

Intracarotid Saline Infusion Improves Outcome From Incomplete Ischemia in Rats

William E. Hoffman, PhD; Lawrence Ferguson, MD;
Chinamma Thomas, MD; and Ronald F. Albrecht, MD

Previous studies suggest that rheological changes associated with ischemia may produce postischemic hypoperfusion. We tested whether intracarotid or intravenous infusions of saline improve neurological outcome from incomplete cerebral ischemia in rats. Rats were anesthetized with 1.4% isoflurane in air, and ischemia was produced by unilateral carotid artery ligation combined with hemorrhagic hypotension to 30 mm Hg for 30 minutes. Intracarotid ($n=10$) or intravenous ($n=10$) saline infusion (0.3 ml/min) decreased hematocrit 20% compared with control rats ($n=10$). Neurological outcome was significantly improved in rats infused with intracarotid ($p<0.05$) but not intravenous saline during ischemia without a change in brain temperature. Cerebral blood flow, measured in a separate study using laser Doppler flowmetry ($n=5$), decreased 70% ($p<0.01$) during carotid ligation and hypotension but was not changed by intracarotid saline infusion ($p>0.30$). These results show that perfusion of ischemic brain with saline improves outcome by factors not related to changes in hematocrit, brain temperature, or inaischemic tissue blood flow. (*Stroke* 1991;22:797-801)

It has been suggested that rheological factors contribute to ischemic injury. Increased blood viscosity, platelet aggregation, and the accumulation of polymorphonuclear leukocytes may worsen ischemic perfusion and produce delayed postischemic hypoperfusion.¹⁻³ Hypervolemic hemodilution has been reported to improve neurological outcome from stroke by improving blood flow and brain tissue oxygenation.⁴ However, these results are controversial.^{5,6} Another possibility is that direct perfusion of the ischemic vasculature with nonblood solutions may improve outcome by removing metabolic by-products and/or pathophysiological blood components. We evaluated whether the intravenous or intracarotid infusion of saline during incomplete ischemia improves neurological outcome in rats and whether this effect is mediated by alterations in brain temperature or brain blood flow.

Materials and Methods

These experiments were performed following approval from the Institutional Animal Care Committee. Thirty-five nonfasted male Sprague-Dawley rats weighing 350-450 g were anesthetized in a bell jar with

isoflurane, their tracheae were intubated, and their lungs were mechanically ventilated with 2% isoflurane in room air. Catheters were inserted into one femoral artery and both femoral veins for continuous blood pressure monitoring, blood sampling, and drug and fluid administration. A catheter was inserted into the right jugular vein for blood withdrawal during ischemia. The right common carotid artery was isolated. Vecuronium was given as a continuous infusion ($0.1 \text{ mg} \times \text{kg}^{-1} \times \text{min}^{-1}$) to maintain paralysis. The rats were equilibrated with 1.4% inspired isoflurane in room air for 30 minutes.

In group 1 ($n=10$) the right carotid artery was ligated. These rats served as controls for the saline groups. In Group 2 ($n=10$) the right carotid artery was isolated and ligated, and these rats were infused with 0.3 ml/min i.v. normal saline during the 30-minute ischemic period. In group 3 ($n=10$) the right carotid artery was isolated and catheterized with a saline-filled PE50 tubing catheter. The catheter extended 5 mm into the artery, and the lumen was placed at the level of the carotid bifurcation. The catheter was tunneled subcutaneously 12 cm and exited from a small incision in the back to equilibrate the saline to body temperature. This catheter was infused with 0.3 ml/min normal saline during the 30-minute ischemic period. In groups 2 and 3, the saline syringes were warmed to body temperature using an overhead heating lamp before infusion. The temperature of the saline infusate was 37°C. In all groups the carotid ligation was not removed.

From the Departments of Anesthesiology (W.E.H., R.F.A.), Neurosurgery (L.F.), and Pathology (C.T.), Michael Reese Hospital, University of Illinois and University of Chicago, Chicago, Ill.

Address for correspondence: Dr. William E. Hoffman, Anesthesiology Department, Michael Reese Hospital, Lake Shore Drive at 31st Street, Chicago, IL 60616.

