Photochemical Stroke Model: Flunarizine Prevents Sensorimotor Deficits After Neocortical Infarcts in Rats

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We produced unilateral photochemical infarcts in the hindlimb sensorimotor neocortex of 186 rats by intravenous injection of the fluorescein derivative rose bengal and focal illumination of the intact skull surface. Infarcted rats showed specific, long-lasting deficits in tactile and proprioceptive placing reactions of the contralateral limbs, mostly the hindlimb. Placing deficits were most prominent during transition to immobility and/or when independent limb movements were required. Administration of flunarizine, a Class IV calcium antagonist, 30 minutes after infarction resulted in marked sparing of sensorimotor function in 30 rats. In contrast to 20 vehicle-treated rats, which remained deficient for at least 21 days, 15 (75%) of the rats treated with 1.25 mg/kg i.v. flunarizine showed normal placing on Day 1 after infarction, whereas the remaining five (25%) recovered within 5 days. Oral treatment of 10 rats with 40 mg/kg flunarizine was also effective. Neocortical infarct volume and thalamic gliosis, assessed 21 days after infarction, did not differ between 30 flunarizine- and 30 vehicle-treated rats. However, when 4-hour-old infarcts were measured in 16 rats, posttreatment with intravenous flunarizine reduced infarct size by 31%. In combination with appropriate behavioral analyses, photochemical thrombosis may constitute a relevant stroke model, in which flunarizine preserved behavioral function during a critical period, corresponding to the spread of ischemic damage.

(Stroke 1989;20:1383-1390)

When the fluorescein derivative rose bengal is intravenously injected into rats and the intact skull surface is focally illuminated, cerebral blood vessels in a confined area sustain photochemical injury. Singlet oxygen molecules generated by the dye/light interaction cause peroxidation of endothelial cell membranes and occlusive platelet aggregation. Subsequent thrombus formation, vascular stasis, extravasation, and cytotoxic edema lead to cerebral infarction and necrosis. This photochemical model of thrombotic stroke is virtually noninvasive, allows for reproducible infarct size and location, and includes endothelial damage/platelet aggregation interactions in a cascade of stroke-like ischemic events. In this model, the time course of changes in cerebral morphology, blood flow, metabolism, and edema formation have been well documented.

Beyond survival, protection of cerebral function (i.e., neurologic/behavioral integrity) constitutes the principal goal and criterion of success for any stroke therapy. Clearly, a model of stroke should also address those clinical priorities. Therefore, we investigated whether photochemical neocortical infarcts reliably produce enduring sensorimotor deficits in rats and, if so, whether those deficits can be mitigated by post-treatment with the Class IV calcium antagonist flunarizine. We selected flunarizine because neuronal pathogenesis following cerebral ischemia has been attributed in part to calcium-mediated processes. Moreover, flunarizine, an antihypoxic and antivasoconstrictive agent, improves cerebral blood flow and protects brain structure and/or function in various models of cerebral ischemia.

Materials and Methods

We used 186 male Wistar rats, weighing 270–320 g, which were anesthetized with halothane in 70% N₂O and 30% O₂. Four percent halothane was used for endotracheal intubation, and anesthesia was maintained by means of 1% halothane. Each rat was
placed in a stereotactic apparatus, the scalp was incised to expose the skull surface, and a catheter was inserted into a lateral tail vein. Upon termination of halothane anesthesia, each rat was mechanically ventilated with the N₂O/O₂ mixture and immobilized with 4 mg/kg i.v. succinylcholine.

