Contribution of Flow-Dependent Vasomotor Mechanisms to the Autoregulation of Cerebral Blood Flow

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Abstract

Regulation of cerebral blood flow (CBF) is the result of multilevel mechanisms to maintain the appropriate blood supply to the brain while having to comply with the limited space available in the cranium. The latter requirement is ensured by the autoregulation of CBF, in which the pressure-sensitive myogenic response is known to play a pivotal role. However, in vivo increases in pressure are accompanied by increases in flow; yet the effects of flow on the vasomotor tone of cerebral vessels are less known. Earlier studies showed flow-sensitive dilation and/or constriction or both, but no clear picture emerged. Recently, the important role of flow-sensitive mechanism(s) eliciting the constriction of cerebral vessels has been demonstrated. This review focuses on the effect of hemodynamic forces (especially intraluminal flow) on the vasomotor tone of cerebral vessels and the underlying cellular and molecular mechanisms. A novel concept of autoregulation of CBF is proposed, suggesting that (in certain areas of the cerebrovascular tree) pressure- and flow-induced constrictions together maintain an effective

autoregulation, and that alterations in these mechanisms may contribute to the development of cerebrovascular disorders. Future studies are warranted to explore the signals, the details of signaling processes and the in vivo importance of these mechanisms.

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Introduction

Regulation of cerebral blood flow (CBF) is of utmost importance to maintain the myriad functions of the brain. It has to ensure an appropriate supply of nutrients and gases for cerebral tissue, fluid and gases exchange in the capillaries and the maintenance of cerebral blood volume (CBV) and intracranial volume and pressure in a very limited range. Obviously, such a complex function requires complex regulatory mechanisms facilitated by the dynamic interaction of mechanotransduction, metabolic (e.g. adenosine), chemical (i.e. changes in PCO₂, pH and PO₂) and other factors; evidence of an important role for glial (astrocytes), pericyte and neural control of CBF has recently emerged [1–14].

All of these mechanisms have to comply with limited space in the closed cranium [15]; historically, the mainte-

nance of a relatively constant CBF, in spite of variations in pressure and flow, has always been at the center of investigations. Although autoregulation of CBF used to be explained by the pressure-sensitive myogenic mechanism (metabolic and other factors are not included here in the notion of autoregulation; we define it as the sum of the mechanisms activated in response to changes in hemodynamic forces) [4–7, 16–19]. Careful analysis of earlier data and recent findings question the concept that it is solely the myogenic mechanism that elicits autoregulation of CBF [20–24].

In this review, we focus on the potential contribution of flow-dependent autoregulatory mechanisms and explain the different results of previous studies with regard to the flow-induced diameter changes of cerebral vessels.

Autoregulation of Cerebral Blood Flow

General Considerations

Total CBF has to be relatively constant in order to allow a stable and continuous supply of blood to the cerebral tissue and to maintain constant intracranial volume and pressure. On the basis of the Hagen-Poiseuille law, it can be assumed that CBF is related to the 4th power of vessel radius, so an increase in the diameter of vessels elicits an exponential increase in blood flow. Therefore, in the closed cranium, general vasodilatation would lead to a substantial increase in CBF and CBV and the elevation of intracranial pressure (and vice versa) [19, 25]. Tight control of CBF and CBV is thus essential for the brain. Indeed, in a wide range (from approx. 60 to 140 mm Hg) of perfusion pressure, CBF increases only slightly in a linear manner measured by different *in vivo* techniques [5, 18]. At this point it has to be noted that in mathematical models, gain = 1 is used to indicate a so-called ‘perfect’ autoregulation [22, 23]. As depicted in figure 1, it is likely that such a perfect horizontal relationship does not exist *in vivo* and that it would not be beneficial in ensuring an appropriate supply of blood to the brain tissue. It is more likely that the slope increases linearly as pressure and flow increases [5, 18, 26, 27]. The linear – not the exponential – increase of CBF in the face of increasing blood pressure is achieved by the mechanisms of cerebral autoregulation. Some authors consider metabolic factors/mechanisms as contributors to the autoregulation of CBF; this interpretation implies that CBF adjusts to metabolic demands and the function of

Fig. 1. Proposed physiological role of flow-induced constriction of cerebral arteries in autoregulation of CBF. Combined effect of changes of intraluminal pressure and intraluminal flow (Δ flow) achieves a more effective autoregulation of CBF, whereas only pressure-induced diameter responses still allow substantial increases in CBF, and thus inefficient autoregulation. It is likely that, *in vivo*, the ‘plateau’ of autoregulation is not perfectly flat, i.e. the gain is less than 1, thus there is a slight increase in CBF as systemic pressure increases. Nevertheless, it is effective enough to prevent an exponential increase of CBF. Also, the range and shape of the autoregulatory curve is likely to be more ‘rounded’ at low- and high-pressure values.

neural tissues. Although metabolic regulation of CBF [2, 5, 13, 14, 28] is obviously an important issue, in this review we define autoregulation as the vasomotor responses to changes in hemodynamic forces achieved by mechanisms intrinsic to the vascular wall and performing cerebral blood perfusion independently of changes in systemic blood pressure without activation of metabolic, chemical, glial, neural and other (e.g. capillary blood-flow regulation by pericytes) regulatory mechanisms [1, 2, 5–11, 18, 19, 29]. Because changes in pressure are accompanied by changes in flow, the *in vivo* responses of cerebral vessels to changes in hemodynamics are most likely a combination of pressure and flow-induced mechanisms [30–33]. Thus, one can hypothesize that changes in flow contribute to the autoregulation of CBF. In other words, when systemic pressure changes *in vivo*, then the diameter responses of cerebral vessels;

thus changes of CBF are determined by the combined effect of pressure and flow.

