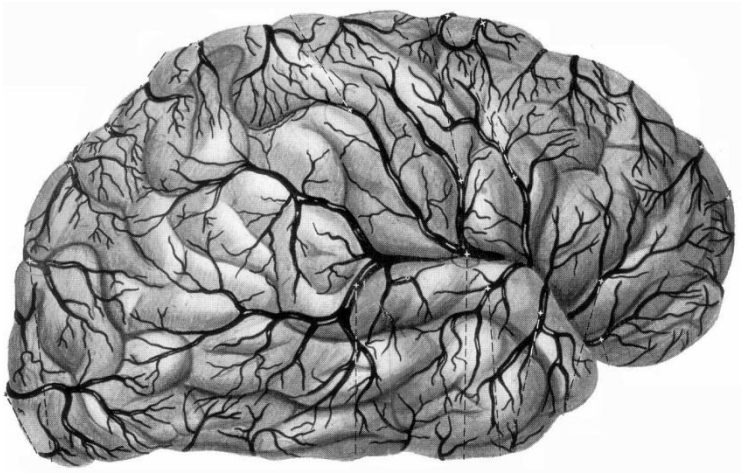
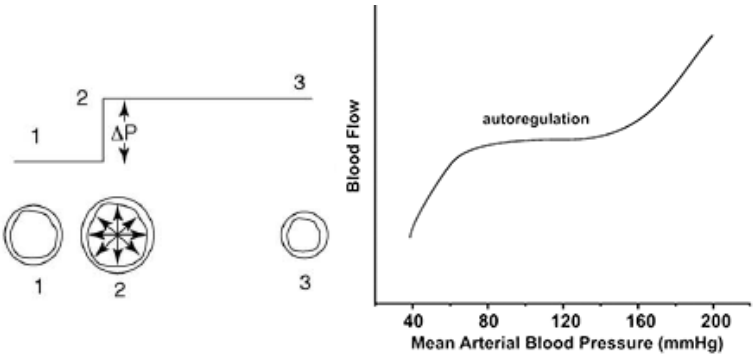
# The cerebrovascular myogenic response

Defects of the myogenic response in cerebrovascular disease and therapeutic modulation of the myogenic tone.



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## Abstract

The myogenic response is the reaction of small arteries and arterioles to changes in intraluminal pressure. By constricting when pressure rises and dilating when pressure drops the blood flow through the organs is kept constant. The vasculature of the brain also shows the myogenic response. This thesis deals with several questions related to the cerebral myogenic response. These questions are answered in six chapters. First, an overview is given of the general myogenic response including its function, its influence on hemodynamics and its independence of the neurohumoral regulatory mechanisms. Second, the cellular mechanisms of the myogenic response are discussed. Several hypotheses on mechanosensation are given and the further molecular signalling pathways leading to the activation of contractile proteins are explained. Third, the cerebral myogenic response is characterized and compared with the general principle. It is discussed whether the endothelium might play a role in the myogenic response, which would be unique to the cerebral vasculature. Fourth, cerebrovascular diseases are described that might be caused by defects of the cerebral myogenic response. These are ischemia/reperfusion injury, cerebral vasospasm after subarachnoid haemorrhage, eclampsia and stroke development. Fifth, potential targets for therapeutic modulation of the general and the cerebral myogenic response are identified such as mediators of mechanosensation, $BK_{Ca}$ channels and the RhoA/Rho-kinase pathway. Finally, therapeutic modulation of the cerebral myogenic response is proposed for the medical conditions mentioned in chapter four. This could be done either by administration of drugs targeting the molecular mechanisms that modulate the cerebral myogenic tone or by treatment of defects of the cerebral myogenic response.

## Introduction

One of the ways through which a constant blood flow through the human organs is maintained is the myogenic response. The myogenic response is defined as the high-pressure-induced constriction and the low-pressure-induced dilation of small arteries and arterioles. In July 2009, one of the leading review journals in pharmacology 'Trends in Pharmacological Sciences' published an article on the myogenic response by M.A. Hill, G.A. Meininger, M.J. Davis and I.Laher, who have studied the myogenic response since the late 1980s. In this article they discussed the therapeutic potential of pharmacological targeting of the arteriolar myogenic tone in cardiovascular diseases. They concluded that with the recent identification of novel candidate signalling pathways in the myogenic response the possibility of distinct pharmacological targets is raised. (Hill, Meininger, Davis, & Laher, 2009) According to Hill et al. this type of therapy would have to target a signalling mechanism specific to the myogenic response, which probably is it's 'mechanosensing' component.

This thesis has two main topics. The first is the role of the vascular myogenic response in cerebrovascular disease. The second is modulation of the myogenic tone as a novel approach in therapy for cerebrovascular disease. The following six chapters of this thesis answer some of the same questions Hill and his colleagues posed in their article on the myogenic response, but with a specific and more extensive attention to the myogenic response in the brain: the cerebral myogenic response. The first chapter gives a general overview of the myogenic response. The second chapter details the hypothesized cellular mechanisms the myogenic response, from the sensation of pressure changes to the activation of contractile proteins. The third chapter compares the cerebral myogenic response with the general principle of the myogenic response. Chapter four discusses four cerebrovascular diseases in which defects of the myogenic response may have a causative role. Chapter five identifies potential pharmacological targets for the modulation of the cerebral myogenic tone for the treatment of cerebrovascular diseases.

## 1. The myogenic response

### *Autoregulation of blood flow*

Autoregulation of blood flow is crucial for maintaining the perfusion requirements of the capillary beds of organs. (Hill et al., 2009) It protects from ischemia when blood pressure drops and it limits over-perfusion when blood pressure is heightened. This makes the delivery of blood, oxygen and nutrients to the organs independent of physiological activity. (Smeda, VanVliet, & King, 1999) The autoregulation of blood flow is done by control of the contraction of the vascular smooth muscle cells surrounding the endothelial vessel wall. The contraction of vascular smooth muscle cells (VSM cells) alters the diameter of the blood vessel and thereby modulates blood flow, because according to Poiseuille's Law the blood flow *Q* is related to the fourth power of the vessel lumen radius *r* : $Q = \frac{\pi \cdot r\hat{}4 \cdot P}{8 \cdot \eta \cdot L}$. (Hill et al., 2009) This explains why small changes in lumen diameter can cause significant changes in blood flow. (Cipolla & Curry, 2002) Vascular blood flow autoregulation consists of different types of regulating mechanisms: neural, endothelial, metabolic and myogenic. The reaction of VSM cells to changes in blood pressure is called the myogenic response.