Rose bengal (15 [in 24 rats] or 20 [in 144 rats] mg/kg, 7.5 mg/ml in 0.9% NaCl) was infused intravenously for 2 minutes in rats with normal hemodynamics and blood gases. Thereafter, the intact skull was illuminated for 20 minutes by means of a fiber-optic bundle, mounted inside a stainless-steel guide with a 1-mm-diam. (in 138 rats) or a 2-mm-diam. (in 30 rats) circular aperture through its base. Cold white light originated from a fiber-optic light source (Schott, Mainz, FRG) electronically regulated to provide a constant 60% of its maximal nominal power (see also Reference 2). The center of the light objective was stereotactically positioned on the flat skull 1.8 mm posterior to the bregma and 2.5 mm right of the midline,23,24 that is, aimed at the hindlimb area of the right parietal sensorimotor neocortex.25 This well-delineated granular-pyramidal type of cortex is also a subfield of the origin of the corticospinal tract in rats.26,27

Thirty minutes after the light was extinguished, 76 rats were alternately treated with either flunarizine or the same volume of its vehicle. In Experiment 1, a single injection of 1.25 mg/kg i.v. flunarizine was compared with vehicle in 24 rats with small infarcts (approximately 1 mm³), produced by the lower dose of rose bengal; in Experiments 2 and 2', 1.25 mg/kg i.v. flunarizine was compared with vehicle in 32 rats with medium-sized infarcts (≥3 mm³), produced by the higher dose of rose bengal; and in Experiment 3, a single oral dose of 40 mg/kg flunarizine was compared with vehicle against medium-sized infarcts in 20 rats, with infarct size intended to be similar to that of Experiment 2. In 30 rats with infarcts, induced with the 2-mm aperture and the higher dose of rose bengal (Experiment 4), 1.25, 2.5, 5.0, or 10.0 mg/kg i.v. flunarizine was compared with vehicle. In 50 other rats, 1.25 mg/kg i.v. flunarizine was injected 1, 3, 6, 12, or 24 hours after infarction produced with the higher dose of rose bengal (Experiment 5). The vehicles for intravenously and orally administered flunarizine were 10% hydroxypropyl-β-cyclodextrine and water, respectively. The intravenous dose of flunarizine corresponds to the high-dose treatment in a clinical pilot study of acute stroke.28 The oral dose was derived from pharmacologic experiments on the antihypoxic and anticonvulsant activities of flunarizine,12,29

We also studied 30 control rats. Six were treated with 1.25 mg/kg i.v. flunarizine and six with 40 mg/kg p.o. flunarizine without previously receiving rose bengal or illumination; six received 20 mg/kg i.v. rose bengal, but no illumination, flunarizine, or vehicle; six received illumination for 20 minutes, but no rose bengal, flunarizine, or vehicle; and six received 20 mg/kg i.v. rose bengal and illumination to the left of the midline, but no flunarizine or vehicle.

Behavioral tests were performed in all 186 rats 5 and 3 days before infarction. In Experiments 1, 2, 3, and 4, behavioral tests were conducted on Days 1, 2, 3, 5, 10, 14, 18, and 21 after infarction. In Experiment 2', rats were tested behaviorally 3 hours after infarction, whereas in Experiment 5, behavioral assessments occurred 2 days after infarction.

Neglect was measured as the absence of head orientation to visual, auditory, and/or light tactile stimuli applied contralaterally to the cortical infarct compared with head orientation after ipsilateral stimuli. (For further methodology, see Reference 30.)

Limbing placing was measured in hand-held, immobile rats and involved separate tests for forward extension, lateral abduction, and adduction. For visual limb placing, a rat was slowly lowered toward a table top and held 10 cm above it with free-hanging forelimbs. Normal rats reached, stretched, and placed both forepaws on the table top. By moving the rat laterally toward the table edge, lateral as well as forward visual limb placing could be assessed. Tactile forward and lateral limb placing were tested by lightly contacting the table edge with the dorsal or lateral aspect of a rat's paw, respectively. Proprioceptive limb placing involved pushing the rat's paw against the table edge to stimulate limb muscles and joints. Visual and tactile (whisker and other facial) contact with the table were avoided by supporting the rat's chin and holding its head 45° upwards. Each rat was also put along the edge of an elevated platform in order to test proprioceptive adduction. A paw was gently pulled down and away from the platform edge and, upon sudden release, was checked for retrieval and placing. For each test, limb placing scores were 0, no placing; 1, incomplete and/or delayed (>2 seconds) placing including interspersed flailing; or 2, immediate and complete placing. For each body side, on a test day, the maximum summed visual limb placing score was 4 and the maximum summed tactile and proprioceptive limb placing score, including the platform test, was 10. The maximum of the latter, summed over all test days, was 80.

