Functional Development of Oligodendrocytes and Open-field Behavior in Developing Rats: An Approach Using Monoclonal Antibody to Immature Oligodendrocytes

Yutaka YAMAMURO¹, Kazunori YOSHIMURA² ³, Kyoko TSUCHIYA¹, Naoto SENSUI¹, and Hiroaki ASOU³

¹Department of Animal Science, College of Bioresource Sciences, Nihon University, Fujisawa, Kanagawa 252-8510, ²Department of Physiology, Saitama Medical School, Moroyama, Saitama 350-0495, and ³Department of Neurobiology, Tokyo Metropolitan Institute of Gerontology, Itabashi-ku, Tokyo 173-0015, Japan

Abstract: To examine the relation between functional development of oligodendrocytes and open-field behavior during the postnatal period, a mouse monoclonal antibody termed 14F7, which predominantly labels stage-specific immature oligodendrocytes, was employed. Antibody 14F7 was administered intraperitoneally into male pups on day 3 and 4 after birth. The open-field test was performed on days 12 and 18 of the postnatal period. Horizontal activity increased remarkably with the growth of pups. On day 18, horizontal activity in the group with 14F7 was significantly higher than the control, while there was no significant difference between treatments on day 12. In contrast to the horizontal activity, the frequency of hind leg rearing, vertical activity, in the group with 14F7 was significantly lower than that in the control. On day 12, choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activities in the cerebral cortex were similar between the groups. These activities increased with the growth of pups in both groups. In the 14F7 group on day 18, ChAT activity was the same as the control, whereas AChE activity was significantly lower compared with the control. These results suggest that neonatal exposure to 14F7 induces abnormal neurotransmission by reducing the degradation of acetylcholine and alters the spontaneous activities in developing rats.

Key words: acetylcholinesterase, oligodendrocytes, open-field behavior, rats

Introduction

In many mammalian species, the greater part of the brain is immature at birth and undergoes developmental changes in its composition and structure in the postnatal period. Studies on the functional maturation of the brain have been performed using several specific markers for neuronal and glial cells [5, 24, 28]. The enzyme activities associated with the synthesis and catabolism of neurotransmitters have been studied and are also considered to be specific markers for the development of the central nervous system [5, 16, 20, 27]. Neuroanatomical
studies have shown that the cerebral cortex receives dopaminergic, noradrenergic, serotonergic and cholinergic afferents from several brainstem structures. Choline acetyltransferase (ChAT), the acetylcholine (ACh)-synthesizing enzyme, and acetylcholinesterase (AChE), an ACh-degradative enzyme, which are considered definitive markers for the cholinergic neural system, have been investigated as indices of the functional maturation of this system in the postnatal period, and a sharp increase in ChAT and AChE activities of rat cerebral cortex occurring after birth, and reaching close to adult levels at three to four weeks of age, was reported [29]. In mice and rats, the development of spontaneous activities during the postnatal period has been investigated by several researchers. All parameters investigated, ambulatory activity, rearing, climbing, sniffing, and grooming, increased continuously or in phase with significant peaks until the postnatal day 27 [1, 2, 8]. Behavioral indices of activation are intimately associated with several neural systems including the cholinergic system [7, 14, 18, 23, 25, 26]. However, the physiological importance of the cholinergic system, especially the cell population participating in neurotransmission of this system, to behavioral maturation during the neonatal period has not yet been investigated in detail.

Recently, Yoshimura et al. generated a monoclonal antibody, termed 14F7, against stage-specific immature oligodendrocytes isolated by a novel oligodendrocyte-lineage cell culture technique [31]. Antibody 14F7 can completely block the expression of myelin basic protein (MBP) and proteolipid protein, specific markers for oligodendrocytes at the last stage of differentiation. 14F7-positive cells were not associated with astrocytes and MBP-positive oligodendrocytes [21]. In the present study, antibody 14F7 was utilized to examine the relation between the aspect of open-field behavior and functional development of oligodendrocytes, and cholinergic-related enzymes during the postnatal period.

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**Materials and Methods**

**Animals and treatments**

Three-month-old primiparous female Wistar rats, that were purchased from Clea Japan, Inc. (Jcl:Wistar; Tokyo, Japan) and were bred in our laboratory with rotation for non-inbreeding, were mated with 4-month-old male Wistar rats and moved into individual plastic cages (310 × 250 × 180 mm) after spermatozoa were found in a vaginal smear (day 1 of gestation). The pregnant rats were housed at 23 ± 1°C with a photoperiod of 14 h light: 10 h dark (lights on at 0600 h) and provided with food and water ad libitum. At 0900 h on day 1 of lactation (the day after parturition), the litter was adjusted to eight pups.

