Protective Effect of Synaptic Inhibition During Cerebral Ischemia in Rats and Rabbits

Patrick D. Lyden, MD, and Brenda Hedges, BS

Background: Excitatory neurotransmitters appear to cause cell death during ischemia by inducing depolarization, influx of ions, and metabolic failure in the postsynaptic neuron. If this hypothesis is correct, then postsynaptic membrane hyperpolarization and inhibition of metabolism may be protective. Antagonists of the excitotoxic amino acid glutamate protect neurons in culture and in animal models of stroke but appear to cause unacceptable side effects in humans. We propose an alternative strategy of protection using agonists of the inhibitory neurotransmitter γ-aminobutyric acid.

Methods: We caused multifocal cerebral ischemia in rats and rabbits by injecting microspheres into the carotid circulation. We administered saline, muscimol, or MK-801 within 5 minutes of stroke onset. We used a bioassay to measure outcome. In rats, we also used learning to assess cortical function, and we performed detailed quantitative brain morphometry 3 months after infarction.

Results: Using the bioassay, we found that muscimol exerted a protective effect in rats (p < 0.01). There was a dose–response effect seen in muscimol-treated rabbits. Rats treated with muscimol or MK-801 exhibited significantly better visual–spatial learning compared with saline-treated subjects (p < 0.001). Hemisphere volume after ischemia was comparable in all groups.

Conclusions: Agonists of γ-aminobutyric acid and antagonists of glutamate appear to protect brain during ischemia. Since agonists of γ-aminobutyric acid are known to have fewer side effects in humans, they may prove more useful in the clinical setting as neuroprotective agents. (Stroke 1992;23:1463–1470)

KEY WORDS • cerebral ischemia • GABA • glutamates • rabbits • rats

The best treatment for acute cerebral vascular occlusion may be thrombolysis, if treatment is given soon after stroke onset.1,2 Occasionally it may not be possible to immediately transport a stroke patient to a hospital. Therefore, it is imperative that cerebral protective agents be developed for use by prehospital personnel to enable delayed transport followed by definitive treatment with a thrombolytic drug. The ideal agent should limit neuronal cell death in an area of low cerebral blood flow and not cause systemic effects or alteration of mental status. Several classes of agents have been proposed for neuroprotection, and recently attention has been focused on antagonists of excitatory neurotransmitters.

Excitatory amino acid neurotransmitters, such as glutamate and aspartate, appear to play a key role in the demise of cells after a variety of cerebral insults including ischemia, trauma, and seizures.3–5 The excitatory amino acid causes depolarization of the postsynaptic membrane, cellular edema, calcium influx, failure of membrane homeostasis, and eventually cell death.6–8 Calcium enters the cell via the N-methyl-D-aspartate (NMDA) receptor itself and via voltage-gated channels. Receptor-specific antagonists limit these effects during ischemia in tissue culture and in some animal models of stroke.9–13 During early trials in human stroke patients, however, these drugs caused side effects including hallucinations and sedation (authors’ unpublished observations). These adverse effects are not surprising, given that most glutamate antagonists behave similarly to the dissociative anesthetics phencyclidine and ketamine.14

We hypothesize that direct postsynaptic inhibition may be an alternative to using receptor antagonists to block the effects of excitatory amino acid.15–18 There are several observations that suggest this prediction. First, in neuronal culture, extracellular sodium and chloride are necessary in physiological quantities for glutamate or NMDA to cause cell death, suggesting that membrane depolarization must occur in this process.6 Altering membrane conductances and the resting membrane potential of the cell, as occurs after synaptic release of γ-aminobutyric acid (GABA), reduces the probability of depolarization due to glutamate release.16,17,19,20 Second, GABA increases chloride conductance, which appears to “clamp” the membrane potential and prevent opening of voltage-gated calcium channels.17 Third, GABAergic neurons are resistant to ischemic injury in culture and in whole brain.19,20,21 Fourth, inhibitory neurotransmitters cause other effects that might be protective, such as a reduction of cellular metabolic demand, and oxygen consumption.22–24 Finally, GABA

From the Department of Neurosciences, University of California, San Diego, and the School of Medicine, Neurology Service, San Diego Veterans Administration Medical Center, San Diego, Calif.

