Neurosurg Focus 3 (3):Article 3, 1997

Molecular biological considerations in cerebral vasospasm following aneurysmal subarachnoid hemorrhage

Jeffrey E. Thomas, M.D.

Department of Neurological Surgery, Thomas Jefferson University and Wills Neurosensory Institute, Philadelphia, Pennsylvania

Chronic delayed cerebral vasospasm (CDCV) remains a serious and often fatal complication of aneurysmal subarachnoid hemorrhage (SAH). The current understanding of its fundamental mechanisms and molecular biological characterization is rudimentary. Two important vasoactive substances have been implicated in CDCV: endothelin-1 (ET-1) and nitric oxide (NO). A 21-amino acid vasoconstrictor peptide, ET-1 has generated interest as a possible important contributor to cerebral vasospasm on the basis of both clinical and experimental evidence suggesting abnormally enhanced production. Nitric oxide is a cell membrane-permeable free radical gas that accounts for the vasodilatory effect of endothelium-derived relaxation factor and is a physiological antagonist of ET-1. As with ET-1, abnormalities of NO production have been implicated in several pathological conditions including cerebral vasospasm. This brief report reviews some of the physiological and regulatory features of these two molecules and explores the possibility of their relationship to cerebral vasospasm.

Key Words * nitric oxide * endothelin-1 * vasospasm * subarachnoid hemorrhage

The chronic delayed type of cerebral vasoconstriction that characterizes vasospasm associated with aneurysmal subarachnoid hemorrhage (SAH) remains the most important cause of mortality and morbidity in those patients surviving an initial SAH.[2,24,30] Many possible causes for the condition have been considered over the past 30 years. Particular interest in erythrocytes (red blood cells [RBCs]), was generated by studies that distinguished acute from chronic delayed cerebral vasospasm (CDCV).[11,12,33,55,56,65,76]

Several facts related to RBCs are noteworthy with regard to CDCV. In studies RBCs were observed to lyse after a period of hours to days, and oxyhemoglobin released during these events was implicated as a cause.[8,42,69] The time course of RBC lysis was consistent with the clinical delay that characterizes CDCV,[74] and the release of oxyhemoglobin from the RBCs during lysis was also consistent with the apparent relationship of CDCV to the volume of SAH.[15] Furthermore, some evidence was offered for the effectiveness of removing blood from the subarachnoid space in warding off CDCV.[21,53,61] The molecular link between oxyhemoglobin and vasospasm, however, remains unknown.

ENDOTHELIN-1

The discovery of endothelin-1 (ET-1) in 1988 provided one of the first important opportunities to explore the pathophysiological mechanisms of CDCV in molecular biological terms.[78] The endothelins were subsequently found to be a family of genes specifying short peptides with diverse functions. Genes for three subtypes of endothelin were mapped and characterized.[79] Endothelin-1 was determined to have very strong vasomotor activity. The **ET-1** gene specifies a 21-amino acid peptide product with several disulfide linkages and potent vasoconstrictor activity restricted to its end-terminal hexapeptide.[78] Its structure very closely resembles that of the snake venom toxin of *Atractaspis engaddensis*, a Middle Eastern asp whose bite rapidly kills rodents by inducing severe coronary vasoconstriction and is lethal in minute amounts.[36,50,68] Endothelin-1 is the most potent vasoconstrictor known and is approximately 10 times as potent as angiotensin II.[78] It is present in many diverse species and has been highly conserved in evolution (porcine ET-1 is identical to human, for example), which implies its biological importance.

Endothelin-1 effects vasoconstriction by a receptor-mediated mechanism that releases Ca^{++} from intracellular stores and also activates Ca^{++} entry via dihydropyridine-sensitive voltage-gated Ca^{++} channels, although the latter (which is the target of nimodipine) does not appear to be a direct effect.[75] The specific binding pattern of ET is not susceptible to Ca^{++} channel blockade.[37] Two specific types of G-protein-coupled receptor have been identified, characterized, and cloned.[62,63] The ET-A receptor is specifically activated by ET-1 and is present on vascular smooth-muscle cells as well as endothelial cells.[40,59] Of importance, and perhaps particularly relevant to CDCV, ET-1 has been well demonstrated to act from the adventitial side.[45,77] Several signal transduction mechanisms linked to receptor activation have been identified.[62,63] Endothelin-1 messenger RNA is extremely unstable and its half-life is short; ET-1 is rapidly eliminated from the bloodstream.[79] The biosynthesis of ET-1 is regulated at the level of transcription.[78] These and other findings indicate that its action is predominantly local.[41] Once bound, however, its dissociation from the receptor is extremely slow, and the vasoconstriction that it causes is characteristically resistant to washout.[79]

