NR2B Subunit Exerts a Critical Role in Postischemic Synaptic Plasticity

Barbara Picconi, PhD; Anna Tortiglione, PhD; Ilaria Barone, PhD; Diego Centonze, MD; Fabrizio Gardoni, PhD; Paolo Gubellini, PhD; Paola Bonsi, PhD; Antonio Pisani, MD; Giorgio Bernardi, MD; Monica Di Luca, MD; Paolo Calabresi, MD

Background and Purpose—We characterized the differential effect of the NR2B subunit antagonist ifenprodil in the induction of activity-dependent long-term potentiation (LTP) and of postischemic LTP as well as in the neuronal damage induced by focal ischemia.

Methods—Intracellular recordings were obtained from rat corticostriatal slice preparations. High-frequency stimulation of corticostriatal fibers was used as a LTP-inducing protocol. In vitro ischemia was induced by oxygen and glucose deprivation. In vivo ischemia was induced by permanent middle cerebral artery occlusion. Intracellular recordings were also performed in the ischemic penumbra.

Results—Antagonists selectively targeting N-methyl-D-aspartate receptors containing the NR2B subunit blocked postischemic LTP without affecting activity-dependent LTP. In a model of focal ischemia, blockade of NR2B subunit in vivo caused reduction of brain damage, amelioration of neurological outcome, and normalization of the synaptic levels of NR2B subunits. Moreover, the antagonism of NR2B subunit was able to rescue the activity-dependent LTP in the ischemic penumbra.

Conclusions—We suggest that NR2B subunits contribute to the striatal damage caused by in vivo and in vitro ischemia and play a critical role in the induction of postischemic LTP as well as in the suppression of activity-dependent LTP in the ischemic penumbra. (Stroke. 2006;37:1895-1901.)

Key Words: corpus striatum ■ electrophysiology ■ ischemia ■ middle cerebral artery occlusion ■ receptors, N-methyl-D-aspartate ■ synapses

Excitotoxicity is a critical mechanism contributing to neuronal death during brain ischemia.1 A major step in the excitotoxic cascade is the overstimulation of N-methyl-D-aspartate (NMDA) glutamate receptors that causes abnormal Ca++ entry, pathological postischemic synaptic plasticity, and subsequent necrosis or apoptosis.1,2

Clinical trials using NMDA antagonists for stroke started despite the fact that in rodent models of stroke the tested NMDA antagonists produced conflicting results when used in the post-insult time window. Discouraging findings began to accumulate, and most of the clinical trials were terminated because of either lack of efficacy or major adverse cognitive effects.1

With the discovery of the basis of heterogeneity of NMDA receptors through molecular approaches, many new potential therapeutic targets have been uncovered.4 Subtype-specific antagonists may allow blockade of specific subsets of NMDA receptors. This would facilitate cell preservation during excitotoxic events while not causing complete synaptic blockade in physiological conditions, likely reducing side effects. The NR2B-selective antagonists, derivatives of ifenprodil, appear to be the best suited for this purpose4 because NR2B-containing receptors have a preferential extrasynaptic location and have been shown to be preferentially involved in apoptotic cell death.5 Notably, animals treated with ifenprodil or its derivatives do not appear to develop the severe side effects seen with agents such as dizocilpine (MK801) or phencyclidine.4 These antagonists acting on NR2B subunits seem to block excitotoxic responses without producing intolerable cognitive side effects.4 NR2B subunits display a unique regional and cell-specific expression profile.6 The regions expressing the highest presence of NR2B mRNA are the cortex, hippocampus, and striatum. Interestingly, these areas are highly vulnerable to ischemia and excitotoxicity and express postischemic pathological synaptic plasticity.2,7 These observations suggest that the presence of NR2B subunits in...
specific neuronal populations might play a critical role in cell type–specific neuronal death.

In this study we selected the striatal spiny neuron, a neuronal subtype highly vulnerable to ischemic insults, to analyze the effect of pharmacological inhibition of NR2B subunits on both physiological and postischemic synaptic plasticity.

Materials and Methods

Animals

Sprague-Dawley male rats (weight, 250 to 270 g; Charles River, Lecco, Italy) were used. All the experiments were conducted in conformity with the European Communities Council Directive of November 1986 (86/609/EEC).

