Cerebral microvascular pathology in aging and Alzheimer’s disease

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Abstract

The aging of the central nervous system and the development of incapacitating neurological diseases like Alzheimer’s disease (AD) are generally associated with a wide range of histological and pathophysiological changes eventually leading to a compromised cognitive status. Although the diverse triggers of the neurodegenerative processes and their interactions are still the topic of extensive debate, the possible contribution of cerebrovascular deficiencies has been vigorously promoted in recent years. Various forms of cerebrovascular insufficiency such as reduced blood supply to the brain or disrupted microvascular integrity in cortical regions may occupy an initiating or intermediate position in the chain of events ending with cognitive failure. When, for example, vasoconstriction takes over a dominating role in the cerebral vessels, the perfusion rate of the brain can considerably decrease causing directly or through structural vascular damage a drop in cerebral glucose utilization. Consequently, cerebral metabolism can suffer a setback leading to neuronal damage and a concomitant suboptimal cognitive capacity. The present review focuses on the microvascular aspects of neurodegenerative processes in aging and AD with special attention to cerebral blood flow, neural metabolic changes and the abnormalities in microvascular ultrastructure. In this context, a few of the specific triggers leading to the prominent cerebrovascular pathology, as well as the potential neurological outcome of the compromised cerebral microvascular system are also going to be touched upon to a certain extent, without aiming at total comprehensiveness. Finally, a set of animal models are going to be presented that are frequently used to uncover the functional relationship between cerebrovascular factors and the damage to neural networks. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Cerebral blood flow; Capillary ultrastructure; Aging; Alzheimer’s disease

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Abbreviations: Aβ, beta amyloid; ACh, acetylcholine; AD, Alzheimer’s disease; APP, amyloid precursor protein; BBB, blood–brain barrier; BM, basement membrane; BMT, basement membrane thickening; CA, hippocampal Ammon’s horn; CAA, cerebral amyloid angiopathy; CAMCOG, Cambridge Cognitive Examination; CBF, cerebral blood flow; CGU, cerebral glucose utilization; CGRP, calcitonin gene-related peptide; ChAT, choline acetyltransferase; CMRO₂, cerebral metabolic rate for oxygen; CNS, central nervous system; EM, electron microscopy; eNOS, endothelial NO synthase; GFAP, glial fibrillary acidic protein; GLUT-1, glucose transporter protein 1; HDS, Hasegawa’s Dementia Scale; HSPG, heparan sulfate proteoglycan; mAChR, muscarinic acetylcholine receptor; MAP2, microtubule-associated protein 2; MCA, middle cerebral artery; MCAO, middle cerebral artery occlusion; MMSE, Mini Mental State Examination; NBM, nucleus basalis magnocellularis; NO, nitric oxide; NOS, nitric oxide synthase; NPY, neuropeptide-Y; PGI₂, prostaglandin I₂; SAH, subarachnoid hemorrhage; SHR, spontaneously hypertensive rat strain; SHR-SP, spontaneously hypertensive stroke-prone rat strain; SI, substantia innominata; SP, substance-P; PHA-L, Phaseolus vulgaris leucoagglutinin; rCBF, regional cerebral blood flow; rCGU, regional cerebral glucose utilization; SMC, smooth muscle cell; VIP, vasoactive intestinal polypeptide; WKY, Wistar–Kyoto rat strain; 2VO, bilateral carotid artery ligation, two vessel occlusion; 3VO, cerebrovascular insufficiency, three vessel occlusion.

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1. Introduction

1.1. The anatomy of the cerebral circulation

1.1.1. The macrovascular supply to the brain

Although the theme of the current review is the age- and dementia-related breakdown of cerebral microvessels, it is essential to have a clear view of the arborization and regional distribution of the larger cerebral blood vessels. The microvascular network of the brain operates strongly dependent on the blood flow and resistance of the large arteries and the smaller, terminal arterioles.

Cerebrovascular research makes use of a range of experimental animal models such as vessel occlusions to unravel the contribution of an optimal cerebral circulation to the physiology and metabolism of the brain. When employing laboratory animal models (the rat and gerbil being the most frequent ones) to tackle the pathophysiology of human cerebrovascular diseases, it should be emphasized that although the organization of the cerebrovascular system is in many respects similar among mammals, some differences between species do exist. For instance, the anterior communicating artery is a well-known anatomical unit in humans but not in rats, while the olfactory artery can be found in rats but not in humans (Fig. 1) (Lee, 1995). Even more remarkable is the incomplete circle of Willis in gerbils (Mayevsky and Breuer, 1992). The description below mainly focuses on the human situation with some remarks related to the cerebral circulation of laboratory animal models popular in cerebrovascular research.

The brain receives its arterial blood supply via two major routes, the internal carotid arteries and the vertebral arteries, the latter forming the unpaired basilar artery at the junction of the medulla and the pons. The carotid system is responsible for the anterior circulation of the brain while the basilar artery provides the blood supply to the posterior cerebral circulation. Obviously, the anterior and posterior circuits are not independent of each other: the two are interconnected by communicating arteries that create the circle of Willis at the base of the brain providing potential shortcuts between the lateral as well as the antero-posterior cerebral circula-
tion (Fig. 1). However, the vertebral and carotid sys-
tems supply distinct brain regions as demonstrated by
McDonald and Potter (1951) in rabbits. Under physi-
ologically optimal circumstances the blood streaming
through the vertebral arteries does not mix with the
blood carried by the internal carotid arteries. This
phenomenon can be demonstrated by infusing vital
dyes in the carotid or vertebral arteries, which will
appear chiefly in the corresponding intracranial vessels.
Nevertheless, if the pressure gradient in the circle of
Willis changes due to an insufficient flow in either the
anterior or posterior circuits, blood from different
origin can be re-distributed via the collateral intercom-
munication in the circle. However, the degree of com-
pensation depends on the individual variation of vessel
diameters and the symmetry of the circle of Willis
(Dickey et al., 1996). The compensatory mechanisms
can play a role when the lumen of an intracranial artery
is narrowed due to severe atherosclerosis (Hartkamp et
al., 1999) or when the common carotid arteries or the
middle cerebral arteries of laboratory animals are ex-
perimentally occluded to create a model for cerebral
ischemia (Weinachter et al., 1990; Coyle and Heistad,

Arteries emanating from the posterior route, that is
from the basilar artery, predominantly furnish the
brainstem and midbrain with fresh blood whereas the
cerebral hemispheres are vascularized from both the
anterior (internal carotid origin) and posterior vessels.
The two large pairs of vessels originating from the
internal carotid arteries are the anterior and the middle
cerebral arteries, the latter carrying 80% of the blood
that reaches the cerebral hemispheres. Without present-
ing a complete and comprehensive list of target areas, it
is worth following the major routes of the larger arter-
ies. The anterior cerebral arteries send their arboriza-
tion to the frontal lobe, the preoptic and supraoptic
areas, the globus pallidus and the amygdala, while the
ramifications of the middle cerebral arteries are respon-
sible for the blood supply to the temporal and parietal
cortex, important subcortical nuclei such as the basal
nuclei and the choroid plexus in the lateral ventricles.
The posterior route reaches the occipital lobe of the
hemispheres and the diencephalon containing the sen-
sory thalamus and the vital autonomic hypothalamic
nuclei.

The major arteries enter the skull at the base of the
brain and their branches consequently advance dorsally
and spread on the surface of the cerebrum in the
subarachnoid space above the pia mater. They perfo-
rate the brain parenchyma perpendicular to the cerebral
surface without establishing anastomoses with each
other. As a narrowed continuum of the subarachnoid
space, the vessels are surrounded by the so-called Vir-
chow–Robin space, which is embraced by lep-
tomeningeal cells. The space gradually disappears as

the artery penetrates deeper in the brain tissue, only the
leptomeningeal cell layer remains to form the first, very
thin layer of the artery, the tunica adventitia. The
second and the thickest layer of the vessel wall, the
tunica media, consists of one or two layers of smooth
muscle cells which are separated from the tunica adven-
titia by elastin and collagen fibers, the lamina elastica
externa. The smooth muscle cells can regulate the flow
in the vessel by contracting or relaxing, which specifies
the most important function of arteries in controlling
blood pressure and flow. Finally, the luminal layer of
the artery is practically equivalent to the endothelial cell
layer and is often referred to as tunica intima.

1.1.2. The microvascular system and the blood–brain
barrier

The network of fine cerebral vessels and capillary
function in the brain inherently differs from that of
arteries. The general notion that arteries regulate blood
pressure while brain capillaries maintain the blood–
brain barrier (BBB) and sustain continuous nutrient,
electrolyte and waste product trafficking between neu-
ral tissue and blood is apparently reflected in the mi-
crovascular anatomy.

Cerebral capillaries represent the finest branches of
the vascular tree and, unlike arteries, they form anasto-

![Fig. 1. The anatomy of the circle of Willis as seen in human (A) and
rat (B). Abbreviations: ACA, anterior cerebral artery; ACOA, ante-
rior communicating artery; AICA, anterior inferior cerebellar artery;
ASA, anterior spinal artery; BA, basilar artery; ICA, internal carotid
artery; MCA, middle cerebral artery; PCA, posterior cerebral artery;
PCOA, posterior communicating artery; SCA, superior cerebellar
artery; VA, vertebral artery.](image-url)
Fig. 2. The ultrastructure of cerebral capillaries observed with electron microscopy. A, an electron microscopic image of a typical cortical capillary from the frontoparietal cortex of a Wistar–Kyoto rat. B, graphic reconstruction of the vessel. Abbreviations: a, astrocytic end feet; bm, basement membrane; em, endothelial mitochondria; en, endothelial nucleus; ep, endothelial cytoplasm; l, capillary lumen; p, pericytes; tj, tight junction.

moses and create a three-dimensional vascular network. The density of this mesh perforating the substance of the brain is highly variable. As a general rule, capillary density in the gray matter was found about three times as much as that of the white matter but it may be more appropriate to note that the observed differences in density apparently correlate with the activity and nutrient demand of the particular brain region. Experimental data supporting this conclusion showed a prominent correlation between capillary length per brain volume and local cerebral blood flow (Gjedde and Diemer, 1985) and between the number of capillaries, local blood flow and glucose utilization in a given brain area (Klein et al., 1986). The phenomenon that metabolically active brain regions are more heavily vascularized than less active zones is supported by the observation that capillary density appears to be most pronounced in areas rich in synapses, followed by cell body populations and finally neural fiber bundles. Furthermore, microvascular density also seems to coincide with the main task of the given brain regions: the sensory and association centers are usually more densely vascularized than motor centers. The laminar structure of the cerebral cortex also displays a typical layer-dependent density pattern where lamina IV followed by lamina I receive the densest vascularization. In addition to this density pattern, the orientation of microvessels can also show a laminar arrangement shown by the cortical capillaries, which run parallel to the surface in lamina I but form a multi-oriented network in lamina IV (Hudetz, 1997).

Fig. 3. Schematic drawing of the cerebral capillary basement membrane.

The cerebral capillaries display a typical ultrastructure crucial to execute BBB function (Fig. 2). The three cellular building blocks that participate in the formation of the capillaries are the endothelial cells, the irregularly occurring pericytes and the astrocytic end feet attached to the vessels’ abluminal surface. The capillary endothelial cells form one layer around the capillary lumen and create tight junctions (also called zonulae occludens) where they are apposed to each other. The tight junctions seal the space between the meeting endothelial surfaces and are considered as the morphological basis for the BBB gaining their full functional integrity with the maturation of the animal (Rubin and Staddon, 1999; Kniesel and Wolburg, 2000; Saunders et al., 2000). Other features that take care of the selective isolation of the brain from the blood are the lack of endothelial fenestrations and an insignificant transport via pinocytic vesicles. The capillary endothelial cells are further characterized by a relatively high number of mitochondria, which can provide the energy needed for the working of the specific BBB transport proteins (e.g. glucose- and amino acid transporters).

The endothelial cells are surrounded by a 30- to 40-nm-thick basement membrane (BM) (Fig. 3) which is often a target of investigation due to its frequently observed malformations under pathophysiological conditions (for example Alzheimer’s disease) (Perlmutter and Chui, 1990; Claudio, 1996; Kalaria, 1996; Farkas et al., 2000b). The extracellular matrix components of
the BM, namely the intrinsic collagen type IV, heparan sulfate proteoglycan (HSPG), laminin and the extrinsic fibronectin are known to be produced by the cell types of the capillaries. These BM constituents are arranged into a trilaminar structure with an endothelial layer (lamina rara interna), an astrocytic layer (lamina rara externa) and a transitory, fused layer in-between the two (lamina densa) (Fig. 3). Collagen type IV, the major structural element of the BM is preferentially located in the lamina densa while the proteins laminin and HSPG are more closely associated with the two laminae rarae, which promote cell adhesion and attachment (Perlmutter and Chui, 1990). Besides the widely cited BM elements, additional proteins that are deposited in the BM have also been identified. Cubin, synthesized by the endothelial and smooth muscle cells, is such a molecule (Charron et al., 1999), suited to cross-link cells and matrix constituents. The BM has been suggested to provide physical support to the microvessels, control cellular migration, filter macro-molecules, influence endothelial function, promote cell adhesion and protect the brain against extravasated proteins (Perlmutter and Chui, 1990).