NITRIC OXIDE

Nitric oxide (NO) began to generate substantial scientific interest among biochemists and vascular physiologists during the late 1970s, when it was proposed as the common intermediate to a class of drugs that caused smooth-muscle relaxation in association with increasing cyclic guanosine monophosphate (cGMP) levels.[3,49] The term "nitrovasodilators" was coined by Murad[49] in this context. Furchgott and Zawadzki, on the basis of earlier work,[18] proposed that the substance derived from endothelium, known as endothelium-derived relaxation factor (EDRF), that was responsible for the phenomenon of vasodilation by agents like acetylcholine, histamine, and bradykinin (agents causing vasoconstriction when the endothelium was removed), was in fact NO. Research interest in this molecule grew exponentially thereafter. More than 4000 publications on NO appeared in 1996,[49] and there is no sign of waning of interest. The more that is discovered about this molecule, the more fascinating it becomes. The smallest known biologically active molecule, NO is a cell membrane-permeable free radical gas that fully accounts for the vasodilator activity of EDRF and is a physiological antagonist of ET-1.[57] Nitric oxide interacts with several intracellular targets including soluble guanylate cyclase, stimulating the conversion of guanosine triphosphate to cGMP. Cyclic guanosine monophosphate activates cGMP-dependent protein kinases leading to vascular smooth-muscle relaxation and inhibition of platelet adhesion and aggregation.[23,64] Thus NO is a powerful vasodilator. Its known functions are extremely diverse, however, and include maintenance of basal vasomotor tone, platelet inhibition,

macrophage-mediated cytotoxicity, neurotransmission, synaptogenesis, intercellular signaling, N-methyl-D-aspartate-mediated neuronal excitotoxicity, selective neuroprotection, penile erection, apoptosis, nonadrenergic noncholinergic intestinal relaxation, and free radical generation.[51,64] The pathological roles are as well-represented as the physiological ones, earning NO its reputation as a "double-edged sword." [52] Nitric oxide is formed by enzymes known as NO synthases (NOS) from L-arginine and molecular oxygen.[46] Like ET-1, it may be produced in endothelial cells and vascular smooth-muscle cells, depending on the circumstances. Also like ET-1, the genes responsible for its generation (the **NOS** genes) are highly conserved in evolution and display a high degree of homology between species.[5,52] The regulation of NOS, and hence NO production, is extremely complex,[48] and the very short biological half-life of NO causes its actions to be highly localized. The NOS are functionally classified into two types, constitutive and inducible. The constitutive NOS are present in unstimulated cells, Ca^{++} dependent, and subdivided into neuronal and endothelial types (after the cells in which they were first identified). The inducible NOS (iNOS) are distinguished by their absence in resting, unstimulated cells. They are generally Ca^{++} independent and their expression may be induced in many cell types, including endothelial and vascular smooth-muscle cells, under certain conditions, particularly exposure to cytokines (tumor necrosis factor-alpha, and gamma-interferon) and lipopolysaccharides.[51] There is recent molecular biological evidence that iNOS subtypes exist.[5] The exact role of the NOS and of NO in cerebrovasculature and ischemia is uncertain, and evidence exists for both protective[27,47,82] and deleterious[9,10,22,28] effects. Recent evidence indicates that the neuronal form of NOS may be deleterious in ischemic conditions, whereas the endothelial type may be protective by means of its vasodilatory effect; the development of relatively selective NOS inhibitors is needed to shed more light on these interactions.[9,80] Upregulation of gene expression of the NOS (both neuronal and inducible) has been demonstrated in models of brain ischemia[29,83] and suggests a pathogenic role on the one hand and a neuroprotective role on the other.[83] Furthermore, iNOS are known to be expressed in blood vessels following many forms of vascular injury.[48] This raises the question of whether they might be similarly expressed in cerebral blood vessels after vascular injury in the form of vasospasm, with its accompanying inflammatory response,[58] and if so, why this expression is apparently ineffective in promoting vasodilation independent of any brain effect, because NO has been demonstrated to increase regional cerebral blood flow.[47,82] It is noteworthy in this regard that the **ET-1** gene is activated in response to exposure to interleukin-1.[81]