Regional and Segmental Differences in Autoregulation of CBF

Autoregulation of CBF has been documented in a variety of animal species and in humans using *in vivo* techniques e.g. clearance of diffusible indicators, observation of pial vessels diameter, measurements of arterial inflow and venous outflow, autoradiography, distribution of radioactive microspheres and, recently, by magnetic resonance imaging, ultrasound and other techniques [18, 34–42].

In the cerebral circulation, the large arteries represent a significant part (approx. 40%) of total cerebrovascular resistance [9, 18, 43–45]. In the late 1970s, Kontos et al. [18], investigating the cat pial circulation, found that large surface vessels from the circle of Willis to pial arterioles up to 200-micrometers contribute approximately 30% of cerebrovascular resistance at 120 mm Hg intraluminal pressure. They proposed that these vessels are exclusively responsible for autoregulation between 120 and 160 mm Hg intraluminal pressure. Similar findings were observed in the rat cerebral circulation [44]. Results showed that, in the cerebral circulation, both large arteries and small arterioles take part in the regulation of CBF, which indicates that there might have been a hierarchy in vascular responsiveness i.e. a basis for more complicated regulation. This also raised the possibility of the spatial characteristics of cerebral autoregulation. Indeed, regional differences in the cerebral autoregulatory capacity were shown: hypertension exceeded autoregulation in the cerebrum of the cat (supplied by the internal carotid system), but not in the brain stem (supplied by the vertebro-basilar system), demonstrating that the autoregulation of flow is more effective in the vasculature of the brain stem [41, 46, 47]. In human cerebral circulation, regional differences have also been shown regarding autoregulatory function [48, 49].

The different magnitudes of response in the cerebral arteries and arterioles to elevation in pressure were already shown by Kontos et al. [18]. Then, it was proposed again and proved by Faraci and Heistad [43] that different segmental responses of cerebral vessels to changes in intraluminal pressure underlie the heterogeneity of autoregulation. They demonstrated that pial arteriolar pressure is greater in the brain stem than in the cerebrum, implying that a drop in pressure is greater on large

er arteries in the cerebrum than in the brain stem. They also found that the resistance of larger arteries increases in the cerebrum and decreases in the brain stem during a moderate increase in blood pressure. They concluded that autoregulation depends primarily on arterioles in the brain stem, whereas in the cerebrum larger vessels play a more important role [50, 51] (in line with the findings of Kontos et al. [18]).

Physiological Role

Collectively, autoregulation ensures that CBF remains relatively constant in a wide range, despite an increase or decrease in systemic blood pressure. *In vivo*, autoregulation shows regional and segmental differences. In the brain stem, (supplied by the vertebro-basilar system) the resistance of larger arteries decreases, whereas that of arterioles increases in response to elevation in pressure. In contrast, in the cerebrum (supplied by the internal carotid artery system), larger arteries increase their resistance to elevation in pressure more than the arterioles, which plays a primary role in autoregulation and protects downstream brain circulation from pressure and volume overload [43, 50, 52].

It is important to note, however, that in these *in vivo* studies of autoregulation of CBF and cerebrovascular response to pressure, the diameter effects of pressure and flow could not be separated and the effect of flow on the diameter of vessels was not even considered [18, 34, 36–43, 50, 53].

Pressure-Induced Myogenic Responses of Cerebral Vessels

Earlier Findings and Conclusions

Until very recently, cerebral autoregulation (relative independence of CBF from changes in systemic blood pressure, especially at higher pressure values) was primarily explained by the pressure-induced myogenic response [52]: the inherent property of vascular smooth muscle to dilate, to decrease and to constrict in order to increase intraluminal pressure. Since its first description by Bayliss [35] early in the 20th century, the myogenic response of different arteries has been widely investigated. In the cerebral circulation it was first shown by Fog [54] and Forbes [55] that pial arterioles of the cat actively dilate and constrict to changes of blood pressure. This

was confirmed later in studies investigating the regulation of CBF in which the simultaneous changes of blood flow and arteriolar pressure were measured.

The presence of myogenic response was shown in vitro in isolated cerebral arteries for the first time in the 1980s [22, 56]. In the in vitro studies investigating the myogenic response, the pressure was changed, but the flow was kept constant. This could be done due to the development of new technology: cannulation of a vessel on both ends and increase of intraluminal pressure by increasing both the inflow and outflow pressure or by closing the outflow end of the vessel and increasing the inflow pressure (in both cases the intraluminal flow remains unchanged) [57–59]. Therefore, the observed diameter responses were due to changes in pressure alone and were not influenced by changes in flow. Interestingly, in many of these studies, cerebral vessels only maintained a constant diameter of 60–140 mm Hg intraluminal pressure [20–22, 24]. This response is referred as the 2nd phase of in vitro arterial myogenic behavior proposed by Osol et al. [21]. If, however, one extrapolated this finding to in vivo conditions, then, because of the constant diameter, increasing pressure would result in an increase in blood flow velocity, thus increasing CBF. In contrast, in vivo measurements of CBF showed that it remained relatively constant while intraluminal pressure (and flow velocity) increased. These observations should have prompted the investigators to hypothesize the existence of a flow-sensitive mechanism which would result in further constriction.

However, in many vascular beds, the myogenic constriction alone likely prevents increases in blood flow during increases in pressure, for example in the cremaster muscle circulation [60]. Although the direct comparison of cerebral vessels to peripheral vessels has to be carried out carefully, Bohlen and Harper [61] and Meininger et al. [62], for instance, found that the magnitude of myogenic constriction in the arterioles of the cremaster muscle is greater than in the comparable-sized arterioles of the rat cerebral cortex.

