

# New developments in the understanding of cerebral vasoregulation and vasospasm: the endothelin-nitric oxide network

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**SUMMARY** Endothelins, which are powerful vasoconstrictors, and nitric oxide, which is a powerful vasodilator, together form a balanced system that regulates blood flow in the brain and in other organs. Ongoing research may yield new drugs that act on this system to prevent or reverse cerebral vasospasm in subarachnoid hemorrhage and other conditions.

**KEY POINTS** Many compounds are involved in cerebral vasoregulation under physiologic and pathologic conditions; of these, endothelins and nitric oxide have attracted considerable attention over the last several years. ■ Endothelins and nitric oxide differ in chemical structure and pharmacological properties: endothelins are potent vasoconstrictor peptides consisting of 21 amino acids; nitric oxide is a free radical with a half-life of only a few seconds and exerts powerful vasodilatory effects. ■ Both are produced by a number of cell types in the brain and interact at various levels to profoundly influence cerebral vessel function.

**INDEX TERMS:** ENDOTHELINS; NITRIC OXIDE; CEREBROVASCULAR CIRCULATION; VASOCONSTRICTION; VASODILATION; CEREBRAL ISCHEMIA, TRANSIENT  
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■ This paper is dedicated to K. Felgenhauer, MD, Professor of Neurology, Director of the Department of Neurology, University of Göttingen, on the occasion of his 60th birthday.

**T**HIS ARTICLE distills new developments that may be of major clinical relevance in understanding cerebral vasoregulation and conditions of vasospasm, briefly summarizes the biology of endothelins (ETs) and nitric oxide (NO), and sketches some of the interactions of a hypothetical ET-NO network in brain vasculature, derived from our own work and that of others. Further, we discuss the pathophysiologic aspects of this network that offer encouraging approaches to future therapy.

Many compounds and mechanisms identified in the last several decades can influence cerebral blood flow under physiologic and pathologic conditions. Most of them have been addressed extensively in a number of excellent reviews.<sup>1-10</sup> Newly discovered factors that have attracted considerable attention in recent years are the ETs and NO, which apparently are components of a well-balanced regulatory arrangement that maintains vascular tone while retaining a high degree of plasticity. In the brain, this counterregulatory system reaches out far beyond blood

vessels to involve neurons and glial cells, thereby potentially reflecting exciting aspects of a functional entity.

#### ENDOTHELINS: A FAMILY OF VASOCONSTRICTORS

ETs, a recently described family of peptides, are among the most potent vasoconstrictors known and possess an extremely long duration of action. At least three ETs have been identified, each consisting of 21 amino acids. ET-2 and ET-3 differ from ET-1 by two and six amino acids, respectively, and also have somewhat different pharmacologic properties.<sup>11-13</sup> The genes encoding these peptides are located on different chromosomes, in humans on chromosomes 6, 1, and 20.<sup>14-18</sup>

Originally isolated from aortic endothelial cells,<sup>11</sup> ETs are produced by a number of other cell types as well, including cerebral endothelial cells,<sup>19,20</sup> heterogeneous populations of neurons,<sup>21-25</sup> glial cells,<sup>26-28</sup> and certain immune cells.<sup>29,30</sup> Recently, even vascular smooth muscle cells were shown to produce ETs upon induction with growth factors.<sup>31</sup> Because of this confusing variety of ET sources, observations of a cell-type-specific regulation of ET-gene expression take on greater importance.<sup>32-34</sup>

ETs derive from prepropeptides approximately 200 amino acids long; specific proteolytic enzymes produce the biologically active ET.<sup>11,35,36</sup> The activity of these "endothelin-converting enzymes" (ECEs) seems to differ among organs and tissues,<sup>37-39</sup> and each ECE mostly cleaves a specific substrate to produce a specific ET.<sup>40</sup> In addition, the distribution of messenger RNA for ET-1, ET-2, and ET-3 is different in different organs.<sup>41</sup> All this may reflect the different physiologic tasks of these peptides. ECEs not only represent important regulatory elements in ET biology but also possess properties attractive for future therapeutic intervention.

ETs act via specific binding sites—G-protein-coupled receptors—distributed throughout the body, not exclusively associated with vascular structures.<sup>42</sup> In the brain, these binding sites have been identified mainly in the cerebellum, basal ganglia, hippocampus, brain stem, and choroid plexus.<sup>25,42-45</sup> Two distinct subtypes of ET receptors have so far been cloned from a number of species. The ET<sub>A</sub> subtype preferentially accepts ET-1 and ET-2 as ligands and appears to be the one predominantly responsible for mediating vasoconstriction. In con-

trast, the ET<sub>B</sub> subtype is considered nonselective, binding ET-1, ET-2, and ET-3 with comparable affinity. Activation of the ET<sub>B</sub> receptor in endothelial cells by low doses of ETs tends to antagonize ET<sub>A</sub>-induced effects and leads to the release of potent vasodilators, mainly NO and prostacyclin.<sup>46-51</sup> Thus, the ratio of ET<sub>A</sub> to ET<sub>B</sub> activation appears to be critical for the net effect of ETs on vascular tone. This ratio can change: any ET-receptor subtype can undergo up- or down-regulation, in turn altering tissue responsiveness to ETs.<sup>52,53</sup>

Signal transduction pathways involved in ET-receptor stimulation include phospholipases C, A<sub>2</sub>, and D, protein kinase C, tyrosin kinase, receptor-gated or voltage-dependent calcium channels, and sodium-hydrogen antiporters.<sup>54-59</sup>

ETs not only are potent vasoconstrictors, but also act on other smooth muscle cells such as those in the bronchial tree.<sup>60</sup> In addition, they display remarkable mitogenic or comitogenic activity in a number of cell types and are able to influence cell differentiation.<sup>61-66</sup> The characteristics and mode of action of ETs may justify their classification as hormones, neuropeptides, or cytokines.

Subtype-selective ET antagonists, monoclonal antibodies against ETs, and ECE inhibitors have been of tremendous help in the search for the physiologic and pathophysiologic role of ETs.<sup>38,67-69</sup> Two peptides, BQ123 (an ET<sub>A</sub> antagonist)<sup>68</sup> and IRL1038 (an ET<sub>B</sub> antagonist)<sup>69</sup> have become available for experimental use, but their pharmacokinetic disadvantages make them unsuitable for clinical application. Recently, RO46-2005, a promising, though non-ET-subtype-selective, nonpeptide antagonist, has been described. A structurally modified pyrimidinyl sulfonamide related to oral antidiabetic agents but devoid of hypoglycemic activity, RO46-2005 can be given by mouth, penetrates the blood-brain barrier, and has a half-life of approximately 8 hours.<sup>70</sup> This or similar compounds may be introduced clinically in the near future.

#### ETs are potent vasoactive mediators in the brain

ETs can provoke extremely potent and long-lasting vasoconstriction, both in vitro and in vivo, in cerebral blood vessels of all sizes and types, including the microcirculation.<sup>71-78</sup> Intracisternal application of as little as 10 pMol of ET-1 in dogs induces a pronounced spastic constriction of the vertebrobasilar arteries, which lasts for more than a day.<sup>71,73</sup> ET-1



can therefore be regarded as a potential mediator of chronic functional narrowing of cerebral vessels. Vasodilatory effects of ETs, apparently concentration-dependent, have also been described for the cerebral circulation and are most likely indirect, ie, they involve other mediators.<sup>48,50,51,78-81</sup>

Under physiologic conditions, ETs do not penetrate the blood-brain barrier or influence its permeability.<sup>42,82</sup> ETs thus either require a damaged endothelial cell layer in order to exert their effect in cerebral vessels via the lumen, or they must act from the adventitial side.<sup>83,84</sup> These observations originally prompted our search for a source of ETs on the outside of cerebral vessels.

In fact, astrocytes (glial cells that profoundly influence the function of both neurons and cerebral endothelial cells) were found to produce ET-1 and ET-3 and, in addition, to express high-affinity binding sites for these peptides.<sup>26-28</sup> Further, ET-1 release by these cells is subject to selective autostimulation: stimulation of astrocytic ET receptors potentiates further ET-1 release while leaving ET-3 unaffected.<sup>28</sup> A similar autostimulation of ETs has been shown in endothelial cells<sup>85,86</sup> and, upon induction, in vascular smooth muscle cells.<sup>31</sup> The amount of ET-1 produced by astrocytes in response to autostimulation with ET-1 greatly exceeds that achieved with other stimulants such as norepinephrine or thrombin.<sup>28,53</sup> Such local autostimulatory amplification within a cerebral microenvironment may be of major pathophysiologic significance in a number of conditions ranging from subarachnoid hemorrhage to cerebral infection.