### *Hemodynamics of the myogenic response*

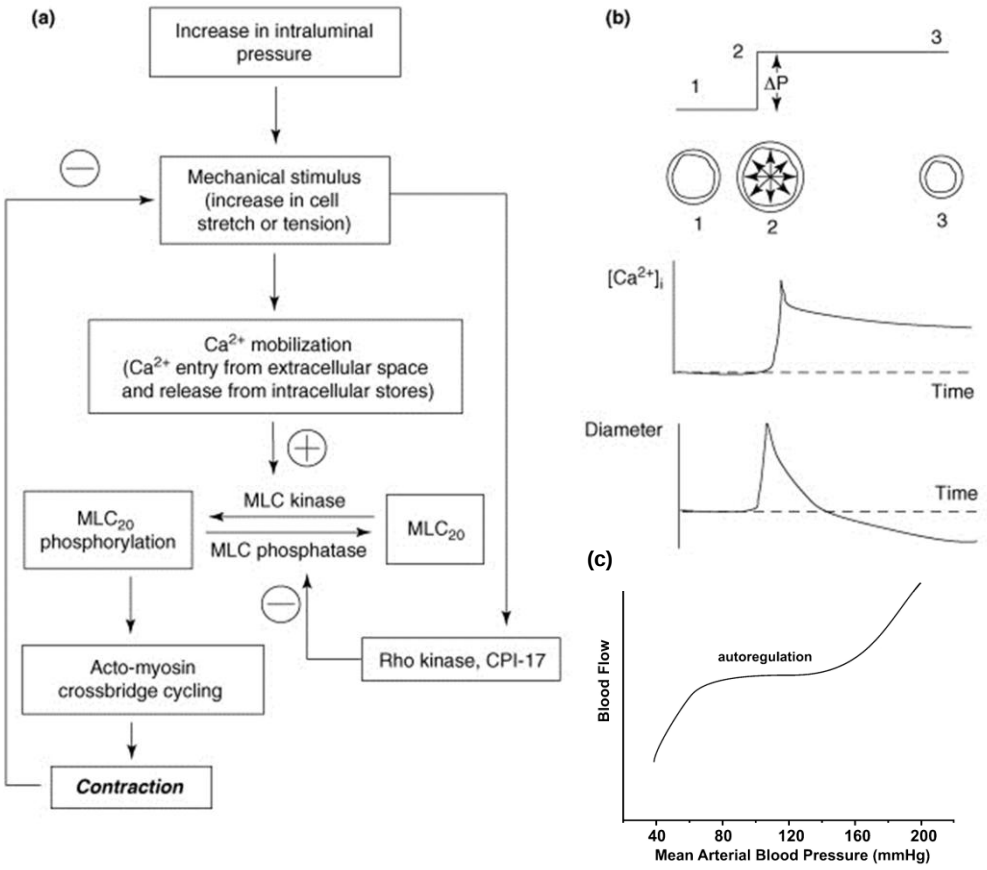
The vascular myogenic response sets the resting tone of blood vessels and acutely reacts to changes in intraluminal pressure by controlling flow resistance in a pressure-dependent manner. (Davis & Hill, 1999) The myogenic response also controls capillary hydrostatic pressure and thereby it modulates fluid transport between the blood and the tissue. (Hill et al., 2009) Vasoconstriction is induced in reaction to elevation of intraluminal pressure (see figure 1b), while vasodilation is induced in reaction to reduction of intraluminal pressure. In this way blood flow and capillary hydrostatic pressure are maintained constant over a range of

intraluminal pressure levels: the autoregulatory range. The blood pressure levels at both ends of the autoregulatory range are the lower and upper limit for adequate autoregulation of blood flow. Someone with a normal blood pressure of 90 mm Hg has a lower limit at 60-70 mm Hg. (Strandgaard, Olesen, Skinhoj, & Lassen, 1973) When the intraluminal pressure exceeds the autoregulatory range the autoregulation of blood flow fails and blood flow becomes linearly dependent on the mean arterial pressure (see the autoregulatory curve in figure 1c). Figure 1c shows that the blood flow strongly drops when the lower limit is passed and it strongly increases when the upper limit is passed. (Faraci, Baumbach, & Heistad, 1989) The autoregulatory curve is not static; it can be altered and shifted. Chronically hypertensive patients show protective remodelling and hypertrophy of arteries that lead to an extended autoregulatory range in both directions and the autoregulatory curve is shifted to the right. (Cipolla & Curry, 2002) This protects hypertensive patients from overperfusion due to autoregulatory failure.

***The myogenic response originates from within vascular smooth muscle cells***

The myogenic response occurs independently of endothelial, neural and hormonal influences. The VSM cells bring forth the myogenic response through an endogenous cellular mechanism based on sensing the vascular wall tension and signalling the molecular message that leads to the appropriate response. (Davis & Hill, 1999) Although the myogenic response occurs independently of influences from outside the vascular smooth cell it is affected by exogenous signals. The myogenic response is enhanced by adrenergic stimulation in skeletal muscle arterioles. (Meininger & Faber, 1991) The myogenic response also interacts with the other regulatory mechanisms since the myogenic tone is the basal tone upon which other regulating mechanisms act. (Hill et al., 2009)

**<u>Figure 1.</u> a + b: Myogenic constriction in response to increased intraluminal pressure. (Hill et al., 2009) c: The autoregulatry curve. (Ishiguro et al., 2002)**



## 2. Cellular mechanisms of the myogenic response

The myogenic response is initiated by a rise in intraluminal pressure through the depolarization of the vascular smooth muscle cell membrane. Pressure-induced membrane depolarization causes an increased $Ca^{2+}$ entry via the voltage-gated $Ca^{2+}$ channels. (Davis & Hill, 1999) The mobilization of $Ca^{2+}$ leads to the activation of contractile proteins, which increases the myogenic tone. (Hill et al., 2009) See figure 1a for an overview of myogenic constriction in response to a rise in intraluminal pressure. This chapter gives a detailed overview of the cellular mechanisms of the myogenic response.