Received December 14, 1990; accepted February 8, 1991.

TABLE 1. Neurological Outcome Scoring for Rats

Score	Description
<i>Consciousness</i>	
0	Normal
1	Restless
2	Lethargic
3	Stuporous
4	Seizures
5	Death
<i>Rope platform</i>	
0	Climbs to platform
1	Pulls up rear legs
2	Hangs on 5 seconds
3	Hangs on <5 seconds
4	No grasp reflex
<i>Limb tone</i>	
0	Normal
1	Weak
<i>Walking</i>	
0	Normal
1	Paw adduction
2	Hypomobility
3	Circling to stroke side
4	Unable to stand
<i>Rotating screen</i>	
0	Grasps to 180° for >5 seconds
1	Grasps to 180° for <5 seconds
2	Grasps to 90°, not 180°
3	Falls from vertical screen
<i>Pain reflex</i>	
0	Normal
1	Hypoactive

Cerebral ischemia was produced by the combination of right common carotid artery occlusion and hemorrhagic hypotension to a mean arterial blood pressure of 30 mm Hg for 30 minutes. A range of 2 mm Hg was allowed for the target pressure. After 30 minutes of ischemia, the withdrawn blood was slowly reinfused over 10 minutes. Rectal and skull temperatures were monitored and maintained at 37°C using an overhead heating lamp. Arterial blood gas tensions, pH, plasma glucose concentration, and hematocrit were measured at baseline, during ischemia, and 20 minutes after reinfusion of the blood during recovery. PaCO₂ was maintained between 35 and 40 mm Hg by adjusting the ventilation. Arterial pH was maintained at normal levels with infusion of bicarbonate. After recovery, the catheters were removed and the incisions were closed. Isoflurane was turned off 20 minutes after the reinfusion of blood. The rats were extubated following the establishment of spontaneous respiration and transferred to their home cages.

Neurological outcome was scored every 24 hours for 3 days, starting 24 hours after ischemia (Table 1). A score of 0 represents no detectable neurological

deficit, and a score of 18 represents stroke-related death. Stroke-related death was determined a minimum of 3 hours following extubation only if the rat showed progressive signs of stroke impairment. The evaluators were blinded to the rat's group.

Those rats surviving the 3-day evaluation period were anesthetized with isoflurane and killed by transcardial perfusion of 20 ml isotonic saline followed by 20 ml 10% buffered formalin. Following removal, the brain was stored in formalin over 8 days for subsequent histological examination. The forebrain was dissected into coronal blocks and imbedded in paraffin, and 7- μ m sections were cut and mounted on slides. The slides were stained using hematoxylin and eosin and examined in a blinded manner by a neuropathologist using light microscopy. Neuronal histopathology was evaluated at the levels of the caudate nucleus and hippocampus. The caudate section was graded on a six-point scale according to the following markers: 0, no observable neuronal death; 1, scattered neuronal death; 2, small focal infarcts in the caudate and cortical areas; 3, large infarcts involving 50% of the ischemic caudate; 4, infarcts involving at least 50% of the total ischemic hemisphere; and 5, total hemispheric infarction. Brain tissue damage of the hippocampal section was graded using a five-point scale according to the following markers: 0, no damage; 1, 0–50% of ischemic hemisphere's hippocampus pyramidal cells injured; 2, 50–100% of ischemic hemisphere's hippocampus pyramidal cells injured; 3, 50% of ischemic hemisphere infarcted; and 4, 100% of ischemic hemisphere infarcted. The histopathology score is the total of the score for the caudate section and the score for the hippocampal section.

To evaluate whether intracarotid saline infusion altered ischemic blood flow or brain temperature, additional studies were performed in five rats. Brain and skull temperatures were measured separately using needle thermistor probes (Yellow Springs Instrument Co., Yellow Springs, Ohio). Brain perfusion was evaluated using a Perimed laser Doppler device (Stockholm, Sweden). The rats were surgically prepared for intracarotid saline infusion and unilateral ischemia as described above. The head of each rat was placed in a Kopf rat head holder. The skull was exposed, and two 1-mm holes were drilled in the skull over the forebrain ipsilateral to the carotid ligation. A thermistor probe and laser Doppler needle probe were inserted 3 mm into the tissue through separate holes. After the rat had stabilized for 30 minutes, hemorrhagic hypotension to 30 mm Hg was induced for 10 minutes. At the end of 10 minutes the intracarotid infusion of saline was started at a rate of 0.3 ml/min and continued for 10 minutes with continued hypotension. At the end of this time, the intracarotid saline infusion was stopped and hypotension was continued for an additional 10 minutes. At the end of this period the withdrawn blood was reinfused, and final brain temperature and perfusion measurements were made 20 minutes later. The rat was then killed

