LABORATORY INVESTIGATION

J Korean Neurosurg Soc 41 : 30-38, 2007

The Role of Aquaporin-4 in Cerebral Edema Formation after Focal Cerebral Ischemia in Rats

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Objective : To elucidate the role of aquaporin-4(AQP4) in cerebral edema formation, we studied the expression and subcellular localization of AQP4 in astrocytes after focal cerebral ischemia.

Methods : Cerebral ischemia were induced by permanent middle cerebral artery(MCA) occlusion in rats and estimated by the discoloration after triphenyltetrazolium chloride(TTC) immersion. Change of AQP4 expression were evaluated using western blot. Localization of AQP4 was assessed by confocal microscopy and its interaction with α-syntrophin was analyzed by immunoprecipitation.

Results : After right MCA occlusion, the size of infarct and number of apoptotic cells increased with time. The ratio of GluR1/GluR2 expression also increased during ischemia. The polarized localization of AQP4 in the endfeet of astrocytes contacting with ventricles, vessels and pia mater was changed into the diffuse distribution in cytoplasm. The interactions of AQP4 and Kir with α-syntrophin, an adaptor of dystrophin complex, were disrupted by cerebral ischemia.

Conclusion : The deranged spatial buffering function of astrocytes due to mislocalized AQP4/Kir4.1 channel as well as increased assembly of Ca^{2+} permeable AMPA receptors might contribute to the development of edema formation and the excitotoxic neuronal cell death during ischemia.

KEY WORDS : Brain edema · Cerebral infarction · Aquaporin 4 · KIR receptors · α-syntrophin · Apoptosis.

Introduction

Cerebral infarction is a major cause of neurological deficits, and results from the reduction of cerebral blood flow due to the occlusion in cerebral blood vessel. If the occlusion is not rapidly reversed, it induces apoptosis and necrosis of cerebral tissue[31]. Although there are several mechanisms of neuronal death in cerebral infarction, the alteration of extracellular ions is considered to be one of the major causes of cellular damage[19,20]. Astrocytes contribute the ionic, neurotransmitter and water homeostasis of brain by uptaking K^+ and glutamate with their accompanying water[33]. There is growing evidence that astrocytes contribute to pathophysiology of neuronal damage during cerebral ischemia[3,17,28,33].

Cerebral edema is associated with many intracranial neuropathological states including cerebral infarction and results from loss of water homeostasis entailing a net increase of water influx into the brain parenchyma. The exact route of water influx in this life-threatening condition is still unknown and no efficient therapy exists.

The movement of water across biological membranes occurs through water channel aquaporins (AQPs)[35]. AQPs are a family of transmembrane proteins that selectively allow the passage of water through the plasma membranes, and AQP4, the predominant form in brain, is highly expressed in astrocytic endfeets near capillaries and in ependymal cells lining the ventricles which are the key sites for water movement between brain and blood or CSF[8,15,25,26]. Recently, it was demonstrated that α-syntrophin, a member of the dystrophin-dystroglycan complex, induces the aggregation and stabilization of inwardly rectifying K^+ (Kir 4.1)/AQP4 channels in astrocyte, which suggests functionally coupling of K^+ buffering and water homeostasis in brain[2,9].

In this study, to elucidate the role of water channels in cerebral edema formation, in particular focusing on functional coupling with K channel, we examined the changes in AQP4

• Received : June 14, 2006 • Accepted : October 2, 2006

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localization and its anchoring by α-syntrophin in astrocytes after focal cerebral ischemia.

Materials and Methods

Experimental animal groups

Twenty nine Sprague-Dawley Rats (8~9 weeks old) with body weights of 250~350gm were participated in the study. Among them, brain tissue of five rats was dissected to serve as a control : to assess the normal findings of rat brain by TTC immersion and Hematoxylin-Eosin(HE) stainings (n=1), and by immunoprecipitation (n=2), and to survey the normal finding of rat brain for immunofluorescent study (n=2). The rest of rats were divided into three groups based on the ischemic duration (2, 6, and 24-hour) and eight rats per group were used : to assess the extent of cerebral infarction (n=2 in each time period), and to examine the change of the expression of AQP4 and the change of the binding of α-syntrophin and AQP4 as well as Kir channel (n=3 in each time period), and for TUNEL staining and the assessment of the distribution of AQP4 and GFAP (n=3 in each time period).

Occlusion of the blood flow in the middle cerebral artery (MCA)

Anesthesia was induced by intraperitoneal injection of chloral hydrate (90mg/Kg), and the maintenance of respiration was carried out by the self-breathing of room air. The body position of rat was changed to the left lateral recumbent position, sterilized with 2% zephanon solution, and under surgical microscope, the skin incision was made from the lower portion of the right auricle to the lower margin of the right eyeball along the zygomatic arch, and then the zygomatic arch was removed. The temporalis muscle and the periosteum were dissected from the skull using microscissors and scalpels. After assessing the trigeminal nerve, the directly antero-lateral area of the foramen ovale was perforated using a diamond drill (Ø 4×4mm). Subsequently, the proximal area of the right middle cerebral artery(MCA) immediately prior to crossing the olfactory bulb was exposed and resected completely from adjacent tissues. Using an electrocautery, the MCA was coagulated, and subsequently resected using microscissors, and the permanent MCA occlusion(MCAO) model was made. During experiments, a probe measuring body temperature was inserted to the rectum of rat and body temperature was maintained at 37±0.5℃ using a homeostatic blanket control unit (Harvard, USA). After suturing the incision area, rats were kept in a conventional breeder, and constant temperature and humidity were maintained until their sacrifice.