The strength of the myogenic constriction or dilation can also be modulated by other effects. For example, hypertension and exercise can enhance the myogenic response, leading to increased constrictions to increase intraluminal pressure [22, 61, 63, 64]. Myogenic dilation can also increase significantly under certain conditions; for instance, arterioles in the cat sartorius muscle dilate significantly more to decrease intraluminal pressure during sympathetic nerve stimulation, enhancing myogenic dilation and providing increased flow [65]. In the case of increasing blood pressure in cerebral vessels, however,

the myogenic constriction is considered to be one of the most important mechanisms of maintaining constant or near constant blood flow [52, 63, 66]. In contrast, as mentioned above, in most cerebral vessels the myogenic response does not reduce the diameter of vessels beyond the basal level in response to an elevation of intraluminal pressure [20–24]. It cannot be excluded, however, that the magnitude of myogenic constriction displays regional and size differences, and that these vessels possess a strong and efficient myogenic response. Currently, the investigation of the myogenic responses of microvessels deeply seeded in the brain is technically challenging and the presence of confounding mechanisms in vivo makes it difficult to interpret the findings. The ratio of the magnitude of pressure (and flow-induced) responses in different brain regions and their contribution to cerebral autoregulation should be clarified in the future.

Molecular Mechanisms of the Myogenic Response of Cerebral Vessels

Although there are several excellent reviews [67–71] of the molecular mechanisms of myogenic response based on these works, we found it appropriate to give a brief overview here.

It is now widely accepted that the primary stimulus for triggering the myogenic response is the pressure-elicited stretch of vascular wall, leading to increased wall tension [72]. However, the sensors are still in question: among others, stretch-activated cation channels, Gq-coupled receptors and interactions between matrix metalloproteinases, extracellular matrix, integrins and the cytoskeleton were proposed [73–76].

The first event in the mechanotransduction is the depolarization of smooth muscle membrane [77]. Although there is no consensus regarding the initiators of membrane depolarization, stretch-activated cation channels (TRPC6 and TRPM4), calcium-activated potassium channels and chloride channels are probably involved [78–83]. The activation of voltage-gated potassium channels limits depolarization as a negative-feedback mechanism [84].

Depolarization of the membrane then leads to the opening of voltage-gated Ca^{2+} channels (which are also capable of responding to stretch directly [85]) and thus elevated inward Ca^{2+} current [86, 87]. Ca^{2+} can be released from sarcoplasmic stores as well, the role of which seems to be minor in the development of myogenic response, however [85, 88]. The increased Ca^{2+} concentration via Ca^{2+} -calmodulin complex leads to myosin light-

chain kinase (MLCK) activation. MLCK phosphorylates myosin light chain (MLC₂₀) leading to increased actin-myosin interaction and the consequent shortening of smooth muscle cells [89–91]. Because of the circumferential orientation of the smooth muscle cells, shortening is translated into constriction of the vessel.

Interestingly, after the initial elevation of pressure, the intracellular Ca²⁺ concentration does not increase, but the vessel is still constricting [21]. This observation resulted in the discovery of novel Ca²⁺-independent mechanisms of the myogenic response, which can sensitize the constrictor apparatus to Ca²⁺. An increased actin-myosin interaction and a consequent shortening of smooth muscle cells depends on the phosphorylated state of MLC₂₀, which is governed by MLCK and myosin light-chain phosphatase (MLCP) [71, 92]. Ca²⁺-independent mechanisms altering the Ca²⁺ sensitivity of smooth muscle cells act through regulation of MLCP. Protein kinase C activation, production of diacylglycerol and RhoA/Rho kinase are involved in these processes [71, 92–94].

As a second messenger, 20-hydroxyeicosatetraenoic acid (20-HETE) was also found to play a role in myogenic response (also in other vascular beds) [3, 95–97]. 20-HETE is capable to inhibit large conductance Ca²⁺-activated K⁺ channels and to increase the influx of Ca²⁺ via L-type Ca²⁺ channels [3, 96, 98]. One of the major molecular targets of 20-HETE is protein kinase C [3, 96, 98, 99].

Collectively, there are two critical mechanisms (Ca²⁺-dependent and independent) contributing to myogenic constriction: (1) an increase in pressure via the increase in wall tension and smooth muscle cell stretch leads to membrane depolarization, Ca²⁺ influx and constriction via MLCK and phosphorylation of MLC₂₀ and (2) Ca²⁺-independent mechanisms involving protein kinase C, diacylglycerol, RhoA/Rho kinase and 20-HETE regulate the activity of MLCP determining the phosphorylated state of MLC₂₀ and thus sensitizing actin-myosin to Ca²⁺.

Flow-Induced Responses of Cerebral Vessels

As mentioned above, in vivo, changes in pressure are accompanied by changes in flow [30–33] and based on theoretical considerations, flow-induced mechanisms may play a role in cerebral autoregulation. Interestingly, flow-induced responses of cerebral vessels vary between species, vessel types and methods used. In theory, flow-induced dilation would reduce the magnitude of myo-

genic constriction of cerebral vessels, which would reduce the gain of autoregulation of CBF, whereas if flow elicited constriction, it could contribute to a more efficient autoregulation of CBF. Next, we summarize the results of studies on the flow-induced diameter responses of cerebral vessels.