TABLE 2. Arterial Blood Pressure, Blood Gas Tensions, pH, Plasma Glucose Concentration, and Hematocrit During Ischemia in Rats

Group	Blood pressure (mm Hg)	Paco ₂ (mm Hg)	PaO ₂ (mm Hg)	pH	Plasma glucose (mg/100 ml)	Hematocrit (%)
Control (n=10)						
Baseline	68±4	37.6±0.8	84±4	7.40±0.01	190±4	42±1
Ischemia	30±1*	38.8±0.8	81±6	7.38±0.01	325±20*	30±1*
Recovery	77±4	39.3±0.8	68±3	7.39±0.01	222±17	39±1
Intravenous saline (n=10)						
Baseline	65±2	39.3±0.8	84±3	7.40±0.01	181±5	42±1
Ischemia	30±1*	38.1±0.9	8.9±3	7.37±0.01	266±15*	24±1*†
Recovery	85±4*	38.0±0.9	69±3	7.41±0.01	169±13	38±1
Intracarotid saline (n=10)						
Baseline	68±3	36.9±0.9	85±3	7.41±0.01	180±7	41±1
Ischemia	30±1*	26.1±0.9	93±3	7.37±0.01	304±18*	23±1*†
Recovery	93±3*	37.2±0.8	67±3	7.38±0.01	213±15	37±1

Data are mean±SEM.

* $p < 0.05$ different from baseline condition by two-way analysis of variance.

† $p < 0.05$ different from control group by two-way analysis of variance.

with an overdose of the anesthetic. Arterial blood gases and pH during ischemia were controlled as described above. Skull and brain temperatures and Doppler perfusion units were measured continuously and recorded at the end of each condition.

Data are reported as mean±SEM. Nonparametric data including neurological outcome score and histopathology score were compared between the control and experimental groups using a Kruskal-Wallis analysis. Physiological data were analyzed using a two-way analysis of variance. A repeated-measures analysis of variance was used to evaluate condition effects for the laser Doppler study. Tukey's tests were used for post-hoc comparisons between groups and conditions. Significance was assumed at $p < 0.05$.

Results

Skull temperature remained between 36.5°C and 37°C throughout the study, with no differences between groups. There were also no differences between groups in physiological variables at baseline (Table 2). During ischemia, plasma glucose concentration increased and hematocrit decreased in each group ($p < 0.05$). The decrease in hematocrit was greater in groups 2 and 3 than in group 1 ($p < 0.05$). Spontaneous respiration and extubation occurred 10–15 minutes after turning off the isoflurane, with no differences between groups.

Neurological outcome scores are shown in Figure 1. Five rats in groups 1 and 2 and two rats in group 3 had stroke-related death. The score in group 3 was significantly better on day 3 than the score in group 1 ($p < 0.05$). The histopathology scores of the five rats that survived in group 1 were 1, 1, 4, 5, and 8; scores in the five rats that survived in group 2 were 0, 1, 2, 5, and 8. Scores for the eight surviving rats in group 3 were 0, 0, 0, 1, 1, 2, 5, and 9. Although the histopathology scores suggested less neuronal damage with intracarotid saline perfusion, this difference