Supported in part by the National Institute of Neurologic Disorders and Stroke, grants NS26783 and N01-NS02377, and by the Veterans Administration.

Address for correspondence: Dr. Patrick D. Lyden, Neurology (127), 3350 La Jolla Village Drive, San Diego, CA 92161.

Received January 6, 1992; final revision received April 27, 1992; accepted April 30, 1992.

See Editorial Comment, p 1469
agonists may increase cerebral blood flow by acting directly on cerebral blood vessels to cause vasodilation, although this finding has been challenged. Muscimol, an alkaloid derived from mushrooms, is an agonist of GABA, the primary inhibitory neurotransmitter in mammalian brain, and is well studied as a potential anticonvulsant.

Materials and Methods

The experimental protocol was approved by the University of California, San Diego, Animal Research Committee following all national guidelines for the care of experimental animals. A similar surgical procedure was used in rats and rabbits, and a full description of the method has been published. To avoid any confounding interaction between anesthesia and ischemia, we implanted catheters in the internal carotid artery and allowed the animals to recover fully from anesthesia before embolization. For the placement of the catheter, animals were anesthetized with halothane 2–4% in oxygen: nitrous oxide 40:60 delivered by face mask. After the catheter was flushed with 0.2 ml heparinized saline, it was secured under the skin and left protruding between the scapulae for external access. Inhaled anesthesia was discontinued after all incisions were closed. We prepared a mixture of unlabeled and iodine-125–labeled plastic microspheres, diameter 25 μm for rats and 50 μm for rabbits (3M Corporation, Minneapolis, Minn.). Each aliquot of spheres was weighed (range, 20–50 μg for rats; 2–7 mg for rabbits), then placed in a gamma counter to measure the radioactivity present, permitting calculation of the specific activity of the mixture. Next, the spheres were suspended in 0.25 ml polyoxyethylene-sorbitan (Tween) 0.05% in normal saline and sonicated at full power for 3 minutes (Branston cell disrupter, Danbury, Conn.) to completely disperse the microspheres. The sonicated mixture was transferred to a gas-tight syringe (Hamilton Company, Reno, Nev.), and another 0.25 ml saline was added. To cause cerebral ischemia 3 hours after implantation of the catheters, a subject was restrained, a Hamilton syringe was inserted through a port on the protruding end of the catheter, and the microspheres were injected. This was followed by three flushes of saline to advance the spheres into the cerebral circulation (each flush was 0.15 ml in rats and 1.0 ml in rabbits). After embolization, each animal was released and examined. A global assessment was made as follows: normal, abnormal (diminished level of consciousness, reduced exploratory behavior, circling, limp, or inability to maintain upright posture), or dead. The animals were judged at 24 and 48 hours by an examiner blinded to the group assignments. The behavioral ratings at 48 hours were used to categorize each animal for the bioassay so that there would be no residual sedative effects from the medications that contaminate the rating. At the end of the observation period, the rabbits were killed and the brains were placed in the gamma counter to measure the trapped radioactivity. Quenching in solid tissue is negligible with a gamma emitter such as 125I. The quantity of microspheres trapped in each brain was then estimated using the specific activity of the injected mixture of microspheres. Rats were allowed to recover from the acute phase of infarction before neurobehavioral testing.

Two weeks after embolization, all surviving rats were tested for visual–spatial learning using a Morris water maze. The water maze is a large circular tank painted matte black, filled with water, and labeled with four starting positions around the outside rim (north, south, east, west). The four cardinal positions divide the tank into imaginary quadrants. In the southeast quadrant there is an escape platform. A video camera suspended above the tank records the path of the animal swimming to the escape platform after it is dropped into the water from one of the start positions. The video signal is digitized and analyzed to compute the time from start to escape. For this study we did not use more detailed maze paradigms, such as a spatial probe or retention test. All animals (and an unlesioned control group) were tested daily using a visible escape platform 1.5 cm above water level. The animal was dropped into the pool from a different sequence of start positions each day, and the swim time was recorded by the computer. If the animal did not climb onto the escape platform after 90 seconds, the operator placed it manually on the platform. After each swim, the animal was allowed to rest for 20 seconds on the platform. On the fourth day of testing with the visible platform all animals should swim directly to the platform, averaging 5 to 8 seconds per swim. This test is used to eliminate animals with severe motor, sensory, or visual deficits that would confound testing of visual–spatial memory. Learning of the visual–spatial information necessary to navigate to the escape platform was then tested by using a hidden platform, which is a matte-black square covered with mesh and suspended 1.0 cm below the surface of the water by a pole. The swimming rat cannot see the platform because it is black (nonreflective) and because of refraction through the water surface. Unlesioned animals learn the location of the platform, and after training swim directly to the platform in 5 to 8 seconds.

