Arq Neuro-Psiquiat (São Paulo) 46(1):90-97, 1988

THE ROLE OF THE ENDOTHELIAL DEPENDENT RELAXING FACTOR IN THE REGULATION OF CEREBRAL CIRCULATION

ANTONIO A. F. DE SALLES *

SUMMARY — It has recently been demonstrated that vessel dilation induced by several physiological agents is dependent on an intact vascular endothelium. In order to explain this endothelium dependence, it has been hypothesized that a still unknown chemical substance, generically named Endothelium Dependent Relaxing Factor (EDRF) is necessary for physiological vasodilation. The role of this EDRF in the cerebrovascular physiology is not yet well understood. In this article the cerebrovascular physiological control is reviewed in relationship with possible EDRF actions. The importance of endothelial lesions in the cerebrovascular responses is discussed.

Papel do fator relaxante endotelial na circulação sanguínea cerebral.

RESUMO — Recentemente foi descoberto que o endotélio vascular deve estar intacto para que vasos sanguíneos dilatem quando estimulados por agentes fisiológicos. Acredita-se que uma substância química ainda desconhecida, genericamente chamada Fator Relaxante Endotelial (FRE), produzida pelo endotélio, é indispensável para o relaxamento vascular. Neste trabalho é revista a fisiologia circulatória cerebral e possíveis ações do FRE. Discute-se também a importância de lesões endoteliais em relação ao controle circulatório cerebral.

Arteries relax in response to various vasodilators: acetylcholine (Ach), substance P, adenosine triphosphate (ATP), adenosine diphosphate (ADP), ionophore A23187, eleidoisin, thrombin, arachidonic acid, histamine, hydralazine, platelet activating factor (PAF), and bradykinin (BKN). However, this will only occur if the endothelium is present [4,19,43]. One hypothesis which would explain this phenomenon is that these compounds stimulate the endothelial cells to release a vasodilator substance which in turn relaxes the underlying smooth muscle [20]. The endothelium dependence of vessel dilation was first described for the dilator effect of Ach in isolated rabbit thoracic aorta [20]. This phenomenon has been extensively studied in several vascular beds of various species. Production and release of an endothelium relaxant factor is thought to be primarily a property of arterial endothelium, although it has been documented in some venous preparations [40,43]. Endothelium dependent relaxing factor (EDRF) appears to be unrelated to the arterial segment and mammalian species studied [43,44]. Ach-induced relaxation of large cerebral arteries of cats and rabbits is also dependent upon an intact endothelium [35,54]. There is not, to date, an agreement as to the chemical nature of the EDRF [19,22,31,44]. Prior to Furchgott's finding of the obligatory role of endothelial cells for relaxation of arteries by Ach [20], it was known that cultured endothelial cells could produce prostaglandins (PG), including prostacyclin (PGI_2), a potent vasodilator of vascular beds and a relaxant of many arteries [41]. Extensive research has been carried out regarding the possibility of a PG derivative as the actual EDRF [16,21,22,31,39,43,53,54]. Since the relaxing effect of muscarinic agonists on many arteries can be reversed by inhibitors of phospholipase A_2

Department of Physiology, Medical College of Virginia: * M.D., Ph.D.

Hawthorne Place 9B — 02114, Boston, Mass. — U.S.A.

and of lipoxygenase, but not of cyclooxygenase, it has been proposed that EDRF might be an unstable oxidation product of the arachidonic acid formed via the lipoxygenase pathway [3,16]. However, in a recent study using aortic preparation from rabbit, alone and in cascade experiments with isolated perfused coronary preparations, Griffith et al.[22] demonstrated that EDRF is not a lipoxygenase derivative or a free radical. They suggested that EDRF is an unstable compound with a carbonyl group at or near its active site. Moreover, by changing the length of the intervening tubing between aortic and coronary preparations, they were able to calculate the half-life of EDRF as 6.3 ± 0.6 sec. These authors concluded that EDRF is probably an aldehyde, ketone or lactone.