Assessment of the range of cerebral infarction

After the peritoneal injection of chloral hydrate, the rats were sacrificed by injecting hypertonic KCl solution to the heart, and the brain was extracted rapidly within 5 minutes. The olfactory lobe of the extracted brain was removed, and using a rodent brain matrix (ASI, Rodent Brain Matrix), the 2mm-interval anterior and posterior coronal sections based on the crossed area of the optic nerve were made and 7~8 sections were prepared. Under the light-blocked condition, these sections were stained with 2% triphenyltetrazolium chloride solution (TTC : Sigma, USA), turning approximately every 10 minutes, and soaked for 30 minutes. After confirming the cerebral infarction area, pictures were taken from a constant distance using a digital camera, washed with distilled water, and fixed with 10% formalin.

Preparation of rat brain protein

After decapitation, rat brain was transferred immediately to ice-cold Hepes buffered saline (HBS; 142mM NaCl, 2.4mM KCl, 1mM MgCl2, 5mM D-glucose, 0.1mM EGTA and 10mM Hepes [pH 7.5]) and left in 20℃ for 10 min. Brain was homogenized with Ultra-Turrax T25 homogenizer (Jandel & Kunkel, Germany) in the homogenization medium (320mM Sucrose, 1mM EGTA, 0.1mM EDTA and 10mM Hepes [pH 7.5]) containing the protease inhibitor mixture (0.3mM phenylmethylsufonyl fluoride, 2g/ml leupeptin, 4g/ml aprotinin and 0.8g/ml pepstatin A). Nuclei and debris were removed after low speed centrifugation (2,500g for 10 minutes) at 4℃ and the postnuclear supernatant was incubated with 2% Tween 20 for 1 hour in ice to extract membranous proteins. The protein concentration of the final suspension was measured using Bradford's method.

Western blotting

The rat brain membrane proteins were lysed in boiling 5x sodium dodecyl sulfate(SDS) sample buffer. The lysates were boiled for 5 minutes, and loaded in each lane (20㎍ per lane) to be separated by SDS-polyacrylamide gel electrophoresis(PAGE). After transferring the proteins onto a nitrocellulose membrane, the membrane was blocked with Phosphate-buffered saline (PBS; 137mM NaCl, 2.7mM KCl, 10mM Na_2HPO_4, 2mM KH_2PO_4 [pH 7.4]) containing 2% skim milk overnight at 4℃ then incubated with primary antibodies (AQP-4 (Serotec, Kidlington, Oxford, UK), GluR1 and GluR2 (Chemicon International Inc, Temecula, CA, USA)) for 1 h at room temperature. After washing three times for 10 minutes each with PBST (PBS, 0.05% Tween 20), the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (3000:1, Amersham Life Science Ltd., Buckingamshire, England) for 1 hour at room temperature. After washing three times with PBST, the membranes were developed and quantified with an enhanced chemiluminescence detection system (LAS-3000, Fuji, Japan).

Immunofluorescent microscopy for AQP4 and GFAP

The localization of AQP4 and GFAP was assessed by double immunolabeling of rat brain sections. Freshly isolated brain was frozen and cut into 8mm sections by vibratome. The frozen sections were fixed with 4% paraformaldehyde for 15 minutes. After permeabilization with 0.2% Triton X-100 in PBS, the sections were blocked with the blocking solution (0.2% Triton X-100 containing 5% BSA, 5% horse bovine serum). The sections were labeled with primary antibodies against GFAP (Dako, Glostrup, Denmark) and AQP4 for 1 hour at room temperature and washed 5 times with PBS. Secondary antibodies of anti-rabbit IgG conjugated Texas Red or anti-mouse IgG conjugated FITC (Molecular Probe, Eugene, OR, USA) were added for 45 minutes at room temperature. After washing 3 times, the slides were mounted with Universial mount and examined under the confocal laser scanning microscope (LSM 510, Carl Zeiss, Germany).

Assessment of neuronal damage with TUNEL and propidium iodide (PI) staining

Neuronal damage was assessed by direct fluorescence detection of digoxigenin labeled genomic DNA in the brain sections with simultaneous nuclear staining using Apop Tag *in situ* apoptosis detection kit (Oncor, MD, USA). Double-stranded DNA breaks identifying apoptotic cells were detected *in situ* by the TUNEL technique on tissue sections which had been fixed in formalin and embedded in paraffin. After deparaffinization with xylene, hydration in graded ethanols and washing in PBS, the sections were treated with 20mg/ml proteinase K (Boehringer Mannheim Gmbh, Mannheim, Germany) for 30 minutes at room temperature. After washing, endogenous peroxidase activity was quenched with 2% hydrogen peroxide (Sigma, St. Louis, MO, USA). The tissue was then reacted with terminal deoxynucleotidyl transferase(TdT) and digoxigenin-labeled UTP, followed by fluorescein-labeled anti-digoxigenin antibody. PI stain was used for counter stain and mounted under a glass coverslip. The slides were observed under the confocal microscope.

Immunoprecipitation

The 500μg of membranous proteins were precleared of immunoglobulin by incubation for 1 hour at 4°C with protein A/G Sepharose beads (Amersham Life Science Ltd., Buckingamshire, England), which were then removed by centrifugation. The supernatants were incubated with 0.2~2μg of anti-α syntrophin overnight at 4°C on a mechanical rotator. Protein A/G Sepharose beads were then added for 1 hour at 4°C, washed four times with RIPA buffer (50mM Tris/HCl [pH7.5], 150mM NaCl, 1% NP-40, 0.5% deoxycholic acid, 0.1% SDS, 1mM pheylmethylsulfonyl fluoride, 1mM EDTA, 1ug/ml leupeptin, 1ug/ml aprotinin, 1ug/ml pepstatin) and resuspended in SDS-PAGE loading buffer. Samples were subjected to gel eletrophoresis and Western blot with anti-AQP4 or anti-Kir using the same method described above.

Results

Infarct volume

After permanent MCAO, location of cerebral infarction was assessed by TTC immersion. The infarction was induced in the area around the optic chiasm and encompassed from 4mm anterior to 6mm posterior area of the optic chiasm in the right hemisphere (Fig. 1). The infarction volume increased in proportion to the ischemic duration. In HE staining, the amount of tissue necrosis was also increased with time after MCAO (Fig. 2). The dilation of blood vessels and sinuses was observed after MCAO, which suggests development of cerebral edema.