The second, heterogeneous cell type of cerebral capillaries, the pericyte is inserted in the BM and covers the vascular wall by its extended processes. Some investigators differentiate granular and filamentous pericytes and attribute a phagocytotic role to the granular type (Tagami et al., 1990). The size and appearance of pericytic profiles seen with the electron microscope is highly variable depending on the level of slicing. When compared to endothelial cells, the density and composition of the cytoplasm looks very similar but the pericytes also contain dense bodies or lysosomes. The pericytes are often considered as a supporting cell type of capillaries, which can regulate capillary tone (Kelley et al., 1987). They also participate in the immune response as shown by their relationship with macrophages and their ability to transform into microglia. These proposals were further substantiated by the demonstration of the presence of macrophage markers on the pericytic surface, their phagocytic activity and antigen presentation (Thomas, 1999). Furthermore, the pericytes can contribute to the regulation of vascular development by inhibiting endothelial cell proliferation and differentiation via chemical signaling (Shepro and Morel, 1993; Hirschi and D’Amore, 1996; Balabanov and Dore-Duffy, 1998; Martin et al., 2000; Rucker et al., 2000).

The cerebral microvessels are supported by astrocytic processes, which are intimately apposed to the abluminal vascular surface. These astrocytic end feet are thought to play a dominant role in the ontogenesis and maintenance of the BBB (Janzer, 1993). In vitro studies have demonstrated that the close apposition of astrocytes to endothelial cells is necessary for the development of typical BBB features such as the formation of tight junctions or the expression of BBB specific proteins (Arthur et al., 1987; Minakawa et al., 1991; Rauh et al., 1992; Hurwitz et al., 1993). The induction of an endothelial BBB phenotype marker, the so-called HT7 surface glycoprotein by an astrocyte-conditioned medium is an adequate example for the latter (Janzer et al., 1993). Furthermore, astrocytes were implicated in the intracerebral regulation of vascular tone and cerebral blood flow indicated by the expression of serotoninergic and cholinergic receptors on the perivascular end feet (Cohen et al., 1996, 1999; Luiten et al., 1996; Elhusseiny et al., 1999) and the close apposition of noradrenergic nerve endings to the vascular astrocytic sheath (Cohen et al., 1997). Besides receiving neuronal innervation, the astrocytes stand in constant biochemical interaction with the endothelial cells (Goldstein, 1988; Abbott et al., 1992) shown for example by their substance-P immunoreactivity (Michel et al., 1986), the presence of endothelial NOS in their cytoplasm (Janigro et al., 1996). The physiology of cerebral blood supply stand in focus.

1.2. The physiology of cerebral blood supply

1.2.1. Flow pattern and rheological factors

The physical pattern of cerebral blood flow (CBF) and its pathological changes in brain microvessels have been reviewed with reference to the general rules of fluid dynamics extended to biologically active systems (de la Torre and Mussivand, 1993). As previously summarized (de la Torre and Mussivand, 1993), a number of major parameters can help characterize the dynamics of blood flow in the cerebral vessels, such as flow velocity, microturbulent flow, viscosity of the blood, shear stress created by the vascular wall and vascular resistance. These factors are inseparably and dynamically interrelated.

The blood flow velocity, which can be routinely determined in larger brain arteries with the use of Doppler sonography (Maulik, 1995) and can also be measured in the cerebral capillary bed with the sophisticated intravital microscopy (Hudetz, 1997), is not equal at all points in the vessel lumen throughout its transversal profile. A flow gradient can be characterized with a
decreasing flow velocity approaching from the midline of a vessel towards the vascular wall when looking at the cross section of the vessel. Moreover, near the vascular wall, the blood flow is reduced to a near standstill where the blood has a cell-free plasma layer (Fung, 1981, 1984). The plasma layer next to the vessel wall also serves a significant biological purpose, namely to allow nutrient and mineral transport from the blood to the brain parenchyma from this slow moving layer thus supplying the brain with energy substrates.

Microturbulent flow can disturb the regular passage of blood and can develop when the usual shape of the vascular lumen becomes irregular, e.g. locally thickened (fibrotic arteries, capillaries with local basement membrane thickening), partially obstructed (atherosclerosis) or compressed (Fig. 4). The flow pattern in this case becomes disrupted and random swirls can build up compromising the slow flow of the cell-free layer near the vessel wall (Fung, 1984). When such abnormalities occur in microvessels, the optimal nutrient transport through the BBB is in jeopardy and can lead to a suboptimal cerebral metabolism.

The third rheological factor of importance is the viscosity of the blood. The viscosity stands in an inverse relationship with flow velocity and CBF meaning that a higher whole blood viscosity is associated with lower flow values. Two major factors having influence on viscosity and thus oxygen-carrying capacity of the blood have been identified as the haematocrit value (Harrison, 1989) and the membrane fluidity and aggregation of erythrocytes (Schmid-Schonbein, 1983). Early indications that an increased haematocrit could contribute to a lowered CBF under neuropathological circumstances were found in clinical studies. For example, an increased haematocrit was shown to coincide with the occlusion of the carotid arteries and associated transient ischemic strokes in humans. In this study, the size of cerebral strokes could be correlated with a decreased CBF, which was suggested to be the result of a high haematocrit value (Harrison et al., 1981). However, claiming a direct causal relationship between an increased haematocrit and the development of ischemic strokes based on these data could well be an overinterpretation of the findings. Yet, a causal relationship between CBF and the haematocrit was convincingly demonstrated in patients in another study: when reducing the haematocrit, a consequent improvement in CBF was measured (Thomas et al., 1977). Supportive animal models experimenting with isovolemic hemodilution also showed that reducing the haematocrit without changing the volume of circulating blood decreased blood viscosity and could consequently enhance cerebral capillary perfusion and oxygen delivery (Lin et al., 1995; Hudetz et al., 1999). Hence, we can conclude with certainty that a lower haematocrit caring for reduced blood viscosity improves CBF. Other properties of erythrocytes like the rigidity of their cell membrane or their affinity to form aggregates can also interfere with CBF. As shown in an experimental rat model, the aggregation of red blood cells compromises microvascular perfusion (Mcbedlishvili et al., 1999). In addition, the rigidity of the erythrocyte membranes can also affect CBF by limiting the rate of capillary perfusion. The inflexibility of the cell membrane can hinder the passage of erythrocytes through capillaries therefore the membrane fluidity of erythrocytes indirectly interferes with CBF.

The contribution of shear stress (due to the above described velocity gradient of flow) to altered CBF can be accomplished through changing viscosity (Kee and Wood, 1984) and/or having an effect on vascular autoregulation (Rubanyi et al., 1990). The alteration of CBF by shear stress plays the most important role in curved blood vessel segments, where the difference in flow velocity between the middle axis and the wall of the vessel is highest. The increased shear stress can present a physical stimulus to the endothelium and may impose slowly regenerating endothelial damage (de la Torre and Mussivand, 1993). On the other hand, shear stress has also been suggested to stimulate mechanoreceptors presumably present on endothelial cells, which would activate inward rectifier K⁺-channels. In turn, NO and PGI2 (prostaglandin I2) could be released initiating an increase of vascular diameter (Rubanyi et al., 1990).

Changes in vascular diameter directly lead to alterations in vascular resistance and CBF, two inversely related physiological parameters. Any change in lumen radius will affect the resistance exponentially. The vascular resistance and CBF can be regulated by myogenic, metabolic, neuronal and biochemical means, which processes are overviewed in the following two sections.
1.2.2. The myogenic and neurogenic regulation of cerebral blood flow

The brain receives probably the most constant blood supply of all body organs maintained by a very finely tuned regulation of CBF. Physiological fluctuations in the cerebral perfusion pressure are normally compensated by the cerebrovascular autoregulation to maintain an optimal, uninterrupted CBF. An intact autoregulation is capable of keeping the CBF independent of perfusion pressure provided the perfusion pressure ranges approximately between 60 and 150 mmHg (Wagner and Traystman, 1985; Paulson et al., 1990). Below or above the given values, the autoregulatory mechanisms become uncoupled from perfusion pressure and lose accurate control of CBF. The dynamic maintenance of CBF is achieved by changes in vascular resistance, which can be controlled by local-chemical factors, endothelial factors, autacoids (e.g. histamine, prostaglandins, leukotrienes) and neurotransmitters (Wahl, 1985; Wahl and Schilling, 1993).

The basic feedback mechanisms of the autoregulatory loop in the brain have been classified as myogenic, chemical/hormonal, neurogenic or endothelial dependent. The myogenic component of cerebral autoregulation was defined as the intrinsic capacity of vascular smooth muscle cells to contract in response to mechanical stress such as an increase in transmural pressure (Ursino, 1991). This contractile response can be visualized by manipulating the transmural pressure in arteries that triggers vasoconstriction when increased. With the help of isolated rat or human brain artery preparations, an increased vascular tone and a decreased lumen diameter were detected when the perfusion pressure was gradually increased (Halpern and Osol, 1985; Wallis et al., 1996). Moreover, increased transmural pressure caused little change in CBF unless the perfusion pressure dropped below 60 mmHg, the lower limit of the autoregulatory capacity (Wagner and Traystman, 1985). Based on these results, one can conclude that stretch-dependent vasoconstriction keeps CBF constant when the perfusion pressure stays within the autoregulatory range. As mentioned above, the cellular components of the myogenic autoregulation were located in the vascular smooth muscle, which depolarizes as mechanical pressure increases (Harder, 1985). Such a pressure-activated contraction of smooth muscle cells was described to depend on the extracellular calcium concentration and to be mediated by an arachidonic acid signal transduction pathway (Harder et al., 1997). A metabolite of arachidonic acid (20-hydroxyecosatetraenoic acid, 20-HETE) in vascular smooth muscle cells serves as a potent vasoconstrictor by inhibiting the opening of calcium activated potassium channels or by activating L-type calcium currents (Harder et al., 1997). However, other endothelial substances such as endothelins released as a response to stimulation of the vascular endothelium, which is the major focus of the next section, can also indirectly elicit vascular contraction.

The neurogenic regulation of the main cerebral arteries differs from that of cerebral microvessels in that the large vessels receive extracranial innervation while the terminal microvascular beds of the brain lack such a neural supply. Similar to the systemic resistance vessels, the large arteries of the brain surface and their parenchymal branches receive sympathetic, parasympathetic and sensory fibers. A fundamental body of information was accumulated by tract-tracing studies which identified the superior cervical ganglion as the major source of sympathetic fibers (Edvinsson et al., 1990) and the sphenopalatine, otic and internal carotid ganglia as the principal origin of parasympathetic fibers (Branston, 1995). The perivascular sympathetic fibers eliciting vasoconstriction were immuno-positive to several compounds including the classical neurotransmitter noradrenaline and neuropeptides like neuropeptide-Y (NPY) (Uddman and Edvinsson, 1989) while smaller pial arteries were also reported to receive serotonergic, vasoconstrictive input from the dorsal raphe nucleus (Lincoln, 1995). On the other hand, the parasympathetic nerves showed the presence of acetylcholine (ACh) and vasoactive intestinal polypeptide (VIP), both potent vasodilators besides nitric oxide (NO), which also emerged as a significant neurogenic relaxing factor (Suzuki and Hardebo, 1993; Branston, 1995). The sensory projection fibers to cerebral arteries were shown to arise from the trigeminal ganglion and to contain additional vasodilatory peptides such as substance-P (SP) and calcitonin gene-related peptide (CGRP) (Uddman and Edvinsson, 1989).

The control of vasoconstriction mediated by autonomic fibers exerts a basic, global and relatively rough modulation of CBF while the finer tuning of regional flow rates involves several additional mechanisms depending on the vascular endothelium. Biochemical signals acting on or released by the endothelial cells can substantially modify cerebrovascular resistance. The receptors and functional involvement of local, chemical factors (adenosine), endothelial factors (thromboxanes, endothelin, endothelium-derived constrictor/relaxing factors and prostacycline), autacoids (histamine, bradykinin, eicosanoids) and hormones (angiotensin, vasopressin) (Wahl and Schilling, 1993) were widely investigated and discussed. Here, we present a selection of the most important findings of this research that are relevant to the physiology and regulation of cerebrovascular blood flow.