Electrophysiology

Preparation and maintenance of rat corticostriatal slices (270 to 300 μm) have been described previously. Slices were kept in artificial cerebrospinal fluid (34°C, O₂/CO₂) with composition as follows (in mmol/L): 126 NaCl, 2.5 KCl, 1.2 MgCl₂, 1.2 NaH₂PO₄, 2.4 CaCl₂, 11 glucose, and 25 NaHCO₃. For synaptic stimulation, bipolar electrodes were used, located in the white matter to activate corticostriatal fibers. Intracellular recording electrodes were filled with 2 mol/L KCl. Magnesium ions were omitted from the medium to better disclose the NMDA-mediated component of the evoked excitatory postsynaptic potential (EPSP). Under this experimental condition, high-frequency stimulation (HFS) (3 trains, 3-second duration, 100-Hz frequency, 20-second intertrain interval) of corticostriatal fibers was used as a long-term potentiation (LTP)–inducing protocol.

In some experiments, 30 μmol/L bicuculline was included in the external medium to avoid contamination of the EPSP by a γ-aminobutyric acidA–mediated component of the synaptic potential, which could depolarize because of the KCl electrodes. Whole-cell patch clamp recordings were made as previously described.

Values represent mean±SEM of changes in the respective cell populations. Student t test was used to compare the means. Drugs were applied by dissolving them to the desired final concentration in the saline solution.

In vitro ischemia was delivered by switching to an artificial cerebrospinal fluid solution in which sucrose replaced glucose, gassed with 95% N₂ and 5% CO₂. Ischemic and drug-containing solutions entered the recording chamber no later than 30 seconds after a 3-way tap was turned. Drugs were D-APV, ifenprodil, NMDA (from Tocris-Cookson), bicuculline (from Sigma). CP 101,606 was a kind gift of Dr Vicini.

Electrophysiological Identification of Ischemic Penumbra

To assess the ischemic penumbra after in vivo ischemia, field potential recordings were performed with the use of extracellular electrodes. To define the ischemic penumbra through electrophysiological criteria, recording electrodes were initially positioned within the striatum, and the stimulating electrodes, constantly held 0.3 mm apart, were progressively placed in up to 8 distinct positions around the recording electrode (360°) to try to elicit extracellular field potentials. We assumed we were in the ischemic core when no field potential (negative deflection from the baseline of at least 0.2 mV) was evocable despite the progressive increase of the stimulation intensity. From the “core” position, the electrodes were progressively (0.5 mm) moved along a single line toward the periphery of the striatum, with the stimulating electrode preceding the recording electrode. When the field potential appeared, we considered we were in the ischemic penumbra. This was confirmed by analyzing the extension of the ischemic core after 2,3,5-triphenyltetrazolium chloride (TTC) staining (see below) of the slices obtained immediately before and after that used for the electrophysiological experiments.

Permanent Middle Cerebral Artery Occlusion and Monitoring of Regional Cerebral Blood Flow

Animals were anesthetized intraperitoneally with chloral hydrate (400 mg/kg), and permanent focal ischemia was induced as reported previously. Each rat was allowed to breathe spontaneously, and rectal temperature was maintained at 37°C with a homeothermic heating blanket.

To ensure that the middle cerebral artery (MCA) was occluded and to validate our methodology, during the experiments, regional cerebral blood flow (rCBF) was monitored in the ipsilateral parietal cortex to the occluded MCA with the use of a laser-Doppler flowmeter (Periflux System 5000).

Infarct Size Analysis

Rats were killed by decapitation 4 hours, 24 hours, and 7 days after permanent MCA occlusion (pMCAO). Brains were removed rapidly and dissected into twelve 500-μm sequential coronal slices. Individual sections were placed for 20 minutes in a solution of 2% TTC (Sigma) in normal saline at 37°C and then transferred in 10% phosphate-buffered formalin for fixation.

Unstained areas on each brain slice, defined as infarction area, were then measured by using an image analyzer (Image-Pro Plus 4.5, Media Cybernetics), and the volume of infarction was calculated by integration of the lesion areas.

Neurological Deficit Scores

Neurological deficits of the experimental animals at 4 hours, 24 hours, 48 hours, and 7 days after MCA occlusion were graded on a scale of 0 to 5. The criteria were as follows: grade 0, no observable neurological deficits; grade 1, failed to extend right forepaw; grade 2, circled to the right; grade 3, fell to the right; grade 4, could not walk spontaneously; and grade 5, dead.