RELATIONSHIP OF ET-1 AND NO TO CDCV

Particularly relevant features of these molecules include evolutionary conservation, high potency, short duration of action, complex physiology and regulation, local action, and physiological antagonism. These features suggest important biological roles including maintenance of normal vasomotor tone. It is tempting to speculate that CDCV represents a disturbance in the equilibrium for which these physiological antagonists are partially responsible.[7,14] To support this hypothesis, clinical features of aneurysmal SAH and CDCV may be invoked: CDCV is related to the volume of SAH; it is always associated with a delay in onset from the initial SAH of several days. Once the condition begins, it is progressive, chronic, and, eventually, self limited. The process appears to burn itself out after a period of days to 2 weeks in most cases. Hypotheses regarding the pathogenesis of CDCV attempt to accommodate these facts. Oxyhemoglobin lends itself to consideration as a pathogen in this context: as the major intracellular protein of the RBC it is liberated after lysis of this cell, and levels of oxyhemoglobin appear to peak in the cerebrospinal fluid (CSF) at 3 to 4 days.[4] Oxyhemoglobin has been demonstrated in vitro to activate the **ET-1** gene and increase levels of ET-1 mRNA;[19,34,54] it is also eventually

enzymatically depleted in the CSF.[60] Independent investigations have demonstrated gene activation in response to platelet products such as thrombin and transforming growth factor- β [38,43] and to cytokines such as interleukin-1 as noted previously.[81] These sorts of mechanisms appear to explain the clinical finding of increased ET-1 levels in CSF following SAH, which have been correlated with the incidence and time course of CDCV.[66,67] Oxyhemoglobin is also known to inactivate NO by directly binding to its heme moiety.[6,17,20,25,32,44,72,73] This phenomenon could explain the finding by some investigators that levels of soluble guanylate cyclase are inappropriately low in CDCV,[35] thus the failure of intrinsic vasodilatory responses to compensate for arterial narrowing. The time course of clinical CDCV is consistent with the delayed and persistent rise in oxyhemoglobin levels that accompany RBC lysis. In short, the presence of oxyhemoglobin is capable of promoting vasoconstriction and inhibiting vasodilation by different but simultaneous mechanisms.

Hypotheses regarding the roles of oxyhemoglobin, ET-1, and NO in CDCV have been put to limited clinical and laboratory tests. Attempts to remove the influence of oxyhemoglobin have included subarachnoid lavage, thrombolysis, and administration of reducing agents to change the oxidation state of ferrous iron.[21,53,61,65] The vasoconstrictor effects of ET-1 have been blunted by various means, but the most effective has been specific ET-A receptor blockade.[16] Administration of NO in various forms has been used successfully to block and rapidly reverse ET-1- and SAH-induced CDCV in animal models.[1,13,31,70] The future of these potential therapies is unknown because the biological roles of these molecules are far reaching, and the effect of removing their influence partially or entirely is unpredictable given the present state of knowledge. A genetic model in which the gene responsible for endothelial NOS has been eliminated, for example, demonstrates a certain resistance to the effects of cerebral ischemia but also demonstrates systemic hypertension.[26] A similar gene knockout mouse with a deficient **ET-1** gene has been shown to manifest blood pressure and craniofacial anomalies.[39] The administration of NO donors via an intravascular route has been discouraged because of effects on systemic hypotension, although recent work in which NO donors were administered intrathecally has met with some success in reversing ET-1-induced vasoconstriction.[70]

CONCLUSIONS

Chronic delayed cerebral vasospasm, the same pathological entity described by Jackson[30] in 1949 and alluded to in the context of aneurysm treatment by Allcock and Drake[2] in 1965, has now emerged as the most important factor in morbidity and mortality rates associated with survival of aneurysmal SAH. Its mysteries have eluded and frustrated clinicians for more than three decades. The technical accomplishments of modern cerebrovascular surgery stand in stark contrast to this complication of aneurysmal SAH, which remains formidable and even deadly. Complex molecular and genetic interactions add to the burden of understanding this phenomenon. Preliminary work has begun to shed light on the molecular biological nature of CDCV implicating, among others, the genes responsible for ET-1 and NO. The intricate and variable physiological roles of these highly conserved biological molecules are slowly being examined in the context of SAH. This work has already begun to have an impact on the clinical research and treatment of CDCV.[71] Continued work will further our understanding of these phenomena and will be ultimately rewarded with a specific and reliable treatment for CDCV.