Ascitic fluids were generated by injecting 5 × 10^6/ml 14F7 hybridoma cells and NS-1 myeloma cells (American Type Culture Collection, Rockville, MD), as the control, into 2-month-old BALB/c mice previously primed with pristane (2,6,10,14-tetramethylpentadecanoic acid). Each protein concentration in the ascitic fluid was NS-1 37.36 mg/ml and 14F7 32.52 mg/ml (BCA Protein Assay Kit; Pierce, Rockford, IL). A volume of 5 µl of 14F7 or NS-1 was administered intraperitoneally to two male pups of each litter on days 3 and 4 after birth. The body weight of pups was measured once daily (0900 h) until the end of the experiment. All experiments conformed to the Guideline for Animal Experiments, College of Bioresource Sciences, Nihon University.

**Determination of open-field behavior**

A 45 × 45 cm open-field apparatus made of acrylic plates with a grid width of 9 cm on the floor (i.e. 25 sections) and a wall height of 8 cm was used. The open-field was placed in an experimental room with normal fluorescent lighting. All procedures were carried out between 1300 and 1600 h. On days 12 (n=8 from 14F7 and NS-1 groups, respectively) and 18 (n=8, respectively) after birth, a pup was moved from its home cage into an acrylic cylindrical opaque tube with a diameter of 9 cm located in the center of the floor for 1 min. After 1 min, the pup was released from the tube and open-field behavior was recorded on videotape with a CCD camera for 15 min. Horizontal activity (i.e. motility) was estimated as the number of grid line crossings from the nose. Other behavioral activities analyzed were as follows.

Latency to movement: The time to the onset of movement (release from the tube to first crossing of the grid line).

Vertical activity (i.e. rearing): The frequency of rearing with hind legs.

After recording, the rat was removed from the open-field and the floor and wall were swept and cleaned with 35% isopropanol.
Assay of ChAT and AChE activities

Following open-field analysis, the cerebral cortex of each pup was obtained, weighed and stored at –80°C. The tissues were homogenized with a Teflon homogenizer in 5 ml of 25 mM sodium phosphate buffer (pH 7.4) per gram of wet weight, and centrifuged at 15,000 g for 60 min at 4°C. The supernatant was obtained as an enzyme solution. The protein concentration was determined using a BCA Protein Assay Kit (Pierce).

The determination of ChAT activity was modified from the methods of Kaneda and Nagatsu [11]. A volume of 100 µl of enzyme solution diluted to 3 mg protein/ml was mixed with 100 µl of substrate solution containing 1 mM choline chloride, 0.4 mM acetyl-CoA, 0.2 mM eserine sulphate, 0.3 mM sodium chloride, and 20 mM EDTA-2Na in 0.1 M sodium phosphate buffer (pH 7.4), and incubated at 37°C for 60 min. The enzyme solution boiled at 95°C for 5 min was used as the negative control. The reaction was stopped with 50 µl of 1 M perchloric acid in an ice bath. After 10 min, 6 µl of 1 mM isopropyl homocholine (IPHC) was added as an internal standard, and the mixture was centrifuged at 1,600 g for 10 min at 4°C. Finally, the supernatant was filtered with a 0.45 µm filter unit to clean the sample.

The determination of AChE activity was modified from methods of Kaneda et al. [12]. A volume of 100 µl of enzyme solution diluted to 0.1 mg protein/ml was mixed with 98 µl of 0.306 M sodium chloride in 0.153 M sodium phosphate buffer (pH 7.0), 2 µl of 3 M magnesium chloride, and 40 µl of 6 mM ACh, and incubated at 37°C for 30 min. For the negative control, the enzyme solution was boiled at 95°C for 5 min. The reaction was stopped with 80 µl of 5% methaphosphoric acid in an ice bath. After 10 min, 40 µl of 1 mM IPHC was added as an internal standard, then treated as described above.

The ACh synthesized by ChAT and choline degraded from ACh by AChE were measured with an HPLC system (HP-1100; Hewlett Packard, Mount View, CA) with post-column enzyme reaction and electrochemical detector. A volume of 10 µl of sample for ACh detection and 5 µl for choline detection were directly injected into a reverse-phase column (Eicompak AC-GEL, 4.6 × 150 mm, Eicom, Kyoto, Japan) where ACh and choline were separated before entering an enzyme reacting column (Eicom AC-Emzympak; Eicom) containing immobilized acetylcholine esterase and choline oxidase, which convert ACh to hydrogen peroxide. The hydrogen peroxide was detected on a platinum electrode (WEP; Eicom) set at 450 mV. The mobile phase consisted of a 100 mM phosphate buffered solution (pH 8.5) containing 0.8 mM sodium 1-decanesulfonate and 1.68 mM tetramethylammonium chloride. The procedure for determination of choline and ACh was the same as described by Yamamuro et al. [30].

