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BACKGROUND AND PURPOSE: **Focal cerebral ischemia results in neuronal changes in remote areas that have fiber connections with the ischemic area. We reported previously that a high-signal-intensity lesion was observed in the substantia nigra after striatal infarction on T2-weighted MR images in both clinical and experimental cases. However, the origin of these changes in signal intensity remains unclear. The aim of this study was to investigate the nigral changes by examining the correlation between the apparent diffusion coefficient (ADC) and the tissue structure.**

METHODS: **Sprague-Dawley rats were subjected to middle cerebral artery occlusion. Four days after the occlusion, when T2-weighted images revealed the presence of an area of high signal intensity in the ipsilateral substantia nigra, diffusion-weighted imaging was performed using a 4.7-T superconductive MR unit, and the ADCs were calculated and imaged. Histopathologic examination by both light and electron microscopy was performed on day 4 after surgery.**

RESULTS: **Diffusion-weighted images showed an area of high signal intensity in the ipsilateral substantia nigra, and the ADC map revealed uniform reduction of the ADC in this area. Swelling of astrocytic end-feet was observed, especially in the pars reticulata.**

CONCLUSION: **These findings suggest that MR changes in the ipsilateral substantia nigra after striatal injury consist mainly of swelling in the astrocytic end-feet.**

Neuronal loss in the ipsilateral substantia nigra is observed after striatal injury both in humans (1, 2) and in animals (3–7). It takes several days before these changes become detectable histopathologically (7, 8). This neuronal death may result from excessive excitation caused by the loss of the inhibitory transmitter γ-aminobutyric acid (GABA) in the striatum (6, 7), as the intraventricular infusion of the GABA-agonist muscimol prevents neuronal loss after destruction of the striatum (6, 9).

Secondary changes in the substantia nigra have been detected by MR imaging (on T2-weighted sequences) in patients with a wide infarction in the territory of the middle cerebral artery (MCA) (10) and in rats with MCA occlusion (11). In both humans and rats, the substantia nigra lies outside the ischemic area. Lesions in the substantia nigra are seen as areas of high signal intensity on T2-weighted images several days after infarction (10, 11).

In a previous study, the high signal intensity in the substantia nigra appeared transiently only at day 4 after MCA occlusion in the rat (11). In our rat MCA occlusion model, light microscopy revealed no pathologic change in the substantia nigra at day 2 after occlusion (7). One week after MCA occlusion, a small number of degenerated dark neurons were apparent, and after 2 weeks or more, neuronal loss, gliosis, and marked atrophy were observed in all animals (7, 12). The question then arises as to what causes the signal change in the substantia nigra at day 4, when neuronal degeneration is barely discernible. In an attempt to answer this question, we examined the apparent diffusion coefficient (ADC) values and the ultrastructural changes in the substantia nigra 4 days after MCA occlusion in the rat. Our findings are presented here, along with a discussion of the cause of the signal change on T2-weighted images.

Methods

Experimental Animals

The experimental and surgical procedures were approved by the animal research committee of the Teikyo University School

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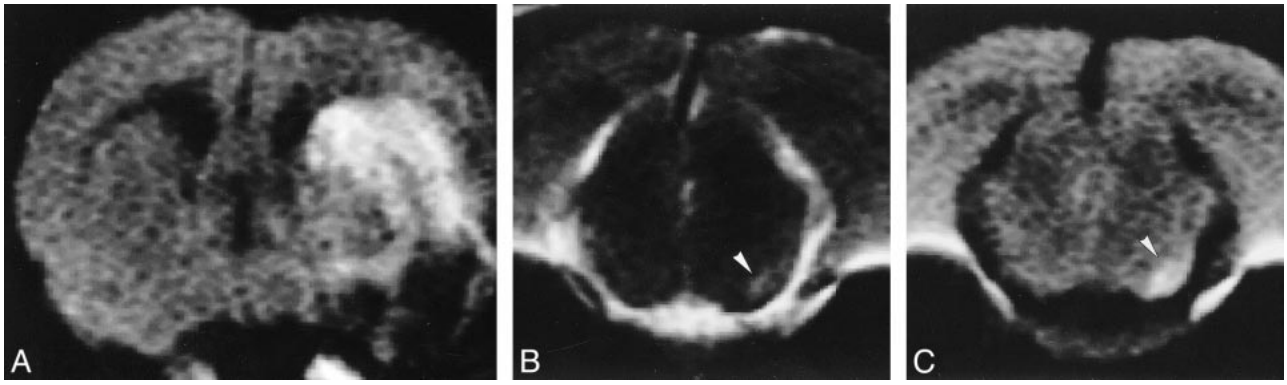

