Immunohistochemical Detection of Laminin and Vimentin in the Thalamic VB Nucleus after Ablation of Somatosensory Cortex in the Rat

By

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—Received for Publication, August 30, 1989—

Key Words: Ventrobasal nucleus, Scar formation, Vimentin, Laminin

Summary: The ventrobasal (VB) nucleus has been studied after ablation of somatosensory cortex in 62 adult rats by the application of both vimentin and laminin immunoreactivity.

Both vimentin- and laminin-immunoreactivity are confirmed in reactive astrocytes (GFAP-positive cells) in the affected VB area and its surroundings. The vimentin immunoreactivity first gives rise in the affected VB at seven days postlesion and appears more active in its surrounding area at fourteen day postlesion. At the twenty-eight days, vimentin-positive astrocytes are reduced in cell volume and their processes become thin. The laminin immunoreactivity is also first detected in the affected area at seven days postlesion and spread in its surrounding area at fourteen days postlesion. At the twenty-eight days, laminin-positive astrocytes are reduced in cell volume and their processes become thin. The time course of both vimentin and laminin immunoreactivity correlates with the degree of astrocytic hypertrophy. Acquisition of vimentin is one of the typical astroglial reactions by brain injury (5). The laminin in the reactive astrocyte contributes to the temporal activation for the regeneration. But the neurons in the affected VB area mostly die. And reactive domain in its surrounding area, will support the repair of affected VB area and form glial scar. The induction of laminin might be involved in scar formation (8).

The gliosis and tissue repair in the brain are unique processes due to the participation of a CNS-specific cell, the astrocyte (5). Within the CNS, vimentin represents the principal constituent of intermediate filaments (1F) found in astrocytes (4, 6, 7, 16, 18, 20). The research for distribution and location of 1F constituents may give some information about the initial period of gliosis. Damage to the brain also induces neuronal survival-promoting factors in the damaged area where such factors may be involved in keeping neurons and glia alive (14). Laminin, a component of extracellular matrix, is a candidate of such factors, which has recently drawn particular attention for its potent stimulating effect on neurite growth in vitro (2, 8, 11) and in vivo (1, 8, 9, 13, 15). Although, laminin induction has not yet been studied in the CNS, when a domain suffers both the deafferentation and the retrograde effect. In the present study, we pursued immunohistochemically the time course of laminin and vimentin induction with reference to astrocytes in the thalamic VB nucleus of the rat following ablation of somatosensory cortex.

Materials and Methods

Sixty-two Wistar strain male rats (7 weeks, body weight 150–170g) were used in this investigation. Sodium-pentobarbital (40mg/kg) was administered into their abdominal cavities. Somatosensory cortical fields were unilaterally removed by suction. The rats were allowed to survive for the periods of 5 (n = 5), 7 (n = 10), 10 (n = 10), 14 (n = 15), 28 (n = 10) and 56 (n = 12) days. Under deep sodium-pentobarbital anaesthesia, the animals were perfused through the left ventricle with periodate-lysine-paraformaldehyde solution (0.01M NaI04-0.075M phosphate buffer-4% paraformaldehyde, pH 6.2). Brains were removed and placed in the fresh perfusate at 4°C for 1 hour. Blocks containing thalamus (5mm in thickness) were dissected out from the brain, dehydrated and embedded in soft paraffin. Serial sections (4 µm) were made. The sections were dried (50°C), deparaffinized in xylene and rinsed in ethanol (100%). Serial four sections were processed with Nissl stain for cytoarchitecture, immunohistochemical technique (ABC method) for laminin, GFAP and vimentin. The thalamic nuclei contralateral to the operation were checked and used to controls.

The immunohistochemical technique (11) was used to identify the presence of laminin, GFAP and vimentin. the primary antibody of laminin was rabbit-antiserum (E-Y Laboratories) 1:100 in PBS for 1 hour. The primary antibody of GFAP was rabbit-antiserum
Results

I. Normal and contralateral VB nuclei (controls)

In light microscopic preparations stained with 0.1% thionin (pH 3.8), various types of neurons, e.g. round and fusiform, were observed in the VB nucleus on the opposite side and the non-operated controls. Normal or unaffected side was negative for laminin and vimentin except astrocytic nuclei (Figs. 2 and 4). GFAP immunoreactivity of control tissue was observed in thin processes of astrocytes, which was faint (Fig. 2).

