Short Communication

Corticofugal Axonal Degeneration in Rats After Middle Cerebral Artery Occlusion

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We used the Fink-Heimer method to study degenerating corticofugal axons after unilateral middle cerebral artery occlusion in 14 adult male Long-Evans hooded rats. Axonal degeneration in the pyramidal tracts was prominent at 1–3 weeks, manifesting in well-defined silver-impregnated axonal bundles coursing from the internal capsule to the pyramids and crossing completely to the contralateral spinal cord. In half of eight rats examined at 1–3 weeks, the cortical infarct included the forelimb region of the sensorimotor cortex, and degenerating corticospinal axons could be traced to the lower cervical segments; in rats with involvement of the hindlimb cortical area as well, axonal degeneration extended to the lumbar sacral segments. Terminal degeneration products were present in the forebrain, midbrain, and brainstem within 2 days after arterial occlusion; the number of degenerating terminals peaked at 7 days and decreased gradually thereafter up to 6 weeks. Dense terminal degeneration was observed in the trigeminal nuclear complex of all seven rats studied at 2 and 7 days. In these seven rats, five had small cortical infarcts, and silver-impregnated terminals were observed in the lateral reticular formation; in two rats with large cortical lesions, terminal degeneration was prominent in the medial reticular formation as well. We conclude that infarcts produced by middle cerebral artery occlusion cause axonal degeneration in the brainstem and spinal cord. The Fink-Heimer method may be useful for evaluating the rat middle cerebral artery occlusion model. (Stroke 1989;20:1396–1402)

Injury to the central nervous system causes axonal (Wallerian) degeneration.1 The Fink-Heimer method is a sensitive technique for detecting early axonal degeneration2,3 and has recently been applied in experimental brain4 and spinal cord5,6 injury. Middle cerebral artery (MCA) occlusion (MCAO)7,8 is a widely used model of focal cerebral ischemia. Occlusion of the MCA below the rhinal fissure typically produces infarcts in the frontoparietal cortex.8 The infarcts involve the motor and sensory cortices, which send axons to the midbrain, brainstem, and spinal cord. We consequently examined the patterns of axonal degeneration in these structures in rats, using the Fink-Heimer method.

Materials and Methods

We anesthetized 14 adult male Long-Evans hooded rats weighing 280–350 g with 40 mg/kg i.p. pentobarbital. To expose the MCA, a right subtemporal craniotomy was carried out without disturbing the zygomatic arch.9 The MCA was occluded and divided 1–2 mm below the rhinal fissure. At 1 day (two rats), 2 days (two rats), 1 week (five rats), 3 weeks (three rats), or 6 weeks (two rats) after MCAO, the rats were again anesthetized with pentobarbital and perfused intra-aortically with 10% buffered formalin. The brains and spinal cords were removed and immersed in the same fixative for >1 week; 40-μm-thick frozen sections of the brain, brainstem, and spinal cord were cut and processed by the Fink-Heimer method.2 For assessing the infarct, forebrain sections containing the infarct were stained by the Klüver-Barrera method. The brain areas were defined according to the atlas of Paxinos and Watson.10 Rats with MCAO tended to circle toward the lesioned side after surgery; however, such symptoms disappeared within 24 hours. Because the zygomatic arch was spared, the rats were able to feed shortly after surgery and regained their preoperative weight 7–10 days after surgery.

Results

Among the 14 rats, infarcts involving all cortical layers were evident in the lesioned hemisphere within 24 hours after MCAO; the infarcts included the lateral parietal area (Par1, Par2), the insular cortex, and the anterior piriform cortex in 100%. The lesion extended into the forelimb (FL) and...
hindlimb (HL) areas of the sensorimotor cortex in seven (50%) and three (21%) rats, respectively. The frontal motor region was affected in three rats (21%), the anterior temporal area in seven (50%) rats, and the occipital cortex in four (29%) rats. The internal capsule was not involved in any rat.

Among the 10 rats examined histologically at 1–6 weeks after MCAO, degenerating pyramidal tract axons were present in 100%, clearly visualized as coarse silver-impregnated fiber bundles coursing from the internal capsule (Figure 1) to the pyramids. The degenerating corticofugal axons formed numerous small bundles that passed through the caudate putamen to the internal capsule, continuing caudally from the ipsilateral cerebral peduncle to the medullary pyramid. All degenerating axons decussated to the contralateral side (Figure 2, top). The silver grains were packed at the ventral tip of the contralateral dorsal funiculus in the spinal cord (Figure 3). No degenerating axons were detected in other white matter areas of the spinal cord.

Among the eight rats examined 1–3 weeks after MCAO, two had infarcts involving both the FL and HL cortical areas, with degeneration of the corticospinal axons extending into the lumbosacral regions (Figure 3, middle and bottom). No lumbosacral corticospinal tract degeneration was seen in the six rats without involvement of the HL area. In the four rats without involvement of the FL area, no corticospinal degeneration was seen in the lower cervical segments.