The regulation of cerebrovascular tonus is closely related to the brain tissue metabolic demands [34]. The adequacy of cerebral blood flow (CBF) is maintained despite large variations in systemic parameters such as arterial blood pressure, blood viscosity, and cardiac output [5,27,34,42]. The mechanism of this strict control is still poorly understood [5,28]. In pathological situations where cerebrovascular endothelium is affected (e.g., arterial hypertension, head injury), the coupling of CBF to brain metabolism is disturbed [21,30,31,49,50,60]. Thus, it seems likely that, in addition to its barrier role, the endothelial layer may play an active role in modulating cerebrovascular reactivity in normal and abnormal circumstances [54]. The following sections will discuss the neurogenic, chemical and metabolic regulation of CBF and their possible relationship to the endothelium dependent relaxing factor.

EDRF AND THE CEREBROVASCULAR NEUROGENIC CONTROL

Two distinct types of nerve ending can be distinguished in the walls of pial vessels by the use of ultrastructural techniques. One type is derived from fibers that originate in the superior cervical ganglion. It contains dense granular vesicles and is assumed to be adrenergic. The other, observed even in animals pretreated with 6-hydroxydopamine or 5-hydroxydopamine, contains agranular vesicles and is generally assumed to be cholinergic [36]. Corresponding with this ultrastructural evidence, functional studies of cerebral vessels *in vitro* have distinguished two types of response to transmural nerve stimulation (TNS). Stimulation of sympathetic nerves produces a contractile response in the pial vessels of rabbit, dog, sheep and monkey. After sympathetic denervation or treatment with guanethidine, nerve stimulation produces dilator response in pial vessels of several species. This dilator response is presumably cholinergically mediated [7]. In addition to the sympathetic and parasympathetic innervation, other systems can be visualized by immunohistochemical techniques. These fibers contain peptides such as vasoactive intestinal polypeptide (VIP)[33] and substance P [10], or monoamines such as serotonin [62].

Adrenergic innervation — Adrenergic fibers originating in the ipsilateral superior cervical ganglion innervate both pial and intracerebral vessels [14,28]. In support of this is the fact that all adrenergic fibers disappear after superior cervical ganglionectomy [9,36]. Also, it has been shown that adrenergic fibers originating in the locus coeruleus may innervate capillaries [24]. However, it is questionable that adrenergic fibers originating in the brain stem truly innervate cerebral vessels, since in many cases the nerve fibers are separated from vessels by a thick basement membrane [28]. With catecholamine fluorescence techniques the density and distribution of adrenergic fibers have been shown to be very similar in pial arteries of several animals and of humans [14]. $\beta-$, α_1- and α_2-adrenergic receptors have been identified and characterized in experiments using pharmacological models [9,14,55]. In the peripheral vessels generally α receptors mediante vasoconstriction while β receptors mediate vasodilation. This appears to be also true in the brain vasculature [5,54,55]. In the cat, stimulation of the cervical sympathetic nerves produces pial arteriolar vasoconstriction, as well as a mild decrease in CBF [28]. The magnitude of CBF decrease caused by sympathetic stimulation depends on the species studied [7]. Also, no significant constrictor response was detectable in smaller arterioles ($<100um$)[61]. The physiological significance of the pial arteries' constriction by sympathetic nerve stimulation is not clear [7]. On the other hand, studies of blood flow suggested that intraparenchymal vessels are generally quite responsive to catecholamine, and that adrenoreceptors in capillaries may modify water permeability as well as blood flow [5]. What is difficult to assess by this approach is the contribution of metabolic changes to vascular changes [14]. Additional problems include wide variability in the respon-

