Annu. Rev. Pharmacol. Toxicol. 1991. 31:229–51

PHARMACOLOGICAL CONTROL OF THE CEREBRAL CIRCULATION*

Oscar U. Scremin

Veterans Affairs Medical Center and Department of Neurology, University of New Mexico, Albuquerque, New Mexico

KEY WORDS: cerebrovascular circulation, drug effects, blood vessels, neurotransmitters

PHYSIOLOGICAL CONCEPTS

This review is about control of a physiological variable; what that variable's function is and how it is regulated must be discussed first to establish a rational basis for its control. The reader is referred to some excellent recent reviews on regulation of cerebral blood flow (CBF)[1] (1–3). We emphasize here some functions of CBF that are not so obvious and have been largely overlooked.

Cerebral blood vessels evolved as the volume of neural structures made diffusion an inefficient process to supply oxygen and nutrients to nerve cells. This being the primary drive, a close correlation can be found between intercapillary distance or capillary surface area and the level of energy exchange of the various brain regions (4–6). This structurally determined

[1]Abbreviations used: acetyl-coenzyme A, Acetyl-CoA; acetylcholine, ACh; adenosine triphosphate, ATP; central nervous system, CNS; cerebral blood flow, CBF; cerebral glucose utilization, CGU; choline, Ch; choline acetyl-transferase, ChAT; chronic obstructive pulmonary disease, COPD; endothelium derived relaxing factor, EDRF; gamma-amino butyric acid, GABA; glutamic-acid-decarboxylase, GAD; magnesium sulfate, $MgSO_4$; partial pressure of CO_2, PCO_2; positron emission tomography, PET; serotonin, 5-HT; single photon emission computed tomography, SPECT.

coupling between neural function and blood flow takes care of the anatomical diversity of the nervous system that imposes extremely variable levels of metabolic requirements. This coupling between blood flow and function is determined during development and remains essentially stable throughout adult life.

On the other hand, adaptation to a dynamic environment imposes varying functional regimes for the whole brain or parts of it. As far as the brain as an ensemble is concerned, we can cite the blood flow adjustments that occur through the sleep-wakefulness cycle (7, 8) and the mental activity-relaxation cycles (9). Temporary shortages of the supply of energy substrates such as during hypoxia also require transient global adjustments of the cerebral circulation (2). Very localized changes on the other hand can be induced by sensory stimulation (10–12). These adjustments require rapid variations in cerebrovascular resistance to make possible a greater rate of local cerebral perfusion to increase the delivery rate of energy substrates. But there are other consequences of an increased tissue circulatory convection. The level of tissue CO_2, for instance, is reciprocally related to the rate of CBF if the production of this metabolite is constant (13). It is well known that PCO_2 is a determinant of neural reflex excitability (14–16). Variations in the rate of blood flow, if induced in the absence of changes in CO_2 production, may therefore be paralleled by proportional changes in reflex function.

Blood flow can also influence synaptic transmission in a more direct way. After a neurotransmitter has been released, it is subjected to a process of elimination from synaptic sites by three main mechanisms: enzymatic degradation, re-uptake into synaptic terminals, and diffusion. The last is facilitated by circulatory convection since the capillaries act as sinks, maintaining a steep gradient that makes the diffusion mechanism efficient. An active synaptic center needs a rate of blood flow in phase with its level of activation to work under optimal conditions, not only to provide adequate energy supply but also to assist in termination of transmitter action. Classic proof of this phenomenon was given by the ganglion perfusion experiments of Feldberg & Gaddum (17) and MacIntosh (18) in which acetylcholine (ACh) was recovered from the venous effluent in amounts commensurate with the level of synaptic activity. This is a pertinent example since diffusion is the main mechanism of termination of ACh action in sympathetic ganglia.

Regulation of acetylcholine synthesis within the central nervous system includes an important role for the vascular system, schematically depicted in Figure 1. ACh is synthesized in the brain from choline (Ch) and acetyl-coenzyme A (Acetyl-CoA) by a reversible reaction catalyzed by choline acetyl-transferase (ChAT) (Figure 1, no 1). The rate of synthesis of ACh appears to be regulated by the availability of the precursors and concentration

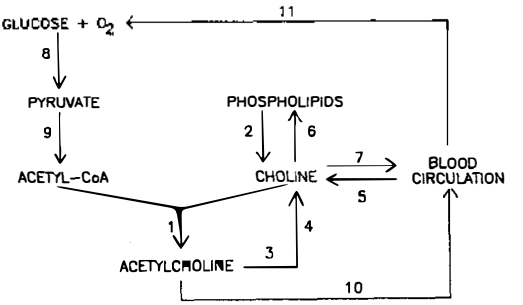

Figure 1 Interactions between ACh synthesis and CBF. Numbers in this scheme correspond to those within parentheses in the text description.

of the products (19–21). Since methylation of phosphatidylethanolamine in brain tissue is very small (20, 22–24), de novo synthesis of Ch can be considered negligible. The sources of Ch for ACh formation are degradation of choline-containing phospholipids within the brain itself (Figure 1, no 2), Ch derived from enzymatic hydrolysis of released ACh at synaptic sites (Figure 1, no 3) and salvaged by a high-affinity uptake system (Figure 1, no 4) (19–21), and plasma Ch (Figure 1, no 5). There is a net production of Ch in the brain from phospholipids (Figure 1, no 7) that is removed by the circulation and produces a standing arteriovenous concentration difference (25, 26). Cerebral venous blood contains about 1.5 times higher concentration of Ch than arterial blood entering the brain (27). Given a constant production rate of Ch, it can be hypothesized that the brain concentration of free Ch will bear an inverse relationship to the rate of CBF. In fact, a linear correlation between tissue free Ch and the reciprocal of local blood flow has been found by measuring Ch concentration and local blood flow in the same brain regions of animals with a middle cerebral artery occlusion (28). Since the rate of ACh synthesis is controlled by Ch availability (21, 23), variations in CBF could induce reciprocal changes in the rate of ACh synthesis through this mechanism.

The other precursor of ACh, acetyl-CoA, is generated by oxidative decarboxylation of pyruvate (Figure 1, no 9) that in turn originates from aerobic degradation of glucose (Figure 1, no 8) (29). This is also a blood flow-dependent process but here the sign is reversed: decreases in blood flow will lead to a decrease of Acetyl-CoA availability and thus slow down the rate of ACh synthesis. Cholinergic function can then be driven in quite opposite ways by changes in CBF. These two sets of processes with opposing influences on brain tissue ACh may balance at CBF levels close to normal, exerting a stabilizing influence on tissue ACh contents. This mechanism

seems to operate even at moderate levels of ischemia, with the result that brain tissue ACh content remains constant in this condition (28). Since the synthesis of ACh obeys the law of mass action (19–21) the excess Ch induced by hypoperfusion compensates for the decrease in acetyl-CoA, the supply of which, as stated above, depends on oxidative metabolism. CBF serves in this case (by virtue of a decreased clearance of Ch) as a means of achieving an "autoregulation" of ACh levels. At a critical level of CBF of approximately 0.02 ml/g/min, however, the limit of operation of this autoregulatory mechanism is reached and tissue ACh concentration falls (28, 30, 31). It is now well established that endogenous ACh can increase CBF (Figure 1, no 10; see detailed discussion below) and we can then envision another feed-back loop that will act to stabilize this variable. A decrease in CBF will increase Ch concentration and by a precursor loading effect enhance availability of ACh, which will, in turn, restore CBF levels.

During the reperfusion following cerebral ischemia on the other hand, oxidative metabolism is restored faster than Ch can be washed out and a condition of high tissue Ch levels is found that leads to a transient excess of tissue ACh, likely brought about by a precursor loading effect (31). In the examples discussed above, the level of blood flow controls the rate of transmitter synthesis, by changing the availability of its precursors.

An analogous phenomenon may operate regarding the synthesis of gamma-amino butyric acid (GABA). The enzyme that synthesizes this transmitter, glutamic-acid-decarboxylase (GAD), like ChAT, does not depend on oxidative metabolism. Degradation of GABA, however, does (32). It is conceivable that this process may underlie the large increase in GABA tissue levels observed in areas of focal cerebral ischemia (33).

CEREBRAL BLOOD FLOW AS A CONTROLLING VARIABLE

In light of the concepts outlined above, we can then visualize two processes. In one, which for the purpose of this discourse we can call the regulator mode, the flow diagram is: {neural function} → {energy demand} → {CBF} → {energy supply}. The role of CBF in this case is that of a regulatory variable, providing enhanced delivery rates of energy substrates to support function. In the other process, the information flow is: {CBF} → {neuronal environment} → {neural function}. Here, CBF operates to determine the functional level of a neural center through a number of mechanisms such as removal of neurotransmitters after their release, changes in the gaseous and ionic environment, and control of precursor availability for transmitter synthesis.