Excitotoxic brain injury

To assess the brain damage after MCAO-induced ischemia, apoptotic level at the various time points was examined by using TUNEL staining (Fig. 3). Apoptosis begined as early as 2 hours after MCAO, was prominent after 6 hours. Up to 80% of nerve cells showed apoptotic pattern at 24 hours after MCAO, indicating that serious cell death was induced by cerebral ischemia.

To investigate whether cell death caused by overstimulation of excitatory glutamate neurotransmitter or its receptors, so called excitotoxicity, play a critical role in ischemic brain injury, the change in expression ratio of AMPA-type glutamate re-

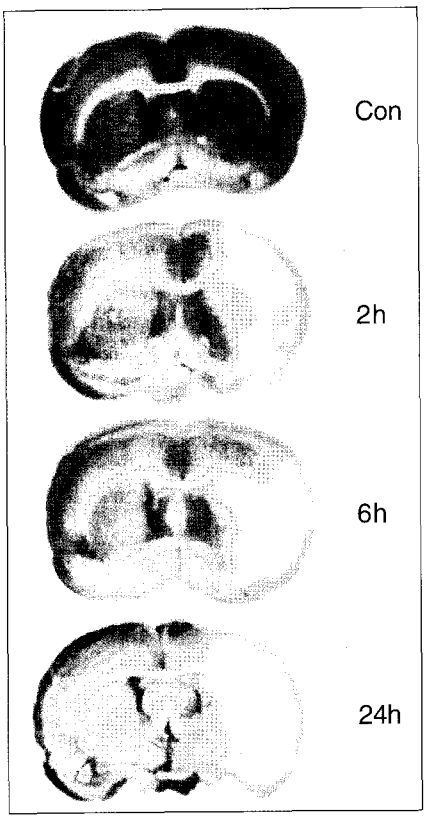

Fig. 1. Cerebral infarct assessed by triphenyl-tetrazolium chloride(TTC) immersion after right middle cerebral artery(MCA) occlusion. Brain sections were obtained after indicated time for TTC immersion. The size of infarct (unstained areas in TTC stained right hemisphere, whitish-yellow color) shows an increase with time after MCA occlusion (Con : control).

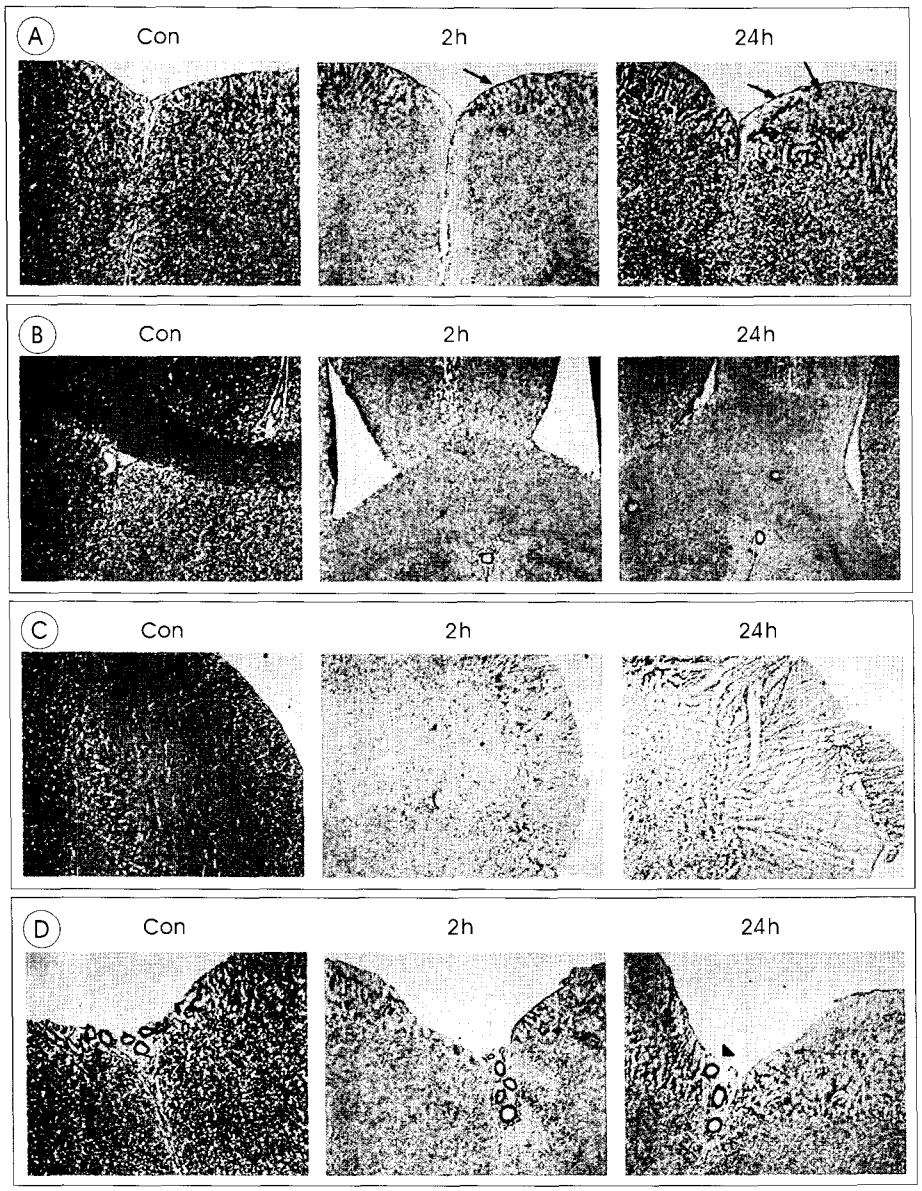

Fig. 2. Cerebral infarct assessed by Hematoxylin–Eosin(HE) stain after right middle cerebral artery(MCA) occlusion. Fresh frozen brain sections were obtained after indicated time and stained by HE dye. The volume of postischemic hemisphere is increased with time(A, arrow). The ventricular size is enlarged at 2hr and then decreased at 24hr. This finding is due to leakage of CSF from ventricular space to subcutaneous space during development of cerebral edema(B). The amount of tissue necrosis in infarct area (C) and the size of vessels(D, arrowhead) are increased with time after MCA occlusion. Note the tissue necrosis and the dilated ventricle and vessels indicative of developing cerebral edema (×20).