#### **NITRIC OXIDE: A VASODILATORY COUNTERBALANCE**

Interest in NO began when Furchgott and Zawadzki<sup>87</sup> discovered that endothelial cells play an obligatory role in mediating vasorelaxation by releasing a chemical compound in response to different stimulants. This compound, initially termed "endothelium-derived relaxing factor" (EDRF), induces relaxation by activating soluble guanylate cyclase in smooth muscle and by increasing the intracellular concentration of cyclic guanosine monophosphate.<sup>88</sup> It was subsequently identified as NO, a free radical with high lipid solubility and an extremely short half-life of only a few seconds in biological fluids.<sup>89,90</sup> However, there is still a debate as to whether a nitroso-thiol compound such as

S-nitroso-cystein eventually accounts for the effect of EDRF.<sup>91,92</sup>

NO is derived from the guanidino group of its precursor, the amino acid L-arginine. This reaction, which is catalyzed by the enzyme NO synthase (NOS), yields citrulline as a by-product, which may be recycled in the cells via an intermediate compound, argininosuccinate, in a partial urea cycle.<sup>93,94</sup> Several isoforms of NOS have been described,<sup>95,96</sup> which are expressed either constitutively (cNOS, eg, in endothelial cells) or upon induction (iNOS). Interestingly, iNOS expression may be triggered in smooth muscle cells of peripheral as well as cerebral arteries by incubation with endotoxin or cytokines.<sup>97-101</sup> Once activated, iNOS results in high amounts of NO, which can contribute, for example, to the pathogenesis of endotoxic shock.<sup>100</sup>

Endothelial cells are not the sole source of NO in the brain, since glial cells and neurons also express cNOS. In neurons, NO release is potently stimulated by the excitatory amino acid glutamate via activation of postsynaptic N-methyl-D-aspartate (NMDA) receptors.<sup>102</sup> NMDA-induced neuronal NO release is most pronounced in the cerebellum,<sup>93,102,103</sup> but also occurs in other brain regions, including the forebrain.<sup>104,105</sup> In the rat cortex, approximately 1% to 2% of the neurons are stained by NOS antibodies or are positive for nicotinamide-adenine-dinucleotide phosphate- (NADPH-) diaphorase, an enzyme that appears to be highly similar or even identical to neuronal NOS.<sup>105-107</sup>

NOS and NADPH-diaphorase have also been identified in nerve fibers surrounding cerebral arteries in rats,<sup>103,108-111</sup> dogs,<sup>112</sup> cats,<sup>113</sup> and humans.<sup>110</sup> This "nitroxidergic" innervation, which appears to originate mainly from parasympathetic ganglia, has been hypothesized to be involved in the pathogenesis of cerebral vasospasm and migraine attacks,<sup>114</sup> via an imbalance (lack or excess) of NO release.

NOS inhibitors can effectively block the relaxation induced by transmural nerve stimulation in isolated basilar or middle cerebral arteries devoid of a functional endothelium,<sup>112-119</sup> indicating that NO from nitroxidergic nerves may mediate the nonadrenergic, noncholinergic relaxation of cerebral blood vessels. Similarly, NO release may be the underlying cause of nonadrenergic, noncholinergic relaxation of smooth muscle cells in the gastrointestinal tract<sup>120</sup> and the genitourinary system, thereby playing a pivotal role in the control of penile erection.<sup>121</sup>

### Vascular NO and cerebrovascular tone

The function of NO can be studied by blocking NOS activity with analogues of arginine such as N<sup>G</sup>-monomethyl-L-arginine (L-NMMA), N<sup>G</sup>-nitro-L-arginine (L-NNA), or its methyl ester (L-NAME).<sup>122</sup> In large cerebral arteries isolated from different species, NOS inhibitors induce contraction, indicating that basal release of NO contributes to the maintenance of resting tone.<sup>123–128</sup> Furthermore, NO mediates relaxation in response to a number of vasoactive compounds such as acetylcholine.<sup>123,125–127,129</sup> However, additional factors important to relaxation may also be released from the endothelium. In rabbit basilar arteries, complete inhibition of muscarinic and histaminergic relaxation can only be achieved by simultaneous application of an NOS inhibitor and indomethacin to block the release of relaxant prostanoids (probably prostacyclin).<sup>127,130</sup>

Similarly, *in vivo*, superfusion with NOS inhibitors decreased the resting diameter of basilar arteries of rats and inhibited acetylcholine-induced dilation.<sup>131–133</sup> NO, therefore, participates in regulating the resting diameter and mediates muscarinic dilation of the basilar artery. However, in small pial arteries of rats, topical application of an NOS inhibitor produced no significant vasomotor effect,<sup>134–138</sup> and comparable results have been obtained in most studies in other species,<sup>139–142</sup> although somewhat different observations have been reported sporadically.<sup>143–146</sup>

Thus, basal release of NO does not appear to be a general prerequisite for the adjustment of resting tone in small pial arteries. Upon topical application of acetylcholine, however, these small arteries dilate in a concentration-dependent manner. This dilation is blocked by simultaneous application of an NOS inhibitor,<sup>133,135,141,143,147</sup> indicating that the apparent noninvolvement of NO in the regulation of resting tension is not due to a lack of NOS activity in the vessel wall.

Topical application of low concentrations of 5-hydroxytryptamine (5-HT) also results in an L-NNA-sensitive dilation of small pial arteries in rats,<sup>136</sup> while the dilating effect of bradykinin is not modified in the presence of L-NNA.<sup>135,144</sup> Taken together, there are pronounced regional and mediator-dependent differences in the function of EDRFs, reflecting this system's high plasticity in the regulation of cerebrovascular resistance.

### NO may link cerebral blood flow to neuronal activity

In most published studies, NOS inhibitors given systemically decreased the resting cerebral blood flow,<sup>129,148–157</sup> indicating an increase in total cerebrovascular resistance. This may partly result from constriction of large arteries such as the basilar artery.<sup>131–133</sup> It may also partly result from constriction of intraparenchymal arteries and arterioles, since superfusion of the parietal cortex with NOS inhibitors decreases regional cerebral blood flow,<sup>155,156,158</sup> although this does not affect the resting diameter of pial arteries appreciably, as discussed above.

Endothelial cells may supply the tonically released NO that influences intraparenchymal resistance vessels, but the presence of cNOS in neurons and glial cells<sup>95,159</sup> suggests that these cells also make substantial contributions. These observations led to the hypothesis, based on computer simulation,<sup>160</sup> that NO released from the parenchyma could couple regional cerebral blood flow to local neuronal activity.

We have recently tested this hypothesis using the spreading cortical depression described by Leao<sup>161</sup> as a model of cortical activation. The spreading depression is characterized by a transient phase of neuronal hyperactivity caused by a massive release of the excitatory transmitter glutamate,<sup>162–165</sup> followed by a more sustained period of hypoactivity travelling over the cortex in a wavelike manner. The wavelike spread of increased neuronal firing is accompanied by transient dilation of pial arteries<sup>166,167</sup> and regional hyperperfusion.<sup>152,168,169</sup> These effects appear to be mostly indirectly induced, since neither glutamate nor NMDA exerts any direct vasomotor effects in isolated cerebral arteries.<sup>145,170,171</sup> Both pial arterial dilation and hyperperfusion during a wave of spreading depression can be reduced considerably by local or systemic application of an NOS inhibitor.<sup>142,152,157</sup> This may point to a role of NO in coupling neuronal activation and arterial dilation under this condition.

Further studies using different methods of cortical activation support the hypothesis that NO links neuronal activity and perfusion. The increase in regional cerebral blood flow induced by electrical stimulation of the tibial nerve can be abolished by intraparenchymal application of an NOS inhibitor.<sup>172</sup> Similarly, cortical hyperperfusion during whisker stimulation can be reduced by systemic application or cortical superfusion with an NOS inhibitor in anesthetized rats.<sup>158</sup> However, in awake rats a

similar degree of hyperperfusion (expressed in percent of resting cerebral blood flow) during whisker stimulation was found in the absence and presence of an NOS inhibitor.<sup>173,174</sup> Whether this lack of effect of NOS inhibitors on metabolic coupling is due to incomplete inhibition of NOS (as suggested by Irikura and coworkers<sup>175</sup>) remains to be established.

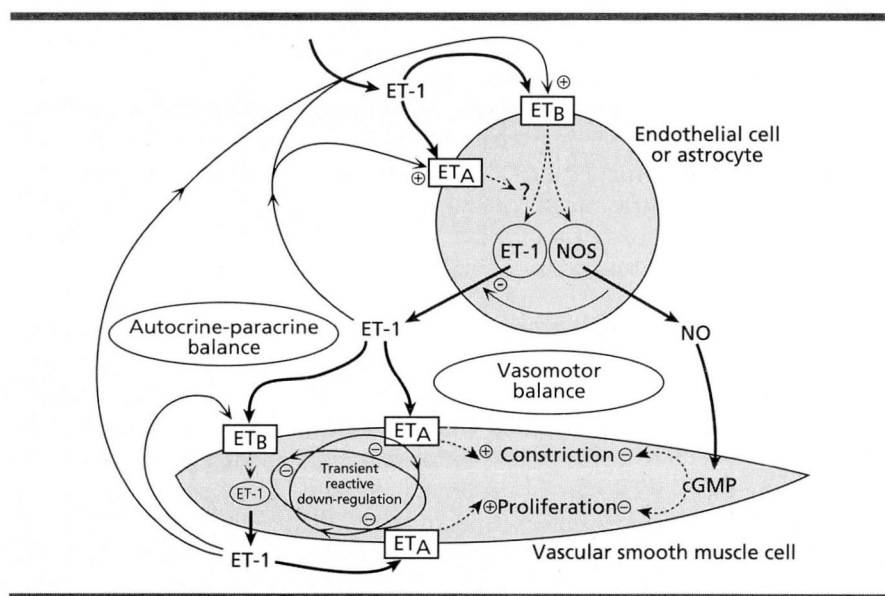
Although the exact role of NO in controlling cerebrovascular resistance is still a matter of speculation,<sup>122,176</sup> it may provide at least part of the link matching metabolic demand with supply.

#### THE ET-NO NETWORK

The ET-NO network, as deduced from the literature as well as from our own work, is presented in the *Figure*. This network provides a basis for understanding the actions and interactions of ETs and NO in cerebral vasoregulation.

