Evolution of Diaschisis in a Focal Stroke Model
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Background and Purpose—Stroke produces diaschisis in adjacent and connected regions. The sequential changes in diaschisis over time and the relationship of regions of diaschisis to functional cortical areas and regions of poststroke neuroplasticity have not been determined.

Methods—Small cortical strokes were produced in the barrel cortex of rats. Relative glucose metabolism was determined in vivo over time with $[^{18}F]$fluorodeoxyglucose small-animal positron emission tomography. Cerebral blood flow was measured with $[^{14}C]$iodoantipyrine. Regions of hypometabolism and hypoperfusion were compared with histological damage in the same animals.

Results—Small cortical strokes produce an initial network of hypometabolism in a broad region of cortex adjacent to the stroke and in the striatum and thalamus on day 1. Cerebral blood flow is diminished only immediately around the cortical infarct on day 1. A substantial area of cortex adjacent to the stroke remains hypometabolic on day 8. This persistent cortical hypometabolism occupies the somatosensory cortex, forelimb motor cortex, and second somatosensory area.

Conclusions—Focal stroke produces ipsilateral diaschisis in connected cortical regions that is clearly distant from subtotal damage and may play a role in poststroke neuroplasticity.

Key Words: cerebral cortex ■ neuronal plasticity ■ somatosensory cortex ■ tomography, emission computed

Stroke produces an area of focal damage and distant areas of reduced blood flow and metabolism, termed diaschisis. Diaschisis may impair functional recovery by preventing effective neural reorganization after injury. Alternatively, diaschisis may be part of a process of structural reorganization after injury, as axonal sprouting and the formation of new cortical connections occur in regions that have been reported to undergo diaschisis after stroke.

Studies of diaschisis in experimental stroke models have been limited. Positron emission tomography (PET), used successfully to serially image glucose metabolism in humans, has not been available to study rodent stroke with adequate resolution. Instead, such studies have relied on the use of single time point autoradiographic methods, with temporal influences only inferred from the analysis of large groups of animals. The study of diaschisis in experimental stroke has also been limited by the large size of many stroke models. These infarcts directly damage a broad range of cortical and subcortical sites and passing axons from remote areas. This precludes the precise localization of areas of diaschisis within functionally identified brain regions and the spatial relationship of diaschisis to remote sources of tissue injury.

In the present study we have overcome these difficulties by using a relatively new but well-characterized model of small focal stroke within the rat somatosensory cortex to determine the location of areas of hypometabolism repeatedly in the same animal within functionally identified cortical areas using $[^{18}F]$fluorodeoxyglucose small-animal positron emission tomography (FDG microPET). Cerebral blood flow was studied with $[^{14}C]$iodoantipyrine (IAP). We show that focal cortical stroke produces hypometabolism in thalamus, striatum, and a large region of cortex at day 1 after stroke but produces hypoperfusion only in a cortical area closely adjacent to the infarct. By day 8 after stroke, the area of hypometabolism is reduced but still occupies specific functional areas of sensorimotor cortex that are significantly larger than the histological infarct.

Materials and Methods

Animals
Twenty-six adult male Sprague-Dawley rats (weight, 250 to 400 g) were used in this study. Twelve animals were given focal cortical strokes and processed for FDG microPET at days 1 and 8 after stroke. Five animals died before both scans were completed. Six animals were given sham operations and processed for FDG microPET. Sham and stroke animals (n=4 per group) were processed for IAP blood flow studies. All procedures were performed in accordance with National Institutes of Health animal protection guidelines and were approved by the University of California at Los Angeles Animal Research Committee.

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Focal Cortical Stroke

Focal cortical stroke was performed as described.\textsuperscript{7} Through a craniotomy, 1 to 2 anterior branches of the middle cerebral artery were permanently occluded, and both common carotid arteries were occluded for 1 hour. Sham animals received a craniotomy and heater probe application over cortex caudal to the middle cerebral artery.\textsuperscript{7}