siveness of different vessels in one species and in vessels from other species [14,17]. For example, norepinephrine-induced contraction of human and monkey cerebral arteries are mediated by α_1 adrenoreceptors, while in dogs this effect appears to be mediated by α_2 adrenoreceptors [55]. Cerebral blood vessels responses to sympathetic nerve stimulation are relatively weak, clearly attributable to the very low responsiveness of their α-adrenergic receptors to norepinephrine [28,55]. This has led some investigators to suggest that cerebrovascular α-adrenoreceptors are different from those found in peripheral arteries [8,14]. Stimulation of β-adrenergic receptors causes cerebrovascular dilation. Evidence derived from a large variety of vascular preparations seems to exclude any dependence of this β-adrenergic dilation on the endothelium [54,55]. Of interest, however, is the fact that lack of endothelial cells potentiates the vasoconstriction effect of adrenergic agents. It is possible that viable endothelial cells could constantly release a specific factor acting on the adrenergic receptor-mediated mechanism of constriction [54]. This finding may be of considerable importance in understanding pathophysiological phenomena involving alterations of endothelial function, whether by mechanical lesion [28] or chemical lesion [50]. This endothelial role in the modulation of adrenergic cerebral vasoconstriction is likely to be related to development of cerebral vasospasm following traumatic brain injury or subarachnoid hemorrhage [26,47]. Also, it appears to be related to the vasoparalysis that follows such pathological states [31,50]. Although the lack of EDRF may not be the only cause of the abnormal vascular behavior in these pathological states, it seems likely that the endothelial cells may play an active role in modulating cerebrovascular responses to adrenergic stimuli [54].

Cholinergic innervation — Pial arteries are supplied with a well developed plexus of nonsympathetic cholinergic nerves. The close association of their endings with the smooth muscle cells at the surface of the media layer fulfills accepted ultrastructural criteria for a functioning neurovascular relationship [9,28]. The presence of muscarine receptors in intracerebral microvessels has been more controversial. It is believed that the cholinergic innervation of the intracerebral vessels, in the case of small arterioles and capillaries, is of intracerebral origin since there is no perivascular space at this vascular level. This system would parallel the adrenergic system arising from the locus coeruleus [13]. Although there is no direct evidence that such a system exists [14,28], indirect evidence supports the presence of a cholinergic control of the cerebral vasculature. For example, administration of acetylcholine intravascularly dilates pial arterioles and increases cerebral blood flow. Carbachol applied locally dilates pial arteries [50]. The autoregulatory cerebral vasodilation following a decrease in systemic arterial blood pressure is blocked by atropine [36]. The currently observed presence of muscarinic binding sites and choline acetyltransferase (ChAT) activity in brain capillary fractions also suggests that there may be a cholinergic innervation of brain capillaries [13,15,51]. However, this ChAT activity could be due to contamination by brain tissue instead of ChAT activity in the actual vessels' wall. Thus, the ChAT data alone is not sufficient to support the idea of cholinergic control of intracerebral arterioles and capillaries [14]. Parasympathomimetic compounds produce either a relaxation or a contraction of the cerebral vasculature. The relaxation occurs at low doses, and the response is inhibited in a competitive manner by atropine. The contraction occurs with high doses and appears to be mediated by muscarinic receptors [9]. When cholinergic fibers are activated by transmural electrical stimulation of cerebral arteries from cats, they induce significant vasodilation [7,28]. This vasodilation is not blocked by atropine [36]. Thus, besides its direct postjunctional vasodilatory action on muscarinic receptors in the vascular smooth muscle, cholinergic innervation can also promote pial vasodilation indirectly, through an inhibition of the norepinephrine release via the nicotinic receptors present on the perivascular sympathetic fibers [9]. Recently, *in vitro* studies have shown that Ach-induced relaxation of large cerebral arteries of cats and rabbits is dependent upon intact endothelium [35,54], suggesting that EDRF may play a role on the cholinergic control of cerebral circulation. The *in vitro* findings may not reflect the true mechanism of cholinergic control of cerebral circulation. As the nerve terminals lie at the arteries' adventitial-medial junction, it would be virtually impossible for neuronally released Ach to traverse the medial mass and reach the endothelium, except perhaps in third and fourth order arterioles where the medial layer is only the width of one or two muscle cells. However, the density of cholinergic innervation is extremely sparse in small arteries and great in larger muscular arteries [43,44]. Demonstration of [$_3H$]-quinuclidinylbenzilate (QNB) binding sites in the particulate fraction

of collagenase-treated cerebral vessel preparation strongly suggests that muscarinic receptors are located in the membrane of capillary endothelial cells. More muscarinic binding sites were found in microvessel fractions than in capillaries, suggesting that in the former preparation, [$_3H$]QNB probably binds to smooth muscle cells as well as to the endothelium [13]. Thus, Ach-induced relaxation of large cerebral vessels may be not dependent only upon EDRF. The presence of Ach receptors in the endothelial cells membrane suggests that EDRF play a role in the intracerebral vessel tonus control and may participate in the capillary blood flow regulation. Lesion of the endothelium of pial microvessels *in vivo* [48] by exposure of the vessels to filtered light from a mercury lamp, in presence of intravascular sodium fluorescein suppressed the Ach dependent relaxation. Moreover, after endothelial lesion the microvessels constricted in presence of Ach. These findings support the importance of EDRF on the control of cerebrosvascular circulation *in vivo* [50].