***Mechanosensation I: mechanosensitive membrane channels***

The myogenic response is the reaction of VSM cells to intraluminal pressure changes so there must be a mechanism for sensation of intraluminal pressure changes. Several membrane channel state changes have been hypothesized to initiate the myogenic response: the opening of nonspecific cation channels, the closing of $K^+$ channels and the opening of $Cl^-$ channels. These mechanosensitive membrane channels are either directly activated by intraluminal pressure changes or activated secondary to an ion channel gating modulating mechanism. (Hill et al., 2009) The identification of the responsible membrane channels currently points to members of the transient receptor potential (TRP) family and the epithelial sodium channel (ENaC). (Hill et al., 2009)

***Mechanosensation II: integrins***

A second mechanosensory mechanism has been proposed that involves integrins. Integrins are a class of membrane-spanning glycoproteins that bind proteins from the extracellular matrix. Integrins might signal the intraluminal pressure changes that exert mechanical force on these bindings. (Hill et al., 2009) Several integrin-specific peptides have been shown to inhibit the myogenic tone and regulate the calcium entry pathway of the VSM cells required in the myogenic response, but concluding that integrins are mediators in the myogenic response is premature. (Davis et al., 2001)

***Mechanosensation III: 20-HETE***

A third hypothesized mechanosensory mechanism involves the cytochrome P-450 4A metabolite of arachidonic acid: 20-hydroxyeicosatetraenoic acid (20-HETE), a potent vasoconstrictor. Its concentration is elevated in the VSM cell in response to pressure. 20-HETE activates protein kinase C (PKC) and thereby inhibits $BK_{Ca}$ channels. (Gebremedhin et al., 2000) This leads to VSM cell contraction through a negative feedback mechanism that is explained in the section below.

***Negative feedback via $BK_{Ca}$ channels***

Large conductance, $Ca^{2+}$-activated K+ channels ($BK_{Ca}$ channels) are part of a negative feedback mechanism of the cerebral myogenic response. The activation of $BK_{Ca}$ channels in response to pressure opposes excessive pressure-induced vasoconstriction. The stretch-induced activation of $BK_{Ca}$ channels in VSM cells causes membrane hyperpolarization, which limits the constriction following a rise of intraluminal pressure by opening voltage-gated $Ca^{2+}$ - channels. (Hill, Yang, Ella, Davis, & Braun, 2010) Calcium enhances the voltage-dependent activation of $BK_{Ca}$ channels so that $Ca^{2+}$ entry during myogenic constriction leads to a higher probability of the $BK_{Ca}$ channel being open. (Hill et al., 2010) $BK_{Ca}$ activation of VSM cells in response to a rise in intraluminal pressure is not seen in all vessels of the body. While it has not been shown in skeletal muscle arterioles it has been shown in cerebral arteries. (Hill, Davis, Meininger, Potocnik, & Murphy, 2006)

***$Ca^{2+}$ entry through voltage-gated $Ca^{2+}$ channels***

The pressure-activated membrane channels depolarize the VSM cell membrane past the activation threshold, which activates the voltage-gated $Ca^{2+}$ channels leading to the entry of extracellular $Ca^{2+}$ and the release of $Ca^{2+}$ from intracellular stores, which in turn leads to myogenic contraction. (Hill et al.,

2009) The voltage-gated $Ca^{2+}$ channels in VSM cells are mainly of the L-type and are activated at an intraluminal pressure at rest. Pressure-induced cell membrane depolarization causes a 10- to 15-fold rise of the probability of the VGC channels of being open. (Davis & Hill, 1999)

***Activation of contractile proteins***

$Ca^{2+}$ mobilization induces smooth muscle contraction through calcium-activated phosphorylation of myosin. $Ca^{2+}$ binds calmodulin. $Ca^{2+}$-bound calmodulin binds and activates myosin light-chain kinase. Activated myosin light-chain kinase phosphorylates the myosin light-chain, which causes the myosin-actin cross-bridges to slide over each other (a process which requires energy from ATP hydrolysis). This results in contraction of the smooth muscle cell.

***$Ca^{2+}$ sensitization***

The contraction of smooth muscle cells is also regulated by myosin light-chain phosphatase (MLCP). Activated MLCP reduces the level of myosin light-chain phosphorylation and thereby it reduces the cycling of actin and myosin cross-bridges seen in smooth muscle cell contraction. MLCP inhibition is called $Ca^{2+}$ sensitization, because here the phosphorylation of myosin is modulated independently of the $Ca^{2+}$ concentration so that the effect of $Ca^{2+}$ is enhanced. (Hill et al., 2009) $Ca^{2+}$ sensitization leads to increased smooth muscle cell contraction due to a higher level of myosin light-chain phosphorylation. Figure 2 gives an overview of $Ca^{2}$ sensitization and its regulation by the RhoA/Rho-kinase pathway.

***The role of RhoA and Rho-kinase in $Ca^{2+}$ sensitization***

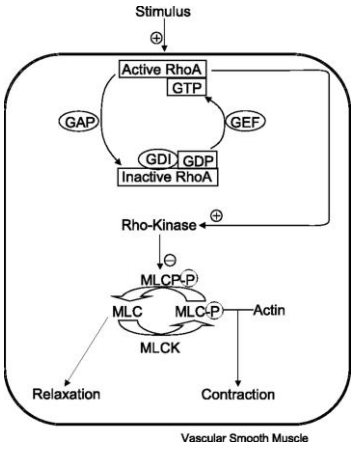
RhoA and Rho-kinase are involved in $Ca^{2+}$ sensitization and may contribute to the myogenic response. (Chrissobolis & Sobey, 2006) RhoA is more active in stretched vascular smooth muscle cells and inhibition of Rho-kinase counteracts pressure-induced constriction of cerebral arteries. (Numaguchi, Eguchi, Yamakawa, Motley, & Inagami, 1999) (Lagaud, Gaudreault, Moore, Van Breemen, & Laher, 2002) RhoA causes relaxation of posterior cerebral arteries and arterioles. (Gokina, Park, McElroy-Yaggy, & Osol, 2005) RhoA is one of the three members of the Rho protein family, which is a subfamily of the RAS superfamily of small GTPases. RhoA is expressed in cerebral vascular tissue and mediates vascular effects. (Chrissobolis & Sobey, 2006) RhoA and Rho-kinase have a significant impact on vascular smooth muscle cell contractility (see figure 2). MLCP activity is inhibited by RhoA-activated Rho-kinase through phosphorylation of the myosin-binding subunit of MLCP. (Kimura et al., 1996) Rho-kinase is activated by GTP-bound RhoA. When activated, the two isoforms of Rho-kinase, ROKα/ROCKII and ROKβ/ROCKI, both phosphorylate the myosin-binding subunit of MLCP and thereby inhibit its activity. The activity of RhoA is mediated by three enzyme classes: GEFs (guanine-nucleotide exchange factors, which activate Rho by exchanging GDP for GTP), GAPs (GTPase-activating proteins, which inactivate Rho by accelerating its GTPAse activity) and GDIs (GDP dissociation inhibitors, which inhibit the dissociation of Rho-bound GDP). (Aspenstrom, 1999) Besides inhibiting MLCP function, active RhoA also induces actin polymerization, which also contributes to the myogenic response. (Chrissobolis & Sobey, 2006)