References

1. Afshar JK, Pluta RM, Boock RJ, et al: Effect of intracarotid nitric oxide on primate cerebral

- vasospasm after subarachnoid hemorrhage. **J Neurosurg** **83**:118-122, 1995
2. Allcock JM, Drake CG: Ruptured intracranial aneurysms--the role of arterial spasm. **J Neurosurg** **22**:21-29, 1965
3. Arnold WP, Mittal CK, Katsuki S, et al: Nitric oxide activates guanylate cyclase and increases guanosine 3',5'-monophosphate levels in various tissue preparations. **Proc Natl Acad Sci USA** **74**:3203-3207, 1977
4. Barrows LJ, Hunter FT, Banker BQ: The nature and clinical significance of pigments in the cerebrospinal fluid. **Brain** **78**:59-80, 1955
5. Bloch KD, Wolfram JR, Brown DM, et al: Three members of the nitric oxide synthase II gene family (NOS2A, NOS2B, and NOS2C) colocalize to human chromosome 17. **Genomics** **27**:526-530, 1995
6. Boje KM, Arora PK: Microglial-produced nitric oxide and reactive nitrogen oxides mediate neuronal cell death. **Brain Res** **587**:250-256, 1992
7. Boulanger C, Luscher TF: Release of endothelin from the porcine aorta. Inhibition by endothelium-derived nitric oxide. **J Clin Invest** **85**:587-590, 1990
8. Boullin DJ, Tagari P, du Boulay G, et al: The role of hemoglobin in the etiology of cerebral vasospasm. An in vivo study of baboons. **J Neurosurg** **59**:231-236, 1983
9. Dalkara T, Moskowitz MA: The complex role of nitric oxide in the pathophysiology of focal cerebral ischemia. **Brain Pathol** **4**:49-57, 1994
10. Dawson VL, Kizushi VM, Huang PL, et al: Resistance to neurotoxicity in cortical cultures from neuronal nitric oxide synthase-deficient mice. **J Neurosci** **16**:2479-2487, 1996
11. Duff TA, Louie J, Feilbach JA, et al: Erythrocytes are essential for development of cerebral vasculopathy resulting from subarachnoid hemorrhage in cats. **Stroke** **19**:68-72, 1988
12. Echlin F: Experimental vasospasm, acute and chronic, due to blood in the subarachnoid space. **J Neurosurg** **35**:646-656, 1971
13. Egemen N, Turker RK, Sanlidilek U, et al: The effect of intrathecal sodium nitroprusside on severe chronic vasospasm. **Neurol Res** **15**:310-315, 1993
14. Emori T, Hirata Y, Ohta K, et al: Secretory mechanism of immunoreactive endothelin in cultured bovine endothelial cells. **Biochem Biophys Res Commun** **160**:93-100, 1989
15. Fisher CM, Kistler JP, Davis JM: Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computed tomography scanning. **Neurosurgery** **6**:1-9, 1980
16. Foley PL, Caner HH, Kassell NF, et al: Reversal of subarachnoid hemorrhage-induced vasoconstriction with an endothelin receptor antagonist. **Neurosurgery** **34**:108-113, 1994
17. Fujiwara S, Kassell NF, Sasaki T, et al: Selective hemoglobin inhibition of endothelium-dependent vasodilation of rabbit basilar artery. **J Neurosurg** **64**:445-452, 1986
18. Furchgott RF, Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial

smooth muscle by acetylcholine. **Nature** **288**:373-376, 1980

19. Fuwa I, Mayberg M, Gadjusk C, et al: Enhanced secretion of endothelin by endothelial cells in response to hemoglobin. **Neurol Medicochirur** **33**:739-743, 1993

20. Gibson QH, Roughton FJW: The kinetics and equilibria of the reactions of nitric oxide with sheep haemoglobin. **J Physiol** **136**:507-526, 1957