II. Ipsilateral VB nucleus to the lesion site

It was clearly demonstrated that positive reactivity to each antiserum occurred in the neuropile of the affected side. Positive reactions of laminin, GFAP and vimentin were evidenced in the VB nucleus by the presence of the reddish-brown precipitates of oxidised DAB. The intensity of these reactions was scored arbitrarily as negative, faint, weak, moderate or strong depending on the stain density of precipitates.

The affected VB areas at five days postlesion, the VB neuron almost looked the same as controls, except for the decrease of basophilic substances in the cytoplasm. The immunoreaction to each antiserum, the stainability of astrocytic nuclei was increased. And astrocytic cell volume was slightly increased. At this time, astrocytes in the VB nucleus were detectable as round or oval and no process. Laminin- and vimentin-positive astrocytes were more numerous than GFAP-positive astrocytes. The immunoreactive astrocytes of each peptide at this time increased than those of controls. At seven days postlesion, having many damaged neurons both round and fusiform, became easily demarcated from the adjacent tissue containing normal neurons. Damaged neurons in the affected area could be distinguished from normal ones by eccentricity of the nucleus and declined stainability of the cytoplasm (Fig. 5). Prominent morphological change was the neovascularization at seven days postlesion (Figs. 5, 6, 7 and 8). The number and volume of each peptide-positive structures at this time, were greater than at five days (Figs. 6, 7 and 8). They were restricted to the affected VB area. Some GFAP-positive astrocytes showed one or two main processes (Fig. 6). At the ten days, GFAP-positive astrocytes became star-shaped and hypertrophied in appearance. And GFAP-positive structures were detectable as some dots in the affected neuropile except astrocytic cell body. Vimentin- and laminin-positive astrocytes became hypertrophy. But vimentin- and laminin-positive structures were confined to the main processes of astrocytes and could not be identified in the delicate framework of glial fibrils demonstrated with GFAP antiserum. The neovascularization in the affected area was in progress and the caliber of their capillaries was enlarged. At this time, the neuropile in the affected VB area was basophilic, that is weak, not well-marked stain. At fourteen days postlesion, an extensive neovascularization was present in the affected site, where was formed innumerable cystic cavities (Figs. 9, 10, 11 and 12). The caliber of their cystic cavities at this time, was rather decreased than that at the ten days. The majority of laminin-positive astrocytes at this time, became star-shaped and more hypertrophied in appearance than at the ten days (Fig. 10). These reactive astrocytes were existent in the affected VB nucleus, but many of them were in its surrounding area. Reactive astrocytes of the peripheral area were observed more hypertrophy than those of the affected VB area. GFAP-positive astrocytes were also star-shaped and its cytoplasmic volume was greater than that at the ten days (Fig. 11). Many GFAP-positive dots were observed in the affected area. GFAP-positive reaction was shown as sharp structures than laminin-positive reaction (Figs. 10 and 11). Vimentin-positive astrocytes at the fourteen days, were more hypertrophied than that at the ten days (Fig. 12). And many patched vimentin-positive structures were observed. It seemed that many of them were vimentin-positive astrocytic nuclei (Fig. 9). Cytoplasmic laminin- and GFAP-immunoreactivity in astrocytes showed both cell body and its processes. But the stainability of astrocytic nuclei was higher than that of its cell body. At this time, many microglial cells and some atrophied nerve cells were seen in the affected area (Fig. 9). The affected area of Nissl stain sections showed basophilia, that is not well-marked stain (Fig. 9). At twenty-eight days postlesion, GFAP-positive astrocytes were still detectable. Although these astrocytes were not observed hypertrophied in appearance and reactive processes became thin. The number of processes at this time was larger than those of controls. Vimentin- and laminin-positive astrocytes were not hypertrophied. By the observation of Nissl stain at this time, there was a clear reduction in VB neurons with further increase in the number of microglial cells. And a clear decrease of budding capillaries in the tissue of the affected site was observed. At fifty-six days postlesion, the immunoreactivity to both laminin and vimentin were decreased

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Abbreviations in this paper are mostly taken from the atlas of Paxinos and Watson.
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to normal level (Figs. 14 and 16). Weak GFAP-positive immunoreactivity was observed in thin processes of reactive astrocytes (Fig. 15). Neovascularization did not appear any longer in the affected site. By the observation of Nissl stain at this time, the affected area was filled up with microglial cells (Fig. 13).