Small-Animal Positron Emission Tomography

Relative metabolic activity was measured with microPET with the use of FDG to evaluate glucose uptake in the awake state as described previously.\textsuperscript{9} Three-dimensional volumetric images were reconstructed with the use of a maximum a posteriori–based 3-dimensional iterative algorithm.\textsuperscript{5,9} Relative glucose metabolism was determined in regions of interest (ROIs) (JANUS; http://eagle.nuc.ucla.edu/). For striatum and thalamus, ROIs were drawn around the structure on the side contralateral to the lesion and superimposed on the ipsilateral corresponding structure. The cortical ROI was established through a vertical line from the medial striatum or thalamus to the overlying cortical surface and through a horizontal line from the ventral base of the striatum or thalamus laterally to the cortical surface. The average intensity signal for the entire ROI was recorded. Each ROI value was an average result of 3 separate measurements on equally spaced sections through the stroke. Relative glucose metabolism was expressed as a percent deficit, as follows: 1−(stroke hemisphere ROI)/(nonstroke hemisphere ROI)×100. Values were expressed as a mean and compared across animals in stroke and sham groups with factorial ANOVA and Bonferroni post hoc testing. To determine the size of the metabolic lesion at days 1 and 8, the cortical area containing pixels below a threshold of 15% less than the cortex in the opposite hemisphere was measured as the lesion area. This threshold distinguishes chronic, nonischemic hypometabolism from the level of hypometabolism seen in regions of diaschisis after stroke.\textsuperscript{10−12} The lesion area in each section through the stroke was multiplied by the distance between sections to produce a total lesion volume in each animal at each time point.\textsuperscript{7} Comparisons between 1 and 8 days were made by paired Student’s t tests.

Cerebral Blood Flow

Cerebral blood flow was measured with \textsuperscript{14}C]IAP autoradiography.\textsuperscript{5,13} Fifty microcuries of 4-iodo-N-methyl-[\textsuperscript{14}C]IAP was injected intravenously. Brains were frozen, and sections were exposed to Kodak MS film. Optical densities were measured in cortex, striatum, and thalamus with the same ROIs as in the microPET experiments (MCID, Image Research). Optical density values in the stroke hemisphere were compared with the same regions in the opposite hemisphere to produce an ischemic/nonischemic ratio.\textsuperscript{13} The ratios in cortex, striatum, and thalamus were determined by dividing ischemic over nonischemic areas and corrected for tissue shrinkage. \textsuperscript{13} The ratios were determined by measuring the pixels in cortex in which the IAP signal was <5% of the contralateral hemisphere, a threshold that clearly identifies ischemic tissue.\textsuperscript{13} These were summed to produce a lesion area in each coronal section and lesion volume calculated as for the PET images.

Histological Lesion Volume

After the final microPET scan animals were decapitated, the brains were frozen, and coronal sections were stained with cresyl violet. Lesion size was measured as that area containing bland tissue devoid of neurons and surrounded by a gliotic rim. Cerebral hemisphere size in Nissl sections was compared with the same sections in the living animal in microPET, and a shrinkage factor of 12% was determined for Nissl tissue processing. Lesion volume was calculated\textsuperscript{7} and corrected for tissue shrinkage.

Flattened Cortical Surface Maps

Orthographic unfolded maps were prepared as described.\textsuperscript{14} The region of diminished glucose metabolism, decreased blood flow, or histological damage was marked on lines through the cortex in each coronal section in microPET, IAP, and Nissl series. Each line was straightened and aligned, with the distance between lines equivalent to the distance between sections in the series. This produces an unbiased, 2-dimensional representation of a coronal data series in the tangential plane of the cortex.\textsuperscript{14} The overall area of each tangential representation was corrected for tissue shrinkage, averaged across animals, and normalized to a composite flattened map of somatosensory cortex in this stroke model.\textsuperscript{7}

Results

Network of Hypometabolism After Small Cortical Stroke

Small strokes were produced within the barrel field and adjacent parietal areas of the rat somatosensory cortex.\textsuperscript{7} Glucose metabolism was measured by FDG microPET on days 1 and 8 after stroke. These time points sample the early, evolving process of necrotic and apoptotic cell death and a later phase of reorganization and tissue repair.\textsuperscript{2,3,7} On day 1 after stroke, there is diminished relative metabolism in ipsilateral cortex, striatum, and thalamus (Figure 1). The most striking decrease in relative metabolism is in the frontal and parietal cortex adjacent to the stroke (Figure 1, arrows). On day 1 glucose metabolism in the cortex ipsilateral to the stroke is reduced by 27±0.03% (Figure 2). Glucose metabolism in the ipsilateral striatum and thalamus is reduced by 17±0.015% and 10±0.015%, respectively (Figure 2). On day 8, glucose metabolism in the cortex ipsilateral to the stroke remains reduced by 13±0.018%. This change in cortical glucose metabolism between days 1 and 8 is statistically significant (Figure 2). The change in cortical glucose metabolism from days 1 and 8 in individual animals is also statistically significant (P<0.008). The hypometabolism in ipsilateral striatum and thalamus returns to control values by day 8 (Figure 2).

