Regulation of the Cerebral Circulation: Role of Endothelium and Potassium Channels

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Faraci, Frank M., and Donald D. Heistad. Regulation of the Cerebral Circulation: Role of Endothelium and Potassium Channels. *Physiol. Rev.* 78: 53–97, 1998.—Several new concepts have emerged in relation to mechanisms that contribute to regulation of the cerebral circulation. This review focuses on some physiological mechanisms of cerebral vasodilatation and alteration of these mechanisms by disease states. One mechanism involves release of vasoactive factors by the endothelium that affect underlying vascular muscle. These factors include endothelium-derived relaxing factor (nitric oxide), prostacyclin, and endothelium-derived hyperpolarizing factor(s). The normal vasodilator influence of endothelium is impaired by some disease states. Under pathophysiological conditions, endothelium may produce potent contracting factors such as endothelin. Another major mechanism of regulation of cerebral vascular tone relates to potassium channels. Activation of potassium channels appears to mediate relaxation of cerebral vessels to diverse stimuli including receptor-mediated agonists, intracellular second messengers, and hypoxia. Endothelial- and potassium channel-based mechanisms are related because several endothelium-derived factors produce relaxation by activation of potassium channels. The influence of potassium channels may be altered by disease states including chronic hypertension, subarachnoid hemorrhage, and diabetes.
I. INTRODUCTION

Vascular tone in the cerebral circulation is regulated by several major mechanisms. One mechanism that has been under intense investigation involves endothelial factors. Endothelium produces and releases potent relaxing and contracting factors that regulate tone of underlying vascular muscle and may also influence vascular growth. A second major area of research involves regulation of cerebral vascular tone by activity of potassium channels. Activation of potassium channels appears to mediate relaxation of cerebral blood vessels in response to a diverse group of important stimuli.

This review summarizes concepts concerning the role of endothelium-derived vasoactive factors and potassium channels in the cerebral circulation. These two mechanisms are related because several endothelium-derived factors produce relaxation by activation of potassium channels. We discuss the functional importance of these mechanisms and, when data are available, review molecular mechanisms that contribute to regulation of cerebral vascular biology. Some abnormalities that occur in cerebral blood vessels under pathophysiological conditions are also reviewed. We focus on these topics because they are areas of intense investigation and because abnormalities in these mechanisms appear to play a key role in brain vascular pathophysiology.

II. DISTINCTIVE CHARACTERISTICS OF CEREBRAL BLOOD VESSELS

There are basic principles of regulation of blood flow that apply in general to all vascular beds. There also, however, are some major differences between cerebral blood vessels and vessels in other organs. We first briefly review some distinctive characteristics of the cerebral circulation.

A. Humoral Stimuli

The endothelial blood-brain barrier limits access of many humoral stimuli to smooth muscle of cerebral blood vessels. It has been assumed that failure of humoral stimuli to alter cerebral blood flow was the result of absence of vasomotor effects of these stimuli on the blood vessels, because the endothelial blood-brain barrier prevents access to vascular muscle. In some cases, however, humoral stimuli can selectively alter resistance of large cerebral arteries, without altering blood flow, because small vessels compensate (presumably via an autoregulatory response) (204). In contrast, humoral stimuli can have major effects on blood flow to regions of the brain such as the choroid plexus, which lack a blood-brain barrier (203, 347, 479). For example, increases in plasma levels of vasopres-sin to concentrations observed under pathophysiological conditions produce marked reductions in blood flow to choroid plexus (203).

Thus the concept that the blood-brain barrier reduces vascular responses to humoral stimuli is sound. A more contemporary view, however, is that some humoral stimuli produce opposing vascular responses in large and small vessels, which result in failure of the stimuli to alter net blood flow. In addition, humoral stimuli may have significant effects on blood flow to circumventricular organs.

B. Neural Stimuli

Cerebral blood vessels have dense innervation from autonomic and sensory fibers. The sources of this innervation include sympathetic nerves (originating predominately in the superior cervical ganglion), parasympathetic fibers (originating primarily in the sphenopalatine, otic, and internal carotid ganglia), and the trigeminal nerve (originating in the trigeminal ganglion) (266). Although perivascular innervation of cerebral vessels is relatively abundant (63), the functional significance of much of this innervation is poorly defined.

Under normal conditions, sympathetic stimulation has little effect on cerebral blood flow, in contrast to far greater effects in other vascular beds (49, 281). Sympathetic stimulation constricts large cerebral arteries, but small vessels downstream dilate (probably an autoregulatory response to a decrease in intravascular pressure), so that blood flow does not decrease (49). Although sympathetic stimuli have little effect under normal conditions, they have profound and physiologically important effects during acute hypertension, because neural stimuli attenuate increases in cerebral blood flow (282). The functional significance of parasympathetic innervation is less clear, although it is known that electrical stimulation of fibers that originate in the sphenopalatine ganglion increase cerebral blood flow (555). Sensory fibers originating in the trigeminal ganglion appear to modulate constrictor responses of cerebral blood vessels (170, 516, 558) and contribute to increase in cerebral blood flow that occur during meningitis, cortical spreading depression, seizures, and reactive hyperemia (134, 557, 699, 798, 807).

C. Metabolic Stimuli

Cerebral circulation, like most other vascular beds (e.g., coronary, mesenteric, and skeletal muscle), but in contrast to some other vascular beds (renal and cutaneous), is characterized by “coupling” of changes in metabolism and blood flow (281, 311). There may be multiple mechanisms by which changes in neuronal activity and metabolism produce a corresponding change in blood
flow. For example, changes in tissue concentration of adenosine, lactate, and tissue Po$_2$, Pco$_2$, and pH may contribute to increases in blood flow during increases in cerebral metabolism. Recent findings strongly support the concept that release of nitric oxide by neurons plays a critical role in producing increases in blood flow during metabolic stimuli (neuronal activation) (183, 190, 191, 193, 195, 312, 315, 530, 596, 640).

D. Hypercapnia and Hypoxia

In some vascular beds (e.g., renal, cutaneous, and skeletal muscle), moderately severe levels of hypercapnia and hypoxia have relatively small effects on blood flow (278). Although hypercapnia and hypoxia have a direct dilator effect in these vascular beds, neurohumoral stimuli (especially the chemoreflex) produce vasoconstriction so that blood flow usually fails to increase (278).

In contrast, hypercapnia and hypoxia are extremely potent vasodilators in the cerebral circulation. The vasoconstrictor response to the chemoreflex is minimal in the cerebral circulation so that the direct vasodilator effects of hypercapnia and hypoxia typically produce large increases in cerebral blood flow. Recent studies suggest that cerebral vasodilation in response to hypercapnia is dependent on formation of nitric oxide (184, 192, 310, 313, 316, 321, 330, 523, 801). Although formation of nitric oxide may contribute to mechanisms that mediate increases in cerebral blood flow during hypoxia (28, 36, 643, 675, 749), additional mechanisms involving formation of adenosine and activation of potassium channels may also be important (28, 226, 412, 675, 676, 711, 750, 811).

E. Autoregulation

Changes in perfusion pressure produce marked changes in cerebrovascular resistance and, therefore, contribute to maintenance of relatively constant levels of blood flow over a wide range of pressures. Autoregulation seems to be particularly effective in brain, renal, and mesenteric vessels and less effective in cutaneous and perhaps coronary vessels (281). Mechanisms that mediate autoregulation of cerebral blood vessels may include myogenic responses, metabolic factors, neural mechanisms, and activation of potassium channels (91, 443, 638).

F. Flow-Mediated Vasodilatation

Large arteries play a surprisingly large role in regulation of vascular resistance in the brain (199). Large arteries play a key role in regulation of the cerebral circulation, a moderate role in the coronary circulation, and little role in mesenteric and skeletal muscle circulation.

Constriction and dilatation of large arteries regulate cerebral vascular resistance and, perhaps most importantly, cerebral microvascular pressure. This effect may be a protective mechanism that attenuates changes in pressure in thin-walled intracranial blood vessels. There is, however, an important "cost" of the large proportion of resistance in large cerebral arteries: the vascular bed is particularly vulnerable to a vascular "steal." This concept has been summarized previously (199).

A major mechanism that likely protects the cerebral circulation against a vascular steal is flow-mediated vasodilatation. The concept is that focal increases in blood flow in one region of the brain produce flow-mediated dilatation of large arteries upstream, and thereby protect against a vascular steal. The concept of flow-mediated vasodilatation has been challenged in other vascular beds (519) but appears to be especially large in magnitude and importance in the cerebral circulation (229).

III. ENDOTHELIUM-DERIVED VASOACTIVE FACTORS

A. Nitric Oxide

1. Nitric oxide synthases

Production and release of potent vasoactive factors by endothelium plays a major role in regulation of vascular tone (Fig. 1). Perhaps, the most important of these substances in relation to regulation of vascular tone is endothelium-derived relaxing factor (EDRF), the labile substance which was initially described by Furchgott and Zawadski (235) in rabbit aorta. Several lines of evidence obtained in subsequent studies have led to the concept that EDRF is nitric oxide (NO) (211, 622) or a closely related compound (429, 485, 566, 684). Although production of NO was first described in vascular endothelium, it is now clear that NO is produced in many cells types by a family of isoenzymes known as NO synthases (81, 224, 225, 547–549, 575, 709).

Nitric oxide is a potent vasodilator that produces relaxation of cerebral blood vessels both in vitro and in vivo (84, 363, 484, 845). After release by endothelium (or other cells), NO stimulates soluble guanylate cyclase in vascular muscle, resulting in an increase in the intracellular concentration of guanosine 3',5'- cyclic monophosphate (cGMP) and relaxation (Fig. 1) (707, 800, 804). Organic nitrovasodilators such as nitroglycerin also produce relaxation of cerebral vascular muscle by activation of guanylate cyclase after biotransformation to NO (5, 454, 800). In addition to this cGMP-dependent mechanism, NO can produce relaxation of some blood vessels from some species via activation of potassium channels (24, 71, 130).

All NO synthase enzymes catalyze a five-electron oxida-
Nitric oxide reacts with superoxide anion at a rate three times faster than the dismutation of superoxide anion by superoxide dismutase (51, 53, 64, 150, 556). Because of the efficiency of this reaction, the local concentration of superoxide dismutase may be an important determinant of activity (the biological half-life) of NO.

Although formation of peroxynitrite (from interaction of NO and superoxide) has the potential to be cytotoxic, the inactivation of superoxide by NO also appears to be protective under some conditions, particularly in vivo if subsequent degradation products are nontoxic (64). Formation of peroxynitrite is not always toxic. For example, under some physiological conditions, peroxynitrite inhibits platelet aggregation and leukocyte adhesion to endothelium (protective effects) with no evidence of cell injury (64, 444). Thus the consequences of interaction of NO and superoxide appear to be dependent in part on factors such as the presence of plasma proteins, reduced thiols, and the ratio of NO to superoxide (64). Formation of peroxynitrite is not always toxic. For example, under some physiological conditions, peroxynitrite inhibits platelet aggregation and leukocyte adhesion to endothelium (protective effects) with no evidence of cell injury (64, 444). Thus the consequences of interaction of NO and superoxide appear to be dependent in part on factors such as the presence of plasma proteins, reduced thiols, and the ratio of NO to superoxide (64). Although peroxynitrite can also cause relaxation of blood vessels, the concentrations of peroxynitrite needed to produce this effect are 50- to 1,000-fold higher than NO (53). Thus peroxynitrite is only modestly effective as a vasodilator. Based on these biochemical characteristics, the concept has emerged that NO is a protective molecule in part because of its ability to react with and inactivate superoxide anion. Relatively little is known regarding the importance of this mechanism in vivo.
Of the three isoforms of superoxide dismutase (Mn-superoxide dismutase, Cu/Zn-superoxide dismutase, and extracellular superoxide dismutase), extracellular superoxide dismutase may be the most important in blood vessels, accounting for up to 70% of total activity of superoxide dismutase in some blood vessels (617, 618, 738). The distribution of extracellular superoxide dismutase in the vessel wall seems ideal for protection from superoxide anion, because NO diffuses from endothelium through vascular muscle (617, 618). Inactivation of NO by superoxide anion may contribute to impaired NO-mediated dilatation of cerebral blood vessels under pathophysiological conditions in which reactive oxygen species are produced. Production of superoxide anion has been reported to occur in brain in response to acute hypertension (814), seizures (30, 47), fluid-percussion injury (359, 410, 765), and perivascular blood (535), as well as during meningitis (446, 520), ischemia with reperfusion (29, 409, 578), and during asphyxia with reventilation (664, 665).