1.2.3. The role of endothelial factors in cerebral blood flow regulation

The vascular endothelium plays a pivotal role in CBF regulation because an important group of vasoactive biochemical compounds are released by and act on the
endothelial cells. These factors are traditionally named as endothelium-derived relaxing factors, nitric oxide (NO) being one of them, and endothelium-derived contracting factors, like endothelins. Most of the data concerning the regulatory function of NO and endothelins were collected from arterial endothelial cells, but the release of these factors from microvascular endothelium was also shown (Yoshimoto et al., 1991; Durieu-Trautmann et al., 1993; Lovick and Key, 1995). In microvessels, the potential target of these factors are the perivascular astrocytes as opposed to the smooth muscle layer in macrovessels (Durieu-Trautmann et al., 1993).

Vascular dilation mediated by nitric oxide (NO) is a well-described phenomenon. NO relaxes vascular smooth muscle and increases regional cerebral blood flow in response to shear stress to the endothelium or stimulation by acetylcholine, bradykinin or other biochemical compounds (Arnal et al., 1999). The mechanical and chemical stimuli can increase the cytosolic calcium concentration and the association of the calcium/calmodulin complex to NO synthase in the endothelial cells (Fleming and Busse, 1999), which in turn modulates NO production by increasing the gene expression and/or the activity of the endothelial NO synthase (eNOS) (Arnal et al., 1999). The origin of NO is, however, not restricted to the endothelium: NO released from neuronal terminals in addition to endothelial sources can also regulate vascular relaxation. In order to visualize the effects of endothelial NO separately from that of neuronal origin, several methods have been applied. The selective blockade of the endothelial NO synthase (eNOS), cell culture of endothelial cells (Weih et al., 1998) or the use of eNOS knockout or mutant mice (Huang et al., 1995; Strauss et al., 2000) all delivered valuable data in NO research. With the help of these models, it was shown that eNOS mediated basal vasodilatation (Huang et al., 1995) and that endothelial NO could buffer blood pressure variability (Strauss et al., 2000). Additional pioneering work using gene therapy to enhance vasorelaxation also made use of eNOS by associating its gene to an adenovirus vector and achieving augmented NO-mediated vasorelaxation in isolated arteries after gene transfer (Ooboshi et al., 1998; Tsutsui et al., 2000).

Endothelins, the very potent vasoconstrictor substances isolated from cultured endothelial cells, were widely investigated for their role in subarachnoid hemorrhage (SAH) Zimmermann and Seifert, 1998). The substances have been held responsible for the delayed cerebral vasospasm after SAH causing considerable damage to the vascular wall. Out of the presently known three endothelin isoforms, ET-1 seems to be the most potent, which probably acts primarily on the endothelin-A receptor (ET-A) (Zimmermann and Seifert, 1998). Although most data on the functional implications of endothelins come from pathological changes after SAH, endothelins can be involved in the control of CBF under physiological circumstances. As supporting evidence, it was demonstrated that when cerebral perfusion pressure was increased with norepinephrine, CBF did not noticeably follow the evoked increase, but when an endothelin-B receptor (ET-B) antagonist, bosentan was administered in combination with norepinephrine, a remarkable rise in CBF was recorded (Mascia et al., 1999). These findings may indicate that ET-B stimulation plays a role in the maintenance of a constant CBF at increasing perfusion pressure under physiological conditions. Because the ET-A and ET-B receptors, as well as the intracellular second messenger of endothelin action, the mitogen-activated protein kinase (MAPK) were identified in the vascular smooth muscle cells (Zimmermann and Seifert, 1998; Zubkov et al., 2000), the suggested regulatory mechanism gains significance in cerebral arteries.

1.2.4. Metabolic cerebral blood flow regulation

Besides the global regulation of cerebral blood supply via changing the diameter of larger brain arteries, CBF is also regulated locally at the level of microvessels, based on the metabolic activity of the particular brain area examined. Since the brain’s fundamental energy source is glucose and its metabolism requires oxygen, the coupling of cerebral glucose utilization (CGU) and cerebral metabolic rate for oxygen (CMRO₂) with CBF has been widely investigated in physiological conditions, as well as in neurodegenerative diseases. CGU is generally considered as an indicator of neuronal activity, taken that glucose is used to maintain resting membrane potential and the restoration of ion gradients after an action potential (Jueptner and Weiller, 1995). This theory may also explain the results of the study where a local administration of glutamate or NMDA to the rat cerebral cortex caused a significant rise in CMRO₂ and CBF (Lu et al., 1997). Besides consuming oxygen and metabolizing glucose, which can regulate CBF, the firing neurons also release K⁺. When the extracellular K⁺ concentration is raised, the ion acts as a vasodilator on nearby vessels and enhances CBF. At the re-establishment of neuronal resting potential, adenosine may also come free and cause an increase in CBF by vasodilatation (Kuschinsky, 1991).

Non-invasive measurements of cerebral CMRO₂ in healthy human volunteers showed a correlation between CBF and CMRO₂ (Leenders et al., 1990; Hoge et al., 1999) but these findings by themselves may not be sufficient evidence to prove a causal, regulatory relationship between CMRO₂ and CBF. Although additional animal studies provided supporting data by demonstrating that reducing blood oxygen concentration elevated CBF proportionally (Jones et al., 1981;
2. Cerebrovascular changes in normal aging

2.1. Cerebral blood flow in the aging brain

A gradual functional decline and a concomitant disintegrating morphology typically characterize the aging central nervous system. The physiological neural changes are also accompanied by a well-defined decline in cerebrovascular parameters. A decreasing CBF, lower metabolic rates of glucose and oxygen and a compromised structural integrity of the cerebral vasculature with special attention to microvessels are representative degenerative features of the vascular system of the aging brain.

Numerous clinical studies employing a range of non-invasive cerebral scanning techniques conducted investigations on blood flow velocity or CBF in healthy aging subjects. The widely used Doppler sonography has been designed to measure flow velocity in larger arteries, and is applied most frequently to monitor the flow in the large basal arteries of the brain, like the middle cerebral artery. On the other hand, methods like PET, SPECT and gas inhalation contrasted CT can detect regional CBF in outlined brain regions like the temporal cortex or basal forebrain structures.

In human aging studies, the cerebral perfusion of healthy volunteers was examined in different age groups ranging from 14 to 100 years old (Tachibana et al., 1984; Iwata and Harano, 1986; Reich and Rusinek, 1989; Vriens et al., 1989; Ackerstaff et al., 1990; Kawamura et al., 1993; Krejza et al., 1999; Schultz et al., 1999). Whereas some studies divided the study population into a young (below 60 years) and an old (above 60 years) group and compared the CBF values of the different groups (Kawamura et al., 1993; Krejza et al., 1999), others were aiming at the investigation of CBF and age in a continuum. This latter approach assessed individual CBF results and correlated them with the age of the subjects instead of comparing age groups (Schultz et al., 1999). The results of both statistical methods pointed to the same direction: when young and old groups were compared, the aged had significantly lower CBF (Reich and Rusinek, 1989; Kawamura et al., 1993) and when a correlation analysis was performed between CBF and life time, CBF negatively correlated to the age of the individuals (Tachibana et al., 1984; Iwata and Harano, 1986; Vriens et al., 1989; Krejza et al., 1999; Schultz et al., 1999).

Several brain areas, such as different cortical regions (prefrontal, frontal, temporal, parietal, occipital and cingulate), the basal ganglia (caudate putamen, lentiform nucleus) and the subcortical white matter were chosen to analyze the local perfusion rate. The CBF of the cerebral cortex and the basal forebrain was found consistently lower with advancing age, while CBF in the subcortical white matter was reported to be either decreased in the elderly (Tachibana et al., 1984) or statistically not different from younger subjects (Reich and Rusinek, 1989). However, when the experimental population was also screened for white matter lesions, the severity of the lesions in the frontal lobe also correlated with a lower CBF (Kawamura et al., 1993) demonstrating a possible causal relationship between the two.

At the same time, Doppler studies focused on the blood flow velocity in the middle-, anterior- and posterior cerebral arteries. The Doppler measurements agreed with the decline observed in regional CBF in that an age-dependent decrease of flow velocity in the basal cerebral arteries was detected (Vriens et al., 1989; Krejza et al., 1999). These data show that both the regional cerebral perfusion rate and the flow velocity in the cerebral resistance vessels decrease in healthy, aging humans.

The descriptive clinical data explained above were supported by further experiments to elucidate the functional mechanisms underlying the age-related CBF changes. The hypothesis that an impaired vasodilatation or enhanced vasoconstriction causes the decrease of CBF appears most plausible. Both possibilities were tested by infusing either the vasodilator adenosine or the dose dependently vasoconstrictive serotonin to young adult and aged experimental animals (Jiang et al., 1992; Hajdu et al., 1993). The adenosine superfusion to the cerebrospinal fluid elicited vasodilatation in both age groups but the increase in vascular caliber was considerably lower in the old animals (Jiang et al., 1992). The application of a high dose of intravascular
serotonin lead to an augmented vasoconstrictor response in the aged animals, which coincided with an additional drop in CBF (Hajdu et al., 1993). These findings indicate that the reduced CBF in aging most probably reflects a shift in vasoregulatory capacity towards the domination of constrictor responses, maybe due to the decline of compensatory/tonic vasodilatory mechanisms. A fine example for a deteriorating vasodilatory capacity was demonstrated by electrical stimulation of the nucleus basalis (Linville and Arneric, 1991; Lacombe et al., 1997). The stimulation caused an increase in regional CBF in the parietal and frontal cortex, as well as the nucleus caudatus and the thalamus in young rats but did so only in the frontal cortex and the thalamus in old animals (Linville and Arneric, 1991). These results show that there can be an age-related impairment in the perivascular neural innervation (thus the cerebrovascular regulatory circuit) in this case originating in the nucleus basalis, which can be held responsible for a lower perfusion rate in the aging brain.

2.2. Cerebral metabolic rates in normal aging

The two characteristic parameters of cerebral metabolism are the cerebral metabolic rate for oxygen (CMRO$_2$) and cerebral glucose utilization (CGU). Human studies conducted to see the correlation between life time and CMRO$_2$ have consistently found that CMRO$_2$ decreased with age though the oldest members of these study populations did not exceed 68 years of age (Yamaguchi et al., 1986; Marchal et al., 1992; Takada et al., 1992).

The surveys conducted on the decline of CGU screened healthy volunteers from 18 to 78 years old and arrived at similar conclusions to those of the CMRO$_2$ studies. Cerebral glucose metabolic rates gradually decreased with advancing age with special attention to the neocortex, the basal ganglia and the thalamus (Kuhl et al., 1984; Eberling et al., 1995; Petit-Taboue et al., 1998). Similar reduction in CGU reported in rats reinforced the clinical findings: 27-month-old Wistar rats showed a significant reduction of CGU in the hippocampus (Tack et al., 1989) and in numerous telencephalic, diencephalic and medullary nuclei (Wree et al., 1991) when compared to 3- to 4-month-old adult controls. In addition, an age-dependent decrease in the level of high energy phosphate metabolites like ATP and phosphocreatine was also detected in line with the reduced CGU values (Hoyer, 1985; Nakayama et al., 1996).

The parallel, synchronized decline of CMRO$_2$, CGU and cellular energy substrates in normal aging probably represents a gradual shift to lower cerebral metabolic activity in general, which may be either a trigger or more likely, the outcome of a similarly lower CBF.

Previous experiments from our lab employing chronic experimental cerebral hypoperfusion as a model associated the decrease in CBF with morphological malformations of cerebral capillaries (Section 4.3) (De Jong et al., 1999; Farkas et al., 2000a). The deformation of the microvascular wall can possibly hinder nutrient transport to and consequent metabolic activity of the brain (de la Torre, 1999). Interestingly, the aging brain also shows a compromised microvascular anatomy, which may interact with cerebral metabolism and brain perfusion, contributing to a suboptimal cognitive performance in the elderly. The specific age-related changes in cerebral capillary structure are the topic of the next section.

2.3. The microvascular ultrastructure in the aging brain

The declining CBF and energy metabolism of the aging brain appear to have well-described morphological correlates. At the level of the cerebral microvessels, both the capillary density of distinct brain regions and the ultrastructure of the capillary walls are prone to age-related alterations. Although increased capillary density attributed to tissue shrinkage in the human neocortex was reported earlier (Meier-Rouge et al., 1984), a number of other groups found reduced cerebral capillary density both in humans (Abernethy et al., 1993) and experimental animals (Amenta et al., 1995a,b; Sonntag et al., 1997). When examining a cohort of 30- to 85-year-old subjects, the density of microvessels in the hypothalamic paraventricular nucleus decreased with age (Abernethy et al., 1993) while the comparison of adult rats to an old group also led to the observation of the rarefaction of capillaries in cortical and hippocampal regions in the old animals (Amenta et al., 1995a,b; Sonntag et al., 1997). Such a reduction in microvascular density could be counteracted with the calcium channel blocker dardopidine (Amenta et al., 1995a,b), or be correlated with the plasma level of insulin-like growth factor (Sonntag et al., 1997) indicating the potential involvement of calcium homeostasis or circulating hormonal factors in determining capillary density in aging (see also Section 4.3).