There are a number of potential sources of ET-1 in the cerebral microenvironment. As mentioned above, endothelial cells,<sup>19,20,177,178</sup> astrocytes,<sup>26-28</sup> and neurons<sup>21-25</sup> all can produce ET-1 and release it upon stimulation with various factors. ET-stimulating factors, such as norepinephrine, thrombin, interleukin-1, endotoxin, and transforming growth factor-beta, are not equally efficient among cell types and also result in a different temporal ET-response pattern.<sup>11,28,31</sup> In addition to being produced in cells that reside in the brain, ET can be produced by macrophages that invade it under pathological conditions, eg, meningitis, ischemia, subarachnoid hemorrhage, or human immunodeficiency virus (HIV) encephalopathy.<sup>29,179-182</sup>

Once ET-1 is present in a certain cerebral microenvironment, it binds not only to vascular smooth muscle cells (causing vasoconstriction), but also to ET<sub>B</sub> receptors and, most likely, to ET<sub>A</sub> receptors located on endothelial cells<sup>85,183,184</sup> and astrocytes.<sup>53,185</sup> Activation of these receptors initiates the



**FIGURE.** The endothelin (ET)-nitric oxide (NO) network: The players are known but the rules of the game are still obscure. ET-1, ET-2, and ET-3 are different peptides; ET<sub>A</sub> and ET<sub>B</sub> are receptors. NOS, nitric oxide synthase; cGMP, cyclic guanosine monophosphate.

autostimulatory amplification of ET-1 on one hand<sup>28,85,86</sup> and, on the other hand, leads to stimulation of NOS and subsequent synthesis of NO.<sup>78,186-188</sup> As illustrated in the *Figure*, NO is capable of inhibiting ET-1 release, thereby serving as a natural control factor of ET autostimulation.<sup>189</sup>

Both NO and ET-1 act on vascular smooth muscle cells, the former inducing vasodilation, the latter provoking vasoconstriction. With respect to smooth muscle proliferation, they also exhibit opposite effects, ET being a stimulator, NO an inhibitor.<sup>61,190</sup> ET-1-induced vasoconstriction appears to be mediated mainly via ET<sub>A</sub>-receptors. There is, however, a high probability that vascular smooth muscle cells, perhaps with regional differences, additionally express an ET<sub>B</sub>-type receptor that is predominantly responsible for autoinduction of ET-gene expression in these cells.<sup>31</sup> Whether ET<sub>B</sub> stimulation can lead to iNOS activation, thereby initiating smooth muscle NO production, is still unknown and has therefore not been integrated into the *Figure*. Nevertheless, vascular smooth muscle ET-1 can contribute to the "autocrine-paracrine balance" within its microenvironment.

An additional level of control over ET action apparently consists of a transient reactive down-



regulation of ET-receptor expression. This homologous down-regulation may selectively affect one receptor subtype and leave the other unaffected, as shown for ET<sub>A</sub> in primary astrocyte cultures in which ET<sub>B</sub> remained unchanged.<sup>53</sup> In principle, ET<sub>B</sub> can also undergo down-regulation.<sup>52,53</sup> Down-regulation may originate at the mRNA level, extend to the expression of the receptor protein in the cell membrane, and could also consist of an internalization of the receptor-ligand complex, perhaps followed by receptor recycling.<sup>191-193</sup>

As one aspect of autostimulatory phenomena, a negative correlation between ET-1 production and ET<sub>A</sub>-receptor expression has been shown for smooth muscle cells and astrocytes.<sup>53,194,195</sup> Interestingly, changes in the ratio of ET<sub>A</sub> to ET<sub>B</sub> receptor expression in human endometrium have been observed during the menstrual cycle, indicating a function-dependent shift in responsiveness to ET.<sup>196</sup>

Removing or adding certain components may, despite the network's considerable plasticity, profoundly disturb vasomotor and autocrine balance. For instance, introducing ET-3 into the network would, considering the low affinity of ET-3 for ET<sub>A</sub> receptors, result in a preferential stimulation of ET<sub>B</sub> receptors. This in turn might lead to a temporary preponderance of vasodilating factors. On the other hand, reducing NOS activity, leading to impairment of NO production, would contribute to exaggerated vascular contraction.

#### DERANGEMENTS OF THE ET-NO NETWORK

Cerebral vasospasm—the functional narrowing of vessels—occurs in a number of conditions, including subarachnoid hemorrhage, cerebral trauma, and meningitis. It may result from increased activity of vasoconstricting agents or decreased vasorelaxing capacities, or both. In either case, a profound disturbance of the basal vasomotor balance in cerebral vessels would result. In a number of species, ETs can produce long-lasting spasm of cerebral vessels upon intracerebroventricular application.<sup>71,73,83,197-199</sup> In patients with subarachnoid hemorrhage-induced vasospasm, elevated levels of immunoreactive ETs have been identified in ventricular cerebrospinal fluid in a temporal pattern paralleling the occurrence of clinically documented vasospasm.<sup>200,201</sup> In addition, an increased sensitivity of cerebral vessels to ETs has been shown following experimental subarachnoid hemorrhage,<sup>202</sup> which may contribute

to the functional preponderance of vasoconstricting agents in this condition.

At the same time, vasorelaxing capacities seem to decrease considerably<sup>203-205</sup>; there is reduced production of NO,<sup>206</sup> reduced NOS immunoreactivity at the adventitial side of cerebral vessels (possibly due to a loss of nitroxidergic innervation), and marked reduction in the level of cyclic guanosine monophosphate,<sup>207,208</sup> which constitutes the effector pathway of NO. Taken together, these events may help to explain the powerful vasospastic reaction of the cerebral vasculature.

Ischemia-induced alterations may follow vasospastic reactions of various origins or may initiate or further enhance them. Both ETs and NO appear to be mediators involved in the pathophysiology of ischemia.<sup>77,209-213</sup> The synthesis of NO is profoundly altered, as shown by transient peaks of NO release immediately after ischemia and, likewise, after reperfusion.<sup>214</sup> Furthermore, experimental ischemia leads to NOS induction.<sup>215,216</sup> Similarly, ischemia has been shown in many ways to affect ET release as well as ET-receptor expression.<sup>41,212,217</sup> Plasma ET levels have been found to be elevated in patients suffering from ischemic stroke.<sup>218</sup> Interestingly, this has also been reported in acute migraine attacks.<sup>219</sup>

Discrete ischemic lesions have further been identified in HIV encephalopathy, characterized by abnormalities appearing early on single-photon emission computed tomography and positron-emission tomography.<sup>220-225</sup> Macrophage-derived multinucleated giant cells in the brains of patients with acquired immunodeficiency syndrome were distinctly positively stained for ETs, as were astrocytes and endothelial cells in their vicinity.<sup>182</sup> This may point to a concerted action *in vivo* of various cell types with respect to ET production in inflammatory conditions, and may be analogous to the autostimulatory amplification of ET levels shown *in vitro*.

#### APPROACHES TO THERAPY

Any disturbance of the ET-NO network, once recognized, could potentially be addressed by agents to restore the preexisting balance. Recently available ET antibodies, ET antagonists, and ECE inhibitors have been reported effective in counteracting cerebral vasospasm in a number of species.<sup>70,226-229</sup> How treatment with ET antagonists will influence ET-receptor expression and, thus, tissue sensitivity to any ET-receptor ligand remains to be determined.

Such alterations will be of therapeutic significance.

The role of NO released during complete or incomplete ischemia as well as during reperfusion is still far from understood.<sup>213</sup> On one hand, vasodilation by NO may help to maintain regional blood flow above the critical threshold; on the other hand, NO may react with superoxide radicals to form peroxynitrite, a harmful free-radical species.<sup>230,231</sup> Accordingly, no effect,<sup>232</sup> an increase,<sup>233</sup> and a decrease<sup>234-236</sup> in ischemic damage have all been observed after application of NOS inhibitors in different experimental models of brain ischemia. Further studies are needed to elucidate the involvement of NO in the pathophysiology of cerebral ischemia.