FIG 1. Representative MR images 4 days after MCA occlusion. *A–C,* The infarction is seen as an area of high signal intensity on diffusion-weighted image (*A*). The ipsilateral substantia nigra shows high signal intensity on T2-weighted (*B*) and diffusion-weighted (*C*) images (*arrowhead*).

of Medicine. Twelve male Sprague-Dawley rats, 9 to 10 weeks of age and weighing 320–350 g, were used. Eight rats were anesthetized with 2% halothane before the proximal part of the left MCA was exposed and permanently cauterized (13, 14). The remaining four rats were subjected to a sham operation and served as controls; in these animals, the MCA was exposed under anesthesia but not cauterized. After surgery, the animals were transferred to observation cages and given free access to food and water for 4 days.

MR Imaging

MR imaging was performed 4 days after surgery in five of the eight MCA-occluded rats and in two of the four sham-operated animals. Images were acquired using a 4.7-T imager/spectrometer system (Unity INOVA, Varian, Palo Alto, CA). An 8-cm quadrature coil was tuned to a radio frequency of 200 MHz for excitation and reception of proton nuclear MR signals. The animals were placed supine on a thermal water blanket maintained at 37.5°C and artificially ventilated with 1.5% isoflurane using a home-built plastic cone device that fit tightly over the nose. To obtain reproducible images, a T1-weighted midsagittal scout image was taken, and nine consecutive coronal sections were selected. The T1-weighted images were obtained with conventional multislice spin-echo sequences (600/20/2 [TR/TE/excitations], 2-mm slice thickness, 128 $\times$ 128 pixel matrix, and 3-cm field of view). The T2- and diffusion-weighted images were also obtained with conventional multislice spin-echo sequences (1500/80/2, 2-mm slice thickness, 128 $\times$ 128 pixel matrix, 3-cm field of view). For diffusion weighting, b-values of 1200 s/mm^2 were used. Diffusion-encoding gradients were irradiated along the *x*-, *y*- or *z*-axes in turn. MR data analyses were performed on a Sun Spark 10 (Sun Microsystems, Palo Alto, CA) workstation using image analyzing software (XSD software, Daris Bioengineering, St. Louis, MO). ADC maps were calculated on a pixel-by-pixel basis using standard equations (15): $ADC = \ln (S_0/S_1)/(b_1 - b_0)$, where S_0 is the signal intensity of the T2-weighted image, S_1 is the averaged signal intensity of three diffusion-weighted images (x, y, and z), and b_0 and b_1 are 0 and 1200 s/mm^2, respectively. The ADCs were assessed in the substantia nigra of both sides. Regions of interest were determined according to a stereotaxic atlas (16) at the level of the substantia nigra. It took about 1 hour to perform the MR examination for one animal.