Three isoforms of NO synthase, which are products of separate genes, have been identified. Based on the cell type from which they were initially identified, these three isoforms are frequently described as neuronal, inducible NO synthase (38, 470, 471), suggesting that endothelium is now known that many substances, in addition to acetylcholine, produce endothelium-dependent relaxation of cerebral arteries in vitro that is endothelium dependent (17, 143, 171, 541, 771). Inhibitors of NO synthase decrease basal levels of cGMP and produce contraction of cerebral arteries in vitro that is endothelium dependent (17, 143, 171, 541, 771). Inhibitors of NO synthase also produce constriction of cerebral blood vessels and decrease cerebral blood flow under basal conditions in several species including nonhuman primates (25, 32, 151, 156, 158, 160, 161, 183, 186, 187, 190, 191, 198, 200, 258, 310, 312, 313, 321, 331, 382, 422, 423, 476, 523–526, 620, 642, 673, 687, 708, 746, 754, 764, 768, 783). This same effect has been observed in midgestation, indicating that the influence on NO in the cerebral circulation may begin early in development (403, 595). Reductions in cerebral blood flow in response to L-NNA are absent in mice that are deficient in expression of the gene for endothelial NO synthase (38, 470, 471), suggesting that endothelium is the primary source of NO that influences basal tone.

In contrast to constriction of cerebral blood vessels in response to inhibitors of NO synthase, exogenous administration of L-arginine, the substrate for NO synthase, has been frequently found to have no effect on cerebral vascular tone (194). Even at relatively high concentrations, L-arginine does not affect tone of cerebral arteries or arterioles in vitro (17, 382, 583), cerebral arterioles (32, 46, 187, 673, 815) and the basilar artery in vivo (186, 187, 386, 505), or cerebral blood flow (197, 198, 258, 275, 422). These findings suggest that availability of L-arginine is not rate limiting for activity of NO synthase in cerebral endothelium. This finding is not surprising because the Michaelis constant (half-saturating concentration of L-arginine) value for L-arginine for endothelial NO synthase is ~3 \( \mu \text{M} \) (224, 225), and levels of L-arginine in plasma and endothelium are in the range of 100–2,200 \( \mu \text{M} \) (68, 225, 277, 342, 822). In contrast to these studies, which indicate that L-arginine has no significant effect on tone of cerebral vessels, some studies have reported very small to moderate levels of vasodilatation in brain in response to L-arginine (94, 261, 351, 352, 552, 553, 620, 678, 687).

Activity of NO synthase in endothelium, and NO synthase in neurons (NOS I), is dependent on the presence of calcium (395, 540). Basal activity of NO synthase can be further stimulated by increases in intracellular calcium in response to receptor-mediated agonists (Fig. 1) (337). Lee (442) first demonstrated that acetylcholine produces endothelium-dependent relaxation of cerebral arteries. It is now known that many substances, in addition to acetylcholine, produce endothelium-dependent relaxation of cerebral vessels (188), which is dependent on production of...
NO (194). Relaxation of cerebral vessels in response to acetylcholine (17, 46, 135, 176, 186, 187, 190, 191, 196, 200, 222, 494, 583, 631, 671, 687, 728, 729, 815), bradykinin (254, 363, 494, 541, 614), arginine vasopressin (143, 361, 364, 620, 746), oxytocin (620), substance P (92, 606, 614, 648, 688), histamine (37, 166, 340, 502, 780), sodium fluoride (a G protein activator) (541), endothelin (via activation of endothelin-B receptors) (390, 391, 396, 702), ADP (370, 404, 405, 498), ATP (337), UK-14304 (an α2-adrenoceptor agonist) (87, 88), UTP (539), and prostaglandin F2α (370) are dependent on production of NO. Basic fibroblast growth factor (348, 681) and some opioids (156) also produce NO-dependent dilation of cerebral vessels in vivo that is presumably endothelium dependent. In addition to these receptor-mediated agonists, relaxation of cerebral blood vessels in response to A-23187 (606), increases in shear stress (583), a product released by cultured astrocytes (564), and 4-hydroxynonenal (a product of lipid peroxidation) is dependent on production of NO (487). Recent studies indicate that endothelium-dependent relaxation that is mediated by NO is present in human cerebral arteries (15, 112, 340, 486, 487, 614, 648) as well as in vessels from experimental animals.

A murine model, which is deficient in expression of the endothelial NO synthase gene, has been developed (307, 713). As might be expected, these mice are chronically hypertensive (307, 713) and exhibit impaired endothelium-dependent relaxation in response to acetylcholine in the aorta (307). In contrast, dilatation of cerebral arterioles in response to acetylcholine is normal in endothelial NO synthase mutants (529). After targeted deletion of a specific gene, it is not uncommon for mutant animals to display no or minimal differences in phenotype, suggesting the presence of redundant or compensatory mechanisms (306, 471). Such a compensatory mechanism in cerebral vascular responses probably explains the observation that responses to acetylcholine are normal in cerebral arterioles (529).

A recent study using antisense oligonucleotides suggested that in addition to expression of endothelial NO synthase, endothelium of cerebral vessels may also express neuronal NO synthase (NOS I) (685). The importance of this finding is unclear, however, because other studies (including the use of the relatively selective inhibitor of neuronal NO synthase 7-nitroindazole) suggest that neuronal NO synthase is not expressed in cerebral endothelium or contribute to endothelium-dependent relaxation (195, 287, 315, 390, 453, 801, 841, 847, 860).

An important property of endothelium is that it provides an antithrombogenic surface for blood vessels (Fig. 1). Aggregation of platelets is inhibited normally by luminal release of NO by endothelium (Fig. 1) (462, 547, 686). The antithrombogenic property of endothelium is due in part to synergistic effects of NO with prostacyclin (another relaxing factor produced by endothelium) (462, 829). Nitric oxide also inhibits neutrophil aggregation, adhesion of leukocytes to endothelium, and proliferation of vascular muscle (428, 462, 829). Nitric oxide inhibits expression of endothelial-leukocyte adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1, an endothelial-leukocyte adhesion molecule) (153, 374, 450). This effect on expression of adhesion molecules appears to be mediated by inhibitory effects of NO on the transcription factor nuclear factor κB (95, 374). Nitric oxide may be an important regulator of expression of redox-sensitive genes such as VCAM-1 in endothelium (374). Inhibition of endogenous production of NO in cerebral endothelium increases expression of VCAM-1 (56). Thus, in addition to influencing vascular tone, release of NO protects cerebral endothelium by inhibiting aggregation and adherence of platelets and leukocytes (455).

3. Regulation of endothelial nitric oxide synthase gene expression

Although endothelial NO synthase is frequently referred to as a constitutive isoform of NO synthase, the level of gene expression for NO synthase in endothelium may be increased or decreased in response to several stimuli and under pathophysiological conditions. Gene expression of endothelial NO synthase (NOS III) may be upregulated by increasing shear stress (540, 588, 769, 776), estrogen (293), cGMP (672), basic fibroblast growth factor (420), transforming growth factor-β1 (328), and in the presence of atherosclerosis (356), cirrhosis (551), and pregnancy (827). Immunocytochemistry suggests that expression of endothelial NO synthase increases in cerebral vessels after ischemia (863) and during experimental allergic encephalomyelitis (864). In contrast, expression of endothelial NO synthase may be downregulated by oxidized low-density lipoprotein (452), hypoxia (225, 527, 654, 865), tumor necrosis factor-α, and lipopolysaccharide (via decreased stability of mRNA) (457, 464, 849) and during heart failure (725). Nitric oxide itself may be involved in regulation of levels of gene expression for endothelial NO synthase via a negative-feedback mechanism (677).

B. Prostacyclin

Prostacyclin is a metabolite of arachidonic acid that is produced, with other prostaglandins and thromboxanes, by the two rate-limiting cyclooxygenase (COX) enzymes [also called prostaglandin H (PGH) synthases] (537, 828). Expression of the two isoforms of cyclooxygenase (COX-1 and COX-2) is determined by separate genes (821, 828). Although COX-1 is expressed constitutively (at varying levels) in most cells including endothelium (726) (Fig. 1), regulatory elements in the promoter region suggest that expression of COX-1 can be modulated by several factors including shear stress (828). The promoter for the
gene which encodes COX-1 (like endothelial NO synthase) does not contain a TATA-like element and thus has one characteristic of a housekeeping gene (726).

Cyclooxygenase-2 is an inducible isoform that is expressed primarily in macrophages, fibroblasts, endothelium, and vascular muscle (327, 726, 828). Although it is characterized as an inducible isoform (and is undetectable in most mammalian tissues under normal conditions), COX-2 is expressed constitutively in some organs including brain (726). Many of the same stimuli that result in expression of inducible NO synthase also stimulate expression of COX-2 (458, 537, 821, 828). These stimuli include lipopolysaccharide, adenosine 3',5'-cyclic monophosphate (cAMP), hypoxia, and some cytokines, growth factors, and hormones (80, 327, 706, 828). Expression of COX-2 is involved in inflammation (537, 828) and may be the predominate source of prostaglandins under these conditions (458). Under basal conditions in adult rats, COX-2 is undetectable using in situ hybridization in cerebral vessels (104). In contrast, under normal conditions in newborn pigs, the predominant source of prostaglandins in cerebral vessels appears to be COX-2 (644). Messenger RNA levels for COX-1 and COX-2 increase in brain in response to ischemia (133, 296, 593, 607, 656), after systemic administration of lipopolysaccharide (80), and in response to Borna disease virus (554). Expression of COX-2 is also increased in brain for prolonged periods of time after repeated episodes of cortical spreading depression (99).

Prostacyclin is a powerful inhibitor of platelet aggregation (Fig. 1) (829). In addition, prostacyclin and stable prostacyclin analogs produce relaxation of cerebral arteries in vitro and cerebral arterioles in vivo (25, 178, 226, 371, 612, 613, 688, 766, 779, 799) (Fig. 1). Relaxation of cerebral vessels in response to prostacyclin is generally thought to be endothelium independent (371, 688, 766) but may be mediated by more than one mechanism. Relaxation of cerebral vessels in response to prostacyclin may be mediated by activation of adenylate cyclase with accumulation of cAMP (626, 762) (Fig. 1) and activation of potassium channels (141, 226). In contrast, recent findings in the newborn pig suggest that dilatation of cerebral arterioles in response to prostacyclin is partially dependent on formation of NO (25).

Factors that regulate COX gene expression and production of prostacyclin in cerebral endothelium are not well defined. Several stimuli, including bradykinin, thrombin, and A-23187, increase prostacyclin production in cerebral arteries and cultured cerebral endothelium (513, 550, 610, 834). Endothelium-dependent relaxation of human cerebral arteries in response to 4-hydroxynonenal, and acetylcholine-induced relaxation of the vertebral artery from newborn humans, is inhibited by indomethacin and thus may be mediated by prostacyclin (112, 487).

Expression of COX-2 in cerebral vessels may influence vascular tone. Messenger RNA for COX-2 is expressed in cerebral microvessels in response to lipopolysaccharide (104) and interleukin-1β (105), and interleukin-1 increases production of prostacyclin in cerebral endothelium (513). Interleukin-1 produces dilatation of cerebral arterioles in newborn pigs that is inhibited by indomethacin (715) and presumably mediated by activation of COX-2.