Diameter Responses of Isolated Cerebral Vessels Differ to Increases in Flow

Dilation of Cerebral Vessels to Flow

First, Fujii et al. [100, 101] showed that the basilar artery of a rat dilated in vivo when intraluminal flow was increased. They used craniotomy to visualize the basilar artery. Metabolic parameters (blood O₂ and CO₂, pH) were maintained at a constant physiological level, and blood pressure was kept constant by controlled bleeding of the animal. Intraluminal flow was elevated in the basilar artery by bilateral carotid artery occlusion. It is of note that among in vivo methods this approach is able to separate the effect of flow and pressure on vessel diameter, while other factors (i.e. metabolic) are controlled. However, it cannot be entirely excluded that occlusion of the carotid arteries which decreases blood flow in the carotid system would generate a signal propagating the basilar artery to elicit dilation. Paravicini et al. [102] confirmed the dilation of the basilar artery using the same method. In 1993, Gaw and Bevan [103] found dilation of rabbit cerebral vessels using a wire myograph, in which, however, flow and pressure cannot be properly controlled. Recently, Drouin et al. [104] and Drouin and Thorin [105] demonstrated flow-induced dilation in cerebral arteries. They studied isolated posterior and anterior cerebral arteries of mice in a pressure-flow chamber in a well-controlled manner. In recent in vitro studies (unpubl. observation), we confirmed that in isolated rat basilar arteries, increases in flow elicit dilation. An original record of such a response is depicted in figure 2, showing substantial dilation of an isolated basilar artery of rat to increases in flow.

Biphasic Response of Cerebral Vessels to Flow

Interestingly, there have been studies in which biphasic response and the dilation and constriction of cerebral vessels to increases in flow was observed. Garcia-Roldan and Bevan [106, 107] showed that isolated rabbit pial arteries dilated to 20 µl/min flow at 30 mm Hg and constricted to the same flow at 90 mm Hg of intraluminal pressure. They used a Halpern perfusion system, in which

Fig. 2. Changes in diameter of an isolated MCA and a basilar artery to changes in intraluminal flow as a function of time, redrawn from original traces [23]. Under this experimental condition, flow is generated by increasing intraluminal pressure differences (e.g. $\Delta 5$, $\Delta 10$ mm Hg) via the vessel by changing the inflow and outflow pressures with the same amount, but in the opposite directions (for details, see [23]). The opposite diameter responses indicate important regional differences in the nature of flow-induced responses of cerebral arteries. Constriction of MCA by increasing cerebrovascular resistance (CVR) contributes to the autoregulation of CBF by the 'pressure-flow' mechanisms and thereby to the overall regulation of intracranial volume and pressure. In contrast, dilation of the basilar artery to increases in flow, similar to other peripheral vessels contributes to the development of functional hyperemia.

flow was generated by pressure difference due to changing the perfusion via the inflow and outflow pumps.

Thorin-Trescases and Bevan [108] confirmed these findings in rabbit cerebral vessels. They found that flow induced dilation at 40 mm Hg in the secondary and tertiary branches of posterior cerebral arteries, dilation and a small constriction at 60 mm Hg and a small dilation followed by constriction at 80 mm Hg intraluminal pressures. Ward et al. [109] observed the similar pressure dependency of the flow-induced response of cerebral vessels: in arteriolar branches (approx. 80 μ m) of the rat posterior cerebral artery flow elicited dilation at 60 mm Hg, and constriction at 120 mm Hg intraluminal pressure.

Ngai and Winn [110] studied the arteriolar branches of rat middle-cerebral arteries (MCA), approximately 35–88 μ m in diameter. They achieved increasing flow at a constant (60 mm Hg) pressure by changing the inflow and outflow pressure to an equal degree, but opposite direction (the same method we used when constrictions were observed in rat MCA and in human intracerebral arteries). They also observed that isolated cerebral arterioles, branches of MCA dilated to flow up to 10 μ l/min, and then restored their diameter at flow rates higher than the initial value.

Similar flow-rate dependency was found by Shimoda et al. [111]. Anterior cerebral arteries and MCA of the neonatal pig constricted to flow between 0.077–0.212 ml/min, and dilated when flow was raised further (up to 1.6 ml/min). There were no differences in the flow-induced responses at 20 and 60 mm Hg intraluminal pressures. In these studies flow was initiated by a syringe pump, and pressure was measured in both the inflow and outflow

cannulas by transducers. When flow increased, a micro-manipulator decreased the outflow pressure adjacent to the increase in pressure due to flow by sensing the pressure difference between the transducers. The luminal pressure was also measured by inserting a cannula into the lumen of the vessel.

Garcia-Roldan and Bevan [107] found the mixture of pressure and the flow-rate dependency of flow-induced response of cerebral vessels. In isolated rabbit pial arterioles they demonstrated that vessels constricted in response to an increase in intraluminal flow from 0 to 20 μ l/min at 90 mm Hg, but did not at the presence of 60 mm Hg of intraluminal pressure. Increasing flow up to 100 μ l/min constricted vessels at both pressure values. It has to be clear that the biphasic response of diameter to flow does not mean that vessels exhibit opposite responses in a temporal manner; instead, it reflects different responses at different pressure values or flow rates.

Constriction of Cerebral Vessels to Increases in Flow

The variation of observed diameter responses of cerebral vessels in response to increases in flow prompted further investigations. Madden and Christman [112] studied isolated cat MCA. They applied 1–4 ml/min flow at 70 and 100 mm Hg intraluminal pressures, and found that increases in flow led to a decrease in diameter and a depolarization of smooth muscle cell membrane. Arteries constricted by flow dilated when PCO_2 was increased, implying that metabolic/chemical signals can override flow-induced constriction. Vessels dilated when flow was stopped, and constriction occurred when flow was suddenly increased to the maximal values. Endothelium denudation did not affect flow re-

sponse, suggesting that flow-induced constriction is endothelium-independent.