The first process, in which CBF acts as a regulator, is expressed by the rapid adjustments of CBF levels to metabolic requirements that occur during phasic changes in brain activity. Although the mechanisms that mediate this dynamic coupling are outside the scope of this review, simplistic explanations, such as that of pH as the universal mediator for this phenomenon, have been abandoned (1). There is probably not a single mechanism for this "coupling", given that the degree of matching between CBF and metabolic expenditure varies for different brain regions and that this phenomenon is sensitive to pharmacological intervention in some regions but not in others. For example, the increase in cerebral cortical blood flow that accompanies arousal can be enhanced by inhibition of acetylcholinesterase and blocked by atropine (34, 35). Changes in the slope of the function that relates CBF to cerebral glucose utilization (CGU) for a large number of brain regions have been described following acidosis (36), or administration of indomethacin (37), hydroxybutirate (38), ergot alkaloids (39), or norepinephrine (40).

The second process mentioned above, in which CBF acts as a controller, requires primary changes in blood flow. This can occur by hemodynamic conditions, such as variations of systemic perfusion pressure outside the limits of autoregulation, or by vascular narrowing or embolism. There are other more interesting possibilities. Changes in cerebrovascular resistance can be induced experimentally by stimulation of discrete regions within the central nervous system (CNS). Both vasodilatation and vasoconstriction have been described (41–44). Both types of responses can be modulated pharmacologically and specific mediators have been proposed for several of these actions. It is then conceivable that certain regions of the CNS might exert their influence over remote ones through blood flow operating in a controller mode, i.e. by acting in anticipation or even in the absence of metabolic variations to induce global changes in neuronal excitability. Although the concept exposed above is still largely hypothetical from the physiological point of view, it is clear that pharmacological intervention can induce primary vascular effects with functional consequences.

THE CHALLENGE OF A COMPLEX SYSTEM

Cerebral resistance vessels are unique in that they are embedded in a neural matrix. In any other organ, neural effects can be ruled out by selective neurectomies. For example, the role of autonomic and dorsal root afferents have been dissected in many organs with great accuracy and detail by selective ablations. No such accurate dissection is possible in the CNS except by the use of neurotoxins, or by electrical stimulation in a volume conductor, both of which offer little selectivity. Furthermore, the brain is the most

heterogenous of all organs and is so structured that unitary analysis is only possible with great limitations.

Measurement of force development by isolated cerebral vessels in vitro is being used increasingly to analyze this element of the complex system, but the results in this situation may have little resemblance to the in vivo ones. Sokoloff referred thus to cerebral metabolism: "The in vitro techniques establish only the existence and potential capacity of the enzyme systems required for the utilization of a given substrate; they do not define the extent to which such a pathway is actually utilized in vivo" (45). This concept can be extended to the cerebral circulation. Receptors present in cerebral vessels are hidden in vivo to most substances, from the blood side by the blood-brain barrier in the endothelium and from the tissue side by a tight covering of glial end feet. These barriers disappear in in vitro preparations that, in addition, are limited to the study of relatively large conducting vessels. Thus, paraphrasing Sokoloff, we can say that such techniques reveal the existence of potential vasoactive mechanisms, only a subset of which may represent the physiological mechanisms. Likewise, pharmacological effects demonstrated in vitro cannot be extrapolated to the in vivo situation.

A clear example can be found in the effects of physostigmine on CBF shown in Figure 2. In vitro techniques tell us that vasodilatation in response to ACh is an endothelium-dependent response that can be found in practically all vessels, including extracerebral ones (46). Moreover, a cholinergic innervation has been demonstrated in extra- and intraparenchymal cerebral vessels throughout the CNS (47, 48) and cholinergic vascular receptors are also widely distributed in the brain (48). All these facts predict a generalized vasodilator effect when the availability of acetylcholine is enhanced through inhibition of the enzyme acetylcholinesterase. However, the result of such intervention in the intact, conscious rat is a very specific increase in CBF in the neocortex, with lack of effect in striatum and olfactory cortex (Figure 2; 49). Other examples can be found in the effects of norepinephrine and serotonin (consistent vasoconstriction in vitro and extremely variable effects on CBF) (50), and of indomethacin, which can decrease CBF although no good model of this phenomenon has been described in vitro (51). These apparent contradictions stress the limitations of reductionistic approaches that, while necessary to analyze the functional elements, tell little about the response of the intact system which is the ultimate objective of therapeutic efforts.

THE IMPACT OF METHODOLOGICAL INNOVATION

Our concepts of CBF function and control have evolved in parallel with the development of CBF measurement methodologies. Before this variable was

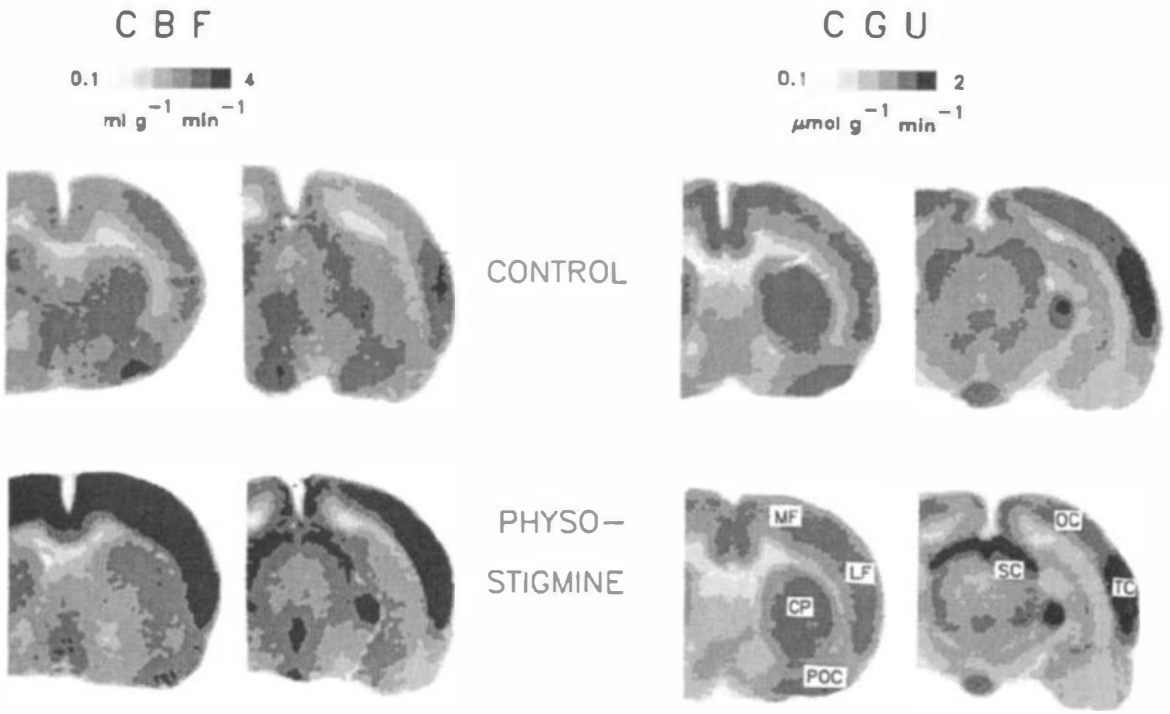

Figure 2 Regional CBF and CGU were measured with Iodo-^{14}C-antipyrine and ^{14}C-2-deoxy-glucose respectively. The figure depicts rat brain autoradiographs at two coronal planes after transformation of optical density to CBF or CGU levels, represented in the corresponding bars. Abbreviations for key structures shown in the right bottom panels are: MF = medial frontal cortex; LF = lateral frontal cortex; CP = caudate-putamen; POC = primary olfactory cortex; OC = occipital cortex; TC = temporal cortex; SC = superior colliculus. Following physostigmine, a marked increase in CBF is observed in frontal, occipital and temporal cortex but without changes in CGU. Superior colliculus shows a parallel increase in CBF and CGU while no changes in CBF or CGU are observed in the rest of the regions. This illustration exemplifies the need for high spatial resolution to reveal regional variations of drug effects on CBF and metabolism.

ever measured, dogma (expressed in the Monro-Kellie doctrine) had it that CBF was invariant. The development of the heat clearance techniques and other measuring devices quickly changed these concepts (52). Not until Kety & Schmidt made (53) the first quantitative measurements of human subjects blood flow, however, were other erroneous theories relating to a determinant role of CBF mental disorders dispelled (54).

The advent of autoradiographic techniques has allowed detection of regional CBF in experimental animals with a spatial accuracy of 10 lines/mm. Since blood flow has practical meaning only in the context of a minimal mass of tissue, certainly greater than a few cells or a single blood vessel, this probably represents the highest resolution worth achieving. In human subjects, positron (55, 56), single photon emission (57), and Xenon-enhanced transmission (58, 59) tomographic procedures have increased accuracy and resolution of CBF measurements over previous bidimensional techniques. This has opened new vistas in pharmacological control of blood flow since this variable can now be explored in very circumscribed areas of the CNS. In keeping with the anatomical and functional diversity of the CNS, wide regional variations in CBF responses to drugs and interventions are now known to occur.