The microanatomical studies from our lab conducted in aging rats depicted the structural abnormalities of the basement membrane (BM) of cerebral microvessels in the frontoparietal motor cortex, the entorhinal cortex and the hippocampus CA1 in groups of 16-, 24-, 30- and 32-month-old rats (De Jong et al., 1990, 1991, 1992). The assessed vascular anomalies typically included perivascular collagen deposits, also referred to as microvascular fibrosis (Fig. 5B,C), and basement membrane thickening (BMT) (Fig. 5A), both starting to appear at 20 months and becoming clearly apparent at
the senescent age of 30 months (Fig. 6B) (De Jong et al., 1991; Luiten et al., 1994). Similar pathological tendencies were reported in the CA1 and CA3 segments of aging rhesus monkeys (Keuker et al., 2000). We have also shown that additional risk factors like chronic hypertension can accelerate the progression of these age-related, degenerative capillary changes, which can develop to a considerable extent already at 60 weeks of age in spontaneously hypertensive stroke-prone rats (SHR-SP) (Fig. 5G–I, Fig. 6D) (Farkas et al., 2000d).

Fig. 5. Electron microscopic photographs of capillary basement membrane pathology in the frontoparietal cortex of 30-month-old Wistar rats (panels A, B & C), in the hippocampus CA1 area of 14-month-old Wistar rats with permanent experimental cerebral hypoperfusion (2VO) (panels D, E & F) and in the frontoparietal cortex of 60-week-old spontaneously hypertensive stroke-prone rats (SHR-SP) (panels G, H and I). A, D & G, basement membrane thickening (BMT) corresponding in the three conditions; B, E and H, fibrosis with well discernible fiber formation in the three conditions; C, F & I, amorphous fibrosis with similar appearance in the three conditions. Abbreviations: e, endothelial cell; l, capillary lumen; p, pericyte; *, basement membrane. Arrowheads are pointing at the site of basement membrane pathology.
The ultrastructural criteria of BMT state that the BM shows focal thickening, which reaches a thickness at least twice as much as a normal segment of the same vessel (Fig. 7B). Splitting and duplication of the BM were also assigned to this category of capillary abnormalities (Farkas et al., 2000c). Besides the careful definition of the electron microscopic appearance of BMT, consensus on the nature of the amorphous material accumulating in the BM has not been reached, yet. Perlmutter and Chui (1990) suggested that BMT could derive from either an increased production or a decreased breakdown of BM components, such as collagen type IV, laminin or HSPG, which was in line with the proposal of De Jong et al. (1990) arguing that microvascular fibrosis and BMT were interrelated by-products of the same degenerative process. Furthermore, additional BM proteins such as perlecan and fibronectin were found responsible for BMT in TGF beta-1 transgenic mice (Wyss-Coray et al., 2000). Others identified β-amyloid accumulating either in a non-fibrillary form or demonstrating delicate fibrils in the capillary BM (Yamaguchi et al., 1992; Inoue et al., 1999; Natte et al., 1999b), but such vascular β-amyloid depositions may rather account for the more pronounced BMT encountered in neurodegenerative diseases (Farkas et al., 2000c) than normal aging. Whatever the molecular constituents of BMT may be, its functional consequences, namely hindered nutrient and electrolyte transport mechanisms through the BBB most probably affect cognitive or other neural processes.

Attempts were also made to elucidate the pathophysiological processes that can cause the above-described BM malformations. The L-type calcium channel blocker nimodipine could successfully prevent the formation of BM deposits in the frontoparietal cortex of 30-month-old Wistar rats after a chronic 6- or 14-month treatment (De Jong et al., 1991). Similarly, administration of nimodipine or nifedipine for 20 weeks to SHR-SP rats protected against BMT in 60-week-old animals (Farkas et al., 2000d). Based on these results, it seems most likely that improvements in the calcium homeostasis significantly contribute to the integrity of the cerebral capillary BM. We suggested earlier that the microvascular protection was probably achieved via a calcium-related improvement in the neural regulation of cerebrovascular function (Farkas et al., 2000d).

Not only the BM but also the vascular cell types of cerebral capillaries can display pathological alterations in aging. The occurrence of membranous inclusions in the BM identified as degenerating pericytes was found to gradually increase with age in rats (De Jong et al., 1991; Peinado et al., 1998; Farkas et al., 2000d). The pericytes of the aging brain were furthermore characterized by an increased size of pericytic mitochondria (Hicks et al., 1983), while the mitochondrial population itself (the total number of mitochondria) remained constant (Stewart et al., 1987). Even though such clear signs of pericytic aging were noted, pericytic aberrations may not be considered as a strictly degenerative property. Taken that the pericytes were suggested to function as immune cells in the cerebral microvasculature (Thomas, 1999), the increased number of cytoplasmic inclusions and enlarged mitochondria may represent an activated, phagocytotic state of these cells. Such an increased activity may denote a combat against potential, age-related leakages of the BBB and, by doing so, could effectively protect microvascular and
neuronal integrity. Other observations also serve as additional support for the claim that pericytic aging should be handled separately from BM pathology. As opposed to BM damage, which was aggravated by hypertension, high blood pressure did not pose an additional accelerating factor for pericytic degeneration (Farkas et al., 2000c) and nimodipine treatment could not prevent the development of pericytic aberrations in aging animals either (De Jong et al., 1991). The latter finding also suggests that the degeneration of pericytes is probably not a calcium concentration-dependent process.

Another prominent cell type of the cerebral microvasculature, which can be affected by the aging process, is the endothelial cell. The common, age-related histological changes include the loss and elongation of the capillary endothelial cells, and a decrease in the number of endothelial mitochondria, but the latter feature was seen only in the monkey (Mooradian, 1988; Shah and Mooradian, 1997). Although the age-dependent ultrastructural changes in the microvascular endothelium cannot be regarded as dramatic, the compromised integrity of the endothelial layer can have its moderate, functional correlates in the barrier and carrier capacity of the BBB. As failure in the barrier function manifests itself in transient leakage of larger serum proteins into the brain parenchyma, BBB permeability became a target of investigation. The outcome of these studies, however, remained controversial and a minimal, if any, BBB leakage associated with aging in the absence of neurological diseases was concluded (Mooradian, 1988; Shah and Mooradian, 1997).

The BBB carrier systems of the aging brain showed more obvious functional decline (Pardridge, 1988). The transport of glucose via the BBB was, for example, reduced in 24-month-old rats compared to 3-month-old controls (Mooradian et al., 1991), and neutral amino acid uptake was also reported to decrease in 8-month-old mice compared to a 3-month-old group (Samuels et al., 1983). The interpretation of these data may not necessarily point to a dysfunction of the transporter proteins themselves, rather, an age-related, decreasing demand of the neural tissue for nutrients or the deviating ultrastructure of the capillary BM may account for the reduced transport.

3. Cerebrovascular pathology in Alzheimer’s disease

3.1. Cerebral blood flow in Alzheimer’s disease

The contribution of vascular factors to the etiology of dementia, with particular attention to Alzheimer’s disease (AD) has become a rapidly extending research field in the last decade. Epidemiological studies emphasized the role of peripheral vascular abnormalities like atherosclerosis or hypertension as risk factors aggravating the progression of cognitive decline (Skoog et al., 1996; Skoog, 1997; Hofman et al., 1997; Breteler, 2000), and a further link has been suggested between the systemic and notably (cardio)vascular pathophysiology and disturbed brain perfusion in AD (de la Torre, 1999; Farkas et al., 2000a,b).

Fig. 7. Typical cerebral capillary wall pathology in the human and rat. A, intact capillary profile; B, basement membrane thickening (BMT); C, perivascular fibrosis; D, pericytic degeneration.
Table 1
Reduced regional cerebral blood flow in selected brain regions of Alzheimer’s disease patients indicated as the percentage of the corresponding values of non-demented, age-matched controls

<table>
<thead>
<tr>
<th>Reference</th>
<th>Brain region (% flow)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Global Cerebral cortex</td>
</tr>
<tr>
<td></td>
<td>Parietotemporal</td>
</tr>
<tr>
<td></td>
<td>Parietal ~83.5</td>
</tr>
<tr>
<td></td>
<td>Temporal ~87.7</td>
</tr>
<tr>
<td>Costa et al., 1988</td>
<td></td>
</tr>
<tr>
<td>Komatani et al., 1988</td>
<td>~72.5</td>
</tr>
<tr>
<td>Montaldi et al., 1990</td>
<td>~88.5 ~89.5 ~82.5</td>
</tr>
<tr>
<td>Eberling et al., 1992</td>
<td>~80 ~80</td>
</tr>
<tr>
<td>O’Brien et al., 1992</td>
<td>~92 ~89.5 ~94.3</td>
</tr>
<tr>
<td>Waldemar et al., 1994</td>
<td>~87.5 ~86.9 ~88.2 ~85.9</td>
</tr>
<tr>
<td>Ohnishi et al., 1995</td>
<td>~73.8</td>
</tr>
<tr>
<td>Ishii et al., 1997</td>
<td>~84.9 ~89.6 ~86.4</td>
</tr>
<tr>
<td>Imran et al., 1999</td>
<td>Reduced Reduced Reduced</td>
</tr>
<tr>
<td>Ishii et al., 1998</td>
<td>~79.3 ~88.3</td>
</tr>
<tr>
<td>Kobari et al., 2000</td>
<td>~73.5 ~73 ~85.7 ~87.3 ~85</td>
</tr>
</tbody>
</table>

The growing literature addressing the issue of an altered CBF in AD established unanimously a decreased global CBF typical of the disease (Table 1). Even though there is a general consensus on a lower cerebral perfusion in AD, the regional distribution and degree of the drop in CBF still appears to be dependent on several factors. To mention the most important ones, the severity and particular symptoms of dementia, the age of the patient and the onset and duration of dementia can, for example, influence regional CBF (rCBF). In addition, the methodological approaches like the application of different imaging techniques (H$_2$O PET, $^{99m}$Tc-HM-PAO SPECT) or the choice of the reference region (occipital lobe, cerebellum, and whole brain) may also deliver some variation in the data. Despite the number of sources that can interfere with rCBF readings, the parietal and temporal cortices were consistently shown to be affected (Costa et al., 1988; Komatani et al., 1988; Montaldi et al., 1990; Eberling et al., 1992; O’Brien et al., 1992; Ohnishi et al., 1995; Ishii et al., 1997; Imran et al., 1999). Reduced rCBF in the frontal cortex in AD was also reported but with less consistency (Montaldi et al., 1990; O’Brien et al., 1992; Imran et al., 1999). In some cases, even though a significant decrease in rCBF in the frontal cortex compared to age-matched controls could not be established, the flow rate in the AD group still correlated with the assessed dementia score (Hasegawa’s Dementia Scale, HDS) (Komatani et al., 1988). Others showed a significant reduction in frontal rCBF when comparing mild AD to moderate AD but not when AD patients and controls were weighed against each other (Eberling et al., 1992). Hence, it seems likely that lowered rCBF in the frontal lobe becomes evident at more advanced stages of AD.

The reported degree of rCBF reduction appeared to be uniform among the temporal, parietal and frontal areas, although the drop of rCBF in the frontal cortex seemed to be slightly though not significantly less than in the parietotemporal lobe. Regional CBF values in the parietotemporal region of AD patients ranged approximately between 80 and 90% in comparison with healthy volunteers (Costa et al., 1988; Komatani et al., 1988; Montaldi et al., 1990; Eberling et al., 1992; O’Brien et al., 1992; Ohnishi et al., 1995; Ishii et al., 1997) while one study reported a decrease even to 71–75% in rCBF of the hippocampus and the parietal cortex in their cohort (Ohnishi et al., 1995).

Regarding the severity of dementia, significant reduction of rCBF in the dorsolateral frontal cortex was
measured in moderate but not in mild AD (Eberling et al., 1992). Furthermore, correlation analysis between the cognitive status of the patients visualized by a variety of dementia evaluating scoring systems and the degree of cerebral hypoperfusion repeatedly provided evidence for the association of the two parameters (Komatani et al., 1988; Montaldi et al., 1990; Eberling et al., 1992; O’Brien et al., 1992; Ohnishi et al., 1995; Imran et al., 1999). Dementia scores obtained by the Hasegawa’s Dementia Scale (HDS) were in proportion with rCBF in the frontal, temporo-parietal and parietal cortices, and in the hippocampus (Fig. 8), as well as with CBF calculated for the whole brain (Komatani et al., 1988; Ohnishi et al., 1995). Mini Mental State Examination (MMSE) scores, in a similar way, correlated to rCBF in the (dorsolateral) frontal cortex, the parietal cortex, and the posterior temporal lobe (DeKosky et al., 1990; Eberling et al., 1992; O’Brien et al., 1992; Imran et al., 1999). The results of the CAMCOG (Cambridge Cognitive Examination) test, a similar but more extensive equivalent of MMSE, were also in a significant relationship with rCBF in the frontal, parietal, (posterior) temporal and parieto-temporal cortical areas (Montaldi et al., 1990; O’Brien et al., 1992).