Sipkema et al. [113] found flow-induced constriction in isolated basilar artery of the Rhesus monkey. In order to make sure that the conditions did not influence the response, in the same set-up, they also used isolated femoral arteries in which they observed dilation to flow. However, in many of these earlier studies, changes in pressure during changes in flow could not be excluded with great certainty. Therefore, constriction to flow could be elicited by the activation of myogenic mechanisms, whereas dilation of femoral artery to flow might be caused by a flow-induced mechanism and/or passive dilation (femoral arteries do not develop spontaneous tone). This conclusion is supported by the finding that the active pressure-diameter curves of these vessels do not differ from the passive diameter obtained in calcium-free conditions. However, to our knowledge, this is the only study investigating the isolated cerebral arteries of monkeys, thus it cannot be excluded that constriction of the basilar artery to flow may be due to a characteristic of the species.

In 2001, Bryan et al. [114] aimed to clarify controversial findings (dilation, constriction and biphasic responses) regarding the flow-induced responses of cerebral vessels. They proposed that controversy and confusion in previous studies were due to different species, different vessel types, different techniques and technical problems causing artifacts, namely the different pH of the extraluminal and intraluminal bath (cerebral vessels are highly sensitive to pH) and the change in pressure accompanying the increase in flow. Under the same experimental conditions, they investigated isolated MCA, penetrating cerebral arterioles (PA) (approx. 70 μm) and cremaster muscle arterioles (which are known to dilate to flow) of rats. They used a syringe pump to generate flow, and decreased the outflow pressure according to the elevation in pressure due to flow, both counted by an algorithm based on the resistance of the tubing and micropipettes and by direct measuring of the intraluminal pressure. They made sure that the flow was laminar, adjusting the necessary length of the vessel. Maximal flow for PA was 40 $\mu\text{l}/\text{min}$, for MCA 300–500 $\mu\text{l}/\text{min}$. They allowed equilibration of the perfusate before it entered the vessel lumen, passing it through gas-permeable tubing in the extraluminal bath in order to avoid pH differences. In this experimental set-up, cremaster muscle arterioles dilated and MCA and PA constricted to increases in flow. In addition, the diameter decreased as a function of calculated shear stress (discussed later).

Recently, we have also investigated isolated MCA of the rat [23]. The sizes of glass pipettes used in this study were matched both to each other and to the diameter of the vessels, in order to achieve equal resistance. In addition, the inflow and outflow reservoirs and the position of the chamber were built in a symmetrical manner, which provided equal pressures or generated flow in the presence of constant pressure in the midsection of the vessels. We raised the pressure and then the flow and then both together, and measured the diameter changes. We found that rat MCA constricted to increases in flow (see also fig. 2), and flow-induced constriction enhanced the only pressure-induced constriction. Importantly, in line with the findings of Bryan et al. [114], flow-induced constriction was also observed in smaller vessels. We found that not only the large arteries (i.e. MCA), but also their smaller side branches (about 50 μm in diameter) constricted to increases in flow (unpubl. observation). Very recently, Kim et al. [115] demonstrated flow-induced constriction of rat cerebral arterioles in brain slices.

It is important to note that isolated MCA maintain a stable steady-state diameter in the presence of a constant flow rate. Upon an increase in flow they constrict, and in response to a decrease in flow they dilate and maintain the new diameter [23]. These findings suggest that in vitro steady-state flow rates activate vasomotor mechanisms that are able to provide a stable diameter. However, it is likely that in vivo, if such a diameter is not sufficient to maintain adequate CBF other mechanisms are activated to provide the sufficient flow to brain tissues. For example, the metabolic dilator adenosine functionally inhibited the flow-induced constriction, proposing that metabolic factors can override the flow-induced responses (similar to the case of increase in CO_2).

Human Cerebral Arteries Also Constrict to Flow

Importantly, we also found that isolated human intracerebral arteries (from the internal carotid circulatory area) constricted to increases in flow, and the flow-induced constriction was mediated by 20-HETE [23]. It has to be noted that the active internal diameters of these vessels were approximately 200–250 μm , equal to the studied rat MCA. This vessel size corresponds to that of the large arteries of the circle of Willis in the rat, but the small arteries in the human brain. Also, the human vessels were isolated from the frontotemporal cortex of patients, thus different responses of human cerebral vessels to flow from different brain regions cannot be excluded.

In conclusion, studies using a well-controlled methodological approach found dilation to flow in rats and mice in the vertebrobasilar circulatory area; constriction was found in cats, rats and importantly, in human isolated cerebral arteries from the internal carotid circulatory area; biphasic responses were observed in rabbit and rat cerebral arterioles showing a pressure and flow-rate dependency (dilated at lower and constricted at higher pressure and flow rates).

What Are the Potential Signals of Flow-Induced Responses of Cerebral Vessels?

Changes in flow are accompanied by changes in many factors e.g. wall shear stress (WSS). It is widely established that in most vascular beds changes of flow are sensed by the endothelium by sensing WSS elicited by increases in flow. The consequent flow-induced dilation reduces WSS which is regulated in a negative-feedback manner [57–59]. This can be described by the following equation:

$$WSS = 4 \eta \times Q \pi \times r^3,$$

where WSS is wall shear stress, η is viscosity, Q is flow and r is vessel radius [59]. It is believed that the overall physiological ‘purpose’ of this mechanism is to optimize the circulatory energy loss. The mechanism is likely to play a role in the development of functional hyperemia, e.g. during exercise (in skeletal and cardiac muscle) [57]. In the case of cerebral vessels, in which flow elicits dilations e.g. in the basilar artery, such WSS-sensitive mechanisms can be operating.

It is more difficult to envision the mechanotransduction of flow-induced constriction. What could be the signal that associated with increasing flow and sensed and converted to mechanical responses? Many previous studies were very careful to exclude any changes in intraluminal pressure during the investigation and observation of flow-induced constriction by keeping pressure constant [112, 114], thus an increase in pressure cannot be the possible trigger.