On Day 1, the rats were evaluated for deficits in spontaneous limb placing, while they traversed a wide (4 cm) or narrow (2.5 cm) wooden beam 2 m long and elevated 30 cm above the table top. The wide beam's width corresponded to the hindlimb gait-width of normal adult rats.31 The narrow beam required more hindlimb adduction. A test session that started with the wide beam, ended when a cumulative total of 2 minutes of forward locomotion (20–40 cm/sec) on each beam was reached.

Additional tests for postural and locomotor reactions included forepaw grasping, limb tone and support, stepping and bracing to imposed displacement, righting from contact and righting in the air, and spontaneous and tail-pinched–induced postures
FIGURE 1. Tactile and/or proprioceptive limb placing deficits contralateral to a photochemical infarct of the right sensorimotor neocortex. Vehicle-treated rat, Experiment 2, 21 days after infarction. a: When visual, whisker, and/or chin contact are allowed, the contralateral forelimb placing deficit is apparently absent; b: but it is unmasked, when cephalic contact stimuli are prevented by turning the rat’s head 45° upwards; c: the contralateral forelimb slips off the edge of the platform as snout and whiskers lose contact with the surface. d: The proprioceptive placing deficit of the contralateral hindlimb, but not forelimb. The forelimb placing deficit is masked by cephalic contact stimuli (e.g., whisker contact with surface), which control forelimb, but not hindlimb placing.

and locomotion. (For detailed methodology, see References 32–34.)

For histologic examination 21 days (for the 60 rats of Experiments 1, 2, and 3) or 4 hours (for the 16 rats of Experiment 2') after infarction, each rat was deeply anesthetized with ether and perfused transcardially with Karnovsky’s fixative. Coronal Vibratome sections (T.P.I., St. Louis, Missouri) 100 μm thick were cut throughout the anteroposterior extent of the cortical infarct and stained with azure A-eosin B. The infarcted area in each section was measured by digital planimetry (Q970, Quantimet, Cambridge, UK), and infarct volume was calculated by integration using the trapezoid rule (see Reference 2). In addition, the dorsal surface area of the infarct was measured from calibrated photographs. Purine nucleoside phosphorylase activity in 100-μm-thick coronal sections through the thalamus served as a histochemical marker for reactive gliosis in thalamic nuclei.35 By means of digital densitometry, the difference between ipsilateral (infarct-induced) and contralateral (background) integrated density in the nucleus ventralis posterolateralis thalami was computed.

Results

When infarcted, vehicle-treated rats moved spontaneously, they did not display postural or locomotor biases in any direction, nor were asymmetries apparent after tail pinching. Limb tone and support were symmetrically normal, and righting reactions occurred from both sides. When pushed sideways, such rats resisted being rolled over as sideways stepping, alternating with bracing and toe-spread, was readily elicited on both sides. Polysensory neglect and weakened grasp contralateral to the infarct occurred in some vehicle-treated rats (i.e., in 20% and 13%, respectively), but only transiently (i.e., during the first 2 postoperative days), whereas visual placing was unaffected.