Degenerating terminals were prominent in many parts of the brain. Among the two rats killed 2 days after MCAO, fine silver grains representing degenerating axon terminals were present in the brainstem, midbrain, and forebrain. The silver-impregnated axon terminals reached a maximum density in these structures among rats killed 1 week after
MCAO, and density decreased gradually in rats killed >1 week after MCAO. In all seven rats studied at 2 and 7 days after MCAO, dense terminal degeneration was observed in the trigeminal nuclear...
FIGURE 3. Photomicrographs of (top) C7 and (middle) L6 segments from rat with infarct involving forelimb and hindlimb sensorimotor cortices 1 week after middle cerebral artery occlusion. Densely packed silver grains, representing degenerating corticospinal axons, can be seen in ventralmost portion of contralateral dorsal funiculus (arrows). Fink-Heimer method, bars indicate 350 μm. Bottom: High-power photomicrograph of site of corticospinal tracts shown in middle. No degenerating axons are present in ipsilateral side. Fink-Heimer method, bar indicates 15 μm.
FIGURE 4. Photomicrographs of caudal pons of rat with large infarct involving forelimb and hindlimb sensorimotor cortices 1 week after middle cerebral artery occlusion. Top: Fine silver grains are distributed in medial portion of pontine reticular formation; degenerating pyramidal tract fibers form bundles separated by fibers of trapezoid body. Fink-Heimer method, bar indicates 150 μm. Bottom: In lateral part of contralateral pontine reticular formation, numerous fine silver grains representing degenerating terminals of corticoreticular fibers can be seen. Fink-Heimer method, bar indicates 50 μm.
complex (Figure 2, bottom). In the reticular formation, the degenerating terminals were distributed predominantly to the contralateral deep mesencephalic reticular nucleus, as well as to the lateral part of the pontine and medullary reticular formations. In two rats with large infarcts involving the FL and HL cortical areas, silver grains were present in the medial reticular formation and more densely distributed to the lateral reticular formation (Figure 4); terminal degeneration products were also present in the contralateral dorsal column nuclei.

In the midbrain, silver grains were scattered in the ipsilateral superior colliculus, the substantia nigra, and the red nucleus. In the forebrain, fine silver grains were present in the contralateral frontoparietal cortex associated with degeneration of callosal fibers. Terminal degeneration products were consistently seen in the ipsilateral thalamic nuclei, particularly the ventro-posteromedial and ventro-posterolateral nuclei. The products were also present in the dorsolateral caudate-putamen bilaterally, although the products were more dense on the ipsilateral side.

Discussion

The rat pyramidal tract originates from layer V pyramidal cells situated in the primary somatosensory cortex (FL, HL, and Par1) and the agranular frontal motor cortex. The FL and HL areas exhibit both motor and somatosensory characteristics and therefore are called the parietal sensorimotor cortex. The pyramidal tract axons penetrate the caudate-putamen in multiple fiber bundles, which join together into larger bundles in the internal capsule. We found no direct capsular involvement in any rat. Thus, pyramidal tract degeneration could result from either death of pyramidal cells or from damage to the axons as they course through the caudate-putamen. Although axons may have been damaged at the caudate-putamen level, where we saw ischemic changes in a few rats, our results strongly suggest that damage at the neocortical level is the primary determinant of pyramidal tract degeneration.

Degenerating corticospinal axons were completely lateralized in the contralateral dorsal funiculus and were not present in any other part of the spinal white matter. Several neuroanatomic studies with classical suppressive silver methods and with recent tracer techniques have shown that corticospinal tract fibers cross to the contralateral side at the decussation. A few uncrossed ventral fibers from the frontal motor cortex have been mentioned. Since the frontal motor cortex was affected in only a few rats in our study, the absence of degeneration in the ipsilateral spinal cord white matter does not rule out uncrossed corticospinal axons. Despite massive degeneration of corticospinal axons in some rats, they did not appear to have gross motor impairment of the limbs.

The MCAO infarct in our study invariably resulted in face sensation and projects to the trigeminal nuclear complex of the pons. Axonal degeneration studies may provide several advantages over counting moribund neurons or measuring lesion volumes. First, the cerebral cortex sends axons to many brain locations in well-defined tracts. By examining degeneration in specific tracts, it may be possible to estimate neuronal loss from different parts of the cortex. Second, the lesion site itself can be concomitantly examined by other methods, that is, ionic or biochemical measurements, for comparison with the degeneration data. Third, degenerating axons in identified tracts can be studied without the complications of tissue shrinkage and histologic artifacts that are frequently present at lesion sites.

References

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