Histologic Examination

Three experimental animals were prepared for histopathologic examination 4 days after MCA occlusion. They were perfused transcardially with a solution of 3% paraformaldehyde and 1% glutaraldehyde in a buffer under pentobarbital anesthesia (50 mg/kg intraperitoneally). The brains were removed and postfixed for more than 24 hours in the same fixative at 4°C. Small blocks including the substantia nigra were cut and postfixed with 1% OsO_4 for 2 hours. After dehydration through a graded alcohol series, the blocks were transferred to propylene oxide and embedded in Epon. Thin sections, stained with 1% toluidine blue, were examined with a light microscope. For electron-microscopic examination, ultrathin sections were stained with uracyl acetate and lead citrate. The two control animals that were subjected to sham operations were prepared similarly.

Statistical Analysis

Data are shown as mean $\pm$ SEM. The values in the ipsilateral substantia nigra were compared with those on the opposite side by a paired Student's *t*-test analysis.

Results

MR Imaging

All the MCA-occluded rats showed cerebral infarction as revealed by high signal intensity in the left caudate putamen and cortex on T2- and diffusion-weighted images (Fig 1A). The ADC map revealed heterogeneous reduction or elevation of the ADC in the ischemic area. T2- and diffusion-weighted images 4 days after MCA occlusion showed an area of high signal intensity in the ipsilateral substantia nigra in all the experimental animals (Fig 1B and C). The ADC map revealed uniform reduction of the ADC in the ipsilateral substantia nigra ($426.2 \pm 19.7 \times 10^{-6}$ mm^2/s), which was significantly lower than that in the contralateral substantia nigra ($637.0 \pm 4.7 \times 10^{-6}$ mm^2/s; $P < .001$, $n = 5$). No significant abnormality in the substantia nigra was observed in the sham-operated rats (data not shown).

Histologic Examination

Histologic analysis was done 4 days after MCA occlusion. Light microscopy revealed perivascular enlargement as the characteristic finding in the pars reticulata. Most of the neurons were normal in appearance (Fig 2). A few neurons in the ipsilateral

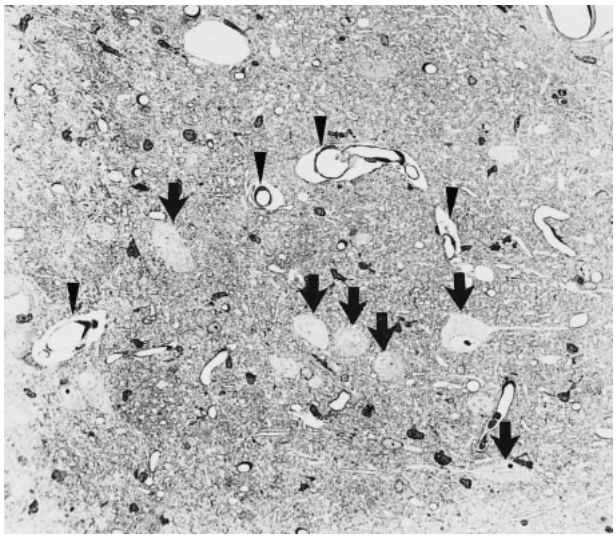

FIG 2. Light micrograph of the left substantia nigra pars reticulata shows perivascular enlargement (*arrowheads*) and intact neurons (*arrows*) (toluidine blue, original magnification ×320).

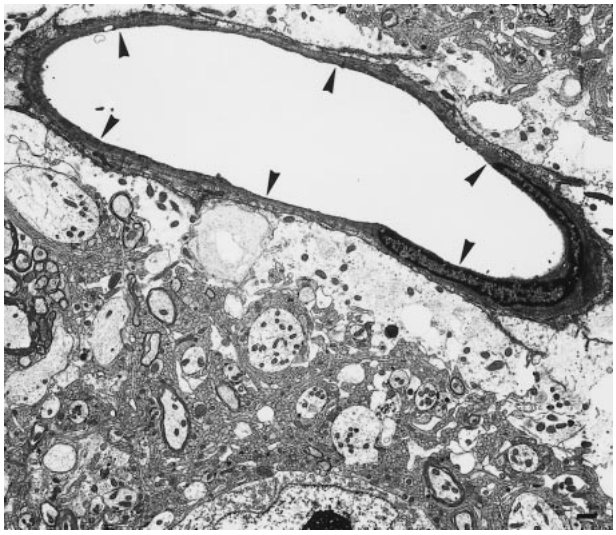

FIG 3. Electron micrograph of the left substantia nigra pars reticulata shows the blood vessel (*arrowheads*) surrounded by lucent swollen astrocytic end-feet. Bar = 1 μm.

