Brain-Derived Neurotrophic Factor Stimulates Production of Prostacyclin in Cerebral Arteries

Anantha Vijay R. Santhanam, PhD; Leslie A. Smith; Zvonimir S. Katusic, MD, PhD

**Background and Purpose**—The role of brain-derived neurotrophic factor (BDNF) and its receptor, tropomyosin receptor kinase B, in control of cerebral circulation is poorly understood. The present study was designed to investigate the cerebral vascular effects of BDNF in vivo.

**Methods**—Replication incompetent adenovirus encoding either rat BDNF (AdBDNF) or green fluorescent protein was injected intracerebrally into rabbits. Forty-eight hours later, animals were euthanized. Plasma and cerebrospinal fluid levels of BDNF were measured by enzyme-linked immunosorbent assay, vasomotor function of isolated basilar arteries was studied in organ chambers, protein expression in the basilar arteries was studied by Western blotting, prostanoid levels were measured by enzyme-linked immunosorbent assay, and cyclic adenosine 3',5'-monophosphate levels were measured by radioimmunoassay.

**Results**—The levels of BDNF in the cerebrospinal fluid were significantly elevated in AdBDNF-treated rabbits as compared with adenovirus encoding green fluorescent protein-treated rabbits (37±5 ng/mL versus 0.006±0.003 ng/mL, respectively; P<0.05; n=14). Western blotting studies revealed that in basilar arteries, AdBDNF increased protein expression of prostacyclin synthase, whereas expression of endothelial nitric oxide synthase and phosphorylated (Ser 1177) endothelial nitric oxide synthase remained unchanged. During incubation with arachidonic acid (1 µmol/L), PGI2 production and levels of cyclic adenosine 3',5'-monophosphate were significantly elevated only in AdBDNF-treated rabbit basilar arteries (P<0.05, n=6). Relaxations to acetylcholine (10-9 to 10-5 mol/L) and arachidonic acid (10-9 to 10-5 mol/L) were significantly potentiated in basilar arteries from rabbits injected with AdBDNF. Potentiation of relaxations to acetylcholine in AdBDNF-treated basilar arteries was inhibited by the nonselective cyclooxygenase inhibitor, indomethacin (10-5 mol/L, P<0.05, n=6) and constitutive phospholipase A2 inhibitor, AACOCF3 (2×10-5 mol/L, P<0.05, n=5).

**Conclusion**—Our results demonstrate that in cerebral arteries, BDNF-induced activation of tropomyosin receptor kinase B receptor signaling in vivo promotes prostacyclin biosynthesis. These findings provide novel mechanistic insight into the vascular protective effect of BDNF in cerebral circulation. *(Stroke. 2010;41:350-356.)*

**Key Words:** basilar arteries ■ neurotrophins ■ neurovascular unit ■ vasodilatation ■ vasomotor function

---

Brain-derived neurotrophic factor (BDNF) is a member of the family of neurotrophic factors, and it participates in nervous system development.1,2 The effects of BDNF are mediated by activation of neurotrophin receptors, which belong to the tropomyosin receptor kinase (Trk) family of tyrosine kinases. BDNF specifically binds to TrkB receptors. In addition, all neurotrophins bind to p75 neurotrophin receptor (p75NTR).3 In the cardiovascular system, expression of BDNF and TrkB have been reported in the aortic wall as well as in the smooth muscle of other large conduit arteries.4,5 Existing evidence suggests that, in the central nervous system, BDNF regulates the homeostatic interaction among neurons, glial cells, and the vasculature, collectively referred to as the “neurovascular unit.” Impairment of cerebral vascular BDNF signaling may cause disruption of the neurovascular unit, thus leading to progressive neuronal dysfunction.6 It is also important to point out that the effects of BDNF on cerebral circulation have not been studied.

Our previous study identified BDNF as a major cytokine produced and released by endothelial progenitor cells.7 More recently, we reported that in cerebral circulation, increased production of prostacyclin (PGI2) stimulated by endothelial progenitor cells is caused by their paracrine effect.8 Based on these observations, we hypothesized that, in the cerebral arterial wall, BDNF may activate metabolism of arachidonic acid resulting in elevated production of PGI2. In this regard, it is important to notice that PGI2 is the primary mediator of endothelium-dependent relaxations in cerebral circulation of infants.9,10 During aging, contribution of PGI2 to endothelial control of vasomotor function decreases so that in adult arteries, nitric oxide becomes the dominant endothelium-derived vasodilator.9,10 Relevant to our study, it has been
suggested that the inhibitory effect of aging on production of PGI₂ increases vulnerability of adult cerebral arteries to vascular injury. Therefore, reactivation of PGI₂ production in adult arteries may help to explain vascular protective effects of endothelial progenitor cells and BDNF.