After the maze testing, rats were killed with halothane and carbon dioxide and perfused through a transcatheter catheter with 100 ml normal saline followed by 100 ml 10% phosphate-buffered formalin. After 24 hours’ fixation by immersion in the same fixative, the brains were removed and stored in a 10% phosphate-buffered formalin solution to measure the trapped radioactivity and then immersed in 30% sucrose for 24–48 hours. To determine cerebral volumes, we followed the method of Cavaleri. From a random start point, we sectioned each brain entirely, selecting a 30-μm section every 400 μm throughout. The sections were mounted and stained with Luxol Fast Blue and cresyl violet. Cerebral hemisphere areas on each section were measured using semiautomated image analysis, and reconstructed volumes were calculated from the area and interval distance between sections, using appropriate corrections for shrinkage.

We randomly divided 74 male Sprague-Dawley rats (weight range, 300–350 g) into three groups. Within 5 minutes of embolization we administered by tail vein a bolus of saline (n=30), MK-801 (a gift from Merck & Co., Inc., New York) 1.0 mg/kg (n=28), or muscimol (Sigma Chemical Co., St. Louis, Mo.) 1.5 mg/kg (n=16). All solutions were prepared so that each animal received an infusion of 1.0 ml/kg over 0.5 minute. We also treated 70 male New Zealand White rabbits (weight range, 3.0–3.5 kg) within 5 minutes of embolization, randomly divided into three groups. We gave saline.
OA SALINE
A B MK-801 1.0 mg/kg
C MUSCIMOL 1.5 mg/kg

10 MICROSPHERES x10^3
100

OA  SALINE
A B  MUSCIMOL 1.5  MG/KG
0 1 4 5 MICROSPHERES X10^3

(n=29), muscimol 1.5 mg/kg (n=24), or muscimol 3.0 mg/kg (n=17) by ear vein over 0.5 minute. The groups were somewhat imbalanced despite random allocation.

The derivation and use of the statistical method for quantal bioassay are completely described elsewhere. For each group of animals we compared the amount of microspheres trapped in the brains to the global behavioral rating. To these data a logistic function was fit to describe the response of the group to increasing amounts of ischemia. From the data, the function estimates the quantity of trapped microspheres that causes 50% of the animals in each group to be abnormal or dead, a parameter called the ED50. In several previous investigations, the ED50 has been shown to be a highly reproducible and efficient measure of the efficacy of cerebral protective agents. A protective drug causes the ED50 to be larger in a treated group of subjects. An intuitive description of this effect is that if the ED50 increases, the treated animals are able to tolerate larger amounts of ischemia without a deterioration in neurological function.

Results

Using the bioassay, muscimol was protective during ischemia in both rats and rabbits (Figure 1). The ED50 values (mean±SE) for saline-treated rats were 3.53±0.96×10^2, for MK-801 7.18±2.07×10^2, and for muscimol 11.19±2.28×10^3 microspheres. The ED50 for muscimol was significantly greater than that for saline (t=3.05, p<0.01), but that for MK-801 was not (t=1.56, p=0.12). There were six deaths in this experiment, three in the saline group and three in the MK-801 group. In the saline-treated animals the numbers of microspheres recovered in the brains were 178×10^2, 7.7×10^2, and 6.1×10^2, and in the MK-801 group we recovered 12×10^2, 22×10^2, and 4.5×10^2 microspheres.