C. Endothelium-Derived Hyperpolarizing Factor

In addition to production and release of NO and prostacyclin, endothelium may also produce relaxation of underlying vascular muscle by release of endothelium-derived hyperpolarizing factor(s) (EDHF) (55, 130, 241, 574) (Fig. 1). In large cerebral arteries, for example, acetylcholine, substance P, and bradykinin produce endothelium-dependent hyperpolarization and relaxation of vascular muscle (77, 142, 546, 590, 648, 740), which appears to be mediated, in part, by an EDHF. Although EDHF is more difficult to bioassay than EDRF, recent evidence indicates that EDHF is a diffusible (transferable) factor that causes relaxation by hyperpolarizing underlying vascular muscle (118, 130, 546, 662). The importance of EDHF as a mediator of endothelium-dependent relaxation has been reported to increase as vessel size decreases (216, 718). For example, expression of endothelial NO synthase and the importance of NO have been reported to decrease, and the importance of EDHF increase, as vessels become smaller in the mesenteric circulation (718).

The identity of EDHF remains a subject of debate and investigation. In some extracranial arteries, NO (sometimes only at relatively high concentrations) produces hyperpolarization of vascular muscle and thus may function as an EDHF (130). Nitric oxide and donors of NO like 3-morpholinosydnonimine (SIN-1) produce marked relaxation but have little or no effect on membrane potential in large cerebral arteries (77, 563, 658, 659). The absence of hyperpolarization suggests that NO is not an EDHF in these blood vessels. In contrast, NO produces glibenclamide-sensitive hyperpolarization of the guinea pig carotid artery, suggesting that the response is mediated by ATP-sensitive potassium channels (141). Because relaxation of cerebral blood vessels in response to prostacyclin is antagonized by inhibitors of potassium channels in some cerebral blood vessels (41, 141, 226), prostacyclin may also act as an EDHF in the cerebral circulation.

In many arteries, however, it seems clear that EDHF is not NO or a prostanoid, because inhibitors of NO synthase and COX do not attenuate endothelium-dependent hyperpolarization or relaxation of vascular muscle (118, 130, 142, 574). A substantial body of evidence, obtained primarily from studies of coronary blood vessels, suggests that an EDHF is a product of cytochrome P-450 monoxygenase.
genase metabolism of arachidonic acid (44, 102, 116, 267, 276, 373, 662). The products of arachidonate that mediate this effect appear to be epoxyeicosatrienoic acids (102, 267). Epoxyeicosatrienoic acids such as 11,12-epoxyeicosatrienoic acid produce hyperpolarization and relaxation of coronary arteries in vitro (102).

Epoxyeicosatrienoic acids are produced in brain (19, 177, 244, 267) and by astrocytes (16), and some epoxyeicosatrienoic acids produce relaxation of cerebral blood vessels (19, 177, 244). For example, both 5,6-epoxyeicosatrienoic acid and 11,12-epoxyeicosatrienoic acid produce relaxation of the middle cerebral artery in vitro (244). Consistent with a possible function as EDHFs, 11,12-epoxyeicosatrienoic acid produces relaxation of the middle cerebral artery, which is inhibited by a high concentration of tetraethylammonium (TEA) ion (244), and 14,15-epoxyeicosatrienoic acid enhances an outward potassium current in smooth muscle isolated from cerebral microvessels (16). In contrast, 5,6-epoxyeicosatrienoic acid (but not 11,12-epoxyeicosatrienoic acid) is a potent dilator of cerebral arterioles in vivo (19, 177). Interestingly, effects of 5,6-epoxyeicosatrienoic acid on cerebral arterioles were inhibited by indomethacin or by superoxide dismutase plus catalase (177), suggesting 5,6-epoxyeicosatrienoic acid does not directly produce relaxation in cerebral arterioles. It is known that 5,6-epoxyeicosatrienoic acid can be metabolized by COX, resulting in formation of reactive oxygen species that mediate vasodilatation (177).

Although it seems clear that some epoxyeicosatrienoic acids produce relaxation of cerebral blood vessels, little is known whether these substances are produced by cerebral endothelium and function as EDHFs in the cerebral microcirculation. A recent study suggests that release of cytochrome P-450 products by endothelium contributes to dilatation of cerebral microvessels during hypoxia in newborn pigs (445). In the carotid artery, acetylcholine causes release of an EDHF that may be a product of cytochrome P-450 monooxygenase metabolism (44, 456). Interestingly, bioassay studies suggest that NO inhibits the formation and/or release of EDHF in this artery (45). The implication of this finding is that when activity of NO is reduced, such as under pathophysiological conditions, synthesis or release of EDHF may increase as a compensatory mechanism. In contrast to these findings, endothelium-dependent hyperpolarization in the carotid artery of the guinea pig does not appear to be a product of cytochrome P-450 monooxygenase, COX, or lipoxigenase and thus may not be a product of arachidonic acid metabolism (142).

Studies of coronary blood vessels that have implicated cytochrome P-450 monooxygenase metabolites as potential EDHFs have frequently used arachidonic acid as a stimulus to produce endothelium-dependent relaxation. In contrast to coronary arteries (102), dilatation of cerebral arterioles in several species in response to arachidonic acid is mediated by COX-dependent generation of reactive oxygen species. Cerebral microvascular responses to arachidonate are inhibited by indomethacin (90, 93, 177, 416, 810, 845) and by scavengers of reactive oxygen species (177, 209, 415, 417, 844). Thus arachidonic acid does not appear to dilate cerebral arterioles by generation of P-450 metabolites unless these metabolites subsequently act as substrate for COX as described for 5,6-epoxyeicosatrienoic acid (177).

Hyperpolarization of vascular muscle in response to EDHF is most likely mediated by activation of potassium channels (102, 130, 241, 546, 662) (Fig. 1). For example, relaxation of cerebral vessels in response to acetylcholine appears to be mediated, in part, by production of an EDHF that activates ATP-sensitive potassium channels (77, 192, 740). Activation of calcium-dependent potassium channels may also mediate EDHF-induced relaxation in some arteries (117, 118, 130, 662), including the canine carotid artery (546). Charybdotoxin, an inhibitor of calcium-dependent potassium channels, produces partial inhibition of relaxation of the middle cerebral artery in response to acetylcholine (817). Endothelium-dependent hyperpolarization, which is not mediated by NO, has been described in human cerebral arteries in response to substance P (648).

The role of cytochrome P-450 monooxygenase metabolites as possible EDHFs has been supported, in part, by the use of cytochrome P-450 inhibitors. Although there is evidence that these inhibitors abolish release of EDHF from endothelium (662), high concentrations of these compounds may also exert nonspecific effects (173, 216). For example, some inhibitors of P-450 including clotrimazole have been reported to directly inhibit activation of potassium channels (independent of effects on activity of P-450 enzymes) (173, 871). Thus interpretation of results obtained with these agents may need to be made with caution (173).

In contrast to studies that implicate a role for EDHFs in regulation of vascular tone, several studies suggest that activation of potassium channels does not contribute to relaxation of cerebral vessels in response to endothelium-dependent vasodilators in vitro and in vivo (17, 78, 200, 631, 751, 817). Thus the overall functional importance of EDHF in cerebral vessels is not clear.

D. Endothelin

In addition to relaxing factors, endothelin can release substances that produce contraction of blood vessels. The endothelium-derived contracting factor (EDCF) that has received the most investigation is endothelin, a peptide originally isolated from aortic endothelium (840) (Fig. 3). There are three isopeptides of endothelin, all 21 amino acids, that are the products of separate genes
Endothelin-B receptors are expressed in smooth muscle in some blood vessels and mediate contraction (Fig. 3). In contrast, activation of ETB receptors (which are commonly expressed in endothelium) produces relaxation of blood vessels through release of prostacyclin or EDRF (257, 290, 692, 774) (Fig. 3). Some studies suggest there may be two distinct subtypes of ETB receptors (receptors that produce vasorelaxation or vasoconstriction) (806).

Endothelin-1 produces potent and long-lasting contraction of cerebral vessels both in vivo and in vitro (1, 2, 14, 31, 152, 185, 212, 217, 219, 265, 338, 390, 600, 625, 632, 680, 697, 698, 701, 757, 820). Endothelin-1 and ET-2 are much more potent than ET-3 in producing contraction (698, 701). Vasoconstriction in response to endothelin is dependent on extracellular calcium (152, 217, 219, 265, 338) and may be mediated by activation of protein kinase C (219, 565).

Endothelin-A receptors are expressed normally in cerebral blood vessels (286, 302, 586, 850), and the predominant mechanism of vasoconstriction in response to endothelin in these vessels is by activation of ETA receptors (1, 2, 154, 212, 217, 350, 359, 390, 391, 586, 632, 647, 695, 701, 820, 869). In contrast to this contractile response, low concentrations of endothelin produce dilatation of cerebral arterioles in vivo that may be mediated by activation of NO synthase and production of NO. NO and cGMP inhibit ET-1 gene expression.

[endothelin (ET)-1, ET-2, and ET-3] (692). All endothelins are synthesized as larger preproforms (~200 amino acids), which are converted by endopeptidases to preproptides (also known as big endothelins). For example, prepro-ET-1 is cleaved to big ET-1, which is then converted to ET-1 by endothelin-converting enzyme (255, 692) (Fig. 3). Endothelin-converting enzymes (ECE-1 and ECE-2) are metalloproteases that are associated with the membrane fraction of cells (255, 257, 785, 803).

Cerebral endothelium can produce endothelin under some conditions. Of the three isopeptides, only ET-1 is produced normally by endothelium (692), and mRNA for ET-1 can be detected in cerebral endothelium (848). Messenger RNA for ECE-1 has also been detected in cerebral endothelium using in situ hybridization (833). Endothelin gene expression can be enhanced by several factors including thrombin, transforming growth factor-β, hemoglobin, and tumor necrosis factor-α (181, 605, 692) and can be inhibited by NO and cGMP (76, 165, 255, 692). A shear stress response element is present in the promoter of the ET-1 gene, but in contrast to the gene for endothelial NO synthase, activation of this element decreases transcription of the ET-1 gene (692).

Endothelins are known to mediate effects in blood vessels through activation of two receptors, endothelin-A (ETA) and endothelin-B (ETB) receptors, which have been cloned (23, 255, 700). In general, ETA receptors are expressed in vascular muscle and mediate contraction to endothelin (257) (Fig. 3).

The response to activation of ETB receptors on vascular tone depends on localization of the receptor. Endothelins-1, 2, and 3 are synthesized as larger preproforms (~200 amino acids), which are converted by endopeptidases to preproptides (also known as big endothelins). For example, prepro-ET-1 is cleaved to big ET-1, which is then converted to ET-1 by endothelin-converting enzyme (255, 692) (Fig. 3). Endothelin-converting enzymes (ECE-1 and ECE-2) are metalloproteases that are associated with the membrane fraction of cells (255, 257, 785, 803).

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E. Other Endothelium-Derived Vasoactive Factors

1. Reactive oxygen species

In addition to the factors described above, cerebral endothelium may produce several additional vasoactive substances including reactive oxygen species, carbon monoxide, and nonendothelin EDCFs. For example, although bradykinin produces NO-dependent relaxation of the basilar artery and the middle cerebral artery (254, 363, 417, 494, 541, 614), another mechanism mediates bradykinin-induced dilatation of pial arterioles that supply the cerebrum. In these microvessels, dilatation in response to bradykinin is endothelium dependent but mediated by reactive oxygen species (either hydroxyl radical or hydrogen peroxide depending on the species) (414, 415, 683, 844). Reactive oxygen species are dilators in the cerebral microcirculation of several species (409, 413, 417, 682,
778, 809, 812, 844, 845). As discussed in section vB, dilatation of cerebral microvessels in response to bradykinin appears to be mediated by activation of potassium channels (208).

2. Carbon monoxide

Recent evidence suggests that carbon monoxide might also function as an EDRF in some blood vessels (853). Carbon monoxide is produced from heme by two isoforms of heme oxygenase (HO-1 and HO-2) (478), and HO-2 is expressed constitutively in endothelium of cerebral arteries (853). The functional significance of carbon monoxide as a regulator of tone in cerebral vessels is unclear however. Carbon monoxide produces relaxation of some blood vessels, including the aorta, but does not produce direct relaxation of cerebral arteries (84). Messenger RNA for HO-1, the inducible form of heme oxygenase, is expressed in cerebral endothelium in response to hemin (a hemoglobin degradation product), but expression is not associated with increased levels of cGMP (790), which provides additional evidence that carbon monoxide may not function as a direct dilator in cerebral blood vessels. Although carbon monoxide may not directly relax cerebral vessels, recent evidence obtained in neurons suggests that carbon monoxide may modulate formation of cGMP in response to NO (326). Thus carbon monoxide may influence cerebral vascular tone indirectly via effects on production of cGMP in smooth muscle in response to NO.