Peptidergic innervation — Research on the innervation of the cerebral blood vessels have disclosed peptidecontaining nerve fibers running along the pial blood vessels (e.g., substance P, VIP, pancreatic polypeptide, gastrin-releasing polypeptide, neurotensin, serotonin, neuropeptide Y, and somatostatin)[37,38]. The origin of these fibers is still unknown. They may be derived from the parasympathetic ganglions around the brain, but the question remains unresolved. Other peptides such as cholecystokinin and proopiomelanocortin related peptides have had their action tested in the pial arterioles. These studies demonstrated that such peptides are not important in cerebrovascular control when applied in physiological concentrations [38,56]. More is known about the action of VIP and substance P on cerebral vasculature. VIP-containing fibers reveal a spiral pattern similar to the muscle cell pattern. VIP-immunoreactive nerve terminals are present primarily in the inner layer of the adventitia. The intraventricular and intra-arterial injection of VIP is followed by increased CBF. In addition, topical application of VIP to the cerebral arterioles and veins causes vasodilation [10,58]. The location of the VIP terminal suggest a direct action of VIP on the muscle itself. The dependence on endothelium for VIP-induced dilation awaits testing. Substance P-containing fibers have a meshwork pattern in the cerebral blood vessels. Ultrastructural observation has shown that substance P immunoreactivity terminal boutons are present in the outer layer of the adventitia apart from the smooth muscle cells. Topical application of substance P to the pial arteriole and vein causes vasodilation [10]. Intracerebral injection of substance P leads to an increase of the local CBF [10]. Thus substance P, as in the systemic circulation, also causes vasodilation in the cerebral vasculature. This effect may very well be endotheliums dependent as is the case in other vascular beds [19,43].

EDRF AND THE CEREBROVASCULAR CHEMICAL REGULATION

The neurogenic control of cerebral circulation is not sufficient to explain physiological changes in CBF that take place under particular circumstances, such as variations in arterial blood pressure, changes in arterial PCO_2 and arterial PO_2, and responses of the cerebral vasculature to oscillations in the brain metabolic status. As we described above, the sympathetic vasoconstriction is rather weak. Furthermore, a generalized sympathetic or parasympathetic discharge would not control regional cerebral blood flow in areas of increased metabolism. Thus, in addition to the factors that externally regulate cerebral circulation, and in addition to the neurogenic control, the cerebral vessels themselves possess the ability to regulate their diameter. In this section we will review these intrinsic control mechanisms influencing cerebral blood flow and their possible relationship with the endothelium dependent relaxing factor.

Arterial PCO_2 and PO_2 cerebrovascular regulation — Arterial hypercapnia dilates cerebral blood vessels [50], increases CBF, and lowers cerebrovascular resistance (CVR) [27]. Arterial hypocapnia causes reverse changes in these variables. The effect of CO_2 is dependent on changes in hydrogen ion concentration of the extracellular fluid in the vicinity of the cerebral blood vessels [1]. Molecular CO_2 and the bicarbonate ion do not appear to have inherent vasoactivity, as shown by the fact that marked changes in cerebrospinal fluid PCO_2 and bicarbonate ion concentration do not alter pial arteriolar caliber unless a change in pH is allowed to occur [32]. The effects of PG in several vascular beds appear to be EDRF mediated [19,43,44]. It has been suggested that PG may participate in the cerebrovascular CO_2 reactivity since