A number of autoradiographic techniques have been designed to obtain pictorial representations of various blood flow-related variables such as glucose utilization (63), tissue pH (61), and protein synthesis (62). The most important of these, in terms of the considerable insight that it has allowed into brain metabolism, is the 2-deoxyglucose methodology developed by Sokoloff and his collaborators (63). An example of the type of information that can be obtained with these techniques is given in Figure 2. Quantitative autoradiography of radioactively labeled compounds permits, in association with CBF autoradiography, a very accurate determination of drug concentration-CBF relationships (64).

All blood flow imaging techniques are based on exchange of a tracer molecule between blood and tissue. This imposes time limitations so that the blood flow measurements obtained in this way represent an average over a period of time in the order of minutes. Rapid adjustments of CBF are then not detected. A number of techniques are available in experimental animals to follow rapid changes in CBF. Most rely on electromagnetic or doppler ultrasound measurements on large vessels supplying or draining brain regions with minimal or no extracerebral contamination. This has been possible in baboons (65) and rabbits (66). These animals possess internal carotid arteries that irrigate almost exclusively the forebrain, whereas other animals require intricate dissections and multiple blood vessel occlusions to isolate the cerebral circulation. In human subjects, the introduction of the duplex doppler ultrasound technique allows a similar time resolution to be achieved by noninvasive measurements of blood flow of the internal carotid artery ex-

tracranially (67) or of intracranial blood vessels (68). These techniques are particularly useful to assess cerebrovascular adaptations in which the time factor is of critical importance, as in the adjustment of cerebrovascular resistance to changes in arterial blood pressure (69). Transcranial doppler ultrasonography has provided information on the dynamic response of regional CBF to sensory activation. A recent elegant study by Conrad & Klingelhöfer (68) has described the dynamic effects of complex visual patterns on local perfusion of the occipital cortex of human subjects and this provides complementary information to that of PET imaging studies (11). Unfortunately, neither in animal experimentation nor in human studies can a single CBF-measuring technique achieve both adequate spatial and temporal resolution.

The tissue hydrogen clearance, first used in the brain by Misrahy & Clark (70), has provided useful insights into the relationships between local blood flow and somatosensory-evoked potentials in experimental animals. This technique is based on the Fick principle. Typically, hydrogen gas is administered by inhalation at a small (3%) nonflammable concentration. A platinum electrode is inserted in the desired region to detect tissue hydrogen concentration amperometrically. When hydrogen gas administration is interrupted, its concentration in tissue decays following first order kinetics. The coefficient of the logistic washout slope represents tissue blood flow (71). The electrodes must be made of small dimensions (typically 25 μm) to avoid tissue injury and artifactual modification of blood flow. Electrode voltage can be recorded in parallel to hydrogen current to analyze electroencephalographic or evoked activity from very small regions within the CNS (34). In this way, Branston et al (72) have correlated somatosensory-evoked activity with blood flow following middle cerebral artery occlusion, and determined the blood flow thresholds for abolition of the different components of this response. It is interesting that certain pharmacological effects on the cerebral cortex, such as the cholinergic inhibition of somatosensory-evoked activity, do not have a metabolic correlate, i.e. are not amenable to analysis by measurement of CGU. We used the H_2 clearance technique to study this phenomenon and thus discovered a dissociation between electrical activity and blood flow under the action of direct cholinergic agonists that induced a substantial increase in local cortical blood flow at a time when the somatosensory response was maximally inhibited (34).

INTRACEREBRAL STEAL, A MYTH?

Focal cerebral ischemia is a situation in which increasing CBF seems an obvious therapeutic option. Two questions must be answered, however, before that task is even attempted: (*a*) can cerebral vasodilators improve blood

flow in ischemic areas? and (*b*) if so, can any benefits be expected from such intervention? There is a long history behind the first question. It was widely held during the first three quarters of this century that CBF was regulated by local pH and that areas of cerebral ischemia were acidotic and maximally dilated. It was believed that the use of cerebral vasodilators under such circumstances could only decrease CBF in ischemic areas that could not dilate further, by diverting it to normally perfused tissue that could (intracerebral steal). The theoretical foundation of this concept rests on the assumption that normal and ischemic tissues constitute two hydraulic resistances in parallel supplied by the same vessel and that neither the parent vessel nor the ischemic tissue arm of the network can decrease its resistance in response to the dilator action.

These two assumptions have been challenged by direct measurements of such resistances in cats. Vasodilator drugs were found to decrease consistently the resistance of the vessels supplying the ischemic tissue and in many cases the vascular resistance of the ischemic tissue itself (73). In spite of early experimental evidence for the existence of intracerebral steal (74–76), there are numerous circumstances in which no intracerebral steal, but rather an increase in blood flow of ischemic areas has been found with the use of cerebral vasodilators such as CO_2 (77, 78), papaverine (79), nimodipine (80), and physostigmine (81) in experimental animals. More evidence against the intracerebral steal concept is provided by studies with acetazolamide, a carbonic anhydrase inhibitor. This agent induces cerebral vasodilatation in normal and ischemic brain (82) by a mechanism that is still poorly understood. The simplicity of its use and the rapid and transient nature of its effect has made this substance a useful tool to explore cerebrovascular responses in humans. Single photon emission computed tomography (SPECT) measurements have shown that CBF increases more in normal than in ischemic regions, enhancing contrast between these areas and facilitating diagnosis. In all cases of a series reported by Chollet et al (83), cerebral vasodilatation in response to acetazolamide was depressed in the symptomatic hemisphere in comparison to the asymptomatic one but no instances of a decrease in CBF in the symptomatic side were observed.

The second question posed above has to be approached with knowledge of the physiological response to cerebral ischemia. When blood flow through one of the main cerebral vessels is interrupted, a gradient of ischemia is observed. A central area of minimal blood flow that may lead to permanent tissue damage is surrounded by a zone of subnormal blood flow. Although electical activity may be suppressed in this zone, commonly referred to as the "ischemic penumbra" (84), ion homeostasis and cellular structure are minimally altered. Strong et al (85) found the levels of CBF in this area to be comparable to those compatible with total restoration of function upon

reestablishment of normal perfusion. This zone of "penumbra", which has been recently detected in cases of human stroke (86–88), may eventually recover full neural function if its vascular resistance can be lowered so as to restore normal blood flow. Trials in human subjects, with measurement of CBF with SPECT techniques, have already shown the possibility of increasing blood flow in this area with hemodilution (89), nimodipine (90), and physostigmine (91). Functional status following these interventions has so far been studied only in the case of hemodilution in which a definite improvement, related to the magnitude of the CBF changes, was found (89, 92). Beneficial effects of nimodipine in ischemic stroke have also been reported (93) although effects of this drug on intracellular calcium may be in part responsible for this outcome.

STRATEGIES TO ENHANCE CBF

Several strategies have been tried to accomplish this task. These can be grouped according to the basic physiological principles involved: Increase in perfusion pressure, decrease in cerebrovascular resistance, and decrease in blood viscosity.

Changes in perfusion pressure When the deficit in cerebral perfusion is the consequence of hemodynamic failure, drugs used to restore peripheral vascular tone should not affect cerebral vascular resistance or metabolism. Both phenylephrine and epinephrine seem to fulfill such requirements (94). Aggressive pharmacological lowering of blood pressure in patients with malignant hypertension may induce cerebral ischemia at blood pressure levels that would be considered satisfactory in normal subjects (95). This is probably due to a reduced autoregulatory capacity of the cerebral circulation in chronic hypertension (96, 97) and highlights the need for careful evaluation of cerebrovascular physiology in these patients before attempting such therapy and for gradual lowering of blood pressure while monitoring brain function.

Cerebrovascular resistance It is not possible to offer a rational classification of drugs capable of inducing a decrease in cerebrovascular resistance. Very few of these drugs show specificity for the cerebrovascular bed and practically none of them are devoid of nonvascular effects on neural function. The effects of drugs on the cerebral circulation need to be analyzed in parallel with an indication of their effect on cerebral metabolism and peripheral vascular resistance. Given the abundance of natural substances with vasomotor activity within the CNS, the possibility of indirect effects of vasodilators, via release of endogenous amines or peptides, has to be considered in every case. The

same applies to the effects of anesthetics and changes in blood gas composition when it comes to their effects on cerebrovascular resistance.