3. Other endothelium-derived constricting factors

In some cerebral arteries such as the canine basilar artery, and in the presence of some pathophysiological conditions, contracting substances that are not endothelin may be produced by cerebral endothelium. These EDCFs are primarily metabolites of arachidonic acid produced through the COX pathway (366), which produce contraction by activation of PGH₂-thromboxane A₂ receptors (144) (Fig. 4). Superoxide anion, which is also produced via the COX pathway, produces contraction of the canine basilar artery, and thus may be an EDCF under some conditions (144), although recent evidence suggests contraction to superoxide anion is due to inhibition of basal effects of NO (362) (Fig. 4). Endothelium-dependent contraction may occur in response to A₂3187 (144, 367, 369, 720), xanthine plus xanthine oxidase (which generates reactive oxygen species) (365), hydrogen peroxide (365), acetylcholine (344, 367, 379, 780), arachidonic acid (379, 780), anoxia (368), angiotensins (480), nicotine (719), and phospholipase A₂ (571).

IV. INDUCIBLE NITRIC OXIDE SYNTHASE

In contrast to endothelial NO synthase (NOS III), which is expressed constitutively, an inducible or "immunologic" isoform of NO synthase (NOS II) can be expressed in many cell types including vascular muscle and endothelium (113, 245, 335, 357, 358, 426, 434, 477, 548). Inducible NO synthase is the product of a separate gene (155, 225, 245, 395, 426, 597). Messenger RNA for inducible NO synthase is typically not present or is present in very low levels in normal cells under basal conditions (225, 556, 576). In response to inflammatory factors and other stimuli, expression of inducible NO synthase occurs over a period of hours (225, 426, 548, 556) (Fig. 5). Thus activity
of inducible NO synthase is regulated primarily at the
level of gene expression (548, 556, 575, 598).

Inducible NO synthase produces much greater amounts of NO than the constitutive forms of NO synthase (endothelial and neuronal NO synthase) (395, 426, 548). Because high concentrations of NO can be toxic to cells, either alone or in combination with superoxide ion that produces peroxynitrite (53) (Fig. 2), inducible NO synthase is often referred to as the “pathological” form of NO synthase (426, 548, 576, 707). Once formed from NO and superoxide, peroxynitrite may be protonated to yield hydroxyl radical, which is extremely reactive (51, 53, 120, 155). Thus, in addition to its direct effects, formation of NO may contribute to formation of toxic reactive species such as hydroxyl radical (Fig. 2).

Stimuli that cause expression of inducible NO synthase and have received the most study are lipopolysaccharide (endotoxin) and some cytokines. Specific cytokines that cause expression of inducible NO synthase vary among species and between cell types but include interleukin-1β, interferon-γ, and tumor necrosis factor-α (225, 548, 575) (Fig. 5). Adenosine 3′,5′-cyclic monophosphate enhances the expression of inducible NO synthase in response to cytokines in several types of cells including vascular muscle (324, 406). S100β, a neurotrophic protein derived from glia, also causes expression of inducible NO synthase in cultured astrocytes (303).

The promoter of the gene for human inducible NO synthase contains a shear-stress response element (113, 225), which suggests that expression of this gene in endothelium might be modulated by blood flow. The 5′-flanking region of the inducible NO synthase gene also contains a hypoxia-responsive element, which suggests that inducible NO synthase is a hypoxia-inducible gene (528). Stimuli that induce expression of inducible NO synthase also induce expression of GTP-cyclohydrolase I, the rate-limiting enzyme in biosynthesis of tetrahydrobiopterin (491). Tetrahydrobiopterin is one of the cofactors required for enzyme activity (225, 395). Lipopolysaccharide also induces mRNA for arginase II (256). Activity of arginase II may play a major role in production of NO by influencing the availability of L-arginine, the substrate for NO synthases.

It is noteworthy that mRNA for interleukin-1β converting enzyme and other genes that encode the interleukin-1 system appear to be expressed constitutively in cerebral microvessels (825). Expression of this “vascular interleukin-1 system” may contribute to regulation of expression of inducible NO synthase and COX-2.

Expression of inducible NO synthase can be inhibited by several factors including interleukin-4, interleukin-10, transforming growth factor-β, basic fibroblast growth factor, aldosterone, heat shock protein 70, and insulin-like growth factor (50, 131, 213, 225, 323, 645, 704) (Fig. 5). Preliminary experiments in mice that lack gene expression for interleukin-10 suggest that endogenous interleukin-10 is a major regulator of expression of inducible NO synthase (206). Inhibitors of tyrosine kinase and glucocorticoids also block expression of inducible NO synthase (225, 575, 670). Expression of the inducible NO synthase in vascular muscle is dependent on binding of nuclear factor κB to the promoter (737) and can be blocked by inhibition of activation of nuclear factor κB (737, 831). As described previously for endothelial NO synthase, NO may modulate the level of gene expression for inducible NO synthase via a negative-feedback mechanism (132, 628, 629). For example, NO inhibits expression of inducible NO synthase in human microglia through a mechanism that may involve decreased availability of nuclear factor κB (132).

With the use of cells in culture, several lines of evidence indicate that glia (110, 140, 214, 215, 228, 232, 236, 237, 284, 285, 294, 407, 440, 441, 544, 545, 589), cerebral endothelium (57–59, 375, 561, 562, 616), cerebral vascular muscle (181, 741), and neurons (533, 534, 601) can be stimulated to express inducible NO synthase. Evidence of expression of inducible NO synthase in these cells includes analysis of mRNA for inducible NO synthase, Western blotting for protein, measurement of activity of inducible NO synthase (calcium-independent formation of L-citrulline), and measurement of nitrite (a breakdown product of NO). Expression of inducible NO synthase occurs in brain in situ in response to ischemia (180, 314, 317, 320, 322, 855). Inducible NO synthase can be detected in the presence of other pathophysiological conditions including meningitis (101), multiple sclerosis (39, 65), immunodeficiency viral and experimental allergic encephalitis (89, 301, 418, 435, 609), herpes simplex virus (418), Borna disease virus (301, 418, 554), encephalomyelitis (784), and rabies virus (301, 418). Inducible NO synthase is expressed in cerebral microvessels in Alzheimer’s disease (162), after ischemia (317, 567, 568), and in brain tumors (175, 264) and endothelium of vessels supplying brain tumors (127). Protein for inducible NO synthase is present in cerebral vascular muscle and infiltrating leukocytes after traumatic brain injury (124). mRNA for inducible NO synthase can be detected in meninges and choroid plexus after systemic administration of lipopolysaccharide (826). Recent studies suggest that NO may modulate permeability of the blood-brain barrier (504), and expression of inducible NO synthase may contribute to increased permeability of the blood-brain barrier after exposure to lipopolysaccharide (69, 721).

Although expression of inducible NO synthase has generally been found to be associated with inflammatory or pathophysiological conditions, it appears that the gene may be active during development. For example, mRNA and protein for inducible NO synthase have been detected in parenchymal microvessels in brain during normal embryonic development and in newborn rats (238). Inducible NO synthase is not
found in vessels in immature or adult animals under normal conditions (124, 238), and the significance of vascular expression of this isoform of NO synthase during early life is not clear. Because NO can influence vascular growth, we speculate that expression of inducible NO synthase early in ontogeny may contribute to vascular remodeling.

Recent evidence suggests that lipopolysaccharide and tumor necrosis factor-α cause expression of inducible NO synthase that affects cerebral vascular tone in vivo (83, 85, 86, 561, 593). Lipopolysaccharide causes marked, progressive dilatation of cerebral arterioles and increases in levels of cGMP in perivascular cerebrospinal fluid that are attenuated by inhibitors of NO synthase, including L-NMMA and aminoguanidine (85, 86). Aminoguanidine appears to be a relatively selective inhibitor of inducible NO synthase (536, 736, 824) and, at appropriate concentrations, does not inhibit cerebral vascular responses that are mediated by endothelial NO synthase (86). Increases in arteriolar diameter in response to these stimuli are also attenuated by dexamethasone (85), which inhibits expression of the inducible NO synthase gene (225, 670). In addition to these findings in cerebral arterioles, lipopolysaccharide causes similar, NO-dependent, dilatation of the basilar artery (755).

V. POTASSIUM CHANNELS

Changes in activity of potassium channels represent a major mechanism that regulates vascular tone. Activation of potassium channels in vascular muscle produces hyperpolarization of the cell membrane, closure of voltage-dependent calcium channels, a decrease in intracellular calcium, and vascular relaxation (387, 579, 582) (Fig. 6). The membrane potential of cerebral vascular muscle measured in vitro has ranged widely from approximately −40 to −70 mV (77, 78, 240, 268, 338, 431, 522, 590, 648, 658, 659, 696, 867). The membrane potential of cerebral vascular muscle in vivo is not known. Changes in membrane potential of only a few millivolts are associated with substantial changes in vascular tone (394, 431, 582, 657). Activation of potassium channels mediates cerebral vascular response to several stimuli including some receptor-mediated agonists, second messengers, and hypoxia (207). Endothelium-derived hyperpolarizing factor produces hyperpolarization and relaxation of vascular muscle by activation of potassium channels (241, 387) (Figs. 6 and 7).

Both electrophysiological and pharmacologically based studies have provided evidence that several types of potassium channels are present in cerebral blood vessels (387, 582). Although the majority of studies have focused on potassium channels in vascular muscle, potassium channels can also be expressed in endothelium (115, 431, 465, 466). In endothelium, membrane hyperpolarization causes mobilization of calcium and may be an important
mechanism for release of NO, prostacyclin, and EDHF (241, 431, 465).

A. ATP-Sensitive Potassium Channels

Adenosine 5′-triphosphate-sensitive potassium channels have been described in vascular muscle, including cerebral vessels (392, 582, 740). These potassium channels are defined based on sensitivity to intracellular ATP, which inhibits activity of the channel (582) (Fig. 6). Dissociation of ATP from the channel results in channel opening and hyperpolarization. In contrast to intracellular ATP, other factors including reductions in Po2 or pH open the channels and produce vasorelaxation (579, 582). These properties support the concept that activity of ATP-sensitive potassium channels may, in part, reflect the metabolic state of cells (579).

Electrophysiological and pharmacological studies of cerebral vascular muscle indicate that ATP-sensitive potassium channels are activated by synthetic compounds including cromakalim (or levcromakalim) and aprikalim (392, 569, 740) (Fig. 6). Activity of these channels is inhibited by sulfonylureas including glibenclamide (392, 582, 657, 740) (Fig. 6). Glibenclamide appears to be a selective inhibitor of ATP-sensitive potassium channels at the most commonly used concentrations (<3 μM) and has been used frequently to examine the role of ATP-sensitive potassium channels in intact cerebral vessels.

Activators of ATP-sensitive potassium channels produce hyperpolarization and relaxation of cerebral arteries (205, 392, 427, 489, 522, 569, 570, 630, 639, 658, 703, 749, 758, 817, 861), including human arteries (282, 648) in vitro. These activators of ATP-sensitive potassium channels also cause dilatation of the basilar artery (200, 389, 499, 569, 729, 732, 770) and cerebral arterioles in vivo (26, 40, 41, 201, 297, 332, 412, 443, 445, 507, 711, 750, 751, 770, 796, 797). Intraparenchymal injection of cromakalim produces a glibenclamide-sensitive increase in local cerebral blood flow (675). Thus ATP-sensitive potassium channels are present and functional in the cerebral circulation. Some evidence suggests that the distribution of ATP-sensitive potassium channels in cerebral vessels is heterogeneous and varies with vessel size and brain region (522, 569).