the increase in CBF during hypercapnia was severely reduced following administration of indomethacin [47]. Several PG (PGI_2, PGE_2, PGG_2 and PGD_2) and their precursor, arachidonic acid, dilate pial arterioles when applied topically. In addtion, large cerebral vessels synthesize PG [11,12,23]. However, the participation of PG in response to CO_2 seems unlikely, since following the administration of cyclooxygenase, a severe inhibitor of the vasodilator effect of arachidonic acid, the responses of pial vessels to arterial hypercapnia, hypocapnia, and hypoxia are not altered [2,28]. The absence of significant involvement of changes in PG synthesis in the responses to CO_2 or to hypoxia do not exclude the possibility that PG may be important mediators of other physiological or of abnormal responses in the brain circulation [58]. There is evidence from experimental studies trat in pathological states such as mechanical brain injury and arterial hypertension, arachidonate is metabolized via the cyclooxyganase or lipoxyganase pathways. Its metabolism by either pathway generates a powerful free oxygen radical which closely resembles the free hydroxyl radical in its reactivity [18,29]. The action of this free radical on cerebral arterioles causes their vasodilator response to topical acetylcholine to be converted to vasoconstriction [25]. The vasodilation to topical acetylcholine is restored by topical application of superoxide dismutase and catalase. These results show that superoxide and other radicals generated in pathological conditions interfere with acetylcholine-induced endothelium dependent vasodilation, probably because they destroy the endothelium derived relaxant factor [59]. Although it is known that following the mentioned pathological states lesions exist in the cerebral arteriole endothelium [28,59,60], that responsiveness to changes in PCO_2 is impaired, and that the Ach-induced vasodilation is converted to vasoconstriction, there is no direct evidence of causal relationship [31]. Further *in vivo* studies using the light/dye model of microvascular endothelial denudation [48,50], with topical pharmacological manipulation and variation of systemic parameters may give insights as to the role of EDRF in cerebrovascular chemical regulation. Arterial hypoxia dilates pial arterioles, increases CBF, and lowers CVR [27]. These effects appear to be related to changes in local metabolism, since they are reverted by local application of oxygenated fluorocarbons [28]. Hypoxia-induced vasodilation is also dependent on the artery's previous tonus and the magnitude of the change in PO_2 [43,44].

Metabolic cerebrovascular regulation — There is a strong relationship between the level of functional activity and metabolic rate of the brain on the one hand, and global or regional CBF on the other [27,34]. Physiological activation of specific areas of the cortex by various types of sensory stimulation, or in association with motor activity, leads to an increased blood flow to the activated areas. These areas also present increased metabolism [34]. It is generally believed that the relationship between CBF and metabolism is dependent on the production of vasodilator metabolites by the neural cells. CBF, in turn, is modulated by these metabolite levels in the perivasular space. There are a large number of candidates that may serve as mediators of metabolic flow regulation. At present, adenosine, hydrogen ions and potassium ions appear to be the most promising candidates. Adenosine is a strong dilator of pial vessels when applied in the perivascular space. Brain adenosine concentration increases under conditions of arterial hypoxia, ischemia, or increased metabolic activity of the brain [28,45,46]. Although dependence on endothelium of the adenosine cerebrovascular relaxation has not been tested, data from other vascular beds suggest that it may not be EDRF mediated [43,44].

Cerebrovascular autoregulation — Two basic mechanisms have been proposed to explain autoregulation in the brain: the myogenic mechanism, which holds that cerebral vessels are responsive to changes in transmural pressure, and the metabolic mechanism, the basic premise of which is that changes in cerebral vascular caliber are the result of alterations in the concentration of vasodilator metabolites. These, in turn, are induced by alterations in blood flow secondary to the changes in pressure. The second theory is more likely [28]. Adenosine concentration in the extracellular brain tissue has been demonstrated to rise quickly during hypotension [28]. Adjustment of pial arteriole diameter during variations in arterial blood pressure is not followed by changes in extracellular H^+ and K^+ activity [57]. Chemical regulation and autoregulation of CBF are not dependent on α and β-adrenoceptors [5]. Thus, adenosine, known to be an endothelium independent vasorelaxant in several vascular beds [43,44], is a strong candidate for mediating of vasodilation during pressure autoregulation [45,46]. It would also be of interest to test whether or not the viscosity autoregulation [42] is EDRF mediated.