**Methods**

**Animals**

Male New Zealand white rabbits (2 to 3 kg, obtained from either Harlan Laboratories, Indianapolis, Ind; or Myrtle’s Rabbitry, Thompsons Station, Tenn) were used for experiments. Rabbits were anesthetized with an intramuscular injection composed of ketamine (35 mg/kg), xylazine (5 mg/kg), and acepromazine (2.3 mg/kg) before intracisternal injections on Day 0. Animals were anesthetized and euthanized on Day 2 with intravenous Sleepaway (sodium pentobarbital, 260 mg/kg; Ft Dodge Animal Health), and basilar arteries were isolated and studied. All procedures were approved by the Institutional Animal Care and Use Committee of the Mayo Clinic.

**Adenovirus and Gene Transfer**

Replication-incompetent adenoviruses constructed using vectors bearing either green fluorescent protein alone (AdGFP; vector control in this study) or rat BDNF and GFP (AdBDNF) were generously gifted by Dr Steve Goldman, University of Rochester Medical Center, Rochester, NY. The detailed methodology for adenoviral construction has been published elsewhere. Three hundred microliters of cerebrospinal fluid was aspirated and mixed with 50 μL vector (10⁸ plaque-forming units) or vehicle and injected aseptically using a 25-gauge needle into the cisterna magna. The transduction titer of 10⁸ plaque-forming units/rabbit was chosen based on previous in vivo gene transfer studies. After injection, animals were maintained in a head-down position for 30 minutes before transfer to postanesthesia recovery.

**Measurement of BDNF Levels**

BDNF levels in the cerebrospinal fluid and plasma were measured by an enzyme-linked immunosorbent assay kit (Catalog BDDB00; R & D Biosciences, Minneapolis, Minn) according to the manufacturer’s instructions.

**Western Blotting**

Soluble proteins were extracted by mincing and homogenizing basilar arteries in lysis buffer as described earlier. Blots were incubated with monoclonal antibodies (1:500 dilution) against phospho Ser/177-endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase (NOS; BD Biosciences), cyclo-oxygenase (COX)-1 (Cayman), COX-2, phospho Trk (BD Biosciences), and polyclonal antibodies to BDNF (Santa Cruz), eNOS (BD Biosciences), and TrkB (Neuromics).

**Confocal Microscopy**

Basilar arteries were embedded in paraffin and 5-μm sections were cut. Staining was performed on deparaffinized sections. Non-specific binding was blocked by incubation of tissue with 10% normal goat serum for 20 minutes. Sections were then incubated with polyclonal antibodies (1:100 dilution) against PGI₂ synthase (Santa Cruz Biotechnology) for 2 hours at room temperature. Texas red conjugated secondary antibody (Invitrogen) was added to sections for 1 hour at room temperature and slides were incubated for 5 minutes with 10 μg/mL of Hoechst 33258 (Sigma) to stain for nuclei. Coverslips were mounted using Prolong Gold mounting medium (Invitrogen), and the arterial sections were visualized using a Zeiss LSM 510 laser scanning confocal microscope.

**Measurement of PGI₂ and Tx₄₂**

Basilar arteries isolated from rabbits injected either with AdGFP or AdBDNF were incubated in Krebs solution in a CO₂ incubator at 37°C for 30 minutes by incubation with arachidonic acid (1 μM/L) at 37°C for 30 minutes. PGI₂ and Tx₄₂ were measured as their stable metabolites, 6-ketoProstaglandin F₁₅₂₀ and TxB₂, respectively, as described earlier.

**Measurement of Cyclic Guanosine 5’-Monophosphate and Cyclic Adenosine 3’,5’-Monophosphate**

Rabbit basilar arteries were incubated in MEM in a CO₂ incubator at 37°C for 30 minutes in 3-isobutyl-1-methylxanthine (10⁻⁴ mol/L; Sigma) to inhibit the degradation of cyclic nucleotides by phosphodiesterases followed by incubation with arachidonic acid (1 μM/L) at 37°C for 30 minutes. After incubation, cyclic guanosine 5’-monophosphate (cGMP) and cyclic adenosine 3’,5’-monophosphate (cAMP) levels were measured by cGMP and cAMP radioimmunoassay kits respectively (Amersham).