Several endogenous substances produce hyperpolarization and relaxation of cerebral vascular muscle that appears to be mediated by activation of ATP-sensitive potassium channels. These substances include EDHF (77), vasoactive intestinal polypeptide (740), calcitonin gene-related peptide (CGRP) (297, 298, 339, 388, 392, 463, 581, 696, 740), adrenomedullin (436), prostacyclin (41, 226), opioids (711), noradrenaline (via β-adrenergic receptors) (385), cAMP (385), and adenosine (28, 392, 569).

Although NO may produce relaxation of some vessels via activation of potassium channels, most studies suggest that NO does not activate ATP-sensitive potassium channels in cerebral vessels. Dilatation of the basilar artery and cerebral arterioles in response to nitrovasodilators (192, 200, 332, 412, 750) and relaxation of the carotid artery in response to acetylcholine and sodium nitroprusside (205) are not inhibited by glibenclamide. In newborn pigs, glibenclamide has been reported to have no effect (40, 445) and to inhibit cerebral vasodilatation in response to nitroprusside (26).

Although it is well known that hypoxia produces marked increases in cerebral blood flow, mechanisms that mediate this response have been elusive. Several recent studies suggest that activation of ATP-sensitive potassium channels is an important mechanism that contributes to cerebral vasodilation during hypoxia (Fig. 6). Relaxation of the carotid artery (749), large cerebral arteries (226), and cerebral arterioles (711, 750) in response to hypoxia is inhibited by glibenclamide. Increases in cerebral blood flow during hypoxia are attenuated by glibenclamide and tolbutamide, another inhibitor of ATP-sensitive potassium channels (675, 676). Activation of ATP-sensitive potassium channels is also one of several mechanisms that may contribute to autoregulatory responses of cerebral arterioles during hypotension (443) and relaxation of cerebral arteries in response to acidosis (383).

Although activation of ATP-sensitive potassium channels appears to be an important mechanism during cerebral vasodilatation in response to several stimuli, these channels do not appear to influence resting tone in the cerebral circulation. In both the basilar artery (200, 385, 388, 732) and cerebral arterioles (26, 28, 40, 41, 192, 298, 332, 412, 436, 445, 463, 507, 711, 797) in vivo, application of glibenclamide has no effect on vessel tone. Intraparenchymal tolbutamide has no effect on local cerebral blood flow (675).

Some progress has been made recently regarding the molecular biology of ATP-sensitive potassium channels (739). The channel is a heteromultimer composed of pore-forming subunits (typically of inward-rectifying potassium channels) and sulfonylurea receptor subunits (667). The sulfonylurea receptor (which in itself cannot form a channel; Ref. 18) has been cloned (4). Subsequent cloning of ion-permeable subunits (739) has allowed coexpression of the sulfonylurea receptor with an inward-rectifying subunit to form a channel that exhibits many properties of native ATP-sensitive potassium channels (259). It is also interesting to note that the sulfonylurea receptor can couple to different types of inwardly rectifying potassium channels (18). This characteristic may confer sensitivity to glibenclamide in potassium channels that are not ATP sensitive (18). Thus the presence of glibenclamide sensitivity does not necessarily indicate the presence of ATP-sensitive potassium channels (667). To what extent this “promiscuous” coupling between the sulfonylurea receptor and inward rectifier potassium channels occurs in...
blood vessels is not clear. In coronary vascular muscle, the sulfonylurea receptor appears to be very predominantly associated with the appropriate subunit or subunits to form ATP-sensitive potassium channels (818). In cerebral arteries, it is noteworthy that a high concentration of glibenclamide (10 μM) has been reported to have no effect on currents carried by inward-rectifier potassium channels (668).

**B. Calcium-Dependent Potassium Channels**

Calcium-activated potassium channels have been described in vascular muscle, including muscle from cerebral vessels (79, 247, 295, 579, 582, 734). These potassium channels are defined based on their activation by increases in the concentration of intracellular calcium (Fig. 7). Activity of calcium-activated potassium channels increases with membrane depolarization (79, 247, 580, 582) and can be affected by other vasoactive stimuli.

Calcium-activated potassium channels consist of α- and β-subunits (346). The α-subunit is a member of the slowpoke (slo) potassium channel gene family. The α-subunit forms the ion conduction pore of the channel (346), and this subunit has been cloned from vascular muscle in humans (hslo) (514). A β-subunit of calcium-dependent potassium channels has also been cloned and is expressed at high levels in aorta (773). This β-subunit is a membrane-spanning protein that appears to act as a regulatory component of the channel (346). Coexpression of the hslo gene cloned from vascular muscle with the β-subunit in *Xenopus* oocytes results in a channel that exhibits many of the properties of calcium-activated potassium channels (calcium sensitivity; inhibition by TEA ion and iberiotoxin) (514). Little is known at present regarding factors that regulate expression of genes that encode α- and β-subunits of calcium-activated potassium channels in vascular muscle.

In comparison with activators of ATP-sensitive potassium channels, development of selective pharmacological activators of calcium-dependent potassium channels has been slow. NS-1619 produces relaxation of cerebral arteries that appears to involve opening of calcium-dependent potassium channels in vascular muscle (295). However, this compound has other vasoactive effects including inhibition of calcium channels (295, 439). Activity of calcium-activated potassium channels can be inhibited with TEA ion, charybotoxin, and iberiotoxin (247, 387, 582) (Fig. 7). Of these inhibitors, iberiotoxin appears to be highly selective, with no known nonspecific effects (387, 582).

In cerebral arteries, application of inhibitors of calcium-activated potassium channels produces contraction in vitro (34, 35, 79, 250) and in vivo (229), suggesting that these channels are active under basal conditions. In cerebral arterioles, inhibitors of calcium-dependent potassium channels have no effect on vessel diameter in vivo (27, 298, 436, 636, 751, 813) or produce very modest vasorelaxation (751). Thus the influence of calcium-dependent potassium channels on basal tone may be more important in large cerebral arteries than in cerebral arterioles.

In addition to influencing basal tone of large cerebral arteries, activation of calcium-dependent potassium channels appears to be an important mediator of vasodilatation in response to several stimuli. Dilatation of cerebral arterioles in response to forskolin (a direct activator of adenylate cyclase), cAMP, isoproterenol (a β-adrenergic agonist that activates adenylate cyclase), CGRP, and adrenomedullin is attenuated by inhibitors of calcium-dependent potassium channels (27, 298, 436, 507, 636, 751). Isoproterenol, forskolin, and cAMP all increase activity of calcium-dependent potassium channels in cerebral vascular muscle, as demonstrated with patch-clamp methodology (734). Thus adenylate cyclase-mediated activation of calcium-dependent potassium channels may be an important mechanism of vasodilatation (Fig. 7). This mechanism may play a major role in regulation of cerebral vascular tone, since cAMP in vascular muscle increases in response to a variety of stimuli including adenosine, prostacyclin, CGRP, vasoactive intestinal polypeptide, pituitary adenylate cyclase activating peptide, and adrenomedullin (22, 168, 169, 174, 304, 339, 398, 572, 585) (Fig. 7). In addition to these stimuli, relaxation of the middle cerebral artery to hypoxia and 11,12-epoxyeicosatrienoic acid, a product of P-450 metabolism of arachidonic acid in brain, is inhibited by TEA ion and thus may be mediated by activation of calcium-dependent potassium channels (243, 244).

It is important to note that NO may activate calcium-dependent potassium channels in some (24, 130, 679, 846), but not all (514), blood vessels (Fig. 7). The functional importance of this mechanism seems to vary with vessel size, species, and tissue (130). In the rat, dilatation of cerebral arterioles in response to nitroprusside and 8-bromo-cGMP (a cGMP analog) is inhibited by TEA ion and iberiotoxin (636). Thus activation of calcium-dependent potassium channels may be an important mediator of relaxation in response to NO and cGMP in cerebral microvessels (636) (Fig. 7).

In contrast, inhibitors of calcium-dependent potassium channels do not attenuate relaxation of rabbit cerebral arterioles (751) or large cerebral arteries from the rabbit and rat (732, 817) in response to nitrovasodilators or acetylcholine (which acts through release of EDRF). These findings are consistent with the observation that NO and SIN-1 do not hyperpolarize vascular muscle in large cerebral arteries (77, 563, 658, 659). One interpretation of these findings is that NO does not activate calcium-dependent potassium channels in some cerebral blood vessels. An alternative interpretation is that NO activates these potassium channels in cerebral vessels, but that NO
that is released under basal conditions activates calcium-dependent potassium channels. Under these conditions, additional NO (applied exogenously or formed endogenously) may not produce further activation of potassium channels. The finding that nitrovasodilators produce membrane hyperpolarization in the endothelium-denuded basilar artery (563) is consistent with this possibility.

Recent electrophysiological studies suggest that reactive oxygen species may relax blood vessels via activation of potassium channels. For example, hydrogen peroxide and oxidizing agents such as 5,5′-dithio-bis(2-nitrobenzoic acid) and oxidized glutathione increase activity of calcium-dependent potassium channels in vascular muscle (425, 627). Dilatation of cerebral arterioles in response to hydrogen peroxide and bradykinin is attenuated by TEA and iberiotoxin (208), suggesting that the response is mediated by activation of calcium-dependent potassium channels. Dilatation of cerebral arterioles in response to bradykinin is mediated by reactive oxygen species (either hydroxyl radical or hydrogen peroxide depending on the species) (414, 415, 683, 844). Cerebral vasodilatation in response to hydrogen peroxide and peroxynitrite may be inhibited by glibenclamide, and thus mediated by ATP-sensitive potassium channels, in some species (813). Thus activation of potassium channels appears to be a major mechanism of relaxation of cerebral vessels in response to reactive oxygen species.

Some endogenously produced substances may influence membrane potential of vascular muscle and produce constriction of cerebral vessel by inhibitory effects on calcium-dependent potassium channels. For example, cerebral microvessels can produce 20-hydroxyeicosatetraenoic acid from arachidonic acid via a P-450 enzyme (269). 20-Hydroxyeicosatetraenoic acid is a potent vasoconstrictor that may produce this effect, at least in part, by inhibition of activity of calcium-dependent potassium channels in cerebral vascular muscle (269).

### C. Voltage-Dependent Potassium Channels

Voltage-dependent potassium channels (also known as delayed rectifier potassium channels) have been described in cerebral vascular muscle (72, 393, 582). Like calcium-dependent potassium channels, the open probability of voltage-dependent potassium channels increases with membrane depolarization (582). When cells are depolarized, these potassium channels are activated, resulting in an outward current that returns the membrane potential toward the resting level (582). Thus voltage-dependent potassium channels appear to be a negative-feedback system, like calcium-dependent potassium channels, which modulates vascular tone. A voltage-dependent potassium channel that is expressed in vascular muscle has been cloned (619).

Voltage-dependent potassium channels can be inhibited fairly selectively by 4-aminopyridine (582). 4-Aminopyridine is often used to distinguish calcium-dependent from voltage-dependent potassium channels; both channels are activated by membrane depolarization. Activity of voltage-dependent potassium channels can also be inhibited by cesium ion and high concentrations of TEA or glibenclamide (582).

Relatively little is known about the physiological importance of these potassium channels in the cerebral circulation. Like calcium-dependent potassium channels, activity of voltage-dependent potassium channels may contribute to regulation of membrane potential and responses of cerebral arteries to changes in arterial pressure (393). One would anticipate that these voltage-dependent potassium channels may limit vasoconstrictor responses to other stimuli that depolarize vascular muscle. Because reduction of intracellular pH increases the current carried by voltage-dependent potassium channels, it has been suggested that these channels may be important in mediation of cerebral vasodilatation during acidosis and/or hypercapnia (72). In pulmonary vascular muscle, NO has been reported to activate voltage-dependent potassium channels (852). 4-Aminopyridine partially inhibits hyperpolarization and relaxation of these arteries in response to NO, which is consistent with activation of these potassium channels by NO (846, 852). It is not known whether a similar mechanism contributes to NO-mediated relaxation of cerebral blood vessels.