CONCLUSIONS

At this point in time there is no direct evidence that EDRF plays a vital role in cerebrovascular regulation. However, indirect evidence suggests that EDRF may be involved in the modulation of cerebral sympathetic vasocontriction and parasympathetic vasodilation. There are no experiments that have tested the known chemical regulators of the cerebrovascular circulation in models of denuded endothelim cerebral vessels. Findings in experimental models of pathological entities such as mechanical head injury, arterial hypertension and subarachnoid hemorrhage, known to have cerebrovascular endothelium lesions, suggest that EDRF serves an important function in regulating cerebral circulation under such circumstances. *In vitro* studies of isolated large cerebral vessels and cultured cerebral microvessels[6], complemented with *in vivo* studies of light/dye denuded pial arteries, may clarify the role of endothelium dependent relaxing factor in the regulation of cerebral circulation.

REFERENCES

1 Betz E, Heuser D — Cerebral cortical blood flow during changes of acid-base equilibrium of the brain. J App Physiol 23:726, 1967.

2. Busija DW — Role of prostaglandins in modulating sympathetic vasoconstriction in the cerebral circulation in anesthetized rabbit. JCBFM 5:17, 1985.

3. Capdevila J, Parkhill L, Chacos N, Okita R, Masters BSS, Estabrook RW — The oxidative metabolism of arachidonic acid by purified cytochromes P-450. Biochem Biophys Res Comm 101:1357, 1981.

4. Cocks TM, Angus JA — Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin. Nature 305:627, 1983.

5. Davis HD, Sundt MT Jr — Relationship of cerebral blood flow to cardiac output, mean arterial pressure, blood volume and α and β blockade in cats. J Neurosurg 52:745, 1980.

6. DeBault LE, Kahn LE, Frommes SI', Cancilla PA — Cerebral microvessels and derived cells in tissue culture: isolation and preliminary characterization. In Vitro 15:473, 1979.

7. Duckles SP — Neurogenic dilator and constrictor responses of pial arteries in vitro. Cir Res 44:482, 1979.

8. Duckles SP, Bevan JA — Pharmacological characterization of adrenergic receptor of a rabbit cerebral artery in vitro. J Pharmacol Exp Ther 197:371, 1976.

9. Edvinsson L, Falck B, Owman Ch — Possibilities for cholinergic action on smooth muscle and on sympathetic axons in brain vessels mediated by muscarine and nicotine receptors. J Pharmacol Exp Ther 200:117, 1977.

10. Edvinsson L, McCulloch J, Uddman R — Substance P: immunohistochemical localization and effect upon cat pial arteries in vitro and in situ. J Physiol 318:251, 1981.

11. Ellis EF, Wei EP, Kontos HA — Vasodilation of cat cerebral arterioles by prostaglandins D2, E2, G2, and 12. Am J Physiol 237 (Heart Cir Phys): H381, 1979.

12. Ellis EF, Nies AS, Oates — Cerebral smooth muscle contraction by thromboxane A_2. Stroke 8:480, 1977.

13. Estrada C, Hamel E, Krause DN — Biochemical evidence for cholinergic innervation of intracerebral blood vessels. Brain Res 266:261, 1983.

14. Ferrari-Dileo G, Potter LT — α-adrenoreceptors and muscarine receptors in human pial arteries and microvessels: a receptor binding study. JCBFM 5:458, 1985.

15. Florence VM, Bevan JA — Biochemical determinations of cholinergic innervation in cerebral arteries. Circ Res 45:217, 1975.

16. Forstermann U, Neufang B — The endothelium-dependent vasodilator effect of acetylcholine: characterization of the endothelial relaxing factor with inhibitors of arachidonic acid metabolism. Eur J Pharmacol 103:65, 1984.

17. Friedman AH, Davis JN — Identification and characterization of adrenergic receptors and catecholamine stimulated adenylate cyclase in hog pial membranes. Brain Res 183:89, 1980.