Taken together, these data can provide compelling evidence for a reduced rCBF in the cerebral cortex, which is proportional to the degree of cognitive decline typical for AD.

Alzheimer’s disease can be more securely established as the specific diagnosis of dementia when the post mortem analysis of the brain delivers neuropathological confirmation. Occasionally it is discovered only in the post mortem material that the subject diagnosed as an AD patient had multiple cerebral infaracts and rather belongs to the group of multi infarct dementia cases, or conversely, dementia patients of miscellaneous origin appear to have typical AD neuropathology. Therefore the correlation between CBF and AD-like neural lesions could add supplementary aspects to the relationship between CBF and AD. Unfortunately, no clinical study performed a correlation analysis between CBF and the severity of neuronal breakdown in AD, but experimental models did aim at unravelling a connection between cerebral hypoperfusion and neuronal damage. The permanent ligation of major arteries supplying the brain has been developed as a model to investigate the histological and behavioral effects of a reduced CBF. The bilateral occlusion of the common carotid arteries of rats (2VO) led to a dramatic initial drop in rCBF which returned to 30–45% of rCBF in the cortex and 20% reduction in the hippocampus 1 week after surgery (Tsuchiya et al., 1992) while the more severe three-vessel occlusion (3VO) ligating the subclavian artery in addition to the common carotid arteries caused a dramatic 25–80% drop in the parietal

![Fig. 8.](image-url) Correlation between regional cerebral blood flow (rCBF) and the severity of dementia in Alzheimer’s disease. A, hippocampal rCBF (modified from Ohnishi et al., 1995); B, frontal cortex; C, temporo-parietal cortex (modified from Komatani et al., 1988; reprinted by permission of the Society of Nuclear Medicine).
cortex and a 40–60% decrease in the hippocampus at 3 or 9 weeks following the occlusion (de la Torre et al., 1992). The histological examination of the 2VO brains after 190 days of hypoperfusion showed a significant loss of hippocampal CA1 neurons and a greater glial fibrillary acidic protein (GFAP) immunoreactivity, the sign of reactive gliosis (Pappas et al., 1996). The hippocampal cell loss was later identified to be the result of apoptotic cell death (Bennett et al., 1998). At the same time, the 3VO condition led to the observations that the hippocampal CA1 damage represented necrosis of pyramidal cells accompanied also with a higher density of GFAP immunoreactivity (de la Torre et al., 1992). The discrepancy in the nature of neuronal injury may stem from the degree of cerebral hypoperfusion created in the two paradigms (2VO or 3VO), since apoptotic and necrotic cell death in neuronal populations can be sequential depending on the severity of the insult. Finally, the extracellular accumulation of amyloid precursor protein (APP) and its cleavage to β-amyloid-like fragments in the hippocampus of aged rats was described in the 2VO paradigm, as well (Pappas et al., 1997; Bennett et al., 2000). Although all these data were obtained by imposing a very dramatic drop in rCBF never encountered in humans (which basically renders the strict comparison with AD limited), these experiments are very valuable from several points of view. First, they reinforce the coincidence of decreased rCBF with neuronal damage. Second, these animal models proposed a possible causal order of cerebral hypoperfusion and the subsequent neuronal injury. Third, the use of the experimental cerebral hypoperfusion models amply demonstrated the sorts of potential neurodegenerative features that may arise as a consequence of chronically reduced CBF. In summary, the experimental findings are arguing for a link between compromised CBF and neuronal histopathology.

3.2. The cholinergic hypothesis of cerebral blood flow regulation and its implications in Alzheimer’s disease

A marked deficiency in cholinergic neurotransmission is one of the distinguishing degenerative features of AD. The loss or shrinkage of cholinergic neurons in the basal forebrain and the medial septum and the disappearance of cholinergic projection fibers from the nucleus basalis (NBM) to the neocortex and from the medial septum to the hippocampus were widely investigated in view of the concomitant memory deficits typical of AD (Gaykema et al., 1992; Muir et al., 1993; Whitehouse, 1998). Whereas studies on the substantial contribution of the cholinergic pathways to cognitive processes have a long history, the capacity of this network to influence CBF has only recently gained more attention. It has been recognized that the activation of the substantia innominata/NBM complex (SI/NBM) could increase regional CBF by inducing vasodilatation in cortical microvessels (Fig. 10A) (Biesold et al., 1989; Vaucher et al., 1994, 1995; Barbelivien et al., 1999). The striking coincidence of a reduced cortical CBF and cholinergic neurodegeneration of the NBM both seen in AD patients has stimulated more extensive research in this field.

The involvement of acetylcholine (ACh) as a neurotransmitter in the control of regional CBF was demonstrated by the administration of cholinergic drugs such as scopolamine, physostigmine or eptastigmine. When a group of human subjects was treated with scopolamine, a non-selective muscarinic ACh receptor blocker, a 20% decrease of CBF in the frontal cortex was detected (Honer et al., 1988). On the other hand, the application of the ACh-esterase inhibitors physostigmine and eptastigmine, which increase the extracellular level of endogenous ACh, led to a significant increase of CBF in a wide variety of brain regions in the rat and man (Scremin et al., 1993; Blin et al., 1997; Peruzzi et al., 2000). Although these experiments provided convincing evidence for the involvement of ACh in CBF regulation, the origin and exact target structures of the neurotransmitter still remained to be clarified.

The source of cholinergic innervation to cortical vessels was identified by either the lesion or the stimulation of the cholinergic basal nuclei, and the consequent measurement of changes in CBF (Biesold et al., 1989; Vaucher et al., 1994, 1995, 1997a; Barbelivien et al., 1999). These experiments can be regarded complementary to the drug treatment studies but it must be kept in mind that although the largest cell population in the SI/NBM complex is cholinergic, other cell types (e.g.
GABAergic) also mingle with them and are represented to a lesser extent. Thus, it should be anticipated that the lesioned or electrically stimulated nucleus basalis neurons might not exclusively employ ACh as their neurotransmitter.

The unilateral lesion of the SI/NBM by ibotenic acid injection in rats offered the possibility to compare CBF in the lesioned side to the intact, contralateral hemisphere. According to the expectations, a significant drop in cortical CBF was detected in the ipsilateral hemisphere 1–5 weeks after the lesion (Fig. 9) (Gomi et al., 1991; Peruzzi et al., 1996, 2000) giving support to the assumption that the basal forebrain can modulate regional CBF in the neocortex. Hence, the loss of cortical cholinergic innervation in AD may contribute to the reported reduced CBF in the disease in a similar fashion. Data accomplished simultaneously by other experiments employing stimulation of the SI/NBM corroborated the same line of reasoning. Electrical stimulation of the SI/NBM via chronically implanted electrodes (Vaucher et al., 1994, 1995, 1997a) or the neurochemical activation of the SI with the cholinergic agonist carbachol (Barbelivien et al., 1999) both resulted in a significant increase of regional CBF in the cerebral cortex and subcortical, extrapyramidal structures. The outcome of these studies thus securely established the concept of a cholinergic neurogenic component of CBF regulation, which originated in the basal forebrain and predominantly affected cerebral cortical areas. Nevertheless, the question was still left open whether projections from the SI/NBM were direct or indirect and whether ACh was the final neurotransmitter at the perivascular sites or an intermediator at preceding synapses.

The physiological studies described above already tackled this problem and arrived at the conclusion that the nature of innervation from the basal forebrain was probably region-dependent. More specifically, the existence of a direct projection to the cortical microcirculation — as opposed to subcortical structures — was suggested based on the pattern of uncoupling between CBF and CGU after electrical stimulation (Vaucher et al., 1997a). In addition, the frontoparietal cortex was pinpointed as the more specific cortical target of cholinergic innervation to microvessels because scopolamine administration preceding chemical activation of the SI could abolish the increase of CBF in the frontoparietal but not in other cortical areas (Barbelivien et al., 1999). Nonetheless, the final evidence for the direct and indirect pathways from the SI/NBM to cortical microvessels was provided by anatomical tract tracing experiments (Vaucher and Hamel, 1995) and the demonstration of cholinergic receptors in the microvascular domain itself (Luiten et al., 1996; Elhusseiny et al., 1999). In the frontoparietal cortex, perivascular nerve terminals arising from the basal forebrain were visualized with an electron microscope after injecting Phaseolus vulgaris leucoagglutinin (PHA-L), the widely used anterograde tracer, into the SI. These PHA-L

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Fig. 10. The cholinergic innervation of cortical cerebral vessels. A, Cholinergic projection from the nucleus basalis/substantia innominata complex to capillaries in the frontoparietal cortex of the rat. B, Cholinergic synaptic elements in close approximation to a cortical capillary. C, Electron microscopic image of a cortical capillary with an astrocytic end foot immunopositive for muscarinic acetylcholine receptors (dark deposits). Abbreviations: a, astrocytic end foot; ACh-ax, axon terminal employing acetylcholine as neurotransmitter; cap, capillary; e, endothelial cell; FRC, frontoparietal cortex; l, capillary lumen; mAChR’s, muscarinic acetylcholine receptors; NBM/SI, nucleus basalis/substantia innominata complex; p, pericyte; *, basement membrane.
positive nerve endings were furthermore comparable with terminals immunoreactive for choline acetyltransferase (ChAT) (Vaucher and Hamel, 1995). The microvascular postsynaptic elements of cholinergic neurotransmission were identified as muscarinic ACh receptors (mAChRs) by conducting receptor binding studies on isolated rat cerebrocortical microvessels (Grammas et al., 1983). Further research localized the mAChR proteins exclusively on the perivascular astrocytic end feet (Fig. 10) (Luiten et al., 1996; Van der Zee and Luiten, 1999) while the mRNA of several mAChR subtypes could be detected in cultured endothelial cells and smooth muscle cells in addition to astrocytes (Elhusseiny et al., 1999).

Putting all these findings together, the outline of a well-defined cholinergic pathway from the basal forebrain to the frontoparietal cortical microvasculature emerges, which is capable of increasing regional CBF in the given cortical area. Projecting the picture of this anatomical and physiological circuitry of neurogenic CBF regulation to AD brains, the attractive theory that cholinergic deficits in AD have pathophysiological cerebrovascular consequences appears to be justified. The cholinergic neurodegeneration in the basal forebrain can therefore account for the decreased CBF at least in the frontal lobe, but the degeneration of indirect projections from the SI/NBM to cortical microvessels via interneurons that release NO (Vaucher et al., 1997b; Tong and Hamel, 1999) may also contribute to the failure of CBF control in other cortical areas, such as the temporal lobe.

3.3. Metabolic parameters and microvascular function in Alzheimer brains

The significantly reduced CBF in AD, which may be the outcome of an impaired vascular regulation was also linked to a depressed cerebral glucose metabolism reflected by cerebral glucose utilization measurements (CGU) (Hoyer et al., 1991; Fukuyama et al., 1994). The affected areas exhibiting suboptimal metabolism coincided with those displaying a marked decrease of rCBF, namely the temporal, parietal and, to a lesser degree, the frontal lobe (Friedland et al., 1985, 1989; Fukuyama et al., 1994). In addition, the lower CGU values acquired from different cortical gyri could be related to particular memory performances: episodic memory failure correlated with CGU in the superior temporal gyrus, the mesial temporal cortex and the cingulate gyrus. Short-term memory disturbance was accompanied by lower CGU in the angular gyrus, the supramarginal gyrus and the superior temporal gyrus, while semantic memory was associated with glucose metabolism in the left inferior frontal gyrus, the temporoparietal junction, the angular gyrus and the supramarginal gyrus (Desgranges et al., 1998).

To specify the sequence of events, which would account for the compromised glucose utilization, a number of factors can be considered, of which the microvascular aspects stand in focus here. Circulating glucose penetrates the brain via the BBB by active transport, which employs a specific glucose transporter protein (GLUT-1) localized in the capillary endothelial membranes at a high density. Immunolabelling and binding experiments of GLUT-1 revealed a decrease of GLUT-1 sites in the hippocampus and the cerebral cortex of AD patients (Kalaria and Harik, 1989; Horwood and Davies, 1994; Simpson et al., 1994). Based on these observations, the diminishing glucose transport through the BBB due to the decreased GLUT-1 density could perform as the limiting step of CGU rate. Such an explanation, however, appears to be contradictory to the proposal that the levels of glucose transporter expression (mRNA for GLUT-1) are regulated according to the metabolic demand and regional CGU of the neural tissue (Vannucci et al., 1998). This theory could mean that the neuronal damage and a concomitantly reduced CGU in AD should precede a decreased concentration of GLUT-1. The conflicting opinions may find a compromise in the results that the significantly reduced GLUT-1 protein concentration was not accompanied with a proportionally lowered GLUT-1 mRNA level in AD (Mooradian et al., 1997) which infers the following. Firstly, the GLUT-1 mRNA concentration does not necessarily indicate the density of the actual transporter protein, and secondly, CGU regulating GLUT-1 mRNA expression is then probably not the only factor responsible for the reduction of GLUT-1 protein density in AD. The latter conclusion is strongly underscored by further evidence for the involvement of brain trophic factors in the control of GLUT-1 gene expression (Boado, 1998). Thus, CGU may not simply be a cause but also a potential result of the reduced GLUT-1 density in the disease.