### D. Inward-Rectifier Potassium Channels

An inward rectifier potassium current has been described in cerebral blood vessels (172, 292, 582, 668). In contrast to calcium-dependent and voltage-dependent potassium channels, inward-rectifier potassium channels are opened by membrane hyperpolarization (582). These potassium channels normally conduct an outward hyperpolarizing current and may play an important role in maintaining resting membrane potential. Inward-rectifier potassium channels are very sensitive to and are inhibited selectively by relatively low concentrations of extracellular barium ions (582). Barium has inhibitory effects on other potassium channels, but these effects occur at much higher concentrations than those required to inhibit inward-rectifier potassium channels (582).

Relatively little is known regarding the functional significance of inward-rectifier potassium channels in cerebral vessels. Recent findings suggest that inward-rectifier potassium channels may play an important role in mediation of vasodilatation in response to extracellular potassium (172, 394, 668). Small elevations in the concentration of extracellular potassium ion produce hyperpolarization and relaxation of cerebral arteries in vitro (394). Both
effects are inhibited by relatively low concentrations of barium ion, but not by a combination of TEA, glibenclamide, and 4-aminopyridine, suggesting that the response is mediated by an inward-rectifier potassium channel (394). This mechanism is potentially important because modest increases in the concentration of extracellular potassium ion, such as those that might occur during neuronal activation, produce marked dilatation of cerebral blood vessels in vivo (231, 432, 515).

VI. HYPERTENSION

A. Acute Hypertension

Acute hypertension produces morphological evidence of damage to endothelium and impairs dilator responses of cerebral blood vessels to endothelium-dependent stimuli such as acetylcholine. This impairment can occur after acute hypertension produced by systemically administered pressor agents (814) or experimentally induced head injury (179, 411). Impaired responses to acetylcholine appear to be specific for endothelium, because vasodilator responses to nitroprusside were relatively normal in this model (179).

Mechanisms that cause impairment of cerebral arterioles to endothelium-dependent stimuli may involve production of reactive oxygen species. Production of superoxide anion in brain has been measured in response to acute hypertension (814) and fluid percussion injury (410, 411, 765). Local application of superoxide dismutase or deferoxamine restores vasodilator responses to acetylcholine toward normal, suggesting that inactivation of NO by superoxide anion or hydroxyl radical accounts for impaired endothelial function (411, 814) (Fig. 4). Experiments examining effects of acute hypertension on vascular responses to acetylcholine by Kontos, Wei, and co-workers (814) were important because they provided the first evidence that superoxide anion inactivates EDRF in vivo.

B. Chronic Hypertension

Endothelium-dependent relaxation is impaired in peripheral blood vessels in experimental animals and humans with chronic hypertension (242, 468, 624, 787, 854). Similarly, we and others have shown that dilatation of the basilar artery (386, 391, 494), middle cerebral artery (781), and cerebral arterioles (48, 497, 509, 510, 678, 843–845) in response to endothelium-dependent agonists including acetylcholine, methacholine, bradykinin, and ADP is impaired in spontaneously hypertensive rats (SHR) and stroke-prone SHR (SHRSP). The finding that cerebral vasodilatation in response to A-23187 is also impaired during chronic hypertension indicates that the impairment extends beyond the receptor level (48, 843, 845). The concept that normal endothelial function is protective in cerebral vessels is supported by a recent genetic analysis demonstrating cosegregation of impaired endothelium-dependent relaxation and stroke-prone phenotype (795).

In contrast to endothelium-dependent stimuli, cerebral vasodilatation in response to the endothelium-independent agonists NO (843), nitroglycerin (494, 497, 495, 509, 510, 843–845), nitroprusside (48, 386, 389, 391, 781), forskolin (389), and adenosine (509, 843) are not impaired during chronic hypertension, which suggests that impairment of vascular function is at the level of endothelium and not smooth muscle. Endothelial dysfunction may contribute to enhanced serotonin-induced constriction, which has been observed in large cerebral arteries of SHRSP (506).

Several studies have attempted to define mechanisms that account for impaired endothelium-dependent relaxation in cerebral blood vessels during chronic hypertension. The impairment appears to be reversible, because antihypertensive treatment restores endothelium-dependent responses to normal (843). In the aorta of SHR, impaired endothelium-dependent relaxation is associated with increased expression of COX-1 (242). This enhanced activity results in production of a COX-derived EDCF (468) that counteracts the normal vasodilator effect of EDRF (Fig. 4). This same mechanism seems to account for altered responses in cerebral arterioles during chronic hypertension, because impaired endothelium-dependent responses can be restored to normal using indomethacin (510) or an inhibitor of PGH2/thromboxane A2 receptors (497) (Fig. 4).

Impaired endothelium-dependent relaxation of the basilar artery, in contrast to cerebral arterioles, does not appear to be due to production of a COX-derived contracting factor because indomethacin does not improve responses to endothelium-dependent agonists (386, 494). Impaired endothelium-dependent responses of the basilar artery during chronic hypertension could potentially be due to reduced production or activity of EDRF. The finding that acute treatment with L-arginine, the substrate for NO synthase, restores responses of the basilar artery to acetylcholine toward normal in older SHRSP provides some evidence to support this possibility (386).

In contrast to agonist-induced endothelium-dependent relaxation, basal release of EDRF does not appear to be impaired during chronic hypertension. This conclusion is based on the findings that inhibitors of NO synthase produce similar constriction of cerebral arteries (391) and similar reductions in cerebral blood flow (336, 842) in SHRSP, SHR, and normotensive Wistar-Kyoto rats (WKY).

The functional influence of potassium channels in cerebral vessels is altered during chronic hypertension. Cerebral vasodilatation in response to activators of ATP-
sensitive potassium channels is impaired in SHRSP (389, 752). Preliminary studies suggest that a high-salt diet impairs membrane hyperpolarization and relaxation of the middle cerebral artery in response to prostacyclin and hypoxia in vitro (460). Relaxation of the middle cerebral artery to both stimuli is inhibited by glibenclamide and thus appears to be mediated by activation of the ATP-sensitive potassium channel (226). Impaired cerebral vascular responses to an activator of ATP-sensitive potassium channels in SHR can be restored largely to normal by chronic antihypertensive therapy (752).

In contrast, activity of calcium-dependent potassium channels appears to be enhanced during chronic hyper-tension. Efflux of $^{86}$Rb, which is an index of potassium channel activity, is greater in carotid arteries from SHR than from WKY (33). Inhibitors of calcium-dependent potassium channels produce greater contraction of the carotid artery in vitro (33) and the basilar artery in vivo (637) in chronically hypertensive rats, suggesting that activity of calcium-activated potassium is enhanced during chronic hypertension. Thus, in contrast to ATP-sensitive potassium channels, which appear to have reduced activity, calcium-dependent potassium channels appear to be activated during chronic hypertension, presumably to compensate for increased intracellular calcium and enhanced myogenic tone.

VII. HYPERCHOLESTEROLEMIA AND ATHEROSCLEROSIS

Endothelium-dependent relaxation of noncerebral arteries is impaired in experimental animals and humans with hypercholesterolemia and atherosclerosis (67, 73–75, 107, 119, 121, 138, 139, 146, 163, 227, 252, 451, 467, 669). Production or activity of EDRF under basal conditions and in response to endothelium-dependent agonists are both impaired. Some studies suggest that hypercholesterolemia alone, in the absence of atherosclerotic lesions, is sufficient to produce endothelial dysfunction (107, 121, 252, 602, 749). Vasorelaxation in response to endothelium-independent stimuli, such as nitrovasodilators, is generally intact in atherosclerotic arteries. One exception is relaxation of the carotid artery to severe hypoxia, which is endothelium independent but nevertheless impaired in atherosclerotic rabbits (749).

Mechanisms that account for impaired endothelium-dependent relaxation during atherosclerosis have not been completely defined, but probably involve generation of reactive oxygen species that inactivate EDRF (67, 372) (Fig. 4). Several studies have demonstrated that vascular production of superoxide anion is increased during hypercholesterolemia and atherosclerosis (68, 559, 602, 603). Immunocytochemistry suggests that peroxynitrite, the reaction product of superoxide anion and NO (Fig. 2), is formed in endothelium and macrophages in atherosclerotic lesions (7, 54, 98). Superoxide dismutase improves endothelium-dependent relaxation during atherosclerosis (560, 748, 819). In addition, plasma levels of ADMA, an endogenous inhibitor of NO synthase, are increased during hypercholesterolemia and might contribute to impairment of endothelium-dependent responses (66, 839, 851). Several studies suggest that administration of L-arginine, the substrate for NO synthase, improves endothelium-dependent relaxation during hypercholesterolemia and atherosclerosis (125, 139, 147, 163). Recent evidence suggests that supplementation with tetrahydrobiopterin, an essential cofactor for NO synthase, also improves endothelium-dependent relaxation using hypercholesterolemia (742). Reductions in plasma cholesterol levels, which produce regression of atherosclerosis, restore endothelium-dependent relaxation toward normal (252, 270, 449, 603).

Recent studies have examined whether gene expression for endothelial NO synthase is altered during atherosclerosis. Oxidized low-density lipoprotein and lysophosphatidylcholine (a lipid component unique to oxidized low-density lipoprotein) impair endothelium-dependent relaxation (20, 145, 722). Lysophosphatidylcholine also impairs responses that are mediated by EDHF (233). Low-density lipoprotein has been reported to both increase (289) and decrease (452) expression of NO synthase in endothelium in culture. Importantly, mRNA and protein for endothelial NO synthase in the vessel wall are increased in atherosclerotic rabbits (356). Recent evidence also suggests that inducible NO synthase is expressed in the vessel wall in atherosclerotic arteries (98). Expression of inducible NO synthase may contribute to formation of peroxynitrite during atherosclerosis (98).

Effects of atherosclerosis on endothelium-dependent responses of cerebral vessels are less clear. Impairment of endothelium-dependent relaxation is observed in the atherosclerotic carotid artery (205, 355, 384, 723, 730). Impaired endothelium-dependent relaxation of the carotid artery can be restored toward normal during regression of atherosclerosis (205, 730). In comparison with the carotid artery, intracranial vessels appear to be resistant to development of atherosclerosis (355). Relaxation of cerebral vessels to endothelium-dependent stimuli can be normal in the same atherosclerotic animals that exhibit marked impairment of endothelium-dependent relaxation in the carotid artery and aorta (355, 384, 723). In contrast, some studies suggest that atherosclerosis or hypercholesterolemia produces endothelial dysfunction in cerebral arteries. Oxidized low-density lipoprotein has been reported to impair relaxation of the basilar artery in response to acetylcholine in vitro (349). Hypercholesterolemia is reportedly associated with impaired endothelium-dependent relaxation of the basilar artery to acetylcholine and ADP (690, 717). In hypercholesterolemic rabbits, administration of L-arginine restored dilator responses of the ba-
silar artery to acetylcholine to normal (690). Mechanisms that account for an apparent relative resistance of intracranial arteries to atherosclerosis are not clear.

The interaction of platelets and leukocytes with the vessel wall is altered during atherosclerosis (788). For example, constrictor responses of coronary arteries in response to serotonin, which is released by platelets during aggregation, are greatly enhanced in patients with coronary artery disease (251, 518). Similarly, constriction of large cerebral arteries and blood vessels supplying the eye in response to serotonin and thromboxane (also released by platelets) and in response to activation of platelets in vivo with collagen is enhanced by atherosclerosis (189, 202, 210, 279, 730, 753, 823). Contraction of human middle cerebral arteries in response to platelets and leukocytes in vitro is enhanced in vessels with atherosclerotic lesions (9). Because NO normally inhibits responses of cerebral arteries to serotonin and other vasoconstrictors (198, 771), impairment of release or activity of NO may contribute to augmented vasoconstrictor responses during atherosclerosis. Inactivation of NO by leukocytes is enhanced in leukocytes obtained from patients with carotid atherosclerosis as compared with leukocytes obtained from control patients (10). In addition to impaired endothelial function, augmented vasoconstrictor responses to serotonin during atherosclerosis may be mediated by increased activity of protein kinase C or changes in expression of serotonin receptor subtypes in vascular muscle (538, 747). Augmented constrictor responses of large cerebral arteries to serotonin are restored largely to normal during regression of atherosclerosis (279).