ceptor GluR1 to GluR2, was investigated after MCAO-induced cerebral ischemia (Fig. 4). Two hours after induction of cerebral ischemia, GluR1 expression in the ischemic right cerebral hemisphere was increased evidently compared to that of left hemisphere. However, there was no significant difference in GluR2 expression between two cerebral hemispheres.

Expression of AQP4

A confocal microscopic analysis showed that AQP4 was strongly expressed at the astrocyte feet processes near the subpial, perivascular and ependymal cell layers adjacent to ischemic region after MCAO (Fig. 5). Similar result was obtained by Western blotting that the expression of AQP4 was significantly increased in the ischemia-induced right cerebral hemisphere than the control left cerebral hemisphere (Fig. 6).

Subcellular localization of AQP4

To examine if there is any change in intracellular distribution of AQP4 after occlusion of the right MCA, colocalization study of AQP4 with the astrocyte markers, GFAP, was performed using a confocal microscope (Fig. 7, 8). In normal condition, AQP4 distributed selectively at the astrocyte feet processes near the subpial, perivascular and ependymal cell layers. However, after ischemia, in spite of increased fluorescence of AQP4 in astrocytes, polarized distribution AQP4 in the endfeet of the vascular contact surface was significantly diminished. AQP4 fluorescence rather showed diffuse pattern in the entire cytoplasm of astrocytes.

AQP4 anchoring to α-syntrophin

To examine whether the altered intracellular distribution of AQP4 after cerebral ischemia is related with the changed binding state of AQP4 with α-syntrophin which maintains the specific location and arrangement of AQP4, immunoprecipitation analysis revealed that AQP4 was present as the bound form to α-syntrophin in normal condition, but the binding of AQP4 and α-syntrophin decreased in a time-dependent manner after cerebral ischemia, and binding of Kir channel to α-syntrophin was also decreased after cerebral ischemia with similar pattern to that of AQP4 (Fig. 9).

Discussion

Cerebral edema is a major cause of death in a variety of common neurological disorders[7], and classified as cytotoxic

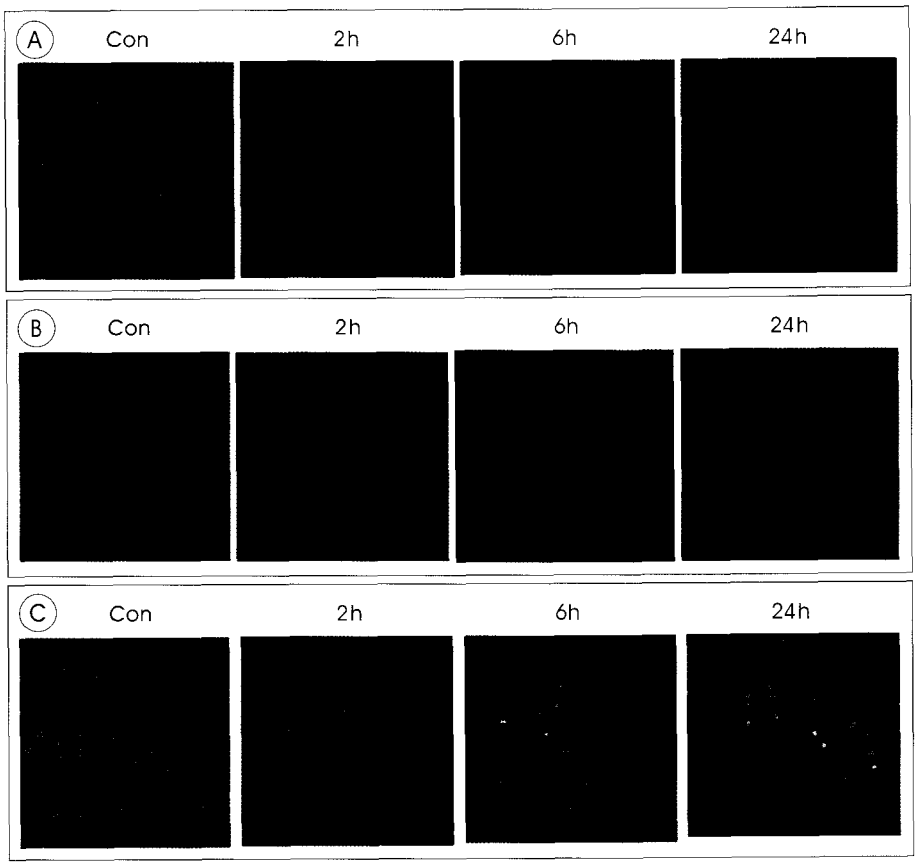

Fig. 3. Cerebral infarct-induced cell death with time course. A : Red color implies the stain of intact nuclei and the amount of stain is decreased with time after middle cerebral artery(MCA) occlusion. B : The green color designates apoptotic nuclei after MCA occlusion. The amount of stain is increased with time. C : Positive apoptotic nuclei are prominent after 6hr and full-blown at 24hr after cerebral ischemia (yellow & orange color) in the merged images.