Muscimol was also protective in rabbits in a dose-dependent manner (Figure 1, bottom). The ED50 values were 3.98±0.50×10^2, 3.18±0.78×10^2, and 5.33±0.59×10^2 microspheres for saline, muscimol 1.5 mg/kg, and muscimol 3.0 mg/kg, respectively. The ED50 for the higher dose of muscimol is probably larger than that of saline, although the test is not quite statistically significant (t=1.726, p=0.09). In the saline group there were four deaths, and we recovered 5.4×10^3, 0.085×10^3, 5.04×10^3, and 0.72×10^3 microspheres in these brains. There were four deaths in the low-dose muscimol group, with 6.72×10^2, 10.3×10^2, and 7.8×10^2 microspheres recovered, and two deaths in the high-dose muscimol group, with 6.12×10^3 and 9.96×10^3 microspheres recovered. We did not test MK-801 in rabbits in this investigation because we have previously shown that a dose of 0.5 mg/kg is protective in this model.
The results of the spatial memory task confirmed the bioassay data (Figure 2). During the first eight trials, all subjects rapidly learned the location of the visible escape platform. This demonstrated that no animals suffered a motor, sensory, or visual deficit severe enough to confound measurement of visual-spatial learning. During the final 12 trials, during which the visible platform was replaced with a hidden platform, we tested the ability of each animal to acquire the location of the escape platform. The control (not embolized) subjects quickly learned the location of the hidden platform so that the mean escape latency was shorter on each day of testing. Saline-treated stroke subjects, on the other hand, did not learn as quickly, so that the escape latency did not improve as much from trial to trial. Subjects treated with muscimol or MK-801 learned the platform location as well as unlesioned control animals (Figure 2, top). From trial 15 to trial 20, it appears that the subjects reached an asymptotic latency, suggesting a plateau level of performance. We averaged the latencies from these plateau trials to compare the learning performance among groups. During the final six trials the control animals swam with a mean±SD latency of 15.3±11.6 seconds, but the corresponding value for saline-treated subjects was 23.5±16.6, for muscimol-treated subjects 13.8±9.4, and for MK-801-treated subjects 13.3±8.2 seconds ($F_{3,111}=7.62$, $p<0.0001$). These plateau phase mean latencies and standard deviations are pictured in the inset in Figure 2. Post hoc testing (Tukey's procedure) revealed that the means for muscimol and MK-801 were both different from saline ($p<0.05$) and were the same as those in unlesioned animals. Latency is also partially dependent on swim velocity, but the data obtained during testing with the visible platform confirmed that none of our test subjects suffered from deficits severe enough to affect swim velocity.

To detect a possible drug effect on the rate of learning, as distinct from plateau latency, we performed a regression analysis on the hidden-platform swim latency data after transformation using the inverse function. The results of the analysis are shown in Figure 2, bottom. The linear model fits the transformed data reasonably well. Alternative regression models might well fit the data, but the linear model is attractive because the slope of the fitted line is easily interpreted as a measure of the rate at which the subjects acquire and store the visual–spatial information needed to navigate to the escape platform.

It could be argued that the bioassay and spatial learning results are due to the fact that the treatment groups were less severely lesioned. We found that the quantity of trapped microspheres and the volume changes in the brains were comparable among the groups (Figure 3). On average, the brains contained $12\times10^3$ microspheres, and there were no differences among the three lesioned groups ($F_{2,32}=0.84$, $p=0.44$). In each lesioned group, the normal hemisphere was significantly larger than the lesioned hemisphere ($p<0.01$ in each, after correction for multiple comparisons). Using multivariate analysis of variance, the volumes of the lesioned hemispheres and the normal hemispheres were not different among groups (Figure 3).
volume of infarction directly, for three reasons. First, the reproducibility of brain volume measurements is very high, but that of the estimates of infarct volumes is not.33,46,47 This is true because it is easier to measure something that is present (brain) than to estimate the area of something that is missing (infarct). Second, the area of infarction is visible months after stroke as a cyst, or area of pannecrosis. Undoubtedly there is also loss of cerebral volume around the zone of necrosis, and the total brain volume includes this additional zone of potential interest. Finally, after infarction the remaining brain may collapse into the cystic area, leading to underestimates of infarct volume.