Adrenergic effects on the cerebral circulation have been extensively studied (50). Enhancement of CBF with adrenergic agonists can only occur through increase of arterial blood pressure when the higher limit of autoregulation is exceeded or in cases of hypotensive shock when blood pressure is raised from levels below the lower limit of autoregulation (94). In conditions of preserved autoregulation, either no effect or a small decrease in CBF is found (50, 94, 98). The absence or small magnitude of the cerebral vasoconstriction observed with adrenergic agents is commonly attributed to restrictions in brain tissue access of the drugs imposed by the blood-brain barrier (99). This may not be the complete explanation, however, because topical application of norepinephrine to the cerebral cortex, bypassing the blood-brain barrier, does not induce changes in cortical blood flow either (100); moreover, infusion of norepinephrine directly into the hypothalamus (101) or intraventricularly (102) enhances rather than decreases CBF. These observations contrast with in vitro experimentation in which isolated cerebral vessels contract in response to norepinephrine (103). The differences between in vitro and in vivo approaches probably do not have a simple explanation and may be related to indirect vasodilatation induced by metabolic effects (102) or the release of vasodilator substances.

The vasodilator effects of ACh and other Ch esters on the cerebral circulation, documented by observation of pial vessels, brain temperature, brain volume changes, and blood flow in the cartoid artery, have been known since 1929 and have been reviewed by Sokoloff (104). In 1973, using the H_2 clearance technique, a quantitative assessment of this phenomenon was obtained in the cerebral cortex of the rat (34). ACh in the presence of physostigmine, carbachol, arecoline, or pilocarpine, applied topically to the exposed cortex to circumvent the blood-brain barrier, markedly increased local CBF (34). The increase in CBF observed during arousal was tested to ascertain whether it could be mediated by ACh, known to be released in this condition (105). The results showed that in the presence of locally applied physostigmine, the increase in local CBF associated with cortical arousal reached 250% of control, as opposed to 150% observed in the absence of this drug. Conversely, in the presence of locally applied atropine, the hyperemia of cortical arousal was completely blocked. This was the first indication of a physiological role of endogenous ACh in the control of cortical blood flow.

There were other possible explanations for these results, however, such as the possible involvement of changes in cortical metabolism in the presence of these drugs. Cholinergic agonists could have increased neuronal metabolic rate, affecting blood flow through release of vasoactive metabolites. To test this possibility, GABA or $MgSO_4$ was applied to the cortex to abolish

neuronal discharges completely, as evidenced by microelectrode recordings. After this was accomplished, cholinergic agonists were still able to enhance local CBF, a fact interpreted as disproving a neuronal mediation of these effects. Years later, we were able to measure the oxygen consumption of the cerebral cortex in rabbits (106) and the brain regional glucose utilization in rats (49) during the vasodilatation elicited by physostigmine and found that none of these variables was affected during this phenomenon.

It is now clear that cholinergic agonists increase CBF by a primary vascular action and that endogenous ACh may play a significant role, still to be elucidated in detail, in cortical vascular physiology. Considerable evidence has accumulated to favor these ideas. The pioneer findings of Molnár et al (41), Shalit et al (107), and Meyer et al (42) implicating central neural mechanisms in the control of CBF have been followed by numerous similar observations (43, 44, 108–110). The early work of Edvinsson et al (111) pointing to a cholinergic innervation of cerebral vessels has also been amply confirmed over the years with more specific techniques (47) and biochemical probes (112–114). The dilator effect of ACh on cerebral blood vessels in vitro (50) is now known to be mediated by an endothelium derived relaxing factor (EDRF) (115). Identity of EDRF with nitric oxide is supported by a large body of work, recently reviewed by Ignarro (116). As discussed above, the role of EDRF in the cerebral vasculature in vivo is still unclear.

Although there is no doubt that endogenous ACh can induce significant changes in CBF, the sites from which this mediator is released to induce its effects are still a matter of controversy. The cranial parasympathetic nerves have been explored extensively with variable results. Either no changes in CBF (42, 117–119) or increases smaller than those seen with physostigmine (120–122) have been reported following electrical stimulation of these nerves. Since cortical arousal, as discussed above, can also induce an atropine-sensitive vasodilatation, the spreading of stimulating current to afferent nerves with secondary arousal can provoke misleading results (119). Very few studies have controlled for this source of artifact by electrical recording or measurements of metabolic variables and this may explain the extreme variability of the results of stimulation of parasympathetic nerves on CBF. The simple expedient of ablating these nerves should abolish the vasodilator effect of cholinesterase inhibition with physostigmine that depends on endogenous ACh. The results of such experiments have been negative, however (119).

The role of an intrinsic cerebral system in cholinergic vasodilatation is suggested by the fact that this phenomenon can be elicited by stimulation of the brain stem (43) or cerebellum (110). Iadecola et al have reported that a lesion in the nucleus basalis can block such effects (123). Recently, a very careful study by Lacombe et al (109) has provided evidence that stimulation of the nucleus basalis of Meynert can increase cortical blood flow and that the

effect is blocked by scopolamine. Similar results were reported by Biesold et al (124). If the nucleus basalis is the sole source of cholinergic cortical nerve fibers that innervate cerebral blood vessels, destruction of this nucleus should prevent the vasodilatation induced by cholinesterase inhibition. This has been recently shown in our laboratory not to be the case (125). Ibotenic acid lesions of this structure did not impede the cortical vasodilatation induced by physostigmine. This result implies that the source of cortical cholinergic vascular afferents is not in the nucleus basalis and stimulation experiments may have activated cortical afferents capable of triggering arousal with the concomitant cholinergic vasodilatation. Alternatively, redundant cholinergic fibers could have reinnervated the cortical vessels in the period between the nucleus basalis lesion and the blood flow experiment (10 days). Further experimentation will be required to explore these possibilities. Alternative potential sources for the endogenous ACh that mediates the regulatory functions described above are other basal forebrain regions, the ponto-mesencephalic tegmentum (126) and intrinsic cortical neurons of the cortex (127); these are worth exploring experimentally.

Serotonin (5-HT) reliably contracts cerebral blood vessels in vitro (128) but it does not cross the blood-brain barrier (129). Although there is no clear indication for a physiological role of 5-HT in CBF regulation, intravenously administered ketanserin, a 5-HT antagonist, has been shown to block remote decreases in CBF associated with cerebral embolization (130), suggesting a role of endogenously released 5-HT in this phenomenon. It has been known for a long time that 5-HT is released in areas of ischemia (131) and these facts may rekindle the interest in therapeutic applications of 5-HT blockers in this condition.

The effects of adenosine on the cerebral circulation and its involvement in CBF regulation have been recently reviewed by Phillis (132). This metabolite induces cerebral vasodilatation upon intravenous administration but it is not very specific since peripheral vasodilatation with a drop of blood pressure also occurs. The effect on CBF is strong enough, however, to induce significant vasodilatation in humans (133). The cerebral vasodilator effect of ATP seems to surpass that of adenosine (134), however, and the Cobalt-ATP salt, with a longer half-life, offers promise as a therapeutic agent (135).

Prostaglandins are synthesized by cerebral blood vessels (136). Numerous observations, reviewed by Walker & Pickard (51), suggest a role of eicosanoids in cerebrovascular regulation. Prostacyclin has been characterized as a cerebral vasodilator both in vitro and in vivo (136) but therapeutic trials in patients with acute cerebral infarction have failed to demonstrate any effects on CBF or neurological status (137). Indomethacin has been reported to attenuate the CBF increase induced by hypercapnia, a phenomenon that seems

to be species-specific (51). A remarkable decrease in CBF, with no apparent ill effects, is known to occur in infants given this drug for the purpose of closing the ductus arteriosus (138–140).

Calcium channel blockers have been extensively studied as cerebral vasodilators of potential use in cerebral ischemia. The better known drug of this group regarding these effects is nimodipine, a 1,4-dihydropyridine. This agent decreases cerebrovascular resistance more than peripheral vascular resistance and effects an increase in CBF at low doses. Dose escalation induces a decrease in arterial blood pressure that offsets the benefits of decreased cerebrovascular resistance (141). No cerebral metabolic changes are induced by nimodipine in intact brains (142, 143) but the normalization of oxidative metabolism after transient ischemia is accelerated (143). Studies in human subjects have shown improvement of CBF in the area of penumbra in stroke patients (90). Beneficial effects of this drug in experimental models of cerebral ischemia remain controversial (144) but clinical trials have been encouraging (93).

UNDESIRABLE DRUG EFFECTS ON CBF

Often drugs given for other therapeutic reasons have a significant effect on CBF, creating a potential for complications in patients at risk. For instance, anticholinergics impair CBF regulatory functions in experimental animals (34) and scopolamine decreases CBF in the cerebral cortex of humans (145). It is then conceivable that drugs with anticholinergic effects commonly used for the treatment of depression may be detrimental to CBF regulation.

The same can be said of nonsteroidal anti-inflammatory drugs. Indomethacin blocks several important CBF regulatory mechanisms in animals and humans (51) and it seems that the use of these agents in patients with cerebrovascular insufficiency should be re-examined. Recently, cimetidine has been reported to block the cerebrovascular vasodilatation in response to hypoxia (146), one of the basic CBF regulatory mechanisms, implying a role of histamine in this response. The use of these drugs should be considered with caution in patients at risk for hypoxic episodes, such as chronic obstructive pulmonary disease (COPD) and sleep apnea.