18. Fridovich I — The biology of oxygen radicals. Science 201:875, 1978.

19. Furchgott RF — Role of endothelium in responses of vascular smooth muscle. Cir Res 53:557, 1983.

20. Furchgott RF, Zasadzki JV — The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288:373, 1980.

21. Goldby FS, Bellin LJ — How an acute rise in arterial pressure damages arterioles. Cardiovasc Res 6:569, 1972.

22. Griffith TM, Ewards DH, Lewis MJ, Newby AC, Henderson AH — The nature of endothelium-derived vascular relaxant factor. Nature 308:645, 1984.
23. Hagen AA, White RP, Robertson JT — Synthesis of prostaglandins and thromboxane B - 2 by cerebral arteries. Stroke 10:306, 1979.
24. Hartman BK, Swanson LW, Raichle ME, Preskorn SH, Clark HB — Central adrenergic regulation of cerebral microvascular permeability and blood flow: anatomical and physiological evidence. Adv Exp Biol 131:113, 1980.
25. Heinle H — Vasoconstriction of carotid artery induced by hydroperoxides. Arch Intern Physiol Biochem 92:267, 1984.
26. Kassell NF, Sasaki T, Colohan ART, Nazar G — Cerebral vasospasm following aneurismal subarachnoid hemorrhage. Stroke 16:562, 1985.
27. Kety SS — Circulation and metabolism of the human brain in health and disease. Am J Med 8:205, 1950.
28. Kontos HA — Regulation of the cerebral circulation. Ann Rev Physiol 43:397, 1981.
29. Kontos HA, Wei EP, Christman CW, Lavasseur J, Povlishock JR, Ellis EF — Free oxygen radicals in cerebral vascular responses. Physiologist 26:165, 1983.
30. Kontos HA, Wei EP, Dietrich D, Navary RM, Povlishock JT, Ghatak NR, Ellis EF, Patterson JL Jr — Mechanism of cerebral arteriolar abnormalities after acute hypertension. Am J Physiol 240 (Heart Circ Physiol 9): H-511, 1981.
31. Kontos HA, Wei EP, Povlishock JT, Christman CW — Oxygen radicals mediate the cerebral arteriolar dilation from arachidonate and bradykinin in cats Cir Res 55:295, 1984.
32. Kontos HA, Wei EP, Raper AJ, Rosenblum WI, Navari RM, Patterson JL Jr — Role of tissue hypoxia in local regulation of cerebral microcirculation. Am J Physiol 234:H582, 1978.
33. Larsson LI, Edvinson L, Fahrenkrug O, Hakanson R, Owman CH, Schaffalitzky de Muckadell O, Sundler F — Immunohistochemieal localization of vasodilator polypeptide (VIP) in cerebrospinal nerves. Brain Res 113:400, 1976.
34. Lassen NA — Brain. In P.C. Johnson (ed): Peripheral Circulation. John Wiley & Sons. New York, 1978, pg 337.
35. Lee TJ-F — Cholinergic mechanism in the large cat cerebral artery. Cir Res 50:870, 1982.
36. Lee TJ-F, Hume WR, Su C, Bevan JA — Neurogenic vasodilation of cat cerebral arteries. Cir Res 42:535, 1978.
37. McCulloch J, Edvinsson L — Cerebrovascular smooth muscle reactivity: a critical appraisal of in vitro and in situ techniques. JCBFM 4:129, 1984.
38. McCulloch J, Kelly PAT — Effects of cholecystokinin octapeptide on pial arteriolar diameter. JCBFM 4:625, 1984.
39. Mey JG De, Claeys M, Vanhoutte PM — Endothelium-dependent inhibition effects of acetylcholine, adenosine triphosphate, thrombin and arachidonic acid in the canine femoral artery. J Pharmacol Exper Therap 222:166, 1982.
40. Mey JG De, Vanhoutte PM — Heterogeneous behavior of the canine arterial and venous wall; importance of the endothelium. Cir Res 51:439, 1982.
41. Moncada S, Herman AG, Higgs EZ, Vane JR — Differential formation of prostacyclin (PGX or $PG/_{o}$) by layers of the arterial wall: an explanation for the anti-thrombotic properties of vascular endothelium. Trom Res 11:323, 1977.
42. Muizelaar JP, Lutz III HA, Becker DP — Effect of mannitol on ICP and CBF and correlation with pressure autoregulation in severely head-injured patients. J Neurosurg 61:700, 1984.
43. Peach MJ, Loeb AL, Singer HA, Saye J — Endothelium-derived vascular relaxing factor. Hypertention 7(Suppl 1):194, 1985.
44. Peach MJ, Singer HA, Loeb AL — Mechanisms of endothelium-dependent vascular smooth muscle relaxation. Biochem. Pharmacol 34:1867, 1985.
45. Phillis JW, DeLong RE, Towner JK — Adenosine deaminase inhibitors enhance cerebral noxic hyperemia in the rat. JCBFM 5:296, 1985.
46. Phillis JW, Preston G, DeLong RE — Effect of anoxia on cerebral blood flow in the rat brain: evidence for a role of adenosine in autoregulation. JCBFM 4:586, 1984.
47. Pickard JD, Vinall PE, Simeone FA — Prostaglandins and cerebral vasospasm: a problem of interpretation. Surg Forum 26:496, 1975.
48. Povlishock JT, Rosenblum WI, Sholley MM, Wei EP — An ultrastrutural analysis of endothelial change paralleling platelet aggregation in a light/dye model of microvascular insult. Am J Path 110:148, 1983.
49. Rosenblum WI, Wei EP, Kontos HA — Platelet aggregation in cerebral arterioles after percussive brain trauma. Tex Heart Inst J 9:345, 1978.