By contrast, tactile and proprioceptive limb placing deficits, contralateral to the infarct, were clearly revealed when vehicle-treated rats were tested adequately. Most (70%) had contralateral forelimb as well as hindlimb placing deficits. The contralateral forelimb placed perfectly when visual, whisker, and/or chin contact were allowed (Figure 1a), but in the absence of cephalic contact stimuli (achieved by turning the head 45° upwards) contralateral forelimb placing failed (Figure 1b). This was also evident when vehicle-treated rats reached or leaned over the platform edge while losing head contact with the surface (Figure 1c). Thus, as long as tactile or proprioceptive stimulation was confined to the forelimb itself, forelimb placing deficits could be unmasked, but the deficits were compensated for when they participated in cephalic reflexes. Forelimb placing deficits recovered, at least partially, in most (78%) vehicle-treated rats within the first 2 weeks after infarction (Figure 2, middle column). The lowest forelimb placing scores (maximum=10) on Day 1 ranged from 4±1 (Experiment 3) to
TABLE 1. Rat Locomotion on Elevated Beams: Effects of Flunarizine on Number of Limb Placing Errors Ipsilateral or Contralateral to 1-Day-Old Unilateral Medium-Sized Photochemical Infarcts of Hindlimb Sensorimotor Cortex

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Side</th>
<th>Beam</th>
<th>Ipsilateral</th>
<th>Contralateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>8</td>
<td>Wide</td>
<td>8.2±1.6</td>
<td>18.4±3.8*</td>
</tr>
<tr>
<td>Flunarizine</td>
<td>8</td>
<td>Narrow</td>
<td>3.2±4.3†</td>
<td>4.5±3.2‡</td>
</tr>
</tbody>
</table>

Data are mean±SEM.
*p<0.01 different from wide beam, Wilcoxon test, two-tailed probabilities.
†p<0.05, 0.01, respectively, different from vehicle, Mann-Whitney U tests, two-tailed probabilities.

5.8±1.6 (Experiment 2). By Day 21, the scores had risen to 10 and 7.5±1.3, respectively; the summed scores, summed over all test days (maximum=80), were 61.2±4.4 and 55.9±10.3, respectively.

Vehicle-treated rats showed contralateral hindlimb placing deficits for at least 21 days after infarction (See Figure 1d and Figure 2, left column). Thus, on Day 21, the mean±SEM hindlimb placing score (maximum=10) ranged from 0.3±0.3 (Experiment 2) to 1.8±0.7 (Experiment 1), and summed over all postoperative days (maximum=80), it reached no higher than 6.3±2.4 (Experiment 1). Recovery of hindlimb placing in vehicle-treated rats was rare (10% of rats) and incomplete (Figure 2, right column). Because hindlimb placing reactions were not controlled by cephalic stimuli, hindlimb dysfunction was more obvious than forelimb placing deficits (see Figure 1d).

Placing deficits on the beams were virtually restricted to the hindlimbs (99.6% of all placing errors in all vehicle-treated rats). On Day 1, vehicle-treated rats made an average of 81% (range 62–91%, depending on infarct size) of all hindlimb placing deficits with the contralateral hindlimb. As summarized in Table 1, the number of errors more than doubled (1.4–2.8) as vehicle-treated rats were shifted from the wide to the narrow beam. In such rats, the contralateral hindlimb slipped off when they decelerated, stopped suddenly, or made small postural shifts, but not during uninterrupted forward locomotion. Transition to immobility and/or independent limb use constituted the major behavioral context for failure to place the contralateral hindlimb.

Flunarizine markedly protected against neurologic deficits. In Experiment 1 (Figure 2, top row), none of the 12 vehicle-treated rats showed hindlimb placing on Day 1. By contrast, only three of 12 flunarizine-treated rats showed deficits in at least one placing test, and their mean±SEM score was 7.9±1.1. From Day 3 on, all 12 flunarizine-treated rats recovered hindlimb placing (maximum scores), whereas all 12 vehicle-treated rats showed sustained deficits. Forelimb placing deficits, clearly present in the vehicle-treated rats during the first week, were virtually absent in the flunarizine-treated rats. In Experiment 2 (Figure 2, middle row), which involved larger infarcts (Table 2), all eight vehicle-treated rats displayed persistent deficits in hindlimb placing. However, only two of eight flunarizine-treated rats showed deficit placing on Day 1, when the mean±SEM score was as high as 8.1±1.2. Furthermore, all eight flunarizine-treated rats recovered hindlimb placing by Day 5, whereas forelimb placing was fully protected from Day 1 on. Except for two of 10 rats (whose placing reactions remained deficient throughout Experiment 3), oral, like intravenous, flunarizine clearly blocked neurologic deficits (Figure 2, bottom row). As shown in Table 1, flunarizine reduced the number of contralateral placing errors during beam traversing and stemmed the increase in number of errors following shifts to the narrow beam in 8 rats.