Aminophylline, a soluble theophylline salt commonly used for patients with COPD, has long been known to decrease CBF in humans (147) and to impair cerebrovascular responses to hypoxia in animals (132). It is surprising that the response of CBF to this drug has been tested only recently in COPD patients. The results have shown significant reductions in CBF at doses customarily used (148) and place a question mark on the use of this drug in COPD patients, particularly in those at risk for cerebrovascular disease.

CONCLUSIONS AND FUTURE DIRECTIONS

CBF should not be viewed merely as a variable that controls the supply of energy substrates and passively follows changes in neuronal metabolism, but rather as a determinant of neuronal function through a number of mechanisms such as removal of released transmitter, control of precursor availability for the synthesis of key molecules, and alteration of composition of the gaseous and ionic environment. All CBF functions mentioned above are amenable to pharmacological control. However, the decision to influence CBF pharmacologically requires knowledge of the particular pathophysiological circumstances and it is there that we lack a solid conceptual framework. Although the last two decades have seen a significant advance in our understanding of the ways in which cerebrovascular resistance is adjusted to meet metabolic demands, the role of CBF in the control of transmitter synthesis and disposal, neuronal electrical functions and regulation of the internal milieu of the brain remains largely unexplored. This configures a vast gap in our knowledge and limits a rational approach to the pharmacological control of CBF. Isolated vessel techniques have been exploited to yield basic information on behavior of cerebrovascular smooth muscle, but the organizational complexities of the nervous system impose limitations to this approach. The ensemble of blood vessels, glial, and neuronal elements possesses properties that cannot be modeled in vitro. There is a need for methodological approaches that would maintain nervous structure intact and yet measure CBF with a spatial resolution commensurate with the organizational principles of the brain. The tracer methodologies for measurement of CBF and metabolism pioneered by Kety & Sokoloff have been followed in recent years by techniques that can produce pictorial representations of a large number of blood flow-related variables, as well as of distribution of pharmacological receptors and enzymes. Adequate combinations of these approaches can provide an integral view of the outcome of pharmacological interventions aimed at modifying CBF and of its impact in neuronal metabolism and function. Local application of drugs to selected nervous structures has been used successfully to circumvent complications created by the blood-brain barrier and peripheral drug effects, but coordination of this approach with the tracer techniques mentioned above has not yet been fully exploited. The analysis of pharmacological effects on cerebrovascular regulations has not gone beyond the descriptive phase. We have gathered an impressive inventory of drug effects on contractility of isolated blood vessels, morphology of pial vessels and CBF levels but only sporadic efforts have been devoted to understanding the impact of a varying CBF on neural function.

Interest in the role of CBF in pathophysiology of the nervous system has been rekindled by the recent availability of imaging techniques for CBF and

metabolism that allow studies in human subjects that were previously accessible in experimental animals. It is now clear that CBF can be modified pharmacologically and the outcome monitored with levels of spatial and temporal resolution that were unsuspected before. The limitations are now more in our ideas than in the tools that we possess. The currently prevalent ideas about CBF functions were laid out with brilliant insight by Roy & Sherrington in 1890 (149). They had no other tools at their disposal than a membrane plethysmograph, a lever, and a smoked drum. In the centennial of that event, the challenge is to make intelligent use of the presently available resources to create a new theoretical framework that will advance beyond the ideas of those pioneer scientists.

ACKNOWLEDGMENTS

I am indebted to Drs. Donald J. Jenden and Ralph R. Sonnenschein for fruitful discussions and thoughtful revision of this manuscript. Supported by the Department of Veterans Affairs.

Literature Cited

1. Siesjö, B. K. 1984. Cerebral circulation and metabolism. *J. Neurosurg.* 60:883–908
2. Heistad, D. D., Kontos, H. A. 1983. Cerebral circulation. In *The Cardiovascular System, Peripheral Circulation and Organ Blood Flow*, ed. J. T. Shepherd, F. M. Abboud, 3:137–82. Bethesda: Am. Physiol. Soc.
3. Wahl, M. 1985. Local chemical, neural and humoral regulation of cerebrovascular resistance vessels. *J. Cardiovasc. Pharmacol.* 7 (Suppl 3):S36–S46
4. Craigie, E. H. 1963. *Craigie's Neuroanatomy of the Rat*, pp. 1–230. New York: Academic
5. Dunning, H. S., Wolff, H. G. 1937. The relative vascularity of various parts of the central and peripheral nervous system of the cat and its relation to function. *J. Comp. Neurol.* 67 (3):433–50
6. Klein, B., Kuschinsky, W., Schrock, H., Vetterlein, V. 1986. Interdependency of local capillary density, blood flow and metabolism in rat brains. *Am. J. Physiol.* 251:H1333–40
7. Reivich, M., Isaacs, G., Everts, E. 1968. The effect of slow wave sleep and REM sleep on regional cerebral blood flow in cats. *J. Neurochem.* 15:301–6
8. Sakai, F., Meyer, J. S., Karacan, I., Derman, S., Yamamoto, M. 1980. Normal human sleep: Regional cerebral hemodynamics. *Ann. Neurol.* 7(5):471–78
9. Maximilian, V. A., Prohovnik, I., Risberg, J. 1980. Cerebral hemodynamic response to mental activation in normo- and hypercapnia. *Stroke* 11:342–47
10. Knopman, D. S., Rubens, A. B., Klassen, A. C., Meyer, M. W. 1982. Regional cerebral blood flow correlates of auditory processing. *Arch. Neurol.* 39(8):487–93
11. Fox, P. T., Miezin, F. M., Allman, J. M., Van Essen, D. C., Raichle, M. E. 1987. Retinotopic organization of human visual cortex mapped with positron-emission tomography. *J. Neurosci.* 7(3):913–22
12. Ginsberg, M. D., Chang, J. Y., Kelley, R. E., Yoshii, F., Barker, W. W. et al. 1988. Increases in both cerebral glucose utilization and blood flow during execution of a somatosensory task. *Ann. Neurol.* 23 (2):152–60
13. Kety, S. S. 1951. The theory and applications of the exchange of inert gas at the lungs and tissues. *Pharmacol. Rev.* 3:1–41
14. King, C. E., Garrey, W. E., Bryan, W. R. 1932. The effect of carbon dioxide, hyperventilation and anoxemia on the knee jerk. *Am. J. Physiol.* 102:305–71
15. Esplin, D. W., Capek, R., Esplin, B. 1973. An intracellular study of the actions of carbon dioxide on the spinal monosynaptic pathway. *Can. J. Physiol. Pharmacol.* 51:424–36
16. Balestrino, M., Somjen, G. G. 1988. Concentration of carbon dioxide, in-