50. Rosenblum WI, Wei EP, Kontos HA — Microvascular endothelial dependent relaxing factor (EDRF) in vivo: interference by minimal endothelial injury. Fed Proc Abst 4980, 1985.
51. Saito A, Wu J-Y, Lee TJ-F — Evidence for the presence of cholinergic nerves in cerebral arteries: an immunohistochemical demonstration of choline acetyltransferase. JCBFM 5:327, 1985.
52. Saye JA, Singer HA, Peach MJ — Role of endothelium in conversion of angiotensin I to angiotensin II in rabbit aorta. Hypertension 6:216, 1984.
53. Singer HA, Saye JA, Peach MJ — Effects of cytochrome P-450 inhibitors on endothelium-dependent relaxation in rabbit aorta. Blood Vessels 21:223, 1984.
54. Sercombe R, Verrechia C, Oudart N, Dimitriadou V, Seylaz J — Pial artery response to norepinephrine potentiated by endothelial removal. JCBFM 5:312, 1985.
55. Toda N — α-adrenergic receptor subtypes in human, monkey and dog cerebral arteries. J Pharm Exp Ther 226:861, 1983.
56. Wahl M — Effects of enkephalins, morphin, and naloxone in pial arteries during perivascular microaplication. JCBFM 5:451, 1985.
57. Wahl M, Kuschinsky W — Unimportance of perivascular H+ and K+ activities for the adjustment of pial arterial diameter during changes of arterial blood pressure in cats. Pflugers Arch 382:203, 1979.
58. Wei EP, Ellis EF, Kontos HA — Role of prostaglandins in pial arteriolar response to CO_2 and hypoxia. Am J Physiol 238 (Heart Cir Physiol 7): H226, 1980.
59. Wei EP, Kontos HA, Christman WC, DeWitt DS, Povlishock JT — Superoxide generation and reversal of acctylcholine-induced cerebral arteriolar dilation after acute hypertention. Cir Res 57, 1985 (in press).
60. Wei EP, Dietrich WD, Povlishock JT, Navari RM, Kontos HA — Functional, morphological, and metabolic abnormalities of the cerebral microcirculation after concussive brain injury in cats. Cir Res 46:37, 1980.
61. Wei EP, Raper AJ, Kontos HA, Patterson JL Jr — Determinants of response of pial arteries to norepinephrine and sympathetic nerve stimulation. Stroke 6:654, 1975.
62. Welch KMA, Helpern JA — Do serotonin and noradrenalin neurons project to cerebral capilaries? In Heistad CC, Marcus ML (eds): Cerebral Blood Flow: Effects of Nerve and Neurotransmitters. Elsevier North Holland, New York. 1981, pg 551.