The time course of both laminin and vimentin immunoreactivity correlated with the degree of astrocytic hypertrophy in the thalamic VB nucleus after ablation of somatosensory cortex in the rat. The degree of astrocytic hypertrophy was determined by both morphological change and cell volume. Both laminin- and vimentin-immunoreactivity in the thalamic VB nucleus was first detected at the seven days and the intensity was maximum at the fourteen days (Table 1). Thereafter, at twenty-eight days postlesion, each antiserum immunoreactivity was negative and/or faint (Table 1). Astrocytic hypertrophy first gave rise in the affected VB nucleus at seven days postlesion and appeared more active in its surrounding area at the fourteen days. At the twenty-eight days, the majority of reactive astrocytes were reduced in cell volume and each antiserum positive processes became thin. At this time, it was difficult to distinguish between affected VB area and its surroundings. And reactive domain became smaller than at the fourteen days. The degree of astrocytic hypertrophy was based on the period of postlesion time, but not the extent of the affected VB area.

In our experiment, some cortical ablation reached the peripheral area containing somatosensory cortex. In such cases, the affected domain was VB and a part of VPL. Although reactive patterns of the affected area in each case were almost the same as described above.

Table 1. Changes in the thalamic VB nucleus of the affected area

<table>
<thead>
<tr>
<th></th>
<th>Nissl</th>
<th>Laminin</th>
<th>Vimentin</th>
<th>GFAP</th>
<th>Neovascularization</th>
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<tr>
<td>5 days</td>
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<td>+/-</td>
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<td>7 days</td>
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<td>10 days</td>
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<td>14 days</td>
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<td>28 days</td>
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<td>56 days</td>
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1) Nissl stain showed basophilic intensity as negative (-), weak (+), moderate (+ +), strong (+ + +) stain in the neuropile.
2) The intensity of these reactions was scored as negative (-), faint (+/-), weak (+), moderate (+ +), strong (+ + +) immunoreactivity of reactive astrocytes.
3) Weak GFAP immunoreactivity was observed in the control tissue. Therefore the intensity of the affected area was scored by reducing the intensity of control. That is as negative (-), faint (+/-), weak (+), moderate (+ +), strong (+ + +).
4) The increase rate of neovascularization is shown as negative (-), very low (+ /), low (+), intermediate (+ +), high (+ + +) rate.

Discussion

The somatosensory cortical fields and the ventro-basal (VB) nucleus of the thalamus have been reported to be connected with each other\(^3\),\(^19\). After ablation of somatosensory cortex in the rat, VB neurons suffer both the deafferentation and the retrograde effect. We examine complex effect as a whole.

Following injury, astrocytes are readily observed in the affected area as GFAP-positive cells with many robust, occasionally branched processes. Our observations with respect to the general morphology of such cells broadly agree with previously published report\(^12\). Intensely GFAP-positive astrocytes are referred as reactive astrocytes in this paper. The present results show that laminin is induced in the thalamic VB nucleus, where it is normally absent from astrocytes, because of the ablation of somatosensory cortex. The induction of laminin coincides both with the morphological changes in glial cells and the expression of GFAP in reactive astrocytes\(^10\). We find that laminin immunoreactivity in the thalamic VB nucleus is first detected at seven days postlesion, becomes a maximum at fourteen days and subsides within twenty-eight days (Table 1). Liesi P. et al. (1984) have reported that laminin appears in glial cells within 24 hours after injury and subsides at the five days in the adjacent area of the kainic acid injection\(^10\). Therefore it may be indicated that laminin induction depends on both kinds of damage and the distance of the affected site. Namely, the laminin induction in our experiment, is directly due to deafferentation and not directly due to retrograde effect. The time course of laminin produc-
tion is also parallel with the time course of neovascularization (Table 1). The increase in size of capillary and potentially the number of cystic cavity induced by degeneration may have a role in the repair of the affected tissue\(^{13}\). Our study also shows that cortical lesion results in the formation of a cystic cavity which is ensheathed by glial cells\(^ {17}\). This region is shown to include astrocytes that secrete laminin for at least seven days postlesion. In our study, laminin-positive astrocytes spread to the peripheral area from the affected VB area at the fourteen days. And at the twenty-eight days, affected area seems to become atrophied. At this time, the adjacent Nissl stain sections of areas within the affected matrix provide evidence of increased gliosis. It is conceivable that continuous expression of laminin in astrocytes correlates with regeneration, and that the laminin may be one of the key-factors allowing neuronal growth in the CNS in vivo\(^ {2,9}\). In some reports, it has been obvious that laminin contributes to the regeneration and/or the repair\(^ {1,2,4,9,10,11,13}\). Simultaneously, laminin-positive reactive astrocytes form basal lamina\(^ {12}\).