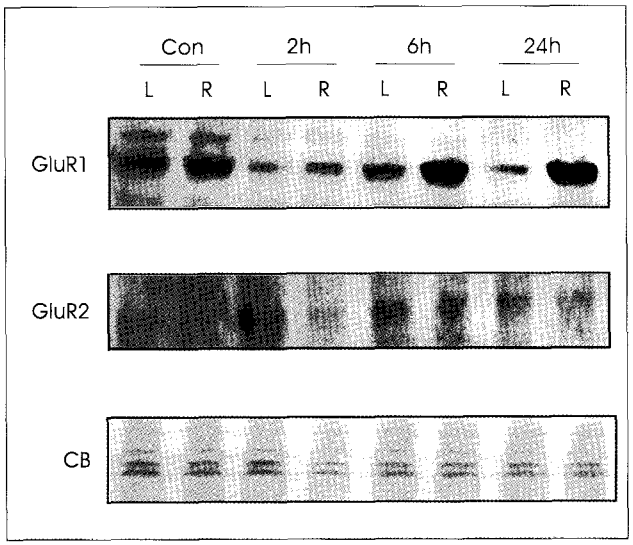

Fig. 4. GluR1 and GluR2 expression after right middle cerebral artery (MCA) occlusion. After right MCA occlusion, whole brains were obtained at the indicated time and membrane protein was extracted for western blot. The expressions of GluR1 in right hemisphere are substantially increased with time after MCA occlusion. The expressions of GluR1 are prominent at 6hr and 24hr(arrowhead). The expressions of GluR2 are not different with both hemisphere at the indicated time (L : left, R : right, CB : Commassie Blue).

and vasogenic brain edema[18]. Cytotoxic edema is an accumulation of intracellular fluid that occurs without disruption of the blood-brain barrier(BBB). In contrast, when the BBB is disrupted, plasma leaks from capillaries into the interstitium and results in vasogenic edema[16]. Cytotoxic and vasogenic edema most likely represents a continum as most pathologic processes may result in both types. For example, ischemia initially leads to cellular swelling (cytotoxic edema) with subsequent disruption of the BBB and resultant vasogenic edema[12]. Although the molecular mechanism underlying cerebral edema formation is not completely known, it is well recognized that water channel protein AQPs are the major pathway of water transport across cell membranes[35]. AQP1, AQP4 and AQP9 are known to play a major role of water formation and reabsorption in CNS[4,34,36]. Especially, AQP4 is expressed in astrocyte foot processes near blood vessels in brain as well as in oligodendrocytes surrounded with ependymal and pial surfaces in contact with CSF[8,15,25,26]. This characteristic localization suggests that AQP4 plays a critical role in brain water balance.

Normally, there is a substantial continuous water efflux out of the neural tissue since the high rate of neuronal ATP synthesis generates a huge amount of water, and since the uptake of metabolic substrates such as glucose is coupled to an influx of water. The normal route for the constitutive water efflux from neurons is via uptake by astrocytes and release into the blood or into the CSF in brain[1]. This water release from astrocyte occurs through their perivascular, subpial-facing endfeet. AQP4 water channels have been crucially implicated in mediating these water fluxes[1,23]. In support of this fact, our study also shows that AQP4 is highly expressed in subpial glial limitans, the pericapillary astrocyte feet processes and ependymal cells.

Recently, concerning the pathophysiological mechanism of cerebral edema induced by cerebral ischemia, the several studies on the role of AQP4 have been carried out[1,21,28,29]. It has been observed that the cytotoxic edema induced by ischemia is more decreased in AQP4 knockout mice and AQP4 down-regulated rat than wild type[1,21,28]. In contrast, the vasogenic

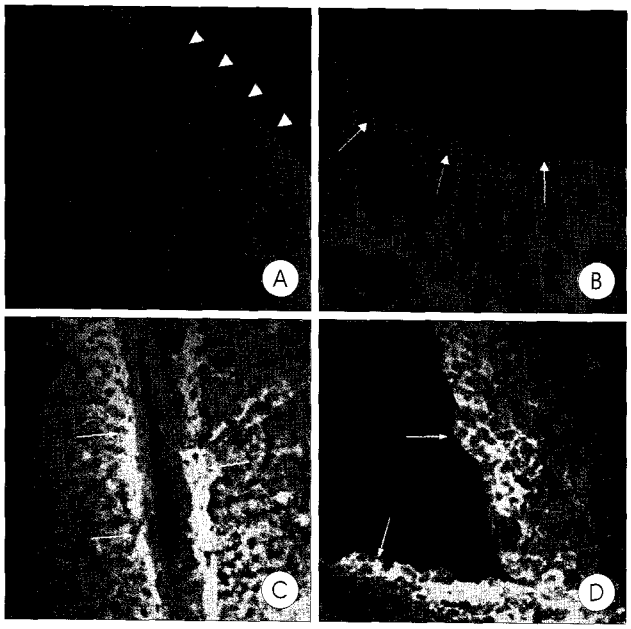

Fig. 5. Localization AQP4 in normal rat brain. AQP4 distribution in rat brain was assessed by immunofluorescent confocal microscopy. In normal rat brain, AQP4 is expressed in subpial glia limitans(A : arrowhead), the pericapillary astrocyte feet processes(B : large arrow) and ependymal cells(C&D, small arrow) (×200).

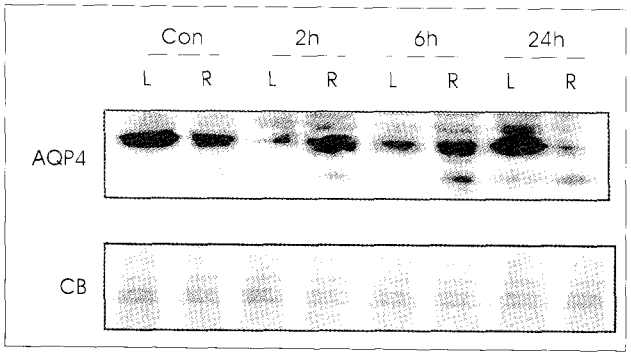

Fig. 6. AQP4 expression at the indicated time after right middle cerebral artery(MCA) occlusion. After right MCA occlusion, whole brains were obtained at the indicated time and membrane protein was extracted for western blot. The expression of AQP4 is upregulated in right hemisphere at 2hr (arrowhead). And this finding sustains for 24hr (L : left, R : right, CB : Commassie Blue).