In some stroke models, glucose metabolism is depressed in cortex contralateral to stroke.\textsuperscript{10,11} To control for this possible confound in our analysis, glucose uptake in ipsilateral cortex was also compared with that of contralateral thalamus on days 1 and 8 after stroke. The thalamus contralateral to the stroke has not been reported to undergo metabolic changes and is not connected to the infarcted cortex. Results with the use of this method are very similar to those obtained with the use of the contralateral cortex as a baseline. Relative glucose metabolism in cortex ipsilateral to the stroke is reduced by 25.7±0.058% on day 1 compared with control. On day 8, relative glucose metabolism in ipsilateral cortex remains reduced, at 9.4±0.022% compared with control. These values are significantly different from control day 1 to stroke day 1 and stroke day 8 and from stroke day 1 to stroke day 8 (F\textsubscript{s,22}=78.36, P≤0.001). There is no significant difference in the relative glucose metabolism in cortex contralateral to the stroke compared with thalamus contralateral to stroke on days 1 and 8 (F\textsubscript{s,22}=2.05, P≥0.39 for all comparisons).

Cerebral Blood Flow

Cerebral blood flow was measured in a separate set of animals on day 1 to determine whether the relative hypometabolism in cortex, striatum, and thalamus on day 1 is a reflection of hypoperfusion in these areas. In sham lesions the ratio of blood flow in ipsilateral to contralateral cortex is 0.89±0.034%. In stroke, the ratio of ipsilateral to contralat-
eral cortex is $0.61 \pm 0.16\%$. This nearly 30% reduction in blood flow signal in cortex ipsilateral to stroke on day 1 is statistically significant ($P=0.0003$; Figure 3). Blood flow values in striatum and thalamus are not significantly different between sham and stroke animals (Figure 3). Thus, the diminished glucose uptake in striatum and thalamus at day 1 is not associated with hypoperfusion in these structures. The ratio of blood flow values in cortex contralateral to the infarct to contralateral striatum or thalamus is not significantly different between stroke and sham animals (data not shown).

### Lesion Size and Location

To directly compare cortical metabolism, perfusion, and infarct size, we measured the volume of cortical hypometabolism at days 1 and 8 with microPET and the final stroke size histologically in the same animals. The metabolic lesion is $111.2 \pm 22.1 \text{ mm}^3$ on day 1 and $70.2 \pm 15.3 \text{ mm}^3$ on day 8. In the same animals the histological lesion size is $5.29 \pm 1.45 \text{ mm}^3$ on day 8. The region of diminished blood flow on day 1 after stroke is $26.3 \pm 2.14 \text{ mm}^3$ (Figure 4). The difference between the metabolic lesion on days 1 and 8 and the final histological lesion volume is statistically significant (Figure 4). This means that a large region of cortex surrounding the stroke is hypometabolic but not infarcted. To determine the topographic relationship of the metabolic, blood flow and histological lesions to functional subdivisions of cortex, orthographic unfolded cortical maps were prepared for each animal and normalized onto a standardized unfolded map of the rat cortical surface for this stroke model. The metabolic defect at day 1 involves much of the frontal and parietal cortex supplied by the middle cerebral artery (Figure 5). On
day 8, the metabolic lesion is smaller and includes the posterior motor cortex and somatosensory cortex, including the somatosensory barrel cortex and forelimb sensorimotor area and second somatosensory area16 (Figure 5). By contrast, the area of histological damage is restricted to a small region of the somatosensory barrel field cortex (Figure 5).

Discussion

In the present study we found that small cortical strokes produce an initial network of hypometabolism in a large region of adjacent cortex, striatum, and thalamus that is not associated with reduced cerebral perfusion. By day 8 after stroke, striatal and thalamic metabolism has normalized, but a substantial area of cortex adjacent to the stroke remains hypometabolic. The region of cortical hypometabolism at day 8 is >13 times larger than the infarct itself and encompasses the functionally related areas of the primary somatosensory and forelimb motor cortex and second somatosensory cortex. This broad region of cortical hypometabolism on day 8 after stroke is clearly distant from ischemic damage or edema in this stroke model7 and is substantially larger than the areas of apoptotic cell death and reperfusion injury. 7 This large area of cortical hypometabolism is thus a region of ipsilateral cortical diaschisis.