## REFERENCES

- Kuschinsky W, Wahl M. Local chemical and neurogenic regulation of cerebral vascular resistance. *Physiol Rev* 1978; 58:656-689.
- Lassen NA, Ingvar DH, Skinhoj E. Brain function and blood flow. *Sci Am* 1978; 239:62-71.
- Kontos HA. Regulation of the cerebral circulation. *Annu Rev Physiol* 1981; 43:397-407.
- Busija D, Heistad DD. Factors involved in the physiological regulation of the cerebral circulation. *Rev Physiol Biochem Pharmacol* 1984; 101:161-211.
- Wahl M. Local chemical, neural, and humoral regulation of cerebrovascular resistance vessels. *J Cardiovasc Pharmacol* 1985; 7 Suppl 3:S36-S46.
- Baumbach GL, Heistad DD. Regional, segmental, and temporal heterogeneity of cerebral vascular autoregulation. *Ann Biomed Eng* 1985; 13:303-310.
- Uddman R, Edvinsson L. Neuropeptides in the cerebral circulation. *Cerebrovasc Brain Metab Rev* 1989; 1:230-252.
- Paulson OB, Strandgaard S, Edvinsson L. Cerebral autoregulation. *Cerebrovasc Brain Metab Rev* 1990; 2:161-192.
- Faraci FM, Heistad DD. Regulation of cerebral blood vessels by humoral and endothelium-dependent mechanisms. Update on humoral regulation of vascular tone. *Hypertension* 1991; 17:917-922.
- Armstead WM, Leffler CW. Neurohumoral regulation of the cerebral circulation. *Proc Soc Exp Biol Med* 1992; 199:149-242.
- Yanagisawa M, Kurihara H, Kimura, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988; 332:411-415.
- Inoue A, Yanagisawa M, Kimura S, et al. The human endothelin family: three structurally related and pharmacologically distinct isoforms predicted by three separate genes. *Proc Natl Acad Sci U S A* 1989; 86:2863-2867.
- Yanagisawa M, Masaki T. Molecular biology and biochemistry of the endothelins. *Trends Pharmacol Sci* 1989; 10:374-378.
- Bloch KD, Friedrich SP, Lee ME, Eddy RL, Shows TB, Quertermous T. Structural organization and chromosomal assignment of the gene encoding endothelin. *J Biol Chem* 1989; 264:10851-10857.
- Bloch KD, Eddy RL, Shows TB, Quertermous T. cDNA cloning and chromosomal assignment of the gene encoding endothelin-3. *J Biol Chem* 1989; 264:18156-18161.
- Arinami T, Ishikawa M, Inoue A, et al. Chromosomal assignments of the human endothelin family genes: the endothelin-1 gene (EDN1) to 6p23-p24, the endothelin-2 gene (EDN2) to 1p34, and the endothelin-3 gene (EDN3) to 20q13.2-q13.3. *Am J Hum Genet* 1991; 48:990-996.
- Bloch KD, Hong CC, Eddy RL, Shows TB, Quertermous T. cDNA cloning and chromosomal assignment of the endothelin 2 gene: vasoactive intestinal contractor peptide is rat endothelin 2. *Genomics* 1991; 10:236-242.
- Rao VVNG, Löffler C, Hansmann I. The gene for the novel vasoactive peptide endothelin 3 (EDN3) is localized to human chromosome 20q13.2-qter. *Genomics* 1991; 10:840-841.
- Yoshimoto S, Ishizaki Y, Kurihara H, et al. Cerebral microvessel endothelium is producing endothelin. *Brain Res* 1990; 508:283-285.
- Bacic F, Uematsu S, McCarron RM, Spatz M. Secretion of immunoreactive endothelin-1 by capillary and microvascular endothelium of human brain. *Neurochem Res* 1992; 17:699-702.
- Giaid A, Gibson SJ, Ibrahim NBN, et al. Endothelin-1, an endothelium-derived peptide, is expressed in neurons of the human spinal cord and dorsal root ganglia. *Proc Natl Acad Sci U S A* 1989; 86:7634-7638.
- Yoshizawa T, Shinmi O, Giaid A, et al. Endothelin: a novel peptide in the posterior pituitary system. *Science* 1989; 247:462-464.
- Lee M-E, de la Monte SM, Ng S-C, Bloch KD, Quertermous T. Expression of the potent vasoconstrictor endothelin in the human central nervous system. *J Clin Invest* 1990; 86:141-147.
- Giaid A, Gibson SJ, Herrero MT, et al. Topographical localisation of endothelin mRNA and peptide immunoreactivity in neurones of the human brain. *Histochemistry* 1991; 95:303-314.
- Takahashi K, Ghatei MA, Jones PM, et al. Endothelin in human brain and pituitary gland: presence of immunoreactive endothelin, endothelin messenger ribonucleic acid, and endothelin receptors. *J Clin Endocrinol Metab* 1991; 72:693-699.
- MacCumber MW, Ross CA, Snyder SH. Endothelin in brain: receptors, mitogenesis, and biosynthesis in glial cells. *Proc Natl Acad Sci U S A* 1990; 87:2359-2363.
- Ehrenreich H, Kehrl JH, Anderson RW, et al. A vasoactive peptide, endothelin-3, is produced by and specifically binds to primary astrocytes. *Brain Res* 1991; 538:54-58.
- Ehrenreich H, Anderson RW, Ogino Y, et al. Selective autoregulation of endothelins in primary astrocyte cultures: endothelin receptor-mediated potentiation of endothelin-1 secretion. *New Biol* 1991; 3:135-141.
- Ehrenreich H, Anderson RW, Fox CH, et al. Endothelins, peptides with potent vasoactive properties, are produced by human macrophages. *J Exp Med* 1990; 172:1741-1748.
- Ehrenreich H, Burd PR, Rottem M, et al. Endothelins belong to the assortment of mast cell derived and mast cell bound cytokines. *New Biol* 1992; 4:147-156.

## SUMMARY

Accumulating evidence indicates an important role for ETs and NO in the regulation of cerebral perfusion. Attempts to understand the physiology and pathophysiology of the ET-NO network as delineated here have opened an exciting and promising area of clinical research with future therapeutic implications.

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31. Hahn AWA, Resink TJ, Scott-Burden T, Powell J, Dohi Y, Bühler R. Stimulation of endothelin mRNA and secretion in rat vascular smooth muscle cells: a novel autocrine function. *Cell Regul* 1990; 1:649-659.
32. Inoue A, Yanagisawa M, Takuwa Y, Mitsui Y, Kobayashi M, Masaki T. The human preproendothelin-1 gene. Complete nucleotide sequence and regulation of expression. *J Biol Chem* 1989; 264:14954-14959.
33. Lee ME, Bloch KD, Clifford JA, Quertermous T. Functional analysis of the endothelin-1 gene promoter. *J Biol Chem* 1990; 265:10446-10450.
34. Simonson MS, Dunn MJ. Endothelins: a family of regulatory peptides. *Hypertension* 1991; 17:856-863.
35. Sawamura T, Kimura S, Shinmi O, Sugita Y, Yanagisawa M, Masaki T. Analysis of endothelin related peptides in culture supernatant of porcine aortic endothelial cells: evidence for biosynthetic pathway of endothelin-1. *Biochem Biophys Res Commun* 1989; 162:1287-1294.
36. Shiba R, Sakurai T, Yamada G, et al. Cloning and expression of rat preproendothelin-3 cDNA. *Biochem Biophys Res Commun* 1992; 186:588-594.
37. Sawamura T, Kimura S, Shinmi O, et al. Characterization of endothelin converting enzyme activities in soluble fraction of bovine cultured endothelial cells. *Biochem Biophys Res Commun* 1990; 169:1138-1144.
38. McMahon EG, Palomo MA, Moore WM, McDonald JF, Stern MK. Phosphoramidon blocks the pressor activity of porcine big endothelin-1-(1-39) in vivo and conversion of big endothelin-1-(1-39) to endothelin-1-(1-21) in vitro. *Proc Natl Acad Sci U S A* 1991; 88:703-707.
39. Webb DJ. Endothelin receptors cloned, endothelin converting enzyme characterized and pathophysiological roles for endothelin proposed. *Trends Pharmacol Sci* 1991; 12:43-46.
40. Oppenorth TJ, Wu-Wong JR, Shiosaki K. Endothelin-converting enzymes. *FASEB J* 1992; 6:2653-2659.
41. Firth JD, Ratcliffe PJ. Organ distribution of the three rat endothelin messenger RNAs and the effects of ischemia on renal gene expression. *J Clin Invest* 1992; 90:1023-1031.
42. Koseki C, Imai M, Hirata Y, Yanagisawa M, Masaki T. Autoradiographic distribution in rat tissues of binding sites for endothelin: a neuropeptide? *Am J Physiol* 1989; 256:R858-R866.
43. Jones CR, Hiley CR, Pelton JT, Mohr M. Autoradiographic visualization of the binding sites for [<sup>125</sup>I] endothelin in rat and human brain. *Neurosci Lett* 1989; 97:276-279.
44. Nambi P, Pullen M, Feuerstein G. Identification of endothelin receptors in various regions of rat brain. *Neuropeptides* 1990; 16:195-199.
45. Hösli E, Hösli L. Autoradiographic evidence for endothelin receptors on astrocytes in cultures of rat cerebellum, brainstem and spinal cord. *Neurosci Lett* 1991; 129:55-58.
46. Arai H, Hori S, Aramori I, Ohkubo H, Nakanashi S. Cloning and expression of a cDNA encoding an endothelin receptor. *Nature* 1990; 348:730-732.
47. Sakurai T, Yanagisawa M, Takuwa Y, et al. Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. *Nature* 1990; 348:732-735.
48. Lidbury PS, Thiemermann C, Korbut R, Vane JR. Endothelins release tissue plasminogen activator and prostanoids. *Eur J Pharmacol* 1990; 186:205-212.
49. Lin HY, Kaji EH, Winkel GK, Ives HE, Lodish HF. Cloning and functional expression of a vascular smooth muscle endothelin-1 receptor. *Proc Natl Acad Sci U S A* 1991; 88:3185-3189.
50. Fukuda N, Izumi Y, Soma M, et al. Effects of indomethacin, endothelium-denudation, methylene blue and L-N<sup>G</sup>-monomethyl arginine on the vasoactive effects of endothelin-3. *Jpn J Pharmacol* 1991; 55:375-380.
51. Hyslop S, de Nucci G. Vasoactive mediators released by endothelins. *Pharmacol Res* 1992; 26:223-241.
52. Sakurai T, Morimoto H, Kasuya Y, et al. Level of ET<sub>B</sub> receptor mRNA is down-regulated by endothelins through decreasing the intracellular stability of mRNA molecules. *Biochem Biophys Res Commun* 1992; 186:342-347.
53. Ehrenreich H, Costa T, Clouse KA, et al. Thrombin is a regulator of astrocytic endothelin-1. *Brain Res* 1993; 600:201-207.
54. Doherty AM. Endothelin: a new challenge. *J Med Chem* 1991; 35:1493-1508.
55. Gulati A, Simal RC. Endothelin mechanisms in the central nervous system: a target for drug development. *Drug Development Research* 1992; 26:361-387.
56. Liu Y, Geisbuhler B, Jones AW. Activation of multiple mechanisms including phospholipase D by endothelin-1 in rat aorta. *Am J Physiol* 1992; 262:C941-C949.
57. Miller RC, Pelton JT, Huggins JR. Endothelins—from receptors to medicine. *Trends Pharmacol Sci* 1993; 14:54-60.
58. Simonson MS, Herman WH. Protein kinase C and protein tyrosine kinase activity contribute to mitogenic signaling by endothelin-1. *J Biol Chem* 1993; 268:9347-9357.
59. Simonson MS, Dunn MJ. Endothelin peptides and the kidney. *Annu Rev Physiol* 1993; 55:249-265.
60. Maggi CA, Giuliani S, Patacchini R, Rovero P, Giachetti A, Meli A. The activity of peptides of the endothelin family in various mammalian smooth muscle preparations. *Eur J Pharmacol* 1989; 174:23-31.
61. Komuro I, Kurihara H, Sugiyama T, Takaku F, Yazaki Y. Endothelin stimulates c-fos and c-myc expression and proliferation of vascular smooth muscle cells. *FEBS Lett* 1988; 238:249-252.
62. Brown KD, Littlewood CJ. Endothelin stimulates DNA-synthesis in Swiss 3T3 cells. *Biochem J* 1989; 263:977-980.
63. Hirata Y, Takagi Y, Fukuda Y, Marumo F. Endothelin is a potent mitogen for rat vascular smooth muscle cells. *Atherosclerosis* 1989; 78:225-228.
64. Simonson MS, Wann S, Mene P, et al. Endothelin stimulates phospholipase C, Na<sup>+</sup>/H<sup>+</sup> exchange, c-fos expression, and mitogenesis in rat mesangial cells. *J Clin Invest* 1989; 83:708-712.
65. Takuwa N, Takuwa Y, Yanagisawa M, Yamashita K, Masaki T. A novel vasoactive peptide endothelin stimulates mitogenesis through inositol lipid turnover in swiss 3T3 fibroblasts. *J Biol Chem* 1989; 264:7856-7861.
66. Tanahashi T, Yamaguchi K, Ishikawa S, Kusuhara M, Adachi I, Abe O. Endothelin-I inhibits adipogenic differentiation of 3T3-L1 preadipocytes. *Biochem Biophys Res Commun* 1991; 177:854-860.
67. Watanabe T, Suzuki N, Shimamoto N, Fujino M, Imada A. Contribution of endogenous endothelin to the extension of myocardial infarct size in rats. *Circ Res* 1991; 69:370-377.
68. Ihara M, Noguchi K, Saeki T, et al. Biological profiles of highly potent novel endothelin antagonists selective for the ET<sub>A</sub> receptor. *Life Sci* 1992; 50:247-255.
69. Urade Y, Fujitani Y, Oda K, et al. An endothelin B receptor-selective antagonist: IRL 1038, [Cys<sup>11</sup>-Cys<sup>19</sup>]-endothelin-1(11-21). *FEBS Lett* 1992; 311:12-16.
70. Clozel M, Breu V, Burri K, et al. Pathophysiological role of endothelin revealed by the first orally active endothelin receptor antagonist. *Nature* 1993; 365:759-761.
71. Asano T, Ikegaki I, Suzuki Y, Satoh S, Shibuya M. Endothelin and the production of cerebral vasospasm in dogs. *Biochem Biophys Res Commun* 1989; 159:1345-1351.
72. Hardebo JE, Kahrström J, Owman C, Salford LG. Endothelin is a potent constrictor of human intracranial arteries and veins. *Blood Vessels* 1989; 26:249-253.
73. Ide K, Yamakawa K, Nakagomi T, et al. The role of endothelin in the pathogenesis of vasospasm following subarachnoid hemorrhage. *Neurol Res* 1989; 11:101-104.
74. Saito A, Shiba R, Kimura S, Yanagisawa M, Goto K, Masaki T. Vasoconstrictor response of large cerebral arteries of cats to endothelin, an endothelium-derived vasoactive peptide. *Eur J Pharmacol* 1989; 162:353-358.