**Analysis of Vascular Reactivity**

Isolated arteries were connected to a force transducer for recording of isometric force and placed in organ baths filled with 25 mL Krebs solution (37°C; 94% O₂/6% CO₂; pH 7.4). Concentration-dependent response curves to acetylcholine, diethyl ammonium (Z)-1-(N,N-diethylamino)diazen-1-1-M1,2-dioleate (DENA-NONoate), and arachidonic acid (10⁻⁹ to 10⁻⁵ mol/L) were cumulatively obtained during submaximal contractions to histamine (3 × 10⁻⁷ to 10⁻⁶ mol/L). In some experiments, either endothelium was mechanically removed or endothelium intact arteries were treated with a constitutive phospholipase A2 (cPLA₂) inhibitor, arachidonyl trifluoromethyl ketone (AA-COCF₃, 20 μmol/L, 30 minutes) before contracting with histamine, and responses to acetylcholine and arachidonic acid were recorded.

**Drugs**

DEA-NONOate was obtained from Cayman Chemical and AA-COCF₃ was obtained from Calbiochem. All other drugs used in the study were obtained from Sigma. The concentration of all drugs is expressed as the final mol/L in the organ chambers. AACOCF₃ was dissolved in dimethylsulfoxide and appropriate control experiments were performed with vehicle.

**Statistical Analysis**

Results of the study are expressed as means ± SEM for “n” (n=the number of rabbits in each group) animals used in each experimental group. Relaxations are expressed as percentage of maximal relaxations induced by 3 × 10⁻⁴ mol/L papaverine on histamine (3 × 10⁻⁷ mol/L to 10⁻⁶ mol/L) contracted arteries. cAMP and cGMP values were analyzed by unpaired Student t test. Densitometric values are expressed as the ratio of studied protein versus actin and comparisons between AdGFP and AdBDNF were assessed by unpaired Student t test. Concentration-response curves were analyzed by nonlinear curve fitting using GraphPad Prism 4.03 software (GraphPad Software Inc, San Diego, Calif) and statistical differences among relaxation values across concentration-response curves were analyzed by 2-way analysis of variance followed by Bonferroni posttests using SigmaStat 3.1 software (Systat Software Inc, San Jose, Calif). A probability value <0.05 was considered statistically significant.

**Results**

In vivo gene delivery of AdGFP or AdBDNF did not affect blood pressure (Supplemental Table I; available at http://stroke.ahajournals.org) and hematologic parameters (Supplemental Table II). Subsequent to gene transfer, levels of BDNF in cerebrospinal fluid were increased from 0.006±0.003 ng/mL in AdGFP-transduced rabbits to 36.95±4.8 ng/mL in AdBDNF-transduced rabbits (P<0.001, n=14), whereas plasma levels of BDNF were
increased from 0.010 ± 0.001 ng/mL in AdGFP-transduced rabbits to 0.026 ± 0.003 ng/mL in AdBDNF-transduced rabbits (P < 0.05, n = 6 to 8).

Western blot analysis demonstrated increased expression of BDNF in AdBDNF-transduced arteries in comparison to AdGFP-transduced rabbit basilar arteries (P < 0.01, n = 8; Figure 1A). Intracisternal administration of AdBDNF activated TrkB receptors, as reflected in phosphorylation of TrkB receptors in AdBDNF-transduced basilar arteries (P < 0.05, **P < 0.01, n = 6 to 8).

Overexpression of BDNF in basilar arteries did not alter the expression of eNOS, inducible NOS, or phosphorylated Ser-1177 eNOS (Figure 2A). However, the protein expression of PGI2 synthase was significantly increased in AdBDNF-transduced arteries (Figure 2B). Immunofluorescence studies confirmed increased expression of PGI2 synthase in smooth muscle cells and adventitial layer of AdBDNF-transduced arteries (Figure 2C). Further characterization of basilar arteries from AdBDNF-treated rabbits demonstrated that expression of COX-1 and COX-2 remained unchanged in comparison to AdGFP-transduced basilar arteries (Figure 2B).