21. Handa Y, Weir BK, Nosko M, et al: The effect of timing of clot removal on chronic vasospasm in a primate model. **J Neurosurg** **67**:558-564, 1987

22. Hara H, Huang PL, Panahian N, et al: Reduced brain edema and infarction volume in mice lacking the neuronal isoform of nitric oxide synthase after transient MCA occlusion. **J Cereb Blood Flow Metab** **16**:605-611, 1996

23. Henry Y, Lepoivre M, Drapier JC, et al: EPR characterization of molecular targets for NO in mammalian cells and organelles. **FASEB J** **7**:1124-1134, 1993

24. Heros RC, Zervas NT, Varsos V: Cerebral vasospasm after subarachnoid hemorrhage: an update. **Ann Neurol** **14**:599-608, 1983

25. Hongo K, Kassell NF, Nakagomi T, et al: Subarachnoid hemorrhage inhibition of endothelium-derived relaxing factor in rabbit basilar artery. **J Neurosurg** **69**:247-253, 1988

26. Huang PL, Huang Z, Mashimo H, et al: Hypertension in mice lacking the gene for endothelial nitric oxide synthase. **Nature** **377**:239-242, 1995

27. Huang Z, Huang PL, Ma J, et al: Enlarged infarcts in endothelial nitric oxide synthase knockout mice are attenuated by nitro-L-arginine. **J Cereb Blood Flow Metab** **16**:981-987, 1996

28. Iadecola C: Bright and dark sides of nitric oxide in ischemic brain injury. **Trends Neurosci** **20**:132-139, 1997

29. Iadecola C, Zhang F, Xu S, et al: Inducible nitric oxide synthase gene expression in brain following cerebral ischemia. **J Cereb Blood Flow Metab** **15**:378-384, 1995

30. Jackson JJ: Aseptic hemogenic meningitis: an experimental study of aseptic meningeal reactions due to blood and its breakdown products. **Arch Neurol Psychiatry** **62**:572-589, 1949

31. Kajita Y, Suzuki Y, Oyama H, et al: Combined effect of L-arginine and superoxide dismutase on the spastic basilar artery after subarachnoid hemorrhage in dogs. **J Neurosurg** **80**:476-483, 1994

32. Kanamaru K, Waga S, Kojima T, et al: Endothelium-dependent relaxation of canine basilar arteries. Part 2: Inhibition by hemoglobin and cerebrospinal fluid from patients with aneurysmal subarachnoid hemorrhage. **Stroke** **18**:938-943, 1987

33. Kapp J, Mahaley MS Jr, Odom GL: Cerebral arterial spasm. 2. Experimental evaluation of mechanical and humoral factors in pathogenesis. **J Neurosurg** **29**:339-349, 1968

34. Kasuya H, Weir BK, White DM, et al: Mechanism of oxyhemoglobin-induced release of endothelin-1 from cultured vascular endothelial cells and smooth-muscle cells. **J Neurosurg** **79**:892-898, 1993