3.4. Microvascular ultrastructure and its implication in blood–brain barrier function in Alzheimer’s disease

3.4.1. Microvascular cell types

The vascular system of the AD brain can exhibit a variety of histopathological properties, which can have serious physiological consequences. Atherosclerosis in the extracranial vessels supplying the brain (Kalaria, 1996; Hofman et al., 1997; Breteler, 2000) or amyloid angiopathy in mid-size brain arteries (Vinters, 1987; Coria and Rubio, 1996; Opeskin, 1996) not only physically thicken the vascular wall, but also contribute to marked blood flow disturbances by narrowing or even blocking the vessel lumen, initiating cerebral hemorrhage or massively increasing the rigidity of the arterial wall. Besides the macrovascular lesions, the malformations of the cerebral microvessels in AD have also been...
known for a relatively long time, but the exact identification of the nature and composition of the artifacts embedded in the microvascular walls has still not been completely agreed upon. In the light microscope, microvascular abnormalities in AD appeared as atrophic thin vessels, glomerular loop formations, fragmented vessels and twisted or tortuous vessels (Bucé et al., 1997). Early ultrastructural studies often mentioned an ‘irregular’ appearance or distorted abluminal surface of the terminal arterioles and capillaries in the AD brain, which referred to the loss of the smooth contour characteristic of healthy vessels (Scheibel, 1987; Hashimura et al., 1991; Kimura et al., 1991). The initial morphological aberrations found in AD can be summarized briefly as follows. The smooth muscle cells (SMC) responsible for vascular contractility were found to show abnormal focal constrictions and a general degeneration (Miyakawa et al., 1988; Hashimura et al., 1991; Kimura et al., 1991), the astrocytic end feet often appeared swollen (Higuchi et al., 1987; Yamashita et al., 1991) and the pericytes displayed either atrophy (Miyakawa et al., 1988) or were encountered at a higher frequency in capillary profiles of AD patients than controls (Stewart et al., 1992). The endothelial cells demonstrated atrophy, swelling or contained irregular nuclei (Miyakawa et al., 1988; Hashimura et al., 1991). The basement membrane (BM) of brain capillaries exhibited a robust thickening and local disruption (Mancardi et al., 1980; Miyakawa et al., 1988; Scheibel et al., 1989; Yamashita et al., 1991) while the perivascular plexus often disappeared (Scheibel, 1987; Scheibel et al., 1989).

Detailed analysis of the morphological abnormalities provided a more precise account of the structural microvascular changes in AD. The endothelium stands in focus for its indispensable participation in creating the BBB, therefore the pathological alterations in the endothelial structure can very well correspond with BBB failure such as transient leakage. The AD-related degeneration of the capillary endothelium pointed out in the earlier studies was verified by the absence of the otherwise obvious immunoreactivity of specific endothelial cell markers (CD34 and CD31) (Kalaria and Hedera, 1995). Furthermore, the cytoplasmic compartments of the endothelial cells were quantitatively investigated, which revealed that although the absolute number of mitochondria was not changed (Mancardi et al., 1985; Stewart et al., 1992), the area of mitochondrial profiles, as well as the density of mitochondria in the endothelial cytoplasm were significantly reduced in AD (Stewart et al., 1992). Because the absolute mitochondrial counts were not deviating from normal, control values, the reduced mitochondrial density could indicate either endothelial swelling or mitochondrial shrinkage, both implying impaired BBB capacity. The number of pinocytic vesicles was reported to remain comparable to controls (Stewart et al., 1992), but later a strong inverse correlation was established between mitochondrial and vesicular content (Claudio, 1996), which may represent failing BBB barrier function for the following reasons. The increased number of pinocytic vesicles in cerebral microvessels was regarded as a form of BBB disruption (Hirano et al., 1994), while the mitochondria represent the endothelial metabolic center providing energy for the maintenance of the BBB. When the number of mitochondria decreases relative to the pinocytic vesicular content in the endothelial cell or inversely, the pinocytic vesicles multiply relative to the number of mitochondria, less energy is available for minimizing the non-specific vesicular ‘leakage’ across the BBB represented by the pinocytic vesicles. An additional sign of a disrupted BBB in AD may be the compromised morphology of tight junctions (Claudio, 1996). The number of tight junctions per standard vessel length was also remarkably decreased but the length of the junctions itself did not alter (Stewart et al., 1992).
Furthermore, the degeneration of microvascular pericytes was noted in AD brains but when the ratio of capillary profiles containing degenerative pericytes was compared to age-matched controls, no difference between the two sample groups was seen (Fig. 11) (Farkas et al., 2000c). Conversely, previous observations showed that the number of pericytic processes increased in AD brains (Stewart et al., 1992). The two sets of data do not stand in conflict, however, because the increase was notable in intact and not degenerating pericytic profiles. These findings together indicate that AD does not enhance the already existing, age-related pericytic pathology, but the proliferation of the healthy perivascular cells can still be amplified in the disease, possibly as a compensatory mechanism, working against BBB disruption.

3.4.2. Microvascular basement membrane pathology in Alzheimer’s disease

The prominent thickening of the basement membrane (BMT) considerably affected the capillaries in the cerebral cortex of AD subjects and was consistently reported by a number of research groups analyzing either biopsy tissue or post mortem material (Fig. 6A, Fig. 12) (Mancardi et al., 1980; Perlmutter and Chui, 1990; Claudio, 1996; Farkas et al., 2000c). The advancement of BMT appeared to be neurodegeneration-rather than AD-specific, since Parkinson’s disease patients and spontaneously hypertensive stroke prone rats also developed microvascular BMT in the cerebral cortex being in many ways comparable to that found in AD subjects (Fig. 6) (Farkas et al., 2000a,c). Furthermore, BMT was locally associated with the neuropathological events in AD. The temporal gyrus with severe AD-like neurodegeneration was shown to selectively contain microvessels with BMT in contrast to healthy microvessels in the intact cerebellum (Zarow et al., 1997). Among the potential candidates as molecular constituents of BMT, collagen can be mentioned first for the following reasons. Firstly, the deposition of collagen fibrils in the BM was noticeable in the form of fiber bundles (microvascular fibrosis) on the electron microscope screen (Farkas et al., 2000c), and the biochemical detection of the collagen content of cerebrocortical microvascular fractions also showed a significant, AD-related increase, particularly of collagen type IV, the basic element of the healthy BM (Kalaria and Pax, 1995).

The other molecular BM constituents such as heparan sulfate proteoglycans (HSPGs) and laminin can also be overexpressed in AD brains. For example, the levels of the HSPG agrin were found altered in association with microvascular damage in AD hippocampus and prefrontal cortex (Berzin et al., 2000). Furthermore, immunocytochemical labeling detected the presence of HSPGs and laminin not only in the vascular walls but also in senile plaques in AD samples (Snow et al., 1988; Perlmutter et al., 1991; Jucker et al., 1996; Fukuchi et al., 1998). The HSPG proteins accumulated in close approximation to capillaries and were colocalized with extravascular amyloid in the frontal, temporal and parietal cortices of AD patients (Perlmutter et al., 1990). Further examination of the different HSPGs showed that typically agrin, less frequently glypicans and syndecans but not perlecan was widely expressed in cerebral blood vessels, senile plaques and neurofibrillary tangles of AD brains (Verbeek et al., 1999). The wide distribution of these extracellular matrix proteins indicates that other cell types than the typical vascular compartments can express HSPGs and laminin. In fact, HSPGs were immunolocalized to a subset of astrocytes and neurons (Snow et al., 1988) and reactive astrocytes were also described to produce laminin (Jucker et al., 1996). Hence, the potential pathological accumulation of HSPGs and laminin in the microvascular wall could very well originate from the perivascular astrocytes or neuronal plexus.

Fig. 12. Electron microscopic images of capillary basement membrane deposits from the cingulate cortex in Alzheimer’s disease. A, basement membrane thickening. B, basement membrane splitting. C, fiber deposits in the basement membrane. Abbreviations: a, astrocytic end foot; e, endothelial cell; er, erythrocyte; p, pericyte; *, basement membrane. Arrowheads are pointing at the sites of basement membrane pathology.
3.4.3. Beta-amyloid peptide deposits in the cerebromicrovascular wall

Besides the intrinsic BM components like collagen, HSPGs and laminin, other sorts of proteins thought to be exogenous to the microvascular wall can also be deposited into the BM. The toxic β-amyloid peptide (Aβ), the hallmark of AD typically occurring in extracellular cerebral plaques, is often encountered as amorphous material or fine fibrils in the capillary BM of AD patients (Yamaguchi et al., 1992; Perlmutter, 1994; Inoue et al., 1999; Natte et al., 1999b). Such Aβ deposits, also designated as cerebral microangiopathy, may alternately account for BMT, though probably not all forms of it since BMT is known to occur in Parkinson's disease and chronic hypertension, as well (Farkas et al., 2000a,d).

Competing theories attribute the origin of Aβ causing cerebral microangiopathy to either the circulating blood, the vessel wall itself or the brain parenchyma (Burgermeister et al., 2000). The blood-borne Aβ hypothesis finds support in the experimental data that the plasma Aβ concentration is markedly increased in AD patients (Kuo et al., 1999), that experimentally infused Aβ can be traced back in the cerebral vascular walls (Mackic et al., 1998), and that transport mechanisms for Aβ via the BBB, such as receptor mediated transport or BBB leakage caused by Aβ toxicity, were also demonstrated (Pluta et al., 1996; Zlokovic, 1996; Janesco et al., 1998; Poduslo et al., 1999; Strazielle et al., 2000). Advocates of the vessel wall hypothesis promoting the concept that perivascular cells can locally produce the accumulating Aβ (Kalaria et al., 1996) have also gathered compelling evidence to confirm their theory. The mRNA of the amyloid precursor protein (APP) was detected with in situ hybridization in endothelial cells, in SMCs and pericytes isolated from AD brains (Natte et al., 1999a), which established the indispensable existence of APP in the vascular domain for potential Aβ production. Another research group identified the APP protein itself in the vascular SMCs of leptomeningeal vessels (Shoji et al., 1990) while further evidence supported the idea of a myocytic origin of Aβ by showing that SMCs of aged dogs accumulated Aβ immunoreactive material of endogenous origin when being kept in cell culture (Wisniewski et al., 1995, 2000). Conflicting results, however, argue for other mechanisms for Aβ deposition in SMCs like the ability of SMCs to readily internalize and assemble Aβ probably via receptor-mediated endocytosis (Urmon et al., 1997). Even if SMCs are indeed able to process Aβ themselves, they can be held responsible only for cerebral amyloid angiopathy (CAA), that is Aβ accumulation in larger leptomeningeal or cortical vessels, and probably not for microangiopathy. Instead, the miscellaneous group of pericytes or the single layer of endothelial cells could presumably participate in the microvascular processing of Aβ. Even though the pericytes were suggested to have the ideal features to take up APP and process the protein to Aβ (Perlmutter, 1994), experimental evidence is scarce to support this claim. The possible endothelial origin of Aβ also seems problematic since no proof other than the expression of the APP gene in the presence of interleukin-1 together with the detection of APP mRNA in the endothelial cells was gathered (Goldgaber et al., 1989; Natte et al., 1999a). Thus, the source of Aβ in the cerebral capillary BM must be rather the result of Aβ-trafficking via the BBB, either from the plasma to the brain, or conversely from the nervous tissue to the cerebral circulation. Furthermore, it also seems likely that even though the cerebrovascular cell types may not produce Aβ themselves, Aβ is still seriously toxic to all the endothelium, the SMCs and the pericytes (Kalaria, 1997; Price et al., 1997; Suo et al., 1997; Thomas et al., 1997; Verbeek et al., 1997; Jancso et al., 1998) and can contribute to the pathological breakdown of the BBB and compromised microvascular integrity seen in AD.