On arachidonic acid stimulation, production of 6-ketoprostaglandin F1alpha, a stable metabolite of prostacyclin (Figure 3A), was selectively elevated in AdBDNF-transduced basilar arteries, whereas levels of thromboxane B2 (Figure 3B) remained unchanged, suggestive of increased activation of PGI2 synthase on BDNF treatment. The levels of cAMP, a second messenger of PGI2, were also significantly elevated in arachidonic acid-stimulated basilar arteries from AdBDNF-transduced rabbits in comparison to AdGFP-transduced rabbit basilar arteries (Figure 3C). However, the levels of cGMP, indicative of nitric oxide production, remained unchanged (Figure 3D).

In histamine-contracted arteries, relaxations to acetylcholine (10^-9 to 10^-5 mol/L) were significantly potentiated in AdBDNF-treated arteries (Figure 4A), whereas relaxations to a nitric oxide donor, DEA-NONOate (10^-9 to 10^-5 mol/L) remained unaltered (Figure 4B). Relaxations to either acetylcholine or DEA-NONOate were not different between non-transduced control arteries and AdGFP-transduced control basilar arteries (data not shown). Removal of endothelium abolished acetylcholine-induced relaxations in both AdGFP- and AdBDNF-treated arteries (Figure 4A).

In AdGFP-treated arteries, relaxations to acetylcholine were reduced in the presence of the eNOS inhibitor, L-NMMA (100 μmol/L), but not in the presence of the COX inhibitor indomethacin (Figure 5A). However, in AdBDNF-transduced arteries, relaxations to acetylcholine were more sensitive to the inhibitory effect of indomethacin as compared with the effect of L-NMMA, suggestive of involvement of PGI2 in mediating potentiation of relaxations in AdBDNF-transduced arteries (Figure 5B). In the presence of both L-NMMA and indomethacin, relaxations to acetylcholine were almost abolished in basilar arteries from both AdGFP- and AdBDNF-treated rabbits (Figure 5A–B). To further examine the role of arachidonic acid metabolism in potentiation of acetylcholine-induced relaxations in AdBDNF-treated rabbit basilar arteries, experiments were performed in the presence of a cPLA2 inhibitor, AACOCF3. Relaxations to acetylcholine remained unaffected by the cPLA2 inhibitor in AdGFP-treated basilar arteries (Figure 5C), whereas the relaxation responses to acetylcholine were inhibited by AACOCF3 (P < 0.05, n = 6) in AdBDNF-treated arteries (Figure 5D).

In agreement with enhanced metabolism of arachidonic acid favoring PGI2 production in AdBDNF-transduced arteries, concentration-dependent relaxations to arachidonic acid (10^-9 to 10^-5 mol/L) were selectively potentiated in AdBDNF-treated arteries (Figure 6A). In basilar arteries of rabbits transduced with AdGFP, removal of endothelium significantly attenuated arachidonic acid-induced relaxations (Figure 6B). In comparison, arachidonic acid-induced relaxations remained potentiated in endothelium-denuded AdBDNF-treated basilar arteries (Figure 6B).
Discussion

To the best of our knowledge, this is the first study to investigate the effects of BDNF on cerebrovascular function. Our study presents several novel findings. First, intracisternal administration of AdBDNF increased BDNF levels in the perivascular space and protein expression of BDNF in the cerebral arterial wall. Second, in arteries transduced with AdBDNF, selectively increased expression of PGI2 synthase was observed. Third, stimulation with arachidonic acid favored production of prostacyclin over thromboxane A2 and increased the levels of cAMP consistent with BDNF-induced activation of PGI2 synthase. Fourth, relaxations to acetylcholine and arachidonic acid were selectively potentiated in AdBDNF-treated arteries, whereas relaxations to a nitric oxide donor, DEA-NONOate, remained unchanged. Fifth, exposure to BDNF amplified the indomethacin-sensitive component of acetylcholine-induced relaxation in rabbit cerebral arteries. Finally, potentiation of relaxations to acetylcholine in basilar arteries exposed to BDNF was sensitive to cPLA2 inhibition.