In Vivo Pharmacological Treatment

Ifenprodil was dissolved in a mixture of ethanol and water and administered intraperitoneally 3 hours after the onset of pMCAO at the dose of 20 mg/kg. Rats evaluated at 48 hours and 7 days received an additional dose of ifenprodil (10 mg/kg) 6 hours after pMCAO. Control rats with pMCAO received vehicle in the same volume and at the same time schedule as ifenprodil-treated animals.

Western Blot Analysis

Subcellular fractionation was performed as previously reported with minor modifications. Tissue was homogenized in ice-cold sucrose 0.32 mol/L containing HEPES 1 mmol/L, MgCl₂ 1 mmol/L, EDTA 1 mmol/L, NaHCO₃ 1 mmol/L, and PMSF 0.1 mmol/L at pH 7.4 in the presence of a complete set of proteases inhibitors (Complete, Roche Diagnostics). Triton insoluble fraction (TIF) was used instead of the classic postsynaptic density (PSD) because the amount of the starting material was very limited.

The following antibodies were used for Western blotting: NR2A and NR2B antibodies were purchased from Chemicon International, Inc (Temecula, Calif), and monoclonal NR1 antibody was purchased from Zymed (San Francisco, Calif). Horseradish peroxidase–conjugated secondary antibodies were purchased from Pierce (Rockford, Ill).

Statistical Analysis

All values are reported as mean±SE. Infarct volumes, neurological scores, and mean residual laser-Doppler flow were analyzed by 1-way ANOVA followed by Newman-Keul test.

Results

NR2B Antagonists Block Postischemic but Not Activity-Dependent LTP

A brief (3 to 5 minutes) episode of in vitro ischemia (oxygen and glucose deprivation) causes a transient depolarization in striatal spiny neurons intracellularly recorded in corticostriatal brain slice preparations. This transient depolarization is followed by a long-term (2 to 4 hours) increase of the
amplitude of glutamate-mediated EPSPs evoked by cortical stimulation. This pathological form of synaptic plasticity has been termed postischemic LTP and requires the activation of NMDA receptors for its induction. Application of the antagonist of the NR2B subunit ifenprodil (3 to 10 μmol/L; n=5; P<0.001 for each concentration) inhibited in a dose-related manner the postischemic LTP, whereas it did not alter the amplitude and the duration of membrane depolarization induced by transient ischemia (Figure 1A). The effect of ifenprodil was mimicked by 10 μmol/L CP 101,606, a derivative of ifenprodil, showing high affinity for NR2B subunits (Figure 1A; n=11; P<0.001). Neither ifenprodil nor CP 101,606 altered membrane potential (−86±4 mV=control; −85±5 mV=ifenprodil; −87±5 mV=CP 101,606; P>0.05) and input resistance (39±8 mΩ=control; 38±8 mΩ=ifenprodil; 39±9 mΩ=CP 101,606; P>0.05) of the recorded striatal spiny neurons. Moreover, the 2 antagonists did not affect per se the amplitude of the EPSP (n=18 for each drug; P>0.05).

HFS of corticostriatal pathway induces activity-dependent LTP. This physiological form of synaptic plasticity has been considered a cellular substrate for motor learning, and its impairment is associated with motor and behavioral abnormalities. Interestingly, the induction of activity-dependent LTP was not altered by 10 μmol/L ifenprodil (n=13; P>0.05), whereas it was fully prevented by 30 μmol/L D-APV (n=12; P<0.001), a competitive NMDA antagonist (Figure 1B).

Postischemic LTP in the Presence of Physiological Concentrations of Magnesium Ions
In physiological conditions, the magnesium-dependent blockade of NMDA receptors is normally relieved by membrane depolarization. By using the whole-cell patch clamp technique, therefore, and even in the presence of physiological concentrations of magnesium ions (1.2 mmol/L), an NMDA-mediated component of corticostriatal excitatory postsynaptic currents (EPSCs) could be unmasked by clamping membrane voltage to −80 mV (Figure 1C). The amplitude of evoked EPSCs was not significantly increased under ischemic conditions (P>0.05). Pretreatment with ifenprodil (10 μmol/L) prevented postischemic LTP (P<0.05). The graphs in the lower part show cumulative data indicating that the ifenprodil-mediated inhibition of postischemic LTP is dose related and is mimicked by CP 101,606, another selective antagonist of NR2B subunits.