terstitital pH and synaptic transmission in hippocampal formation of the rat. *J. Physiol.* 396:247–66
17. Feldberg, W., Gaddum, J. H. 1934. The chemical transmitter at synapses in a sympathetic ganglion. *J. Physiol.* 81: 305–19
18. MacIntosh, F. C. 1938. Liberation of acetylcholine by the perfused superior cervical ganglion. *J. Physiol.* 94:155–69
19. Jenden, D. J. 1979. The neurochemical basis of acetylcholine precursor loading as a therapeutic strategy. In *Brain Acetylcholine and Neuropsychiatric Disease*, ed. K. L. Davis, P. A. Berger. pp. 483–513. New York: Plenum
20. Tucek, S. 1984. Problems in the organization and control of acetylcholine synthesis in brain neurons. *Prog. Biophys. Mol. Biol.* 44:1–46
21. Freeman, J. J., Jenden, D. J. 1976. The source of choline for acetylcholine synthesis in the brain. *Life Sci.* 19:949–62
22. Lakher, M., Wurtman, R. J. 1987. In vivo synthesis of phosphatidylcholine in rat brain via the phospholipid methylation pathway. *Brain Res.* 419:131–40
23. Blusztajn, J. K., Wurtman, R. J. 1983. Choline and cholinergic neurons. *Science* 221:614–20
24. Zeisel, S. H. 1985. Formation of unesterified choline by rat brain. *Biochem. Biophys. Acta* 835:331–43
25. Dross, K., Kewitz, H. 1972. Concentration and origin of choline in the rat brain. *Naunyn Schmiedeberg's Arch. Pharmacol.* 274:91–106
26. Spanner, S., Hall, R. C., Ansell, G. B. 1976. Arterio-venous differences of choline and choline lipids across the brain of rat and rabbit. *Biochem. J.* 154:133–40
27. Choi, R. L., Freeman, J. J., Jenden, D. J. 1975. Kinetics of plasma choline in relation to turnover of brain choline and formation of acetylcholine. *J. Neurochem.* 24:735–41
28. Scremin, O. U., Jenden, D. J. 1989. Effects of middle cerebral artery occlusion on cerebral cortex choline and acetylcholine in rats. *Stroke* 20:1524–30
29. Tucek, S. 1984. Problems in the organization and control of acetylcholine synthesis in brain neurons. *Prog. Biophys. Mol. Biol.* 44:1–46
30. Scremin, O. U., Jenden, D. J. 1989. Focal ischemia enhances choline output and decreases acetylcholine output from rat cerebral cortex. *Stroke* 20:92–95
31. Scremin, O. U., Jenden, D. J. 1989. Brain choline and acetylcholine levels in forebrain global ischemia and recirculation. *J. Cereb. Blood Flow Metab.* 9 (Suppl. 1):S655
32. Sicsjö, B. K. 1978. *Brain Energy Metabolism*, pp. 1–607. New York: Wiley
33. Graham, S. H., Shiraishi, K., Panter, S. S., Simon, R. P., Faden, A. I. 1990. Changes in extracellular amino acid neurotransmitters produced by focal cerebral ischemia. *Neurosci. Lett.* 110:124–30
34. Scremin, O. U., Rovere, A. A., Raynald, A. C., Giardini, A. 1973. Cholinergic control of blood flow in the cerebral cortex of the rat. *Stroke* 4:232–39
35. Pearce, W. J., Scremin, O. U., Sonnenschein, R. R., Rubinstein, E. H. 1981. The electroencephalogram, blood flow, and oxygen uptake in rabbit cerebrum. *J. Cereb. Blood Flow Metab.* 1:419–28
36. Kuschinsky, W., Suda, S., Sokoloff, L. 1981. Coupling between metabolism and blood flow in the rat brain during metabolic acidosis. *J. Cereb. Blood Flow Metab.* 1 (Suppl. 1):S88–S89
37. McCulloch, J., Kelly, P. A. T., Grome, J. J., Pickard, J. D. 1982. Local cerebral circulatory and metabolic effects of indomethacin. *Am. J. Physiol.* 243:H416–23
38. Kuschinsky, W., Suda, S., Sokoloff, L. 1985. Influence of gamma-hydroxybutyrate on the relationship between local cerebral glucose utilization and local cerebral blood flow in the rat brain. *J. Cereb. Blood Flow Metab.* 5:58–64
39. Beck, T., Vogg, P., Krieglstein, J. 1988. Uncoupling of cerebral blood flow and glucose utilization by dihydroergocristine in the conscious rat. *Naunyn Schmiedeberg's Arch. Pharmacol.* 338(1):82–87
40. Kuschinsky, W., Suda, S., Bunger, R., Yaffe, S., Sokoloff, L. 1983. The effects of intravenous norepinephrine on the local coupling between glucose utilization and blood flow in the rat brain. *Pflügers Arch.* 398:134–38
41. Molnár, L. 1967. *Sur le Contrôle Nerveux de la Circulation Sanguine Régionale des Centres Cérébraux*, pp. 1–127. Budapest: Akademiai Kiado
42. Meyer, J. S., Teraura, T., Sakamoto, K., Kondo, A. 1971. Central neurogenic control of cerebral blood flow. *Neurology* 21:247–62
43. Scremin, O. U., Rubinstein, E. H., Sonnenschein, R. R. 1980. Role of central

pathways and cranial nerves in cerebral cholinergic vasodilatation. In *Advances in Physiological Sciences, Vol. 9, Cardiovascular Physiology, Neural Control Mechanisms,* ed. A. G. B. Kovach, P. Sandor, M. Kollai. New York: Pergamon

44. Reis, D. J., Iadecola, C., Nakai, M. 1985. Control of cerebral blood flow and metabolism by intrinsic neural systems in brain. In *Cerebrovascular Diseases,* ed. F. Plum, W. Pulsinelli. pp. 1–25. New York: Raven
45. Sokoloff, L. 1989. Circulation and energy metabolism of the brain. In *Basic Neurochemistry,* ed. G. J. Siegel, B. W. Agranoff, R. W. Albers, P. Molinoff, pp. 565–90. New York: Raven Press. 4th ed.
46. Furchgott, R. F., 1990. The 1989 Ulf von Euler lecture: Studies on endothelium-dependent vasodilation and the endothelium-derived relaxing factor. *Acta Physiol. Scand.* 139:257–70
47. Saito, G. A., Wu, J. Y., Lee, T. J. F. 1985. Evidence for the presence of cholinergic nerves in cerebral arteries: An immunohistochemical demonstration of choline acetyltransferase. *J. Cereb. Blood Flow Metab.* 5:327–34
48. Estrada, C., Hammel, E., Krause, D. N. 1983. Biochemical evidence for cholinergic innervation of intracerebral blood vessels. *Brain Res.* 266:261–70
49. Scremin, O. U., Allen, K., Torres, C. D., Scremin, A. M. E. 1988. Physostigmine enhances blood flow metabolism ratio in neocortex. *Neuropsychopharmacology* 1 (4):297–303
50. Edvinsson, L., MacKenzie, E. T. 1977. Amine mechanisms in the cerebral circulation. *Pharmacol. Rev.* 28(4):275–348
51. Walker, V., Pickard, J. D., 1985. Prostaglandins, thromboxane, leukotrienes and the cerebral circulation in health and disease. *Adv. Tech. Stand. Neurosurg.* 12:3–90
52. Kety, S. S. 1964. The cerebral circulation. In *Circulation of the Blood: Men and Ideas,* ed. A. P. Fishman, D. W. Richards. pp. 703–42. New York: Oxford Univ. Press
53. Kety, S. S. 1951. The theory and applications of the exchange of inert gas at the lungs and tissues. *Pharmacol. Rev.* 3:1–41
54. Kety, S. S., Woodford, R. B., Harmel, M. H., Freyhan, F. A., Appel, K. E., Schmidt, C. F. 1948. Cerebral blood flow and metabolism in schizophrenia: The effects of barbiturate semi-narcosis, insulin coma and electroshock. *Am. J. Psychiatr.* 104:765
55. Phelps, M. E., Mazziotta, J. C., Huang, S. C. 1982. Study of cerebral function with positron computed tomography. *J. Cereb. Blood Flow Metab.* 2:113–62
56. Baron, J. C., Frackowiak, R. S. J., Herholz, K., Jones, T., Lammertsma, A. A., et al. 1989. Use of PET methods for measurement of cerebral energy metabolism and hemodynamics in cerebrovascular disease. *J. Cereb. Blood Flow Metab.* 9 (6):723–42
57. Devous, M. D., Stokely, E. M., Bonte, F. J. 1985. Quantitative imaging of regional cerebral blood flow by dynamic single-photon tomography. In *Radionuclide Imaging of the Brain,* ed. B. L. Holman. pp. 135–61. New York: Churchill-Livingstone
58. Meyer, J. S., Hayman, L. A., Amano, T., Hakajima, S., Shaw, T., et al. 1981. Mapping local blood flow of human brain by CT scanning during stable xenon inhalation. *Stroke* 12:426–36
59. Gur, D., Wolfson, S. K. Jr., Yonas, H., Good, W. F., Shabason, L., et al. 1982. Progress in cerebrovascular disease: Local cerebral blood flow by xenon enhanced CT. *Stroke* 13 (6):750–58
60. Eriksson, S., Hagenfeldt, L., Law, D., Patrono, C., Pinca, E., Wennmalm, Å. 1983. Effect of prostaglandin synthesis inhibitors on basal and carbon dioxide stimulated cerebral blood flow in man. *Acta Physiol. Scand.* 117:203–11
61. Kobatake, K., Sako, K., Izawa, M., Yamamoto, Y. L., Hakim, A. M. 1984. Autoradiographic determination of brain pH following middle cerebral artery occlusion in the rat. *Stroke* 15:540–47
62. Dwyer, B. E., Donatoni, P., Wasterlain, C. G. 1982. A quantitative autoradiographic method for the measurement of local rates of brain protein synthesis. *Neurochem. Res.* 7:563–76
63. Sokoloff, L., Reivich, M., Kennedy, C., Desrosie, M. H., Patlak, C. S., et al. 1977. The [C14]-deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure and normal values in the conscious and anesthetized albino rat. *J. Neurochem.* 28:897–916
64. Scremin, O. U., Scremin, A. M. E., Somani, S., Giacobini, E. 1990. Brain regional distribution of physostigmine and its relation to cerebral blood flow