In our results, astrocytes in the affected area also accumulate vimentin when they react to injury. All reactive astrocytes contain various amount of GFAP and many of them express vimentin\(^ {10}\). And the time course of vimentin induction is the same as that of laminin induction. Namely, vimentin immunoreactivity in the thalamic VB nucleus is first detected at seven days postlesion, reaches a maximum at fourteen days and subsides within twenty-eight days (Table 1). The cytoplasm of laminin- and GFAP-positive reactive astrocytes, is shown to be more intensively stainable than its nucleus. But the cytoplasm of vimentin-positive reactive astrocytes, is shown to be more weakly stainable than its nucleus. And many vimentin-positive nuclei are observed as patched stains in the affected neuropile. Ciesielski-Treska J. et al. (1988) have observed that vimentin appears primarily close to nuclei, and that filaments of vimentin extend into proximal segments of the cell processes by immunofluorescence procedure\(^ {9}\). Perinuclear accumulation of vimentin filaments may be considered indicative of association with the nuclear function\(^ {20}\). The rapid accumulation of GFAP, the acquisition of vimentin, the increase in the diameter of cell bodies and the resumption of proliferation, are typical of the astroglial reaction in adult lesioned brain\(^ {5}\). And 1F composed of vimentin and GFAP are related to microtubules with respect to their cytoplasmic organization\(^ {9}\). Presently, the cell biological role of 1F is still speculative. 1F may serve as the mechanical integrators of cytoplasmic components, as a system for organelle movement, or as a structural pathway for cellular metabolism such as protein synthesis\(^ {20}\).

In conclusion, it is assumed that astrocytes in the affected area are temporarily activated for the regeneration of its area, but the neurons in the affected area mostly die. And reactive astrocytes in its surrounding area, will support the repair of the affected VB area and form glial scar. The induction of laminin in reactive astrocytes might be involved in scar formation\(^ {9}\).

Acknowledgement

We thank Dr. M. Fujii for her many useful suggestions on this study.

References

15) Rogers, S. L., Edson, K. J., Letourneati, I., and Niel, oon, 1., and Edson, K. J., Letourneati, I., and Niel, oon, 15)
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Explanation of Figures

Plate I

Four serial sections for Nissl stain (Fig. 1), laminin (Fig. 2), GFAP (Fig. 3) and vimentin (Fig. 4) in normal adult rat brain. Astrocytes in normal thalamic VB nucleus, are negative for both laminin and vimentin. In normal astrocytes, faint GFAP immunoreactivity is observed in some thin processes (Fig. 3 arrows). Asterisk shows the same capillary. Bar: 100 µm.
Plate II

Four serial sections for Nissl stain (Fig. 5), laminin (Fig. 6), GFAP (Fig. 7) and vimentin (Fig. 8) in the affected VB nucleus at the seven days. The volume of each peptide-positive structures at this time is slightly increased (Figs. 6, 7 and 8 arrows). Prominent morphological changes at the seven days is the neovascularization (Figs. 5, 6, 7 and 8). Asterisk shows the same capillary. Bar: 100 μm.
Plate III

Four serial sections for Nissl stain (Fig. 9), laminin (Fig. 10), GFAP (Fig. 11) and vimentin (Fig. 12) in the affected VB nucleus at the fourteen days. The majority of laminin-, GFAP- and vimentin-positive astrocytes become hypertrophied (Figs. 10, 11 and 12 arrow 1). The affected area at this time, shows basophilia, that is not well-marked stain (Fig. 9). Vimentin-positive astrocytic nuclei (Fig. 12 arrow 2). Asterisk shows the same capillary. Bar: 100 μm.
Plate IV

Four serial sections for Nissl stain (Fig. 13), laminin (Fig. 14), GFAP (Fig. 15) and vimentin (Fig. 16) in the affected VB nucleus at the fifty-six days. At fifty-six postlesion, there is a clear reduction in VB neurons with further increase in the number of microglial cells (Fig. 13). Both laminin- and vimentin-immunoreactivity are negative (Figs. 14 and 16). Weak GFAP-positive immunoreactivity is observed in thin processes of reactive astrocytes (Fig. 15 arrows). Asterisk shows the same capillary, Bar: 100 μm.