Figure 1. Antagonists at the NR2B subunit of NMDA receptors block postischemic but not activity-dependent LTP. A, A brief in vitro ischemic episode induces a membrane depolarization whose amplitude and time course are similar in 2 striatal spiny neurons recorded either in control medium (top tracing) or in the presence of 10 μmol/L ifenprodil (bottom tracing). The upward deflections reflect evoked EPSPs (0.1 Hz) that are significantly increased after ischemia in the control neuron but not in the cell exposed to ifenprodil. The insets shown at higher sweep speed are averages of EPSP amplitude before and after the ischemic insult in both experimental conditions. The graphs in the lower part show cumulative data indicating that the ifenprodil-mediated inhibition of postischemic LTP is dose related and is mimicked by CP 101,606, another selective antagonist of NR2B subunits. B, Activity-dependent LTP is not altered by ifenprodil, whereas it is fully prevented by D-APV, a competitive NMDA receptor antagonist. Tracings in the upper part are taken either 5 minutes before HFS or 30 minutes after HFS. C, Cumulative data obtained in the presence of 1.2 mmol/L magnesium by clamping the neurons at different voltage levels. Postischemic LTP was evident only when the ischemic insult was applied holding the cell at −50 mV but not −80 mV. Preincubation with ifenprodil (10 μmol/L) prevented postischemic LTP. Tracings on the right show superimposed synaptic currents recorded 5 minutes before and 30 minutes after the ischemic insult in the 3 experimental conditions. Data presented in A and B are from intracellular recordings; data in C are from whole-cell patch clamp recordings.
potential of the recorded striatal cells at membrane values significantly positive to their resting membrane potential. Accordingly, when recorded at holding potentials of $-50 \text{ mV}$ but not at $-80 \text{ mV}$, striatal spiny neurons responded to single stimulation of corticostratal fibers by producing excitatory postsynaptic currents partially blocked by $30 \mu\text{mol/L D-APV}$ (22±3%; $n=4$; $P<0.05$).

As described, a postischemic LTP was observed in striatal neurons held at $-50 \text{ mV}$ even in the presence of 1.2 mmol/L magnesium ($n=5$; $P<0.01$), whereas this synaptic phenomenon was absent in neurons recorded at $-80 \text{ mV}$ ($n=4$; $P>0.05$). According to the results obtained with intracellular recordings, 10 μmol/L ifenprodil fully blocked postischemic LTP in striatal neurons clamped at $-50 \text{ mV}$ ($n=4$; $P>0.05$; Figure 1C).

Blockade of NR2B Subunit on Brain Damage Induced by Focal Ischemia

The neuroprotective effect of ifenprodil was also evaluated using a rat model of focal ischemia such as the pMCAO model. Ifenprodil was administered at a dose of 20 mg/kg IP 3 hours after the onset of pMCAO. Brain infarct volume was evaluated 4 hours, 24 hours, 48 hours, and 7 days after the ischemic insult. When cerebral infarct damage was evaluated 4 hours after pMCAO, no statistical significance was found between ifenprodil-treated and vehicle-injected rats (Figure 2A; $n=8$; $P>0.05$). In contrast, in the ifenprodil-treated group the damage was markedly and significantly reduced when evaluated 24 hours, 48 hours, or 7 days after pMCAO (75% at 24 hours, $n=8$; 68% at 48 hours, $n=8$; 59% at 7 days, $n=8$; $P<0.05$ compared...
with vehicle-treated rats for the 3 time points; Figure 2A and 2B). The reduction of infarct volume by ifenprodil was associated with an improvement in neurological deficits, which was significant \((P<0.05)\) in those animals monitored 48 hours \((n=8)\) and 7 days \((n=8)\) after vascular occlusion, whereas no difference was seen in animals monitored 4 hours \((n=8)\) and 24 hours \((n=8)\) after pMCAO (Figure 2C).

Because it was important to establish that comparable levels of regional ischemic severity were achieved in all experimental groups, during MCAO we monitored rCBF with laser-Doppler flowmetry. pMCAO resulted in an immediate reduction of rCBF to \(\approx 25\% - 30\%\) of baseline in the territory supplied by the ipsilateral artery and remained at this level until the end of the 2-hour observation period. There was no significant difference \((P>0.05)\) in cortical rCBF between rats included in the vehicle-treated group and those receiving ifenprodil 3 hours after pMCAO (Figure 2D).