4. Animal models for cerebral hypoperfusion

4.1. Cerebral blood flow after vascular occlusion

4.1.1. Frequently used animal models

Since it has been anticipated that there is a dynamic interaction between hypertension, reduced CBF, cerebral microvascular pathology, cognitive performance and memory capacity, experimental models were established to investigate the causal relationship between these factors. The laboratory animal models offer the possibility to take the correlation analysis between CBF, vascular parameters and cognitive performance accomplished in human studies one step further since correlation analysis offers only a description of the coincidence of particular factors, but not causality per se. Our theory presented in the Introduction contemplates that chronically reduced CBF can trigger the degeneration of the capillary ultrastructure in the brain. Creating a reduction of cerebral blood flow in laboratory animals can test such a presumed sequence of events best. The ligation of the different large arteries that supply the brain is routinely applied under experimental conditions to achieve various degrees of cerebral hypoperfusion (Fig. 13). The bilateral occlusion of the common carotid arteries (two-vessel occlusion, 2VO) is a well characterized method in rats and gerbils, but the gerbil model is more suitable for ischemia research for the following reasons. The posterior communication between the basilar artery and the carotid artery system is often missing in gerbils, meaning an incomplete circle of Willis (Mayevsky and Breuer, 1992). This anatomical peculiarity has substantial functional implications such
as the incapability of the basal cerebral circulation to compensate for a reduced blood supply via either the carotid or the basilar arterial system. Therefore 2VO in gerbils severely affects the anterior cerebral circulation causing acute ischemic attacks. Due to this phenomenon the rat rather than the gerbil has been chosen to reconstruct chronic cerebral hypoperfusion comparable to the human situation. The 2VO surgery in rats produces a less marked hypoperfusion compared to the cerebrovascular insufficiency imposed by the three-vessel occlusion technique (3VO), the ligation of the subclavian artery combined with the occlusion of the two common carotid arteries (de la Torre et al., 1992; Tsuchiya et al., 1992; Ohta et al., 1997). The ligation of the middle cerebral artery (MCAO) is also in practice but has been more regularly applied as an ischemic stroke model due to its acute, severe consequences (Nagahiro et al., 1998).

4.1.2. The time course of experimental cerebral hypoperfusion

When the cerebral blood supply through the carotid arteries is experimentally shut off, a compensatory redistribution of blood in the circle of Willis and perhaps other processes that counteract the sudden drop in CBF can be expected. In order to estimate how long it takes for the cerebral circulation to normalize CBF and to see to what degree blood flow can be restored following 2VO, it is worth following the temporal dynamics of cerebral perfusion after the surgery. The expected recovery of CBF can be carefully reconstructed based on the data available in literature (Fig. 14). The earliest research paper on the topic reported a striking 90–95% drop in cortical rCBF at 1 or 3 h after the ligation (Sadoshima et al., 1983), which, however, could not be reproduced by later studies. Most follow-up studies point to the following time course of CBF alteration after bilateral carotid ligation (Fig. 14). The cortical...
rCBF was 34% of the control value at 2.5 h after surgery, increased to about 52% 2 days later, reached roughly 64–66% at 1 week and 80% 3 months after 2VO. Although the latest cortical rCBF value obtained 3 months after the ligation was still lowered, it did not differ significantly from the control values any more (Tsuchiya et al., 1992, 1993; Ohta et al., 1997). The decrease of rCBF in the hippocampus appeared to be less dramatic than in the cortex but the degree of hypoperfusion was still considerable. The hippocampal perfusion rate fell to 52% of the control 2.5 h after the 2VO surgery, 70% 2 days later, a still significant 74% after 1 week and a non-significant 75% at 3 months survival time (Tsuchiya et al., 1992, 1993; Ohta et al., 1997). When the hippocampal Ammon’s horn (CA region) and the dentate gyrus were separately examined, the CA area showed a slightly lower rCBF than the dentate gyrus (Tsuchiya et al., 1992, 1993). The blood flow of the same regions in the above-introduced cerebrovascular insufficiency (3VO) model exhibited the following alterations. The rCBF in the hippocampus CA1 section reached only 38% of the control value 3 weeks after imposing 3VO but recovered to normal level 9 weeks after the surgery. The cortical flow suffered a setback to 85% at 3 weeks but also returned to control level at 9 weeks (de la Torre et al., 1992).

The comparison of young and aged rats in the 3VO paradigm delivered interesting observations. While the CBF of 1-month-old rats recovered to normal values already at 9 weeks after the surgical intervention as presented above, 14-month-old animals could not compensate for the reduced cerebral blood supply in the given time frame; what is more, their hippocampal and cortical rCBF decreased even further. In particular, the cortical hypoperfusion was striking with its very low rates of 26% at 3 weeks and only 20% at 9 weeks survival time while the hippocampal rCBF stagnated from 66 to 40% (de la Torre et al., 1992). The possible explanation may point out the loss of regulatory capacity in the aging brain which can extend to delayed or even impaired compensatory mechanisms against a lowered cerebral perfusion pressure and blood supply. The regulation of rCBF would normally sustain the gradual return of rCBF to control values as seen in young animals.

As mentioned in Section 3.1, longitudinal clinical studies on the etiology of AD raised the issue of the involvement of cardiovascular factors in the disease process. Chronic hypertension in particular is a condition, which was recently associated with cerebrovascular deficiencies in AD. Hypertension could accomplish its detrimental effects on cerebral vessels via creating cerebral hypoperfusion, an existing condition in hypertensive patients, as well as in experimental animals with spontaneously high blood pressure (Nobili et al., 1993; Fujishima et al., 1995; Nakane et al., 1995; Katsuta, 1997). In particular, the results obtained with spontaneously hypertensive rats (SHR) demonstrated that the cortical rCBF was reduced in the SHR animals compared to normotensive controls (WKY), and the cerebral hypoperfusion affected more extensive regions with the advancement of age, observed in groups of 4- and 16- to 17-month-old SHR rats (Katsuta, 1997). Furthermore, a long-term antihypertensive treatment of SHR rats restored the cortical rCBF to values of normotensive controls, which also showed a significant linear correlation with the decreasing mean arterial pressure (Fujishima et al., 1995). Though the SHR rat strain is not strictly a cerebral hypoperfusion model, the coincidence of chronic hypertension and the reduced CBF in this rat strain can reinforce the idea that peripheral vascular factors or enhanced vasoconstriction contribute to the disturbance of cerebral perfusion, which phenomenon can be extrapolated to the aging brain and dementia cases. Finally, the intriguing resemblance of deteriorated cerebral microvascular morphology and memory disturbances assessed in SHR and 2VO rats encourage this line of reasoning further (Farkas et al., 2000 a,b), and are discussed in detail below.

4.2. Metabolic markers in experimental cerebral hypoperfusion

In addition to the degree of cerebral hypoperfusion, the regional cerebral glucose utilization (rCGU) in separate brain regions was also measured in 2VO animals. The data obtained in the experiment showed that rCGU in cortical regions followed the reduction in rCBF 1 week after the vascular ligation but no significant changes occurred in the hippocampus. In fact, the cerebral cortex emerged as the most sensitive area, which is reflected by the constantly reduced rCGU measurements in its six separate parts measured, while only five out of the other 18 brain regions examined demonstrated a significant reduction in glucose utilization. However, correlation analysis between CGU and CBF averaged to the whole brain showed a very significant linear relationship in both SHAM control and 2VO animals (Tsuchiya et al., 1993).

Others examined the activity of cytochrome oxidase, the terminal enzyme of the mitochondrial electron transport chain, which generates ATP via oxidative phosphorylation. Quantitative histochemistry revealed that 4 weeks after the 2VO surgery cytochrome oxidase activity was selectively reduced in the hippocampal CA1 area and the posterior parietal cortex (de la Torre et al., 1997). The diminishing mitochondrial enzyme activity probably reflected impaired ATP synthesis and a suboptimal neuronal energy metabolism. The fact that the changes were detected in areas deeply involved in spatial memory processing (hippocampus CA1)
probably accounts for the compromised spatial learning performance of these animals tested in the Morris water maze (de la Torre et al., 1997).

4.3. Cerebral microvascular damage due to experimental cerebral hypoperfusion

The 2VO model proved to be a very suitable method to determine the effects of a decreased CBF on the ultrastructure of cerebral capillaries. Unlike the acute MCAO often imposed in stroke research, where after a short blockage of blood flow the subsequent reperfusion phenomenon is also studied, the chronically applied 2VO paradigm induces a relatively mild but permanent brain hypoperfusion. Further difference between the MCAO and 2VO is that, in contrast with MCAO, stroke does not normally occur in normotensive animals as a result of 2VO.

In one of our previous studies (De Jong et al., 1999), the microanatomy of brain capillaries was investigated with electron microscopy (EM) 14 months after creating cerebral hypoperfusion by 2VO. The hippocampus was chosen as the area of interest due to its selective neuronal and metabolic vulnerability represented by the neuronal damage and the reduced cytochrome oxidase activity observed here under 2VO condition (Pappas et al., 1996; de la Torre et al., 1997). Within the hippocampus two distinct regions, the CA1 and the dentate gyrus were analyzed and compared with each other. The microvascular histopathological features that we quantified were basement membrane (BM) deposits and pericytic degeneration. The category of BM deposits represented a merged group of basement membrane thickening (BMT) and microvascular fibrosis described in detail above (Sections 2.3 and 3.4.2), while pericytic degeneration was defined as irregular membranous inclusions in the pericytic cytoplasm. The ratio of capillaries displaying either of the two types of abnormalities (that is BM deposits or degenerating pericytes) was calculated and expressed as the percentage of the total number of capillaries analyzed. Out of the two hippocampal regions, only the CA1 exhibited a significant increase in both capillary BM deposits and degenerating pericytes in the 2VO animals. When the ratio of intact capillaries was expressed, a 20% decrease of healthy vessels was found in the CA1 region of the 2VO rats (Fig. 6C). On the other hand, no significant changes in microvascular ultrastructure emerged in the dentate gyrus indicating the selective vulnerability of the CA1 microvasculature to a reduced cerebral perfusion in the hippocampus.

As a technical consideration to the 2VO experiments, it must be noted that the microvascular consequences of 2VO are not always restricted to the brain but can affect the eye as well (Slakter et al., 1984). The shrinkage of the optic nerves or the extensive disruption of the microvasculature in the eyes associated with pathological retinal appearance reported in 2VO rats (Slakter et al., 1984; Ohta et al., 1997) gains special importance when the animals are trained in a learning paradigm that involves visual cues. The problem can be circumvented by evaluating the visual aptitude of the animals in the given learning task in order to prove that the expected cognitive failure indeed correlates to the cerebral capillary damage and not to a potential retinal impairment.

The cerebral capillary breakdown demonstrated under the 2VO condition and previously in spontaneously hypertensive stroke prone rats (SHR-SP) (Farkas et al., 2000d). The functional link between these three different conditions sharing the common pattern of cerebral capillary pathology may well be cerebral hypoperfusion although firm and direct evidence is still lacking to support this view. Similar to the studies with aged rats (De Jong et al., 1990), our SHR-SP experiments also focused on the frontoparietal cortex where BMT significantly increased at the age of 60 weeks with 10%, microvascular fibrosis at 40 and 60 weeks with 3 and 9%, respectively, while the occurrence of degenerative pericytes remained the same comparing the hypertensive animals to a normotensive age-matched group. The damage to the basement membrane expressed as basement membrane deposits (BMT and/or fibrosis) is shown in Fig. 6D. The finding that the amount of capillary wall deposits correlated to the systolic blood pressure in the hypertensive SHR-SP group (Fig. 15) supports the theory of a causal relationship between physiological peripheral and cerebral morphological vascular factors.

The disruption of cerebral capillary integrity could be prevented by the chronic application of nimodipine or nifedipine both in normotensive, aging Wistar and in hypertensive SHR-SP rats (Fig. 16) (De Jong et al., 1990, 1991; Farkas et al., 2000d). Nimodipine and nifedipine are two L-type calcium channel antagonists from the dihydropyridine family that can block excessive calcium influx either at vascular smooth muscle cells, or at neural elements when penetrating the brain parenchyma. Dihydropyridines moderate vasoconstriction and blood pressure by targeting the vascular smooth muscle layer (Katz et al., 1985; Fleckenstein and Fleckenstein-Grun, 1988; Alborch et al., 1995) while in the brain they can also serve as potent neuroprotective agents by preventing the building up of an extreme intracellular calcium concentration in neurons and astrocytes (Scriabine et al., 1989; Schuurman and Traber, 1994; Disterhoft et al., 1996). The latter possibility requires that the drugs penetrate the BBB, which was shown for both nimodipine and nifedipine (Janicki et al., 1988; Larkin et al., 1992; Uchida et al., 1997).