The easiest hypothesis would be that in some cerebral vessels, increasing WSS is converted to constriction instead of dilation by an unknown mechanism. For example, 20-HETE, the mediator of flow-induced constriction in MCA can cause dilation in basilar arteries known to dilate to flow, because it is converted into as of yet unknown dilator metabolites [116]. In this case, constriction can be the function of WSS, as calculated by Bryan et al. [114, 117] in rat MCA and in PA elevated

by either increasing flow or viscosity of the perfusate solution. We have also found similar results in MCA i.e. the magnitude of constriction could be plotted as a function of WSS (unpubl. observation). However, there are theoretical considerations that argue against the idea that WSS acting on the vascular endothelium is the signal for cerebral vessels in flow-induced constriction. (1) If WSS is sensed, how does it relate to changes in CBF and CBV? How can a positive-feedback mechanism ‘serve’ a negative-feedback mechanism i.e. autoregulation? Because constriction increases WSS further, it should result in further constriction of cerebral vessels and a further increase in WSS (in a positive-feedback manner), while CBF and CBV would decrease continuously. Such changes in CBF and CBV have not been observed, however.

(2) Madden and Christman [112] and Bryan et al. [114, 117] found that constriction of cerebral vessels to flow is independent of the presence of a functioning endothelial layer, and that the mechanisms responsible for the constriction are localized in the smooth muscle layer of cerebral arteries. This finding also argues against WSS being the signal. The intriguing question is: how can WSS be sensed by the smooth muscle if the flowing blood is in contact with the intraluminal side of the endothelium and not with the smooth muscle? Bryan et al. [117] proposed that the endothelium plays a role in attenuating the WSS-induced constrictions by an unknown dilating factor, a dilating process or by attenuating the mechanical force of the WSS as it is transmitted to the abluminal side of the vessel. This dilator mechanism may be present, but it needs further experimental support. This could be nitric oxide (NO), since inhibition of NOS (NO synthase) in zero-flow conditions reduced the diameter of cerebral vessels [117, 118].

(3) One can assume that WSS is sensed by the smooth muscle through interstitial flow driven by the transvascular pressure difference, which was shown to be capable of causing smooth muscle constriction [119]. This idea is supported by the findings that integrins play a role (as sensors) in interstitial flow-related mechanotransduction and that blocking integrins inhibits the flow-induced constriction of cerebral arteries [112, 114, 119]. This hypothesis could also explain why intact endothelium attenuates flow-induced constriction, because, in the case of endothelial injury or denudation the magnitude of transvascular flow is much greater than in intact arteries [119].

It is of note that no significant amount of fluid can leave the vessels in the closed cranium, although it is un-

likely that a larger amount of fluid would leave the wall of larger vessels due to the presence of well-developed internal elastic lamina and several layers of smooth muscle cells [52, 120]. Moreover, the amount of transmural fluid movement is likely to be minimal, because of the tight connections between endothelial cells [121]. Thus, it is unlikely to lead to 'leakiness' or increased filtration of plasma into the brain parenchyma. Nevertheless, the exact nature of the 'transmural fluid signal' proposed by Tarbell et al. [119] still remains obscure.

(4) Flow velocity increases as diameter decreases if flow (and pressure) is constant. The flow velocity itself could be a candidate for the trigger parameter as it is related to changes in CBF and CBV, but it is difficult to envision a sensor for it since velocity is not a force that can be sensed by cells.

(5) Another possibility is that increasing flow (mass transport) washes vasoactive substances in or out, providing a tonic effect on vascular smooth muscle. For instance, NO has been proposed to perform a tonic dilator effect on cerebral vessels [29, 117, 118]. We have also shown a significant reduction in the basal diameter of cerebral vessels when NOS was inhibited. It is also known that NO can inhibit the production of 20-HETE [122], which was shown to mediate the flow-induced constriction of cerebral arteries [23]. Thus, hypothetically, a decreased effect of NO could lead to an enhanced constrictor effect of 20-HETE. Reactive oxygen species (ROS) were also shown to play a role in flow-induced constriction in rat MCA [23], and they could be a potential candidate to decrease the bioavailability of NO leading to enhanced 20-HETE production. Endothelial-derived dilator arachidonic acid metabolites by lipoxygenase, epoxyeicosatrienoic acids, are known to be produced by the endothelium in response to shear stress [123], and they are capable of inhibiting TXA₂ receptor [124] which was shown to mediate the constriction to flow by 20-HETE [23]. They could also therefore play a role in the 'wash-out' theory. However, presently existing data question these possibilities, showing that flow-induced constriction of cerebral vessels still exists in the presence of NO synthase inhibitor L-NAME and after the denudation of endothelium [114, 117], and such constriction (instead of dilation) was not observed in basilar arteries. In addition, at a constant flow rate, increases in the viscosity of the perfusing solution could constrict the vessels while increasing WSS [114].

These ideas and other controversial observations need to be addressed by future studies. Such 'physiological' investigations would be very important since the precise

regulation of CBF has important clinical implications, for preventing and treating headache of vascular origin, transient ischemic attack, stroke, vascular cognitive impairment (and mixed vascular dementia with Alzheimer disease) and edema formation after brain injury or associated with tumor formation and other fatal cerebral pathologies. Without knowing the exact nature of the mechanical vasomotor response, 'signaling' studies cannot yield valuable information.

Cellular and Molecular Signaling of Flow-Induced Responses of Cerebral Vessels

In this section we briefly summarize the molecular mechanisms that have been shown to play a role in the development of flow-induced responses of cerebral vessels (fig. 3).