### Antagonism of NR2B Subunit Blocks Postischemic LTP and Preserves Physiological Plasticity in Ischemic Penumbra

It has been postulated that energy deprivation in the ischemic penumbra could induce postischemic LTP and occlude activity-dependent LTP.\(^2,8\) Thus, we tested the hypothesis that blockade of NR2B subunits could mitigate the brain damage by preventing the induction of postischemic LTP and sparing physiological LTP in the ischemic penumbra.

As shown in Figure 3A, the ischemic penumbra was detected by morphological and electrophysiological analyses (see Materials and Methods) in the early phase after the focal ischemia (4 hours after pMCAO). A set of slices was obtained from animals that received the systemic administration of ifenprodil at a dose of 20 mg/kg 3 hours after pMCAO. Striatal spiny neurons recorded from the ischemic penumbra of these slices showed activity-dependent LTP whose amplitude and time course were identical to the physiological synaptic plasticity recorded from control sham-operated rats (Figure 3B; \(n=9; P>0.05\)). Conversely, in cells recorded from ischemic animals receiving vehicle, the activity-dependent LTP was not observed (Figure 3B; \(n=19; P<0.001\)). The differential induction of physiological synaptic plasticity in the ischemic penumbra of these 2 groups of animals is not caused by differences in the intrinsic membrane properties of the recorded cells because both membrane potential \((n=12, -87 \pm 4 mV=pMCAO+vehicle; n=13, -86 \pm 5 mV=pMCAO+ifenprodil; P>0.05)\) and input resistance \((n=12, 38 \pm 8 \text{ mol/L} \times \text{m}^2=pMCAO+vehicle; n=13, 39 \pm 9 \text{ mol/L} \times \text{m}^2=pMCAO+ifenprodil; P>0.05)\) were similar in the 2 groups. Moreover, these 2 experimental groups showed a similar pattern of current-evoked firing discharge (Figure 3C).

### Ifenprodil Selectively Rescues NR2B Subunit Downregulation Induced by Focal Ischemia

A biochemical approach was used to measure protein concentrations of NMDA receptor subunits into the neuronal postsynaptic compartment 4 and 48 hours after pMCAO. TIF\(^{10}\) was obtained from ischemic (core) and penumbral tissue. The contralateral side was used as control. pMCAO produced a decrease of NR2B protein level in TIF from ischemic core and penumbral...
tissue 4 hours after pMCAO (Figure 4A; n=6 for each experimental group). Similar data were found at 48 hours. pMCAO produced a decreased NR2A protein level in the ischemic tissue only after 48 hours. No significant effect on NR2A level in the penumbra was found (*P<0.01; #P<0.05).

Discussion

It has already been reported that ifenprodil and other NR2B antagonists decrease infarction volume after experimental focal cerebral ischemia. However, the bases of the neuroprotective action of these drugs at cellular and synaptic level are still unknown. To our knowledge, this study represents the first demonstration that NR2B subunit antagonists block posts ischemic LTP, favoring the recovery of activity-dependent LTP in the ischemic penumbra.

By using an in vitro model of ischemia, we demonstrated that selective antagonists of the NR2B subunit block the induction of posts ischemic LTP at concentrations that do not affect physiological synaptic plasticity. This finding suggests that the neuroprotective properties of ifenprodil in vitro are mediated by the involvement of the extrasynaptic NMDA receptor. It is known, in fact, that the subunit composition of extrasynaptic and synaptic NMDA receptors is not identical. Extrasynaptic NMDA receptors are composed predominantly of NR1 and NR2B subunits, whereas synaptic NMDA receptors preferentially express NR2A subunits.

We found that ifenprodil, given 3 hours after focal ischemia, reduced the infarct volume evaluated 24 hours, 48 hours, and 7 days after the insult. This effect was associated with an improvement in neurological deficits. A time window of 3 hours is similar to that used in most clinical studies dealing with neuroprotection and thrombolysis. Ifenprodil not only protected against the enlargement of the infarct area, but it also showed a tendency to reduce the infarct volume from that originally observed at 4 hours. Thus, future studies should also investigate a larger therapeutic window for the use of ifenprodil.