The data presented here indicate that synaptic inhibition with a GABA agonist protects the brain during cerebral ischemia. We demonstrated the protective effect of muscimol in rats and rabbits, and the effect is at least as potent as an effect of the NMDA receptor antagonist MK-801, as we have demonstrated previously.9 A dose–response effect was suggested in the rabbits we tested. The spatial learning data collected from the rats confirm the findings from the quantal bioassay. During the first eight trials, all subjects quickly learned the location of the platform, suggesting that the embolization did not cause a severe impairment of motor, sensory, or visual function and that swim velocity was the same in all groups (Figure 2). During the final 12 trials, the platform was hidden below water level, forcing the subjects to rely on visual–spatial clues in the testing room to find the platform.30 We have shown previously that embolized rats suffer a delay in learning, manifested as a longer escape latency during testing with the hidden platform.29 This delay was not seen in rats treated with muscimol or MK-801, suggesting that visual–spatial learning was preserved in the treated animals. Using quantitative brain morphometry we confirmed that all groups suffered a similar degree of ischemic insult, so the protective effect is not an artifact of selection bias.

It has been suggested that the vulnerability of a neuron to ischemia may reflect a balance of excitatory and inhibitory inputs.17,18,48 GABAergic cells in culture and tissue slices resist NMDA toxicity,15,16,21 and ischemia appears to cause an upregulation in GABA binding sites.49 Synaptic inhibition is mediated through two GABA receptor subtypes, A and B.17 The GABA-A receptor is found throughout the brain and is a ligand-gated chloride channel that mediates a fast inhibitory response.50 The GABA-B receptor is not well studied, but among other effects, GABA-B stimulation appears to reduce presynaptic release of several neurotransmitters and to mediate the late inhibitory postsynaptic potential.16,51

Muscimol crosses the blood–brain barrier and exhibits a very high affinity for the GABA-A receptor but little or no physiological affinity for the GABA-B receptor.20,52 Muscimol appeared to be protective in a gerbil model when given before the onset of global ischemia.53 Also, muscimol infusion into the ventricle for 2 weeks prevented ibotenic acid–induced degeneration of the substantia nigra after cortical ablation.54 However, longer infusions of diazepam or muscimol, started several hours after cortical injury, may retard recovery.54–57 In contrast, the GABA-B agonist baclofen, administered to rats after forebrain ischemia, was not protective, supporting the concept that the protective effect of
muscimol is mediated by GABA-A receptors. Other GABA-A agonists did not block potassium- or ischemia-induced release of aspartate, as measured by hippocampal microdialysis, further suggesting that muscimol is not acting to reduce presynaptic release of neurotransmitters but rather to protect the postsynaptic cell by hyperpolarization.

One might question why the neuroprotective agents we tested did not show a measurable effect on the pathological outcome measures. Many stroke investigators have noted previously that histopathological outcome variables tend to be insensitive to pharmacological effects. The variance associated with measuring cerebral volumes is considerable, and very large numbers of subjects are necessary to clearly demonstrate protective effects. We attempted to reduce the variance by exhaustively sectioning the entire brain, selecting sections for study following a stratified sampling scheme, and by measuring the hemisphere areas using a semiautomated process that reduces operator decision making. The remaining variance is attributable to at least three sources: 1) Only survivors could be studied, since quantitative morphometry is possible only if the brain is properly perfused at the time of death. Treatment allowed several subjects to survive to time of death with very large infarctions, and inclusion of these data biased the morphometry results away from showing a protective effect. 2) Individual response to an insult is variable, as is well known in all animal models of cerebral infarction. Thus, even if all subjects had received the identical number of microspheres, the pathological response would still be variable. 3) Residual measurement error is attributed to effects of fixation, freezing, dehydration, and staining.

We conclude that the GABA-A agonist muscimol is at least as protective as a glutamate antagonist, MK-801, in this animal model of stroke. There are several reasons why histopathological outcome variables tend to be insensitive to pharmacological effects. The variance associated with measuring cerebral volumes is considerable, and very large numbers of subjects are necessary to clearly demonstrate protective effects. We attempted to reduce the variance by exhaustively sectioning the entire brain, selecting sections for study following a stratified sampling scheme, and by measuring the hemisphere areas using a semiautomated process that reduces operator decision making. The remaining variance is attributable to at least three sources: 1) Only survivors could be studied, since quantitative morphometry is possible only if the brain is properly perfused at the time of death. Treatment allowed several subjects to survive to time of death with very large infarctions, and inclusion of these data biased the morphometry results away from showing a protective effect. 2) Individual response to an insult is variable, as is well known in all animal models of cerebral infarction. Thus, even if all subjects had received the identical number of microspheres, the pathological response would still be variable. 3) Residual measurement error is attributed to effects of fixation, freezing, dehydration, and staining.