pars reticulata showed cytoplasmic condensation; however, the nuclear membranes were preserved. The ultrastructural characteristics of the pars reticulata are shown in Figure 3. Swelling of the astrocytes was observed around blood vessels, but it was restricted to the perivascular end-feet. There was no remarkable change in the pars compacta.

Discussion

Focal cerebral infarction results in delayed neuronal changes in remote areas that have fiber connections with the infarcted area (7, 10, 11, 17). In this study, we found MR imaging changes reflective of the secondary changes in the substantia nigra, which might be caused by transneuronal mechanisms.

MR imaging is a noninvasive and powerful tool for detecting cerebral edema in experimental animals. Diffusion-weighted images can reveal changes of cerebral ischemia as early as 30 minutes after the onset of ischemia, and more clearly than T2-weighted images (18). T2- and diffusion-weighted images reflect differences in water content and water diffusion, respectively (15, 19). The development of cellular edema is associated with a reduction of the ADC (20). ADC mapping is a sensitive method for detecting ischemic edema, and ADC reduction was found to occur as early as 15 minutes after the onset of ischemia (21).

In one recent study, reduction of the ADC at the substantia nigra was detected after temporary hypoxia-ischemia in rats (22). In this model, the substantia nigra was exposed to hypoxia; therefore, the change in the substantia nigra was not delayed, and might have derived from direct injury. Conversely, there was no ischemia of the substantia nigra in our model (23); hence, the changes in the substantia nigra are considered to represent remote effects of striatal injury.

It has been reported that either hypoxic-ischemic injury or excitotoxic striatal injury during development induces apoptotic cell death of the dopaminergic neurons in the pars compacta in rats (24, 25), and that neuronal death does not occur in the pars reticulata as a result of combined lesions of the striatum and globus pallidus in rats younger than postnatal day 20 (26). DeGiorgio et al (27) reported that neuronal loss in the pars reticulata began 4 days after neurotoxic injury of the caudate-putamen and globus pallidus in rats, followed by significant loss (50%) at 6 days and a plateau after 8 days. Substantia nigra lesions induced by striatal injury are likely to differ depending on the age or the model used. Further studies are needed to explore the mechanism of neuronal death in the substantia nigra.

We focused on the findings at day 4 after MCA occlusion in this study because the substantia nigra lesion is reproducibly observed as an area of high signal intensity on T2-weighted images at this time point. The lesion was not seen on either T2- or proton–density–weighted images at days 1, 7, 14, or 28 after MCA occlusion (11), or on diffusion-weighted images at days 1 and 7 in our preliminary study (data not shown). It is unclear why the lesion is not detectable on MR images at days 7 and 14, when neuronal degeneration in the substantia nigra is observed neuropathologically (7).

Conclusion

Four days after MCA occlusion, cell swelling in the pars reticulata took place not in the neurons, but in the astrocytes. This finding was consistent with the high signal intensity seen on T2- and diffusion-weighted images, as well as with the ADC reduction, but we did not expect to observe uniform ADC reduction attributable mainly to astro-

cytic swelling in the perivascular end-feet. This is new knowledge regarding secondary degeneration. We are still unclear as to the process of subsequent neuronal loss. Nonetheless, we can confirm that the abnormality of the substantia nigra observed on T2-weighted images was due to astrocytic swelling. Careful examination by MR imaging thus enables us to detect degeneration that occurs in remote areas from the primary lesion.

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