Short communication

Regional and temporal progression of reactive astrocytosis in the brain of the myelin mutant taiep rat

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Abstract

Reactive astrocytosis in taiep rats was shown by glial fibrillary acidic protein (GFAP) immunoreactivity measured by means of enzyme-linked immunosorbent assay and indirect immunofluorescence. Increased GFAP immunoreactivity was first observed in the brainstem of 15-day-old taiep rats and was widespread throughout all brain regions at 6 months of age. Characteristically, astrocytes were hypertrophic and displayed strong GFAP fluorescence. The pattern of these reactive cells may correlate with the process of dysmyelination in the taiep rat.

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The taiep (acronym of tremor, ataxia, tonic immobility episodes, epilepsy and paralysis) mutant rat was obtained by selective inbreeding of the Sprague–Dawley strain [7]. From the onset of the myelination process, taiep rats exhibit hypomyelination and suffer progressive demyelination resulting in a highly hypomyelinated central nervous system (CNS) as they reach adulthood [3]. It has been described that the myelination defect found in the taiep rat is due to an abnormal link of microtubules with smooth endoplasmic reticulum membrane [2], thus leading to microtubule accumulation in the oligodendrocyte cytoplasm [3,11]. A common feature in demyelinating diseases is an increase of glial fibrillary acidic protein (GFAP) immunoreactivity, condition commonly known as reactive astrocytosis [4,12]. It has been suggested that reactive astrocytosis reflect the active cellular response that preserves the tissue integrity against a variety of neuronal insults [12]. Based on these considerations, it is expected to find reactive astrocytosis in those brain regions of taiep rats where the myelination defect has been found. Due to the progressive nature of taiep defect, it is further expected that reactive astrocytosis might follow a temporal course. The present study addressed those issues by measuring GFAP immunoreactivity in brain regions of taiep rat during its postnatal development.

Brains were obtained from different age (15 days, 1 month, 3 months and 6 months) normal Sprague–Dawley rats and homozygous affected taiep rats to dissect out the following regions: cerebral cortex (Cx), cerebellum (Ce), brainstem (BS) and diencephalon (Dienc). These regions were individually homogenized in phosphate buffer saline, pH 7.4 (PBS) and centrifuged at 3000 rpm for 10 min, at 4°C. The supernatants were re-centrifuged at 12 000 rpm for 30 min at 4°C (Sorvall RMC 14), pellets discarded and protein content was determined in supernatants by the Bradford method. Aliquots containing 1 \( \mu \)g of total protein per brain region were placed into individual wells of an enzyme-linked immunosorbent assay (ELISA) plate. One
hundred microliters of 0.1 M carbonate buffer were added to each well and the plate was incubated for 18 h at 4°C. To block non-specific binding sites, 100 μl of 0.5% bovine serum albumin, IgG free (Sigma), was added to each well at room temperature. After an incubation of 30 min, this solution was removed and wells were washed thrice with PBS. A rabbit anti-cow GFAP polyclonal antibody (1:3400 dilution; Dako, Denmark) was added to each well, and incubated for 1 h at room temperature. After three washings with PBS, a horseradish peroxidase-conjugated goat anti-rabbit IgG (1:1000 dilution; Pierce) was added and incubated for 1 h at room temperature. The antibody–antigen complex was revealed by adding 100 μl of 2',2'-azidine-bis(3-ethylbenzyl-tiazolin-6-sulfonic) containing 0.3% H₂O₂ into each well. After 10 min, optical density (OD) in each well was determined using a multplate reader (BioRad, Model 550) at 414 nm.

GFAP immunoreactivity was assessed by indirect immunofluorescence in brain sagittal slices of 6-month-old control and taiep rats. Rats were deeply anesthetized with chloral hydrate and perfused through the ascending aorta with 100 ml of PBS, followed by 100 ml of 4% paraformaldehyde in PBS. The brain was then removed and maintained in the fixative for 48 h at 4°C. After overnight maintenance in PBS containing 10% sucrose at 4°C, the brain was frozen and sectioned in 30-μm slices on the sagittal plane using a Leitz cryostat. Slices were individually collected in a 24-well plate containing PBS and processed for immunofluorescence. Slices were first incubated with 10% IgG-free bovine serum albumin in PBS containing 0.2% Triton X-100 for 20 min at room temperature. The primary antibody was a rabbit anti-cow GFAP (1:400 dilution; Dako) and the secondary antibody was a fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (1:60 dilution; Pierce). Slices were mounted on glass slides using an antiquenching medium (Vector Laboratories) and then scanned in a Nikon microscope equipped with confocal imaging system (Bio-Rad MRC-600, Watford, UK). The fluorescence was detected with 40× and 60× oil-immersion objectives at excitation/emission wavelengths of 488/522 nm (green channel). Ten to twenty consecutive 1-μm optical sections were obtained in the z-series and the resulting images were projected in a bidimensional plane on the screen monitor using the color

Fig. 1. Progression of reactive astrocytosis in the brain of taiep rats. ELISA assay was used to evaluate GFAP immunoreactivity in brain homogenates of Sprague–Dawley or taiep rats at the following ages: 15 days (A), 1 month (B), 3 months (C), and 6 months (D). BS, brainstem; Ce, cerebellum; Cx, cerebral cortex; Dienc, diencephalon. Values are the mean±S.E.M. of four rats evaluated by duplicate. *P<0.05, **P<0.01, ***P<0.001 when compared with the respective control group, Student’s t-test.
green for FITC. For each region, the number of pixels obtained by the confocal microscopy computational tool was counted for the measure of the fluorescence intensity.