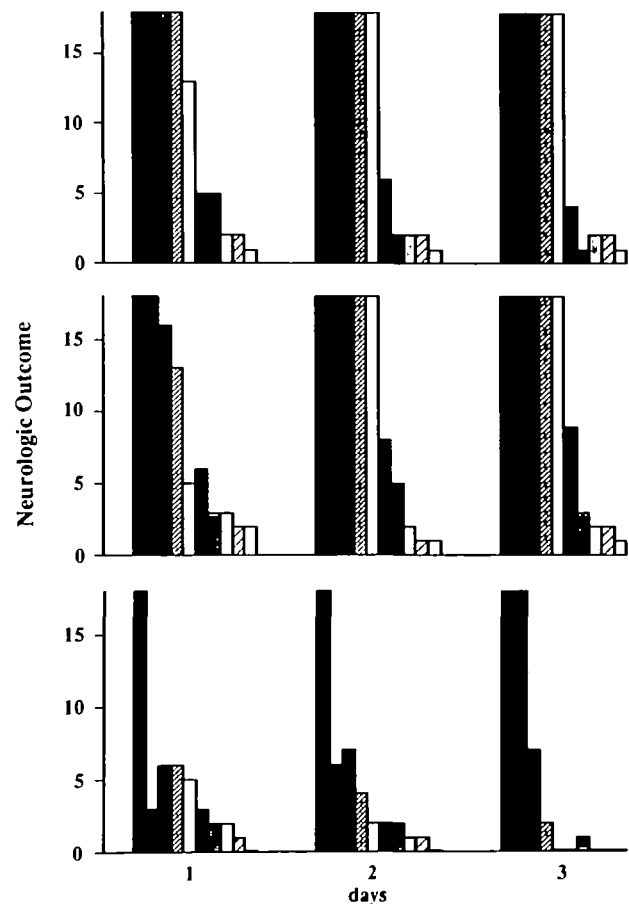


FIGURE 1. Bar graphs of neurologic outcome score in control (top), intravenous saline-perfused (middle), and intracarotid saline-perfused (bottom) rats. Individual scores for each rat are shown for 3 days. There was significant difference between control group and group receiving intracarotid saline over scoring period ($p < 0.05$).

TABLE 3. Brain Perfusion and Temperature During Ischemia and Intracarotid Saline Infusion in Five Rats

Condition	Perfusion		Temperature	
	Duration (minutes)	(units)	Brain (°C)	Skull (°C)
Baseline	30	71±15	37.4±0.3	36.9±0.1
Ischemia alone	10	24±6*	37.1±0.5	36.6±0.1
Ischemia + saline	10	22±6*	37.3±0.3	36.5±0.1
Ischemia alone	10	18±3*	37.3±0.2	36.5±0.1
Recovery	15	51±20	37.2±0.3	36.7±0.1

Data are mean±SEM.

* $p < 0.05$ different from baseline condition by repeated-measures analysis of variance.

was not significant ($p > 0.05$). There was no neuronal injury in the nonischemic hemisphere in rats from any group. There was a significant correlation between neurological outcome score and histopathology score in the ischemic hemisphere ($r = 0.46$, $p < 0.05$).

In the study of brain temperature and blood flow during ischemia and intracarotid saline infusion, blood gas tensions and pH were maintained at baseline levels. The results shown in Table 3 indicate that ischemia reduced brain perfusion by 70%, which was not significantly altered by intracarotid saline infusion. Brain and skull temperatures did not change significantly during any condition.

Discussion

The results of this study show that an intravenous saline infusion during incomplete cerebral ischemia in rats decreases hematocrit but does not improve neurological outcome compared with control rats. This is consistent with clinical reports that normovolemic or hypervolemic hemodilution does not improve outcome from stroke.^{5,6} The intracarotid infusion of saline during ischemia significantly improved neurological outcome compared with the control group, without changes in brain temperature or tissue perfusion. Since hematocrit was decreased to the same levels in groups 2 and 3, we conclude that the improvement in outcome in group 3 was due to the infusion of saline into the ischemic zone rather than to hemodilution. Intracarotid saline infusion may improve outcome by increasing local perfusion pressure in the ischemic zone. Although this treatment does not increase cerebral blood flow, it may improve blood flow distribution by mechanically opening ischemic blood vessels. This may also wash out ischemic metabolic by-products and ameliorate postischemic hypoperfusion by inhibiting platelet aggregation and the accumulation of polymorphonuclear leukocytes.^{2,3} These actions may be important in limiting postischemic neuronal injury.