35. Kim P, Schini VB, Sundt TM Jr, et al: Reduced production of cGMP underlies the loss of endothelium-dependent relaxations in the canine basilar artery after subarachnoid hemorrhage. **Circ Res** **70**:248-256, 1992
36. Kloog Y, Ambar I, Sokolovsky M, et al: Sarafotoxin, a novel vasoconstrictor peptide: phosphoinositide hydrolysis in rat heart and brain. **Science** **242**:268-270, 1988
37. Koseki C, Imai M, Hirata Y, et al: Autoradiographic distribution in rat tissues of binding sites for endothelin: a neuropeptide? **Am J Physiol** **256**:R858-R866, 1989
38. Kurihara H, Yoshizumi M, Sugiyama T, et al: Transforming growth factor- β stimulates the expression of endothelin mRNA by vascular endothelial cells. **Biochem Biophys Res Commun** **159**:1435-1440, 1989
39. Kurihara Y, Kurihara H, Suzuki H, et al: Elevated blood pressure and craniofacial abnormalities in mice deficient in endothelin-1. **Nature** **368**:703-709, 1994
40. Lin HJ, Kaji EH, Winkel GK, et al: Cloning and functional expression of a vascular smooth muscle endothelin 1 receptor. **Proc Natl Acad Sci USA** **88**:3185-3189, 1991
41. MacCumber MW, Ross CA, Glaser BM, et al: Endothelin: visualization of mRNAs by in situ hybridization provides evidence for local action. **Proc Natl Acad Sci USA** **86**:7285-7289, 1989
42. MacDonald RL, Weir BK: A review of hemoglobin and the pathogenesis of cerebral vasospasm. **Stroke** **22**:971-982, 1991
43. Marsen TA, Simonson MS, Dunn MJ: Thrombin induces the preproendothelin-1 gene in endothelial cells by a protein tyrosine kinase-linked mechanism. **Circ Res** **76**:987-995, 1995
44. Martin W, Villani GM, Jothianandan D, et al: Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. **J Pharmacol Exp Ther** **232**:708-716, 1985
45. Mima T, Yanagisawa M, Shigeno T, et al: Endothelin acts in feline and canine cerebral arteries from the adventitial side. **Stroke** **20**:1553-1556, 1989
46. Moncada S, Palmer RM, Higgs EA: Nitric oxide: physiology, pathophysiology, and pharmacology. **Pharmacol Rev** **43**:109-142, 1991
47. Morikawa E, Moskowitz MA, Huang Z, et al: L-arginine infusion promotes nitric oxide-dependent vasodilation, increases regional cerebral blood flow, and reduces infarction volume in the rat. **Stroke** **25**:429-435, 1994
48. Morris SM Jr, Billiar TR: New insights into the regulation of inducible nitric oxide synthesis **Am J Physiol** **266**:E829-E839, 1994
49. Murad F: Signal transduction using nitric oxide and cyclic guanosine monophosphate. **JAMA** **276**:1189-1192, 1996
50. Nakajima K, Kumagaye S, Nishio H, et al: Synthesis of endothelin-1 analogues, endothelin-3 and sarafotoxin S6b: structure-activity relationships. **J Cardiovasc Pharmacol** **13** (Suppl 5):S8-S12, 1989

51. Nathan C: Nitric oxide as a secretory product of mammalian cells. **FASEB J** **6**:3051-3064, 1992
52. Nathan C: Nitric oxide: roles, tolls, and controls. **Cell** **78**:915-918, 1994
53. Nosko M, Schulz R, Weir B, et al: Effects of vasospasm on levels of prostacyclin and thromboxane A2 in cerebral arteries of the monkey. **Neurosurgery** **22**:45-50, 1988
54. Ohlstein EH, Storer BL: Oxyhemoglobin stimulation of endothelin production in cultured endothelial cells. **J Neurosurg** **77**:274-278, 1992
55. Osaka K: Prolonged vasospasm produced by the breakdown products of erythrocytes. **J Neurosurg** **47**:403-411, 1977
56. Ozaki N, Mullan S: Possible role of the erythrocyte in causing prolonged cerebral vasospasm. **J Neurosurg** **51**:773-778, 1979
57. Palmer RMJ, Ferrige AG, Moncada S: Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. **Nature** **327**:524-526, 1987
58. Peterson JW, Kwun BD, Hackett JD, et al: The role of inflammation in experimental cerebral vasospasm. **J Neurosurg** **72**:767-774, 1990
59. Resink TJ, Scott-Burden T, Weber E, et al: Phorbol ester promotes a sustained down-regulation of endothelin receptors and cellular responses to endothelin in human vascular smooth muscle cells. **Biochem Biophys Res Commun** **166**:1213-1219, 1990
60. Roost KT, Pimstone NR, Diamond I, et al: The formation of cerebrospinal fluid xanthochromia after subarachnoid hemorrhage: enzymatic conversion of hemoglobin to bilirubin by the arachnoid and choroid plexus. **Neurology** **22**:973-977, 1972
61. Saito I, Ueda Y, Sano K: Significance of vasospasm in the treatment of ruptured intracranial aneurysms. **J Neurosurg** **47**:412-429, 1977
62. Sakurai T, Yanagisawa M, Takuwa Y, et al: Cloning of a cDNA encoding a non-isotype-selective subtype of the endothelin receptor. **Nature** **348**:732-735, 1990
63. Simonson MS, Dunn MJ: The molecular mechanisms of cardiovascular and renal regulation by endothelin peptides. **J Lab Clin Med** **119**:622-639, 1992
64. Snyder SH, Brecht DS: Biological roles of nitric oxide. **Sci Am** **266**:68-77, 1992
65. Sonobe M, Suzuki J: Vasospasmogenic substance produced following subarachnoid hemorrhage, and its fate. **Acta Neurochir** **44**:97-106, 1978
66. Suzuki H, Sato S, Suzuki Y, et al: Endothelin immunoreactivity in cerebrospinal fluid of patients with subarachnoid hemorrhage. **Ann Med** **22**:233-236, 1990
67. Suzuki R, Masaoka H, Hirata Y, et al: The role of endothelin-1 in the origin of cerebral vasospasm in patients with aneurysmal subarachnoid hemorrhage. **J Neurosurg** **77**:96-100, 1992
68. Takasaki C, Tamiya N, Bdolah A, et al: Sarafotoxins S6: several isotoxins from *Atractaspis engaddensis* (burrowing asp) venom that affect the heart. **Toxicon** **26**:543-548, 1988