**The influence of endogenous estrogen on the cerebral myogenic tone**

It has been found that female Sprague-Dawley rats possess a lower Rho-kinase function in cerebral arteries then males, which is consistent with the lower myogenic tone in female cerebral arteries compared to those from males. (Chrissobolis, Budzyn, Marley, & Sobey, 2004) The female cerebral Rho-kinase expression was found to be supressed by the higher level of endogenous estrogen in females compared to males. (Chrissobolis et al., 2004) (Hiroki, Shimokawa, Mukai, Ichiki, & Takeshita, 2005) Estrogen increases the bioavailability of nitric oxide (NO) and thereby supresses Rho-kinase expression in cerebral arteries. (Geary, Krause, & Duckles, 1998) This role of estrogen might explain why the incidence of stroke and cerebrovascular disease is relatively low among premenopausal females compared to age-

matched men and postmenopausal women. (Chrissobolis et al., 2004)

**Figure 2. $Ca^{2+}$ sensitization and the role of RhoA/Rho-kinase. (Chrissobolis et al., 2004)**




## 3. The myogenic response in the cerebral circulation

Like the mesenteric, skeletal muscle, renal and coronary circulation, the cerebral circulation also shows the myogenic response. (Paulson, Strandgaard, & Edvinsson, 1990) The cerebrovascular myogenic response prevents brain ischemia during blood pressure decrease and capillary damage and edema formation during blood pressure increase. The rate of cerebral blood flow (CBF) equals cerebral perfusion pressure divided by vascular resistance. (Shahsavari, McKelvey, Ritzen, & Rydenhag, 2011) Normal cerebral blood flow in adults is about 50 mL per 100 gram of brain tissue per minute if the mean arterial blood pressure is between 60 and 150 mm Hg. (Johansson, Li, Olsson, & Klatzo, 1970) These two pressure levels are the limits of the autoregulatory range of the cerebral arteries. As explained in chapter one, when cerebral arterial pressure exceeds the autoregulatory range cerebral blood flow becomes linearly dependent on mean arterial pressure. (Faraci et al., 1989)

The cerebral vessels maintain a relatively dilated state. (Hill et al., 2006) This could be caused by activation of the constriction-limiting $BK_{Ca}$ channels as explained in chapter two. (Hill et al., 2006) The strength of the myogenic response of cerebral vessels is relatively high compared to mesenteric vessels. (Osol, Laher, & Cipolla, 1991) Furthermore, the cerebral myogenic response is an exception to the rule that larger vessels have smaller myogenic responsiveness. (Osol et al., 1991)

### *The role of the endothelium in the cerebral myogenic response*

As explained in chapter two, the myogenic response is generally independent of the endothelium, but in the cerebral arteries it might play a role. Harder et al. proved that the pressure-induced constriction of middle cerebral from cats is dependent on intact endothelium. They hypothesized that the cerebrovascular endothelium of the cat releases a transferable contractile factor in response to pressure that induces VSM cell membrane depolarization. (Harder, Sanchez-Ferrer, Kauser, Stekiel, & Rubanyi, 1989) While other studies supporting a role for the endothelium in the myogenic response can be explained by VSM-cell damage after chemical removal of the endothelium the study from Harder et al. cannot. This leaves open the possibility that the endothelium specifically amplifies the cerebral myogenic response in order to maintain optimal blood flow in this crucially important organ. (Meininger & Davis, 1992)

## 4. Defects of the myogenic response in cerebrovascular disease

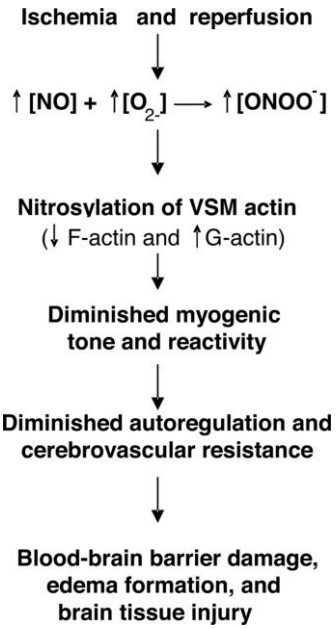
Several medical conditions coincide with impaired myogenic tone and myogenic reactivity like ischemia/reperfusion, eclampsia and stroke development in stroke-prone rats. In contrast, other conditions coincide with increased myogenic tone and reactivity like cerebral vasospasm after subarachnoid hemorrhage. In this chapter it is explained for each condition what defects and how these defects of the myogenic response play a role in these conditions.

***Ischemia/reperfusion-induced impairment of cerebrovascular myogenic tone and reactivity***

During reperfusion after occlusion of cerebral arteries, autoregulatory failure occurs. This often results in reperfusion injury and edema formation. (Cipolla & Curry, 2002) Cipolla et al. assessed the myogenic response of middle cerebral arteries of male Wistar rats after different periods of reperfusion by measuring the myogenic tone and the myogenic reactivity. They discovered that the myogenic tone and reactivity are normal after short durations of reperfusion, but longer periods of reperfusion cause both a diminished tone and reactivity. After 30 minutes myogenic tone is diminished and after six hours, myogenic reactivity is impaired. Reperfusion periods longer than six hours progressively worsen the reactivity, which makes six hours the threshold duration for reperfusion-induced impairment of myogenic reactivity. Whether after six hours the autoregulatory capacity is diminished is unknown. Ischemia also impairs the myogenic activity of cerebral arteries. The duration threshold for ischemia-induced impairment of myogenic activity is between 15 and 30 minutes and exceeding this treshold causes a sharp drop in myogenic tone. (Cipolla & Curry, 2002)