In this model of incomplete cerebral ischemia in rats, neurologic outcome worsens until the second day after ischemia and neuronal death progresses for several days after ischemia.⁷ This is consistent with other models of ischemia in which neuronal injury

increases for several days after the insult.⁸ Neuronal injury may continue after ischemia because of an accumulation of polymorphonuclear leukocytes in the ischemic zone that inhibits postischemic reperfusion. Polymorphonuclear leukocytes are viscoelastic cells that normally adhere to the endothelium. The deformation and passage of these cells through the capillaries require 1,000 times longer than for red blood cells.⁹ During low-flow states such as ischemia, polymorphonuclear leukocytes may accumulate, acting as plugs in the ischemic microcirculation.^{10,11} The cells may also degranulate within the capillary, releasing tissue-damaging substances. This is consistent with the results of Groggaard et al,³ who showed that depletion of circulating polymorphonuclear leukocytes before the induction of ischemia improved postischemic reperfusion compared with a control treatment. Mechanical or biochemical obstruction of capillaries during ischemia may be an important mechanism of progressive postischemic neuronal injury.

Besides washout of polymorphonuclear leukocytes, there are other possible mechanisms for the improvement of ischemic outcome with intracarotid saline infusion. Although there was no difference in hematocrit between groups 2 and 3, it is likely that the local hematocrit in ischemic tissue was decreased with intracarotid saline infusion. This may improve metabolite and ion or fluid exchange between the vascular and tissue spaces and decrease edema formation. Intracarotid saline infusion may also improve tissue perfusion by increasing the local perfusion pressure. We did not see a significant change in ischemic tissue perfusion with intracarotid saline infusion using laser Doppler flowmetry. The perfusion units so measured are a function of the number of red blood cells and their velocity within the illuminated area (1–2 mm²). This technique does not measure absolute blood flow but produces a close correlation of changes in regional blood flow with other techniques such as [¹⁴C]iodoantipyrine autoradiography.¹² Our results indicate that intracarotid saline infusion does not change blood flow within the ischemic zone.

Another possibility is that intracarotid saline infusion may improve the oxygenation of ischemic tissue. Blood flow to the ischemic forebrain (1 g tissue) is approximately twice the saline infusion rate.^{13,14} However, not all of the saline infused would reach the ischemic brain since we are infusing into the common carotid artery. Oxygen dissolved in the saline would provide <1% of the oxygen carried by the blood flow to the ischemic hemisphere. This indicates that the oxygen carried by the saline is probably not an important factor for decreasing ischemic injury. However, an increase in the intravascular filling pressure may enhance oxygenation by opening more capillaries for perfusion without changing red blood cell velocity.

It is unclear whether hemodilution improves outcome following acute stroke. Koller et al⁴ reported that hypervolemic hemodilution in patients with

acute ischemic stroke improved outcome up to 90 days after ischemia. Harrison et al¹⁵ also saw a positive correlation between hematocrit and cerebral infarct size following ischemia. In contrast, other studies have shown no improvement in the stroke outcome of patients treated with normovolemic or hypervolemic hemodilution.^{5,6} Lowering the hematocrit may improve cerebral blood flow and enhance oxygen delivery.^{16,17} Experimental data suggest that a hematocrit of 30–35% provides optimal rheological and oxygen-carrying properties of the blood.¹⁸ However, this may not relate to ischemic stroke. We saw a decrease in hematocrit to 23–24% during ischemia in the saline-perfused rats compared with 30% in the control rats. However, no difference in outcome was seen between the intravenous saline-treated and control rats. We conclude that decreases in hematocrit occur in this model of hypotensive ischemia but that decreases below 30% do not improve outcome.

In conclusion, a significant improvement in neurological outcome from ischemia was observed with intracarotid infusion of saline during ischemia compared with the control treatment. Another group of rats treated with an intravenous saline infusion showed no change in outcome compared with the control rats. The improvement in outcome with intracarotid saline infusion cannot be explained by a difference in measured physiological variables or hematocrit between groups. Intracarotid saline infusion may facilitate the washout of ischemic metabolic by-products or decrease platelet aggregation or the accumulation of polymorphonuclear leukocytes in ischemic tissue. Ischemic perfusion may also be improved by a mechanical effect of increased local perfusion pressure, which may keep blood vessels open and improve blood flow distribution and oxygenation.