In normal Sprague–Dawley rats, OD_{414\text{ nm}} values of GFAP immunoreactivity were similar in all brain regions and remained constant after the first month of age (Fig. 1B–D). Conversely, OD_{414\text{ nm}} values of GFAP immunoreactivity corresponding to a particular brain region of \textit{taiep} rats significantly increased depending on the age. GFAP immunoreactivity was significantly higher in brainstem of 15-day-old \textit{taiep} rats (\(P<0.05\), Student’s \(t\), \(n=4\)) than in normal rats (Fig. 1A). An increased GFAP immunoreactivity was also observed in the cerebellum and cerebral cortex of \textit{taiep} rats at 1 and 3 months of age (\(P<0.05, P<0.01\), Student’s \(t\), \(n=4\) for both) (Fig. 1B.C). In the diencephalon, statistical difference (\(P<0.001\), Student’s \(t\), \(n=4\)) in GFAP immunoreactivity was only observed in 6-month-old rats (Fig. 1D).

Confocal microscopy analysis confirmed the increment in GFAP immunoreactivity in all brain regions of \textit{taiep} rats as compared with those of normal 6-month-old Sprague–Dawley rats (Fig. 2). In addition to the increased fluorescence in cerebellum white matter, \textit{taiep} rat astrocytes showed hypertrophy due to process outgrowth (Fig. 3). However, no difference in immunofluorescence intensity was found in the cerebellum cortex of \textit{taiep} rats. Increased GFAP immunoreactivity was also observed in myelin-rich brain regions such as corpus callosum and brainstem (data not shown).

Our results showed that \textit{taiep} mutant rats suffer progressive reactive astrogliosis as assessed by GFAP immunoreactivity affecting those brain regions where hypomyelination has shown to be more severe. It has been demonstrated that the myelination defect in \textit{taiep} rats is evident in the spinal cord ventral columns at birth and widespread throughout all brain regions during the first year of life [11]. Our results suggest that axon demyelination in \textit{taiep} rats would lead to marked astrogliosis, beginning in those brain regions first myelinated such as the brainstem and spreading to other regions as the myelin defect progress. This suggestion is supported by the finding of a correlation between reactive astrogliosis and oligodendrocyte cytoskeleton alteration in the optic nerve of \textit{taiep} rats [8].

Since the number of astrocytes in the brain of \textit{taiep} rats has been shown to be similar to that of normal rats [8,11], the increased GFAP immunoreactivity could then be due to both the increment in cytoskeleton proteins and the hypertrophy of astrocytes. In line with this suggestion, the astrocyte population and morphometric parameters were found unaltered in the cerebral cortex of \textit{taiep} rats (data not shown). It has been reported that motor alterations of \textit{taiep} rats progress with the age of the animal, but the typical signs such as tremor, ataxia, tonic immobility episodes and audiogenic epilepsy are only observed in rats.
older than 6 months [7]. At this age, the presence of reactive astrocytosis was found here in diencephalic structures such as the striatum thus suggesting the association of reactive astrocytosis with functional alterations in those structures involved in the motor control.

The presence of reactive astrocytosis in taiep rats is in good agreement with that found in other dysmyelination animal models, such as jimpy and shiverer mutant mice [1]. It has been suggested that reactive astrocytosis reflects astrogial activation, process involving the expression of many new molecules undetectable in quiescent astrocytes as well as the upregulation of a variety of molecules, in addition to GFAP, that are found in resting astrocyte at lower levels [4]. In vitro studies have shown that taiep astrocytes release nitric oxide and tumor necrosis factor α (TNFα) in response to lipopolysaccharide stimulation significantly higher than do normal astrocytes [9]. Nitric oxide or pro-inflammatory cytokines such TNF-α released by activated astrocytes might aggravate the demyelination process and contribute to microglia-derived neurotoxicity [13]. However, it has also been suggested that reactive astrocytes could attenuate microglia-derived neurotoxicity by releasing oligodendrotrrophic factors such as insulin-like growth factor, ciliary neurotrophic factor and platelet-derived growth factor thus contributing to the remyelination [5,6,10]. Due to the long survival of taiep rats, these animals could be useful to further understand the functional significance of reactive astrocytosis in the demyelinating diseases.

References