following intravenous administration in rats. *J. Neurosci. Res.* 26:188–95
65. Meyer, J. S., Yoshida, K., Sakamoto, K. 1967. Autonomic control of cerebral blood flow measured by electromagnetic flowmeters. *Neurology* 17:638–48
66. Scremin, O. U., Sonnenschein, R. R., Rubinstein, E. H. 1982. Cerebrovascular anatomy and blood flow measurements in the rabbit. *J. Cereb. Blood Flow Metab.* 2:55–66
67. Greene, E. R. 1982. Noninvasive measurement of blood flow using pulsed doppler ultrasound. In *Oxygen Transport to Human Tissues,* ed. J. A. Loeppky, M. L. Riedesel, pp. 135–50. Holland: Elsevier
68. Conrad, B., Klingelhöfer, J. 1989. Dynamics of regional cerebral blood flow for various visual stimuli. *Exp. Brain Res.* 77:437–41
69. Jorch, G., Jorch, N. 1987. Failure of autoregulation of cerebral blood flow in neonates studied by pulsed Doppler ultrasound of the internal carotid artery. *Eur. J. Pediatr.* 146 (5):468–72
70. Misrahy, G. A., Clark, L. C. 1956. Use of the platinum black cathode for local blood flow measurements in vivo. *20th Int. Physiol. Congr.* Aug:650–51 (Abstr.)
71. Auckland, K., Bower, B. F., Berliner, R. W. 1964. Measurement of local blood flow with H_2 gas. *Circ. Res.* 14:164–87
72. Branston, N. M., Symon, L., Crockard, H. A., Pasztor, E. 1974. Relationship between the cortical evoked potential and local cortical blood flow following acute middle cerebral artery occlusion in the baboon. *Exp. Neurol.* 45:195–208
73. Date, H., Hossmann, K. A. 1984. Effect of vasodilating drugs on intracortical and extracortical vascular resistance following middle cerebral artery occlusion in cats. *Ann. Neurol.* 16:330–36
74. Fieschi, C., Agnoli, A., Battistini, N., Bozzao, L., Prencipe, M. 1968. Derangement of regional cerebral blood flow and of its regulatory mechanism in acute cerebrovascular lesions. *Neurology* 18:1166–79
75. Symon, L., Khodadad, G., Montoya, G. 1971. Effect of carbon dioxide inhalation on the pattern of gaseous metabolism in ischemic zones of the primate cortex. An experimental study of the "intracebral steal" phenomenon in baboons. *J. Neurol. Neurosurg. Psychiatr.* 34:481–86
76. Waltz, A. G. 1970. Effect of $PaCO_2$ on blood flow and microvasculature of ischemic and non-ischemic cerebral cortex. *Stroke* 1:27–37
77. Nakagawa, Y., Yamamoto, L., Meyer, E., Hodge, C. P., Feindel, W. 1981. Effects of hypercapnia on enhancement of decreased perfusion flow in non-infarcted brain tissues. *Stroke* 12:85–92
78. Kogure, K., Fujishima, M., Scheimberg, P., Reinmuth, O. M. 1969. Effects of changes in carbon dioxide pressure and arterial pressure on blood flow in ischemic regions of the brain in dogs. *Circ. Res.* 24:557–65
79. Gogolak, I., Gotoh, F., Tomita, M., Tanahashi, N., Kobari, M., et al. 1985. No intracerebral steal phenomenon in the ischemic brain following papaverine administration. *Stroke* 16:114–17
80. Mohamed, A. A., Gotoh, O., Graham, D. I., Osborne, D. A., McCulloch, J., et al. 1985. Effects of pretreatment with the calcium antagonist nimodipine on local cerebral blood flow and histopathology after middle cerebral artery occlusion. *Ann. Neurol.* 18 (6):705–11
81. Scremin, O. U., Scremin, A. M. E. 1986. Physostigmine induced reversal of ischemia following middle cerebral artery occlusion in the rat. *Stroke* 17:1004–9
82. Posner, J. B., Plum, F. 1960. The toxic effects of carbon dioxide and acetazolamide in hepatic encephalopathy. *J. Clin. Invest.* 39:1246–58
83. Chollet, F., Celsis, P., Clanet, M., Guiraud-Chaumeil, B., Rascol, A., Marc-Vergnes, J. P. 1989. SPECT study of cerebral blood flow reactivity after acetazolamide in patients with transient ischemic attacks. *Stroke* 20:458–64
84. Astrup, J., Siesjö, B. K. 1981. Thresholds in cerebral ischemia: The ischemic penumbra. *Stroke* 12:723–25
85. Strong, A. J., Venables, G. S., Gibson, G. 1983. The cortical ischemic penumbra associated with occlusion of the middle cerebral artery in the cat: 1. Topography of changes in blood flow, potassium ion activity, and EEG. *J. Cereb. Blood Flow Metab.* 3:86–96
86. Olsen, T. S., Larsen, B., Herning, M., Skriver, E. B., Lassen, N. A. 1983. Blood flow and vascular reactivity in collaterally perfused brain tissue: Evidence of an ischemic penumbra in patients with acute stroke. *Stroke* 14 (3):322–41
87. Raynaud, C., Rancurel, G., Samson, J. C., Baron, J. C., Soucy, J. P., et al. 1987. Pathophysiologic study of chronic infarcts with I-123 Isopropyl Iodo-

Amphetamine (IMP): The importance of periinfarct area. *Stroke* 18:21–29
88. Raynaud, C., Rancurel, G., Tzourio, N., Kieffer, E., Soucy, J. P., et al. 1987. The rCBF in subacute stroke studied by SPECT. *J. Cereb. Blood Flow Metab.* 7(Suppl. 1):S107
89. Ueda, M., Matsumoto, Y., Omiya, N., et al. 1987. Evaluation of hemodilution therapy in acute ischemic stroke by single photon emisson CT. *J. Cereb. Blood Flow Metab.* 7:S203
90. Fieschi, C., Argentino, C., Toni, D., Pozzilli, C. 1988. Calcium antagonists in ischemic stroke. *J. Cardiovasc. Pharmacol.* 12 (Suppl. 6):S83–S85
91. Scremin, A. M. E., Kunkel, C., Scremin, O. U., Eisenberg, B., Elliot, T. 1989. Physostigmine effects on human cerebral blood flow. *Arch. Phys. Med. Rehabil.* 70:A54
92. Strand, T., Asplund, K., Eriksson, S., Hagg, E., Lithner, F., Wester, P. O. 1984. A randomized controlled trial of hemodilution therapy in acute ischemic stroke. *Stroke* 15:980–89
93. Gelmers, H. J., Gorter, K., De Weerdt, C. J., Wiezer, H. J. A. 1988. A controlled trial of nimodipine in acute ischemic stroke. *New Engl. J. Med.* 318 (4):203–7
94. Schleien, C. L., Koehler, R. C., Gervais, H., Berkowitz, I. D., Dean, J. M., et al. 1989. Organ blood flow and somatosensory-evoked potentials during and after cardiopulmonary resuscitation with epinephrine or phenylephrine. *Circulation* 79 (6):1332–42
95. Haas, D. C., Streeten, D. H. P., Kim, R. C., Naalbandian, A. N., Obeid, A. I. 1983. Death from cerebral hypoperfusion during nitroprusside treatment of acute angiotensin-dependent hypertension. *Am. J. Med.* 75:1071–76
96. Baumbach, G. L., Heistad, D. D. 1988. Cerebral circulation in chronic arterial hypertension. *Hypertension* 12:89–95
97. Paulson, O. B., Waldemar, G., Schmidt, J. F., Strandgaard, S. 1989. Cerebral circulation under normal and pathologic conditions. *Am. J. Cardiol.* 63:2C–5C
98. Drummond, J. C., Oh, Y. S., Cole, D. J., Shapiro, H. M. 1989. Phenylephrine-induced hypertension reduces ischemia following middle cerebral artery occlusion in rats. *Stroke* 20:1538–44
99. Oldendorf, W. H. 1971. Brain uptake of radiolabeled aminoacids, amines and hexoses after arterial injection. *Am. J. Physiol.* 221:1629–39
100. Rovere, A. C., Raynald, A. C., Scremin, O. U. 1977. On the mechanism of amphetamine induced vasodilatation of the rat's cerebral cortex. *Experientia* 33:1461–62
101. Rosendorff, C., Cranston, W. I. 1971. Effects of intrahypothalamic and intraventricular norepinephrine and 5-hydroxytryptamine on hypothalamic blood flow in the conscious rabbit. *Circ. Res.* 28:492–502
102. MacKenzie, E. T., McCulloch, J., O'Keane, M., Pickard, J. D., Harper, A. M. 1976. Cerebral circulation and norepinephrine: Relevance of the blood-brain barrier. *Am. J. Physiol.* 231:483–88
103. Edvinsson, L., Owman, C. 1974. Pharmacological characterization of adrenergic alpha and beta receptors mediating the vasomotor responses of cerebral arteries in vitro. *Circ. Res.* 35:835–49
104. Sokoloff, L. 1959. The action of drugs on the cerebral circulation. *Pharmacol. Rev.* 11:1–85
105. Metz, B. 1966. Hypercapnia acetylcholine release from the cerebral cortex and medulla. *J. Physiol.* 186:321–32
106. Scremin, O. U., Sonnenschein, R. R., Rubinstein, E. H. 1982. Cholinergic cerebral vasodilatation in the rabbit: Absence of concomitant metabolic activation. *J. Cereb. Blood Flow Metab.* 2:241–47
107. Shalit, M. N., Reinmuth, O. M., Shimoyo, S., Scheimberg, P. 1967. Carbon dioxide and cerebral circulatory control. III The effects of brain stem lesions. *Arch. Neurol.* 17:342–53
108. Mraovitch, S., Seylaz, J. 1987. Metabolism-independent cerebral vasodilation elicited by electrical stimulation of the centromedian-parafascicular complex in rat. *Neurosci. Lett.* 83:269–74
109. Lacombe, P., Sercombe, R., Verrecchia, C., Philipson, V., MacKenzie, E. T., Seylaz, J. 1989. Cortical blood flow increases induced by stimulation of the substantia innominata in the unanesthetized rat. *Brain Res.* 491:1–14
110. Arneric, S. P., Iadecola, C., Underwood, M. D., Reis, D. J. 1987. Local cholinergic mechanisms participate in the increase in cortical cerebral blood flow elicited by electrical stimulation of the fastigial nucleus in rat. *Brain Res.* 411:212–25
111. Edvinsson, L., Nielsen, K. C., Owman, C., Sporrong, B. 1972. Cholinergic mechanisms in pial vessels: Histochemistry, electron microscopy and pharmacology. *Z. Zellforsch. Mikrosk. Anat.* 134:311–25