75. Martin de Aguilera E, Irurzun A, Vila JM, Aldasoro M, Galeote MS, Lluch S. Role of endothelium and calcium channels in endothelin-induced contraction of human cerebral arteries. *Br J Pharmacol* 1990; 99:439-440.
76. Papadopoulos SM, Gilbert LL, Webb RC, D'Amato CJ. Characterization of contractile responses to endothelin in human cerebral arteries: implications for cerebral vasospasm. *Neurosurgery* 1990; 26:810-815.
77. Robinson MJ, Macrae IM, Todd M, Reid JL, McCulloch J. Reduction of local cerebral blood flow to pathological levels by endothelin-1 applied to the middle cerebral artery in the rat. *Neurosci Lett* 1990; 118:269-272.
78. Feger GI, Schilling L, Ehrenreich H, Wahl M. Endothelin-induced contraction and relaxation in rat isolated basilar artery: effect of BQ-123. *J Cereb Blood Flow Metab* 1994; 14:845-852.
79. de Nucci G, Thomas R, D'Orleans-Juste P, et al. Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc Natl Acad Sci U S A* 1988; 85:9797-9800.
80. Kauser K, Rubanyi GM, Harder DR. Endothelium-dependent modulation of endothelin-induced vasoconstriction and membrane depolarization in cat cerebral arteries. *J Pharmacol Exp Ther* 1990; 252:93-97.
81. Faraci FM. Regulation of the cerebral circulation by endothelium. *Pharmacol Ther* 1992; 56:1-22.
82. Faraci FM. Effects of endothelin and vasopressin on cerebral blood vessels. *Am J Physiol* 1989; 257:H799-H803.
83. Mima T, Yanagisawa M, Shigeno T, et al. Endothelin acts in feline and canine cerebral arteries from the adventitial side. *Stroke* 1989; 20:1553-1556.
84. Ogura K, Takayasu M, Dacey RG. Differential effects of intra- and extraluminal endothelin on cerebral arterioles. *Am J Physiol* 1991; 261:H531-H537.
85. Yokokawa K, Kohno M, Yasunari K, Murakawa K, Takeda T. Endothelin-3 regulates endothelin-1 production in cultured human endothelial cells. *Hypertension* 1991; 18:304-315.
86. Saijonmaa O, Nyman T, Fyhrquist E. Endothelin-1 stimulates its own synthesis in human endothelial cells. *Biochem Biophys Res Commun* 1992; 188:286-291.
87. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980; 288:373-376.
88. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 1991; 43:109-142.
89. Ignarro LJ, Buga GM, Wood KS, Byrns RE. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci U S A* 1987; 84:9265-9269.
90. Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987; 327:524-526.
91. Myers PR, Minor RL, Guerra R, Bates JN, Harrison DG. Vasorelaxant properties of the endothelium-derived relaxing factor more closely resemble S-nitrosocysteine than nitric oxide. *Nature* 1990; 345:161-163.
92. Marshall JJ, Kontos HA. Endothelium-derived relaxing factors. A perspective from in vivo data. *Hypertension* 1990; 16:371-386.
93. Garthwaite J. Glutamate nitric oxide and cell-cell signalling in the nervous system. *Trends Neurosci* 1991; 14:60-67.
94. Arnt-Ramos LR, O'Brien WE, Vincent SR. Immunohistochemical localization of argininosuccinate synthetase in the rat brain in relation to nitric oxide synthase-containing neurons. *Neuroscience* 1992; 51:773-789.
95. Förstermann U, Schmidt HHHW, Pollock JS, et al. Isoforms of nitric oxide synthase. Characterization and purification from different cell types. *Biochem Pharmacol* 1991; 42:1849-1857.
96. Feldman PL, Griffith OW, Stuehr DJ. The surprising life of nitric oxide. *Chemical Engineering News* 1993; Dec 20:26-38.
97. Busse R, Mülsch A. Induction of nitric oxide synthase by cytokines in vascular smooth muscle cells. *FEBS Lett* 1990; 275:87-90.
98. Fleming I, Gray GA, Julou-Schaeffer G, Parratt JR, Stoclet JC. Incubation with endotoxin activates the L-arginine pathway in vascular tissue. *Biochem Biophys Res Commun* 1990; 171:562-568.
99. Auget M, Guillon J-M, Delafloette S, Etienne E, Chabrier P-E, Braquet P. Endothelium independent protective effect of N<sup>G</sup>-monomethyl-L-arginine on endotoxin-induced alterations of vascular reactivity. *Life Sci* 1991; 48:189-193.
100. Wright CE, Rees DD, Moncada S. Protective and pathological roles of nitric oxide in endotoxin shock. *Cardiovasc Res* 1992; 26:48-57.
101. Ueno M, Lee TJ-F. Endotoxin decreases the contractile responses of the porcine basilar artery to vasoactive substances. *J Cereb Blood Flow Metab* 1993; 13:712-719.
102. Garthwaite J, Charles SL, Chess-Williams R. Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature* 1988; 336:385-388.
103. Brecht DS, Hwang PM, Snyder SH. Localization of nitric oxide synthase indicating a neural role for nitric oxide. *Nature* 1990; 347:768-770.
104. Knowles RG, Palacios M, Palmer RMJ, Moncada S. Formation of nitric oxide from L-arginine in the central nervous system: a transduction mechanism for stimulation of the soluble guanylate cyclase. *Proc Natl Acad Sci U S A* 1989; 86:5159-5162.
105. Schmidt HHHW, Gagne GD, Nakane M, Pollock JS, Miller ME, Murad F. Mapping of neural nitric oxide synthase in the rat suggests frequent co-localization with NADPH diaphorase but not with soluble guanylyl cyclase, and novel paraneural functions for nitrinergic signal transduction. *J Histochem Cytochem* 1992; 40:1439-1456.
106. Dawson TM, Brecht DS, Fotuhi M, Hwang PM, Snyder SH. Nitric oxide synthase and neuronal NADPH diaphorase are identical in brain and peripheral tissue. *Proc Natl Acad Sci U S A* 1991; 88:7797-7801.
107. Dawson TM, Dawson VL, Snyder SH. A novel neuronal messenger molecule in brain: the free radical, nitric oxide. *Ann Neurol* 1992; 32:297-311.
108. Poeggel G, Müller M, Seidel I, Reichardt L, Bernstein H-G. Histochemistry of guanylate cyclase, phosphodiesterase, and NADPH-diaphorase (nitric oxide synthase) in rat brain vasculature. *J Cardiovasc Pharmacol* 1992; 20 Suppl 12:S76-S79.
109. Iadecola C, Beitz AJ, Renno W, Xu X, Mayer B, Zhang F. Nitric oxide synthase-containing neural processes on large cerebral arteries and cerebral microvessels. *Brain Res* 1993; 606:148-155.
110. Nozaki K, Moskowitz MA, Maynard KI, et al. Possible origins and distribution of immunoreactive nitric oxide synthase-containing nerve fibers in cerebral arteries. *J Cereb Blood Flow Metab* 1993; 13:70-79.
111. Tomimoto H, Akiguchi I, Wakita H, Nakamura S, Kimura J. Distribution of NADPH-diaphorase in the cerebral blood vessels of rats: a histochemical study. *Neurosci Lett* 1993; 156:105-108.
112. Toda N, Ayajiki K, Yoshida K, Kimura H, Okamura T. Impairment by damage of the pterygopalatine ganglion of nitroxidergic vasodilator nerve function in canine cerebral and retinal arteries. *Circ Res* 1993; 72:206-213.
113. Estrada C, Mengual E, Gonzalez C. Local NADPH-diaphorase neurons innervate pial arteries and lie close or project to intracerebral blood vessels: a possible role for nitric oxide in the regulation of cerebral blood flow. *J Cereb Blood Flow Metab* 1993; 13:978-984.
114. Toda N, Okamura T. Regulation by nitroxidergic nerve of arterial tone. *News in Physiological Sciences* 1992; 7:148-152.
115. Toda N, Okamura T. Possible role of nitric oxide in transmitting information from vasodilator nerve to cerebroarterial muscle. *Biochem Biophys Res Commun* 1990; 170:308-313.
116. Toda N, Okamura N. Mechanism underlying the response to vasodilator nerve stimulation in isolated dog and monkey cerebral arteries. *Am J Physiol* 1990; 259:H1511-H1517.