There are several advantages to the use of FDG microPET in the analysis of hypometabolism after experimental stroke. The same animals are sequentially imaged and then processed for histological study. This eliminates interanimal variability as a source for the changing regions of hypometabolism in cortex adjacent to the infarct. In addition, the use of the same animals for sequential metabolic imaging and then histological analysis clearly identifies the topographic relationship of the infarct to the larger region of cortical hypometabolism. With FDG microPET, glucose uptake occurs in the awake state so that, unlike small-animal MRI, cortical metabolic changes are not confounded by anesthesia. However, there are several limitations to FDG microPET as used in this study. With the current scanner and reconstruction algorithm, the in-plane image resolution is 1.2 mm17; this is roughly the size of 3 barrels in the posteromedial barrel subfield (Figure 5). This resolution limits the certainty of our quantitative areal measurements and the mapping of metabolic data (Figure 5). Second, the need to repeatedly scan the same animals over 8 days after stroke precludes multiple episodes of repeated anesthetization and arterial catheterization for quantitative glucose measurements. Instead, we measured glucose metabolism and blood flow in relation to the contralateral hemisphere. Because the cortex contralateral to stroke may be hypometabolic,10,11 this technique may have introduced error into our measurements. However, we carefully evaluated the possibility of contralateral metabolic and
blood flow changes in our data analysis and did not find these changes. This finding is likely explained by the lack of contralateral cortical connections of the area of this infarct\[16,18\] and is consistent with other studies of small cortical strokes.\[11\]

Local cerebral glucose utilization is a direct reflection of the amount of synaptic activity in the observed region. The ipsilateral cortical diaschisis in this study corresponds to the region of dense cortical connections of the infarct core and thus might reflect neuronal inactivity resulting from a loss of input. Recently, acute cerebellar diaschisis was related to a diminished neuronal firing rate, through the loss of afferent drive from infarcted cortex.\[19\] In crossed cerebellar diaschisis, this diminished afferent drive corresponds to the known topography of corticopontocerebellar projections.\[19\] The present study uses the well-known anatomy of the barrel cortex as a template to map the areas of diaschisis and cell damage and finds a similar result: the topography of ipsilateral cortical diaschisis corresponds to the known pattern of ipsilateral and contralateral connections of the infarct core. The infarct is located in the anterolateral and a portion of the postero medial barrel field\[7\] (Figure 5). These connections very closely match the topography of cortical hypometabolism at day 8 after stroke\[16,18,20\] (Figure 5). In contrast, the region of the infarct core in this stroke model does not have significant connections with contralateral cortex.\[16,18,20\] This may explain the lack of a contralateral diaschisis effect in this study. The region of the infarct core also has less substantial connections with distant brain areas, such as posterior parietal cortex, rostral motor cortex, and thalamus,\[16,18,20\] These areas were not hypometabolic at day 8 after stroke. The lack of observable diaschisis in these remote sites may reflect a threshold in the size of a cortical projection that must be lost to produce diaschisis, or the small size of these areas may have been below the resolution of the FDG microPET technique.

Recent studies have suggested that, while cerebral glucose utilization is a reflection of synaptic activity, the actual glucose uptake responsible for the signal observed on PET may occur in astrocytes within the vicinity of synapses.\[21\] Thus, a mechanism for the decrease in metabolism observed here may be a disruption of the normal glial-neuronal relationships in the cortex adjacent to the infarct. This idea is consistent with the present data. The location of cortical hypometabolism corresponds to the region of nestin expression in cortical astrocytes in this stroke model.\[7\] Nestin expression is induced in astrocytes that are remote from cell damage by axotomy.\[22\] Thus, nestin expression is a marker both of distant astrocyte activation after focal injury and of regions of deafferentation from the injury site.\[22\] The present data and our previous observations\[7\] suggest that focal stroke disconnects adjacent cortical areas, activates astrocytes within these areas, and, through these 2 effects, subsequently alters cortical metabolism to produce ipsilateral cortical diaschisis.

The region of ipsilateral cortical diaschisis is clearly different from areas of cell death and damage in this model. The ischemic penumbra defines a region of tissue initially adjacent to the infarct that ultimately progresses to infarction.\[23\] In this model, a region of cortex within 500μm of the infarct core undergoes delayed apoptotic cell death, oxidative DNA damage, and significant gliosis.\[7\] This cortical region, which is closely adjacent to the infarct, is thus a penumbral area. The penumbra is both smaller and more spatially related to the infarct core than the large area of cortical hypometabolism.

Instead, the region of ipsilateral cortical diaschisis is coextensive with an area of poststroke neuroplasticity. Levels of the growth-promoting proteins GAP-43 and tenasin are increased within the area medial to the infarct in this stroke model\[24\] and in cortex medial to the infarct in other stroke models.\[2,3\] Furthermore, substantial axonal sprouting occurs in a 3- to 4-mm region of cortex medial to barrel field infarcts.\[4\] This is the same region of ipsilateral cortical diaschisis in the present study. Axonal sprouting has also been demonstrated in cortex medial to other types of ischemic lesions.\[25\] The overlap of axonal sprouting and cortical hypometabolism suggests that ipsilateral cortical diaschisis may be part of a process of neuronal reorganization and reconnection after stroke.

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