Acknowledgments

We wish to thank Susan Anderson for her technical assistance in this study. We thank Dr. Verna L. Baughman for editing the manuscript.

References

1. Fischer EG, Ames A III: Studies on mechanisms of impairment of cerebral circulation following ischemia: Effect of hemodilution and perfusion pressure. *Stroke* 1972;3:538–542
2. Safar P, Stezoski W, Nemoto EM: Amelioration of brain damage after 12 minutes cardiac arrest in dogs. *Arch Neurol* 1976;33:91–95
3. Groggaard B, Schürer, Gerdin B, Afors KE: Delayed hypoperfusion after incomplete forebrain ischemia in the rat. The role of polymorphonuclear leukocytes. *J Cereb Blood Flow Metab* 1989;9:500–505
4. Koller M, Haenny P, Hess K, Weniger D, Zangger P: Adjusted hypervolemic hemodilution in acute ischemic stroke. *Stroke* 1990;21:1429–1434
5. Scandinavian Stroke Study Group: Multicenter trial of hemodilution in acute ischemic stroke: I. Results in the total patient population. *Stroke* 1987;18:691–699
6. Italian Acute Stroke Study Group: Haemodilution in acute stroke. Results of the Italian Haemodilution Trial. *Lancet* 1988;1:318–321
7. Baughman VL, Hoffman WE, Thomas C, Albrecht RF, Miletich DJ: The interaction of nitrous oxide and isoflurane with incomplete cerebral ischemia in the rat. *Anesthesiology* 1989;70:767–774
8. Pulsinelli WA, Brierley JB, Plum F: Temporal profile of neuronal damage in a model of transient forebrain ischemia. *Ann Neurol* 1982;11:491–498
9. Gaethtgens P, Ley K, Pries AR, Müller R: Mutual interactions between leukocytes and microvascular blood flow. *Prog Appl Microcirc* 1985;7:15–28
10. Schmid-Schönbein GW: Capillary plugging by granulocytes and the no-reflow phenomenon in the microcirculation. *Fed Proc* 1987;46:1297–2401
11. Engler RL, Schmid-Schönbein GW, Pavelec RS: Leukocyte capillary plugging in myocardial ischemia and reperfusion in the dog. *Am J Pathol* 1972;111:98–111
12. Dirnagl U, Kaplan B, Jacewicz M, Pulsinelli W: Continuous measurement of cerebral cortical blood flow by laser-Doppler flowmetry in a rat stroke model. *J Cereb Blood Flow Metab* 1989;9:589–596
13. Baughman VL, Hoffman WE, Miletich DJ, Albrecht RF, Thomas C: Neurologic outcome in rats following incomplete cerebral ischemia during halothane, isoflurane, or N₂O. *Anesthesiology* 1988;69:192–198
14. Baughman VL, Hoffman WE, Thomas C, Miletich DJ, Albrecht RF: Neurologic outcome in aged rats after incomplete cerebral ischemia. *Anesth Analg* 1988;67:677–682
15. Harrison MJG, Pollock S, Kendall BE, Marshall J: Effect of haematocrit on carotid stenosis and cerebral infarction. *Lancet* 1981;2:114–115
16. Thomas DJ, Du Boulay GH, Marshall J, Pearson TC, Ross Russell RU, Symon L, Wetherley-Mein G, Zilkha E: Effect of haematocrit on cerebral blood-flow in man. *Lancet* 1977;2:941–943
17. Wade JPH: Transport of oxygen to the brain in patients with elevated haematocrit values before and after venesection. *Brain* 1983;106:513–523
18. Messer K, Gornandt L, Jesch F, Sinagovitz E, Sunder-Plassmann L, Kessler M: Oxygen transport and tissue oxygenation during hemodilution with dextran. *Adv Exp Med Biol* 1973;37:669–680

KEY WORDS • cerebral ischemia • hemodilution • rats

Intracarotid saline infusion improves outcome from incomplete ischemia in rats.

W E Hoffman, L Ferguson, C Thomas and R F Albrecht

Stroke. 1991;22:797-801

doi: 10.1161/01.STR.22.6.797

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Copyright © 1991 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/22/6/797>

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/>