117. Lee TJ-F, Sarwinsky SJ. Nitric oxidergic neurogenic vasodilation in the porcine basilar artery. *Blood Vessels* 1991; 28:407-412.
118. Toda N, Okamura N. Role of nitric oxide in neurally induced cerebroarterial relaxation. *J Pharmacol Exp Ther* 1991; 258:1027-1032.
119. Chen F-Y, Lee TJ-F. Role of nitric oxide in neurogenic vasodilation of porcine cerebral artery. *J Pharmacol Exp Ther* 1993; 265:339-345.
120. Sanders KM, Ward SM. Nitric oxide as a mediator of nonadrenergic noncholinergic transmission. *Am J Physiol* 1992; 262:G379-G392.
121. Burnett AL, Lowenstein CJ, Brecht DS, Chang TSK, Snyder SH. Nitric oxide: a physiologic mediator of penile erection. *Science* 1992; 257:401-403.
122. Iadecola C, Pelligrino DA, Moskowitz MA, Lassen NA. Nitric oxide synthase inhibition and cerebrovascular regulation. *J Cereb Blood Flow Metab* 1994; 14:175-192.
123. Schilling L, Parsons AA, Mackert JRL, Wahl M. Effect of L-arginine on haemoglobin-induced inhibition of endothelium-dependent relaxation of isolated cerebral arteries. *Acta Neurochir Suppl (Wien)* 1990; 51:341-343.
124. Schilling L, Mackert JRL, Parsons AA, Wahl M. Investigation of the modulatory role of the endothelium in rat isolated basilar artery: effects on K<sup>+</sup> channel activator-induced relaxation. *Pflügers Arch* 1991; 418 Suppl 1:R102.
125. Parsons AA, Schilling L, Wahl M. Analysis of acetylcholine-induced relaxation of isolated middle cerebral artery: effects of inhibitors of nitric oxide synthesis, Na,K-ATPase, and ATP-sensitive K channels. *J Cereb Blood Flow Metab* 1991; 11:700-704.
126. Alafaci C, Salpietro F, Tomasello F, Ross-Smith M, Angus JA. Nitric oxide synthesized from L-arginine regulates vascular tone in rat cerebral vessels. *J Cereb Blood Flow Metab* 1991; 11 Suppl 2:S261.
127. Schilling L, Parsons AA, Mackert JRL, Wahl M. Is K<sup>+</sup> channel activation, EDRE, or cyclooxygenase products involved in acetylcholine-induced relaxation of rabbit isolated basilar artery. *J Cereb Blood Flow Metab* 1991; 11 Suppl 2:S256.
128. Alonso MJ, Salas M, Sanchez-Ferrer CF, Marin J. Predominant role for nitric oxide in the relaxation induced by acetylcholine in cat cerebral arteries. *J Pharmacol Exp Ther* 1992; 261:12-20.
129. Kovach AGB, Szabo C, Benyo Z, Csaki C, Greenberg JH, Reivich M. Effects of N<sup>G</sup>-nitro-L-arginine and L-arginine on regional cerebral blood flow in the cat. *J Physiol* 1992; 449:183-196.
130. Ea Kim L, Javellaud J, Oudart N. Endothelium-dependent relaxation of rabbit middle cerebral artery to a histamine H<sub>3</sub>-agonist is reduced by inhibitors of nitric oxide and prostacyclin synthesis. *Br J Pharmacol* 1992; 105:103-106.
131. Faraci FM. Role of nitric oxide in regulation of basilar artery tone in vivo. *Am J Physiol* 1990; 259:H1216-H1221.
132. Mayhan WG. Impairment of endothelium-dependent dilatation of basilar artery during chronic hypertension. *Am J Physiol* 1990; 259:H1455-H1462.
133. Faraci FM. Role of endothelium-derived relaxing factor in cerebral circulation: large arteries vs. microcirculation. *Am J Physiol* 1991; 261:H1038-H1042.
134. Busija DW, Leffler CW, Wagerle LC. Mono-L-arginine-containing compounds dilate piglet pial arterioles via an endothelium-derived relaxing factor-like substance. *Circ Res* 1990; 67:1374-1380.
135. Parsons AA, Wang Q, Schilling L, Lassen NA, Wahl M. Effects of N<sup>G</sup>-nitro-L-arginine (NOLAG) on rat pial arterioles in situ. *Pflügers Arch* 1991; 419 Suppl 1:R112.
136. Parsons AA, Schilling L, Wahl M. Nitric oxide as a modulator of 5-hydroxytryptamine-induced responses in rat pial arterioles in situ. In: Olesen J, Saxena PR, editors. 5-Hydroxytryptamine mechanisms in primary headaches. New York: Raven Press, 1992:157-161.
137. Morikawa E, Rosenblatt S, Moskowitz MA. L-arginine dilates rat pial arterioles by nitric oxide-dependent mechanisms and increases blood flow during focal cerebral ischaemia. *Br J Pharmacol* 1992; 107:905-907.
138. Mayhan WG. Endothelium-dependent responses of cerebral arterioles to adenosine 5'-diphosphate. *J Vasc Res* 1992; 298:353-358.
139. Haberl RL, Decker PJ, Piepgras A, Einhüpf K. Is L-arginine the precursor of an endothelium-derived relaxing factor in the cerebral microcirculation? *J Cardiovasc Pharmacol* 1991; 17 Suppl 3:S15-S18.
140. Bauknight GC, Faraci FM, Heistad DD. Endothelium-derived relaxing factor modulates noradrenergic constriction of cerebral arterioles in rabbits. *Stroke* 1992; 23:1522-1526.
141. Wei EP, Kukreja R, Kontos HA. Effects in cats of inhibition of nitric oxide synthesis on cerebral vasodilation and endothelium-derived relaxing factor from acetylcholine. *Stroke* 1992; 23:1623-1629.
142. Wahl M, Schilling L, Parsons AA, Kaumann A. Involvement of calcitonin gene related peptide (CGRP) and nitric oxide (NO) in the pial artery dilatation elicited by cortical spreading depression. *Brain Res* 1994; 637:204-210.
143. Rosenblum WI, Nishimura H, Nelson GH. Endothelium-dependent L-Arg- and L-NMMA-sensitive mechanisms regulate tone of brain microvessels. *Am J Physiol* 1990; 259:H1396-H1401.
144. Rosenblum WI, Nishimura H, Nelson GH. L-NMMA in brain microcirculation of mice is inhibited by blockade of cyclooxygenase and by superoxide dismutase. *Am J Physiol* 1992; 262:H1343-H1349.
145. Faraci FM, Breese KR. Nitric oxide mediates vasodilatation in response to activation of N-methyl-D-aspartate receptors in brain. *Circ Res* 1993; 72:476-480.
146. Faraci FM, Breese KR. Nitric oxide contributes to dilatation of cerebral arterioles during seizures. *Am J Physiol* 1993; 265:H2209-H2212.
147. Wang Q, Parsons AA, Schilling L, Wahl M, Paulson OB, Lassen NA. The role of endothelium-derived relaxing factor in the regulation of resting cerebral blood flow and basal cerebrovascular tone. In: Moncada S, Higgs EA, editors. *Biology of nitric oxide*. Colchester: Portland Press, 1992:122-123.
148. Tanaka K, Gotoh F, Gomi S, et al. Inhibition of nitric oxide synthesis induces a significant reduction in local cerebral blood flow in the rat. *Neurosci Lett* 1991; 127:129-132.
149. Kozniowska E, Oseka M, Stys T. Effects of endothelium-derived nitric oxide on cerebral circulation during normoxia and hypoxia in the rat. *J Cereb Blood Flow Metab* 1992; 12:311-317.
150. Wang Q, Paulson OB, Lassen NA. Effect of nitric oxide blockade by N<sup>G</sup>-nitro-L-arginine on cerebral blood flow response to changes in carbon dioxide tension. *J Cereb Blood Flow Metab* 1992; 12:947-953.
151. Raszkiewicz JL, Linville DG, Kerwin JE, Wagenaar F, Arneric SP. Nitric oxide synthase is critical in mediating basal forebrain regulation of cortical cerebral circulation. *J Neurosci Res* 1992; 33:129-135.
152. Goadsby PJ, Kaube H, Hoskin KL. Nitric oxide synthesis couples cerebral blood flow and metabolism. *Brain Res* 1992; 595:167-170.
153. Pelligrino DA, Koenig HM, Albrecht RF. Nitric oxide synthesis and regional cerebral blood flow responses to hypercapnia and hypoxia in the rat. *J Cereb Blood Flow Metab* 1993; 13:80-87.
154. Tanaka K, Fukuuchi Y, Gomi S, et al. Inhibition of nitric oxide synthesis impairs autoregulation of local cerebral blood flow in the rat. *Neuroreport* 1993; 4:267-270.
155. Niwa K, Lindauer U, Villringer A, Dirnagl U. Blockade of nitric oxide synthesis in rats strongly attenuates the CBF response to extracellular acidosis. *J Cereb Blood Flow Metab* 1993; 13:535-539.
156. Dirnagl U, Lindauer U, Villringer A. Nitric oxide synthase blockade enhances vasomotion in the cerebral microcirculation of anesthetized rats. *Microvasc Res* 1993; 45:318-323.