112. Florence, V. M., Bevan, J. A. 1979. Biochemical determination of cholinergic innervation in cerebral arteries. *Circ. Res.* 45:212–18
113. Duckles, S. P. 1977. Evidence for a functional cholinergic innervation of cerebral arteries. *J. Pharmacol. Exp. Ther.* 200:117–26
114. Arneric, S. P., Honig, M. A., Milner, T. A., Greco, S., Iadecola, C., Reis, D. J. 1988. Neuronal and endothelial sites of acetylcholine synthesis and release associated with microvessels in rat cerebral cortex: Ultrastructural and neurochemical studies. *Brain Res.* 454: 11–30
115. Furchgott, R. F., Zawadzki, J. V. 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288:373–76
116. Ignarro, L. J. 1990. Biosynthesis and metabolism of endothelium-derived nitric oxide. *Annu. Rev. Pharmacol. Toxicol.* 30:535–60
117. Busija, D. W., Heistad, D. D. 1981. Effects of cholinergic nerves on cerebral blood flow in cats. *Circ. Res.* 48:62–69
118. Linder, J. 1981. Effects of facial nerve section and stimulation on cerebral and ocular blood flow in hemorrhagic hypotension. *Acta Physiol. Scand.* 112: 185–93
119. Scremin, O. U., Sonnenschein, R. R., Rubinstein, E. H. 1983. Cholinergic cerebral vasodilatation: lack of involvement of cranial parasympathetic nerves. *J. Cereb. Blood Flow Metab.* 3:362–68
120. James, I. M., Millar, R. A., Purves, M. J. 1969. Observations on the extrinsic neural control of cerebral blood flow in the baboon. *Circ. Res.* 25:77–93
121. D'Alecy, L. G., Rose, C. J. 1977. Parasympathetic cholinergic control of cerebral blood flow in dogs. *Circ. Res.* 41:324–31
122. Pinard, E., Purves, M. J., Seylaz, J., Vasquez, J. V. 1979. The cholinergic pathway to cerebral blood vessels: II. Physiological studies. *Pflügers Arch.* 379:165–72
123. Iadecola, C., Mraovitch, S., Meeley, M. P., Reis, D. J. 1983. Lesions of the basal forebrain in rat selectively impair the cortical vasodilation elicited from cerebellar fastigial nucleus. *Brain Res.* 279:41–52
124. Biesold, D., Inanami, O., Sato, A., Sato, Y. 1989. Stimulation of the nucleus basalis of Meynert increases cerebral cortical blood flow in rats. *Neurosci. Lett.* 98:39–44
125. Scremin, O. U., Torres, C. D., Scremin, A. M. E., O'Neal, M., Heuser, D., Blisard, K. S. 1990. The role of the nucleus basalis in cholinergic control of cortical blood flow. *J. Neurosci. Res.* In Press
126. Vincent, S. R., Satoh, K., Armstrong, D. M., Fibiger, H. C. 1983. Substance P in the ascending cholinergic reticular system. *Nature* 306:688–91
127. Eckenstein, F., Thoenen, H. 1983. Cholinergic neurons in the rat cerebral cortex demonstrated by immunohistochemical localization of choline acetyltransferase. *Neurosci. Lett.* 36:211–15
128. McCulloch, J., Edvinsson, L. 1984. Cerebrovascular smooth muscle reactivity: A critical appraisal of in vitro and in situ techniques. *J. Cereb. Blood Flow Metab.* 4 (2):129–39
129. Hardebo, J. E., Owman, C. 1980. Barrier mechanisms for neurotransmitter monoamines and their precursors at the blood-brain barrier. *Ann. Neurol.* 8:1–11
130. Dietrich, W. D., Busto, R., Ginsberg, M. D. 1989. Effect of the serotonin antagonist ketanserin on the hemodynamic and morphological consequences of thrombotic infarction. *J. Cereb. Blood Flow Metab.* 9:812–20
131. Welch, K. M. A., Meyer, J. S., Teraura, T., Hashi, K., Shinmaru, S. 1972. Ischemic anoxia and cerebral serotonin levels. *J. Neurol. Sci.* 16:85–92
132. Phillis, J. W. 1989. Adenosine in the control of the cerebral circulation. *Cerebrovasc. Brain Metab. Rev.* 1 (1):26–54
133. Sollevi, A., Ericson, K., Eriksson, L., Lindqvist, C., Lagerkranser, M., Stone-Elander, S. 1987. Effect of adenosine on human cerebral blood flow as determined by positron emission tomography. *J. Cereb. Blood Flow Metab.* 7 (6):673–78
134. Forrester, T., Harper, A. M., MacKenzie, E. T., Thomson, E. M. 1979. Effect of adenosine triphosphate and some derivatives on cerebral blood flow and metabolism. *J. Physiol.* 296:343–55
135. Molnár, L., Hegedüs, K., Fekete, I. 1989. Effect of ATP and physostigmine on blood vessels. *J. Cereb. Blood Flow Metab.* 9 (Suppl. 1):S486
136. Moncada, S. 1983. Progress in cerebrovascular disease: Biology and therapeutic potential of prostacyclin. *Stroke* 14 (2):157–68
137. Martin, J. F., Hamdy, N., Nicholl, J., Lewtas, N., Bergvall, U., et al. 1985. Double-blind controlled trial of

prostacyclin in cerebral infarction. *Stroke* 16 (3):386–90
138. Cowan, F. 1986. Indomethacin, patent ductus arteriosus and cerebral blood flow. *J. Pediatr.* 109:314–44
139. Pryds, O., Greisen, G., Johansen, K. H. 1988. Indomethacin and cerebral blood flow in premature infants treated for patent ductus arteriosus. *Eur. J. Pediatr.* 147 (3):315–16
140. Van Bel, F., Van de Bor, M., Stijnen, T., Baan, J., Ruys, J. H. 1989. Cerebral blood flow velocity changes in preterm infants after a single dose of indomethacin: Duration of its effect. *Pediatrics* 84 (5):802–7
141. Mohamed, A. A., McCulloch, J., Mendelow, A. D., Teasdale, G. M., Harper, A. M. 1984. Effect of the calcium antagonist nimodipine on local cerebral blood flow: relationship to arterial blood pressure. *J. Cereb. Blood Flow Metab.* 4:206–11
142. Mohamed, A. A., Mendelow, A. D., Teasdale, G. M., Harper, A. M., McCulloch, J. 1985. Effect of the calcium antagonist nimodipine on local cerebral blood flow and metabolic coupling. *J. Cereb. Blood Flow Metab.* 5:26–33
143. Heffez, D. S., Passonneau, J. V. 1985. Effect of nimodipine on cerebral metabolism during ischemia and recirculation in the Mongolian gerbil. *J. Cereb. Blood Flow Metab.* 5 (4):523–28
144. Scriabine, A., Schuurman, T., Traber, J. 1989. Pharmacological basis for the use of nimodipine in central nervous system disorders. *FASEB J.* 3:1799–806
145. Honer, W. G., Prohovnik, I., Smith, G., Lucas, L. R. 1988. Scopolamine reduces frontal cortex perfusion. *J. Cereb. Blood Flow Metab.* 8 (5):635–41
146. Clozel, J. P., Amend, P., Saunier, C., Hartemann, D. 1985. Cimetidine inhibits the hypoxia-induced increase in cerebral blood flow in dogs. *Crit. Care Med.* 13 (11):976–81
147. Magnussen, I., Hoedt-Rasmussen, K. 1977. The effect of intraarterial administered aminophylline on cerebral hemodynamics in man. *Acta Neurol. Scand.* 55:131–36
148. Bowton, D. L., Alford, P. T., McLees, B. D., Prough, D. S., Stump, D. A. 1987. The effect of aminophylline on cerebral blood flow in patients with chronic obstructive pulmonary disease. *Chest* 91 (6):874–77
149. Roy, C. S., Sherrington, C. S. 1890. On the regulation of the blood supply of the brain. *J. Physiol.* 11:85–108