The diminished myogenic tone and myogenic reactivity during reperfusion might be caused by the increased production of nitric oxide during ischemia and reactive oxygen species during reperfusion. (Kumura et al., 1996) The increased NO production in endothelial cells during ischemia is available for reaction with reactive oxygen species generated in endothelial cells during reperfusion. This results in increased formation of peroxynitrite ($ONOO^-$). The reactive peroxynitrite decreases the myogenic tone, impairs the low-pressure-induced dilation of arteries and leads to vasomotor paralysis at high concentrations.
These experimental findings lead to the hypothesis that peroxynitrite causes diminished myogenic tone and myogenic reactivity during reperfusion. (Cipolla & Curry, 2002) Ischemic injury is associated with a loss of filamentous F-actin, the polymer form of actin, which slides alongside myosin in smooth muscle cell contraction. (Cipolla et al., 2002) It has been show that $ONOO^-$ induces F-actin depolymerization and inhibits monomeric globular G-actin polymerization. This is possibly mediated through nitrosylation of F-actin, but how nytrosylation could lead to F-actin depolymerization is unknown. (Maneen & Cipolla, 2007) Impairment of cerebral myogenic tone and reactivity in ischemia/reperfusion may be caused by an increase of $ONOO^-$ and subsequent $ONOO^-$-induced nytrosylation of F-actin and F-actin depolymerization. See figure 3 for a schematic view of this hypothesis on the role of the myogenic response in ischemia/reperfusion.

**Figure 3. Ischemia/reperfusion-induced impairment of cerebrovascular myogenic tone and reactivity (Maneen et al. 2006)**




***Increased myogenic tone and reactivity in SAH-induced cerebral vasospasm***

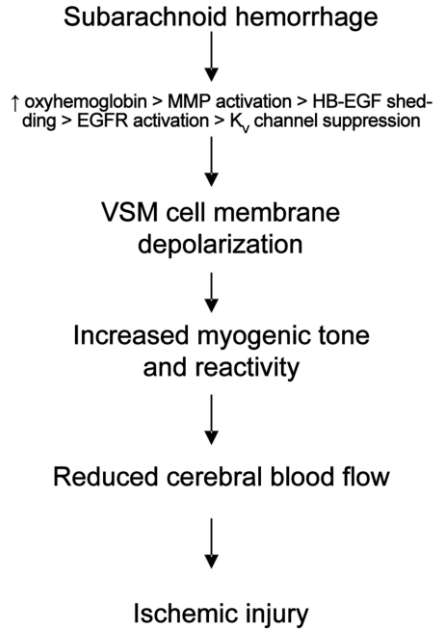
In the days following subarachnoid hemorrhage (SAH), some patients experience delayed and sustained vasoconstriction of cerebral arteries. This is called cerebral vasospasm. It is a major cause of death and disability in patients who suffered a subarachnoid hemorrhage after rupture of a cerebral aneurysm. (Ishiguro et al., 2002) Vasospasm causes reduced cerebral blood flow and thereby can lead to ischemic injury.

Studies of cerebral vasospasm have relied on vasospasm assessment through angiography of large diameter (>1 mm) arteries. This is why fewer studies have been done on vasospasm in small diameter arteries and the role of the myogenic response in these small arteries. (Ishiguro et al., 2002) Ishiguro et al. showed enhanced myogenic tone in small diameter cerebral arteries from a rabbit model of SAH compared to control animals. Also in small diameter cerebral arteries the SAH model showed increased pressure-induced vasoconstriction in the physiological range (60 – 100 mmHg) compared to control animals. (Ishiguro et al., 2002) According to Ishiguro et al. this means that the severe narrowing of cerebral arteries in cerebral vasospasm could be caused by the increased myogenic tone and reactivity of small cerebral arteries.

The etiology of cerebral vasospasm following subarachnoid hemorrhage is unclear. It might be induced by an increased level of oxyhemoglobin. Oxyhemoglobin induces MMP activation, which subsequently causes heparin-binding EGF-like growth factor shedding, activation of the tyrosine kinase EGF receptor and suppression of voltage-dependent rectifier ($K_v$) channels. Suppression of $K_V$ channels leads to membrane depolarization, which leads to vasoconstriction as explained in chapter two. (Koide, Penar, Tranmer, & Wellman, 2007) These findings suggest that defects of the myogenic response contribute to cerebral vasospasm following subarachnoid hemorrhage. See figure 4 for a schematic view of this hypothesis on the role of the myogenic response in SAH-induced cerebral vasospasm. The expression of R-type voltage-dependent calcium channels in cerebral VSM cells after SAH might also contribute to SAH-induced cerebral vasospasm. (Ishiguro et al., 2005) More on R-type VDCCs can be found in chapter six.

RhoA and Rho-kinase, two proteins involved in $Ca2^+$ sensitization and the myogenic response, are thought to be involved in oxyhemoglobin-induced vasoconstriction. (Ishiguro et al., 2002) The hypothesis that increased RhoA/Rho-kinase activity might contribute to the increased contractility of vascular smooth muscle cells in cerebral vasospasm holds biological significance, since the high RhoA/Rho-kinase activity seen in cerebral vasospasm is expected to increase myosin light-chain phosphorylation. (Chrissobolis & Sobey, 2006) As explained in chapter two, the RhoA/Rho-kinase pathway plays an important role in the myogenic response through its role in $Ca^{2+}$ sensitization. A high RhoA/Rho-kinase activity increases phosphorylation of the myosin binding subunit of MLCP, which renders MLCP inactive and thereby increases phosphorylation of the myosin light-chain. This leads to cycling of actin and myosin cross-bridges seen in vascular smooth muscle cell contraction and finally to vasoconstriction. (Chrissobolis & Sobey, 2006)