edema is more aggravated in AQP4 null mice compared with wild type[29]. These opposing actions of AQP4 in cytotoxic and vasogenic edema are probably related to the bidirectional water transport through the AQP4 channel. In the cases of cytotoxic edema, overexpresssion of AQP4 in astrocyte endfeet may induce the cytotoxic edema in the early stage of ischemia, because the influx and efflux of water movement is produced through the intact BBB and AQP4 located in perivascular astrocyte endfeet. But in the vasogenic edema, water movement into the extracellular space is induced through disrupted BBB, which is not related with AQP4, and then efflux of water is produced to the subarachnoid space, ventricular systems and blood vessels through the AQP4 which is expressed in

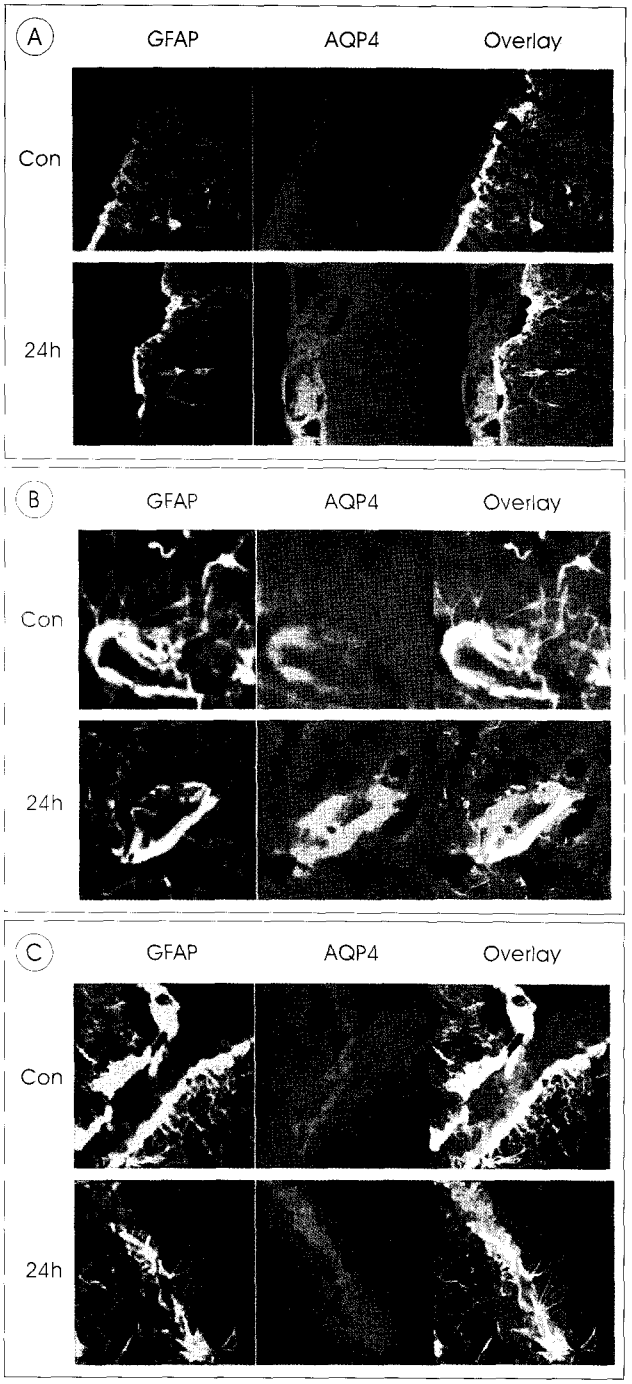

Fig. 7. Co-localization of AQP4 and Glial fibrillary acidic protein(GFAP) in rat brain after middle cerebral artery(MCA) occlusion. After right MCA occlusion, rat brain was isolated after 24hr and distribution patterns of AQP4 (FITC, green color) and GFAP (Texas-red, red color) fluorescence were assessed by immunofluorescent confocal microscopy. At the normal rat brain, AQP4 is concentrated at the astrocyte foot processes near the subpial (A), perivascular (B) and ependymal cell (C) layers. The merged images show that the colocalized AQP4 and GFAP are prominent at the interfaces of endfeet of astrocytes contacting with pia mater, capillary endothelial cell, ependymal cells (orange color). At 24hr after MCA occlusion, AQP4 is overexpressed at the subpial, perivascular and ependymal cell layers but can not be detected selectively at the astrocyte foot process and is expressed across the entire cellualar surface of astrocyte. At 24hr after MCA occlusion, the merged images show more diffuse locations of the colocalized AQP4 and GFAP signals (×200).