157. Duckrow RB. A brief hypoperfusion precedes spreading depression if nitric oxide synthesis is inhibited. *Brain Res* 1993; 618:190-195.
158. Dirnagl U, Lindauer U, Villringer A. Role of nitric oxide in the coupling of cerebral blood flow to neuronal activation in rats. *Neurosci Lett* 1993; 149:43-46.
159. Murphy S, Simmons ML, Agullo L, et al. Synthesis of nitric oxide in CNS glial cells. *Trends Neurosci* 1993; 16:323-328.
160. Gally JA, Montague PR, Reeke GN Jr, Edelman GM. The NO hypothesis: possible effects of a short-lived, rapidly diffusible signal in the development and function of the nervous system. *Proc Natl Acad Sci U S A* 1990; 87:3547-3551.
161. Leao AAP. Spreading depression of activity in the cerebral cortex. *J Neurophysiol* 1944; 7:359-390.
162. Curtis DR, Watkins JC. Analogues of glutamic and  $\gamma$ -amino-butyric acids having potent actions on mammalian neurones. *Nature* 1961; 191:1010-1011.
163. Fikova E, van Harreveld A. Glutamate and spreading depression. *J Neurobiol* 1974; 5:469-473.
164. Marrannes R, Willems R, DePrins E, Wauquier A. Evidence for a role of the N-methyl-D-aspartate (NMDA) receptor in cortical spreading depression in the rat. *Brain Res* 1988; 457:226-240.
165. Lauritzen M, Hansen AJ. The effect of glutamate receptor blockade on anoxic depolarization and cortical spreading depression. *J Cereb Blood Flow Metab* 1992; 12:223-229.
166. Leao AAP. Pial circulation and spreading depression of activity in the cerebral cortex. *J Neurophysiol* 1944; 7:391-396.
167. Wahl M, Lauritzen M, Schilling L. Change of cerebrovascular reactivity after cortical spreading depression in cats and rats. *Brain Res* 1987; 411:72-80.
168. Hansen AJ, Lauritzen M. The role of spreading depression in acute brain disorders. *An Acad Bras Cienc* 1984; 56:457-479.
169. Lauritzen M. Cerebral blood flow in migraine and cortical spreading depression. *Acta Neurol Scand* 1987; 113 Suppl 76:1-40.
170. Takayasu M, Dacey RG. Effects of inhibitory and excitatory amino acid neurotransmitters on isolated cerebral parenchymal arterioles. *Brain Res* 1989; 482:393-396.
171. Hardebo JE, Wieloch T, Kahrström J. Excitatory amino acids and cerebrovascular tone. *Acta Physiol Scand* 1989; 136:483-485.
172. Northington FJ, Matherne GP, Berne RM. Competitive inhibition of nitric oxide synthase prevents the cortical hyperemia associated with peripheral nerve stimulation. *Proc Natl Acad Sci USA* 1992; 89:6649-6652.
173. Wang Q, Kjaer T, Jorgensen MB, et al. Nitric oxide does not act as a mediator coupling cerebral blood flow to neural activity following somatosensory stimuli in rats. *Neurol Res* 1993; 15:33-36.
174. Sokoloff L, Kennedy C, Adachi K, Wang F, Takahashi S, Melzer P. Effects of inhibition of nitric oxide synthase on resting local cerebral blood flow and on changes induced by hypercapnia or local functional activity. In: Krieglstein J, Oberpichler-Schwenk H, editors. *Pharmacology of cerebral ischemia* 1992. Stuttgart: Wissenschaftliche Verlagsgesellschaft, 1992:371-381.
175. Irikura K, Maynard KI, Moskowitz MA. Importance of nitric oxide inhibition to the attenuated vascular responses induced by topical L-nitroarginine during vibrissal stimulation. *J Cereb Blood Flow Metab* 1994; 14:45-48.
176. Iadecola C. Regulation of the cerebral microcirculation during neural activity: is nitric oxide the missing link? *Trends Neurosci* 1993; 16:206-214.
177. Saito A, Shiba R, Yanagisawa M, et al. Endothelins: vasoconstrictor effects and localization in canine cerebral arteries. *Br J Pharmacol* 1991; 103:1129-1135.
178. Loesch A, Domer FR, Alexander B, Burnstock G. Electron-immunochemistry of peptides in endothelial cells of rabbit cerebral vessels following perfusion with a perfluorocarbon emulsion. *Brain Res* 1993; 611:333-337.
179. Liszczak TM, Varsos VG, Black PMcL, Kistler JP, Zervas NT. Cerebral arterial constriction after experimental subarachnoid hemorrhage is associated with blood components within the arterial wall. *J Neurosurg* 1983; 58:18-26.
180. Jackowski A, Crockard A, Burnstock G, Ross Russell R, Kristek F. The time course of intracranial pathophysiological changes following experimental subarachnoid haemorrhage in the rat. *J Cereb Blood Flow Metab* 1990; 10:835-849.
181. del Zoppo GJ, Schmid-Schönbein GW, Mori E, Copeland BR, Chang C-M. Polymorphonuclear leukocytes occlude capillaries following middle cerebral artery occlusion and reperfusion in baboons. *Stroke* 1991; 22:1276-1283.
182. Ehrenreich H, Rieckmann P, Sinowatz F, Weih KA, Arthur LO, Goebel F-D, et al. Potent stimulation of monocytic endothelin-1 production by HIV-1 glycoprotein 120. *J Immunol* 1993; 150:4601-4609.
183. Emori T, Hirata Y, Marumo F. Specific receptors for endothelin-3 in cultured bovine endothelial cells and its cellular mechanism of action. *FEBS Lett* 1990; 263:261-264.
184. Vigne P, Ladoux A, Frelin C. Endothelins activate  $\text{Na}^+/\text{H}^+$  exchange in brain capillary endothelial cells via a high affinity endothelin-3 receptor that is not coupled to phospholipase C. *J Biol Chem* 1991; 266:5925-5928.
185. Couraud P-O, Durieu-Trautmann O, Mahe E, Marin P, Le Nguyen D, Strosberg AD. Comparison of binding characteristics of endothelin receptors on subpopulations of astrocytes. *Life Sci* 1991; 49:1471-1476.
186. Fukuda N, Izumi Y, Soma M, et al. L-N<sup>G</sup>-monomethyl arginine inhibits the vasodilating effects of low dose of endothelin-3 on rat mesenteric arteries. *Biochem Biophys Res Commun* 1990; 167:739-745.
187. Namiki A, Hirata Y, Ishikawa M, Moroi M, Aikawa J, Machii K. Endothelin-1- and endothelin-3-induced vasorelaxation via common generation of endothelium-derived nitric oxide. *Life Sci* 1992; 50:677-682.
188. Karaki H, Sudjarwo SA, Hori M, Takai M, Urade Y, Okada T. Induction of endothelium-dependent relaxation in the rat aorta by IRL 1620, a novel and selective agonist at the endothelin ET<sub>B</sub> receptor. *Br J Pharmacol* 1993; 109:486-490.
189. Boulanger C, Lüscher TF. Release of endothelin from the porcine aorta. Inhibition by endothelium-derived nitric oxide. *J Clin Invest* 1990; 85:587-590.
190. Wink DA, Kasprzak KS, Maragos CM, Elespuru RK, Misra M, Dunams TM, et al. DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. *Science* 1991; 254:1001-1003.
191. Supattapone S, Simpson AWM, Ashley CC. Free calcium rise and mitogenesis in glial cells caused by endothelin. *Biochem Biophys Res Commun* 1989; 165:1115-1122.
192. Durieu-Trautmann O, Couraud PO, Foignat-Chaverot N, Strosberg AD. cAMP-dependent down-regulation of endothelin-1 receptors on rat astrocytoma C6 cells. *Neurosci Lett* 1991; 131:175-178.
193. Marsault R, Feolde E, Frelin C. Receptor externalization determines sustained contractile responses to endothelin-1 in the rat aorta. *Am J Physiol* 1993; 264:C687-C693.
194. Nambi P, Pullen M, Wu H-L, Nuthulaganti P, Elshourbagy N, Kumar C. Dexamethasone down-regulates the expression of endothelin receptors in vascular smooth muscle cells. *J Biol Chem* 1992; 267:19555-19559.
195. Roubert P, Viossat I, Lonchampt M-O, et al. Endothelin receptor regulation by endothelin synthesis in vascular smooth muscle cells: effects of dexamethasone and phosphoramidon. *J Vasc Res* 1993; 30:139-144.
196. O'Reilly G, Charnock-Jones DS, Davenport AP, Cameron IT, Smith SK. Presence of messenger ribonucleic acid for endothelin-1, endothelin-2, and endothelin-3 in human endometrium and a change in the ratio of ET<sub>A</sub> and ET<sub>B</sub> receptor subtype across the menstrual cycle. *J Clin Endocrinol Metab* 1992; 75:1545-1549.
197. Asano T, Ikegaki I, Satoh S, et al. Endothelin: a potential modulator of cerebral vasospasm. *Eur J Pharmacol* 1990; 190:365-372.