Atherosclerosis enhances constriction of large cerebral arteries and blood vessels supplying the eye in response to activation of leukocytes in vivo (202). Activated leukocytes can release several vasoactive substances that may contribute to augmented cerebral vasoconstrictor during atherosclerosis, including reactive oxygen species, thromboxane, leukotrienes, platelet-activating factor, and endothelin (13).

Increases in plasma levels of homocysteine, produced by either genetic or dietary factors, are associated with increased risk for complications of atherosclerosis (492, 517). Severe hyperhomocysteinemia predisposes to venous and arterial thrombosis, and moderate hyperhomocysteinemia has been proposed to be an independent risk factor for peripheral vascular disease, myocardial infarction, and stroke (62, 517, 646). Furchgott and co-workers (343) provided evidence that acute exposure to high levels of homocysteine (100 \( \mu \)M) impairs endothelium-dependent relaxation of the aorta in vitro by a mechanism that involves generation of superoxide anion. Preliminary evidence suggests that high levels of homocysteine, in the presence of \( Cu^{2+} \), impair endothelium-dependent responses in the cerebral circulation via a mechanism that also involves generation of superoxide anion (858). A recent study suggests that modest hyperhomocysteinemia impairs endothelium-dependent relaxation and antithrombotic activity in the carotid artery and produces increases in platelet-mediated vasoconstriction (447). In humans, moderate hyperhomocysteinemia is associated with impaired endothelium-dependent relaxation in the forearm (759). Thus, in addition to hyperlipidemia, hyperhomocysteinemia may contribute to cerebral vascular dysfunction.

**VIII. DIABETES**

Diabetes is associated with endothelial dysfunction in extracranial blood vessels in experimental animals (157, 274, 437, 542, 655, 760) and humans (345, 521, 663, 694). Similarly, impaired dilatation of cerebral arterioles (493, 512, 641) and the basilar artery (230, 408, 496, 505) has been observed during diabetes mellitus. Increases in cerebral blood flow in response to activation of muscarinic receptors are also impaired during diabetes (642).

Mechanisms that mediate endothelial cell dysfunction during diabetes are not completely defined. Some evidence suggests that production of endothelium-derived NO is normal during diabetes (437, 655). A recent study reported that hyperglycemia does not alter levels of endothelial NO synthase mRNA, protein, or enzyme activity in endothelium (481). Under these conditions, endothelium-dependent relaxation may be impaired by excess generation of reactive oxygen species that destroy NO (157, 274, 760, 761). Levels of mRNA for Mn-superoxide dismutase and Cu/Zn-superoxide dismutase in aorta have been reported to be decreased during diabetes (353). Preliminary findings suggest that mRNA for COX-1 in aorta is also decreased during diabetes (100).

Impairment of endothelium-dependent relaxation during diabetes may also be due to simultaneous production of an EDCF (129, 760). In cerebral arterioles, activation of protein kinase C (641) and/or production of an EDCF that activates a PGH2/thromboxane A2 receptor (512) appears to account for endothelial dysfunction during diabetes (Fig. 4). Hyperglycemia per se produces impairment of endothelium-dependent dilatation of cerebral arterioles, which also seems to involve activation of protein kinase C (511). In contrast to cerebral arterioles, impaired responses to endothelium-dependent stimuli in the basilar artery are not due to production of an COX-derived EDCF (496) and are not improved by acute treatment with L-arginine (505), the substrate for NO synthase.

Diabetes mellitus is not associated with generalized impairment of vasodilator mechanisms. Cerebral vasodilatation in response to several endothelium-independent stimuli including nitroglycerin, nitroprusside, and forskolin is normal during diabetes, indicating that nonspecific impairment of vascular muscle is not present (230, 493, 496, 499–501, 505, 507). In contrast, cerebral vasodila-
tation in response to some stimuli that act directly on vascular muscle is impaired during diabetes. This impairment includes responses to isoproterenol and norepinephrine (via activation of β-adrenergic receptors) (500, 501, 641) as well as activators of ATP-sensitive potassium channels (499, 507).

IX. AGING

Normal aging is associated with several changes in cardiovascular function and is sometimes associated with impairment of endothelium-dependent responses (246, 482, 772). Relaxation of the basilar artery from humans (271) and cerebral arterioles (508) and the carotid artery (635) in rats in response to endothelium-dependent agonists is impaired with aging.

In contrast to changes that occur during chronic hypertension and diabetes mellitus, impaired dilatation of cerebral arterioles in response to endothelium-dependent stimuli during aging does not appear to be related to the production of a COX-derived EDCF (508). Direct measurements indicate that release of NO in response to endothelium-dependent stimuli is impaired in the aorta, but not the pulmonary artery (772). Bioassay studies suggest that luminal release of NO from the carotid artery is not impaired with aging, although the artery itself does not relax normally to acetylcholine (635). These findings have led to the suggestion that impaired endothelium-dependent relaxation may be due to generation of reactive oxygen species in the vessel wall (635) (Fig. 4).

Deposits of β-amyloid in brain and cerebral vessels are seen in aging individuals and in Alzheimer’s disease patients. Recent data suggest that β-amyloid may impair endothelium-dependent relaxation by generation of superoxide anion (763). β-Amyloid produces oxidative stress in cerebral endothelium and neurons via an effect mediated by receptors for advanced glycation end products (838). Expression of these receptors is enhanced in cerebral vessels during Alzheimer’s disease (838). Thus the presence of β-amyloid protein near cerebral vessels may contribute to impairment of endothelium-dependent responses that occurs with aging. In transgenic mice that overexpress amyloid precursor protein, preliminary studies suggest cerebral vasodilatation in response to acetylcholine is impaired (856). Inducible NO synthase has been detected in cerebral microvessels during Alzheimer’s disease (162), and β-amyloid has been reported to enhance expression of inducible NO synthase in response to proinflammatory cytokines (689).

Relaxation of cerebral vessels in response to nitroprusside and activators of ATP-sensitive potassium channels is not altered with aging (201, 271, 482, 635, 770), with the exception of one study in which dilatation of the basilar artery and cerebral arterioles that supply brain stem in response to activators of ATP-sensitive potassium channels was impaired in old rats (770). Impairment of vasoconstrictor responses to several stimuli has been reported in the human basilar artery (272).

X. ISCHEMIA

Ischemia can impair responses of cerebral blood vessels to several stimuli. Dilatation of cerebral arterioles in response to the endothelium-dependent agonist acetylcholine is impaired after ischemia with reperfusion (123, 126, 503, 578). Formation of reactive oxygen species including superoxide anion, which is known to occur during reperfusion after ischemia (29, 578), seems to mediate impaired endothelium-dependent relaxation in cerebral arterioles (578) (Fig. 4) because impaired vasodilatation can be restored toward normal with superoxide dismutase and catalase (578).

There is potentially more than one source of reactive oxygen species during reperfusion after ischemia. Superoxide anion may be generated through the COX pathway (29, 578) or by NO synthase in the absence of adequate levels of L-arginine (149, 194, 830). Expression of both COX-1 and COX-2 has been reported to increase in response to ischemia with reperfusion (296, 656). Increased production of NO in combination with superoxide anion may result in formation of peroxynitrite, which produces vasodilatation but may also produce vascular injury (791) (Fig. 2). Cerebral endothelium in culture produces hydroxyl radical in response to anoxia (430) and thus is also a potential source of reactive oxygen species during ischemia.

Brain injury during reperfusion after ischemia is associated with activation of leukocytes and adherence of leukocytes to endothelium (13). Intercellular adhesion molecule-1 (ICAM-1), an important mediator of leukocyte adhesion (450), is expressed in cerebral endothelium in response to ischemia (136, 220, 608). Studies using mice that are deficient in expression of the gene for ICAM-1 suggest that neutrophils invade and accumulate in ischemic tissue, and these accumulating cells contribute to injury of the brain and possibly vessels (136, 735). Invading leukocytes may be a source of reactive oxygen species and may express inducible NO synthase (13, 317, 320). Leukocytes can also impair endothelium-dependent relaxation of cerebral arteries (8, 11, 13) through a mechanism that may involve platelet-activating factor (11, 12).

Several lines of evidence, including the use of mice that are deficient in expression of the genes for endothelial and neuronal NO synthase, suggest that NO produced by NO synthase in endothelium plays a protective role during and after cerebral ischemia (308, 309, 459, 552, 623, 857, 859). Endothelium-derived NO may be protective against ischemia by contributing to vasodilatation and by
inhibition of aggregation and adherence of platelets or leukocytes (194, 462, 829). The recent finding that physiological concentrations of peroxynitrite inhibit interaction of endothelium and leukocytes raises the possibility that a portion of the protective effect of endothelium-derived NO during ischemia (or other situations in which superoxide is generated) relates to its inactivation of superoxide and perhaps inhibition of leukocyte adhesion by physiological concentrations of peroxynitrite (444).

Immunocytochemical analysis of protein levels suggests that expression of the gene for NO synthase (NOS system) has been reported to be normal after subarachnoid hemorrhage (360, 72). Several mechanisms have been proposed to account for this vascular dysfunction. One possibility is that subarachnoid hemorrhage causes a reduction in production or activity of NO. Levels of NO synthase in endothelium (determined by Western blotting) and activity of NO synthase in large cerebral arteries have been reported to be relatively unchanged after subarachnoid hemorrhage (360, 577). In addition, release of NO (detected using a bioassay system) has been reported to be normal after subarachnoid hemorrhage (377, 378). In contrast, another study suggests that mRNA for endothelial NO synthase is decreased in cerebral ischemia (863). Measurements of calcium-independent enzyme activity and immunocytochemistry suggest that inducible NO synthase is expressed in cerebral microvessels in response to ischemia (317, 567, 568). Mice that lack expression of inducible NO synthase are resistant to focal cerebral ischemia (318, 319). Overall, it is not clear whether vascular expression of inducible NO synthase is beneficial or detrimental.

Cerebral ischemia may also impair responses of cerebral vessels to activation of ATP-sensitive potassium channels. For example, dilatation of cerebral arterioles in response to CGRP and prostacyclin, which produce glibenclamide-sensitive dilatation of cerebral vessels (41, 297, 388, 463), and aprikalim (an activator of ATP-sensitive potassium channels; Fig. 6) is impaired after ischemia with reperfusion (41, 463).

In addition to NO, production of other vasoactive products may change in response to ischemia. Levels of endothelin in brain tissue (42, 792, 837) and brain extracellular fluid (42) are increased after ischemia, and increased production of endothelin may contribute to brain injury and limit vasodilatation in response to ischemia (632). A combined ETA/ETB receptor antagonist protects against neuronal injury after cerebral ischemia (604). Gene expression of adrenomedullin is also increased in response to ischemia (802). It is not known whether enhanced production of adrenomedullin, a potent vasodilator (43, 436), plays a protective role during cerebral ischemia.

XI. SUBARACHNOID HEMORRHAGE

A. Endothelium-Dependent Relaxation

Subarachnoid hemorrhage is often associated with cerebral vasospasm and, as a consequence, stroke (137, 221). Several mechanisms may contribute to vasospasm after subarachnoid hemorrhage including impaired endothelium-dependent relaxation, production of EDCF's including endothelin, and impaired activity of potassium channels in cerebral blood vessels.

Endothelium-dependent relaxation is impaired in large cerebral arteries in experimental models of subarachnoid hemorrhage (171, 299, 354, 364, 377–379, 732, 794, 836) and in the basilar artery from patients after subarachnoid hemorrhage (273, 612). Several mechanisms have been proposed to account for this vascular dysfunction. One possibility is that subarachnoid hemorrhage causes a reduction in production or activity of NO. Levels of NO synthase in endothelium (determined by Western blotting) and activity of NO synthase in large cerebral arteries have been reported to be relatively unchanged after subarachnoid hemorrhage (360, 577). In addition, release of NO (detected using a bioassay system) has been reported to be normal after subarachnoid hemorrhage (377, 378). In contrast, another study suggests that mRNA for endothelial NO synthase is decreased in cerebral ischemia (863). Measurements of calcium-independent enzyme activity and immunocytochemistry suggest that inducible NO synthase is expressed in cerebral microvessels in response to ischemia (317, 567, 568). Mice that lack expression of inducible NO synthase are resistant to focal cerebral ischemia (318, 319). Overall, it is not clear whether vascular expression of inducible NO synthase is beneficial or detrimental.