Over the last 2 decades, research on BDNF had been focused on neuronal differentiation and neuroprotection. Despite the identification of BDNF receptor TrkB on vascular endothelial and smooth muscle cells, knowledge of the functional role of BDNF had been primarily restricted to cell survival and angiogenesis. More importantly, in vivo studies on cerebrovascular function of recombinant BDNF were hindered by its short half-life and inability to cross the blood–brain barrier. Our laboratory has extensive experience with adenovirus-mediated gene transfer in the cisterna magna, and we have reported that adventitial gene delivery results in persistent expression of recombinant proteins in adventitial fibroblasts in the cerebral arterial wall. In the present in vivo study, using adenovirus-mediated gene transfer, we achieved high perivascular concentrations of recombinant BDNF (approximately 35 ng/mL). Consistent with our findings, in vitro studies have reported BDNF-mediated neuroprotection or antiapoptotic effect on cultured endothelial cells in concentration range of 10 to 100 ng/mL. We also observed that intracisternal injections of AdBDNF re-
sulted in increased cerebral arterial expression of BDNF and subsequent activation of TrkB receptors.

By screening vascular protective proteins expressed in the cerebral arterial wall exposed to BDNF, we identified selective upregulation of PGI2 synthase. Increased activity of PGI synthase was confirmed by selective increase in production of PGI2 as well as elevation of cAMP levels and enhanced vasodilatation mediated by PGI2. In the rabbit basilar artery, endothelium-dependent relaxation to acetylcholine is primarily mediated by nitric oxide. COX blockade by indomethacin has a minimal effect on vasodilatation induced by acetylcholine.18,19 Our results with AdGFP-transduced control basilar arteries are in agreement with these reports because indomethacin did not significantly inhibit the maximal relaxations to acetylcholine. Lack of inhibition with the cPLA2 inhibitor on acetylcholine-induced relaxations further confirmed nitric oxide playing a predominant vasodilator role in basilar arteries of rabbits exposed to AdGFP. It is likely that, under physiological conditions, activation of cholinergic receptors on endothelium does not couple to the production and release of vasodilator prostanoids. However, on BDNF treatment, acetylcholine-induced relaxations were sensitive to indomethacin as well as to cPLA2 inhibition, suggestive of involvement of arachidonic acid-derived metabolites in the augmentation of relaxations. Inhibition of acetylcholine-induced relaxations in endothelium-denuded arteries from BDNF-treated rabbits suggests a role of PGI2 derived by activation of muscarinic receptors on the endothelium. Nevertheless, it is possible that upregulation of PGI2 synthase is not limited to the endothelium but occurs in smooth muscle cells as well. Mor-
phological studies by confocal microscopy indeed demonstrated that upregulation of PGI2 synthase was observed in the endothelial, medial, and adventitial layers of cerebral arteries obtained from AdBDNF-treated rabbits. Arachidonic acid induces endothelium-dependent dilation of cerebral blood vessels, through endothelial lipoygenase, endothelium-dependent hyperpolarization and by stimulating production of the vasodilator prostaglandin E2 (PGE2).

Figure 5. A, Relaxations to acetylcholine in histamine-contracted basilar arteries from rabbits injected intracisternally with AdGFP were inhibited by L-NMMA (100 μmol/L). Data are expressed as percentage of maximal relaxation induced by 3×10−4 mol/L papaverine; 100% = 1.28 ± 0.17 g (AdGFP), 1.45 ± 0.30 g (AdGFP + L-NMMA), 1.32 ± 0.17 g (AdGFP + indomethacin), and 1.96 ± 0.29 g (AdGFP + L-NMMA + indomethacin), respectively (*P < 0.05, **P < 0.01, ***P < 0.001, n = 6). B, Relaxations to acetylcholine in histamine-contracted basilar arteries from rabbits injected intracisternally with AdBDNF demonstrated sensitivity to L-NMMA as well as to indomethacin (10−5 mol/L). 100% = 1.42 ± 0.08 g (AdBDNF), 1.62 ± 0.14 g (AdBDNF + L-NMMA), 1.51 ± 0.19 g (AdBDNF + indomethacin), and 2.28 ± 0.38 g (AdBDNF + L-NMMA + indomethacin), respectively (*P < 0.05, **P < 0.01, ***P < 0.001, n = 6). C, Relaxations to acetylcholine remained unchanged in basilar arteries from AdGFP-treated rabbits on incubation with the cPLA2 inhibitor, AACOCF3 (20 μmol/L, 30 minutes). 100% = 1.44 ± 0.20 g (AdGFP) and 1.51 ± 0.19 g (AdGFP + AACOCF3), respectively (P = nonsignificant, n = 5). D, Potentiation of relaxations in AdBDNF-treated arteries was abolished in the presence of the cPLA2 inhibitor, AACOCF3. 100% = 1.60 ± 0.22 g (AdBDNF) and 1.36 ± 0.14 g (AdBDNF + AACOCF3), respectively (*P < 0.05, **P < 0.01, n = 5).