198. Kobayashi H, Hayashi M, Kobayashi S, Kabuto M, Handa Y, Kawano H. Effect of endothelin on the canine basilar artery. *Neurosurgery* 1990; 27:357-361.
199. Macrae I, Robinson M, McAuley M, Reid J, McCulloch J. Effect of intracisternal endothelin-1 on blood flow to the lower brain stem. *Eur J Pharmacol* 1991; 203:85-91.
200. Ehrenreich H, Lange M, Near KA, et al. Long term monitoring of immunoreactive endothelin-1 and endothelin-3 in ventricular cerebrospinal fluid, plasma, and 24-h urine of patients with subarachnoid hemorrhage. *Res Exp Med (Berl)* 1992; 192:257-268.
201. Suzuki R, Masaoka H, Hirata Y, Marumo F, Isotani E, Hirakawa K. The role of endothelin-1 in the origin of cerebral vasospasm in patients with aneurysmal subarachnoid hemorrhage. *J Neurosurg* 1992; 77:96-100.
202. Alafaci C, Jansen I, Arbab MA-R, Shiokawa Y, Svendgaard N-A, Edvinsson L. Enhanced vasoconstrictor effect of endothelin in cerebral arteries from rats with subarachnoid hemorrhage. *Acta Physiol Scand* 1990; 138:317-319.
203. Kim P, Sundt TM, Vanhoutte PM. Alterations in endothelium-dependent responsiveness of the canine basilar artery after subarachnoid hemorrhage. *J Neurosurg* 1988; 69:239-246.
204. Kanamaru K, Weir BKA, Findlay JM, Krueger CA, Cook DA. Pharmacological studies on relaxation of spastic primate cerebral arteries in subarachnoid hemorrhage. *J Neurosurg* 1989; 71:909-915.
205. Hatake K, Wakabayashi I, Kakishita E, Hishida S. Impairment of endothelium-dependent relaxation in human basilar artery after subarachnoid hemorrhage. *Stroke* 1992; 23:1111-1117.
206. Kim P, Lorenz RR, Sundt TM, Vanhoutte PM. Release of endothelium-derived relaxing factor after subarachnoid hemorrhage. *J Neurosurg* 1989; 70:108-114.
207. Kim P, Schini VB, Sundt TM, Vanhoutte PM. Reduced production of cGMP underlies the loss of endothelium-dependent relaxations on the canine basilar artery after subarachnoid hemorrhage. *Circ Res* 1992; 70:248-256.
208. Edwards DH, Byrne JV, Griffith TM. The effect of chronic subarachnoid hemorrhage on basal endothelium-derived relaxing factor activity in intrathecal cerebral arteries. *J Neurosurg* 1992; 76:830-837.
209. Fuxe K, Kurosawa N, Cintra A, et al. Involvement of local ischemia in endothelin-1 induced lesions of the neostriatum of the anesthetized rat. *Exp Brain Res* 1992; 88:131-139.
210. Macrae IM, Robinson MJ, Graham DI, Reid JL, McCulloch J. Endothelin-1 induced reductions in cerebral blood flow: dose dependency, time course, and neuropathological consequences. *J Cereb Blood Flow Metab* 1993; 13:276-284.
211. Sharkey J, Ritchie IM, Kelly PAT. Perivascular microapplication of endothelin-1: a new model of focal cerebral ischaemia in the rat. *J Cereb Blood Flow Metab* 1993; 13:865-871.
212. Yamashita K, Kataoka Y, Niwa M, et al. Increased production of endothelins in the hippocampus of stroke-prone spontaneously hypertensive rats following transient forebrain ischemia: histochemical evidence. *Cell Mol Neurobiol* 1993; 13:15-23.
213. Pelligrino DA. Saying NO to cerebral ischemia. *J Neurosurg Anesthesiol* 1993; 5:221-231.
214. Malinski T, Bailey F, Zhang ZG, Chopp M. Nitric oxide measured by a porphyrinic microsensor in rat brain after transient middle cerebral artery occlusion. *J Cereb Blood Flow Metab* 1993; 13:355-358.
215. Endoh M, Maiese K, Pulsinelli WA, Wagner JA. Reactive astrocytes express NADPH diaphorase in vivo after transient ischemia. *Neurosci Lett* 1993; 154:125-128.
216. Zhang ZG, Chopp M, Zaloga C, Pollock JS, Förstermann U. Cerebral endothelial nitric oxide synthase expression after focal cerebral ischemia in rats. *Stroke* 1993; 24:2016-2022.
217. Kourembanas S, Marsden PA, McQuillan LP, Faller DV. Hypoxia induces endothelin gene expression and secretion in cultured human endothelium. *J Clin Invest* 1991; 88:1054-1057.
218. Ziv I, Fleming G, Djaldetti R, Achiron A, Melamed E, Sokolovsky M. Increased plasma endothelin-1 in acute ischemic stroke. *Stroke* 1992; 23:1014-1016.
219. Färkkilä M, Palo J, Saijonmaa O, Fyhrquist F. Raised plasma endothelin during acute migraine attack. *Cephalalgia* 1992; 12:383-384.
220. Pohl P, Vogl G, Rössler H, Zangerle R, Gerstenbrand F. Single photon emission computed tomography in AIDS dementia complex. *J Nucl Med* 1986; 29:1382-1386.
221. LaFrance ND, Pearlson GD, Schaerf FW, McArthur JC, Pold BF, Links JM, et al. I-123 IMP-SPECT in HIV-related dementia. *Advances in Functional Neuroimaging* 1988; 1:9-15.
222. Mizusawa H, Hirano A, Llena JE, Shintaku M. Cerebrovascular lesions in acquired immune deficiency syndrome (AIDS). *Acta Neuropathol (Berl)* 1988; 76:451-457.
223. Engstrom JW, Lowenstein DH, Bredesen DE. Cerebral infarctions and transient neurological deficits associated with acquired immunodeficiency syndrome. *Am J Med* 1989; 86:528-532.
224. Smith TW, DeGirolami U, Henin D, Bolger F, Hauw J-J. Human immunodeficiency virus (HIV) leukoencephalopathy and the microcirculation. *J Neuropathol Exp Neurol* 1990; 49:357-370.
225. Xiu R-J, Jun C, Berglund O. Microcirculatory disturbance in AIDS patients—a first report. *Microvasc Res* 1991; 42:151-159.
226. Matsumura Y, Ikegawa R, Suzuki Y, et al. Phosphoramidon prevents cerebral vasospasm following subarachnoid hemorrhage in dogs: the relationship to endothelin-1 levels in the cerebrospinal fluid. *Life Sci* 1991; 49:841-848.
227. Yamaura I, Tani E, Maeda Y, Minami N, Shindo H. Endothelin-1 of canine basilar artery in vasospasm. *J Neurosurg* 1992; 76:99-105.
228. Clozel M, Watanabe H. BQ-123, a peptidic endothelin ET<sub>A</sub> receptor antagonist, prevents the early cerebral vasospasm following subarachnoid hemorrhage after intracisternal but not intravenous injection. *Life Sci* 1993; 52:825-834.
229. Nirei H, Hamada K, Shoubo M, Sogabe K, Notsu Y, Ono T. An endothelin ET<sub>A</sub> receptor antagonist, FR139317, ameliorates cerebral vasospasm in dogs. *Life Sci* 1993; 52:1869-1874.
230. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci U S A* 1990; 87:1620-1624.
231. Beckman JS. The double-edged role of nitric oxide in brain function and superoxide-mediated injury. *J Dev Physiol* 1991; 15:53-59.
232. Dawson DA, Kusumoto K, Graham DI, McCulloch J, Macrae IM. Inhibition of nitric oxide synthesis does not reduce infarct volume in a rat model of focal cerebral ischaemia. *Neurosci Lett* 1992; 142:151-154.
233. Yamamoto S, Golanov EV, Berger SB, Reis DJ. Inhibition of nitric oxide synthesis increases focal ischemic infarction in rat. *J Cereb Blood Flow Metab* 1992; 12:717-726.
234. Nowicki JP, Duval D, Pognet H, Scatton B. Nitric oxide mediates neuronal death after focal cerebral ischemia in the mouse. *Eur J Pharmacol* 1991; 204:339-340.
235. Nagafuji T, Matsui T, Koide T, Asano T. Blockade of nitric oxide formation by N<sup>G</sup>-nitro-L-arginine mitigates ischemic brain edema and subsequent cerebral infarction in rats. *Neurosci Lett* 1992; 147:159-162.
236. Buisson A, Plotkine M, Boulu RG. The neuroprotective effect of a nitric oxide inhibitor in a rat model of focal cerebral ischaemia. *Br J Pharmacol* 1992; 106:766-767.