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B. Endothelin

In addition to impaired endothelial production of NO, recent observations suggest that endothelial production of endothelin may be a major mechanism that contributes to vasospasm after subarachnoid hemorrhage. As discussed in section III, endothelin is a potent constrictor of cerebral arteries that normally produces vasoconstriction by activation of ET<sub>α</sub> receptors (1, 2, 154, 212, 217, 350, 390, 586, 647, 695, 701, 820, 869) (Fig. 3). Levels of ET-1 are increased in the basilar artery and in cerebrospinal fluid after subarachnoid hemorrhage (291, 397, 399, 424, 488, 691, 744, 866). Several studies in experimental animals, including nonhuman primates, suggest that vasospasm after experimental subarachnoid hemorrhage can be significantly attenuated by antagonists of ET<sub>α</sub> receptors such as BQ-123 (223, 288, 291, 333, 334, 376, 587, 820, 835) or combined ET<sub>α</sub>/ET<sub>β</sub> receptor antagonists (691, 716, 820, 866, 868, 870). Endothelin-1 is produced from its precursor, big ET-1, by ECE (692, 833). Phosphoramidon and CGS-26303, inhibitors of ECE (103, 692, 833), attenuate vasospasm after subarachnoid hemorrhage (490). Anti-sense oligonucleotides for prepro-ET-1 mRNA inhibit contraction of the basilar artery in response to hemolysate (611).

In addition to the ET-1 gene, expression of other ET-1-related genes appears to change after subarachnoid hemorrhage. Levels of mRNA for ET<sub>α</sub> receptors are increased in cerebral arteries after subarachnoid hemorrhage (333). In normal cerebral arteries, both functional studies (1, 2, 154, 212, 350, 390, 586, 695, 701, 820, 869) and competition binding analysis (691) suggest that contraction of cerebral arteries to endothelin is mediated very predominantly by ET<sub>α</sub> receptors. Interestingly, ET<sub>β</sub> receptors are expressed to a greater extent, presumably in vascular muscle, after subarachnoid hemorrhage (286, 691). Augmented expression of ET<sub>β</sub> receptors in vascular muscle may contribute to vasoconstriction that occurs with subarachnoid hemorrhage (691) (Fig. 3). Activity of ECE in the basilar artery also increases threefold after subarachnoid hemorrhage (691), which may also contribute to vasospasm.

Stimuli that cause endothelin gene expression after subarachnoid hemorrhage are not well defined. Candidates include hemoglobin and thrombin, factors which are known to enhance endothelin gene expression in endothelium (128, 605, 692, 745), and are present in the thrombus and cerebrospinal fluid after subarachnoid hemorrhage. Leukocytes, which infiltrate the basilar artery after subarachnoid hemorrhage, are immunoreactive for ET-1 (390) and thus represent another potential source of endothelin.

C. Potassium Channels

A third major mechanism that may contribute to vasospasm after subarachnoid hemorrhage involves changes in activity of potassium channels in cerebral vessels. Cerebral vascular muscle is depolarized after subarachnoid hemorrhage, and this depolarization probably contributes to vasoconstriction and perhaps vasospasm (268, 865, 867). Depolarization of vascular muscle after subarachnoid hemorrhage is most likely due to inhibition of potassium channels (268). Nicorandil, which produces relaxation of cerebral vessels in part by activation of potassium channels (332), partially reverses vasospasm in an experimental model of subarachnoid hemorrhage (268). Recent studies suggest that, although several dilator mechanisms are impaired after subarachnoid hemorrhage, dilatation of the basilar artery in response to activators of potassium channels is preserved or enhanced after subarachnoid hemorrhage (731, 732, 867). Dilatation of the large cerebral arteries in response to synthetic activators of potassium channels (aprikalin and cromakalim) and CGRP (which hyperpolarizes cerebral vascular muscle) (392, 696) is preserved or enhanced after experimental subarachnoid hemorrhage (6, 167, 300, 325, 329, 731, 732, 867). These findings suggest that activators of potassium channels in vascular muscle may have beneficial effects during vasospasm after subarachnoid hemorrhage.

XII. MENINGITIS

Brain inflammation and bacterial infections of the central nervous system (meningitis) are associated with dilatation of cerebral blood vessels and increases in cerebral blood flow (60, 260, 400–402, 650–652, 754). Lipo-polsaccharide and proinflammatory cytokines such as tumor necrosis factor-α cause cerebral vasodilatation (83, 85, 86, 714) and probably contribute to these vascular changes. Some of the pathophysiological changes that occur during meningitis include brain edema and increases in intracranial pressure (400, 401).

Recent studies have provided insight into mechanisms that produce cerebral vasodilatation in the early phase of meningitis. One mechanism may involve expression of inducible NO synthase (Fig. 5). The level of nitrite in cerebrospinal fluid is increased markedly in patients and experimental animals with meningitis (97, 419, 532, 649, 786, 793). Messenger RNA for inducible NO synthase has been detected in brain during viral meningitis but seems to be confined primarily to inflammatory cell infiltrates (101). Cerebral endothelium produces nitrite in response to pneumococcal stimulation (401), and increases in cerebral blood flow that occur early in the pathogenesis of bacterial meningitis are mediated by NO (260, 401, 724).

Stimuli that could cause expression of inducible NO synthase in brain during meningitis include cell wall components of bacteria, including lipopolysaccharide, local production of tumor necrosis factor-α or interleukin-1β, and reactive oxygen species (650, 652). Increased produc-
tion of superoxide anion has been observed in brain and along cerebral microvessels during experimental meningitis (520, 446). Pneumococcal cell wall components increase production of nitrite and tumor necrosis factor-α in astrocytes and endothelium in culture (61, 228, 400), which are inhibited by L-NNA, aminoguanidine, dexamethasone, and interleukin-10 (61, 228, 400). Tumor necrosis factor-α and interferon-γ cause expression of inducible NO synthase in cerebral endothelium (561).

Leukocytes enter the subarachnoid space and cerebrospinal fluid early in the development of meningitis and may contribute to its pathophysiology. Expression of adhesion molecules is critical for accumulation of leukocytes at sites of bacterial infection (182), and recruitment of leukocytes contributes to development of brain edema and increases in cerebral blood flow (21). Because leukocytes can express inducible NO synthase, it is possible that attachment and migration of these cells contribute to increases in permeability of the blood-brain barrier and vasodilatation during meningitis (69, 70, 97, 721). Inhibition of adhesion molecules reduces leukocyte rolling and inflammation in experimental meningitis (21, 808). Interleukin-10 inhibits increases in cerebral blood flow, intracranial pressure, and leukocyte migration into cerebral spinal fluid during meningitis (400). Infusion of leukocytes and disruption of the blood-brain barrier in response to tumor necrosis factor-α and interleukin-1β are reduced in mice that are deficient in expression of leukocyte adhesion molecules (756).

In addition to expression of inducible NO synthase, other mechanisms may contribute to cerebral vasodilatation during meningitis or in response to proinflammatory cytokines. For example, many of the same stimuli that produce expression of inducible NO synthase also cause expression of COX-2 (537, 828), and expression of COX-2 is considered to be important in inflammation (537, 828). Activation of trigeminal C fibers (807), receptors for CGRP (86), and substance P (653) may also contribute to cerebral vasodilatation in response to lipopolysaccharide or during meningitis. Lipopolysaccharide may cause stimulation of trigeminal sensory fibers that release CGRP locally. The observation that meningitis is commonly associated with headache is consistent with the possibility that trigeminal fibers are activated. In addition, recent findings suggest that activation of receptors for bradykinin also contributes to increases in cerebral blood flow and brain edema during meningitis (461).

It is also possible that adrenomedullin, produced in response to lipopolysaccharide, contributes to vasodilatation during meningitis. The peptide adrenomedullin is structurally related to CGRP (666). Adrenomedullin is produced in both endothelium and vascular muscle, and expression of the gene for adrenomedullin is enhanced by lipopolysaccharide (727). Adrenomedullin produces marked relaxation of cerebral blood vessels (436, 802). Because adrenomedullin can act via stimulation of receptors for CGRP (666), it is possible that cerebral vasodilatation in response to lipopolysaccharide that is inhibited by a CGRP antagonist is mediated, in part, by adrenomedullin.

Although some laboratories have had difficulty demonstrating expression of inducible NO synthase in human monocytes or macrophages, the gene for inducible NO synthase can be expressed under some conditions in these human cells (164, 584). In brain, inducible NO synthase is expressed in microglia (a monocyte/macrophage-like cell) during multiple sclerosis in humans (39). Immunocytochemical evidence suggests that peroxynitrite is also formed in the brain in multiple sclerosis (39).

**XIII. CONCLUSIONS AND FUTURE DIRECTIONS**

In summary, there have been many advances in our understanding of cerebral vascular biology and pathophysiology in recent years. These advances include demonstration of major roles for both endothelium and potassium channels in regulation of cerebral vascular tone. These mechanisms can be altered, often in a selective manner, under pathophysiological conditions.

Additional insight in cerebral vascular biology in the future may be possible with incorporation of molecular approaches in studies of cerebral vessels. Although these approaches have been used to only a limited extent to date, molecular biological methods offer considerable promise for providing new insight in studies of the cerebral circulation.

A potentially valuable approach is the study of animal models that are deficient in expression of specific genes (gene knockouts). Gene mutation approaches have been advanced recently to include the possibility of inducible knockouts and cell-specific targeting. An alternative approach to examine the role of specific genes is to inhibit gene expression using antisense oligonucleotides or oligonucleotides that inhibit availability of transcription factors that are necessary for gene expression.

Several murine models have been developed that are deficient in expression of genes that are important for vascular biology, including neuronal NO synthase (305, 308, 330, 623), endothelial NO synthase (307, 309, 713), and inducible NO synthase (438, 475, 816). Mice that are doubly mutant in endothelial and neuronal NO synthase have also been produced (733). Such models may prove valuable for several reasons. First, although inhibitors of NO synthase have been very useful in providing insight into the role of NO in the cerebral circulation, many of these inhibitors are not completely selective for a single isoform of NO synthase. Thus defining the role of a specific isoform of NO synthase can sometimes be difficult. One value of NO synthase mutants is that they allow stud-
ies to be performed in a model in which a single isofrom of NO synthase is selectively deleted. Studies of mice that are deficient in expression of superoxide dismutases have also been produced (106, 109) and may be useful in defining the role of superoxide anion in destruction of NO and formation of peroxynitrite in cerebral vessels.

A similar approach may be useful in studies of potassium channels where some commonly used inhibitors may have actions on more than one potassium channel. To examine the role of potassium channels in a selective manner, it may be possible to produce mice that are deficient in expression of the genes for the ion-conducting component of potassium channels or important modulators of channel function such as the β-subunit of the calcium-activated potassium channel. Presumably, deletion of these components may alter channel function and provide novel models to examine the role of potassium channels in blood vessels.

After selective deletion of a specific gene, it is not uncommon for mutant animals to display no or minimal differences in phenotype. Such findings suggest the presence of redundant or compensatory mechanisms after selective gene deletion (306, 330). Such compensatory mechanisms in cerebral vascular responses probably explain the normal responses to acetylcholine that have been observed in cerebral arterioles in mice in which the gene for endothelial NO synthase has been deleted (529).

It may also be of value to overexpress genes in transgenic animals (in the whole animal or in a cell-specific manner) or by using gene transfer techniques. Examples of these experimental models include the recently developed renin-angiotensinogen double transgenic mouse (531) and methods of gene transfer to cerebral vessels using liposomes or more efficient viral vectors such as retroviruses, adeno-associated viruses, or adenoviruses (280, 775). Successful gene transfer to cerebral vessels has recently been achieved in vitro (114, 122) and in vivo (615). Initial studies suggest that local overexpression of genes whose products are vasoactive such as endothelial NO synthase can alter function of cerebral arteries (114).

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