**Figure 4.** ***Increased myogenic tone and reactivity in SAH-induced cerebral vasospasm***



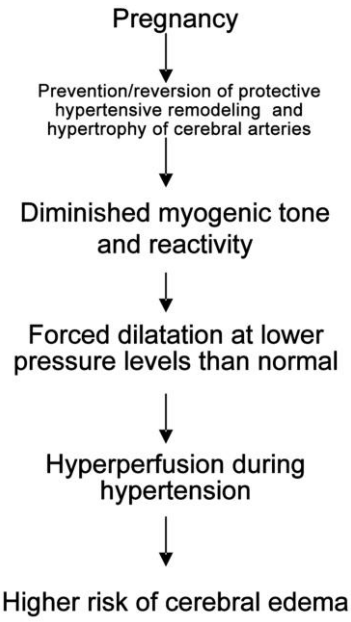

***Diminished myogenic reactivity in eclampsia***

Eclampsia is a leading cause of maternal death during late pregnancy and the postpartum period. (Cipolla, Vitullo, & McKinnon, 2004) Eclampsia patients can suffer hemorrhage, cerebral edema and vasculopathy. It is associated with headaches, nausea, visual disturbances, vomiting, convulsions and loss of consciousness. Days or weeks postpartum, the neurological signs are reversed due to recovery of normal blood pressure. These

neurological symptoms are similar to those seen in hypertensive encephalopathy, where an acute rise in blood pressure that exceeds the autoregulatory range causes forced dilation of arteries and arterioles. Eclampsia patients also suffer from this loss of autoregulatory capacity in blood vessels. (Cipolla et al., 2004)

The women who develop eclampsia are generally normotensive before pregnancy. This notion and the similarities to hypertensive encephalopathy led Cipolla et al. to the hypothesis that pregnancy leads to vascular changes that increase the risk of eclampsia. Cipolla et al. suggest that pregnancy and the postpartum period predispose the cerebral circulation to forced dilatation (auto regulatory breakthrough) at lower pressure levels. This may lower cerebrovascular resistance and promote hyperperfusion during hypertension as happens during eclampsia. The resulting forced dilatation and edema might lead to eclampsia. Because forced dilatation occurs during autoregulatory breakthrough, women with normal pressure might be unaffected. (Cipolla et al., 2004) Cipolla et al. also found that pregnancy prevents protective hypertensive remodeling and hypertrophy of cerebral arteries and fails to increase the pressure at which forced dilatation occurs. (Cipolla, DeLance, & Vitullo, 2006) Remodeling and hypertrophy are seen in hypertension in non-pregnant women and are thought to have a protective effect by normalizing wall stress, shifting the autoregulatory range to higher pressure levels and making cerebral arteries more resistant to forced dilatation during hypertension, protecting the blood-brain barrier from disruption. (Cipolla, Smith, Bishop, Bullinger, & Godfrey, 2008) More recently Cipolla et al. showed pregnancy reverses preexisting hypertensive remodeling. This could explain why pre-pregnancy hypertension is a risk factor for eclampsia, since the blood pressure remains high while the upper limit of autoregulation is lowered. (Cipolla et al., 2008) See figure 5 for an overview of this hypothesis on the role of the myogenic response in eclampsia.

**Figure 5.** ***Diminished myogenic reactivity in eclampsia***




***Loss of myogenic reactivity prior to stroke in stroke-prone rats***

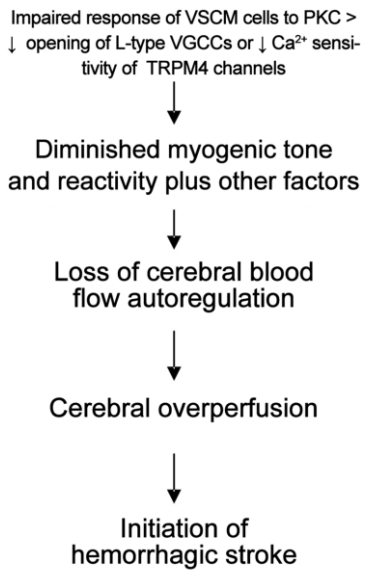
Smeda et al. have studied the autoregulation of the cerebral blood flow in Wistar Kyoto stroke-prone spontaneously hypertensive rats (SHRsp). (Smeda, VanVliet et al., 1999) They showed attenuated CBF autoregulation by middle and posterior cerebral arteries of SHRsp during the period of a highly likely onset of stroke. Attenuated CBF autoregulation of these two regions coincide with impaired myogenic constriction in small isolated arteries of the middle cerebral region, but myogenic constriction of isolated arteries of the posterior cerebral region was not impaired. The experiments done by Smeda et al. led to their conclusion that the loss of cerebral blood flow autoregulation prior to stroke in SHRsp could cause cerebral overperfusion and facilitate the initiation of hemorrhagic stroke. They note that the inability of the middle cerebral arteries to constrict in response to pressure is not due to a general inability to constrict, because they do constrict in response to agonists. This implicates a possible defect of the myogenic response in stroke development, although the defects in pressure-dependent constriction cannot fully account for the pattern of CBF

autoregulation loss observed in post-stroke SHRsp. (Smeda, VanVliet et al., 1999)

Smeda et al. also studied the constriction of cerebral arteries in response to protein kinase C (PKC) activation in SHRsp, because earlier studies suggest that PKC activation contributes to pressure-induced constriction. (Smeda, King, & Harder, 1999) Smeda et al. showed that the loss of myogenic reactivity in SHRsp was associated with the loss of middle cerebral arteries constriction in response to PKC activation. Other studies suggest that PKC might contribute to myogenic constriction by opening L-type voltage-gated $Ca^{2+}$ channels. Also the PKC products IP3 and diacylglycerol seem to be elevated in response to pressure, which suggests that activation of PKC could be initiated by a pressure-induced increase of phospholipase C activity. (Smeda et al., 1999) This role for PKC in pressure-induced constriction implicates an alternative mechanism for the myogenic response, but these mechanisms might also work in conjunction to facilitate the myogenic response. (Smeda et al., 1999) These studies by Smeda et al. and others show the ability of PKC to induce constriction, but do not show activation of PKC in response to a rise in intraluminal pressure. Given the above, these studies do not prove that PKC really plays a role in the myogenic response and stroke development. See figure six for an overview of theis hypothesis on the role of the myogenic response in stroke development.

Earley et al. have shown that PKC stimulates TRPM4-dependent currents in VSM cells through increasing the $Ca^{2+}$ sensitivity of these TRPM4 channels. This mechanism functions as a critical mediator of myogenic tone in cerebral arteries. (Earley, Straub, & Brayden, 2007) TRPM4 is a member of the TRP family of the membrane channels mentioned in chapter 2, which might be involved in mechanosensation of the myogenic response. (Earley, Waldron, & Brayden, 2004) This gives more credibility to a role for PKC in the myogenic response and stroke development due to impaired constriction in response to PKC.

