speed of this wave. If one estimates the length of the fibers in a young chick, one can plot the time course of the peak CST activity against the distance along the fibers (Fig. 3). It appears, then, that the wave of peak CST activity progresses at a speed of roughly 1 mm/day. It is thus of the same order of magnitude as the slow phase of axonal flow (2, 3, 9). The data suggest the possible existence of a second wave traveling in the nerve on the fourth and fifth days, and reaching the tract eight days later (Figs. 1 and 3).

DISCUSSION

The results indicate that on about the fourteenth or fifteenth day of incubation, a "background" CST activity appears diffusely throughout the optic fibers, indicating a relatively simultaneous start of sulfatide synthesis. Since CST activity parallels myelination (3), this indicates the beginning of myelination. This is in agreement with the ultrastructural observations made by Rager (10, 11), who observed the first myelinated fibers in the chick optic nerve around the sixteenth day, as well as in the first layer of the tectum (Rager, personal communication). Thereafter, a population of axons undergoes intense myelination, which progresses as a wave from the eye toward the brain at the same

![Fig. 3. Progression of the waves of peak CST specific activity along the developing optic pathway. Each point is taken from the peak activities in Figure 1. The lengths of the segments correspond to those of a one-week-old animal.](http://jnen.oxfordjournals.org/)

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speed as the slow axonal flow. A second wave, corresponding to a second fiber population, may follow the first (Fig. 3).

One might assume that what triggers this wave originates in the ganglion cells and travels at about 1 mm/day. Therefore, the wave would have started in the retina around the fourteenth or fifteenth day of embryonic life (Fig. 3). This coincides with the beginning of the diffuse background CST activity (Fig. 1). In order to substantiate this hypothesis, work is now in progress to measure biochemically the sequence of myelin deposition along the optic pathway. During the period immediately preceding, from the eleventh day on, the synapses of the optic nerve develop in the tectum (7, 11). It is tempting to speculate that, when the ganglion cells have established contact with postsynaptic elements in the tectum, a message is sent to the cell bodies, indicating that the searching phase is over and that the functional transmission phase can begin.

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