Flow-Induced Dilation of Cerebral Vessels

Only few data exist in the literature regarding the mechanisms of flow-induced dilation of cerebral vessels. In the rat basilar artery, Paravicini et al. [102] found that the dilation is mediated by H₂O₂ and NO generated by NADPH oxidase and eNOS (endothelial NOS), and that phosphatidylinositol 3 kinase activation is important in activation of NADPH oxidase. Recently, Drouin et al. [104] and Drouin and Thorin [105] similarly demonstrated that H₂O₂ mediates flow-induced dilation, which however derives from NO synthase activated by an Akt-dependent pathway (fig. 3).

Biphasic Response of Cerebral Vessels to Flow

In line with the observations of flow-induced dilation, in some of the studies founding biphasic response to flow the dilation was endothelium-dependent and was blocked by the inhibition of NO synthase (L-NAME) [108, 110]. Ward et al. [109] also found that indomethacin blocked the response and proposed a role for cyclooxygenase-derived metabolites. The constrictor response was found to be dependent on increases in intracellular (smooth muscle) Ca²⁺ concentration [106, 107].

Flow-Induced Constriction of Cerebral Vessels

Madden and Christman [112] found that administration of integrin-binding peptides, scavenging ROS by superoxide dismutase and inhibition of tyrosine kinase blocked the constriction, suggesting that integrin signaling, free radicals and tyrosine kinase all play a role in the mediation of flow-induced constriction in an endotheli-

Fig. 3. Proposed intracellular and molecular mechanisms eliciting flow-induced responses of cerebral vessels. Flow induces dilation, biphasic responses or constriction of cerebral vessels, depending on the regional and segmental localization of the vessels. We propose that, in the internal carotid system, larger arteries (i.e. MCA) constrict to increases in flow. The flow-induced constriction is mediated by 20-HETE (a metabolite of arachidonic acid (AA) produced by cytochrome P450 4A enzymes (CYP450 4A) acting via thromboxane A₂/prostaglandin H₂ (TP) receptors and requires COX activity. CYP450 4A also produces ROS, which contribute to the constriction. The vertebro-basilar system larger arteries (supplying the brain stem), such as the basilar artery, dilate to flow. Dilation is mediated by NADPH oxidase (activated by phosphatidylinositol 3 kinase (PI3-K)-derived H₂O₂ and/or eNOS-derived NO. eNOS is activated in an Akt-dependent pathway, and probably generates H₂O₂ as well (based on [23, 102, 104]). The exact nature of signals and sensors are still unknown (see detailed description in the text).

um-independent manner. Similarly, Bryan et al. [114, 117] observed that an integrin blocker specific for β_3 -integrin and superoxide dismutase abolished the flow-induced constriction. They also measured Ca^{2+} concentration in the vessel wall and demonstrated that flow-induced constriction is accompanied by increases in intracellular Ca^{2+} . Denudation of the endothelium did not affect flow-induced constriction.

Recently, we found that the flow-induced constriction of cerebral vessels was blocked by inhibition of 20-HETE synthesis, thromboxane A₂ receptor, COX activity and scavenging ROS. Therefore, we proposed that increases in flow activate arachidonic acid cascade, and that the metabolites are further metabolized by cytochrome P450 4A enzymes (CYP450 4A) into 20-HETE. It is known that CYP450 4A also produces ROS, which also contribute to the constriction. Thus, it seems that the flow-induced constriction of MCA and of human cerebral arteries is mediated by 20-HETE and ROS via thromboxane A₂/prostaglandin H₂ receptors and requires COX activity (fig. 3) [23].

Physiological Significance of Flow-Induced Responses of Cerebral Vessels of Different Sizes and Regions: Implications for Heterogeneity of Autoregulation

From the observed responses, one can estimate the change in CBF by using diameter values induced by changes in pressure only and then by simultaneous changes in pressure and flow using the Hagen-Poiseuille equation [23]. A 'gain factor (G)' can be calculated indicating the strength or efficacy of the autoregulation of blood flow ($G = 1$ indicates perfect autoregulation, whereas $G < 1$ means inefficient autoregulation, when CBF increases as a function of intraluminal pressure) [22, 23]. In the case of flow-induced constriction, we found that a simultaneous increase of pressure and flow enhanced the pressure-induced decrease in diameter. The gain of autoregulation (G) calculated using diameters induced by pressure alone was below 1, whereas G was approximately 1 when pressure and flow were increased simultaneously i.e. indicating a more efficient autoregulation. When only pressure was increased the estimated CBF showed a linear increase, but when pressure and flow increased simultaneously the estimated CBF decreased significantly and remained relatively constant [23]. Based on

this, we propose that in the circulatory areas where vessels constrict to flow a simultaneous operation of pressure- and flow-induced constrictions is necessary to explain an effective autoregulation of CBF (fig. 1).

In the internal carotid circulatory area of the cerebrum, resistance is primarily determined by larger arteries [18, 43, 44, 50]. In line with this, larger arteries (i.e. MCA) constrict to increases in flow, flow-induced constriction enhances the pressure-induced tone of cerebral vessels leading to a more efficient autoregulation of CBF and, via this flow-induced constriction of cerebral arteries, plays a role in regulating CBV and intracranial pressure. Therefore, in addition to myogenic response, flow-induced constriction (or dilation) may also participate in the development of segmental resistance of the cerebral circulation, because both large arteries and arterioles respond to changes in flow with either constriction or dilation [23, 100, 105–107, 110, 114, 125].