We increased infarct sizes (>10 mm³) by means of a 2-mm objective, so that the infarcts encompassed the entire hindlimb area, with or without destruction of the underlying corpus callosum or hippocampus (Experiment 4). In that condition, up to 10 mg/kg i.v. flunarizine no longer prevented persisting hindlimb placing deficits.

When 1.25 mg/kg i.v. flunarizine was administered 1, 3, 6, 12, or 24 hours after medium-sized infarcts and tactile/proprioceptive limb placing was evaluated on Day 2 (Experiment 5), 70%, 40%, 30%, 10%, and 0%, respectively, of the rats (n=10 per time) showed intact placing reactions.

The locus of neocortical infarction, which was similar for flunarizine- and vehicle-treated rats, was centered inside the parietal hindlimb area, with slight encroachment upon the adjacent forelimb area in 57% of the rats.

Pretreatment with flunarizine has been reported to reduce photochemical infarct size when measured 4 hours after infarction. As shown in Table 2, in our experiments cortical infarct volume or amount of thalamic gliosis did not differ significantly between flunarizine- and vehicle-treated rats. Likewise, the dorsal surface areas of the infarcts did not differ (results not shown). However, those results were obtained on Day 21, by which time infarcts undergo necrotic atrophy, contain cystic cavities, and are surrounded by fibrogliotic reaction tissue (e.g., compare Figure 2 of Reference 2 with our Figure 3a). Therefore, we repeated the conditions of Experiment 2, but measured neocortical infarct sizes 4 hours after infarction (Experiment 2'). As mentioned in Table 2, treatment with intravenous flunarizine 30 minutes after infarction significantly reduced 4-hour-old infarct volume by an average of 31.4% (Mann-Whitney U test, two-tailed p=0.038).

The pattern of thalamic gliosis was comparable in all groups and involved mainly the ipsilateral nucleus ventralis posterolateralis (see Figure 3b), with occasional gliosis in the posterior nucleus complex and in the nucleus dorsalis lateralis (45% of the rats).
1. Hindlimb placing reactions
2. Forelimb placing reactions
3. Rats with hindlimb placing

**Figure 2.** Time course of mean±SEM contralateral tactile and proprioceptive limb placing reactions following unilateral photochemical infarction of sensorimotor neocortex in rats. A: Experiment 1, 1.25 mg/kg i.v. flunarizine (●, shaded bars) versus vehicle (cyclodextrin, □, open bars) injected 30 minutes after induction of small infarcts (approximately 1 mm³). B: Experiment 2, 1.25 mg/kg i.v. flunarizine (●, shaded bars) versus vehicle (cyclodextrin, □, open bars) injected 30 minutes after induction of medium-sized infarcts (≥3 mm³). C: Experiment 3, 40 mg/kg p.o. flunarizine (●, shaded bars) versus vehicle (water, □, open bars) injected 30 minutes after induction of medium-sized infarcts. Mann-Whitney U tests, *p<0.05, **p<0.01, ***p<0.0001, two-tailed probabilities.