We conclude that the GABA-A agonist muscimol is at least as protective as a glutamate antagonist, MK-801, in this animal model of stroke. There are several GABA-A agonists available for use in humans, and they tend to have a low incidence of side effects. Thus, this category of drug may ultimately prove useful in treating acute stroke.

Acknowledgment

We are grateful to Fred Gage, PhD, for the use of the water maze.

References

5. Auer RN, Siesjo BK: Biological differences between ischemia, freezing, dehydration, and staining.
8. Auer RN, Siesjo BK: Biological differences between ischemia, freezing, dehydration, and staining.
10. Simon RP, Swan JH, Griffiths T: Blockade of N-methyl-D-aspartate receptors may protect against ischemic damage in the brain. Science 1984;226:850-852
15. Tecumse ES, Chao DW: GABAergic neocortical neurons are resistant to NMDA receptor-mediated injury. Neurology 1989;39:676-682
36. Lyden PD, Zivin JA, Kochhar A, Mazzarella V: The effects of calcium channel blockers on neurological outcome following


---

**Editorial Comment**

The Promethean task of identifying critically vulnerable processes accounting for irreversible ischemic brain damage, with the hope of developing neuroprotective strategies, has long eluded investigators until the role of excitotoxins and the protective effects of their inhibitors were delineated (Rothman and Olney and Choi). Like a veritable life-giving nectar, these findings have galvanized a broad assault on detailing these complex mechanisms. In parallel with complementary schemes to restore occluded circulation with thrombolytic agents, optimize microcirculation using hemorheological approaches, modify tissue pH changes, minimize free radical damage, stabilize membrane/intracellular structures, and regulate gene expression to augment regrowth and stabilization, efforts to inhibit excitotoxins have now gone beyond animal research with the planning of clinical trials. As Lyden and Hedges demonstrate, they have seized upon the known dual influences on neural tissue of both facilitatory and inhibitory input to optimize neuroprotection during ischemia. The complex responses of neurons to massive and bewildering electrical traffic of both varieties, accounting for the highest evolutionary developments of the human mind and consciousness, cannot, however, be simply reduced to excitation and inhibition. Nonetheless, this form of reductionism allows an operational approach to activities of brain in which neural excitation is equated with energy utilization, while cellular tranquility suggests energy preservation. Thus, the strategy of Lyden and Hedges to hyperpolarize neurons with agonists of the inhibitory neurotransmitter γ-aminobutyric acid (GABA), namely with muscimol, can be viewed as achieving the complementary action of excitotoxin inhibition.

In addition to providing a fresh revisit to the use of GABA agonists, Lyden andHedges emphasize the distressing side effects of sedation and hallucinations in human trials with glutamate antagonists, which may limit their usefulness clinically and hence justify the impetus to find alternative means of maximizing neuroprotection.

The first use of GABA agonists in an ischemic model of stroke to assess neuroprotection was by Sternaul et al, who found that five agents with GABA agonist activity were effective in averting delayed neuronal death. However, Rosenbaum et al found no benefit with one GABA agonist, baclofen. In the study by Sternaul et al, gerbils received temporary 5-minute ischemia from bilateral carotid occlusion, and the various GABA agonists used included pentobarbital, diazepam, baclofen, valproic acid, and muscimol. Four days after recirculation, the animals were killed, and particular histological attention was given to the hippocampus, particularly the CA1 neurons. It is worth noting that each of these GABA agonists were protec-
Protective effect of synaptic inhibition during cerebral ischemia in rats and rabbits.

P D Lyden and B Hedges

*Stroke*. 1992;23:1463-1469
doi: 10.1161/01.STR.23.10.1463

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://stroke.ahajournals.org/content/23/10/1463

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org/subscriptions/