This segmental vascular resistance is thought to provide constant blood flow when the latter is altered locally (e.g. due to metabolic factors), and to protect downstream brain circulation from pressure and volume overload [43, 50, 52]. Regarding flow-induced responses, the importance of the segmental function of cerebrovascular resistance and the role of large arteries are underlined by the findings of Fujii et al. [100, 101] and, Garcia-Roldan and Bevan [106], which show that isolated cerebral arterioles constrict when the pressure or flow-rate are high, and dilate when they are low.

So the vasomotor tone 'set' by the two hemodynamic forces can be modulated or overridden by other factors sensitive to the needs of neural tissues. Whereas flow-induced constriction may play an important role (together with the pressure-sensitive myogenic tone) in the regulation and maintenance of CBV and intracranial pressure, local neural needs can increase CBF regionally via neural, glial and other regulatory mechanisms, which can also be propagated to upstream vessels [1, 17]. This concept is in line with the suggestion that metabolic dilation could overcome the constrictor effect of pressure or flow [23, 112, 126, 127]. Conversely, in the brain stem (vertebrobasilar system) arterioles are the major site of resistance [50, 51], thus, larger arteries (e.g. the basilar artery) 'can' dilate to flow and participate in reactive hyperemia [125]. The heterogeneity of autoregulation is likely due to the different efficacy of pressure- and flow-sensitive vascular mechanisms and/or the contribution of metabolic mechanisms and neurovascular coupling. The proposed concept should be further clarified in the future by in vitro investigations and comparisons of pressure-

and flow-induced responses of cerebral arteries and arterioles from different regions of the cerebrovascular tree [128]. More importantly, in vivo imaging of CBF in experimental animals and humans could be performed by means of MRI, laser-speckle imaging and other novel techniques in order to clarify the idea of the role of hemodynamic forces in the regulation of CBF, in which the pharmacological intervention of signaling mechanisms may reveal further details of its autoregulation.

All in all, regulation of CBF is the result of multilevel, interacting complex mechanisms to maintain an appropriate blood flow providing a supply of nutrients and gases to the brain tissues. To simplify, the tone of cerebral vessels is affected by both intraluminal pressure-induced constriction and flow-induced dilation or constriction, 'setting a basal vasomotor tone', which is then modulated by metabolic, astrocytic-glial, neural signals providing the complex multilevel regulation of CBF. The sum of these mechanisms is capable of matching the requirement of maintenance of an appropriate blood flow for the brain tissue at the same time as complying with the limited space in the closed cranium.

Pathophysiological and Clinical Relevance of Pressure- and Flow-Induced Autoregulatory Mechanisms of CBF

Changes in Hypertension

Previous studies described that the myogenic response of cerebral vessels is enhanced in hypertension [22, 63, 129, 130]. This is logically considered to be an adaptation extending the range of CBF autoregulation to higher systemic blood pressure. Based on the concept described in this review (fig. 1), enhanced flow-dependent constriction may also contribute to the functional adaptation of cerebral vessels in order to 'protect' brain tissue and intracranial space from high pressure and volume. Indeed, flow-induced constriction seems to be enhanced [131] and mediated by the upregulation of 20-HETE signaling Toth et al. [manuscript in preparation]. This could be due to the chronic elevation of blood flow due to the greater pressure drop across the cerebral circulation in hypertension.

Possible Contribution to Stroke and Other Cerebrovascular Disorders

As mentioned above, myogenic response is enhanced in hypertension, protecting the cerebral circulation from higher blood pressure. The importance of this mecha-

nism was demonstrated by Smeda [132] and Smeda et al. [133], showing that the enhanced myogenic response of rat cerebral arteries is attenuated before and lost after the onset of cerebral hemorrhage. Parallel with the myogenic response, we found that enhanced flow-induced constriction in hypertension is attenuated before and lost after the onset of hemorrhagic stroke in stroke-prone spontaneously hypertensive rats (unpubl. observation). On the other hand, enhanced constriction to flow in hypertension can cause a decreased ability to adapt to decreases in blood pressure, thereby probably contributing to ischemic damage of the brain. This idea is supported by the findings that the inhibition of 20-HETE production significantly reduces the infarct size after MCA occlusion [134–136]. All of these ideas need to be investigated in the future.

Summary

Although autoregulation of CBF has been explained solely by the pressure-sensitive myogenic mechanism, more and more data show that this explanation is insufficient. It is logical to assume that changes in flow also play a role, because flow changes occur during changes in pressure. This was probably the reason for conducting many studies over the past 30 years: to elucidate the nature, underlying mechanisms and physiological importance of the flow-induced responses of cerebral vessels. These studies showed that an increase in intraluminal flow is sensed by the vascular tissues of cerebral vessels and is converted into changes in diameter: constriction, dilation or biphasic response. It seems that the nature of

response depends on the region of the brain or vascular segment, size or localization. Species and gender differences can not be ruled out either. These differences are likely to play a physiological role by modulating myogenic mechanisms and in the regulation of CBF and CBV and intracranial pressure, at the same time providing an appropriate amount of blood flow to the brain tissue.

Although the precise signaling of the mechanotransduction of flow signal is not yet known, arachidonic metabolites, especially 20-HETE, NO, potassium and TRP channels seem to play important roles. In addition, ROS produced by these and other enzymes, such as NAD(P)H oxidase, may also contribute to the modulation of vasomotor tone.

It is clear, however, that there are still many controversial observations, unexplained signals and unknown mechanisms that could all contribute to the diameter response of cerebral vessels of different size and origin to changes in hemodynamic forces and thus the regulation of CBF. Because cerebrovascular diseases (e.g. stroke, vascular dementia, cognitive impairment and aging) that are associated with the impaired regulation of CBF are still leading causes of human morbidity and mortality, it is imperative to further investigate the responses and the underlying mechanisms of cerebral vessels to rheological and hemodynamic forces.

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