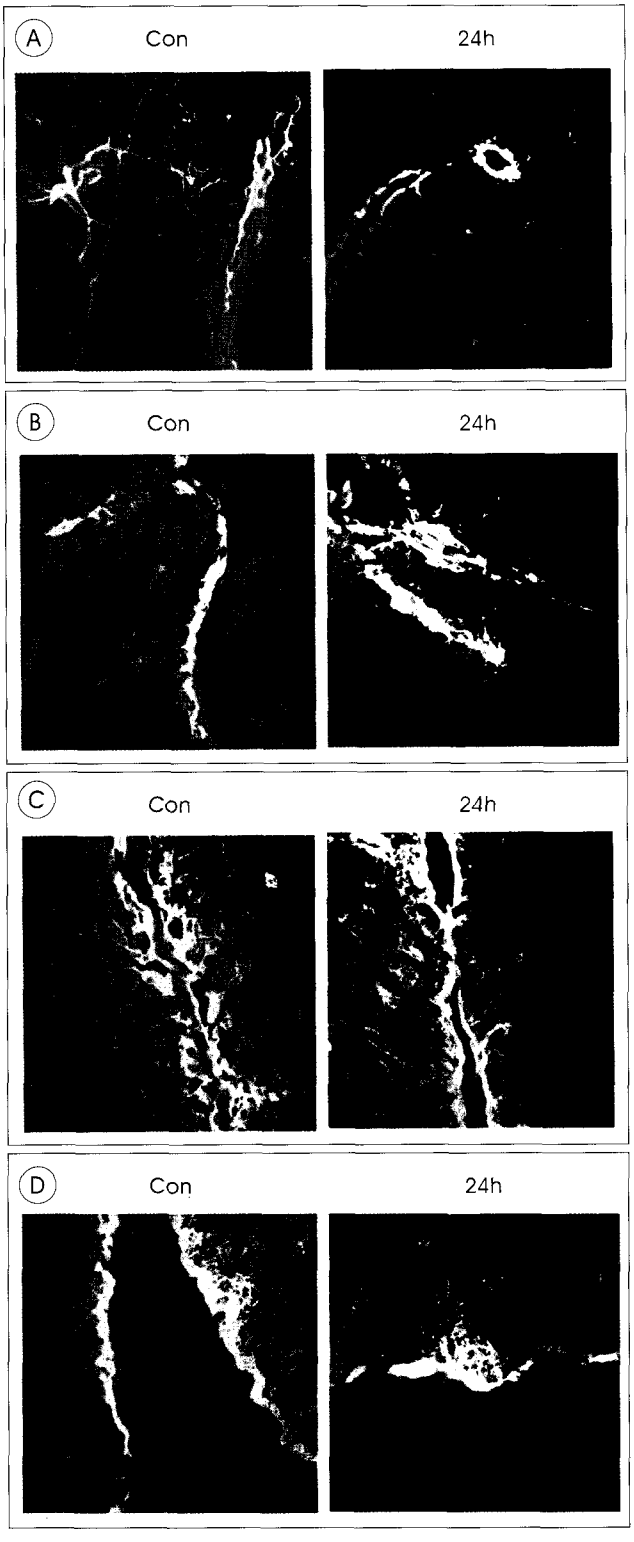

Fig. 8. Mis-localization of AQP4 after cerebral infarct. After right middle cerebral artery(MCA) occlusion, rat brain was isolated after 24hr. Co-localized AQP4 (FITC, green color) and Glial fibrillary acidic protein (Texas-red, red color) fluorescence were assessed by immunofluorescent confocal microscopy. Polarized localization of AQP4 at the interfaces of endfeet of astrocytes contacting with the small and large capillary endothelial cells(A, B), ependymal cells(C) and subpial cell(D) layers are disrupted after 24 hr of MCA occlusion at the merge images compared with those of control group (×800).

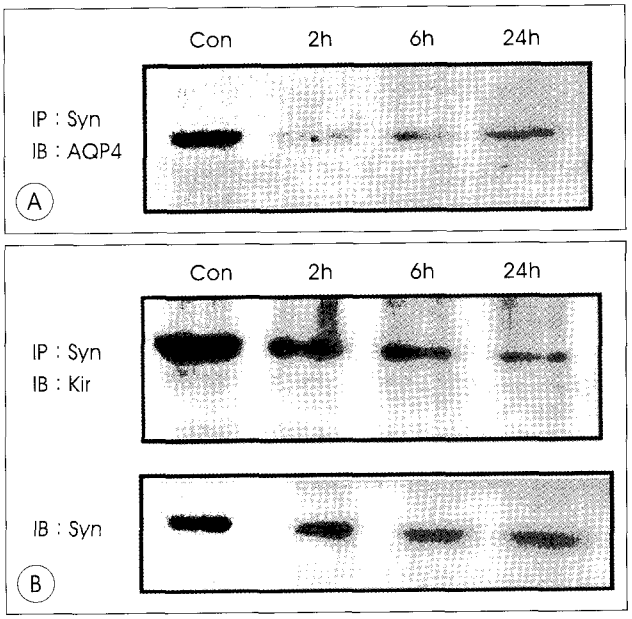

Fig. 9. Interaction of AQP4 and Kir with syntrophin of after right middle cerebral artery(MCA) occlusion. After right MCA occlusion, whole brains were obtained at the indicated time and membrane protein was extracted for immunoprecipitation. The bindings of AQP4/Syn(A) and Kir/Syn(B) are decreased with time after 2hr(arrowhead) and these findings(A & B) sustain for 24hr (IP : immunoprecipitation, IB : immunoblot, Syn : α-snytrophin).

the perivascular astrocyte endfeet. Therefore, it was suggested that the AQP4 participates in the resolution of the vasogenic edema as the pathways of the excessive fluid into the subarachnoid space, ventricles and blood vessels, considering that the vasogenic edema is aggravated in the AQP4 null mice during the ischemia[1,22,30]. In our study, the overexpression of AQP4 in the ischemic hemisphere observed during the early stage of ischemia is speculated to contribute to the formation of cytotoxic edema, and the mislocation of AQP4 in astrocytes after 24 hours might interfere with the absorption of vasogenic brain edema.