The beneficial effect of the two drugs on cerebral capillaries thus promoted the concept that a dysregula-
Fig. 15. Correlation between capillary basement membrane deposits in the frontoparietal cortex and systolic blood pressure in normotensive (WKY) (A) and spontaneously hypertensive stroke-prone rats (SHR-SP) (B). The graphs include animals from the following age groups and pharmacological treatment: WKY-40, 40-week-old WKY \((n=6)\); WKY-60-plac, 60-week-old WKY without treatment \((n=6)\); WKY-60-nimo, 60-week-old WKY with chronic, oral nimodipine treatment for 20 weeks \((n=6)\); WKY-60-nife, 60-week-old WKY with chronic, oral nifedipine treatment for 20 weeks \((n=6)\); SHR-SP-40, 40-week-old SHR-SP \((n=6)\); SHR-SP-60-plac, 60-week-old SHR-SP without treatment \((n=8)\); SHR-SP-60-nimo, 60-week-old SHR-SP with chronic, oral nimodipine treatment for 20 weeks \((n=6)\); SHR-SP-60-nife, 60-week-old SHR-SP with chronic, oral nifedipine treatment for 20 weeks \((n=6)\).

Fig. 16. The vascular protection in cortical microvessels of 20 weeks chronic treatment with the calcium channel blockers nimodipine and nifedipine. Abbreviations: WKY, Wistar Kyoto normotensive rat strain; SHR-SP, spontaneously hypertensive stroke-prone rat strain; 40, 40-week-old animals; 60-plac, 60-week-old animals fed by placebo diet for 20 weeks; 60-nimo, 60-week-old animals fed by nimodipine containing diet for 20 weeks; 60-nife, 60-week-old animals fed by nifedipine containing diet for 20 weeks.
creased endothelial permeability (Wei et al., 1986; Imaizumi et al., 1996; Lagrange et al., 1999). The microvascular pathology in SHR-SP animals presented here can be associated with such a chain of events. Dihydropyridine treatment can supposedly prevent the intracellular calcium accumulation in astrocytes and/or neurons innervating the cerebral microvascular domain, thus moderating free radical production (Zhu et al., 1999) and consequent cerebral capillary injury. The improvement of the calcium homeostasis of these neural elements also controlling capillary blood flow may also explain the improvement in brain perfusion, which was shown to increase in SHR rats and rabbits after nimodipine or nifedipine treatment (Tanaka et al., 1986; Thoren et al., 1989; Ooboshi et al., 1990; Weinstein, 1995).

Hence, we can draw the conclusion that cerebral capillary damage can be initiated by an imbalance in the intracellular calcium homeostasis of astrocytes and neurons, which reaches its effect on microvascular ultrastructure via the release of toxic free radicals, the disturbance of the neurogenic regulation of CBF and the degree of brain perfusion.

4.4. Learning capacity and neuropathology under experimental cerebral hypoperfusion

4.4.1. Learning and memory impairment after carotid occlusion

The testing of learning and memory capacity of 2VO animals, and the detection of neuronal damage complete the major fields of investigation on the degenerative features that may arise as a consequence of cerebral hypoperfusion. The main focus of the behavioral and histological experiments has been on the hippocampus and the cerebral cortex for their selective vulnerability shown by CBF measurements and metabolic activity assessed in the 2VO paradigm. The learning tests most frequently used in cerebral hypoperfusion studies had been specially designed to unravel mainly hippocampus-related spatial learning processes (Kesner et al., 2000).

The eight-arm radial maze task (Fig. 17A) makes use of the motivation of food-deprived animals to search for food pellets in the maze. The rats must learn through consecutive trials which arms of the maze are baited and enter those arms only once. The animals commit an error if they make revisits to the baited arms in case not all the other pellets have been found, or if they make non-baited arm visits when non-baited arms are also included in the paradigm. When 2VO rats were trained to learn the test starting either 3 days or 3 months after surgery, they committed significantly more errors than their controls and were not able to improve their performance to control levels even after 15 trials (Table 2) (Ni et al., 1994; Ohta et al., 1997; Nanri et al., 1998). However, when the animals had mastered the task before the onset of 2VO (pre-trained rats), and they were placed back on the maze 3, 7 or 21 days after the surgery as a retention test, they quickly managed to perform like the SHAM controls. Surprisingly these pre-trained animals demonstrated a marked setback between 63 and 168 days after 2VO, when they made significantly more mistakes than the controls (Ni et al., 1994; Pappas et al., 1996; Bennett et al., 1998). The extensive behavioral data on learning performance indicated that the acquisition of spatial memory rather than the memory retention phase was disturbed shortly after 2VO in the eight-arm radial maze, and that the long-term effect of cerebral hypoperfusion severely impaired retention from approximately 2 months following the onset of 2VO.

The Morris water maze spatial learning paradigm (Fig. 17B) also tests spatial memory acquisition and retention, but exploits another sort of motivation, namely the urge to escape from an unpleasant situation. Solving the maze involves considerably more stress for the animals than the eight-arm radial maze, possibly enhancing group differences further (Pappas et al., 1997). The Morris water maze protocols also vary between research groups but the basic concept of the
test remains the same: the acquisition period includes a set of trials when the rats must learn the location of a hidden platform submerged under the water surface in a circular swimming pool. The escape latency or the distance covered from introducing the rat into the water till finding the platform is measured at each trial to visualize learning performance. During the retention trial (also called probe trial) successive to the training sessions the platform is removed from the pool, and the time the animals spend in the area previously containing the platform is taken as indicative of retention capacity. The acquisition of the task in the Morris water maze showed significant impairment at different time points ranging from 2 weeks to 12 months after the carotid occlusion (Fig. 18A, Table 2) (Pappas et al., 1996; Ohta et al., 1997; De Jong et al., 1999). The probe trial executed at 2 weeks or 3 months after 2VO invariably confirmed compromised spatial orientation skills (Ohta et al., 1997). Hence, the results collected in the Morris water maze paradigm also revealed that cerebral hypoperfusion markedly compromised spatial learning and memory retention.

4.4.2. Cerebral histopathology in experimental cerebral hypoperfusion

Parallel with the memory deficits, the neuronal integrity under the 2VO condition also degenerated. Histological examination of brain slices revealed white matter rarefaction (Ohta et al., 1997; Nanri et al., 1998) and a progressive neuronal damage in the hippocampus and the cerebral cortex (Tsuchiya et al., 1992; Ni et al., 1994; Pappas et al., 1996, 1997; Ohta et al., 1997; Bennett et al., 1998; Nanri et al., 1998). More specifically, the hippocampus CA1 area displayed neuronal shrinkage (Ni et al., 1994; Nanri et al., 1998), biochemical and morphological signs of apoptotic cell death such as DNA strand breaks, chromatin condensation and cytoplasmic shrinkage (Pappas et al., 1997; Bennett et al., 1998), pyramidal cells loss (Ni et al., 1994; Pappas et al., 1996; Ohta et al., 1997; Bennett et al., 1998) and a decreased immunoreactivity for microtubule-associated protein 2 (MAP2), a marker protein of neuronal dendrites (Nanri et al., 1998). Reactive gliosis accompanying the neuronal damage in the hippocampal CA1 segment was also enhanced (Tsuchiya et al., 1992; Ohta et al., 1997), also shown by the significant increase in glial fibrillary acidic protein (GFAP) immunoreactivity (Pappas et al., 1996; Nanri et al., 1998). The listed abnormalities also characterized — although to a lesser degree — the neurodegenerative processes in the cerebral cortex (Tsuchiya et al., 1992; Ni et al., 1994; Nanri et al., 1998). In addition to the marked neurodegeneration as a consequence of cerebral hypoperfusion, recent investigation disclosed extracellular deposits of the amyloid precursor protein (APP) (Pappas et al., 1997), and a progressive accumulation of Aβ peptides in aging 2VO rats detected by Western blot analysis (Bennett et al., 2000).

4.4.3. Correlation between learning skills and neuronal/vascular pathology in the cerebral hypoperfusion model

Correlation analysis between the memory data and the histopathological changes following chronic cerebral hypoperfusion highlighted the hippocampal CA1 area as the central site where neuronal damage coincided with impaired learning capacity. This is in agreement with the well-established notion that the hippocampus CA1 is the specific structure where spatial

<table>
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<th>Reference</th>
<th>Age of animals at the onset of 2VO</th>
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<tr>
<td>Ni et al., 1994</td>
<td>7 weeks–9 months</td>
<td>Eight-arm radial maze</td>
<td>3 days–4 months</td>
<td>More errors, impaired learning</td>
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<td>Pappas et al., 1996</td>
<td>9–10 months</td>
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<td>14, 28, 70, 112 and 161 days</td>
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<tr>
<td>Ohta et al., 1997</td>
<td>9 weeks</td>
<td>Eight-arm radial maze</td>
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<tr>
<td>De Jong et al., 1999</td>
<td>3 months</td>
<td>Morris water maze</td>
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<tr>
<td>de la Torre et al., 1997</td>
<td>14 months</td>
<td>Morris water maze</td>
<td>3 months</td>
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</tr>
<tr>
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<td>19 months</td>
<td>Morris water maze</td>
<td>1 month/2 months/1 year</td>
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<tr>
<td>de la Torre et al., 1997</td>
<td>14 months</td>
<td>Morris water maze</td>
<td>1, 2 and 3 weeks</td>
<td>Longer escape latencies, impaired learning</td>
</tr>
<tr>
<td>de la Torre et al., 1997</td>
<td>14 months</td>
<td>Morris water maze</td>
<td>2, 4 and 6 weeks</td>
<td>No significant difference</td>
</tr>
</tbody>
</table>
learning takes place (Van der Zee et al., 1992; Shapiro and Eichenbaum, 1999; Kesner et al., 2000). The increasing number of apoptotic cells and GFAP immunoreactivity in the CA1 segment was significantly related to the eight-arm radial maze performance of the 2VO animals (Pappas et al., 1996; Bennett et al., 1998). Furthermore, the data obtained in the Morris water maze indicated a marked relationship between spatial learning and hippocampal microvascular breakdown also selectively in the CA1 region but not in the dentate gyrus. The BM deposits significantly correlated with impaired memory in the hippocampus CA1 (Fig. 18B,C). The coincidence of neuronal damage and microvascular breakdown in the hippocampus CA1, accompanied by compromised spatial learning skills suggests that these three read-outs of cognitive status and cellular integrity are functionally related in the chronic 2VO model, where the onset of cerebral hypoperfusion acts as the trigger for microvascular pathology, neuronal damage and impaired learning. These degenerative changes, which develop as the consequence of cerebral hypoperfusion, also find correlates in human dementia such as cerebral capillary breakdown, compromised neuronal integrity and failing cognition. Although the neuropathology of AD is a lot more complex, cerebral hypoperfusion probably augments the advancement of the aspects of dementia as shown by the 2VO model.

5. Conclusions

Cerebral microvascular pathology in aging and to a markedly pronounced degree in AD include the physiological changes in cerebral perfusion — particularly the decrease of regional cerebral blood flow in cortical areas, a reduction of cerebral glucose utilization, the loss of vascular innervation with special focus to the cholinergic breakdown typical of AD and the ultrastructural damage to capillaries in the cerebral cortex represented by extensive basement membrane pathology. The coincidence of these changes contributes to an improper blood–brain barrier function in an additive manner. A wide range of comprehensive laboratory animal experiments attempted to unravel a causal interaction between these pathological vascular changes. Fig. 19, a scheme produced by integrating the data from our lab and those of others, shows a hypothetical chain of events rooted in vascular deficiency, which can augment compromised memory capacity.

Variable conditions, which extend from chronic hypertension through the breakdown of neurogenic cere-
brovascular regulation to the effect of circulating toxic molecules such as the β-amyloid peptide, can initiate general vasoconstriction. As such a vasoconstrictive reaction obviously affects the resistance vessels supplying the cerebral circulation, the cerebral perfusion rate (CBF) gradually decreases causing insufficient flow in the cerebral microvascular network. As a next step, the reduced regional CBF can give rise to well-defined microanatomical aberrations in the capillary ultrastructure of cortical areas (BMT, perivascular collagen deposits). Consequently, capillary basement membrane pathology will either physically hinder passive nutrient and electrolyte trafficking from blood to brain or interfere with active transport processes by possibly deforming the biochemical structure of transporter proteins. The neural compartments will be thus deprived of essential nutrients with a major focus on glucose, thus compromising their energy status and metabolic activity. A decline in mitochondrial cytochrome oxidase activity and ATP production detected in experimentally hypoperfused rats are persuasive signs of such neuronal metabolic crisis. If the condition persists for long, the neurons exhaust their capacity to sustain the function of carrier proteins responsible for setting the homeostatic intracellular ion balance, respond with delay to extracellular stimuli, and the neural circuits lose plasticity. In the end, inevitable structural neuronal disintegration, even cell death may follow. The outcome of the process can be detected at the behavioral level as cognitive failure and memory dysfunction.

The connected pathological changes are obviously interrelated, implying that the sketched circle of events is under no circumstances a one-way route, but a more complex network ushered by potent feedback mechanisms. Nevertheless, the theory presented here can be regarded as the backbone of the pathological cerebrovascular incidents associated with aging and AD, which connects to suboptimal cognitive capacity. The
breakdown of the cerebral circulation and the condition of cerebral microvessels thus seem to enhance the characteristic memory deficits that typify dementias like AD.

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