**Table 2.** Neocortical Infarct Volume and Gliosis in Nucleus Ventralis Posterolateralis Thalami After Photochemical Infarction in Rats Treated with Flunarizine 30 Minutes After Infarction

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>Infarct volume (mm³)</th>
<th>Gliosis (integrated density ×10⁶)</th>
<th>Infarct volume (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle (cyclodextrine)</td>
<td>0.84±0.12</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Flunarizine (1.25 mg/kg i.v.)</td>
<td>0.96±0.10</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2 and 2'</td>
<td>Vehicle (cyclodextrine)</td>
<td>3.57±0.59</td>
<td>1.59±0.25</td>
<td>8 7.01±0.71</td>
</tr>
<tr>
<td></td>
<td>Flunarizine (1.25 mg/kg i.v.)</td>
<td>3.41±0.39</td>
<td>1.53±0.14</td>
<td>8 4.81±0.74*</td>
</tr>
<tr>
<td>3</td>
<td>Vehicle (cyclodextrine)</td>
<td>2.71±0.26</td>
<td>0.38±0.16</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Flunarizine (40 mg/kg p.o.)</td>
<td>2.61±0.32</td>
<td>0.39±0.12</td>
<td>ND</td>
</tr>
</tbody>
</table>

Data are mean±SEM. ND, not determined.

*p=0.038 different from vehicle, Mann-Whitney U test, two-tailed probability.
FIGURE 3. a: A 21-day-old photochemical infarct in hindlimb sensorimotor cortex of vehicle-treated rat (coronal section stained with azure A-eosin B). b: Thalamic gliosis in ipsilateral nucleus ventralis posterolateralis, marked histochemically by purine nucleoside phosphorylase. Photomicrographs from Experiment 2.

The following observations of the control rats are also pertinent: intravenous or oral flunarizine did not produce behavioral effects on its own; rose bengal or skull illumination alone caused neither brain damage nor behavioral effects; and following comparable left-sided infarcts, sensorimotor deficits were similar to those reported above.

Discussion

Photochemical damage to the sensorimotor cortex of rats produced selective and lasting deficits in tactile and proprioceptive limb placing. Thus, a stroke-relevant method of inflicting focal brain damage, that is, photochemical thrombosis, can be fruitfully employed to analyze cortical involvement in sensorimotor functions. In this regard, our findings confirm other well-established evidence that damage to the parietal sensorimotor neocortex specifically yields deficits in limb usage.

Our analysis is new in that it specifies at least some of the behavioral conditions that govern the tactile/proprioceptive limb placing deficit in neocortically damaged rats. For instance, persistent deficits were evident when limbs were tested one at a time. Further, deficits were masked when limb placing was integrated into other behavioral patterns. Thus, the forelimb placing deficit could be overcome by sensory stimuli arising from the head (e.g., visual, whisker, and/or chin contact), whereas tactile/proprioceptive stimuli limited to the deficient forelimb itself were inadequate. In our tests for neglect we elicited head-turning reactions, which may have been responsible for the minimal, transient neglect observed. Indeed, when neglect is made head movement-independent, sensorimotor cortical damage selectively results in a prominent, long-lasting deficit. Likewise, deficits in tactile/proprioceptive placing of the hindlimb disappeared as soon as limb placing was incorporated into a locomotor automatism, such as during trotting across an elevated beam. However, deficiencies in hindlimb placing became obvious as the rats became immobile and/or when isolated limb movements were required (e.g., during small postural shifts). Therefore, distinct features of such behavioral dysfunctions (in particular, abnormalities in independent limb movement) make for a potentially useful animal analogue of neurologic disorders in human stroke. The hindlimb placing deficits in our rats are robust in that they persist (e.g., for at least 6 months) when an infarct of at least 3 mm³ is produced inside the hindlimb sensorimotor cortex (observed in 20 rats). However, the validity of our behavioral results as a predictor for functional protection in human stroke is presently unknown.