A few researches have been performed about the interaction of AQP4 and inwardly rectifying K^+(Kir) channels[11,23,27]. Recently Nagelhus et al.[23] demonstrated the coenrichment of Kir4.1 and AQP4 at the endfeet membranes of retinal Muller cells and brain astrocytes, suggesting that these molecules serve as molecular correlates for the K^+ buffering and associated water flux. The direction of water movement, associated with the osmotic gradient through AQP4 channel, is determined by the movement of K^+ ions through Kir4.1 channel[27]. Therefore, the mechanism of postischemic cytotoxic edema induced by the disturbance of water movement is associated with the dysfunction of the Kir channel in astrocyte[2,5,11,23,27,32,33]. During cerebral ischemia, in which AMPA glutamate receptors are excessively activated, the function of outwardly rectifying K^+ currents in astrocytes is also impaired[14]. Through such process, the increase of the extracellular glutamate concentration

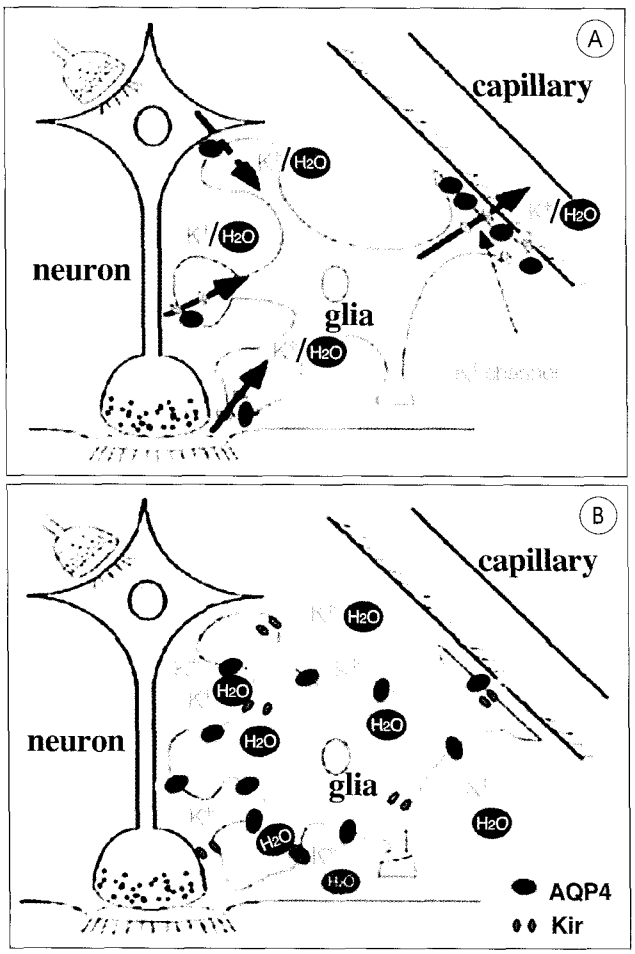

Fig. 10. Hypothetical scheme of the coupling between water and K^+ ions transport across glial membranes. A : In normal rat brain, AQP4 shows a precise colocalization with Kir channel and this coupling is predominantly expressed at the astrocyte foot process near the perivascular layer. The glial cell aspirates the excess water/K^+ ions from the extracellular space and extrudes them to vessels via AQP4/Kir channels. It is hypothesized that this mechanism may maintain the regional microenvironment around neuronal cells. B : In permanent middle cerebral artery occlusion(MCAO) model, AQP4 is overexpressed across the entire cellular surface of astrocyte, not at the astrocyte foot process. The colocalization of AQP4/Kir channels and the interaction of these channels are disrupted at permanent MCAO model. It is suggested that the accumulation of the excess water/K^+ ions at the extracellular space may deteriorate the regional microenvironment around neuronal cell and contribute to the development of edema formation and the hyperexcitability of neuronal cells.

and the transient inhibition of Kir current in astrocytes might respresent a further mechanism serving the formation of the edema in astrocyte endfeet and resultant neuronal excitability. It is thought that such edema in perivascular astrocyte endfeet is a marker of the formation of cerebral edema and the disruption of ion homeostasis induces the damage with vasogenic brain edema more exacerbated. In our study, the size of infarct is prominent after 2 hours and increases with time after right MCAO. In the TUNEL stain, positive apoptotic neclei are detected up to 80% at 24 hours after cerebral ischemia. In addition, the ratio of GluR1/GluR2 expression increased with the increase of the cellular damage during ischemia. These findings indicate that neuronal excitotoxicity was induced by the increase of Ca^{2+} permeable AMPA receptors and that deranged function in spatial buffering of glutamate, ions and water by astrocytes might also contribute the brain injury and edema formation

Recently it was reported that coexpression and stabilization of AQP4/Kir4.1 in astrocytic endfeet requires α-syntrophin, a adaptor of the dystrophin-glycoprotein complex(DGC) and stabilized AQP4/Kir4.1 channels[1,2,5,9,24]. It was shown that Kir4.1, AQP4, and α-syntrophin all have a tendency to increase in a parallel fashion in human astrocytes in various pathological brain states[32]. They have also shown that Kir4.1/AQP4 and AQP4/α-syntrophin are tightly co-localized in the end feet of astrocytes[13,23] and that K^+ buffering is coupled to changes in the volume of the extracellular space, suggesting a functional relationship between Kir4.1 and AQP4[6,10]. These results indicate that AQP4, Kir4.1 and α-syntrophin are directly linked at the perivascular astrocytic endfeet and these pattern may contribute the control of extracellular K^+ ion concentration and water movement. In our study of colocalization, AQP4 expression of astrocyte was increased, but polarized localization of AQP4 at the astrocyte endfeet in normal condition was disrupted with time after MCA occlusion. In addition, immunoprecipitation assay shows deranged interaction of AQP4 and Kir4.1 with α-syntrophin with time after MCA occlusion. Based on these findings, it might be predicted that disrupted colocalization of AQP4/Kir4.1 channel during ischemia and resultant deterioration of the regional microenvironment around neuronal cell contribute to the development of edema formation and the excitotoxicity of neuronal cells (Fig. 10).

Conclusion

This study was aimed to elucidate the role of water channels in cerebral edema formation, in particular focusing on functional coupling with K channel its anchoring by α-syntrophin in astrocytes. We found that the deranged spatial buffering function of astrocytes due to mislocalized AQP4/Kir4.1 channel as well as increased assembly of Ca^{2+} permeable AMPA receptors might contribute to the development of edema formation and the excitotoxic neuronal cell death during ischemia. In the future, the detailed investigation of the mechanism involved in functional coupling of astrocytic AQP4/Kir4.1 and its effect on neuronal microenvironment could help in the design of therapies targeted on the specific molecules in cerebral edema.

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