**Figure 6.** ***Loss of myogenic reactivity prior to stroke in stroke-prone rats***




## 5. Pharmalogical targets for modulation of the myogenic tone

In order to modulate the myogenic tone without interfering with the neurohumoral regulatory mechanisms the pharmacological target would have to be unique to the signaling pathway of myogenic response. Recent identification of candidate signaling pathways have raised the possibility of findings such targets. This could include targets in the mechanism of mechanosensation such as TRP, ENaC and integrins. Since mechanosensation is specific to the myogenic response targeting signaling pathways of mechanosensation would be preferred over targeting general signaling pathways like voltage-gated $Ca^{2+}$ entry, intracellular $Ca^{2+}$ release or actin myosin cross-bridge cycling. (Hill et al., 2009)

***$BK_{Ca}$ channels***

$BK_{Ca}$ channels (large conductance, $Ca^{2+}$-activated K+ channels) are a possible pharmacological target for modulation of the myogenic tone, because they are part of a negative feedback mechanism of the myogenic response as explained in chapter two. The VSM cell's large conductance, $Ca^{2+}$-activated K+ channels ($BK_{Ca}$ channels) oppose excessive pressure-induced vasoconstriction.

Although the cellular mechanisms of mechanosensation are unclear $BK_{Ca}$ channels have been implicated in different hypothesized mechanisms. (Hill et al., 2010) This makes $BK_{Ca}$ channels an interesting subject of further research. Doing tissue and species specific experiments is important for studying $BK_{Ca}$ channels, because of vascular heterogeneity of $BK_{Ca}$ channel expression and activity. (Hill et al., 2010)

***RhoA and Rho-kinase***

The RhoA/Rho-kinase pathway has also been identified as a possible therapeutic target for modulation of the myogenic tone due to its role in $Ca^{2+}$ sensitization as explained in chapter two. (Jarajapu & Knot, 2005) The Rho-kinase inhibitors Y-27632 and HA-1077 decrease the myogenic tone of rat cerebral arteries. Also, the pressure-induced increase in myogenic tone was significantly decreased by the Rho-kinase inhibitor Y-27632. (Jarajapu & Knot, 2005) Rho-kinase inhibitors increase the activity of MLCP, which subsequently leads to a lower level of myosin light-chain phosphorylation, reduced VSM cell contraction and vasodilatation. Another name for the Rho-kinase inhibitor HA-1077 is fasudil. In chapter six the therapeutic potential of fasudil for treatment of cerebral vasospasm is discussed.

***Tissue-specific intervention***

Tissue-specific intervention of the myogenic tone could be a possibility if there is vascular heterogeneity. This means specific modulation of the cerebral myogenic tone could be done if a signaling mechanism of the myogenic response specific to the cerebral circulation exists. (Hill et al., 2009) This could be beneficial for the medical conditions that might be caused by defects of the cerebral myogenic response as discussed in chapter four. Targeting $BK_{Ca}$ channels has the potential to specifically modulate the cerebral myogenic tone since $BK_{Ca}$ channels mediate the negative feedback mechanism specific to the cerebral myogenic response as explained in chapter two. The mechanosensative membrane channels TRPM4 and TRPC6 could also be good targets for specific modulation of the cerebral myogenic tone if the critical role of these TRPs in the myogenic response is specific to cerebral arteries. (Earley et al., 2004) (Welsh, Morielli, Nelson, & Brayden, 2002)

## 6. Therapeutic modulation of the cerebrovascular myogenic tone

In their article on the therapeutic potential of pharmacologically targeting the arteriolar myogenic tone Hill et al. proposed that the ability to reset the myogenic tone could enable modification of systemic vascular resistance and pressure without interfering with the neurohumoral regulatory mechanisms. Increasing the myogenic tone would be advantageous in medical conditions associated with a depressed cardiovascular system such as shock. Decreasing the myogenic tone could be beneficial in some hyperdynamic states. (Hill et al., 2009)

***Protein nitrases for recovery from actin nytrosilation due to ischemia/reperfusion***

In chapter four defects of the myogenic response are shown in different medical conditions, including ischemia/reperfusion-induced impairment of cerebrovascular myogenic tone and reactivity. It is explained how peroxynitrite formed during ischemia/reperfusion leads to impairment of cerebrovascular myogenic tone and reactivity due to nytrosylation of filamentous actin. Protein nitrases might exist that can denitrify nytrosylated proteins. (Maneen & Cipolla, 2007) If actin can be denitrified by protein nitrases, these enzymes could be a good pharmacological target for prevention or treatment of ischemia/reperfusion-induced impairment of cerebrovascular myogenic tone and reactivity.

***Modulation of the myogenic tone as therapy for SAH-induced cerebral vasospasm and potential of the rho-kinase inhibitor fasudil***

Cerebral vasospasm after subarachnoid hemorrhage (SAH) also coincides with a defect in the myogenic response. Standard treatment for cerebral vasospasm is nimodipine, which blocks L-type voltage gated calcium channels that mediate the myogenic response as

discussed in chapter two. Another therapy for prevention and treatment of cerebral vasospasm is called Triple H therapy. Triple H therapy combines hypervolemia, hemodilution and hypertension to increase the systemic systolic pressure to 180-220 mm Hg to recover normal levels of blood flow. Although the therapy works against the symptoms of vasospasm, it also increase the health risks such as heart failure, cerebral edema, electrolyte imbalances and intracranial bleeding due to forced dilation, autoregulatory breakthrough and subsequent increased cerebral blood flow. (Ishiguro et al., 2002) If therapeutic modulation of the cerebral myogenic tone could recover cerebral blood flow by reducing the myogenic tone of vasospastic cerebral arteries to normal levels this therapy could be more beneficial than Triple H therapy.

As explained in chapter four, an increase of RhoA/Rho-kinase activity might cause cerebral vasospasm. In chapter five, the RhoA/Rho-kinase pathway is identified as a possible therapeutic target for modulation of the myogenic tone. These two findings give reason to target the RhoA/Rho-kinase pathway in therapy of cerebral vasospasm. The protein kinase inhibitor and potent vasodilator fasudil can be used as an intraarterial agent against SAH-induced cerebral vasospasm. Fasudil specifically inhibits cerebrovascular myosin light-chain kinase, protein kinase C and most potently Rho-kinase. Investigation of treatment with this drug led to the conclusion that it is safe and effective, but more clinical trials are needed to verify this. (Sayama, Liu, & Couldwell, 2006) Fasudil has been in clinical use in Japan since 1995.