The blockade of ischemic LTP likely contributes to explain the peculiar neuroprotective activity of ifenprodil because ischemic LTP has been claimed to represent a synaptic correlate of the apoptotic process on the basis of several pharmacological similarities between the 2 phenomena. Interestingly, apoptosis is initiated by the stimulation of extrasynaptic NMDA receptors containing the NR2B subunit, a finding that is in agreement with our results.

Along with its effect on infarct enlargement, ifenprodil was able to preserve activity-dependent LTP in the ischemic penumbra, a result fully compatible with the idea that systemic administration of this agent inhibits ischemic LTP (and delayed neuronal death) in ischemic brains. Physiological LTP and ischemic LTP are mutually occlusive. Thus, we can speculate that in ischemic vehicle-treated animals the physiological LTP is occluded by postischemic LTP. Conversely, it is likely that in ischemic ifenprodil-treated animals physiological LTP is preserved because ischemic LTP is prevented by NR2B antagonism.

Interestingly, it has been reported that ifenprodil also acts as a relatively potent antagonist of the 5-hydroxytryptamine (5-HT₃)
receptor. This effect might have potential implications in the interpretation of our data. In fact, activation of 5-HT3 receptors stimulates striatal dopamine release. Because induction of postischemic LTP requires activation of endogenous dopamine, it is possible that part of the neuroprotective effect of ifenprodil may depend on the activation of 5-HT3 receptors. Future studies to address this issue are required.

The reduction of infarct volume by ifenprodil was associated with a restoration of the physiological ratios existing among the different subunits of the NMDA receptors at synaptic sites. In fact, we observed specific molecular alterations in the glutamatergic sites of ischemic areas. Our biochemical and molecular data on TIF demonstrated a reduction of NR2B subunit of NMDA receptor in the postsynaptic compartment 48 hours after pMCAO. Interestingly, the depletion of NR2B was restricted to the TIF and not found in the homogenate, suggesting that pMCAO does not affect NR2B protein expression but rather the correct targeting of this subunit and its assembly to the NMDA receptors at synapses. Ifenprodil treatment for 48 hours selectively affected the NR2B subunit protein levels, restoring NR2B levels to control values. This is in agreement with several reports indicating that chronic blockade of NMDA receptor activity induced a dramatic reversible increase in the number of NMDA receptor clusters and a shift to a more synaptic distribution. Previous observations showed that ischemia induces changes in the protein interactions that occur within the PSD and alters the association between NMDA receptor subunits and PSD-interacting proteins. In particular, recent observations showed that modulation of NR2B interactions with PSD proteins, ie, PSD-95, attenuates ischemic neuronal damage in vivo after stroke onset.

Conclusions

We have shown that the NMDA receptors containing the NR2B subunit play a critical role in the induction of postischemic synaptic plasticity, whereas they do not have a major function in the activity-dependent corticostriatal LTP. This selective role of the NR2B subunit on LTP induced by energy deprivation may explain both the ifenprodil-mediated reduction of postsynaptic neuronal damage and the rescue of physiological LTP in the ischemic penumbra obtained by antagonizing the NR2B subunit.

Acknowledgments

We wish to thank M. Tolu for his technical assistance.

Sources of Funding

This work was supported by Ministero dell’Istruzione, dell’Università e della Ricerca FIRB 2001, Progetti Finalizzati Ministero della Sanità, Telethon grant GGP02035 (Drs Pisani and Calabresi), Ministero dell’Istruzione, dell’Università e della Ricerca FIRB 2001, PRIN2004 (Drs Pisani and Bernardi), and a European Community grant (LSHM-CT-2004-511995, Synaptic Scaffolding Proteins Orchestrating Cortical Synapse Organization during Development).

Disclosures

None.

References

NR2B Subunit Exerts a Critical Role in Postischemic Synaptic Plasticity
Barbara Picconi, Anna Tortiglione, Ilaria Barone, Diego Centonze, Fabrizio Gardoni, Paolo Gubellini, Paola Bonsi, Antonio Pisani, Giorgio Bernardi, Monica Di Luca and Paolo Calabresi

Stroke. 2006;37:1895-1901; originally published online June 1, 2006;
doi: 10.1161/01.STR.0000226981.57777.b0
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 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/37/7/1895

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/