69. Tanishima T: Cerebral vasospasm: contractile activity of hemoglobin in isolated canine basilar arteries. **J Neurosurg** **53**:787-793, 1980
70. Thomas JE, Nemirovsky A, Zelman V, et al: Rapid reversal of endothelin-1-induced cerebral vasoconstriction by intrathecal administration of nitric oxide donors. **Neurosurgery** **40**:1245-1249, 1997
71. Thomas JE, Rosenwasser RH: Intrathecal administration of sodium nitroprusside in the treatment of chronic delayed cerebral vasospasm associated with aneurysmal subarachnoid hemorrhage. **Fed Drug Admin** **52**:307, 1997
72. Toda N: Hemolysate inhibits cerebral artery relaxation. **J Cereb Blood Flow Metab** **8**:46-53, 1988
73. Toda N, Ayajiki K, Okamura T: Endothelium modulation of contractions caused by oxyhemoglobin and NG-nitro-L-arginine in isolated dog and monkey cerebral arteries. **Stroke** **24**:1584-1589, 1993
74. Tourtellotte WW, Metz LN, Bryan ER, et al: Spontaneous subarachnoid hemorrhage. Factors affecting the rate of clearing of the cerebrospinal fluid. **Neurology** **14**:301-306, 1964
75. Wallnofer A, Weir S, Ruegg U, et al: The mechanism of action of endothelin-1 as compared with other agonists in vascular smooth muscle. **J Cardiovasc Pharmacol** **13** (Suppl 5):S23-S31, 1989
76. Weir B, Grace M, Hansen J, et al: Time course of vasospasm in man. **J Neurosurg** **48**:173-178, 1978
77. Willette RN, Sauermelch CF: Abluminal effects of endothelin in cerebral microvasculature assessed by laser-Doppler flowmetry. **Am J Physiol** **259**:H1688-H1693, 1990
78. Yanagisawa M, Kurihara H, Kimura S, et al: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. **Nature** **332**:411-415, 1988
79. Yanagisawa M, Masaki T: Molecular biology and biochemistry of the endothelins. **Trends Pharmacol Sci** **10**:374-379, 1989
80. Yoshida T, Limmroth V, Irikura K, et al: The NOS inhibitor, 7-nitroindazole, decreases focal infarct volume but not the response to topical acetylcholine in pial vessels. **J Cereb Blood Flow Metab** **14**:924-929, 1994
81. Yoshizumi M, Kurihara H, Morita T, et al: Interleukin 1 increases the production of endothelin-1 by cultured endothelial cells. **Biochem Biophys Res Commun** **166**:324-329, 1990
82. Zhang F, White JG, Iadecola C: Nitric oxide donors increase blood flow and reduce brain damage in focal ischemia: evidence that nitric oxide is beneficial in the early stages of cerebral ischemia. **J Cereb Blood Flow Metab** **14**:217-226, 1994
83. Zheng ZG, Chopp M, Gautam S, et al: Upregulation of neuronal nitric oxide synthase and mRNA, and selective sparing of nitric oxide synthase-containing neurons after focal cerebral ischemia in rat. **Brain Res** **654**:85-95, 1994
-

Manuscript received July 15, 1997.

Accepted in final form August 19, 1997.

Address reprint requests to: Jeffrey E. Thomas, M.D., Cerebrovascular Division, Department of Neurological Surgery, Thomas Jefferson University and Wills Neurosensory Institute, 834 Walnut Street, Suite 650, Philadelphia, Pennsylvania 19107.