Figure 6. A, Concentration-dependent relaxations to arachidonic acid in histamine-contracted arteries were potentiated in endothelium-intact basilar arteries of rabbits transduced with AdBDNF. 100% = 1.64 ± 0.15 g (AdGFP, endothelium-intact [E+]) and 1.78 ± 0.16 g (AdBDNF, E+), respectively (*P < 0.05, **P < 0.01, n = 5). B, Relaxations to arachidonic acid remained potentiated in endothelium-denuded basilar arterial rings obtained from rabbits injected intracisternally with AdBDNF. 100% = 1.01 ± 0.18 g (AdGFP, endothelium-denuded [E−]) and 1.11 ± 0.09 g (AdBDNF, E−), respectively (*P < 0.05, **P < 0.01, n = 5 to 6).
prostacyclin. In agreement, relaxations to arachidonic acid in AdGFP-transduced arteries were significantly reduced on endothelium denudation in the present study. However, in arteries exposed to BDNF, relaxations to arachidonic acid remained potentiated despite endothelium removal. It is likely that, in agreement with our morphological studies, upregulation of PGI2 synthase in medial and adventitial layers of arteries exposed to BDNF contributed to the observed potentiation. Production of 6-keto PGF1α and cAMP levels were increased on arachidonic acid treatment consistent with increased activation of PGI2 synthase and production of prostacyclin. The fact that relaxation to the nitric oxide donor, DEA-NONOate, was not affected in AdBDNF-treated arteries suggests the effect of BDNF is selective toward PGI2-adenylate cyclase signaling and resulting elevation of cAMP.

Our results significantly expand understanding of the mechanisms underlying cerebrovascular protective effects of BDNF, because PGI2 may also prevent platelet aggregation and thrombus formation, inhibit smooth muscle proliferation, and stimulate therapeutic angiogenesis.23,24 On binding to TrkB receptors, BDNF could activate one or more of these 3 pathways: Ras/Rap-MAPK, PI3K-Akt, and the PLCγ-PKC cascades.24 The molecular targets stimulated by BDNF, mediating production of PGI2, and cerebrovascular protection, require further investigation.

We would also like to point out that in cerebral circulation, high production of PGI2 has been detected in arteries obtained from young children and newborn pigs.9,10 Moreover, with maturation, production of PGI2 in cerebral arterial wall is decreasing thereby increasing reactivity to vasoconstrictors.9,10 Results of the present study suggest that in adult cerebral arteries, BDNF favors production of PGI2, thus rejuvenating the cerebral arterial wall by enhancing vasodilator capacity and protecting against vasoconstrictor stimuli. In addition, increased local concentration of PGI2 in the arterial wall is known to activate prosurvival signaling by activation of perteoxisome proliferator-activated receptor delta.25 This, in turn, may increase resistance of cerebral circulation against injury.

Findings of the present study are the first to demonstrate that, in cerebral arteries, BDNF promotes vasodilatation. PGI2 biosynthesis appears to be the most likely mediator promoting vasodilatation. In addition to its existing trophic role in the “neurovascular unit,” our study adds substantial evidence to the concept that in cerebral circulation, BDNF exerts beneficial effects by favoring arachidonic acid metabolism through PGI2 synthase.

Sources of Funding
This work was supported in part by National Heart, Lung, and Blood Institute Grants HL-53524 and HL-91867 (Z.S.K.), the American Heart Association Scientist Development Grant 0835436N (A.V.R.S.), and the Mayo Foundation.

Disclosures
None.

References
Brain-Derived Neurotrophic Factor Stimulates Production of Prostacyclin in Cerebral Arteries

Anantha Vijay R. Santhanam, Leslie A. Smith and Zvonimir S. Katusic

Stroke. 2010;41:350-356; originally published online December 17, 2009;
doi: 10.1161/STROKEAHA.109.564492

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/41/2/350

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/