Statistical analysis

Statistical significance was based on repeated-measures ANOVA. Analyses between the two experimental groups at the same stage were tested its contrasts, means comparisons, after ANOVA (super ANOVA). Criterion for significance was P<0.05 in all cases.

Results

The body weight of pups increased linearly with the age in both treated groups. There was no difference between the groups (Fig. 1).

Latency to the onset of movement tended to shorten with the growth of the pups. There was no significant difference between the treatment groups on day 12 and day 18 (Fig. 2). Horizontal activity increased remarkably with the growth of the pups. On day 18 of the postnatal period, horizontal activity in the group treated with antibody 14F7 was significantly higher (F(1,7) = 9.069, P = 0.0196) than the control, while there was no significant difference between treatments on day 12. On day 12 no pups showed rearing with hind legs. However, the frequency of rearing, vertical activity, in the group treated with antibody 14F7 on day 18 was significantly lower (F(1,7) = 7.609, P = 0.0282) than that in the control (Fig. 3).

The weight and protein content of the cerebral cortex increased with the weight of the pups in both groups. There were no significant differences between treatments at each stage (data not shown).

On day 12 of the postnatal period, ChAT and AChE activities of the cerebral cortex in the group treated with antibody 14F7 were similar to those of the control. These activities increased with the growth of pups in both groups. In the 14F7 group on day 18, ChAT activity was the same as the control, whereas AChE activity was significantly decreased (F(1,7) = 7.451, P = 0.0294) compared with the control (Fig. 4).
Physical and physiological conditions change drastically with the progression of postnatal development. The postnatal stages investigated in the present study are quite different, since the eye-lids open and gait by the limbs is generally established on days 14 to 16 of the postnatal period in rats. Accordingly, rats on day 12 did not display rearing behavior and locomotion was low compared with that on day 18. In the present study, neonatal exposure to antibody 14F7 resulted in a reduction of cerebral AChE activity in rats on the 18th postnatal day, whereas no difference was seen in ChAT activity between treatments. AChE, a secreted enzyme that is associated with the cell membrane, is not only present at a high concentration in the terminal of cho-
linergic neurons, but is also present at a moderately high concentration in certain noncholinergic neurons that receive cholinergic inputs. The existence of an antigen being recognized by antibody 14F7 is limited to oligodendrocytes expressing the progenitor marker A2B5 to O4 antigen [31], and the antigen suppresses the expression of MBP by binding with antibody 14F7 [21]. Our preliminary analysis confirmed that antibody 14F7 recognizes two different antigens in immature oligodendrocytes, and these were nearly identical with drebrin A and E. However, the role of drebrins on the functional and morphological development of oligodendrocytes is yet unknown. The present results suggest that oligodendrocytes, proximal to the terminal of neurons, are concerned with the biosynthesis of AChE in cholinergic and noncholinergic neurons. In addition to its role in inactivating released ACh, AChE appears to function as a chemical messenger in the central nervous system [3, 9]. Consequently, the block of an antigen against antibody 14F7 induces the abnormal neurotransmission in the cholinergic systems.

Recently, numerous studies using psychopharmacological and neurotoxic drugs have reported the influence of AChE activity on the central nervous system [4, 6, 10, 13], and the disorder of catalysis by AChE induces behavioral abnormality. Diclorvos, an organophosphorous pesticide, reduced AChE activity of the rat brain, increased horizontal activity, and decreased vertical activity and defecation in the open-field test [22]. These findings are consistent with the relationship between AChE activity in the cerebral cortex and behavioral parameters during the postnatal period determined in the present study. Thiamine, vitamin B1, deficiency also induced a decrease of AChE and altered some parameters of open-field performance [19]. Furthermore, cholinergic immunotoxin 192 IgG-saporin increased AChE activity in several brain regions and decreased motor activity in the open-field test [15]. Clomipramine-induced behavior depression was associated with an increase of AChE in the hippocampus and a decrease in the frontal cortex of adult rats [17]. Antibody 14F7 exposure to the neonate plays a specific response in the same manner as other AChE inhibitors reported previously. Lack or excess of AChE activity in the central nervous system, along with numerous psychiatric and neurodegenerative syndromes, alters behavioral reactions, but the corresponding molecular processes and signal transduction pathways are yet unknown.

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References