***Antagonists of R-type VDCCs against SAH-induced cerebral vasospasm***

R-type voltage-dependent calcium channels (R-type VDCCs) may represent a novel therapeutic target for prevention of cerebral vasospasm. (Ishiguro et al., 2005) Ishiguro et al. found that after subarachnoid hemorrhage cerebral VSM cells express R-type VDCCs (gene $Ca_v$ 2.3) in addition to the normal expression of L-type VDCCs (gene $Ca_v$ 1.2). This leads to enhanced $Ca^{2+}$ entry and increased myogenic tone seen in cerebral vasospasm. It was found that SNX-482, a specific R-type VDCC blocking peptide dilates cerebral arteries of an animal model of SAH, but not of healthy animals. (Ishiguro et al., 2005) Wang et al. conclude that the R-type channel antagonist SNX-482 has more potential than the L-type channel antagonist nimodipine for the improvement of cerebral blood flow in cerebral vasospasm. (Wang, Yin, Jia, & Jiang, 2010) Suppression of R-type VDCC activity may help to treat SAH-induced cerebral vasospasm.

***$K_V$7 activating drugs against SAH-induced cerebral vasospasm***

$K_V$ channels are a possible pharmacological target for treatment of SAH-induced cerebral vasospasm due to their role in oxyhemoglobin-induced vasospasm, as explained in chapter four. (Koide et al., 2007) Zhong et al. have proposed $K_V$7 activating drugs as candidates for cerebral vasospasm therapy. Zhong et al. have shown that activation of $K_V$7 channels with S-1 reversed the enhanced myogenic tone and myogenic reactivity due to ScTx1 treatment of rat middle cerebral arteries and this effect was pressure-dependent. (Zhong et al., 2010) The ongoing search for $K_V$7 channel activators might result in a novel therapy for the excessive myogenic tone seen in SAH-induced cerebral vasospasm.

***Inhibition of 20-HETE synthesis to reduce cerebral infarct size or reverse cerebral vasospasm***

As explained in chapter two, 20-HETE is a potent vasoconstrictor that possibly plays a role in pressure-induced constriction of cerebral arteries. Inhibition of 20-HETE synthesis has been proposed as a therapy for size reduction of ischemia-induced cerebral infarcts. (Renic et al., 2009) This beneficial effect of 20-HETE synthesis inhibition is unrelated to the role of 20-HETE in regulating cerebral blood flow. Inhibition of 20-HETE synthesis actually impairs autoregulation of cerebral blood flow. (Gebremedhin et al., 2000) The beneficial effect of 20-HETE synthesis inhibition is probably mediated

through a direct neuroprotective effect. Examples of 20-HETE synthesis inhibitors are HET0016 and TS-011. HET0016 can block 20-HETE synthesis on the long term, while TS-011 can block acutely. TS-011 is the most potent and specific inhibitor of the 20-HETE synthesis. It crosses the blood-brain barrier and selectively inhibits cerebral 20-HETE synthesis. (Renic et al., 2009) It was shown that TS-011 reduces infarct volume in middle cerebral artery occlusion model of ischemic stroke and collagenase induced intracerebral hemorrhage models. (Miyata & Roman, 2005)

20-HETE might be involved in the development of cerebral vasospasm after subarachnoid hemorrhage. The 20-HETE levels rise in the cerebrospinal fluid of rats after SAH. (Kehl et al., 2002) (Cambj-Sapunar, Yu, Harder, & Roman, 2003) It has been hypothesized that the release of 5-HT after subarachnoid hemorrhage activates 5-HTB1 receptors and 20-HETE synthesis. (Miyata & Roman, 2005) This leads to cerebral vasospasm, because 20-HETE is a vasoconstrictor. Inhibition of 20-HETE synthesis with 17-ODYA or HET0016 prevents the fall in cerebral blood flaw after subarachnoid hemorrhage. Treatment with TS-011 has been shown to reverse cerebral vasospasm in rat and models of SAH. (Takeuchi et al., 2005) (Hacein-Bey et al., 2006) This suggests 20-HETE synthesis inhibition is a potential therapy for SAH-induced cerebral vasospasm.

## Conclusion

The cerebral myogenic response maintains autoregulation of the cerebral blood flow. Cerebral arteries respond to pressure elevations by vasoconstriction and to pressure drops by vasodilation. This prevents harmful effects of changes in cerebral blood flow due to pressure changes. The cerebral myogenic response does not differ much from the general principle. Except that the endothelium may play a role in it and that cerebral arteries show a negative feedback mechanism in the form of pressure-induced activation of $BK_{Ca}$ channels. The differences result in relatively dilated cerebral arteries a strong cerebral myogenic response. These are important characteristics of such a crucial organ as the brain since they keep cerebral blood flow constant and the risk of ischemia low. Some medical conditions coincide with defects of the cerebral myogenic response. Ischemia/reperfusion, eclampsia and stroke development show impaired myogenic tone and reactivity. In contrast, cerebral vasospasm after subarachnoid hemorrhage shows increased myogenic tone and reactivity. The myogenic response could be modulated to prevent or treat disease. The preferred pharmacological targets are specific to the myogenic response instead of the general signaling pathways of vascular smooth muscle contraction in order to prevent interference in the neurohumoral regulatory mechanisms. Targets specific to the myogenic response could be cellular mechanisms of mechanosensation, but it is not yet fully understood how this works. They could include pressure-sensitive membrane channels, integrins or 20-HETE. Due to the role of $BK_{Ca}$ channels in the negative feedback of the cerebral myogenic response $BK_{Ca}$ channels could be good pharmacological targets for the specific modulation of the cerebral myogenic response. Also the RhoA/Rho-kinase pathway has been identified as a possible target, so Rho-kinase inhibitors could lower the myogenic tone and vasodilation to prevent or treat cerebral vasospasm. Vascular heterogeneity must be studied in order to determine tissue-specific reaction to pharmacological intervention in the mentioned mechanisms of the myogenic response. Also other affected mechanisms in medical conditions that coincided with an altered myogenic response could be therapeutically targeted such as ischemia/reperfusion induced actin nytrosylation, subarachnoid hemorrhage-induced R-type VDCC emergence and oxyhemoglobin-induced $K_V$ channel suppression. So both treatment of defects of the cerebral myogenic response and modulation of the myogenic tone by targeting molecular mechanisms of the myogenic response could help in countering cerebrovascular disease.

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