Our findings point to possible mechanisms underlying the protective effects of flunarizine in this stroke model. Measured 21 days after infarction, gross thalamocortical structural damage did not differ between flunarizine- and vehicle-treated rats. By contrast, when infarct size was assessed 4 hours after infarction (i.e., corresponding to the time of maximal expansion of the ischemic zone), posttreatment with intravenous flunarizine reduced infarct size by 31%, confirming a similar effect obtained with flunarizine pretreatment. There may
be several reasons why an early reduction in infarct size does not show as a smaller infarct several weeks later. In contrast to the clear delineation of a 4-hour-old infarct,2 cavitation, shrinkage, and deformation of tissue in 21-day-old infarcts (see also Reference 7) may render it difficult to demonstrate the residue of early alterations in infarct volume, particularly when the volumes are relatively small. On the other hand, the necocortical infarct, which we produced, may differ from that brought about by middle cerebral artery occlusion (MCAO). In that model, neocortical damage corresponds to a peri-infarct, oligemic, penumbral40,41 in which collateral blood flow may still occur. On the contrary, in our photochemical stroke model, which produces endarterial–like thrombosis,42 the cortical lesion consists of both the irreversible infarct core and the penumbra. The latter feature, combined with a possibly smaller penumbra-to-core ratio than in the MCAO model, may make the photochemical lesion more resistant to drug-induced size reductions. On the other hand, as our photochemical infarcts produced subtotal damage to the hindlimb cortical area, only a small reduction in infarcted area may suffice to produce functional protection.

The site of flunarizine-induced protection conceivably lies outside the primary focus of irreversible destruction. In this respect, two findings are relevant. First, flunarizine spared hindlimb function when damage to the hindlimb cortex was subtotal, but flunarizine was ineffective when the entire hindlimb area was infarcted. Therefore, viable cortical tissue surrounding the irreversibly thrombotic core, such as in the penumbra, appears to be of primary importance in functional recovery and its promotion by flunarizine. Secondly, flunarizine displayed a postinfarct critical period of efficacy that overlaps the first 4 hours after infarction, when the ischemic zone has been shown to expand.1,3–7

Neural tissue within the penumbra is forced into a precarious metabolic state of low blood flow and enhanced glucose utilization.1,40 This peri-infarct, oligemic area is characterized by blood–brain barrier dysfunction, mechanical compression due to vasoogenic edema, extravasation of vasoactive substances, release of neurotoxins, and disturbed cellular ion homeostasis.1,41 Repeated transient increases in extracellular potassium concentration have been demonstrated in the penumbra.40,41 When sufficiently elevated (e.g., 15–20 mM), such increases in potassium concentration are not only responsible for vasospasm,13 but they also trigger and sustain excessive neuronal depolarization. This is evidenced by successive waves of spreading depression as well as by more protracted bouts of depolarization, tending toward terminal depolarization, which is typical of irreversible hypoxia–ischemia. Whereas spreading depression does not appear to cause irreversible neuronal damage in otherwise intact brain,43 such focal ischemia–related waves of depolarization cannot yet be excluded as a factor contributing to delayed recovery of function or to long-term functional loss, when (however transiently) they act on weakened neural systems.

Flunarizine exerts inhibitory effects on spreading depression, particularly in previously damaged tissue.44,45 Most importantly, following arrest of blood flow to the brain, flunarizine doubles the latency of the rapid rise in cerebral extracellular potassium concentration, indicating an increased resistance of cerebral tissue to ischemia-induced depolarization.46 Along with its antihypoxic12 and antivasoconstrictive13 properties, these cerebral effects of flunarizine may enable the penumbra to cope with hemodynamic, metabolic, and ionic derangements during the early phase of expanding ischemic damage. On the other hand, extravasation and metabolic changes distal to the primary focus,1,47 spread of vasoactive compounds,1,47 transsynaptic depression of subcortical as well as cortical areas,48 and altered neurotransmitter/receptor mechanisms49–51 following infarction require more study. In particular, their contribution to the early pathophysiology of evolving stroke, as well as their role in the cerebrovascular mechanisms that enable flunarizine to spare neurologic function in this experimental stroke model, need further elucidation.

Acknowledgments

We gratefully acknowledge the technical assistance of Bruno Van Deuren, Hilde Duyschaever, and William Melis, and we thank Lambert Leijssen for artwork and photography.

References


Key Words: animal models • cerebral cortex • flunarizine • rats • cerebral ischemia
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Stroke. 1989;20:1383-1390
doi: 10.1161/01.STR.20.10.1383
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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